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ROZPRAWA DOKTORSKA

mgr Sebastian Makuch

Cukrowa pochodna cynkowej ftalocyjaniny jako innowacyjne narzędzie terapii fotodynamicznej w leczeniu łuszczycy

Promotor: Prof. dr hab. n. med. Piotr Ziółkowski

Promotor pomocniczy: dr n. med. Marta Woźniak

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Pragnę serdecznie podziękować wszystkim osobom, które w jakikolwiek sposób przyczyniły się do powstania tej pracy doktorskiej.

Na samym początku chciałbym wyrazić głęboką wdzięczność moim promotorom, prof. dr hab. Piotrowi Ziółkowskiemu oraz dr Marcie Woźniak. Ich nieoceniona pomoc oraz wsparcie merytoryczne były kluczowe dla mojego rozwoju naukowego. Dziękuję za wszystkie cenne uwagi oraz wskazówki, które motywowały mnie do dalszej pracy i doskonalenia swoich umiejętności badawczych.

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Nie mogę zapomnieć też o mojej siostrze, Patrycji. Twoje wsparcie oraz wiara w moje możliwości były dla mnie bardzo ważne.

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Spis treści

1. Cykl prac stanowiących rozprawę doktorską

1. **Makuch Sebastian**, Woźniak Marta, Krawczyk Monika, Pastuch-Gawołek Gabriela, Szeja Wiesław, Agrawal Siddarth: Glycoconjugation as a promising treatment strategy for psoriasis, Journal of Pharmacology and Experimental Therapeutics, 2020, vol. 373, nr 2, s. 204-212, DOI:10.1124/jpet.119.263657, 140 punktów, IF(4,03)

IF = 4,03; Punkty MEiN = 140

2. **Makuch Sebastian**, Kupczyk Piotr, Makarec Alicja, Chodaczek Grzegorz, Ziółkowski Piotr, Woźniak Marta: The impact of proinflammatory cytokines and imiquimod on GLUT1 in HaCaT keratinocytes - a potential anti-psoriatic therapeutic target?, Cellular Physiology and Biochemistry, 2023, vol. 57, nr 2, s. 54-62, DOI:10.33594/000000615, 140 punktów, IF(2,5)

IF = 2,5; Punkty MEiN = 140

3. **Makuch Sebastian**, Kupczyk Piotr, Woźniak Marta, Makarec Alicja, Lipińska Maja, Klyta Magdalena, Sulecka-Zadka Joanna, Szeja Wiesław, Gani Mariachiara, Rapozzi Valentina, Ziółkowski Piotr, Smoleński Piotr: In vitro and in vivo antipsoriatic efficacy of protected and unprotected sugar-zinc phthalocyanine conjugates, Pharmaceutics, 2024, vol. 16, nr 6, art.838 [20 s.], DOI:10.3390/pharmaceutics16060838, 100 punktów, IF(4,9)

IF = 4,9; Punkty MEiN = 100

4. **Makuch Sebastian**, Dróżdż Mateusz, Makarec Alicja, Ziółkowski Piotr, Woźniak Marta: An update on photodynamic therapy of psoriasis - current strategies and nanotechnology as a future perspective, International Journal of Molecular Sciences, 2022, vol. 23, nr 17, art.9845 [18 s.], DOI:10.3390/ijms23179845

IF = 5,6; Punkty MEiN = 140

Sumaryczny IF: 17,03
Sumaryczna ilość punktów MEiN: 520

2. Wykaz skrótów

PDT - Terapia fotodynamiczna (Photodynamic Therapy)

ALA - Kwas 5-aminolewulinowy (5-Aminolevulinic Acid)

GLUT1 - Transporter glukozy typu 1 (Glucose Transporter 1)

IL - Interleukina (Interleukin)

TNF-α - Czynnik martwicy nowotworów alfa (Tumor Necrosis Factor alpha)

IMQ - Imikwimod (Imiquimod)

HaCaT – Unieśmiertelniona linia komórkowa ludzkich keratynocytów (Human Adult Low Calcium Temperature keratinocytes)

DMEM - Zmodyfikowane podłoże Eagle'a Dulbecco (Dulbecco's Modified Eagle Medium)

qPCR - Ilościowa reakcja łańcuchowa polimerazy (Quantitative Polymerase Chain Reaction)

ZnPc - Cynkowa ftalocyjanina (Zinc Phthalocyanine)

FT-IR - Spektroskopia w podczerwieni z transformatą Fouriera (Fourier Transform Infrared Spectroscopy)

NMR - Rezonans magnetyczny jądrowy (Nuclear Magnetic Resonance)

MALDI-TOF-MS - Spektrometria mas z laserową desorpcją i jonizacją wspomaganą matrycą w czasie przelotu (Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry)

HPLC - Wysokosprawna chromatografia cieczowa (High-Performance Liquid Chromatography)

MTT - Test cytotoksyczności (MTT assay - [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide])

ROS - Reaktywne formy tlenu (Reactive Oxygen Species)

DCFH-DA - Dichlorodihydrofluoresceina diacetate (2′,7′-Dichlorodihydrofluorescein Diacetate)

DCF - Dichlorofluoresceina (Dichlorofluorescein)

PASI - Wskaźnik powierzchni i nasilenia łuszczycy (Psoriasis Area and Severity Index)

DMSO - Dimetylosulfotlenek (Dimethyl Sulfoxide)

ELISA - Test immunoenzymatyczny (Enzyme-Linked Immunosorbent Assay)

SD - Odchylenie standardowe (Standard Deviation)

IC50 – Medialne stężenie powodujące 50% inhibicji (half maximal inhibitory concentration)

3. Omówienie rozprawy doktorskiej

3.1 Wstęp

Łuszczyca jest przewlekłą chorobą skóry o podłożu immunologicznym, charakteryzująca się nadmierną proliferacją keratynocytów. Szacuje się, że częstość jej występowania waha się od 0,14% do 5,32% populacji, dotykając około 125 milionów ludzi na całym świecie. Objawy łuszczycy, takie jak zaczerwienienie, łuszcząca się skóra, świąd czy uczucie napięcia skóry, mają negatywny wpływ na jakość życia pacjentów. Etiologia i patogeneza łuszczycy są złożone i wciąż nie do końca poznane. Paradoksalnie, pomimo znaczącego postępu, szczególnie w obszarze leków biologicznych, wielu pacjentów z umiarkowaną i ciężką postacią łuszczycy nadal potrzebuje nowych leków, które zagwarantują wyższą skuteczność, bezpieczeństwo, oraz niższe koszty leczenia.

Jedną z najbardziej obiecujących metod leczenia zmian łuszczycowych jest forma fototerapii znana jako terapia fotodynamiczna (PDT), która polega na ogólnoustrojowym lub miejscowym podaniu fotouczulacza, a następnie selektywnym naświetlaniu zmiany skórnej światłem widzialnym. PDT opiera się na interakcji pomiędzy fotouczulaczem, światłem o określonej długości fali oraz tlenem. Początkowo PDT była stosowana w leczeniu chorób skóry, a następnie ewoluowała jako terapia przeciwnowotworowa.

Wiele fotouczulaczy stosowanych w PDT jest obecnie intensywnie badanych w kontekście nowotworów, jednak w leczeniu łuszczycy badania pod tym kątem są znacznie bardziej ograniczone. Skuteczność stosowanych klinicznie fotouczulaczy w leczeniu łuszczycy, takich jak kwas 5–aminolewulinowy (ALA), jest niedostateczna, a pacjenci często zgłaszają działania niepożądane, takie jak ból lub uczucie pieczenia. Fakt ten wyraźnie wskazuje na potrzebę poszerzenia badań nad fotouczulaczami drugiej generacji. Pomimo obiecujących wyników, PDT pozostaje pod kątem klinicznym niedostatecznie wykorzystywana, co podkreśla konieczność przetestowania szerszego spektrum fotouczulaczy na pacjentach z łuszczycą.

W porównaniu do fotouczulaczy pierwszej generacji i ich prekursorów, ftalocyjaniny, będące fotouczulaczami drugiej generacji, wykazują wysoki profil bezpieczeństwa i skuteczności. Charakteryzują się wysoką fotostabilnością oraz optymalną długością fali wzbudzenia (670 nm), umożliwiającą głębszą penetrację tkanek biologicznych. Pomimo obiecujących efektów

w PDT, zastosowanie ftalocyjanin jest ograniczone głównie z powodu niskiej specyficzności tkankowej oraz słabej rozpuszczalności. W celu obejścia tych ograniczeń, w publikacjach składających się na rozprawę zaproponowano strategię glikokoniugacji ftalocyjanin, która oferuje poprawę biodostępności, rozpuszczalności oraz selektywnej akumulacji skoniugowanych z cukrami leków w komórkach charakteryzujących się nadekspresją transporterów glukozy.

3.2 Główny cel badań i problem badawczy

Głównym celem badań była ocena metody glikokoniugacji cynkowej ftalocyjaniny oraz jej potencjalnego zastosowania w terapii fotodynamicznej łuszczycy, poprzez wykorzystanie nadekspresji transportera glukozy GLUT1, a także zbadanie wpływu cytokin prozapalnych na ekspresję GLUT1 w keratynocytach, z wykorzystaniem łuszczycopodobnego modelu in vitro oraz in vivo. Głównym problemem badawczym była ocena, czy nadekspresja transportera glukozy GLUT1 w keratynocytach, stymulowana przez cytokiny prozapalne, może być skutecznie wykorzystana w celu zwiększenia selektywności glikokoniugatów cynkowej ftalocyjaniny.

3.3 Cele szczegółowe pracy

a) Przedstawienie metody glikokoniugacji jako potencjalnej strategii terapii celowanej łuszczycy, mającej na celu zwiększenie selektywności leków poprzez wykorzystanie transportera glukozy GLUT1 w proliferujących keratynocytach.
b) Ocena wpływu cytokin prozapalnych (IL-6, IL-17, IL-23, IL-36, TNF-α) oraz imikwimodu (IMQ) na ekspresję transportera GLUT1 w keratynocytach HaCaT.
c) Analiza mechanizmów transportu glikokoniugatów cynkowej ftalocyjaniny w keratynocytach HaCaT stymulowanych cytokinami
d) Ocena skuteczności terapii fotodynamicznej z wykorzystaniem glikokoniugatów cynkowej ftalocyjaniny na łuszczycopodobnym modelu *in vitro* oraz *in vivo*.

e) Przedstawienie aktualnego stanu wiedzy na temat terapii fotodynamicznej w leczeniu łuszczycy oraz omówienie potencjalnych przyszłych zastosowań nanotechnologii w tej dziedzinie.

3.4 Materiały i metody

Pierwszą pracą w cyklu rozprawy doktorskiej jest artykuł przeglądowy, który jako pierwszy proponuje strategię glikokoniugacji w leczeniu łuszczycy i przedstawia ją jako potencjalną metodę terapii celowanej. Celem tej strategii jest poprawa farmakokinetyki oraz selektywności leków przeciwłuszczycowych poprzez wykorzystanie nadekspresji transportera glukozy GLUT1 w komórkach hiperproliferujących. W artykule przedstawiono perspektywy w projektowaniu leków przeciwłuszczycowych, proponując możliwości przyłączania do nich cukrów.

Druga publikacja ocenia wpływ stymulacji cytokinami prozapalnymi, takimi jak IL-6, IL-17, IL-23, IL-36 i TNF-α (wszystkie w stężeniu 100 ng/ml), a także imikwimodem (IMQ, 1 μM) na ekspresję transportera GLUT1. Do badań wykorzystano unieśmiertelnioną linię komórkową ludzkich keratynocytów HaCaT, hodowanych w warunkach wysokiej zawartości glukozy (medium DMEM) z dodatkiem 10% surowicy płodowej bydlęcej, antybiotyków oraz L-glutaminy, w temperaturze 37°C. Komórki stymulowano cytokinami lub imikwimodem przez 48 godzin, po czym sprawdzono ekspresję genów za pomocą qPCR, natomiast poziom białka GLUT1 oceniono przy użyciu immunofluorescencji oraz metody Western blot. Dodatkowo, sprawdzono poziom wychwytu glukozy przez komórki HaCaT po stymulacji i porównano go z niestymulowanymi komórkami kontrolnymi.

Trzecia praca z cyklu przedstawia syntezę chemiczną, charakterystykę oraz ocenę aktywności biologicznej cukrowych pochodnych cynkowej ftalocyjaniny na łuszczycopodobnym modelu komórkowym oraz mysim. Podstawowe odczynniki i rozpuszczalniki niezbędne do syntezy chemicznej pochodziły z firm Sigma-Aldrich (St. Louis, MO, USA) oraz Thermo Scientific (Waltham, MA, USA), natomiast 4-nitroftalonitryl oraz cynkową ftalocyjaninę (ZnPc) zakupiono od TCI Chemicals (Tokyo, Japan). Charakterystyka związków była przeprowadzana za pomocą spektroskopii FT-IR (Bruker IFS 1113v), spektroskopii NMR (Bruker 600 AMX), analizy elementarnej (Vario ELCube), spektrofluorymetrii (FSL980) oraz spektrometrii mas (MALDI-TOF-MS). Czystość związków określano za pomocą HPLC z użyciem kolumny Phenomenex Aeris. Syntezy cukrowych pochodnych ftalocyjaniny cynku: a) glukozowej

pochodnej cynkowej ftalocyjaniny z zabezpieczeniami acetylowymi (Glu-4-ZnPc-P); b) galaktozowej pochodnej cynkowej ftalocyjaniny z zabezpieczeniami acetylowymi (Gal-4-ZnPc-P); c) glukozowej pochodnej cynkowej ftalocyjaniny z niezabezpieczoną częścią cukrową (Glu-4-ZnPc), przeprowadzono zgodnie z opublikowanymi procedurami.

Do badań *in vitro* wykorzystano unieśmiertelnioną linię komórkową ludzkich keratynocytów HaCaT, którą stymulowano przez 24 godziny cytokinami prozapalnymi (IL-6, IL-17A, IL-23). Oceniono fotocytotoksyczność cukrowych koniugatów cynkowej ftalocyjaniny za pomocą testu MTT, mierząc aktywność metaboliczną komórek po naświetlaniu światłem czerwonym o fluencji 0,9 J/cm². Sprawdzono również poziom wychwytu wewnątrzkomórkowego badanych fotouczulaczy za pomocą cytometrii przepływowej oraz lokalizację subkomórkową z użyciem mikroskopii fluorescencyjnej. Ponadto, poziom reaktywnych form tlenu (ROS) po naświetleniu oceniano za pomocą barwnika DCFH-DA, który po utlenieniu przekształca się w fluorescencyjny DCF, a jego sygnał fluorescencyjny mierzono za pomocą czytnika płytek Bio-Tek (Corning Incorporated, New York, USA) (długość fal wzbudzenia i emisji odpowiednio 488 nm oraz 525 nm). Aktywność biologiczna koniugatów była porównywana z wolną ftalocyjaniną cynku we wszystkich eksperymentach przeprowadzonych na komórkach.

Badania *in vivo* przeprowadzono na modelu mysim z wywołanym zapaleniem skóry przy użyciu 62,5 mg 5% imikwimodu (IMQ) przez 7 dni. Łącznie 60 myszy (połowa samców i połowa samic) podzielono na sześć grup: a) grupa kontrolna; b) IMQ z DMSO; c) kontrola naświetlania; d) grupa z niską dawką Glu-4-ZnPc-P-PDT (0,30 mg/kg); e) średnią dawką (0,60 mg/kg) i f) wysoką dawką (1,20 mg/kg). Myszom C57BL/6, w wieku 8-11 tygodni i wadze 15-29 g, podawano dożylnie koniugaty cynkowej ftalocyjaniny rozpuszczone w DMSO, a następnie po 4h naświetlano ogolony obszar skóry czerwonym laserem o fluencji 15 J/cm² przez 7 minut. Zmiany skórne po terapii oceniano za pomocą skali PASI codziennie przez 7 dni, a następnie pobrano do dalszych badań próbki skóry, śledziony, wątroby oraz krwi, w tym histologii i pomiaru poziomów cytokin (IL-6, IL-17A, IL-23) metodą ELISA.W celu przeprowadzenia badań in vivo, pozyskano zgodę Lokalnej Komisji Etycznej we Wrocławiu (nr zgody: 035/2023).

Analiza statystyczna wyników została przeprowadzona z użyciem oprogramowania Prism 8.0.1 (GraphPad Software Inc., San Diego, CA, USA). Zastosowano test t dla prób niezależnych, a wyniki badań *in vitro* oraz pozyskanych z materiału biologicznego myszy przedstawiono jako średnią ± SD z trzech niezależnych eksperymentów. Do określenia liczby zwierząt zastosowano

obliczenia wielkości próby i analizę mocy testu; wartości *p* mniejsze niż 0,05 uznawano za statystycznie istotne.

3.5 Podsumowanie wyników

Pierwszym etapem badań zrealizowanych w ramach niniejszej pracy doktorskiej była ocena wpływu cytokin prozapalnych na poziom ekspresji transportera GLUT1. Wyniki analizy qPCR wykazały, iż stymulacja komórek HaCaT IL-6, IL-17. IL-23 oraz IL-36, prowadziła do wzrostu ekspresji genu *SLC2A1* kodującego GLUT1, podczas gdy TNF-α nie wpłynął znacząco na jego ekspresję. Analiza immunofluorescencyjna dodatkowo potwierdziła uzyskany wynik. Ocena densytometryczna analizy Western blot z kolei wykazała, iż wszystkie z badanych cytokin oraz imikwimod, zwiększają poziom białka GLUT1. Na koniec oceniono wpływ egzogennej stymulacji cytokinami oraz imikwimodem na poziom wychwytu 2-deoksy-D-glukozy (2-DG) przez komórki HaCaT. Najwyższy wychwyt 2-DG zaobserwowano po stymulacji IL-23 (1.93x), a najniższy po stymulacji TNF-α (1.07x).

Wyniki badań przedstawionych w kolejnej pracy jednoznacznie potwierdziły, iż tetra-substytuowana glukozowa pochodna cynkowej ftalocyjaniny z zabezpieczeniami acetylowymi (Glu-4-ZnPc-P) wykazuje najwyższą fotocytotoksyczność wobec komórek HaCaT stymulowanych cytokinami (IC_{50} = 2.55 μM), w porównaniu do Glu-4-ZnPc (IC_{50} = 22.7 μM), Gal-4-ZnPc-P (IC_{50} = 7.13 μM) oraz ZnPc (IC_50 = 5.842 μM) po 24 godzinach. Ponadto, po 24-godzinnej inkubacji komórek ze związkami w ciemności bez naświetlania, aktywność cytotoksyczna koniugatów ZnPc z cukrami była niższa w porównaniu do ZnPc.

Dalsze badania biologiczne dowiodły, iż stymulacja cytokinami prozapalnymi IL-6, IL-17A i IL-23, zwiększa wewnątrzkomórkową akumulację związków Glu-4-ZnPc i Glu-4-ZnPc-P, podczas gdy selektywny dla GLUT1 inhibitor BAY-876 zmniejsza akumulację obu związków w komórkach HaCaT. Z pośród wyżej wymienionych cytokin, IL-17A wykazała największy wpływ na akumulację związków przez keratynocyty, powodując wzrost wychwytu Glu-4-ZnPc o 56.3% oraz Glu-4-ZnPc-P o 27.6%. W przeciwieństwie do Glu-4-ZnPc i Glu-4-ZnPc-P, średnia intensywność fluorescencji komórek HaCaT traktowanych związkami Gal-4-ZnPc-P oraz ZnPc nie uległa zmianie, zarówno po stymulacji cytokinami, jak i po inkubacji z inhibitorem BAY-876. Zgodnie z oczekiwaniami, wychwyt wewnątrzkomórkowy koniugatu Glu-4-ZnPc z niezabezpieczoną częścią cukrową był znacznie wydajniejszy od pozostałych związków.

Obserwacje za pomocą mikroskopii fluorescencyjnej potwierdziły, że wszystkie z badanych ftalocyjanin, a w szczególności Glu-4-ZnPc, lokalizowały się preferencyjnie w mitochondriach komórek HaCaT. Pozostałe pochodne, czyli Glu-4-ZnPc-P oraz Gal-4-ZnPc-P, wykazały niższą wewnątrzkomórkową akumulację w mitochondriach, w porównaniu do Glu-4-ZnPc oraz nieskoniugowanej ZnPc. Subkomórkowa kolokalizacja wszystkich testowanych związków z barwnikiem specyficznym dla lizosomów była znikoma. Zgodnie z hipotezą badawczą, glukozowa pochodna z niezabezpieczonymi resztami cukrowymi charakteryzowała się najwyższą wydajnością wychwytu komórkowego, co było spójne z wynikami uzyskanymi metodą cytometrii przepływowej. Paradoksalnie, koniugat, który najefektywniej był transportowany do wnętrza komórki, nie wykazywał najwyższego efektu fotocytotoksycznego.

Naświetlanie badanych ftalocyjanin, zgodnie z założeniami, prowadziło do produkcji reaktywnych form tlenu (ROS), jednego z głównych mediatorów śmierci komórkowej indukowanej przez PDT. W badaniu określającym poziom produkcji ROS wykazano, iż preinkubacja komórek HaCaT z IL-17A znacząco zwiększała produkcję ROS po zastosowaniu Glu-4-ZnPc-P.

Kolejne badania dotyczyły oceny działania koniugatu Glu-4-ZnPc-P na mysim modelu łuszczycy indukowanej imikwimodem (IMQ). Myszy, które poddano naświetlaniu po podaniu dożylnym Glu-4-ZnPc-P, wykazywały istotną redukcję wskaźnika PASI w porównaniu do grup kontrolnych. Poprawa ta była również zauważalna w postaci redukcji splenomegalii wywołanej przez IMQ, a analiza histopatologiczna potwierdziła przywrócenie prawidłowej morfologii skóry, a także zmniejszenie nacieków leukocytarnych i grubości naskórka. Ponadto, poziom IL-6 oraz IL-17A był istotnie niższy w surowicy myszy poddanych naświetlaniu po zastosowaniu Glu-4-ZnPc-P, co dodatkowo potwierdziło działanie przeciwzapalne tego związku na łuszczycopodobnym modelu zapalenia skóry indukowanym imikwimodem.

Podsumowując, wyniki pracy doktorskiej podkreślają wysoki potencjał terapeutyczny cukrowych pochodnych cynkowych ftalocyjanin, w szczególności Glu-4-ZnPc-P, w kontekście celowanej terapii fotodynamicznej łuszczycy. Opisane w niniejszej rozprawie wyniki mogą stanowić solidną podstawę dla przyszłych badań klinicznych nad zastosowaniem koniugatów glukozy w leczeniu łuszczycy oraz innych chorób skóry.

3.6 Wnioski

- Stymulacja keratynocytów HaCaT cytokinami prozapalnymi (IL-6, IL-17, IL-23, IL-36) oraz imikwimodem (IMQ) zwiększa poziom ekspresji transportera GLUT1
- Glukozowa pochodna cynkowej ftalocyjaniny z zabezpieczeniami acetylowymi Glu-4-ZnPc-P wykazuje wyższą fotocytotoksyczność, w porównaniu do wolnej cynkowej ftalocyjaniny (ZnPc)
- Glukozowa pochodna cynkowej ftalocyjaniny z niezabezpieczoną resztą cukrową Glu-4-ZnPc charakteryzuje się najefektywniejszym transportem wewnątrzkomórkowym spośród badanych ftalocyjanin oraz preferencyjną akumulacją w mitochondriach
- Selektywny dla GLUT1 inhibitor BAY-876 hamuje transport koniugatów glukozy w komórkach HaCaT
- Koniugat Glu-4-ZnPc-P, po podaniu dożylnym i naświetlaniu, prowadzi do redukcji wskaźnika PASI, zmniejszenia poziomu cytokin prozapalnych (IL-6, IL-17, IL-23) we krwi oraz do ogólnej poprawy kondycji skóry u myszy z łuszczycopodobnym zapaleniem skóry indukowanym imikwimodem, co świadczy o wysokim potencjale terapeutycznym tego związku

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Minireviews

Glycoconjugation as a Promising Treatment Strategy for Psoriasis

Sebastian Makuch, Marta Woźniak, Monika Krawczyk, Gabriela Pastuch-Gawołek, Wiesław Szeja, and Siddarth Agrawal

Department of Pathology, Faculty of Medicine, Wroclaw Medical University, Wroclaw, Poland (S.M., M.W., S.A.); Department and Clinic of Internal Medicine, Occupational Diseases, Hypertension and Clinical Oncology, Wroclaw Medical University, Wroclaw, Poland (S.A.); and Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Faculty of Chemistry (M.K., G.P.-G., W.S.) and Biotechnology Centre (M.K., G.P.-G., W.S.), Silesian University of Technology, Gliwice, Poland

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ABSTRACT

Despite the progress in the development of novel treatment modalities, a significant portion of patients with psoriasis remains undertreated relative to the severity of their disease. Recent evidence points to targeting the glucose transporter 1 and sugar metabolism as a novel therapeutic strategy for the treatment of psoriasis and other hyperproliferative skin diseases. In this review, we discuss glycoconjugation, an approach that facilitates the pharmacokinetics of cytotoxic molecules and ensures their preferential influx through glucose transporters. We propose pathways of glycoconjugate synthesis to increase effectiveness, cellular selectivity, and tolerability of widely used antipsoriatic drugs. The presented approach exploiting the heightened glucose requirement of proliferating keratinocytes bears the potential to revolutionize the management of psoriasis.

SIGNIFICANCE STATEMENT

Recent findings concerning the fundamental role of enhanced glucose metabolism and glucose transporter 1 overexpression in the pathogenesis of psoriasis brought to light approaches that proved successful in cancer treatment. Substantial advances in the emerging field of glycoconjugation highlight the rationale for the development of glucose-conjugated antipsoriatic drugs to increase their effectiveness, cellular selectivity, and tolerability. The presented approach offers a novel therapeutic strategy for the treatment of psoriasis and other hyperproliferative skin diseases.

Introduction

An increasing body of evidence is accumulating, suggesting a significant surge in the frequency of autoimmune diseases in the last decades (Lohi et al., 2007; Lerner and Matthias, 2015a). Psoriasis, rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, inflammatory bowel diseases, and systemic lupus erythematosus are several examples (Lerner and Matthias, 2015a,b). All of the mentioned conditions are a consequence of chronic activation of T and B lymphocytes in the absence of an infection or other detectable cause (Davidson and Diamond, 2001). The overall prevalence of autoimmunity is approximately 3%–5% in the general population, but the effects on mortality and morbidity are significant (Jacobson et al., 1997; Eaton et al., 2007). Ironically, despite enormous advances in the molecular sciences, diagnostic methods, and clinical classification, there is still an urgent need to improve the therapeutic outcome of patients with an autoimmune disease.

One of the most prevalent autoimmune diseases is psoriasis, which is a chronic inflammatory skin condition that affects 2%–3% of the population worldwide (Langley et al., 2005). Symptoms of psoriasis, which include redness, scaling, flaking, pruritus, skin tightness, pain, and bleeding, have a significant impact on patients' physical and mental well-being (Feldman et al., 2014). Patients with psoriatic diseases cannot only have skin and joint impairment but can also experience decreased quality of life and work productivity as well as severe medical comorbidities, such as increased cardiovascular events and depression (Armstrong et al., 2013; Lebwohl et al., 2014). The dominant feature of psoriasis is an aberrant hyperproliferation of keratinocytes (Lowes et al., 2007), which are the primary cell type of the epidermis and vital participants of the immune system, recruiting T cells to the skin (Lowes et al., 2014). Activated by interleukin (IL)-12 and IL-23, IL-17–producing T cells (Gerosa et al., 2008) produce abundant key psoriatic cytokines, such as tumor necrosis factor (TNF-α), interferon-gamma, and IL-17 and IL-23, that

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ABBREVIATIONS: GLUT1, glucose transporter 1; IL, interleukin; JAK, Janus activated kinases; TNF, tumor necrosis factor.

mediate effects on keratinocytes to intensify skin inflammation (Kim and Krueger, 2015). Exaggerated proliferation of keratinocytes is a hallmark of psoriasis and results from overexpression of the previously mentioned cytokines and growth factors (Hiebert and Werner, 2018). Currently used treatment agents, such as calcineurin inhibitors, anti-interleukin monoclonal antibodies against proinflammatory cytokines, fumaric acid esters, glucocorticoids, retinoids, and vitamin D derivatives, tend to suppress chronic inflammation (Boehncke and Schön, 2015). Unfortunately, despite the progress in the development of novel treatment modalities over the past decades, studies reveal that a significant proportion of patients with psoriasis remain undertreated relative to the severity of their disease (Lebwohl et al., 2014). Patients experience treatment failure with, or intolerance to, traditional systemic therapies and/or phototherapy and switch to biologic agents as second-line therapy. Side effects are the main reason for discontinuing traditional systemic therapies, whereas lack of efficacy is the main reason for discontinuing biologic agents (Lambert et al., 2017). Biologics were reported to lose their effectiveness after long-term use (Levin et al., 2014). The toxicity of traditional compounds such as methotrexate prevents many patients from obtaining a favorable outcome from the treatment because of the development of multiple toxic effects, including bone marrow suppression and gastrointestinal ulceration (Roenigk et al., 1988; Pearce and Wilson, 1996). Only 36%–58% of patients with moderate to severe psoriasis treated according to the guidelines reach current therapeutic targets of PASI75 (Psoriasis Area and Severity Index - represents the percentage of patients who have achieved a 75% or more reduction of the score and PASI90 (Psoriasis Area and Severity Index - represents the percentage of patients who have achieved a 90% or more reduction of the score) at 1 year, highlighting a gap in efficacy between selective clinical trials and the real-world setting. A population-based survey of patients, dermatologists, and rheumatologists found a high treatment dissatisfaction among the patients and a need for safe, effective, and easy-to-use therapies for psoriasis (Lebwohl et al., 2016). Thus, new, rationally designed agents are needed to replace or complement currently applied therapies. Glycoconjugation is a strategy that offers improved water solubility and stability and the potential for a selective accumulation of conjugated drugs by targeting glucose transporter 1 (GLUT1) receptors (Fig. 1), whose expression is highly abundant in proliferating psoriatic keratinocytes (Crunkhorn, 2018). A recently published paper by Zhang et al. (2018) in Nature Medicine revealed that glucose metabolism is essential for proliferating keratinocytes, highlighting a potential therapeutic target for pathologic hyperproliferation. In this review, we propose the conjugation of glucose to antipsoriatic drugs as a novel strategy for targeted delivery through GLUT1 receptors, which may potentially increase the effectiveness, cellular selectivity, and tolerability of antipsoriatic drugs and, thus, revolutionize the management of psoriasis.

Current Treatment Options and Their Limitations

The choice of psoriasis treatment depends on a wide variety of factors, such as disease severity, its effects on the patient's life, comorbidities, and health care access. Psoriatic patients are often assigned into two groups: those with mild psoriasis and those with moderate to severe psoriasis (Mrowietz et al., 2011). Mild psoriasis affects less than 3% of body surface area and responds well to topical application of vitamin D analogs or corticosteroids as well as localized UVB phototherapy (O'Neill and Feldman, 2010). Combinations of potent topical corticosteroids and vitamin D analogs or UVB phototherapy are commonly prescribed by dermatologists. With proper adherence, considerable improvement with these therapies may be seen in as little as 1 week, although several weeks may be required for full benefits (Kleyn et al., 2019). However, treatments including vitamin D analogs as well as UVB phototherapy may cause undesirable side effects, such as mild irritant dermatitis, hypercalcemia, burning, or photoaging (Singh et al., 2016; Kim et al., 2017). On the other hand, moderate to severe psoriasis is defined as involvement of more than 3%–10% of the body surface area and usually requires the addition of systemic treatments in combination with topical therapy. The effectiveness of topical therapy alone is limited in cases with severe symptoms, extensive skin lesions, and poor quality of life. Additionally, long-term use of topical corticosteroids induces side effects of local skin ulceration or suppression of the hypothalamic-pituitary-adrenal axis (Castela et al., 2012). Notably, methotrexate continues to be used as a first-line systemic agent since 1971 despite the fact that it yields an improvement in only 20%–30% of all patients with moderate-to-severe psoriasis (Maybury et al., 2014). Toxicity prevents many patients from obtaining a favorable outcome from the drug. It has been reported that multiple toxic effects occur in two-thirds of patients, and up to roughly 30% of patients discontinue methotrexate therapy within the first year of treatment because of hepatotoxicity (Bookstaver et al., 2008; Conway et al., 2015). New biologic and small-molecule therapies have been developed to complement or replace traditional drugs (Table 1). Biologic molecules that are being currently used for the treatment of psoriasis have been thoroughly described in several reviews (Lowes et al., 2007; Rønholt and Iversen, 2017; Rendon and Schäkel, 2019). Unlike earlier psoriatic treatments, biologic agents were designed to target T cells and specific inflammatory mediators, such as TNF or IL. The major concern of biologic therapeutics is the impact of long-term chronic immunosuppression, which may potentially lead to increased infection and cancer risk (Lowes et al., 2007). Only conclusions obtained from long-term clinical studies are able to distinguish whether the new therapies are successful. Biologics are also costly, inaccessible for the general public, and require repeated injections. Thus, the development of novel drugs to increase effectiveness and reduce toxicity is desirable.

Zhang et al. (2018) have recently found that targeting the glucose transporter GLUT1 and sugar metabolism offers a novel therapeutic strategy for the treatment of psoriasis and other hyperproliferative skin diseases. According to their results, excessively proliferating keratinocytes require glucose uptake through GLUT1, which is not a requisite for healthy skin development and function. The findings indicate that targeting elevated glucose intake and GLUT1 overexpression, which provide clinically corroborated strategies for cancer treatment (Bronstein et al., 2011; Vander Heiden, 2011; Cantor and Sabatini, 2012), could lead to the

development of safe and effective therapies for skin diseases (Fig. 1).

Glycoconjugation for Selective Glucose Transporter Targeting

Glucose is a fundamental energy source that is absorbed by cells through the plasma membrane. The transporters that allow cellular uptake of glucose can be classified into two distinct families: 1) active and energy-dependent sodium/glucose cotransporters and 2) passive, facilitative transporters (GLUTs) that use electrochemical gradients to transport glucose. There are 14 mammalian facilitative glucose transporters, among which GLUT1, the most common glucose transporter, is widely overexpressed in many human cancers, including hepatic, pancreatic, breast, esophageal, brain, renal, lung, cutaneous, colorectal, endometrial, ovarian, and cervical (Calvo et al., 2010; Szablewski, 2013; Liu and Auguste, 2015). To maintain cellular homeostasis, growth, and proliferation, cancer cells significantly increase glucose uptake and the flux of metabolites through glycolysis. This phenomenon, termed "the Warburg effect," arises from mitochondrial metabolic changes and is a characteristic trait of cancer (Warburg, 1956). Elevated glucose uptake and GLUT overexpression are frequent in neoplasms and provide clinically corroborated strategies for cancer treatment (Bronstein et al., 2011; Vander Heiden, 2011; Cantor and Sabatini, 2012). In general, glycoconjugation offers improved water solubility and stability and the potential for selective targeting. Therefore, it becomes an appealing strategy for targeted delivery of clinically prescribed drugs (Medina and Owen, 2002; Calvaresi and Hergenrother, 2013; Srinivasarao et al., 2015; Ashley, 2016), with significant advances in the field, reaching as far as late-stage human clinical trials (Pohl et al., 1995; Medina and Owen, 2002; Calvaresi and Hergenrother, 2013; Liu and Auguste, 2015; Granchi et al., 2016; Patra et al., 2016; Srinivasarao and Low, 2017).

Historically, it was suggested that the heightened requirement for glucose, constitutive overexpression of GLUT1 and the persistent metabolism of glucose to lactate, is an adaptation to a stressful and dynamic microenvironment, which is characteristic exclusively for solid tumors, in which concentrations of crucial nutrients and oxygen are spatially and

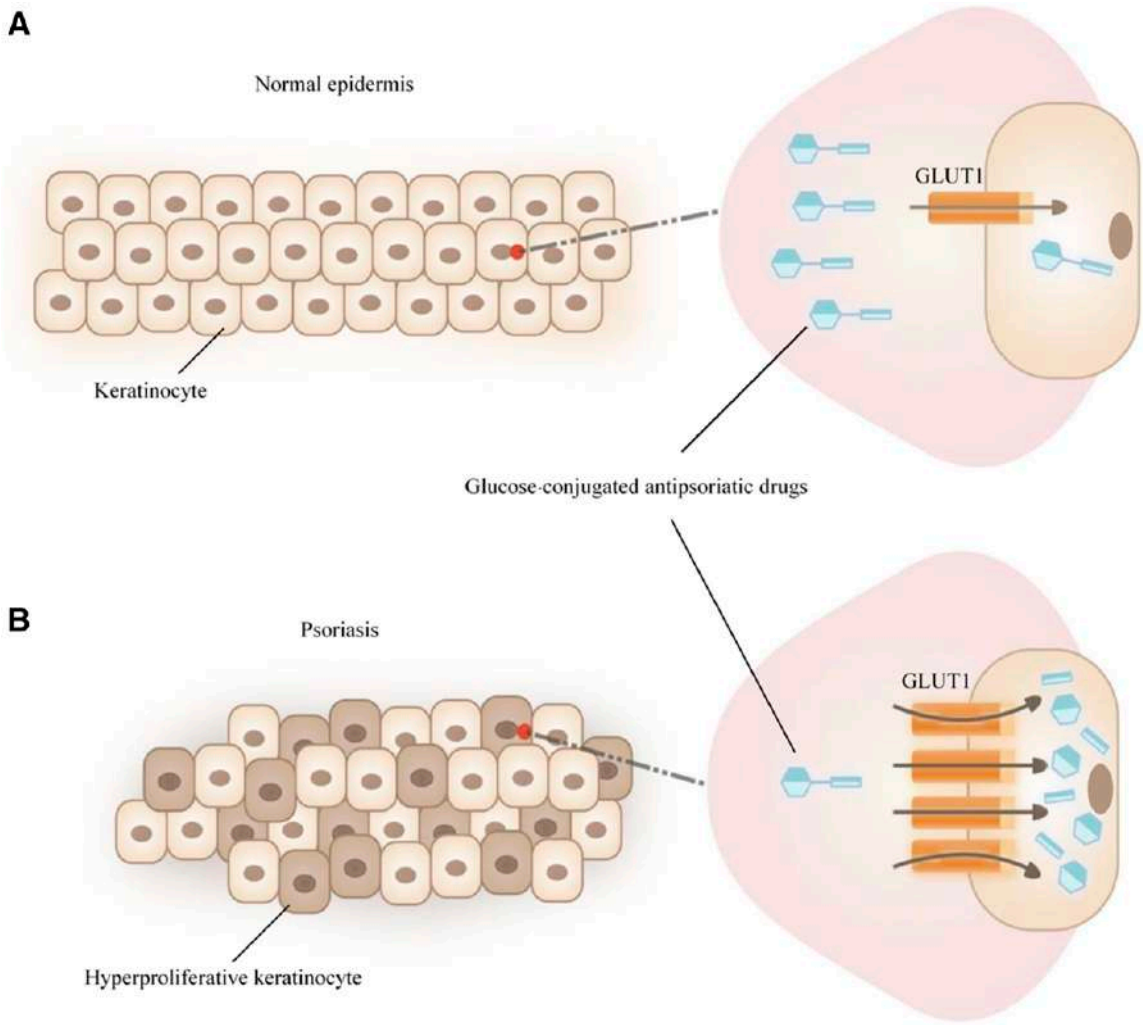

Fig. 1. Transport of glucose-conjugated antipsoriatic drugs through GLUT1 in keratinocytes. In contradistinction to healthy keratinocytes (A), hyperproliferative keratinocytes are composed of overexpressed GLUT1 transporters, which contribute to significantly higher uptake of glucose-conjugated drugs (B). As a result, in chronic inflammation, the glucose-conjugated antipsoriatic drugs may lead to reduced inflammation and hyperproliferation of keratinocytes. The intracellular cleavage of acid-labile linkers in the more acidic environment results in controlled drug release in hyperproliferative keratinocytes.

TABLE 1

FDA-approved traditional and biologic therapies for psoriasis

Agents have been classified into two main groups: 1) systemic immunosuppressives used for the treatment of moderate to severe psoriasis; and 2) biologic agents divided into TNF-α inhibitors, IL-17 inhibitors, and IL-23 and related cytokine inhibitors.

Class		Compound	Mechanism of action
Traditional agents commonly used in treatment of moderate to severe psoriasis		Methotrexate	A folate antimetabolite that inhibits T-cell activation as well as DNA synthesis and repair (Chan and Cronstein, 2013)
		Cyclosporine	A calcineurin inhibitor that leads to reduced production of interleukin-2 (Matsuda and Koyasu, 2000)
		Apremilast	A phosphodiesterase 4 inhibitor that leads to increased intracellular cAMP levels to regulate various inflammatory mediators (e.g., decreases levels of TNF-α and interleukin-23, increases level of interleukin-10) (Schafer, 2012)
		Tofacitinib	An inhibitor of interleukin-2-induced phosphorylation of JAK3 and STAT5, which are involved in immune cell function (Hodge et al., 2016)
		Fumaric acid esters	Fumarate derivatives that activate Nrf2 to inhibit the production of proinflammatory cytokines, such as IL-12 and IL-23 (Balak, 2015)
		Acitretin	A retinoid that binds to and activates retinoid receptors to normalize keratinocyte differentiation in the epidermis (Tippmann et al.,2009)
Biologic agents	TNF-α inhibitors	Etanercept	A recombinant protein that binds to the Fc portion of IgG and blocks soluble TNF-α interaction with receptors on the cell surface (Goffe and Cather, 2003)
		Infliximab	A chimeric monoclonal antibody that interferes with endogenous TNF-α (Guo et al., 2013)
		Adalimumab	A recombinant monoclonal antibody against TNF-α (Mease, 2007)
		Certolizumab pegol	A pegylated Fab' fragment of humanized monoclonal antibody against TNF-α; selectively binds and neutralizes the activity of human TNF-α (Acosta-Felquer et al., 2016)
	Interleukin 17 inhibitors	Secukinumab	A human IgG1κ monoclonal antibody that selectively binds to interleukin-17A and inhibits the interaction of this cytokine with the IL-17 receptor (Fala, 2016)
		Ixekizumab	A humanized IgG4κ monoclonal antibody against IL-17A, that inhibits the release of proinflammatory cytokines and chemokines (Monin and Gaffen, 2018)
		Brodalumab	A human monoclonal IgG2 antibody that acts as an antagonist of IL-17 receptor A (IL-17RA) to block the release of proinflammatory cytokines and chemokines (Monin and Gaffen, 2018)
	Interleukin 23 and related cytokines inhibitors	Ustekinumab	A human IgGκ monoclonal antibody that binds with high affinity to p40 subunit of both IL-12 and IL-23 to reduce the expression of key cytokines, such as MCP-1, TNF-α, IP-10, and IL-8 (Benson et al., 2011)
		Guselkumab	A human IgG1λ monoclonal antibody that selectively blocks the IL-23 receptor and reduces the serum levels of IL-17A, IL-17F, and IL-22 (Al-Salama and Scott, 2018)
		Tildrakizumab	A human IgG1κ monoclonal that binds to the p19 subunit of IL-23 and consequently inhibits its interaction with the IL-23 receptor (Papp et al., 2015)
		Risankizumab	A human IgG1 monoclonal antibody against the p19 subunit of IL-23, resulting in inhibition of its interaction with the IL-23 receptor (Haugh et al., 2018)

FDA, Food and Drug Administration. STAT5, Signal transducer and activator of transcription 5. Nrf2, Nuclear factor erythroid 2-related factor 2. MCP-1, Monocyte chemoattractant protein-1.

temporally heterogeneous (Gatenby and Gillies, 2004; Gatenby et al., 2006). However, recent studies reveal that GLUT1 upregulation is considered to be one of the most immediate events in the pathogenesis of psoriasis by promoting epidermal hyperproliferation, inflammation, and angiogenesis (Tao et al., 2008; Tochio et al., 2013; Hodeib et al., 2018). Moreover, it plays a significant role in the evolution of the disease as well as the development of associated comorbidities. It is now well-established that GLUT1 is strongly upregulated in psoriatic lesions, epidermal hyperplasia, and wound healing when compared with healthy skin (Tao et al., 2008; Tochio et al., 2013).

The results of the published data provide a strong rationale for the design and evaluation of glucose-conjugated antipsoriatic drugs to improve their pharmacokinetic and pharmacodynamic properties. Glycoconjugation of antipsoriatic drugs could generate a valuable treatment option for moderate to severe forms of psoriasis as well as comorbidities associated with the disease. In the case of classic comorbidities associated with psoriasis, such as psoriatic arthritis, evidence suggests that the uptake of radiolabeled glucose correlates with the degree of arthritis activity, which may allow the preferential accumulation of glucose-conjugated drugs in the inflamed regions (Mehta et al., 2011; Rose et al., 2014). Moreover, comorbidities related to treatment, such as nephrotoxicity (cyclosporine) and hepatotoxicity (methotrexate, leflunomide, and acitretin), may be limited because of the expected pharmacokinetic properties of these glucose conjugates.

Perspectives in Antipsoriatic Drug Design

Glycoconjugation generally offers improved water solubility and stability and, if the glycoside of choice is a GLUT substrate, the potential for selective targeting to pathologic cells. Glycoconjugation may facilitate the pharmacokinetics of cytotoxic exogenous molecules and ensure their facilitated transport through glucose receptors. Alternatively, compounds that have a sugar moiety may interact with the machinery for the intake and metabolism of glucose. Antimetabolites, which build on sugar structures, may, through competitive inhibition, disrupt the cellular metabolism to cause cell death (Harjes et al., 2012; Zhang et al., 2014).

Current evidence has shown that flavonoids, such as genistein, suppress psoriasis-related inflammation (Wang

et al., 2019). Of the many possible structural modifications of isoflavones, those that form glycoconjugate derivatives deserve special attention. Previous results have shown that sugar derivatives of genistein were not only able to significantly facilitate the transport of polyphenol into the intracellular compartment but also to modify its mechanism of action and exert a dozen-times higher therapeutic effect than genistein alone (Rusin et al., 2011; Gogler-Pigłowska et al., 2012). These results provide a strong rationale to further develop and explore the biologic effect of glycoconjugates of active substances used in clinical practice. Thus, we propose methods for the synthesis of conjugates of clinically used antipsoriatic drugs, namely, cyclosporine, acitretin, and tofacitinib with glucose. It is expected that at least some of the undesirable effects of these drugs can be diminished by chemical modification because of preferential influx into the target cells. Moreover, it is assumed that the derivatives may exhibit increased cellular selectivity and a more significant therapeutic effect. The design of specific targeting ligands has been presented in several excellent review publications (Calvaresi and Hergenrother, 2013; Granchi et al., 2016), and our experience in the synthesis of glycoconjugates of biologically active compounds (Pastuch-Gawołek et al., 2016) is the starting point for planning complex glucose derivatives of selected drugs used in psoriasis therapy.

Glycoconjugate of Acitretin. Acitretin is a second-generation synthetic retinoid administered for moderate to severe psoriasis (Boehncke and Schön, 2015). Although acitretin is a widely used systemic agent for the treatment of psoriasis, the efficacy of the drug is notoriously variable. As reported, there was a dose-response trend, with the highest doses of acitretin (50–75 mg/day) proving more effective than lower doses (10–25 mg/day) (Goldfarb et al., 1988). Hyperlipidemia is an obvious side effect of acitretin, particularly hypertriglyceridemia (Orfanos et al., 1997). Further side effects include arthralgia, mucocutaneous dryness, and photosensitivity. These factors limit acitretin's clinical use, especially in patients with other risk factors for cardiovascular disease.

Retinoic acid analogs have emerged as important therapeutic agents for the treatment of various skin diseases (e.g., acne and psoriasis) and as potential cancer chemopreventive agents. Therefore, synthesis of acitretin derivatives with increased solubility as well as stability toward hydrolysis and oxidation was critical. Balakrishnan et al. (1997) reported that glucuronide conjugates of retinoids were active and exhibited improved hydrolytic stability. However, it was found that retinoid glucuronides do not bind to retinoid receptors, and their potential application in psoriasis was not reported. We propose the synthesis of D-glucose conjugates, potential ligands of GLUT1, that will undergo intracellular hydrolysis into acitretin and glucose. The strategy for the synthesis of the conjugate is shown in Fig. 2A. Amide formation is among the most widely studied and used transformation in synthetic chemistry. A vast range of coupling agents and carboxyl-activating species, therefore, exist for undertaking such reactions (Valeur and Bradley, 2009; Koniev and Wagner, 2015). The known 2-aminoethyl- or 3-aminopropyl-β-D-glucoside was treated with acitretinamide in the presence of carbon diimides as a coupling agent [e.g., *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide, di-cyclohexylcarbodiimide] (Valeur and Bradley, 2009; Koniev and Wagner, 2015). The reaction is routinely conducted according to proposed procedures. Deprotection of the conjugate sugar portion is performed under standard conditions (basic conditions) by treatment with sodium methanolate. Moreover, taking into account the higher stability of *S*-glycosides in comparison with *O*-glycosides, the synthesis of acitretin glycoconjugate derivatives of 1-thioglycosides, containing an amino group in their aglycon, is shown in Fig. 2A (and follows a similar route as in the case of *O*-glycoside).

Glycoconjugate of Cyclosporin. Cyclosporin is a widely used immunosuppressant. Despite its everyday clinical use, it is associated with several side effects that include high blood pressure, headache, nephrotoxicity, increased hair growth, and vomiting as well as increased risk of infection, lymphoma, and liver problems (Tedesco and Haragsim, 2012). The increased blood pressure can cause cardiovascular events; it is thus recommended that the lowest effective dose for people requiring long-term treatment be used (Robert et al., 2010). Cyclosporin exhibits very poor solubility in water, and, as a consequence, suspension and emulsion forms of the medication have been developed for oral administration and injection. We expect that glycoconjugates may increase the bioavailability of the drug and optimize the formulation. The attachment of the sugar to result in a cyclosporine derivative glycopeptide is possible because of the functionalization of a double C=C bond. The attractiveness of designed compounds stems from the belief that *C*-glycosides are better potential drug candidates because, compared with their *O*-glycoside parents, they are not only more stable to acid but also to glycosidases and thus will have better biologic half-lives. We describe here a route to a C-glycoside analog of cyclosporin. Given its robustness and general ease of implementation, transition metal-catalyzed olefin metathesis has become an increasingly ubiquitous method for generating C−C double bonds in a wide variety of fields, including organic synthesis, green chemistry, and biochemistry (Grubbs and Khosravi, 2015). In the method reported here, the critical step is an olefin cross-metathesis of allyl derivatives of D-glucose and peptide. Planned substrates are methyl 6-*O*-allyl-D-glucoside and allyl-D-glucopyranoside prepared according to known and effective procedures. The critical issue is to preserve the E-configuration of substituents on the cyclosporin derivatives. Recently, ruthenium-based olefin metathesis catalysts bearing dithiolate ligands have been employed to generate olefins with high selectivity, which made it possible to obtain almost exclusively the E isomer (>99% E) (Ahmed and Grubbs, 2017). These catalysts demonstrate significantly improved initiation, resulting in considerably increased activity of these catalysts in reactions of trans olefins and demonstrating higher yields at shorter reaction times while maintaining high stereoselectivity of products (>99% E). The synthesis project is shown in Fig. 2C. In the planned reaction of olefin cross-metathesis leading to cyclosporine glycoconjugates, it was decided to use 6-*O*-allyl-D-glucoside, allyl-β-D-glucopyranoside, and 1C-allyl-β-D-glucopyranoside as the sugar substrates.

Glycoconjugate of Tofacitinib. Janus activated kinases (JAKs) are a family of receptor-associated tyrosine kinases involved in several physiologic functions such as immune responses and are related to autoimmune and inflammatory diseases (Babon et al., 2014; Schwartz et al., 2016). Tofacitinib is a JAK inhibitor approved for the treatment of active psoriasis as well as rheumatoid arthritis.

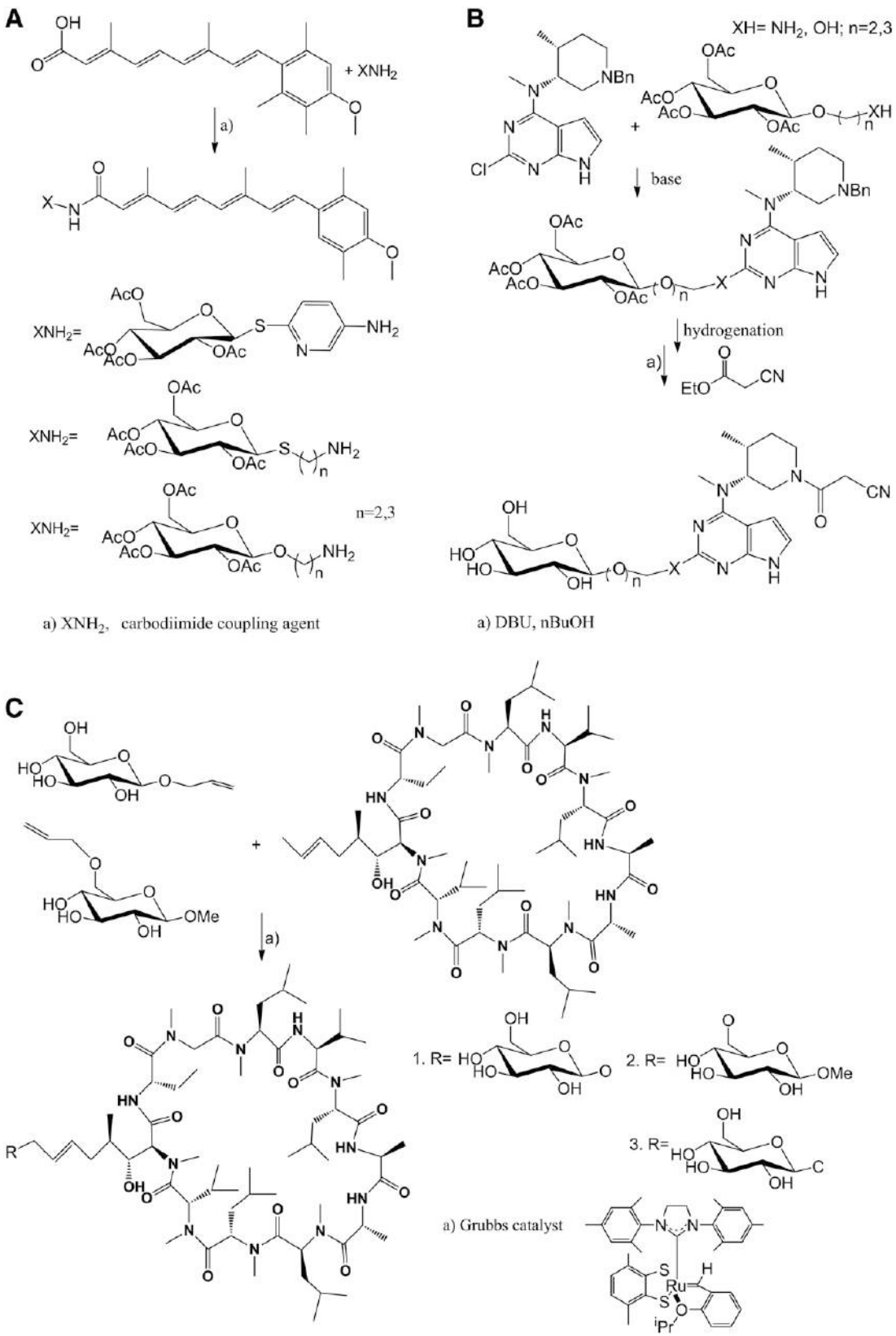

Fig. 2. Synthesis of glucose-conjugated antipsoriatic drugs. Synthesis of (A) acitretin glycoconjugate, (B) tofacitinib glycoconjugate, and (C) cyclosporin glycoconjugate.

A comprehensive analysis of the different synthetic methods used to prepare this active pharmaceutical ingredient was reported (Carvalho et al., 2019). The synthons (the building blocks) used in the synthesis of tofacitinib are 4-methyl 3-*N*-methyl–*cis*-piperidine and 2,4-dichloro-7*H*-pyrrolo[2,3-day] pyrimidine. The nucleophilic substitution of chlorine at the

C-4 pyrimidine of the derivative with the amine group from the piperidine derivative according to a aromatic nucleophilic substitution mechanism is performed in the presence of a base such as K_2CO_3 or Na_2CO_3 (Renom-Carrasco et al., 2016).

In our proposal (Fig. 2B), the compound obtained in this reaction was a substrate for a second nucleophilic substitution reaction with a sugar derivative. Displacement of a chlorine atom in this compound by treatment with per-*O*-acetylated D-glucose derivatives leads to the formation of the glycoconjugate. After removing the protecting benzyl group from the piperidine nitrogen atom by simple hydrogenolysis, this atom can be further functionalized. Price et al. (2009) described a single-step process for direct amidation of alkyl cyanoacetates using 1,8-diazabicyclo[5.4.0]undec-7-ene in n-butanol (nBuOH). Under the proposed conditions, deacetylation of the sugar unit also takes place, and as a result, the desired drug is obtained.

Assessment of Chemical Properties, the Bioactivity, and the Biodistribution of Glycoconjugates

After synthesis and traditional liquid chromatography separation, the compound must be characterized to confirm the desired structure. It is advisable to perform the validation with both chromatographic and spectroscopic methods (1H nuclear magnetic resonance, 13C nuclear magnetic resonance, high-resolution mass spectrometry) as well as to determine the optical purity using polarimetry. Compound stability, solubility, cellular uptake, biodistribution, and bioactivity need to be comprehensively evaluated to determine the potential clinical application of the conjugate. There are several well-established methods to evaluate the cellular uptake of glycoconjugates. Barnett et al. (1973) reported that GLUT-mediated drug entry could be measured using radiolabeled glucose, whereas Kim et al. (2012) suggest the use of 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-d-glucose (2-NBDG), a fluorescent analog of d-glucose, as a marker for visualization of cellular uptake and biodistribution of a fluorescent deoxyglucose derivative. For confirmation as to whether glucose transporters are involved in the uptake of the glucose-conjugated compound, cytochalasin B and phloretin can be used as known inhibitors of GLUTs (Tomaszowski et al., 2017). After evaluation of the optimal dosage of glucose conjugates in vitro, it is advisable to perform in vivo studies on psoriasis mouse models to determine the pharmacokinetics, pharmacodynamics, and, in particular, the maximum tolerated dose as well as anti-inflammatory properties. Although there is a scarcity of data regarding the administration of glucose derivatives in psoriatic animal models, protocols for the evaluation of glucose conjugates in cancer therapy could be used as a reference (Liu et al., 2017). Moreover, drug serum stability, biodistribution in collected blood and urine, and visualization of the localization in the whole animal can be assessed using radiolabeled glucose (Stüben et al., 1996) or real-time whole-body near-infrared reflectance spectroscopy optical imaging (Zhou et al., 2009).

Conclusions

Notable advances in our understanding of psoriasis, courtesy of basic science observations, has opened new avenues for the treatment of skin diseases. Recent findings concerning the fundamental role of glucose metabolism and GLUT1 expression in the pathogenesis of psoriatic lesions brought to light approaches that have garnered much attention and proved successful in cancer treatment. Substantial advances in the emerging field of glycoconjugation highlight the rationale for the development of glucose-conjugated antipsoriatic drugs to increase their effectiveness, cellular selectivity, and tolerability. The presented approach that exploits the heightened glucose requirement of keratinocytes may revolutionize the management of psoriasis.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Makuch, Woźniak, Krawczyk, Pastuch-Gawołek, Szeja, Agrawal.

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Address correspondence to: Siddarth Agrawal, K. Marcinkowskiego 1, 50-368 Wrocław, Poland. E-mail: siddarth@agrawal.pl

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Original Paper

The Impact of Proinflammatory Cytokines and Imiquimod on GLUT1 in HaCaT Keratinocytes – a Potential Anti-Psoriatic Therapeutic Target?

Sebastian Makuch[a] Piotr Kupczyk[a] Alicja Makarec[b] Grzegorz Chodaczek[c] Piotr Ziółkowski[a] Marta Woźniak[a]

[a]Department of Clinical and Experimental Pathology, Wroclaw Medical University, 50-368 Wroclaw, Poland, [b]Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland, [c]Bioimaging Laboratory, Łukasiewicz Research Network - PORT Polish Center for Technology Development, Wroclaw, Poland

Key Words
Psoriasis • Cytokines • Glucose transporters • GLUT1 • Inflammation

Abstract
Background/Aims: Glucose metabolism has been proven as an essential process for proliferating keratinocytes, which highlights the importance of glucose transporter-1 (GLUT1) not only in the onset of psoriasis but also in the progression and severity of this inflammation-driven disease. In this study, we attempted to find a connection between proinflammatory cytokines (IL-6, IL-17, IL-23, IL-36, TNF-α), a skin inflammation inducing agent – imiquimod (IMQ) and GLUT1 expression. ***Methods:*** Human keratinocyte HaCaT cell line was incubated with exogenous cytokines: IL-6, IL-17A, IL-23, IL-36, TNF-α at a final concentration of 100 ng/ml, or with 1 μM of IMQ, for 48 h. Following the stimulation, glucose uptake and GLUT1 expression were evaluated. The activity of GLUT1 was measured in the presence of a selective GLUT1 inhibitor, BAY-876. The expression of GLUT1 was examined by immunofluorescence and quantified by qPCR, Western blotting and densitometry. ***Results:*** The results from qPCR analysis showed that the administration of exogenous IL-6, IL-17, IL-23 and IL-36 to HaCaT cells resulted in upregulation of GLUT1-encoding SLC2A1 gene, while TNF-α had no significant effect. The same results were confirmed by immunofluorescence analysis, as the fluorescent intensity of GLUT1 was elevated following cytokine and IMQ stimulation. Western blot and densitometry showed that all examined cytokines, as well as IMQ, increased GLUT1 expression. HaCaT cells displayed an improved intracellular 2-deoxy-D-glucose (2-DG) uptake and GLUT1 activity after stimulation by exogenous cytokines and IMQ. The highest uptake of 2-DG was observed after IL-23 stimulation (1.93x) and the lowest after TNF-α stimulation (1.07x). BAY-876 inhibited the 2-DG uptake compared to control. ***Conclusion:*** Our findings suggest that

Sebastian Makuch
Department of Clinical and Experimental Pathology, Wroclaw Medical University, Karola Marcinkowskiego 1 str., Wroclaw, 50-368, Poland
Tel. +48737901267, E-Mail sebastian.mk21@gmail.com

cytokines and IMQ may play a key role in regulating GLUT1 expression in HaCaT cells. We believe that GLUT1 overexpression could potentially be utilized in the targeted treatment of psoriasis.

Introduction

Psoriasis is an immune-mediated skin disease manifested by skin inflammation [1, 2]. Typical clinical signs of the disease include increased incidence of plaques and scales, which initiate associating comorbidities such as pain or itch [3]. Similarly to other chronic proinflammatory diseases of the cutaneous system, psoriasis can have a significant impact on both physical and mental health quality [4]. The prevalence of this disease varies from 0.27% to 11.40% and affects approximately 55.8 million adults around the world [5, 6].

The hallmark of psoriasis is constant inflammation that leads to keratinocyte hyperproliferation and disordered differentiation. The early phase of the pathogenesis of psoriasis consists of the activation of autoreactive T cells, which secrete a wide variety of cytokines that play a key role in the development of inflammation. Additionally, various triggers, including Toll-like receptor (TLR) agonists and autoantigens may contribute to the activation of the pathogenic cascade resulting in enhanced production of proinflammatory and proliferation-inducing mediators such as IL-6, IL-17, IL-22, IL-23, IL-36 and TNF-α by immune cells [7, 8]. Among these important cytokines lie potential therapeutic targets for the treatment of psoriasis.

Current treatments for psoriasis seek to minimize inflammation and remove scales [9]. Therapeutic guidelines include topical treatments (mainly corticosteroids, retinoids, vitamin D analogues), phototherapy (UVA and UVB), systemic treatments (immunosuppressants) and systemic biologic treatments [10, 11]. Biopharmaceuticals target specific immune cells that are responsible for psoriasis. The FDA-approved biologic therapies include TNF-α inhibitors (*Etanercept*, *Infliximab*, *Adalimumab*, *Certolizumab*), IL-17 inhibitors (*Secukinumab*, *Ixekizumab*, *Brodalumab*) and IL-23 inhibitors (*Guselkumab*, *Tildrakizumab*, *Risankizumab*) [12, 13]. There is currently no FDA-approved IL-6 inhibitor therapy for psoriasis, but clinical data have shown the potential [12, 13]. Limited long-term outcome data show that biologics are safe for prolonged use and well-tolerated; however, side effects such as infections, malignancies, cardiac disorders, hepatotoxicity and nerve demyelination have been reported [14]. Thus, novel therapeutic approaches are required to minimize the adverse effects of currently applied therapies.

Glucose is the primary source of energy for all cells, and it is involved in every metabolic cycle and pathway. Due to the high metabolic activity of rapidly proliferating cells in psoriasis, the glucose uptake facilitated by glucose transporters (SGLTs and GLUT) is elevated [15, 16]. A study conducted by Zhang et al. showed that glucose metabolism is crucial for proliferating keratinocytes [17]. Moreover, the genetic deletion of GLUT1 ameliorated psoriasiform hyperplasia induced by imiquimod (IMQ) and IL-23 [17]. Other studies have also found that increased GLUT1 expression in psoriasis lesions promotes keratinocyte proliferation [18] and causes elevated epidermal hyperproliferation, inflammation, and angiogenesis [19]. Interestingly, overexpressed GLUT1 transporters in hyperproliferative keratinocytes may lead to enhanced uptake of selective glucose-conjugated pharmaceuticals [20].

The above-mentioned studies have highlighted GLUT1 as a potential therapeutic target for pathologic hyperproliferation, however, we have failed to find studies examining the impact of cytokines and IMQ on GLUT1 expression and function in psoriasis *in vitro*. The aim of our study was to find a connection between proinflammatory cytokines (IL-6, IL-17, IL-23, IL-36, TNF-α), IMQ and glucose transporter's (GLUT1) activity.

Materials and Methods

Cell culture

The HaCaT cell line was obtained from Cell Lines Service (CLS Cell Lines Service, Germany; 300493) and maintained in Dulbecco's modified Eagle's medium (DMEM, high glucose, no glutamine) supplemented

with 10% v/v fetal bovine serum (FBS), 1% v/v antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin), 1% v/v L-Glutamine at 37°C and 5% CO_2 in a humidified incubator. Cell culture reagents were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). Cells were passaged at 80% confluence. The culture medium was renewed every 3 days. For all experiments, HaCaT cells were incubated with exogenous cytokines: IL-6, IL-17A, IL-23, IL-36, TNF-α (all from Peprotech, USA) for 48 h at a final concentration of 100 ng/ml, or 1 µM imiquimod (IMQ, InVivoGene, USA) for 48 h [21].

Table 1. SLC2A1, ACTB and G3PDH genes forward and reverse primers

Genes	Forward (F) and Reverse (R) Primers Sequences	Gene Accession Number
Target Gene		
SLC2A1	F: GGTTGTGCCATACTCATGACC R: CAGATAGGACATCCAGGGTAGC	NM_006516.4
Housekeeping Genes (HKGs):		
ACTB	F: CAACCGCGAGAAGATGAC R: GTCCATCACGATGCCAGT	NM_001101.5
G3PDH	F: TGGTATCGTGGAAGGACTCA R: TGGTATCGTGGAAGGACTCA	NM_002046.7

RNA extraction and quantitative real-time RT-PCR

The total RNA from cells was isolated using GeneMATRIX Universal RNA Purification Kit (EURx, Gdansk, Poland) according to the manufacturer's protocol. For first-strand cDNA synthesis we used 1 µg of RNA and then qPCR with SG qPCR Master Mix (EURx) from smART RT-qPCR Kit (EURx) was performed following the manufacturer's instructions, using Light Cycler 480 instrument (Roche, Basel, Switzerland). The glyceraldehyde 3-phosphate dehydrogenase (*G3PDH*) and β-actin (*ACTB*) genes were used as housekeeping gene (HKG) standards for GLUT1 gene (*SLC2A1*) expression. Each sample was duplicated, the mean value was used for calculations. Primers for the human *SLC2A1* target gene and HGKs were designed using ProbeFinder 2.48 (Roche). The synthesized (Genomed, Poland) forward and reverse sequences of target and HKGs are listed in Table 1.

Glucose uptake assay

HaCaT cells were seeded in a 96-well culture plate (15x10^3 per well). The next day, cells were stimulated with 100 ng/ml of exogenous cytokines or 1 µM IMQ. After 48 h, cells were washed 3x with phosphate-buffered saline (PBS) and treated with 1 µM BAY-876 [22] (Selleckchem, Houston, TX, USA) and 1 µM insulin [23] (Thermo Fisher Scientific), dissolved in serum-free culture medium, for 2 h. Next, 2-Deoxy-D-glucose (2-DG) (ab136955, Abcam) was added for 20 min and cells were washed 3x with PBS. Cells in each well were lysed with Extraction Buffer from the Glucose Uptake Assay Kit (Colorimetric) (ab136955, Abcam, Cambridge, UK). Further steps were performed according to the manufacturer's instruction. Samples were diluted by adding 45 µl Assay Buffer to 5 µl of sample. Absorbance (OD) was measured using a microplate reader (Biotek, Winooski, VT, USA) at 412 nm wavelength in a kinetic mode.

Immunofluorescence

For fluorescence confocal microscopy, HaCaT cells were seeded in a 96-well culture plate at 1x10^6 cells/well and after reaching 70% confluence underwent fixation using 4% paraformaldehyde (4% PFA) in PBS for 10 min at room temperature (RT) followed by rinsing (3xPBS) for 3 min (the scheme of rinsing was applied in all steps). After that, a blocking step was performed using a blocking solution (BS) containing 3% Bovine Serum Albumin (Sigma-Aldrich, Saint Louis, MO, USA), 5% Normal Donkey Serum (Abcam), 0.01% Triton X-100 (Sigma-Aldrich), 0.01% Tween 20 (Sigma-Aldrich), 0.3 µM glycine in PBS for 1 h at 4°C. Next, cells were incubated with a primary rabbit anti-human GLUT1 antibody (1:200, clone 16D21, monoclonal, Sigma-Aldrich) diluted in BS overnight at 4°C. The next day, cells were rinsed and incubated with a donkey anti-rabbit DyLight 488 antibody (dilution 1:500, Novus Biologicals, USA) for 2 h at RT in dark conditions. The negative controls were prepared with the omission of the primary antibody. After rinsing, cells were additionally incubated with a phalloidin-Alexa Fluor 555 conjugate (Cytotek, USA) at 37°C for 45 min on a plate rotor for the detection of cellular distribution of actin filaments. Moreover, for the nucleus counterstaining, cells were incubated with 100 µl of PBS containing DAPI (1:10000, Sigma-Aldrich).

Confocal microscopy and image processing

The imaging was performed on a spinning-disk confocal microscope (Carl Zeiss, Oberkochen, Germany) equipped with a dry 20x objective (NA 0.4) and a QImaging Rolera EM-C2 EM-CCD camera. The laser wavelengths used for excitations were 405 nm for DAPI, 488 nm for DyLight 488 (GLUT1), and 561 nm for Alexa Fluor 555 (phalloidin). Emission filters were as follows: BP 450/50 (DAPI), FE01-520/35 (DyLight 488) and BP 600/52 (Alexa Fluor 555). Each condition was imaged in triplicates, and five randomly

selected areas per well were analyzed for GLUT1 protein expression. Maximum intensity projections were created from confocal Z-stacks and exported to TIFF files (Zen Microscopy Software, Zeiss). Further steps of fluorescence intensity (FI) analysis were performed in Fiji-ImageJ software (National Institute of Health, Bethesda, USA). In all the channels, Subtract Background and Median filter algorithms were applied to reduce background noise, and images were converted to the 8-bit grey scale. Next, the Huang Threshold algorithm was used on the phalloidin channel to obtain a binary image showing cell bodies, followed by the Watershed algorithm to separate joint objects. Cells were detected using the Analyze particles function and identified regions of interest were transferred onto the GLUT1 channel in order to calculate the FI within them.

Western blot analysis

Following the cell lysis, protein samples (30 µg) were analyzed on 10% SDS-PAGE under non-reducing conditions. Gels were blotted onto a nitrocellulose membrane and transferred at 70 V for 2 h. The membrane was blocked with 5% non-fat dried milk in PBS with 0.1% Tween 20 (Merck Life Science, MI, Italy) for 1 h at RT. Target proteins were detected with the following primary antibodies: anti-GLUT1 polyclonal rabbit IgG (diluted 1:250, Merck, New Jersey, USA) and anti-β-actin monoclonal mouse IgG (diluted 1:5000, Merck). The nitrocellulose membrane was incubated at 4°C overnight with the primary antibody, then 3x washed in PBS with 0.1% Tween 20. Following the washing, the membrane was incubated for 1 h with a secondary anti-rabbit IgG (diluted 1:10000, Merck) conjugated to horseradish peroxidase. Western blot bands were detected with Super Signal® West PICO (Thermo Fisher Scientific) and visualized with ChemiDOC XRS, Quantity One 4.6.5 software (Bio-Rad Laboratories, Segrate, Milano, Italy). Western blot semi quantitative calculations were prepared using ImageJ software (version 2.1.4.7 i1).

Statistical analysis

All experiments were conducted in triplicates. Data were presented as means ± SD (as stated in figure legends). Statistical significance was determined by ANOVA with post-hoc Holm-Sidak's multiple comparisons test using GraphPad Prism 9 software. $p < 0.05$ was considered as statistically significant.

Results

The expression of GLUT1 is significantly induced by IL-6, IL-17, IL-23, IL-36 and IMQ in HaCaT keratinocytes

To investigate a potential connection between proinflammatory cytokines, IMQ and glucose metabolism in keratinocytes, we examined whether GLUT1 expression is altered upon IL-6, IL-17, IL-23, IL-36, TNF-α and IMQ stimulation. We treated HaCaT cells with exogenous cytokines and IMQ and analyzed their impact on GLUT1 expression. Briefly, RNA from control and treated keratinocytes were reverse-transcribed into cDNA and analyzed by qPCR using primers specific to GLUT1. The results showed that the administration of exogenous IL-6, IL-17, IL-23 and IL-36 to HaCaT cells resulted in the upregulation of the *SLC2A1* gene, while TNF-α had no significant effect (Fig. 1). A statistical difference in the *SLC2A1* mRNA expression level was also found between IMQ-treated cells and control. These observations demonstrate that GLUT1, which is the primary glucose transporter expressed in keratinocytes, is upregulated upon cytokine and IMQ-induced inflammation.

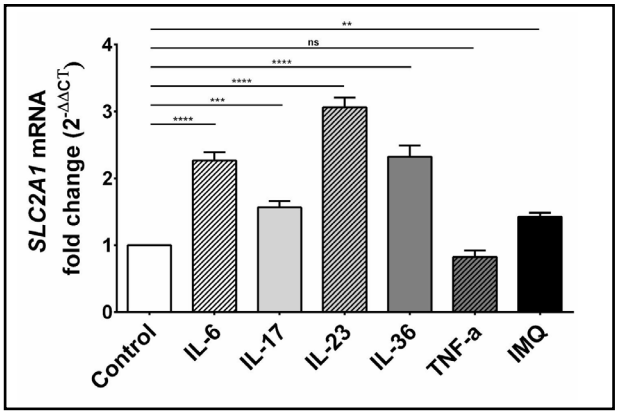

Fig. 1. Expression level of SLC2A1 mRNA in HaCaT cells after stimulation with exogenous cytokines and IMQ. The statistical significance level was set at p = 0.01-0.05 (*), 0.001-0.01 (**), 0.0001-0.001 (***), p ≤ 0.0001 (****), p > 0.05 = not significant (ns).

Incubation of HaCaT cells with exogenous cytokines and IMQ increases the intracellular uptake of glucose

To evaluate the effect of proinflammatory cytokines and IMQ on the glucose uptake by HaCaT cells, we measured the concentration of 2-DG in the intracellular compartment by using the Glucose Uptake Assay. Our results showed enhanced intracellular glucose uptake and GLUT1 activity in HaCaT cells stimulated by exogenous cytokines and IMQ. The highest uptake of 2-DG was observed after IL-23 stimulation (1.93x) and lowest after TNF-α stimulation (1.07x). 2-DG concentration was increased 1.47x for IL-6, 1.44x for IL-17, 1.62x for IL-36 and 1.6x for IMQ (Fig. 2). Additionally, the stimulation of cells with a selective GLUT1 inhibitor (BAY-876) led to a decrease of 2-DG uptake compared to control (Fig. 2). These results demonstrate that IMQ and proinflammatory cytokines, except for TNF-α, significantly regulate the intracellular glucose uptake and metabolism during the inflammation.

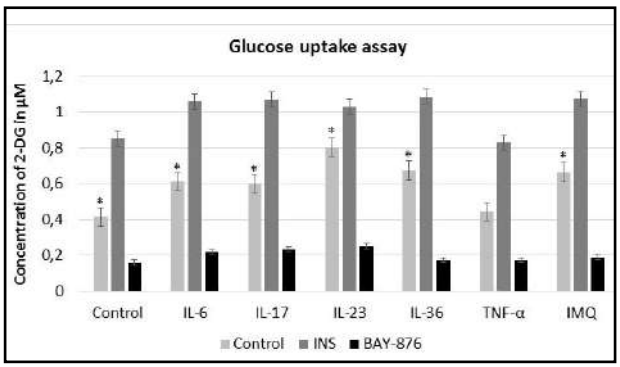

Fig. 2. 2-DG uptake in HaCaT cells after stimulation with insulin (INS), BAY-876 inhibitor, exogenous cytokines and IMQ. Insulin significantly increased the uptake of 2-DG, while GLUT1 selective inhibitor BAY-876 decreased the concentration of 2-DG in HaCaT cells. The intracellular glucose uptake and GLUT1 activity in HaCaT cells stimulated with IMQ and proinflammatory cytokines was enhanced. The experiment was conducted thrice. Data represent mean ± SD; *p < 0.05, compared to untreated control.

Exogenous cytokines and IMQ increase the fluorescence intensity of GLUT1 in HaCaT cells

To investigate the impact of cytokines and IMQ on the GLUT1 protein level in HaCaT cells, we performed a quantitative analysis of the fluorescence intensity (FI) of the GLUT1 signal following the stimulations. GLUT1 protein localization was visualized using confocal microscopy. The immunostaining revealed that GLUT1 localized primarily to the cell membrane but was also visible in the perinuclear region, which is clearly visible on the overlay with DAPI staining (Fig. 3). Most importantly, the fluorescence intensity of GLUT1 in HaCaT cells was higher after incubation with IL-6, IL-17, IL-23, IL-36 and IMQ, while TNF-α did not significantly affect the GLUT1 level (Fig. 4).

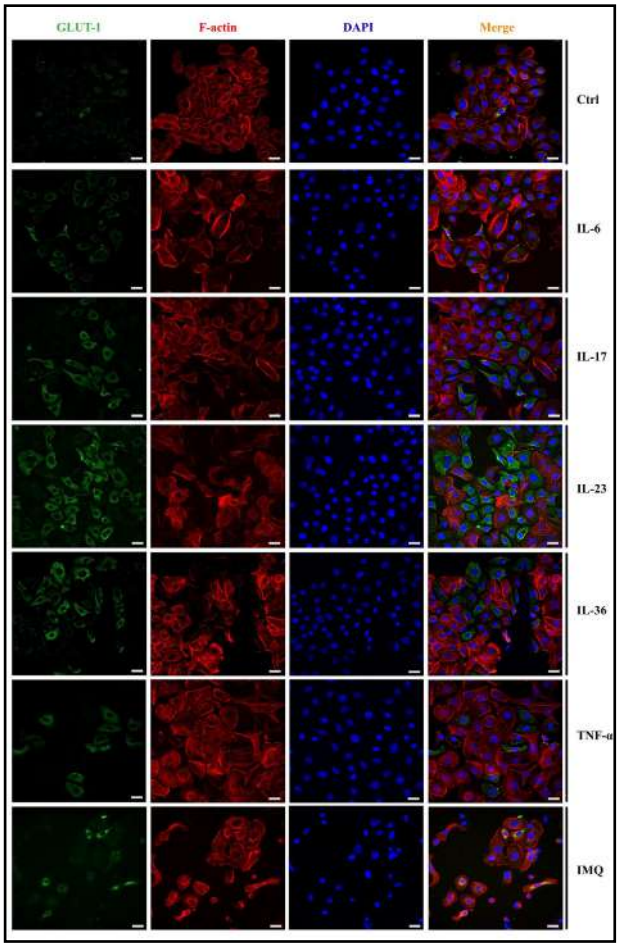

Fig. 3. Immunofluorescence staining of GLUT1 in HaCaT cells after the stimulation with exogenous proinflammatory cytokines and IMQ. Scale bar = 20 µm.

Western blot and densitometry prove the correlation between proinflammatory cytokines and the increase of GLUT1 expression

Using Western blot method, it was shown that all of the examined cytokines, as well as IMQ, increased the GLUT1 expression (Fig. 5). The relative expression analysis revealed that HaCaT cells, cultured with IL-36 and IMQ expressed almost 80% ($p < 0.05$) more of GLUT1 protein, compared to control cells cultured in the standard medium, while IL-23 and TNF-α increased GLUT1 levels by ~40% ($p < 0.05$). IL-6 and IL-17 treated cells present ~60% higher relative expression than control cells.

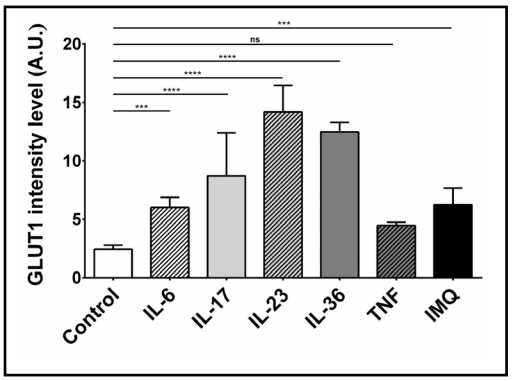

Fig. 4. Fluorescence intensity analysis of the GLUT1 signal in HaCaT cells after stimulation with exogenous cytokines and IMQ. The analysis was performed in the Fiji-ImageJ software. The statistical significance level was set at $p = 0.01$-0.05 (*), 0.001-0.01 (**), 0.0001-0.001 (***), $p \leq 0.0001$ (****), $p \geq 0.05$ = ns = not significant.

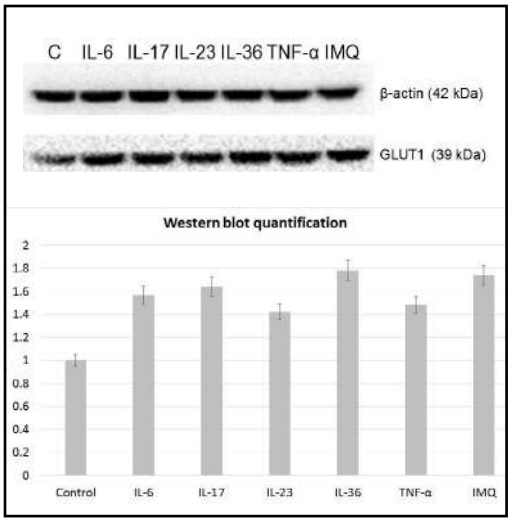

Fig. 5. Western Blot analysis of the GLUT1 signal in HaCaT cells after stimulation with exogenous cytokines and IMQ.

Discussion

Glucose is the major energy source for rapidly proliferating keratinocytes during the psoriasis progression. Its uptake is mediated and precisely regulated by glucose transporters (GLUTs). Among thirteen GLUT family members known to date, only GLUT1 has been identified as a significant hexose transporter expressed in keratinocytes and activated T lymphocytes [17]. In this study, we found that the exogenous administration of pro-inflammatory cytokines (IL-6, IL-17, IL-23, IL-36) and IMQ increases GLUT1 expression and results in elevated glucose uptake in inflamed keratinocytes. Surprisingly, stimulation of cells with TNF-α played a minor role in the expression of GLUT1. The pathogenesis of psoriasis involves precise communication between keratinocytes, immune cells, and other skin-related cells. A variety of different inflammatory cytokines are involved in this process, including IL-4, IL-6, IL-23, IL-17, IL-22, IL-36, and TNF-α. Their increased secretion triggers multiple cell signaling pathways, resulting in excessive keratinocyte proliferation and production of antimicrobial peptides, chemokines, and growth factors contributing to the amplification of inflammation [24–26]. The discovery that cytokines provide signals to promote glucose uptake has already been shown in several studies [27–29], but only few of them have highlighted this phenomenon in the case of psoriasis. Hodeib et al. found that rapidly proliferating keratinocytes express high levels of GLUT1, leading to an increased epidermal thickness, inflammatory cell density, and microvessel density [19]. Furthermore, as suggested by Ebeling et al., the upregulation of GLUT1 expression increases glucose transport, which seems to have an impact on insulin resistance, especially in severe psoriasis cases [30]. The positive correlation between GLUT1 expression and progressive keratinocyte proliferation, shown by increased Ki-67 expression in psoriasis lesions, has also been indicated by Abdou et al. [31]. Consistently with our results, Huang et al. also observed membrane-

enriched GLUT1 in keratinocytes from the psoriatic skin [32]. These findings reinforce our suggestions regarding the potential of glucose metabolic-targeted therapy. Furthermore, according to Zhang et al. study, GLUT1 deletion or inhibition abolished glucose transport by 95% in keratinocytes and impaired their proliferation *in vitro*. However, despite targeted GLUT1 deletion in keratinocytes, there was no apparent difference in skin morphology *in vivo*, suggesting that alternative substrates may be responsible for complementing the requirement for glucose, such as amino acids, fatty acids, or other hexoses. Moreover, the same study showed that GLUT1 downregulation renders the skin resistant to IMQ- or IL-23-induced psoriasiform hyperplasia [17]. This finding, in accordance with our results, suggests that cytokines and IMQ may play a key role in regulating GLUT1 expression. Taking into account that GLUT1 deletion in keratinocytes dampens psoriasiform hyperplasia and the GLUT1 overexpression is mediated by immune cells, we believe that inflammatory cytokines should be taken into account while precisely designing pharmaceuticals targeting GLUT1. Based on our data, we believe that GLUT1 overexpression could potentially be utilized by using selective glucose-conjugated pharmaceuticals against psoriasis. Thus, we propose a new drug development path that seems to alleviate psoriasis symptoms by their selective glucose-conjugated uptake by cytokine-induced GLUT1 overexpression. However, further studies are required to precisely dissect the utility of GLUT1 in the potential treatment of psoriasis.

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Author Contributions

Sebastian Makuch was responsible for the conceptualization of the study, original draft preparation, conduction of the experiments and manuscript editing. Piotr Kupczyk and Alicja Makarec conducted the experiments and edited the manuscript; Grzegorz Chodaczek analyzed the data, prepared some of the figures and edited the manuscript. Piotr Ziółkowski received the funding and supervised the study. Marta Woźniak edited the manuscript and supervised the study. All authors have read and agreed to the published version of the manuscript.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflicts of interest to declare

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Article

In Vitro and In Vivo Antipsoriatic Efficacy of Protected and Unprotected Sugar–Zinc Phthalocyanine Conjugates

Sebastian Makuch [1,*], Piotr Kupczyk [1], Marta Woźniak [1], Alicja Makarec [1], Maja Lipińska [2], Magdalena Klyta [2], Joanna Sulecka-Zadka [3], Wiesław Szeja [4], Mariachiara Gani [5], Valentina Rapozzi [5], Piotr Ziółkowski [1] and Piotr Smoleński [6,*]

[1] Department of Clinical and Experimental Pathology, Wroclaw Medical University, 50-368 Wroclaw, Poland; piotr.kupczyk@umw.edu.pl (P.K.); marta.wozniak@umw.edu.pl (M.W.); alicja.makarec@umw.edu.pl (A.M.); piotr.ziolkowski@umw.edu.pl (P.Z.)
[2] Experimental Animal Facility, Wroclaw Medical University, 50-368 Wroclaw, Poland; maja.lipinska@umw.edu.pl (M.L.); magdalena.klyta@umw.edu.pl (M.K.)
[3] Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, 50-375 Wroclaw, Poland; joanna.sulecka-zadka@upwr.edu.pl
[4] Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Silesian University of Technology, B. Krzywoustego 4, 44-100 Gliwice, Poland; wieslaw.szeja@adres.pl
[5] Department of Medicine, Laboratory of Biochemistry, P.le Kolbe 4, 33100 Udine, Italy; gani.mariachiara@spes.uniud.it (M.G.); valentina.rapozzi@uniud.it (V.R.)
[6] Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wroclaw, Poland
* Correspondence: s.makuch@umw.edu.pl (S.M.); piotr.smolenski@uwr.edu.pl (P.S.)

Abstract: Psoriasis, a chronic immune-mediated skin disorder affecting over 125 million people globally, is characterized by abnormal keratinocyte proliferation and immune cell infiltration. Photodynamic therapy (PDT) remains underutilized in the treatment of psoriasis despite its potential as a promising and effective therapeutic approach. This study aimed to explore the efficacy of zinc phthalocyanine (ZnPc) and its sugar conjugates as potential antipsoriatic agents. We successfully synthesized protected and unprotected sugar-conjugated zinc phthalocyanines and evaluated their potential against cytokine-stimulated HaCaT keratinocytes, as well as an established IMQ psoriasis-like in vivo model. Tetrasubstituted protected glucose–ZnPc (Glu-4-ZnPc-P) demonstrated superior phototoxicity (IC50 = 2.55 μM) compared to unprotected glucose conjugate (IC50 = 22.7 μM), protected galactose–ZnPc (IC50 = 7.13 μM), and free ZnPc in cytokine-stimulated HaCaT cells (IC50 = 5.84 μM). Cellular uptake analysis revealed that IL-17A, a cytokine that plays a central role in the pathogenesis of psoriasis, enhanced unprotected Glu-4-ZnPc uptake by 56.3%, while GLUT1 inhibitor BAY-876 reduced its accumulation by 23.8%. Intracellular ROS generation following Glu-4-ZnPc-P-PDT was significantly increased after stimulation with IL-17A, correlating with in vitro photocytotoxicity. In vivo PDT using Glu-4-ZnPc-P exhibited significant improvement in Psoriasis Area and Severity Index (PASI), inhibiting splenomegaly and restoring normal skin morphology. This study highlights sugar-conjugated zinc phthalocyanines as potential candidates for targeted PDT in psoriasis, providing a basis for further clinical investigations.

Keywords: zinc phthalocyanine; sugar conjugates; psoriasis; cytokines; drug uptake

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1. Introduction

Psoriasis is an immune-mediated, chronic, and noncontagious skin disease. It was estimated that the prevalence varies from 0.14% to 5.32% of the population [1], affecting approximately 125 million people worldwide [2]. It is characterized by abnormal proliferation of keratinocytes, infiltration of immune cells, and epidermal hyperplasia of dermal vessels. The most common form is plaque psoriasis, which can appear as raised areas of inflamed skin covered with red and silvery scales [3,4]. The pathogenesis of psoriasis is a

complex and multi-factorial process that can be mediated by proinflammatory cytokines, such as IL-6, IL-17, and IL-23 [2,5].

One of the recommended treatments from the guidelines of care for the management of psoriatic skin is phototherapy [6]. Studies showed promising effects of photodynamic therapy (PDT) for psoriasis, a form of phototherapy in which inappropriate cells are selectively destroyed by reactive oxygen species (ROS) [7]. PDT is based on the interaction between photosensitizer, light at a specific wavelength, and oxygen [8]. Originally, PDT was used to treat cutaneous disorders and later evolved as antitumoral therapy [9]. Many photosensitizers for PDT are widely researched, such as first-generation photosensitizer aminolevulinic acid (ALA). However, patients who received ALA-PDT frequently experienced intense pain and burning sensations, which in many cases were intolerable for them [10]. This underscores the need to explore second-generation photosensitizers in scientific investigations [11].

Phthalocyanines (Ps), developed over a span of 75 years primarily as blue and green pigments, have emerged as pivotal organic dyes [11]. In recent times, their applications have expanded to include optoelectronics and photoelectronics, notably in LCD displays, along with serving as a photoconductor in laser printers [11]. The basis for exploring additional applications lies in the relative simplicity of synthesis, coupled with the extensive potential for tailoring properties through the strategic selection of molecular structures [12]. The photophysical properties of phthalocyanine dyes are significantly influenced by the presence and nature of the coordinated central metal. Phthalocyanines with closed-shell, diamagnetic ions such as Zn^{2+}, Al^{3+}, and Ga^{3+} exhibit both high triplet yields and extended excited triplet state lifetimes [13]. Consequently, these macrocycles, particularly those involving zinc, demonstrate favorable photochemical and photodynamic characteristics attributed to their efficient generation of reactive oxygen species. Owing to their low toxicity, these compounds have proven to be a promising class of photosensitizers, finding application in biological and medical research, particularly in photodynamic therapy [14]. They are characterized by long excitation wavelength (670 nm), especially compared to ultraviolet light therapy, which allows deeper penetration into the skin [15,16]. Moreover, phthalocyanine displays better safety and effectiveness [15]. Studies have demonstrated a positive effect of zinc phthalocyanine (ZnPc) on melanoma cancer [17], as well as a photosensitizer for PDT of esophageal cancer [18].

However, there are limited data on the effectiveness of second-generation photosensitizers in the treatment of psoriasis [19]. Liu et al. performed an in vivo study showing that α-(8-quinolinoxy) zinc phthalocyanine (ZnPc-F7)-mediated PDT reduced the psoriatic symptoms. The metallophthalocyanine compound was characterized by suitable solubility, a strong effect, and low toxicity [20]. Another study introduced an amphiphilic zinc phthalocyanine polymer conjugate (ZPB) with a core-shell nanostructure, demonstrating a positive effect of photodynamic therapy against psoriasis in a guinea pig model [15].

It has been proven that glucose metabolism is essential for the proliferation of keratinocytes, hence the overexpression of glucose transporters, especially GLUT1 [21,22]. A study conducted by Zhang et al. showed that overexpressed GLUT1 transporters and increased glucose uptake may be the new potential target for the treatment of psoriasis and other hyperproliferative skin diseases [21]. The aim of our study was to confirm if sugar conjugation with zinc phthalocyanine leads to enhanced uptake of the conjugated drug through GLUT1 by rapidly proliferating HaCaT keratinocytes and verify the antipsoriatic activity of the conjugates in vivo.

2. Materials and Methods

2.1. Materials and Analytical Methods

All standard chemicals and solvents were obtained from commercial suppliers (zinc acetate dehydrate, 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose—Sigma-Aldrich, St. Louis, MO, USA; 1,8-diazabicyclo-[5.4.0]undec-7-ene—Thermo Scientific, Waltham, MA, USA; and 4-nitrophthalonitrile—

TCI Chemicals, Tokyo, Japan). Free zinc phthalocyanine (ZnPc) was obtained from TCI Chemicals, Tokyo, Japan.

Bruker IFS 1113v (Ettlingen, Germany) was used to measure the FT-IR spectra (range of 4000–400 cm^{-1}) abbreviations: vs, very strong; s, strong; m, medium; w, weak; br., broad). NMR spectra were recorded in DMSO-d_6 and DMF-d_7 solvents using a Bruker 600 AMX spectrometer (Bruker BioSpin MRI GmbH, Ettlingen, Germany) at ambient temperature (abbreviations: br, broad, m, multiplet). ^{1}H chemical shifts (δ) are expressed in ppm relative to $Si(Me)_4$. The Elemental Analyser Vario ELCube (Elementar Analysen Systeme GmbH, Langenselbold, Germany) was used for the determination of C, H, and N contents (Laboratory of Elemental Analysis at Faculty of Chemistry, University of Wrocław). Luminescence spectra, as well as at 300 K, were recorded on an FSL980 spectrofluorometer (Edinburgh Instruments, Livingston, UK). The H_2O solution (10^{-3} M) of (**Glu-4-ZnPc**) was used for the MALDI-TOF-MS experiment that was carried out on a JEOL JMS-S3000 SpiralTOF™-plus Ultra-High Mass Resolution MALDI-TOFM mass spectrometer (JEOL Ltd., Tokyo, Japan) at the Faculty of Chemistry, University of Wrocław). HPLC analyses were performed using the HPLC system Dionex with a UV–Vis detector (Dionex Corporation, Sunnyvale, CA, USA). Column: Phenomenex Aeris PEPTIDE XB-C18, 250 × 4.6 mm, particle diameter 5 μm (Phenomenex, Torrance, CA, USA). Mobile phase: gradient from 0% B in A to 80% B in A in 30 min, then 100% B for 2 min and 0% B for 5 min. A: water containing 0.1% TFA. B: acetonitrile/water (80/20, *v*/*v*) containing 0.1% TFA (TFA: trifluoroacetic acid). All reagents were HPLC-gradient grade.

4-(1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranosyl)phthalonitrile, 4-(1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranosyl)phthalonitrile, [1(4),8(11),15(18),22(25)-tetrakis(1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranosyl)phthalocyaninato]zinc(II) (**Glu-4-ZnPc-P**) and [1(4),8(11),15(18),22(25)-tetrakis(1,2:3,4-di-O-isopropylidene-α-D-galactopyranosyl) phthalocyaninato]zinc(II) (**Gal-4-ZnPc-P**) were synthesized according to the reported procedure [23], while compound (**Glu-4-ZnPc**) based on procedure described for synthesis of [1,2,3,4-Tetrakis(α/β-D-galactopyranos-6-yl)-phthalocyaninato]zinc(II) by Cavaleiro et al. [24]. All of the compounds were dissolved in DMSO, as **Glu-4-ZnPc-P, Gal-4-ZnPc-P,** and **ZnPc** are insoluble in water.

2.2. Synthesis of [1(4),8(11),15(18),22(25)-Tetrakis(1,2:5,6-tetrahydroxy-α-D-glucofuranosyl)-phthalocyaninato]zinc(II) (Glu-4-ZnPc)

100 mg (62.1 μmol) of [1(4),8(11),15(18),22(25)-Tetrakis(1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranosyl)phthalocyaninato]zinc(II) was dissolved in 25 mL of TFA/water mixture (*v*:*v*, 9:1). The dark-green mixture was stirred in the absence of light for four hours at room temperature under nitrogen. The volatile components were removed through evaporation under vacuum, and the remaining residue was dissolved in a solution of 1% acetic acid in water. The mixture was stirred at ambient conditions for one day, after which it was again dried under vacuum. It was then washed twice with 10 mL of chloroform and finally dried in vacuo. **Glu-4-ZnPc** was obtained as a dark-green solid with a yield of 90%, based on **Glu-4-ZnPc-P**. IR (KBr, cm^{-1}): 3428 (br, vs), 2919 (m), 2851 (m), 2365 (w), 2333 (w) 1678 (br, s), 1612 (br, s), 1485 (m), 1402 (m), 1336 (m), 1286 (m), 1228 (s), 1206 (s), 1130 (m) 1084 (m), 1047 (m), 944 (w), 840 (w) 801 (w), 745 (m), 724 (m), 669 (w). ^{1}H NMR (600.15 MHz, DMSO-d_6): δ, ppm, 9.30–9.25 (m, 4 H, Pc-H_α), 9.12–9.07 (m, 4 H, Pc-H_α), 7.98–7.94 (m, 4 H, Pc-H_β). 5.55–3.25 (set of multiplets, 44 H, sugar-*H*, partially overlapped in the range of 3.5–3.0 ppm). ^{1}H NMR (600.15 MHz, DMF-d_7): δ, ppm, 9.33–9.29 (m, 4 H, Pc-H_α), 9.25–9.13 (m, 4 H, Pc-H_α), 8.15–8.00 (m, 4 H, Pc-H_β, partially overlapped by DMF). 5.76–3.65 (set of multiplets, 44 H, sugar-*H*, partially overlapped in the range of 3.75–2.8 ppm). $C_{56}H_{56}N_8O_{24}Zn$ (**Glu-4-ZnPc**) (MW 1290.5 + $8H_2O$): C, 46.88; N, 7.81; H, 5.06. Found: C, 46.46; N, 7.61; H, 5.48. MALDI-TOF-MS: *m*/*z* 1289.275 (calcd. for $C_{56}H_{56}N_8O_{24}Zn$, $[M]^+$ 1289.49). The purity of the **Glu-4-ZnPc** was determined based on HPLC measurement and found to be around 95% (Figures S6 and S7).

2.3. Cell Culture

The human keratinocytes cell line HaCaT was obtained from Cell Lines Service (CLS Cell Lines Service, Eppelheim, Germany; 300493) and maintained in Dulbecco's modified Eagle's medium (DMEM, high glucose) supplemented with 10% v/v fetal bovine serum (FBS), 1% v/v antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin), 1% v/v L-Glutamine (2 mM) at 37 °C and 5% CO_2 in a humidified incubator. Cell culture reagents were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). The culture medium was renewed every 3 days. For the experiments, HaCaT cells were incubated with exogenous cytokines IL-6, IL-17A, and IL-23 (all from PeproTech, Cranbury, NJ, USA) for 24 h at a final concentration of 100 ng/mL each.

2.4. Dark Cytotoxicity and Cell Proliferation Assay

Cell proliferation was evaluated by utilizing the conversion of the yellow tetrazolium salt (MTT) into violet formazan insoluble crystals in the mitochondria of active cells. To assess the cytotoxicity of zinc phthalocyanine–sugar conjugates, HaCaT cells were seeded at a density of 1×10^4 per well in a 96-well plate and stimulated the following day with IL-6, IL-17A, and IL-23 for 24 h at a final concentration of 100 ng/mL each (PeproTech, Cranbury, NJ, USA). The next day, phthalocyanine solutions dissolved in DMSO and diluted in a culture medium at concentrations from 0.1 to 10 µM were added for 4 h and kept in the dark. Then, cells were irradiated at a fluence of 0.9 J/cm^2 using a halogen lamp (Penta Lamps, Teclas, Lugano, Switzerland). The following day, the photosensitizer solutions were removed from each well, and an MTT assay was performed. After 4 h of incubation with 0.5% MTT solution, the medium was removed, and the dye was dissolved by dimethyl sulfoxide (DMSO, Sigma-Aldrich, Munich, Germany), creating the color, whose intensity is proportional to the viable cells. The optical absorbance was measured at 490 nm using a BioTek ELX800 multi-well reader (BioTek, Winooski, VT, USA). Dark cytotoxicity was evaluated under the same conditions but without light irradiation. Each compound concentration was tested in four replicates and repeated at least three times. Based on the results, the dose of 2.5 µM was selected for the rest of the experiments for comparison, as higher concentrations were displaying dark cytotoxicity.

2.5. Cellular Uptake by Flow Cytometry

The cellular uptake of investigated photosensitizers was determined by using flow cytometry and quantified based on phthalocyanines' red fluorescence. HaCaT cells were seeded on a 12-well culture plate at a density of 15×10^4 per well. The next day, cells were incubated with 100 ng/mL of exogenous cytokines (IL-6, IL-17A, or IL-23) for 24 h or BAY-867, a selective GLUT1 inhibitor, for 2 h before drug administration, at a concentration of 1 mM. Then, cells were washed 3× with phosphate-buffered saline (PBS) and incubated with phthalocyanines at 2.5 µM for 24 h in darkness. After incubation, cells were washed two times with PBS and harvested for analysis. Then, cells were collected by centrifugation, resuspended in 200 µL of PBS, and analyzed by flow cytometry using Cytoflex (Beckman Coulter, Brea, CA, USA). The detection of the fluorescent signal originating from phthalocyanines involved the use of a 638 nm laser for excitation and a 660/20 nm emission filter and was detected using the APC channel in the logarithmic scale. Each test evaluated a total of ten thousand cells. Data were subjected to analysis using CytExpert 2.0 software (Beckman Coulter, Brea, CA, USA) and presented as mean fluorescence.

2.6. Fluorescent Microscopy

HaCaT cells were cultured at a density of 2×10^4 cells per well on Millicell® EZ Slide (Merck KGaA, Darmstadt, Germany) at 37 °C and allowed to incubate for 24 h. Subsequently, phthalocyanine–sugar conjugates were administrated, with a final concentration of 2.5 µM. Following 24 h incubation, cells were washed with PBS to eliminate unbound phthalocyanines, and a serum-free medium containing MitoTracker Green (40 nM) for 30 min or LysoTracker Green (75 nM) for 10 min was added, then washed 3 times with PBS,

and placed in fresh medium. Cell imaging was performed using a fluorescent microscope Axio Observer 7 (Zeiss, Oberkochen, Germany). The argon-ion laser light (wavelength 488 nm or 633 nm) excited the fluorescence of MitoTracker Green, LysoTracker Green, or phthalocyanine–sugar conjugates in the cells on the chamber slides, while emitted fluorescence was filtered using barrier filters (517 nm or 664 nm, respectively). Colocalization of the phthalocyanines with mitochondria and lysosomes was measured using Just Another Colocalization Plugin (JACoP) on Fiji (ImageJ distribution, version 1.54g, National Institutes of Health, Bethesda, MD, USA) to obtain Pearson's correlation coefficients [25].

Throughout the entire imaging experiment, parameters such as laser-line intensity and photometric gain remained constant.

2.7. ROS Generation In Vitro after PDT

ROS levels were assessed by monitoring the intracellular peroxide-dependent oxidation of DCFH-DA, resulting in the formation of the fluorescent compound 2,7-dichlorofluorescein (DCF). HaCaT cells were seeded onto a 96-well plate at a density of 15×10^3 cells per well and cultured overnight. The next day, cells were stimulated with 100 ng/mL of exogenous cytokines (IL-6, IL-17A, or IL-23). Subsequently, fresh medium containing the studied phthalocyanines (2.5 μM) was introduced, and the cells were incubated for 24 h in darkness. After three washes with PBS, 50 μL of DCFH-DA (10 μM) was added, and the cells were further incubated for 30 min. Following the removal of the medium and three additional PBS washes, the cells were exposed to laser light (670 nm) with an illumination dose of 0.9 J/cm^2. Following illumination, the cells were lysed with 1% SDS (100 μL) for 10 min on a table concentrator, and the DCF fluorescence was quantified using a Bio-Tek microplate reader (Corning Incorporated, New York, NY, USA) with excitation/emission wavelengths of 488/525 nm.

2.8. Animal Experiments

C57BL/6 mice, aged 8 to 11 weeks, with a body weight range of 15 g to 29 g, received a daily topical dose of 62.5 mg of 5% imiquimod (IMQ) (Aldara; Meda AB, Solna, Sweden) on a shaved skin area measuring 3 cm × 4 cm on the back for seven days. A total of 60 mice (half male and half female) were divided into six groups: (a) control; (b) IMQ treated with DMSO (0.625 mL/kg, which is 1.25%); (c) light exposure control; (d) Glu-4-ZnPc-P-PDT low dose (0.30 mg/kg), Glu-4-ZnPc-P-PDT medium dose (0.60 mg/kg), and Glu-4-ZnPc-P-PDT high dose (1.20 mg/kg). Each experimental group consisted of 10 mice, a number determined by statistical methods (sample size calculation, power analysis) to ensure that the anticipated minimum animal numbers for each group are sufficient for a reliable estimation of the efficacy of the applied therapy. After IMQ application, Glu-4-ZnPc-P dissolved in DMSO was administered intravenously via the tail vein using 0.5 mm syringes with a needle diameter not exceeding 25–30 G at a volume of 25 μL per body mass (20 g)—resulting in a final DMSO concentration of 0.625 mL/kg–1.25%. A total of 4 h after the administration of Glu-4-ZnPc-P, the mouse skin was illuminated with a red laser at an intensity of 15 J/cm^2 for 7 min. The output power, spot diameter, and irradiation time were set at 1.00 W, 6 cm, and 420 s, respectively. Every day, from the first IMQ application until 7 days after illumination, the Psoriasis Area and Severity Index (PASI) was assessed. PASI index was used to evaluate erythema, plaque thickness, and scaling, all on a scale from 0 (no changes) to 4 (very severe changes), to a total score of 12. After IMQ treatment and 7 days following irradiation, the skin, spleen, liver, and blood samples were collected for further experiments. The blood for the ELISA experiment was collected by cardiac puncture.

The study was approved by the Local Ethics Committee on Animal Experiments (LKE) in Wroclaw, Poland (No. 035/2023).

2.9. Histology

Mice skin fragments were excised and prepared for fixation in 10% buffered formalin (Chempur, Piekary Śląskie, Poland) for 24 h. On the following day, the skins underwent

standard dehydration processing in an escalating ethanol solution (50%, 70%, 80%, 95%, and 100%) for 10 min each, followed by two incubations with xylene for 10 min and subsequent paraffin embedding. The formalin-fixed paraffin-embedded (FFPE) tissue blocks were sliced into 5 µm sections using a microtome (Leica, Wetzlar, Germany). These sections were subjected to the routine hematoxylin and eosin (H&E) staining protocol. The confirmation of the psoriasis-like skin inflammation model was performed independently by two histopathologists. The specimens were analyzed for epidermal thickness, determination of the intensity and type of inflammatory cell infiltration in all skin compartments using an optical microscope Olympus BX53 with a digital camera ColorView IIIu (Olympus, Toyko, Japan), and cellˆA picture analysis software (Olympus Soft Imaging Solution GmbH, Münster, Germany).

2.10. Determination of Serum Cytokine Levels (ELISA)

The serum levels of IL-6 (cat. no. KMC0061), IL-17A (cat. no. BMS6001), and IL-23 (cat. no. BMS6017) were determined using sandwich ELISA kits obtained from GIBCO® Invitrogen GmbH (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol.

2.11. Statistical Analysis

Statistical analyses of in vitro and in vivo data were performed using Prism 8.0.1 (GraphPad Software Inc., San Diego, CA, USA) by applying the unpaired *t*-test. The in vitro results were presented as the mean ± SD from three independent experiments. Sample size calculation and power analysis were used to determine the number of animals; *p* values lower than 0.05 were considered statistically significant.

3. Results and Discussion

Synthetic methodology. The reaction of 4-nitrophthalonitrile with 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose or 1,2:3,4-di-O-isopropylidene-α-D-galactopyranose in the presence of K_2CO_3 gave the corresponding glycosubstituted phthalonitriles, by Choi et al. procedure [23]. Self-cyclization of the precursors produced tetra-glycosylated zinc(II) phthalocyanines Glu-4-ZnPc-P and Gal-4-ZnPc-P (Figure 1). Additionally, a water-soluble derivative, [1(4),8(11),15(18),22(25)-tetrakis(1,2:5,6-tetrahydroxy-α-D-glucofuranosyl)phthalocyaninato]zinc(II) (Glu-4-ZnPc), can be obtained by deprotecting the hydroxyl groups of connected sugar molecules in the reaction of tetrakis(1,2:5,6-di-O-isopropylidene-α-D-glucofuranosyl)phthalocyaninato]zinc(II) (Glu-4-ZnPc-P) with a solution of TFA in water (*v*:*v*, 9:1) with high yield. The resulting product is a dark-green solid that is soluble and stable in the presence of air in polar solvents such as DMSO, water, and methanol. To remove the propylidene protections, an adapted procedure from Ribeiro et al. for a comparable class of compounds was employed [24].

The ^{1}H NMR spectra of Glu-4-ZnPc-P in DMSO-d_6 (Figure S2) and DMF-d_7 (Figure S3) indicate deprotection of the carbohydrate groups as evidenced by the absence of signals from the isopropylidene protons. The analysis reveals three resonances centered between 9.30 and 7.94 ppm, which are due to the eight Pc-α and four Pc-β protons. The signals corresponding to the protons of the glycosylated moieties are found between 5.76 and 3.25 ppm and are partially overlapped by signals of water, especially in DMSO-d_6 solvent. Although some impurities are detected in ^{1}H NMR spectra, the HPLC technique confirms the purity of Glu-4-ZnPc-P at approximately 95% (Figures S6 and S7). The MALDI-TOF-MS mass spectrum also confirms the molecular structure of the obtained water-soluble glycosylated derivative of phthalocyanine (Figures S4 and S5).

The emission spectra of **Glu-4-ZnPc** are shown in Figure S8, while Figure S9 presents the UV–Vis spectra of this compound in DMSO and water. The excitation spectrum in DMSO is well defined, indicating no intermolecular aggregation. The sharp Q band at 687 nm, which originates from the $S_0 \rightarrow S_1$ transition, indicates that the compound exists as a monomeric species in the DMSO solution [26]. However, its optical properties differ

significantly between water and DMSO. In water, the Q band intensity is much lower than in DMSO, suggesting that the molecules aggregate due to the cofacial arrangement of the Glu-4-ZnPc [24]. The B-band of the water solution is slightly shifted to a shorter wavelength compared to the spectrum in DMSO (340 and 357 nm for DMSO and water spectra, respectively). Additionally, the Q band in the case of the water solution is also blue-shifted and split into two absorptions at 642 nm and in the range of 660–680 nm.

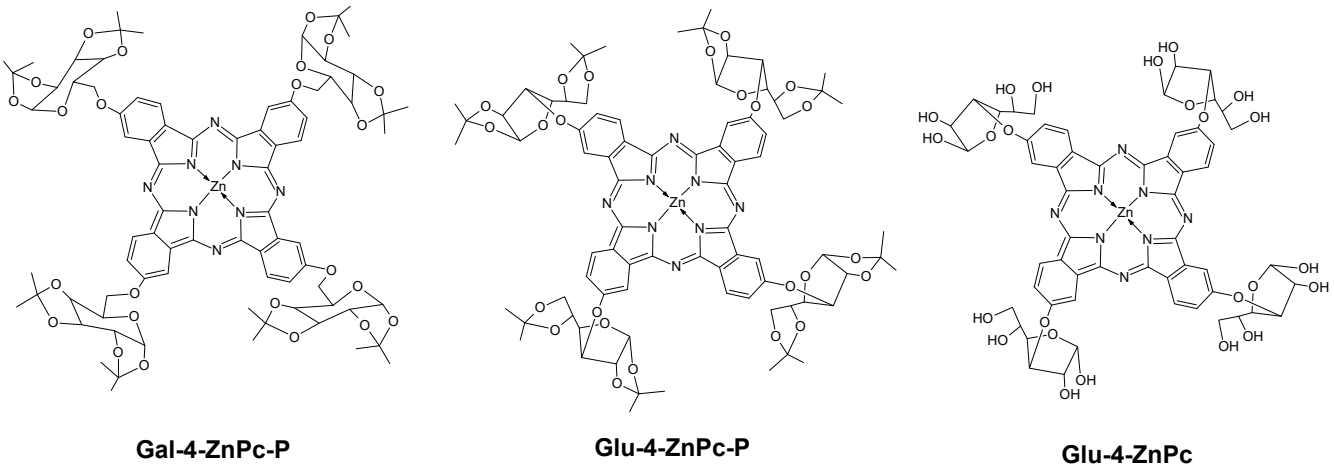


Figure 1. Structural formulas of glycosubstituted zinc phthalocyanines.

3.1. Glu-4-ZnPc-P Shows Better Photocytotoxic Activity Compared to Glu-4-ZnPc, Gal-4-ZnPc-P, and ZnPc

To evaluate the efficacy of sugar-conjugated zinc phthalocyanines, their dark and phototoxicity toward HaCaT cells stimulated with exogenous cytokines such as IL-6, IL-17A, and IL-23, which play a key role in the pathogenesis of psoriasis, was assessed using MTT assay. The photosensitizers were incubated with cells for 4 h in the dark and then irradiated with red light at a fluence of 0.9 J/cm^2. The viability was tested 24 h after irradiation. Dark cytotoxicity was evaluated under the same conditions but without the red light irradiation (after 28 h in total). Glu-4-ZnPc-P showed the highest phototoxicity toward cytokine-stimulated HaCaT cells, with IC_{50} = 2.55 μM, compared to Glu-4-ZnPc (IC_{50} = 22.7 μM), Gal-4-ZnPc-P (IC_{50} = 7.13 μM), and ZnPc (IC_{50} = 5.842 μM) after 24 h (Figure 2). The photocytotoxic efficacy of the studied compound is shown in Figure 3A. At a dose of 2.5 μM, Glu-4-ZnPc-P emerged as the most potent cytotoxic compound in our study, underscoring its potential efficacy in inducing cellular cytotoxicity. Interestingly, after 24 h of dark incubation without irradiation, the cytotoxic activity of the ZnPc–sugar conjugates was lower at higher concentrations compared to ZnPc (Figure 3B). Our findings align with several studies conducted on breast cancer cells [27,28], demonstrating that acetyl-protected conjugates exhibiting superior photodynamic activity exhibit lower intracellular uptake, thereby implying that uptake alone is not the predominant factor influencing photodynamic damage. One potential explanation could involve the intracellular localization of the compound, where it may accumulate in non-cytotoxic compartments or fail to effectively interact with critical cellular targets. Additionally, the compound's chemical properties or structural characteristics might hinder its ability to induce cytotoxic effects despite efficient cellular internalization. Moreover, the administered light dose in our study, at 0.9 J/cm^2, was comparatively smaller than those previously utilized for the activation of other metal-based photocytotoxic compounds in HaCaT cells, such as the α-(8-Quinolinoxy) zinc phthalocyanine (ZnPc-F7), with a fluence of 5.37 J/cm^2 [20].

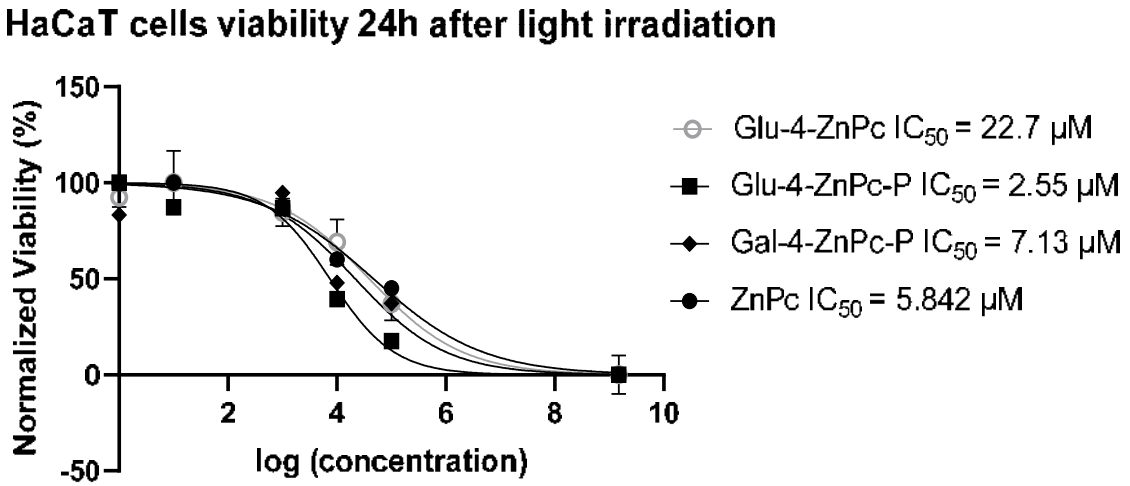


Figure 2. IC_{50} values of sugar-conjugated ZnPc compared to free ZnPc against HaCaT cells at a light dose of 0.9 J/cm^2 24 h after light irradiation.

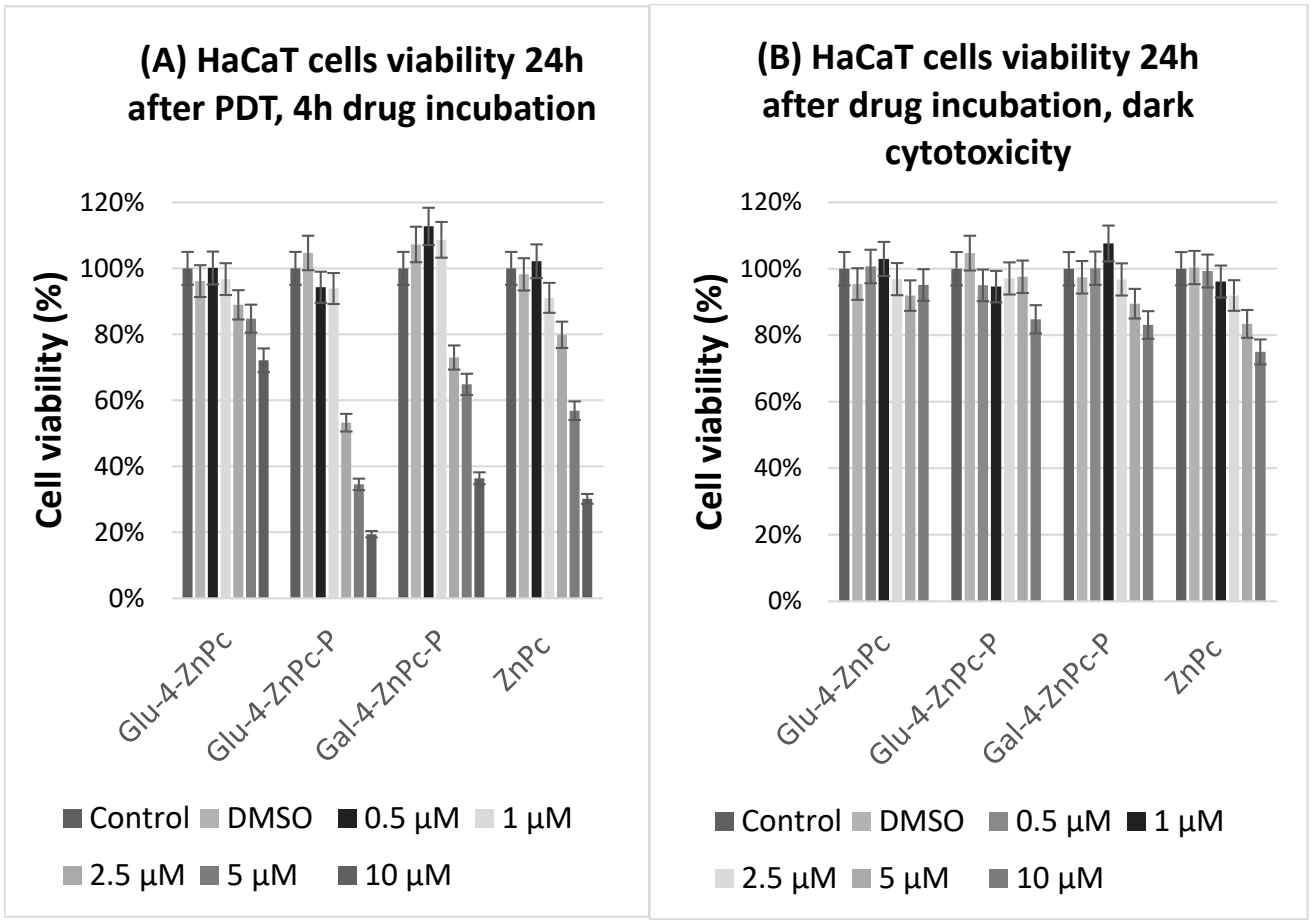


Figure 3. Cell viability of HaCaT cells stimulated with proinflammatory cytokines following 4 h or 24 h incubation with sugar–ZnPc conjugates or free ZnPc; (**A**) photocytotoxicity at 0.9 J/cm^2; (**B**) dark cytotoxicity.

To better illustrate the phototoxicity of the studied photosensitizers, we also examined the morphological changes in HaCaT cells after PDT using optical microscopy (Figure 4). The presented images revealed that the treated cells were partially or completely dead 24 h after photodynamic treatment. The residual cells primarily manifested characteristics such

as cellular shrinkage, detachment, diminished refractivity, and morphological changes indicative of cell death processes, including apoptosis and necrosis. In summary, these findings provide evidence of the cytotoxic impact exerted by the conjugates on HaCaT cells stimulated with IL-6, IL-17A, and IL-23.

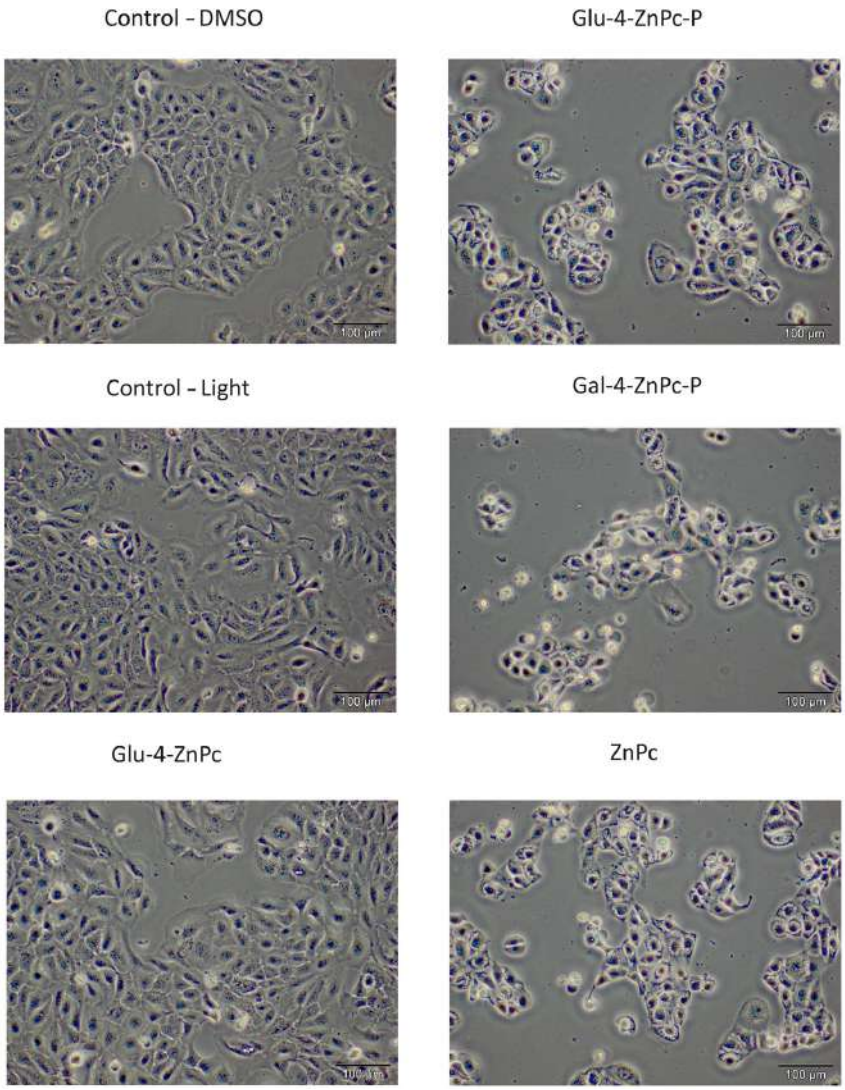


Figure 4. The morphology of cytokine-stimulated HaCaT cells 24 h after photodynamic effect with sugar-conjugated ZnPc and free ZnPc at a drug dose of 2.5 μM and light dose of 0.9 J/cm^2.

3.2. Proinflammatory Cytokines IL-6, IL-17A, and IL-23 Enhance the Cellular Uptake of Glu-4-ZnPc and Glu-4-ZnPc-P, While the Selective GLUT1 Inhibitor BAY-876 Reduces the Accumulation of the Compounds in HaCaT Cells

The cellular uptake of Glu-4-ZnPc was significantly influenced by the presence of proinflammatory cytokines IL-6, IL-17A, and IL-23. Specifically, IL-17A demonstrated the most substantial enhancement, with a mean fluorescence intensity increase of 56.3%. IL-6 and IL-23 also increased the cellular uptake by 46.6% and 42.2%, respectively (Figure 5A). The selective GLUT1 inhibitor BAY-876 exhibited a notable reduction in the accumulation of Glu-4-ZnPc. The mean fluorescence intensity was decreased by 23.8% for the cells treated with the inhibitor before treatment. Additionally, the results show a time-dependent progressive increase in Glu-4-ZnPc uptake over a 48 h period (Figure 5B).

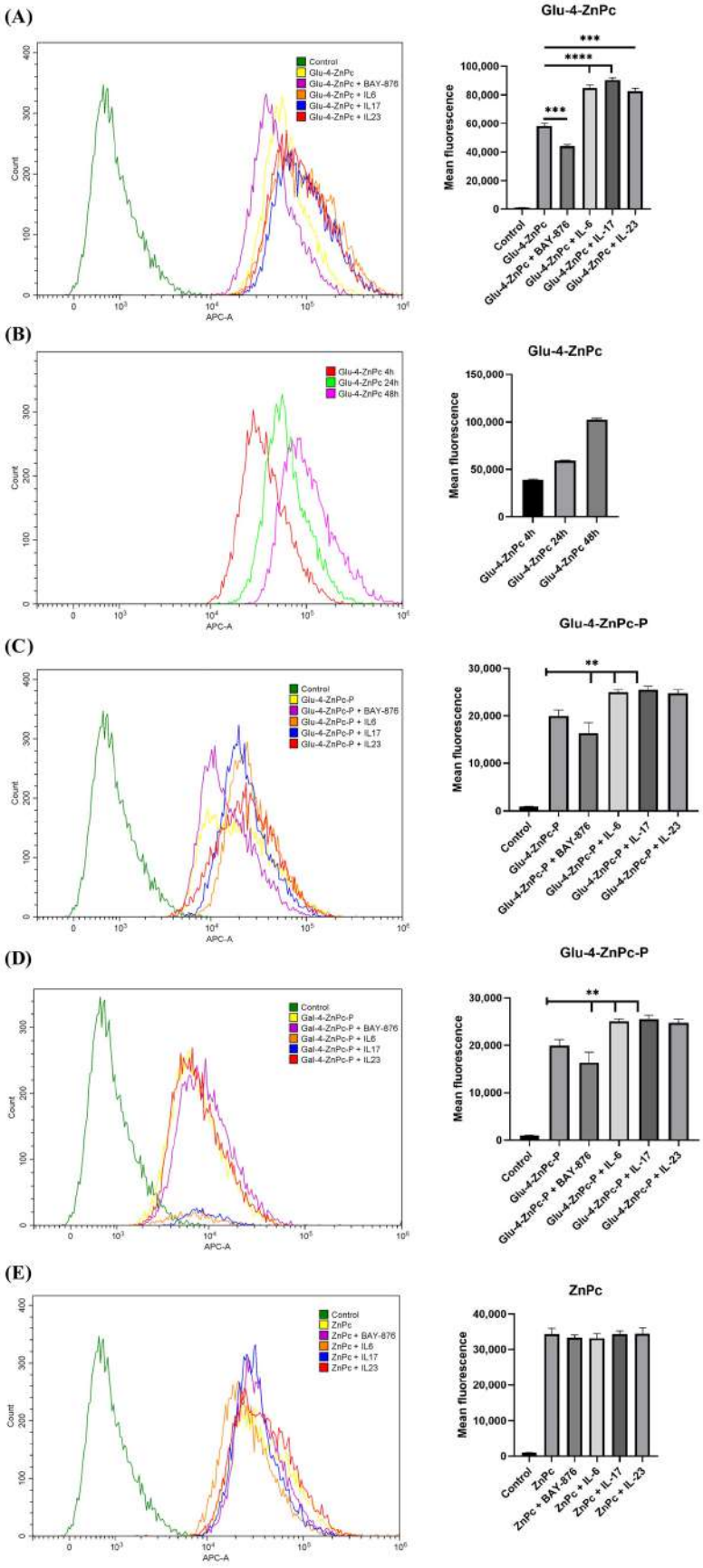


Figure 5. Flow cytometry analysis of uptake of sugar-conjugated zinc phthalocyanines compared to free ZnPc, after stimulation with proinflammatory cytokines IL-6, IL-17A, and IL-23 or inhibition with

BAY-876; (**A**) Glu-4-ZnPc after 24 h; (**B**) Glu-4-ZnPc after 4 h, 24 h and 48 h; (**C**) Glu-4-ZnPc-P after 24 h; (**D**) Gal-4-ZnPc-P after 24 h; (**E**) ZnPc after 24 h. ** ($p < 0.01$); *** ($p < 0.001$); **** ($p < 0.0001$).

In addition to Glu-4-ZnPc, we investigated the cellular uptake of the protected glucose–ZnPc conjugate Glu-4-ZnPc-P under the same conditions. After treatment with IL-6, the uptake increased significantly by 25.4% from 19,919 to 24,961 mean fluorescence. Similarly, IL-17A and IL-23 treatments resulted in elevated uptake, with percentage differences of 27.6% and 23.9%, respectively ($p < 0.01$ for all cytokines) (Figure 5C).

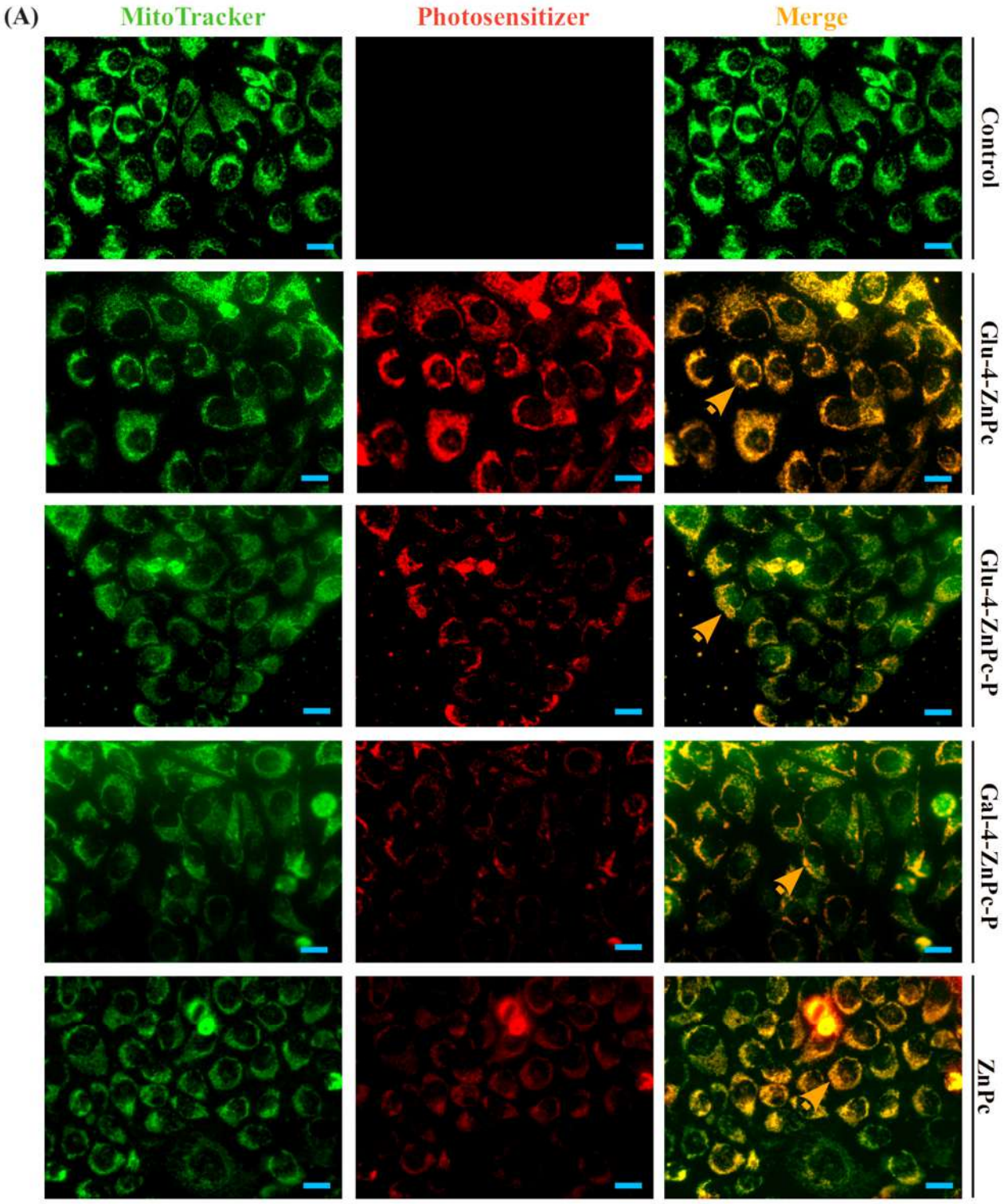
BAY-876 treatment led to a reduction in Glu-4-ZnPc-P uptake, with a mean fluorescence intensity decrease of 17.9%. However, this reduction was not statistically significant ($p = 0.072$).

Unlike Glu-4-ZnPc and Glu-4-ZnPc-P, the mean fluorescence intensity of HaCaT cells treated with Gal-4-ZnPc-P or ZnPc did not increase upon stimulation with proinflammatory cytokines IL-6, IL-17A, and IL-23, nor decrease following incubation with BAY-876 (Figure 5D,E). In summary, the mean fluorescence intensity analysis reveals a distinct hierarchy in cellular uptake among the studied compounds: Glu-4-ZnPc > ZnPc > Glu-4-ZnPc-P > Gal-4-ZnPc-P.

In our previous study, we showed that the incubation of HaCaT cells with proinflammatory cytokines, such as IL-6, IL-17A, IL-23, and IL-36, enhances the expression of GLUT1 transporter [29]. Here, for the first time, we noticed that sugar-conjugated zinc phthalocyanines are preferentially accumulated in the intracellular compartment of the hyperproliferating keratinocytes and that the unprotected Glu-4-ZnPc is partially transported by GLUT1 inhibitor. In the pursuit of prodrugs selectively targeted to treated cells, emphasis has been placed on constructs containing hexoses, including D-glucose, D-galactose, D-mannose, and D-fructose. Utilizing the overexpression of GLUT1 in hyperproliferating cells, as opposed to normal cells, has emerged as an appealing strategy for the controlled delivery of prodrugs formed by conjugating biologically active aglycons with sugar derivatives [30].

3.3. Sugar–Zinc Phthalocyanine Conjugates Are Preferentially Accumulated in Mitochondria

To confirm the targeted specificity of sugar-conjugated zinc phthalocyanines toward HaCaT cells, a medium containing 2.5 μM of each compound was administrated for 24 h. The fluorescence images showed that all of the examined drugs were predominantly localized in mitochondria, with a clear colocalization with MitoTracker®, contrary to the lysosome marker LysoTracker® (Figure 6). Glu-4-ZnPc showed a significantly higher colocalization with mitochondria compared to other phthalocyanines ($p < 0.001$) (Figure 6C). Acetyl-protected Glu-4-ZnPc-P and Gal-4-ZnPc-P displayed a lower intracellular accumulation compared to unprotected Glu-4-ZnPc and free ZnPc. The results are in line with those obtained by flow cytometry. These findings prove the increased intracellular accumulation of unprotected glucose–zinc phthalocyanine in HaCaT cells. In contrast to previous findings suggesting inadequate localization of phthalocyanines within mitochondria due to a dispersed distribution [27], our study demonstrates successful accumulation of glucosylated zinc phthalocyanines within the mitochondria. Despite differences in cytotoxic mechanisms and the observed effects of the investigated sugar-conjugated phthalocyanines, all of the studied compounds exhibited a preference for subcellular localization in the mitochondria. These organelles have been extensively studied as focal points for optimizing the efficacy of PDT due to their vital roles in cellular metabolism [31–33]. Targeted PDT for mitochondria is achieved using phthalocyanine photosensitizers facilitated by carrier delivery and structural modification involving aromatic nitrogen heterocycles, ammonium, TPP, or polypeptides [34]. However, despite certain significant findings [35], the association between mitochondria and psoriasis has remained unclear until now.



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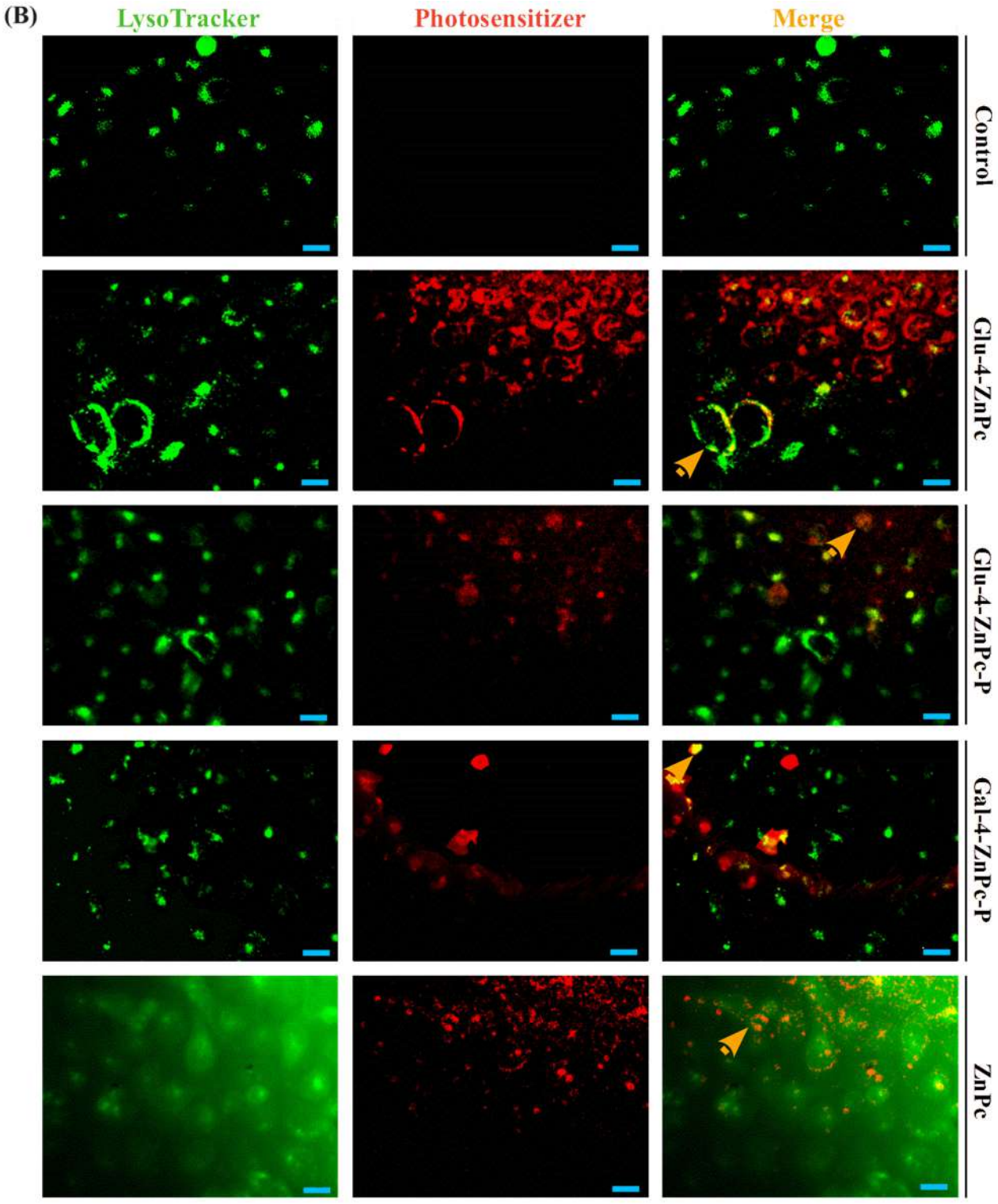

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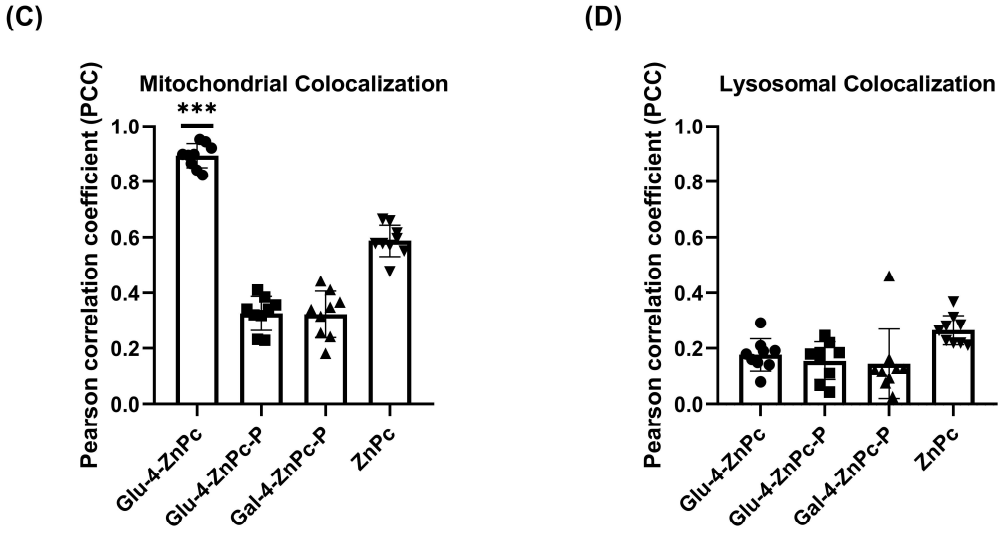

Figure 6. Subcellular localization of sugar-conjugated zinc phthalocyanine and free ZnPc in HaCaT cells after 24 h incubation. The red fluorescence of the compounds was mainly colocalized with (**A**) MitoTracker®. In contrast, the red fluorescence of the compounds displayed scant colocalization with (**B**) LysoTracker® green fluorescence; Pearson's correlation coefficients (PCCs) of images (n = 9) of phthalocyanines and green MitoTracker® (**C**,**D**) LysoTracker® in HaCaT cells using Pearson's correlation coefficient and one-way ANOVA. n = 9 images for each group. *** $p < 0.001$. Orange arrows show areas of colocalization of autofluorescent signal with mitochondrial or lysosomal probes. Scale bars = 20 μm.

3.4. Glu-4-ZnPc-P Photogenerates Intracellular ROS the Most Efficiently in HaCaT Cells

The irradiation of the sugar-conjugated zinc phthalocyanines led to the production of ROS, one of the main mediators of cellular death induced by PDT. The intracellular ROS generation efficiency of the studied compounds against control HaCaT cells or stimulated with exogenous cytokines was examined by using DCF, an oxidized green fluorescent product of 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). We attempted to compare the formation of ROS between different phthalocyanines. The results show that all of the analyzed phthalocyanines can induce the formation of ROS under illumination, with the efficiency at generating ROS in the following order: Glu-4-ZnPc-P > Gal-4-ZnPc-P > ZnPc >> Glu-4-ZnPc ($p < 0.001$, Figure 7), which is strongly correlates the with in vitro photocytotoxicity. Moreover, we observed that the preincubation of HaCaT cells with IL-17A significantly increases the intracellular ROS generation following Glu-4-ZnPc-P-PDT treatment ($p < 0.001$). Our study aligns with the results obtained by Liu et al. [27] and Kimani et al. [28], who showed that acetyl-protected conjugates generate higher ROS levels compared to unprotected sugar-conjugated zinc phthalocyanines on MCF-7 breast cancer cells. One possible explanation for these results could be the presence of subcellular localization or compartmentalization of the photosensitizer within specific cellular organelles, leading to localized ROS production even in the context of overall low cellular uptake. Additionally, the efficiency of ROS generation might be influenced by the photosensitizer's interaction with intracellular targets. It is worth noting that apart from IL-17, which increased the intracellular ROS generation following Glu-4-ZnPc-P-PDT treatment, the presence of proinflammatory cytokines did not affect the intracellular ROS generation. This could be attributed to the inherent photostability and quantum yield of Glu-4-ZnPc, which remained unaffected by cytokines. Additionally, factors such as enhanced antioxidant defenses or cellular stress responses might mitigate ROS production, leading to low cell cytotoxicity despite the higher intracellular concentration of Glu-4-ZnPc. Further investigations into the subcellular distribution, intracellular trafficking, and molecular interactions of the photosensitizer may provide insights into the mechanisms underlying high ROS

levels despite low cellular uptake, contributing to a comprehensive understanding of its therapeutic potential.

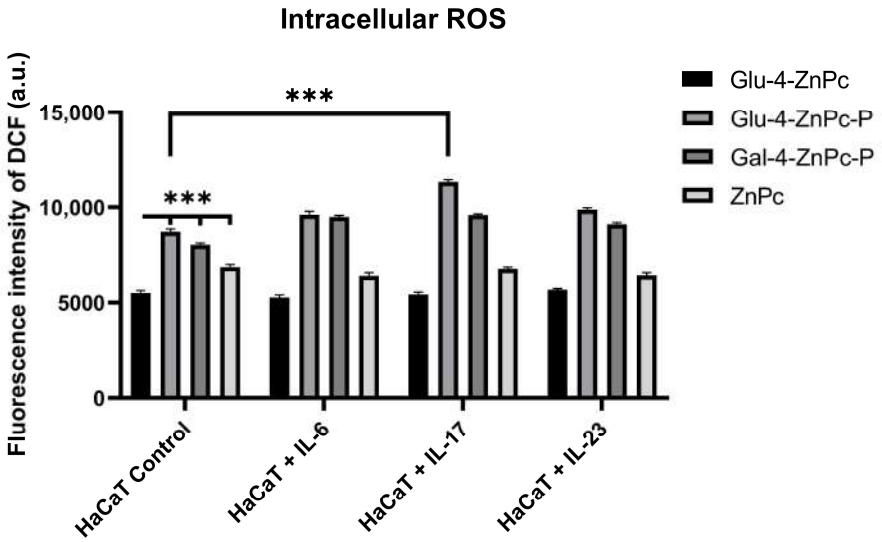

Figure 7. Cellular ROS generation efficiency Glu-4-ZnPc, Glu-4-ZnPc-P, Gal-4-ZnPc-P, and ZnPc (all at 2.5 μM) with the light dose of 0.9 J/cm^2; *** ($p < 0.001$).

3.5. In Vivo Antipsoriatic Activity of Glu-4-ZnPc-P

In light of the more promising in vitro results observed with Glu-4-ZnPc-P, we attempted to verify its safety and therapeutic efficacy in vivo. The skin of mice from each group was monitored daily: Control skin (B1), IMQ-Psoriasis-Like Skin (B2), IMQ-Psoriasis-Like Skin with Red Light irradiation (B3), and PDT: IMQ-Psoriasis-Like Skin following intravenous administration of Glu-4-ZnPc-P and red light irradiation (B4) (Figure 8). The PASI index was calculated based on the daily monitoring of skin condition. The PDT-treated group demonstrates significant improvement in PASI index compared to the skin of the IMQ-Psoriasis-Like group ($p < 0.0052$) (Figure 8(B9)). Moreover, the impact of PDT was analyzed by macroscopic assessment of spleens in all groups, as well by calculation of spleen/body weight ratio index (Figure 8(B5–B8,B10)). Spleens of animals after Glu-4-ZnPc-P-PDT have significantly inhibited IMQ-related splenomegaly in relation to IMQ-Psoriasis-Like Skin ($p < 0.001$) and Light Irradiated IMQ-Psoriasis-Like Skin group, respectively ($p < 0.001$). Additionally, we evaluated the impact of Glu-4-ZnPc-P-PDT therapy on the level of proinflammatory cytokines in mice serum. We found that IL-6 ($p < 0.05$) and IL-17A ($p < 0.001$) were significantly lower 7 days after irradiation (Figure 8(B11)). Finally, hematoxylin and eosin (H&E) staining and assessment by experienced histopathologists confirm the effectiveness of Glu-4-ZnPc-P-PDT (Figure 8(C1–C4)). The analysis confirmed the restoring normal morphology, skin barrier and thickness, as well as the visible reduction in leukocyte infiltration in all skin compartments, including the epidermis, dermis, subcutaneous dermis, and muscle dermis in PDT animals (C4). The skin of the PDT group demonstrates comparable morphological and cellular parameters to the skin of the control group (C1) but opposite to incomplete only light irradiated (C3) and IMQ-Psoriatic-like skin (C2) groups of animals, respectively. Our study shows the promising realm of PDT in psoriasis treatment in comparison with previous investigations that have explored zinc phthalocyanines as potential photosensitizers. A study conducted by Liu et al. [20] revealed the anti-psoriasis effects of α-(8-quinolinoxy) zinc phthalocyanine (ZnPc-F7)-mediated PDT, showcasing its inhibitory impact on hyperproliferation and immune regulation in both in vitro and in vivo models. This aligns with our findings, where Glu-4-ZnPc-P exhibited significant inhibitory effects on HaCaT cell proliferation and attenuated the psoriasis-like inflammation in mice.

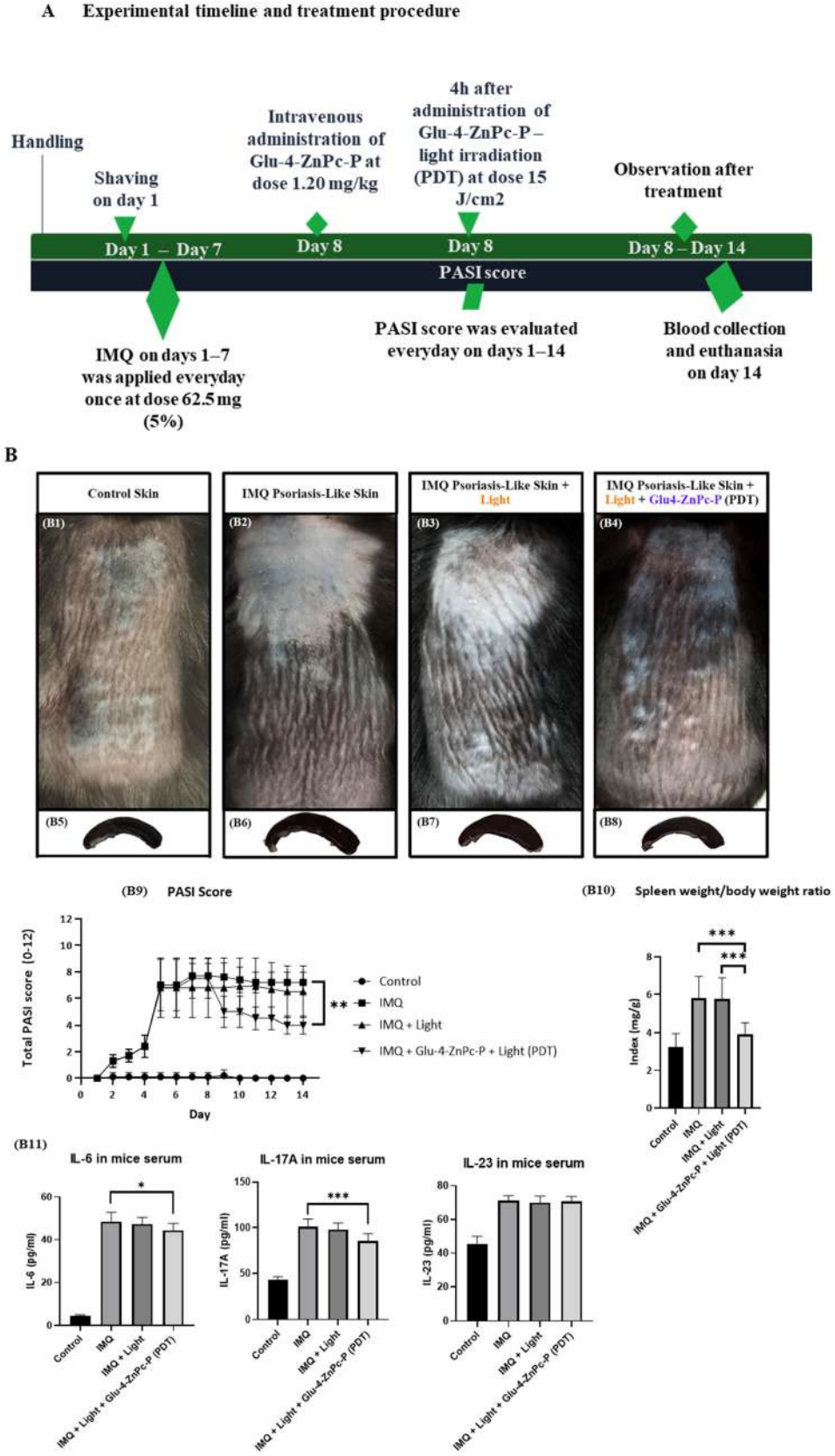

Figure 8. *Cont.*

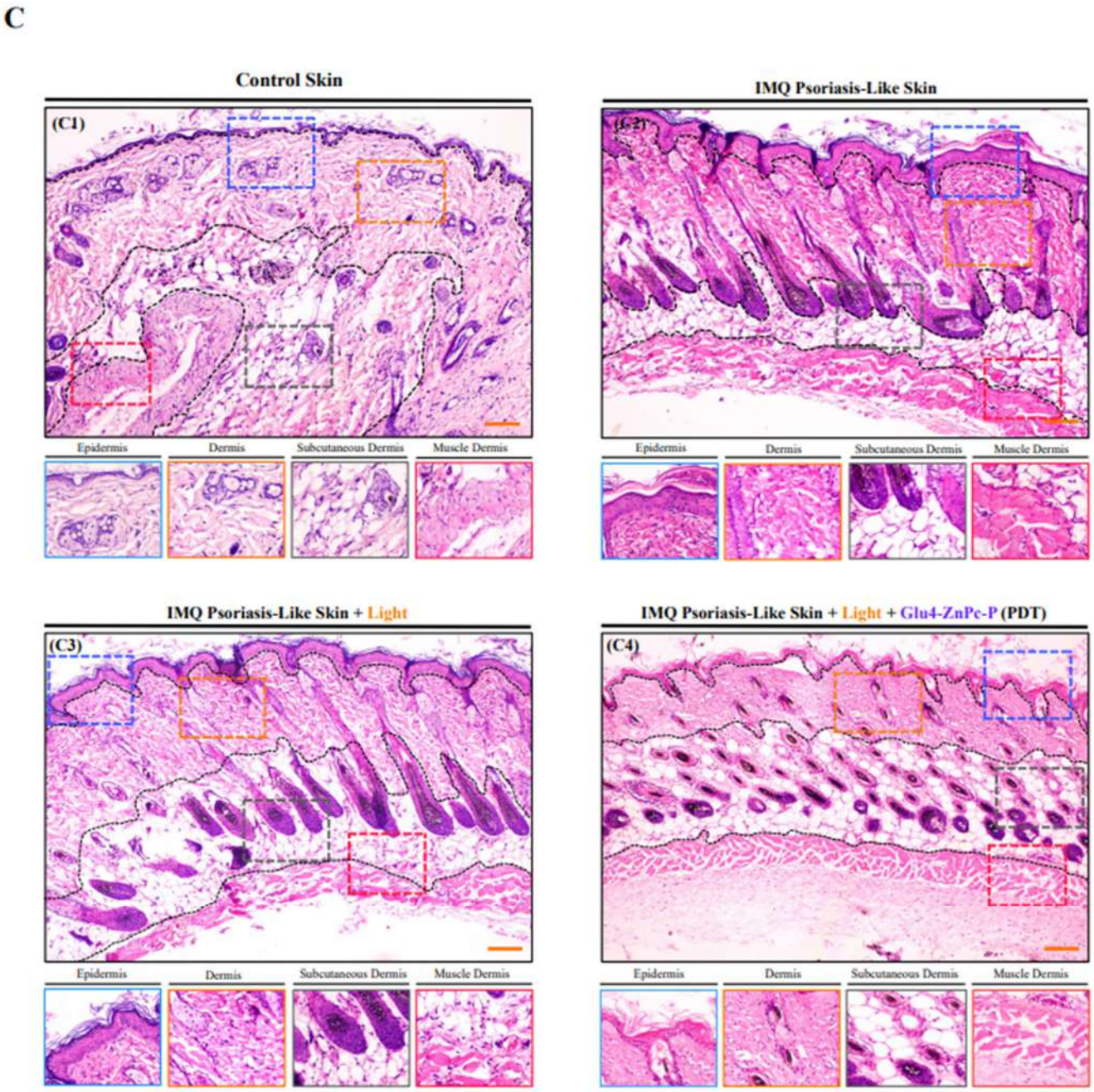

Figure 8. (**A**) The diagram illustrates the steps of experimental design in time. (**B**) The safety and therapeutic effects of PDT were analyzed in several ways. The daily imaging of skin backs of (**B1**): control skin; (**B2**): IMQ-Psoriasis-Like Skin; (**B3**): IMQ-Psoriasis-Like Skin with Light irradiation; (**B4**): complete PDT: IMQ-Psoriasis-Like Skin following Light Irradiation and intravenous administration of Glu-4-ZnPc-P; (**B5**–**B8**): macroscopic assessment of spleens in the studied groups; (**B9**): Total PASI score; (**B10**): calculation of spleen/body weight ratio index; (**B11**): determination of IL-6, IL-17A and IL-23 levels in mice serum by ELISA; (**C**) hematoxylin and eosin (H&E) staining of all skin compartments, including the epidermis, dermis, subcutaneous dermis and muscle dermis in (**C1**): control group; (**C2**): IMQ-Psoriatic-like skin; (**C3**) IMQ-Psoriasis-Like Skin with Light irradiation; (**C4**): Glu-4-ZnPc-P-PDT. The whole skin tissue from all four groups was additionally selected as squares and enlarged below main images for epidermis (blue), dermis (orange), subcutaneous (gray), and muscle (red) dermis for better visualization of skin compartments. * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$); the main images are under magnification of ×10, scale bars = 100 μm.

4. Conclusions

In conclusion, our study underscores the potential of sugar-conjugated zinc phthalocyanines, particularly tetrasubstituted protected glucose–ZnPc (Glu-4-ZnPc-P), as promising antipsoriatic agents. Demonstrating superior phototoxicity and enhanced cellular uptake in cytokine-stimulated keratinocytes, Glu-4-ZnPc-P proved effective in inhibiting abnormal keratinocyte proliferation. Moreover, the in vivo application of Glu-4-ZnPc-P photodynamic therapy demonstrated significant improvement in Psoriasis Area and Severity Index (PASI), effectively inhibiting splenomegaly and restoring normal skin morphology in an established IMQ psoriasis-like model. Despite a few promising outcomes, the clinical utilization of PDT in psoriasis treatment is still limited. This emphasizes the importance of conducting additional research to assess the effectiveness of a broader range of photosensitizers and explore the potential of targeted photodynamic therapy in the treatment of psoriasis.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/pharmaceutics16060838/s1, Figure S1: FT-IR spectrum of Glu-4-ZnPc in KBr; Figure S2: ^{1}H NMR (600.15 MHz) of Glu-4-ZnPc in DMSO-d_6; Figure S3: ^{1}H NMR (600.15 MHz) of Glu-4-ZnPc in DMF-d_7; Figure S4: MALDI-TOF of Glu-4-ZnPc. Inset: Experimental isotopic distribution pattern for Glu-4-ZnPc; Figure S5: Simulated isotopic distribution pattern for Glu-4-ZnPc; Figure S6: HPLC analysis of Glu-4-ZnPc using 280 nm detection; Figure S7: HPLC analysis of Glu-4-ZnPc using 670 nm detection; Figure S8: Emission spectra of Glu-4-ZnPc in H_2O and DMSO; Figure S9: Excitation spectra of Glu-4-ZnPc in H_2O and DMSO.

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Institutional Review Board Statement: The study was approved by the Local Ethics Committee on Animal Experiments (LKE) in Wroclaw, Poland (No. 035/2023).

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Review

An Update on Photodynamic Therapy of Psoriasis—Current Strategies and Nanotechnology as a Future Perspective

Sebastian Makuch [1,*], Mateusz Dróżdż [2], Alicja Makarec [3], Piotr Ziółkowski [1] and Marta Woźniak [1]

[1] Department of Clinical and Experimental Pathology, Wroclaw Medical University, 50-368 Wroclaw, Poland
[2] Laboratory of RNA Biochemistry, Institute of Chemistry and Biochemistry, Freie Universität Berlin, Takustraße 6, 14195 Berlin, Germany
[3] Faculty of Biotechnology, University of Wroclaw, 50-383 Wroclaw, Poland
* Correspondence: sebastian.mk21@gmail.com

Abstract: Psoriasis (PS) is an immune-mediated skin disease with substantial negative effects on patient quality of life. Despite significant progress in the development of novel treatment options over the past few decades, a high percentage of patients with psoriasis remain undertreated and require new medications with superior long-term efficacy and safety. One of the most promising treatment options against psoriatic lesions is a form of phototherapy known as photodynamic therapy (PDT), which involves either the systemic or local application of a cell-targeting photosensitizing compound, followed by selective illumination of the lesion with visible light. However, the effectiveness of clinically incorporated photosensitizers in psoriasis treatment is limited, and adverse effects such as pain or burning sensations are frequently reported. In this study, we performed a literature review and attempted to provide a pooled estimate of the efficacy and short-term safety of targeted PDT in the treatment of psoriasis. Despite some encouraging results, PDT remains clinically underutilized. This highlights the need for further studies that will aim to evaluate the efficacy of a wider spectrum of photosensitizers and the potential of nanotechnology in psoriasis treatment.

Keywords: photodynamic therapy; psoriasis; nanotechnology; PDT

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1. Introduction

Psoriasis is an immune-mediated and chronic skin disease characterized by the abnormal proliferation of skin cells, known as keratinocytes. It is estimated that its prevalence varies from 0.14% (East Asia) to 5.32% (Central Europe) [1]. This disease is associated with genetic predisposition, autoimmune disorders, mental health, and environmental factors, including infections, stress, alcohol, smoking, obesity, physical trauma, or certain medications, such as lithium and beta-blockers [2,3]. Its pathogenesis is defined as a multi-factorial process that depends on uncontrolled increased proinflammatory cytokine expression, among the most important being IL-17, IL-21, IL-22, IL-23, and IL-26. For instance, IL-17 and IL-23 stimulate keratinocyte proliferation and increase the secretion of TNF-α and chemokines, which enhance the activation of dendritic cells, leading to inflammation [4–6].

For many years, psoriasis has been treated by many traditional approaches, including acupuncture, hydrotherapy, and dietary treatment, which can help in controlling the progress of the disease and alleviating some of the symptoms. Scientists have implemented a wide range of therapeutic tools customized according to the type of psoriasis, its location, extent, and severity, including corticosteroids, calcipotriene, coal tar, oral systemics (e.g., acitretin, cyclosporine, methotrexate), biologics (etanercept, infliximab, alefacept, efalizumab, and ustekinumab), as well as phototherapy, with the use of ultraviolet B light (UVB), psoralen ultraviolet A light (PUVA), or photodynamic therapy (PDT) [7,8].

In contrast to biological agents and other drugs, phototherapy is an effective and safe method that does not cause any systemic side effects. This technique was originally developed with the idea of using broadband ultraviolet B light (BB-UVB, 290–230 nm).

However, subsequent studies proved the better efficacy of the narrow-band ultraviolet B (NB-UVB, 311 nm), and even an excimer laser/lamp (308 nm) used as a monochromatic UVB source [9]. All these methods are currently used as first-line therapies for stable plaque psoriasis. The next key achievement turned out to be the development of psoralen ultraviolet A (PUVA) treatment, which combines a photosensitizing drug (PS) and UVA radiation. This method is currently the first-line therapy for refractory psoriatic plaques [10]. The other light source that proved to be efficient in psoriasis treatment was the flash lamp pumped pulsed dye laser (585–595 nm), which is preferred for nail psoriasis [2]. Nevertheless, because of its efficacy and cost-effectiveness, PDT seems to be an attractive option for psoriasis treatment [7]. This technique is based on a photochemical reaction used to destroy diseased cells selectively. It comprises three elements: a light-sensitive dye (photosensitizer), which accumulates in the intracellular compartment of the cells, a light with an appropriate wavelength that is capable of exciting the photosensitizer, and oxygen dissolved in the tissue being treated. Target sites for PS include different organelles, such as the plasma membrane, mitochondria, lysosomes, Goldi apparatus, or endoplasmic reticulum [11]. Under specific conditions, PS is activated by absorbing the photons of light energy and transferring them to molecular oxygen, leading to the formation of other reactive oxygen species (ROS), including hydroxyl radicals (OH) or superoxides (O^2). These compounds produce strong reactivity with lipids, nucleic acids, proteins, and other biochemical substrates, leading to the activation of pathogenetic mechanisms, which have been thoroughly described in various reviews [8,12,13]. Photodamage leads to cell death through apoptosis, necrosis, or autophagy, depending on the cell type, PDT dosimetry (light intensity), and PS type. Photosensitizers localized in the mitochondria affect apoptosis after irradiation, whereas those in the plasma membrane stimulate necrosis [14]. However, the effectiveness of clinically incorporated photosensitizers is limited, and adverse effects such as pain or burning sensations are frequently reported [15,16]. Therefore, it is desirable to develop novel photosensitizers with a convincing safety profile and potent, selective activity.

In this study, we aimed to review the literature in order to provide a pooled estimate of the efficacy and short-term safety of targeted PDT in the treatment of psoriasis. We focused on describing clinical trials evaluating photosensitizers in the PDT of psoriasis. Due to significant disagreement on the use of this method in the scientific community, our goal is to estimate whether PDT treatment is worth further consideration for psoriasis treatment. Reported differences may be explained by variables in terms of patient selection criteria, drug concentrations, the light spectrum, or dosages [17]. Therefore, such a juxtaposition of the latest achievements in this regard seems to be very beneficial for further analysis.

2. Efficacy of ALA-PDT Therapy

The use of PDT in psoriasis treatment is subject to many discussions. To the best of our knowledge, there has been no study showing a 100% recovery of psoriasis PDT-treated patients. Nevertheless, several studies showed the prominent role of PDT in blocking the uncontrolled production of inflammatory cytokines that lead to the apoptosis of T-lymphocytes and inflammation during psoriasis development [18,19].

One major advancement in the use of PDT in psoriasis treatment was the development of 5-aminolevulinic acid (ALA) as a photosensitizing agent, localizing in the abnormal epithelium and causing cytotoxic effects [20]. In physiological conditions, ALA is an early intermediate in the heme biosynthesis pathway. However, this compound can be further metabolized by the cells into protoporphyrin IX (PpIX), which may be subsequently activated by visible light [21]. The accumulation of PpIX in the intracellular compartment of the cells results from the lack of conversion to heme because of the limiting enzyme known as ferrochelatase. This condition leads to oxidative damage and induces cytotoxicity. Various reviews have extensively analyzed the metabolic pathways of ALA and its derivatives in PDT therapy [21–23].

Including the clinical trials, ALA-PDT was first mentioned in 1994, when three patients with chronic plaque-stage psoriasis were administered 10% ALA. The clearance of the psoriatic lesion with PDT was comparable to those treated with dithranol; all patients experienced a mild burning sensation [24]. This study was further continued after four years on a larger study group of 22 patients with chronic plaque psoriasis. Among 80 treatment sites, there was a clear reduction in 14 sites (18%), and a 30–50% reduction in the scale, erythema, and induration (SEI) index. There was no improvement observed in 60 sites (75%) [25]. Following this study, many other clinical trials described some beneficial effects of ALA-PDT in psoriasis and found that the apoptosis of T-lymphocytes within inflammatory plaques was associated with reduced inflammatory cytokine production (such as TNF- α, IL-1β, and IL-6) and the normalization of keratinocyte proliferation. The potential mechanism of PDT-action was described recently by Wang et al. who found that ALA-PDT excites the MAPK pathway, promoting the expression of p38, JNK, and ERK kinases [26]. This activation leads to the upregulation of the apoptotic genes PARP and caspase 3, which enhance cell apoptosis [24]. Furthermore, Yi et al. showed the role of SOCS1 and SOCS3 in ALA-PDT-mediated psoriasis treatment. ALA-PDT activates SOCS1/3 productivity by increasing the intracellular oxidative stress in keratinocytes. Therefore, SOCS1/3, which is known as a potential blocker of the JAK pathway, attenuates the proliferation of keratinocytes in psoriasis [27]. In another in vitro study, Chen et al. demonstrated that ALA-PDT reduced the number of abnormal T cells and the mRNA expression of IL-17 and IFN-γ involved in a psoriasis lesion [28]. Nevertheless, information on the molecular mechanism of ALA-PDT action in psoriasis treatment is still scarce and requires further investigation.

Considering the recently published studies, the use of systemic ALA-PDT for psoriasis was also addressed by Liu et al., who described the case of a 49-year-old male patient with chronic plaque psoriasis. The patient had a psoriasis lesion on his finger with pyogenic granuloma (PG). After one week of ALA-PDT treatment, signs of improvement were demonstrated. There were also no signs of reoccurrence after one month of treatment [29]. However, although this case shows the treatment success of ALA-PDT, it relates only to one patient, which may be not sufficient to statistically estimate the efficiency of ALA-PDT. Other clinical researchers noticed a quite low efficiency of ALA-PDT. In independent studies, Le Pillouer-Prost et al., Almutawa et al., and Choi et al. found that due to the variability in clinical responses and severe pain, ALA-PDT was not sufficient for chronic plaque psoriasis [15,16,30]. The most common side effect experienced with topical ALA-PDT was pain at the irradiation site, which lasted up to 2 days in some patients [31]. The pain was described as a stinging or burning sensation in the treatment sites, sometimes leading to discontinuation of treatment with this method. Moreover, low photodynamic doses (5–10 J/cm^2) and topical anesthetics such as 3% lidocaine hydrochloride cream, capsaicin cream, or lidocaine-prilocaine 5% cream (EMLA) turned out to be ineffective in alleviating the pain caused by ALA-PDT [32]. Taken together, these findings present a major challenge for researchers to improve the efficacy of ALA-PDT in psoriasis treatment. The solution seems to be the development of novel photosensitizers or using PDT in combination with other techniques (e.g., nanotechnology); this concept is discussed later in this review.

Considering the fact that ALA-PDT has more advantages than chemotherapy or radiation (presenting little or no systemic toxicity) and surgery (healing without scarring) to treat psoriasis, this method has significant potential for improvement [33]. Maytin et al. performed a pilot study assessing the use of vitamin D (VD) combined with ALA-PDT. The concept appeared due to the common prescription of vitamin D analogs (such as calcitriol and calcipotriol) against psoriasis by dermatologists. Seven patients with chronic plaque psoriasis were enrolled in this study; each plaque was treated with topical 0.005% calcipotriol (cream or ointment) or a matched vehicle control applied twice daily for 6 days. On day 7, patients underwent one PDT session with topical 20% ALA, red or blue light, and a power density of 100 mW/cm^2. In findings inconsistent with the researchers'

hypothesis, no clinical improvement in psoriasis was observed for plaques treated with calcipotriol cream. However, in the calcipotriol ointment-treated plaques, clinical success became apparent due to VD preconditioning. A psoriasis symptom inventory (PSI) index revealed a greater decline on the VD-preconditioned side than on the vehicle control side. Moreover, a remarkable decline in the severity of itching was reported within the VD-pretreated plaques compared to the control plaques. Thus, these observations highlighted the promising potential of ALA-PDT in alleviating psoriatic lesions pretreated with vitamin D ointment [33]. Following this concept, calcipotriol ointment was also administered to the psoriatic area of the leg of a patient suffering from squamous cell carcinoma (SCC). Together with skin dermabrasion and the PDT technique, the patient was successfully treated [34].

Furthermore, it has been shown that 80% of patients with psoriasis exhibit symptoms in their nails [35]. Their involvement is associated with a high prediction of psoriatic arthritis. Therefore, it is crucial to treat nail psoriasis, which is challenging due to therapy resistance. In an open-trial study with 69 nails from 8 patients, Tehranchinia et al. compared the efficacy of clobetasol 0.05% ointment and ALA-PDT in the treatment of severe nail psoriasis. The mean nail psoriasis severity index (NAPSI) scores in the nails treated with clobetasol were significantly less than those in the nails treated with ALA-PDT. However, this finding changed diametrically 6 months after the last treatment session. There was a significant time-effect improvement in all the nail matrix, nail bed, and total NAPSI scores in both treatment groups. Although more data are needed for further evidence, these initial results suggest that the efficacy of ALA-PDT at a 24-week follow-up was greater than that of clobetasol [36].

The effects of ALA-PDT on keratinocytes and in vivo model of psoriasis have been summarized and shown in Figure 1.

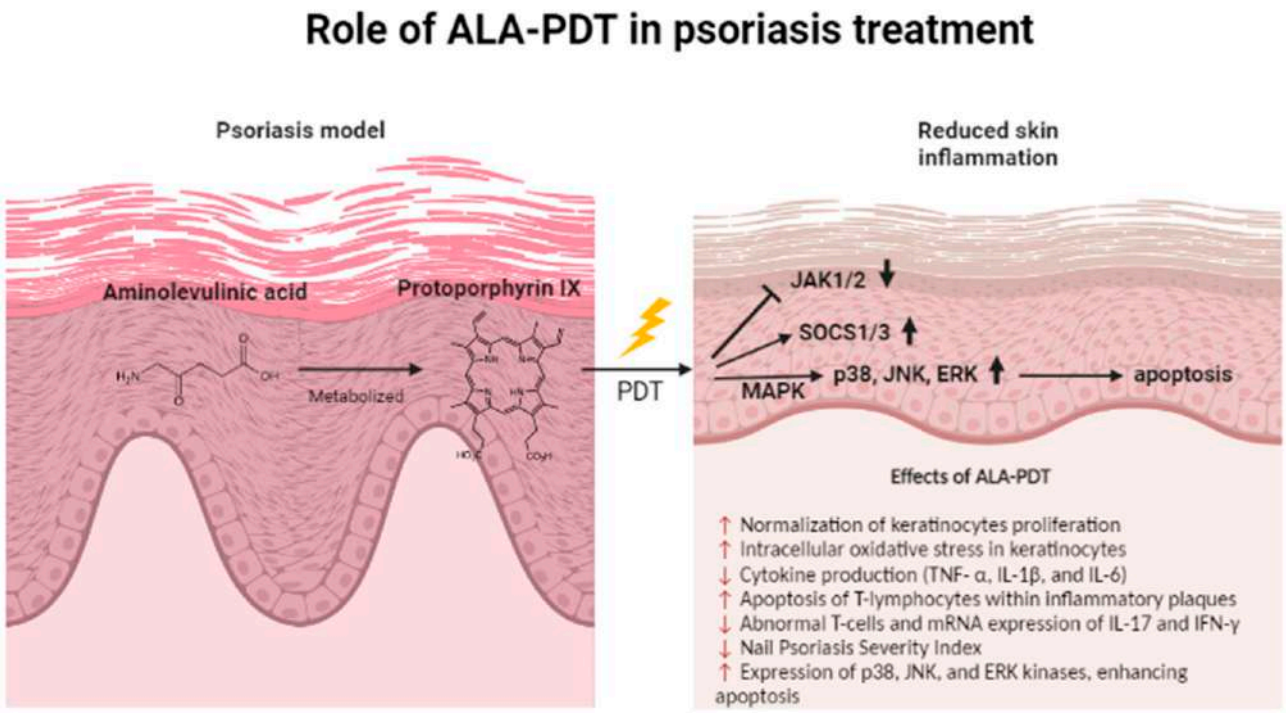

Figure 1. The role of ALA-PDT in psoriasis treatment. Aminolevulinic acid (ALA) is metabolized by cells into protoporphyrin IX (PpIX), which may be subsequently activated by visible light. The effects of ALA-PDT on a psoriasis model and the affected genes have been summarized.

3. Efficacy of Non-ALA-PDT Therapy

To improve selective targeting, localized action, and the stimulation of immune responses, the majority of the current studies aimed to design the most efficient porphyrin-based photosensitizers for PDT. These compounds contain photophysical properties suit-

able for PDT application, such as the high quantum yield of singlet oxygen and light absorption at 600–750 nm, increasing light penetration to deeper areas of psoriatic lesions [37]. It is already discovered that the number and location of the positively charged groups in the macrocyclic structure play a key role in porphyrin uptake by the target cells and for appreciable $^1O_2$ production [38]. For instance, Calzavara-Pinton et al. performed a study with a modified version of ALA, known as methyl aminolevulinate (MAL), exhibiting more lipophilic properties. Thanks to this structural change, MAL may penetrate deeper into the skin than ALA. In this retrospective observational study, including 221 patients from 20 Italian hospital centers, 17 of them had psoriatic plaques. Four patients (4/17, 23.5%) developed a marked inflammatory reaction, and 5 patients (5/17; 29.4%) had severe pain and/or a burning sensation. Six (6/17; 35.3%) patients showed marked improvement [17]. Furthermore, Slomp et al. analyzed the photodynamic effect of different porphyrin derivates on HaCaT keratinocytes. They found that those porphyrins presenting at least two adjacent positively charged groups showed the most anti-inflammatory and anti-hyperproliferating effects, leading to the reduction of edema, cellular infiltration, and hyperproliferation of the epidermis [39]. Similarly, an immunosuppressive effect of a porphyrin form (5,10-diphenyl-15,20-di(N-methylpyridinium-4-yl) porphyrin) was recently reported in a mouse psoriasis model. PDT diminished several inflammatory indicators, including proinflammatory cytokine secretion and neutrophil infiltration, and led to reduced keratinocyte proliferation [40]. Another photosensitizer that has been proven to be active against skin inflammation is the α-(8- quinolinoxy) zinc phthalocyanine (ZnPc-F7). This compound is characterized by good solubility and low toxicity. Due to its excitation at 670 nm, it may penetrate more deeply into the skin compared to the PUVA-based treatment. Liu et al. witnessed a significant reduction in HaCaT cell proliferation and IL-17 mRNA expression after ZnPc-F7-mediated PDT, indicating a therapeutic effect in psoriasis [41]. The modified version of phthalocyanine tested in the psoriatic lesion was the silicon phthalocyanine (Pc) 4, coupled with red light. Pc4-PDT elicited cell death through apoptosis on activated CD3+ T cells in the psoriatic lesion, in a dose-response manner [42]. Furthermore, a recent study identified another potential therapeutic candidate for the PDT of psoriasis. Lin et al. investigated the anti-inflammatory effects of novel NNO-tridentate vanadium (IV) complexes in a psoriasis-like skin disease mouse model. These compounds reduced the expression of IL-17 and IL-22 cytokines, suggesting a promising role in relieving psoriatic symptoms [43].

While ALA-PDT seems to have been more extensively researched, there is a gap in the knowledge regarding the efficacy of other photosensitizers in psoriasis treatment. Table 1 summarizes the most critical in vivo and clinical studies since the onset of PDT in psoriasis treatment. This table includes all details about the treatment methods, i.e., the method of delivery, wavelength, treatment parameters, and the total number of treatment sessions; all are involved in the final success of a complete recovery.

Table 1. A summary of in vivo studies and clinical trials evaluating the effect of PDT in psoriasis treatment.

Study	Photosensitizer	Way of Delivery	Wavelength	Treatment Parameters	Pre-Treatment with Drug	Number of Patients	Total Number of Treatment Sessions	Results	Side Effects	Reference
Treatment of psoriasis by topical photodynamic therapy with polychromatic light [24].	5-aminolevulinic acid (ALA, 10%)	Topical	600–700 nm	Light dose: 25 J/cm^2 Dose rate: 70 mW/cm^2	5 h	3	Max: - 3 times per week	Dithranol and topical PDT were comparable.	Burning sensations during irradiation	Boehncke et al. (1994)
The variable response of plaque psoriasis after a single treatment with topical 5-aminolaevulinic acid photodynamic therapy [25].	ALA (20%)	Topical	400–650 nm	Light dose: 2–16 J/cm^2, Dose rate: 10–40 mW/cm^2	4 h	22	Max: 12 Once a week	Thirty-five percent of patients' psoriasis was cleared. 80 treatment sites: 14 cleared, 6 showed a 30–50% reduction in SEI score, 60 showed little or no improvement.	Stinging, tingling, burning sensations during and after illumination	Collins et al. (1997)
Improved response of plaque psoriasis after multiple treatments with topical 5-aminolaevulinic acid photodynamic therapy [44].	ALA (20%)	Topical	broad-band visible radiation	Light dose: 8 J/cm^2 Dose rate: 15 mW/cm^2	4 h	10	Max: 12 3 times per week	Eighty percent of patients responded to ALA-PDT. 19 treatment sites: 4 cleared, 10 responded but did not clear, 5 did not change.	Pain and discomfort (80% patients during treatment and 50% during and between treatments, respectively)	Robinson et al. (1999)
Systemic photodynamic therapy with aminolaevulinic acid induces apoptosis in lesional T lymphocytes of psoriatic plaques [45].	ALA (5,10 or 15 mg/kg)	Oral	Blue light, maximum at 417 nm	Light dose: for 5 or 10 mg/kg ALA: 1, 3, 6, 12, or 20 J/cm^2 for 15 mg/kg ALA: 1, 2, 4, 8 or 10 J/cm^2 Dose rate: 9–11 mW/cm^2	1, 3 or 6 h	12	Max: 1 1 time per week	A 5 or 10 mg/kg ALA dose did not show improvement. 15 mg/kg ALA dose showed improvement.	Mild burning during light exposure	Bissonnette et al. (2002)
Lack of efficacy and tolerability of topical PDT for psoriasis in comparison with narrowband UVB phototherapy [46].	ALA (20%)	Topical	630 nm	Light dose: 10 J/cm^2 Dose rate: 120 mW/cm^2	4 h	4	Max: 12 1–3 times per week	SEI score reduced by 5% in 2 patients, 17% in 1 patient and was unchanged in one patient. These results were lower than the NB-UVB values.	Pain during treatment	Beattie et al. (2004)
Topical aminolaevulinic acid-based photodynamic therapy as a treatment option for psoriasis? Results of a randomized, observer-blinded study [47].	ALA (1%)	Topical	600–740 nm	Light dose: 5, 10, 20 J/cm^2 Dose rate: 60 mW/cm^2	4–6 h	29	Max: 12 2 times per week	Eight patients were excluded. Sixty-three treatment sites: 8 cleared, 53 from substantial to minimal improvement, 2 with no improvement.	Pain, stinging, burning during irradiation, lasting up to several hours	Radakovic-Fijan et al. (2005)

Table 1. *Cont.*

Study	Photosensitizer	Way of Delivery	Wavelength	Treatment Parameters	Pre-Treatment with Drug	Number of Patients	Total Number of Treatment Sessions	Results	Side Effects	Reference
Clinical and immunohistochemical evaluation of psoriatic plaques treated with topical 5-aminolaevulinic acid photodynamic therapy [48].	Δ -ALA hydrochloride (20%)	Topical	630 nm	Light dose: 10–30 J/cm^2 Dose rate: 20–315 mW/cm^2	4–5 h	12	Max: 5 Once a week	Psoriatic plaques improved. The SEI score decreased.	Pain and discomfort during treatment	Fransson et al. (2005)
Disappointing results and low tolerability of photodynamic therapy with topical 5-aminolaevulinic acid in psoriasis. A randomized, double-blind phase I/II study [49].	ALA (0.1%, 1%, or 5%)	Topical	600–740 nm	Light dose: 20 J/cm^2 Dose rate: 60 mW/cm^2	4–6 h	12	Max: 12 2 times per week	Three patients were excluded. In the 0.1%, 1%, and 5% ALA-treated groups, the mean percentage improvement was 37.5%, 45.6%, and 51.2%, accordingly.	Pain and burning during and after irradiation	Schleyer et al. (2006)
A placebo-controlled randomized study on the clinical effectiveness, immunohistochemical changes and protoporphyrin IX accumulation in fractionated 5-aminolaevulinic acid-photodynamic therapy in patients with psoriasis [50].	ALA (10%)	Topical	600–750 nm	Light dose: 2 and 8 J/cm^2 Dose rate: 40 mW/cm2	4 h + 2 h of dark interval	8	Max: 4 Once a week	Psoriatic lesions and plaques cleared, and plaque severity score decreased.	Burning and stinging during irradiation	Smits et al. (2006)
Topical 5-aminolaevulinic acid photodynamic therapy for intractable palmoplantar psoriasis [51].	ALA (20%)	Topical	630 nm	Light dose: 15 J/cm^2 Dose rate: 30 mW/cm^2	4 h	3	Max: 10 Once a week	The patients showed partial improvement in psoriatic lesions and plaques.	-	Kim et al. (2007)
Methylene blue mediated photodynamic therapy for resistant plaque psoriasis [52].	MB (0.1%)	Topical	670 nm	Light dose: 5 J/cm^2 Dose rate: 565 mW/cm^2	-	16	-	Sixteen patients showed improvement. Sixty-eight percent of the patients achieved a seventy-five percent reduction in severity score.	-	Salah et al. (2009)

Table 1. *Cont.*

Study	Photosensitizer	Way of Delivery	Wavelength	Treatment Parameters	Pre-Treatment with Drug	Number of Patients	Total Number of Treatment Sessions	Results	Side Effects	Reference
Pulsed dye laser vs. photodynamic therapy in the treatment of refractory nail psoriasis: a comparative pilot study [52].	Methyl-aminolaevulinic acid (MAL)	Topical	595 nm	Light dose: 9 J/cm^2 Dose rate: -	3 h	14	Max: 6 Monthly	Fourteen patients showed lower NAPSI scores. Both nail matrix and bed nail bed involvement cleared.	Slight pain during treatment	Fernandez-Guarino et al. (2009)
The effects of keratolytic pretreatment prior to fluorescence diagnosis and photodynamic therapy with aminolevulinic acid-induced porphyrins in psoriasis [53].	ALA (10%)	Topical	600–750 nm	Light dose: 10 J/cm^2 Dose rate: 40 mW/cm^2	6 h	10	Max: 6 Once daily	It was observed that psoriasis decreased, as well as clinical severity score.	Stinging, burning during irradiation	Kleinpenning et al. (2010)
A phase II placebo-controlled study of photodynamic therapy with topical hypericin and visible light irradiation in the treatment of cutaneous T-cell lymphoma and psoriasis [54].	Hypericin (0.05%, 0.1%, 0.25%)	Topical	590–650 nm	Light dose: 8 to 20 J/cm^2 Dose rate: -	24 h	11	Max: 6 2 times per week	There was an improvement in skin lesions.	Mild burning and itching during treatment	Rook et al. (2010)
The Vitamin D Analog Calcipotriol Combined with Aminolevulinate-Mediated Photodynamic Therapy for Human Psoriasis: A Proof-of-Principle Study [33].	ALA (20%)	Topical	417 nm (blue light), 635 nm (red light)	Light dose: 10, 20 or 40 J/cm^2 Dose rate: 100 mW/cm^2	2 h	7	Max: 7 Twice daily	No clinical improvement in psoriasis was observed.	Stinging and pain during illumination	Maytin et al. (2012)
A retrospective analysis of real-life practice of off-label photodynamic therapy using methyl aminolevulinate (MAL-PDT) in 20 Italian dermatology departments. Part 1: Inflammatory and aesthetic indications [17].	MAL (160 mg/g)	Topical	635 nm	Light dose: 37 J/cm^2 Dose rate: -	3–4 h	17	Max: 3.6 9.9 ± 5.6 days between treatments	Two patients experienced worsening psoriatic lesions. Three patients showed poor or no clinical improvement. Twelve patients showed a moderate or marked clinical response.	Pain or burning sensations during the treatment	Calzavara-Pinton et al. (2013)

Table 1. *Cont.*

Study	Photosensitizer	Way of Delivery	Wavelength	Treatment Parameters	Pre-Treatment with Drug	Number of Patients	Total Number of Treatment Sessions	Results	Side Effects	Reference
Pyogenic granuloma in a patient with psoriasis successfully treated by 5-aminolevulinic acid photodynamic therapy: A case report [22]	ALA (20%)	Topical	633 ± 10 nm	Dose rate: 90 mW/cm^2	3 h	1	1 session of ALA-PDT treatment followed-up weekly for 1 month	One week following the ALA-PDT treatment, the erosions had dried up, and the PG lesion was encrusted. No signs of recurrence were demonstrated 1 month after treatment	-	Liu et al., (2016)
Systemic ALA-PDT effectively blocks the development of psoriasis-like lesions and alleviates leucocyte infiltration in the K14-VEGF transgenic mouse [28].	ALA (65 mg/kg)	Injection	633 nm	Light dose: 108 J/cm^2 Dose rate: 90 mW/cm^2	2 h	6 (mice)	Max: 2 Weekly	ALA-PDT blocked the development of psoriasis-like lesions. The scores lowered.	-	Chen et al. (2017)
Anti-Psoriasis Effects and Mechanisms of A-(8-Quinolinoxy) Zinc Phthalocyanine-Mediated Photodynamic Therapy [41]. 1) ZnPc-F7-PDT effects on propranolol-induced psoriatic lesions in cavy 2) ZnPc-F7-PDT effects on IMQ-induced psoriatic lesions in Nu/Nu mice	1) ZnPc-F7 (1% or 5%)	Topical	630, 670 nm	Light dose: 14.15 J/cm^2 Dose rate: 300–1500 mW/cm^2	24 h	70 (cavies), n = 20	Max: 1	After 2 weeks of recovery, the ears exhibited no discernible abnormalities compared to normal animals. The histopathological traits remained, except for inflammatory cell infiltration	-	Liu et al. (2017)
	2) ZnPc-F7 (0.30, 0.60, 1.20 mg/kg)	Injection		Light dose: 19.10 J/cm^2 Dose rate: -	6 h	70 (mice), n = 20	Max: 1	ZnPc-F7-PDT reduced the psoriatic symptoms caused by IMQ.		
ALA-PDT alleviates the psoriasis by inhibiting JAK signaling Pathway [27].	ALA (20%)	Topical	635 nm	Light dose: 12 J/cm^2 Dose rate: 30 mW/cm^2	4 h	Group (mice)	-	In IMQ-induced mice ALA-PDT reduced scaling, redness, erythema, scales, thickness and cumulative scores	-	Yi et al. (2019)
Photodynamic Therapy Combined with Dermabrasion in Cutaneous Squamous Cell Carcinoma Concomitant with Psoriasis [27]	Dermabrasion conjugated with ALA (20%)	Topical	635 nm He–Ne laser	Light dose: 100 J/cm^2 Dose rate: 0.083 W/cm^2 for 1200 s	5 h	1	four applications of PDT	The ulcer and plaque completely disappeared, and there were no obvious scars after treatment	-	Xu et al. (2019)

Table 1. *Cont.*

Study	Photosensitizer	Way of Delivery	Wavelength	Treatment Parameters	Pre-Treatment with Drug	Number of Patients	Total Number of Treatment Sessions	Results	Side Effects	Reference
A Comparison of The Effects of Clobetasol 0.05% and Photodynamic Therapy Using Aminolevulinic Acid With Red Light in the Treatment of Severe Nail Psoriasis [36]	ALA (20%) and clobetasol propionate 0.05% ointment	Topical	630 nm (range 600–730 nm); red light-PDT	Light dose: 120 J/cm^2 Dose rate: 200 mW/cm^2	3 h	8	Every 3 weeks for 5 cycles	Six months after the last treatment session, the mean NAPSI scores in the nails treated with ALA-PDT were greater than those in the nails treated with clobetasol 0.05% ointment.	Slight pain during irradiation	Tehranchinia et al. (2020)
Effective topical treatments using innovative NNO-tridentate vanadium (IV) complexes-mediated photodynamic therapy in a psoriasis-like mouse model [43]	0.001% vanadium complex—PDT	Topical	Blue light 430 nm	Light dose: 0.1 J/cm^2 Dose rate: -	4 h	imiquimod (IMQ)-induced psoriasis mouse model	30 min irradiation for 8 consecutive days	A higher phototoxicity index with non-toxicity under dark conditions, efficient skin morphological recovery according to the PASI score decrease in the percentage of IL-17A and IL-22 in the spleen.	-	Lin et al., 2022

4. Nanotechnology Combined with PDT in Psoriasis Treatment—Future Perspectives

Over the past few years, one of the most significant prospects in therapeutics is nanotechnology. This field has provided the development of novel nanostructured drug release systems and has enabled the application of novel therapeutic agents against psoriasis. While nanotechnology combined with PDT has been widely developed in other skin disorders, in terms of psoriasis treatment, it has been inadequately researched. Compared with conventional drug delivery systems, the use of nanotechnology allows scientists to modify the solubility of hydrophobic materials, execute the controlled or sustained release of a drug to a specific site, and enhance drug stability, with reduced undesirable effects [55]. Nanocarriers may penetrate the skin, enhancing the concentration gradient at the skin surface, and can provide physical and chemical properties allowing the release of the drug at the delivery site [56].

Several studies have evaluated novel nanocarriers in PDT, including polymeric, lipidic, and metallic nanocarriers. Among the polymer-based nanoparticles, dendrimers, polymeric micelles, nanogels, nanospheres, and nanocapsules have been utilized in the PDT of various skin diseases [57,58]. Therefore, it is reasonable to investigate the role of these nanosystems in psoriasis treatment. Nanocarriers that have been used in PDT of psoriasis and those with high potential have been summarized in Figure 2.

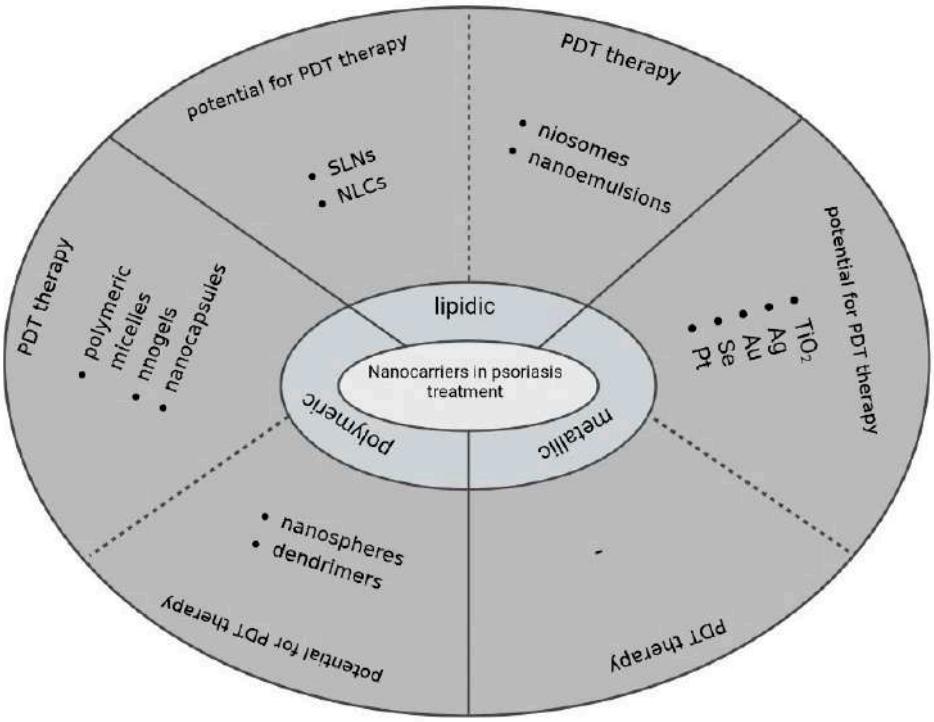

Figure 2. Diagram showing the use of analyzed nanocarriers in psoriasis treatment. Nanocarriers were classified by various studies reporting their use in PDT-based psoriasis treatment and studies proving their great potential in the treatment of other skin diseases.

Dendrimers have gained more interest as nanocarriers over the past decade. They are synthesized from polyfunctional monomers, forming a three-dimensional architecture with unique physicochemical features, such as high reactivity, good solubility, and biocompatibility. Due to their simple modification, the ability to target a delivery site, and guaranteed reproductive pharmacokinetic behavior, dendrimers are widely studied as drug delivery systems for skin disorders. For instance, in 2001, Batah et al. [59] and, eight years later, Casas et al. [60] undertook attempts to encapsulate 5-ALA for dendrimers. These independent studies showed the improvement of 5-ALA stability and its pharmacokinetic profile, suggesting their potential use as macromolecular prodrugs for PDT. Consistently, in 2018, Zhou et al. synthesized a series of iron-chelating agents with ALA conjugated to

dendrimers, to improve ALA-PDT efficiency by enhancing PpIX accumulation. Their findings on human cancer cell lines revealed a higher efficacy of PpIX generation in ALA-HPO dendrimers than with ALA alone. This system provides a promising option to enhance the treatment effect of ALA-PDT in cancer [61]. However, the potency of the abovementioned agents has never been tested on psoriasis models.

Polymeric micelles are nanosized molecules that are synthesized from amphiphilic copolymers. Due to their low toxicity, core-shell architecture, nano-size, and relatively high stability, these compounds are often used in drug delivery systems [62]. Considering their use in PDT, Yin et al. designed an amphiphilic zinc phthalocyanine polymer conjugate in the form of a monomolecular micelle. This nanocarrier showed efficient anti-psoriasis activity on psoriasis guinea pig models [63]. To further investigate these findings, zinc phthalocyanine was loaded into nanocapsules, including chitosan nanocapsules, poly-ε-caprolactone (PCL), and PCL-coated chitosan nanocapsules to test their photodynamic activity in psoriasis treatment. These formulations increased skin permeation and drug release rates. All forms of ZnPc conjugated to micelles exhibited a more sustained drug release with no burst effect, suggesting their beneficial role in PDT for psoriasis treatment [64].

Nanogels are hydrophilic crosslinked polymeric nanoparticles that are characterized by a high capacity to incorporate molecules and drug encapsulation capacity, as well as tunable size, ease of preparation, and minimal toxicity [65]. Considering PDT, Wang et al. constructed a chitosan/hyaluronan-based nanogel to co-load methotrexate (MTX) and 5-aminolevulinic acid (ALA) for combined chemo-photodynamic therapy for psoriasis. The results showed enhanced penetration and retention of MTX and ALA passing through and into the skin of psoriatic mice [66]. A drug-targeting delivery system with MTX-loaded nanoparticle was also developed by Yin et al., who observed increased cancer cell apoptosis [63]. These results seem promising against psoriatic lesions, due to their potential ability to induce apoptosis in hyperproliferating keratinocytes. Furthermore, Freag et al. synthesized a hydrogel consisting of a liquid crystalline nanoparticulate (LCNP) reservoir of berberine, blended with oleate (Brb-OL). Berberine is one of the most promising agents derived from natural plants for the management of psoriasis. A study revealed a threefold increase in drug accumulation and a tenfold augmentation of drug permeation compared to crude berberine, leading to alleviation of the inflammatory cytokines exhibited in psoriasis [67]. More studies are required to investigate the role of nanogels as drug delivery systems in psoriasis treatment.

Due to their ability to enhance skin permeation while entrapping pharmaceutical ingredients, nanospheres and nanocapsules are more likely to be tested as topical drug administration systems for psoriasis therapy [68]. These vesicles differ from each other in their morphology and architecture. Nanocapsules contain either an oily or aqueous core, surrounded by a polymeric shell, usually in combination with a mixture of lipophilic and hydrophilic surfactants. In contrast, nanospheres are dense polymer-based matrix systems [69]. Considering their application in the PDT of other skin diseases, Shi et al. synthesized multifunctional hybrid nanospheres that consisted of $Fe^{3+,}$ aggregation-induced emission (AIE) PS and the Bcl-2 inhibitor (sabutoclax). Due to the presence of Fe^{3+}, intracellular O_2 concentration was increased in parallel to the increase in the nanosphere concentration taken up by tumor cells. Both in vitro and in vivo results show the potential of multifunctional hybrid nanospheres in PDT therapy [70]. A promising therapeutic effect in PDT therapy was also shown by Liu et al., who formed biodegradable cancer cell membrane-coated mesoporous copper/manganese silicate nanospheres [71]. In the case of nanocapsules, Amantino et al. developed a nanocapsule containing a poly(lactide-co-glycolide) (PLGA) coating for the encapsulation of an anthraquinone derivative. This nanoparticle increased the drug's uptake and efficacy, suggesting its promising use in PDT [72].

The first attempts to use nano-sized particles in drug discovery systems were based on lipids. They are natural carriers, which makes them easily available and cost-effective. Among the lipid-based nanoparticles tested in combination with PDT for psoriasis treat-

ment, we can distinguish poly-amphiphiles (such as niosomes), solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs), as well as nanoemulsions [58].

Niosomes are non-ionic surfactant-based vesicles that have been proved to increase the residence time of drugs in the epidermis while reducing systemic absorption [73]. Parnami et al. formulated MTX- and trioxysalen-loaded niosomes and found some beneficial effects while performing experiments on a mouse model. Their application to the epidermis while using narrow-band UV radiation increased the local concentrations of encapsulated drugs and reduced the systemic side effects [74]. Relatively similar findings were reported by Abu et al., who found promising therapeutic effects from acitretin-based niosomes. The developed system inhibited the proliferation of HaCaT cells in vitro and in vivo, showing decreased epidermal thickness without detectable side effects [75].

In general, NLCs are very similar to SLNs, both being forms of carriers of hydrophobic drugs that are dispersed within the core of the lipid particles. The only difference is in the structure; NLCs do not have a perfect crystal structure, therefore, they enable better drug encapsulation with less drug leakage. Viegas et al. developed a multifunctional nanostructured lipid carrier (NLC) that allowed the co-delivery of both tacrolimus and siRNA for the TNF-α to the psoriatic plaques. Tacrolimus, as the inhibitor of calcineurin, interacts with some of the cytokines involved in psoriasis pathogenesis, including TNF-α, while antisense therapy using siRNA is one of the more promising strategies to silence the different cytokines and pro-inflammatory molecules involved in psoriasis pathogenesis. This complex showed the high encapsulation efficiency of TAC and effective TNF-α siRNA complexation, providing drug distribution to some extent in the deeper layers of the skin, with low toxicity associated with the uptake of NLC in 4 h, and an approximately 7-fold reduction of TNF-α expression after topical application in psoriatic mice [76]. The co-delivery of other molecules, including drugs and siRNA, through nanocarriers was also evaluated in other studies aiming to control the psoriatic process [77,78]. High encapsulation efficiency was also determined by Wang et al., who constructed N3-labeled cell membrane-derived nanovesicles coated with IR-780-PLGA nanoparticles (N3-NV-INPs). This platform inhibited keratinocyte proliferation and the release of cytokines such as IL-17, IL-22, and TNF-α, indicating its potential in clinical application [79]. Taking into account SLNs, no study has determined their efficacy in PDT-mediated psoriasis treatment. However, Goto et al. have successfully synthesized aluminum chloride phthalocyanine-loaded SLNs and have shown promising signs of application in the treatment of melanoma [80].

Among the emulsion-based nanosystems, nanoemulgels are gaining more and more popularity. These consist of a nanoemulsion that is further incorporated into a water-based gel using different polymers. With its usage, water softens and removes the hyperkeratinized scales formed due to the deposition of the dead cells on the skin that hinder controlled drug release and transport through the skin layers [81]. By a low-energy emulsification method, Algahtani et al. formed a curcumin nanoemugel with increased solubility and skin penetrability in psoriatic mice. Curcumin is a natural compound with inhibitory effects on NF-Kß and MAPK, as well as the STAT3 pathways, and the ability to downregulate the proinflammatory cytokines involved in psoriasis pathogenesis [82]. To further investigate these findings, Gomez et al. determined that curcumin-loaded chitosan/alginate nanoparticles (Cur-CS/Alg NPs), together with a blue light emitting diodes (LED) light irradiation, repressed the hyperproliferation of TNF-α-induced HaCaT cells. Curcumin was also tested in a study conducted by Woźniak et al., in which an encapsulated compound in liposomes increased phototoxicity and decreased malignant cell motility following PDT in skin malignancies compared to healthy keratinocytes [83].

Among the metal-based nanoparticles used in psoriasis treatment, titanium dioxide, silver, gold, selenium, and platinum nanoparticles have garnered interest from the scientific community [84]. Although the knowledge about their use in PDT for psoriasis treatment is scarce, several studies have reported their beneficial application for topical administration in other epidermic cells. For instance, Feng et al. developed a folic acid-conjugated silica-coated titanium dioxide and assessed its biocompatibility in two cell lines: fibroblast

cells (L929) and the human nasopharyngeal epidermoid cancer (KB) cells. After 24 h of incubation, the significantly increased uptake of folic acid-conjugated silica-coated TiO2 to L929 and KB cells was observed. After UV radiation, the system was non-toxic and led to the increased mortality of cells, confirming the photokilling ability of TiO2-based nanoparticles [85]. Regarding gold nanoparticles, Fereig et al. aimed to conjugate gold nanoparticles with chitosan nanoparticles and examined whether this hybridization would enhance the anti-psoriatic efficacy in vivo. As shown via a transmission electron microscope (TEM) and X-ray diffraction (XRD) analysis, the anti-inflammatory effect of the conjugate was evident from a lower spleen-to-bodyweight ratio and better histopathological skin condition compared to the other analyzed formulations [86]. Consistently, several other studies reported the potential option of gold-loaded nanoparticles for topical treatment [87–89]. Considering silver nanoparticles, David et al. aimed to evaluate the anti-inflammatory effects of these structures when conjugated with European black elderberry fruit extracts (*Sambucus nigra*—SN, in the Adoxaceae family). In vitro studies showed a decrease in cytokine production induced by UVB irradiation. In addition, in vivo studies revealed a reduction in edema and cytokine levels in paw tissues, suggesting their potential in the treatment of psoriasis lesions [90]. Taken together, the use of metal nanoparticles has the perspective to improve the efficacy of psoriasis treatment; however, more studies are required to draw further conclusions.

5. Conclusions

After a thorough review of the literature, we suggest that PDT seems to have the potential to alleviate psoriasis. However, despite the ambiguous role of ALA-PDT and the fact that it has been extensively tested in psoriasis treatment, there is a gap in the research regarding other photosensitizers, the efficacy of which could have been promising. This highlights the need to evaluate more non-ALA photosensitizers on psoriatic in vitro and in vivo models, as well as in clinical trials. Another limiting factor observed in most of the reviewed studies was the side effects associated with PDT; however, we believe that this obstacle could be tackled by the appropriate utilization of nanotechnology.

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5. Streszczenie w języku polskim

Łuszczyca to przewlekła choroba zapalna o podłożu immunologicznym, charakteryzująca się hiperproliferacją naskórka i dotykająca około 125 milionów ludzi na całym świecie. Objawy łuszczycy, takie jak zaczerwienienie, łuszczenie się skóry i świąd, znacząco obniżają jakość życia pacjentów. Pacjenci chorzy na łuszczycę, korzystający z terapii systemowej i/lub fototerapii, często przechodzą na leczenie drugiego rzutu lub leki biologiczne z uwagi na pojawiające się skutki uboczne. Pomimo znacznych postępów w rozwoju leków biologicznych, są one kosztowne, trudno dostępne dla ogółu społeczeństwa i wymagają wielokrotnych podaży. Z racji tego potrzebne są nowo zaprojektowane związki, które zastąpią lub uzupełnią obecne terapie.

Jedną z najbardziej obiecujących metod jest terapia fotodynamiczna (PDT), która od dawna stosowana jest w leczeniu różnych chorób skóry, takich jak rogowacenie słoneczne, rak podstawnokomórkowy oraz rak kolczystokomórkowy. Niestety obecnie, skuteczność stosowanych klinicznie fotouczulaczy w leczeniu łuszczycy jest niedostateczna, a pacjenci często zgłaszają działania niepożądane, takie jak ból lub uczucie pieczenia. Pomimo obiecujących wyników badań przedklinicznych, PDT pozostaje pod kątem klinicznym niedostatecznie przebadana, co podkreśla konieczność przetestowania szerszego spektrum fotouczulaczy na pacjentach z łuszczycą.

Ftalocyjaniny, będące fotouczulaczami drugiej generacji, charakteryzują się wysokim profilem bezpieczeństwa i skuteczności, jednak ich zastosowanie ograniczają niska specyficzność tkankowa oraz słaba rozpuszczalność. W odpowiedzi na te wyzwania, w niniejszej rozprawie zaproponowano strategię glikokoniugacji ftalocyjanin, mającą na celu poprawę ich biodostępności i selektywnej akumulacji w keratynocytach charakteryzujących się nadekspresją transporterów glukozy. Proponowane jest innowacyjne podejście leczenia łuszczycy, wykorzystując fakt, iż glukoza jest niezbędnym bioenergetycznym i syntetycznym substratem dla szybko proliferujących komórek naskórka.

Na rozprawę doktorską składa się cykl 4 monotematycznych artykułów opublikowanych w międzynarodowych czasopismach naukowych uwzględnionych na liście Journal Citation Reports (JCR) oraz na wykazie czasopism naukowych Ministerstwa Edukacji i Nauki (MEiN).

W pierwszej publikacji po raz pierwszy zaproponowano strategię glikokoniugacji w kontekście potencjalnego zastosowania w leczeniu łuszczycy, mającą na celu poprawę farmakokinetyki oraz selektywności związków przeciwłuszczycowych. Kolejna praca badała wpływ cytokin prozapalnych, takich jak IL-6, IL-17, IL-23, IL-36, oraz imikwimodu, na poziom ekspresji GLUT1 w keratynocytach HaCaT. Wyniki wykazały, że stymulacja tymi czynnikami prowadzi do znacznego wzrostu ekspresji transportera glukozy typu 1 (GLUT1), co sugeruje, iż cytokiny oraz IMQ mogą odgrywać kluczową rolę w jego regulacji. W trzeciej publikacji oceniono działanie cynkowej ftalocyjaniny (ZnPc) oraz jej cukrowych pochodnych na łuszczycopodobnym modelu komórkowym oraz mysim, wykazując, iż tetra-substytuowana pochodna glukozy z zabezpieczeniami acetylowymi (Glu-4-ZnPc-P) charakteryzuje się najwyższą fototoksycznością w porównaniu do innych pochodnych. Ponadto, naświetlanie myszy po podaniu dożylnym Glu-4-ZnPc-P doprowadziło do redukcji wskaźnika PASI i ogólną poprawę kondycji skóry, co świadczy o wysokim potencjale terapeutycznym tego związku. Ostatecznie, czwarta publikacja cyklu ogranicza się do przeglądu literaturowego, w którym przedstawiono aktualny stan wiedzy na temat terapii fotodynamicznej w leczeniu łuszczycy, opisano kluczowe badania przedkliniczne i kliniczne, oraz omówiono perspektywy zastosowania nanotechnologii w kontekście terapii celowanej łuszczycy.

Podsumowując, strategia przyłączenia cukrów do fotouczulaczy wykazuje wysoki potencjał w kontekście celowanej terapii fotodynamicznej łuszczycy. Opisane w niniejszej rozprawie wyniki mogą stanowić solidną podstawę dla przyszłych badań klinicznych nad zastosowaniem koniugatów glukozy w leczeniu łuszczycy oraz innych chorób skóry. Niemniej jednak, konieczne są dalsze badania przedkliniczne w celu pełnego zrozumienia mechanizmów działania tych związków.

6. Streszczenie w języku angielskim

Psoriasis is a chronic immune-mediated inflammatory disease, characterized by hyperproliferation of the epidermis, and affecting approximately 125 million people worldwide. Symptoms such as redness, skin peeling, and itching significantly reduce the quality of life of patients. Psoriatic patients receiving systemic therapy and/or phototherapy often switch to second-line treatments or biologics due to side effects. Despite significant progress in the development of biological therapies, these treatments are costly, hardly accessible, and require multiple administrations. Therefore, newly designed compounds are necessary to either replace or complement current therapies.

Photodynamic therapy (PDT) has been a reliable treatment option for various skin diseases such as actinic keratosis, basal cell carcinoma, and squamous cell carcinoma over the past few years. Unfortunately, the efficacy of clinically used photosensitizers in the treatment of psoriasis is inadequate, with patients often reporting side effects such as pain and burning sensations. Despite promising preclinical finding, PDT remains underutilized in clinical settings, for psoriasis, highlighting the need to test a broader spectrum of photosensitizers on patients.

Despite their high safety and efficacy profiles, phthalocyanines, which are second-generation photosensitizers, face several limitations, mostly because of poor tissue specificity and low solubility. In response to these challenges, this dissertation proposes a strategy of glycoconjugation of phthalocyanines in order to improve their bioavailability and selective accumulation in keratinocytes, which are characterized by the overexpression of glucose transporters on their surface. The proposed approach leverages the fact that glucose is a vital bioenergetic and synthetic substrate for rapidly proliferating epidermal cells.

The doctoral dissertation consists of a collection of four thematically-related articles published in international scientific journals included in the Journal Citation Reports (JCR) list and the Polish Ministry of Education and Science (MEiN) registry.

The first publication described for the first time the strategy of glycoconjugation with potential applications in psoriasis treatment, which attempts to improve the pharmacokinetics and selectivity of antipsoriatic drugs. The second study examined the effect of pro-inflammatory cytokines such as IL-6, IL-17, IL-23, IL-36, and imiquimod on GLUT1 expression in HaCaT keratinocytes. The findings revealed that stimulation by these factors causes a considerable increase in GLUT1 expression, suggesting that cytokines and IMQ may play a key role in

regulating this transporter. The third publication evaluated the activity of zinc phthalocyanine (ZnPc) and its sugar derivatives on a psoriasis-like *in vitro* and *in vivo* model, demonstrating that the acetyl-protected, tetra-substituted glucose derivative (Glu-4-ZnPc-P) exhibited the highest photocytotoxicity compared to other derivatives. Furthermore, the irradiation of mice following intravenous administration of Glu-4-ZnPc-P resulted in a reduction of the PASI index and an overall improvement of the condition of the skin, indicating the therapeutic potential of this compound. Finally, the fourth article is a literature that outlines the current state of knowledge about photodynamic therapy in the treatment of psoriasis by highlighting preclinical and clinical studies, and exploring the potential of nanotechnology in targeted therapies for psoriasis.

In summary, the strategy of conjugating sugars to photosensitizers bears the potential to revolutionize current targeted treatments for psoriasis. The results presented in this dissertation could serve as a strong foundation for future clinical trials exploring the use of glucose conjugates in treating psoriasis and other skin diseases. However, further preclinical studies are needed to fully understand the mechanisms of action of these compounds.

7. Zgoda Lokalnej Komisji Etycznej

UCHWAŁA NR 035/2023/P2

z dnia 19.07.2023

Lokalnej Komisji Etycznej do spraw doświadczeń na zwierzętach we Wrocławiu

§ 1

Na podstawie art. 48 ust. 1 pkt. 1 / art. 48 ust. 1 pkt. 2[1] ustawy z dnia 15 stycznia 2015r. o ochronie zwierząt wykorzystywanych do celów naukowych lub edukacyjnych (Dz. U. poz. 266), zwanej dalej „ustawą" po rozpatrzeniu wniosku pt.: *„Ocena działania glukozowej pochodnej cynkowej ftalocyjaniny na mysim modelu imitującym łuszczycę"* z dnia 06.07.2023 r., złożonego przez Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu, Centrum Badań Przedklinicznych, adres: ul. Marcinkowskiego 1/3, 50-368 Wrocław,[2] zaplanowanego przez mgr inż. Magdalenę Klytę[3], przy udziale[4] nie dotyczy, Lokalna Komisja Etyczna:

WYRAŻA ZGODĘ[5]

Na przeprowadzenie doświadczeń na zwierzętach w zakresie wniosku.

§ 2

W wyniku rozpatrzenia wniosku o którym mowa w § 1, Lokalna Komisja Etyczna ustaliła, że:

1. ~~Wniosek należy przypisać do kategorii: [PB9] Badania podstawowe: narządy zmysłów (skóra,~~ oczy i uszy);
2. Najwyższy stopień dotkliwości proponowanych procedur to: umiarkowana;
3. Doświadczenia będą przeprowadzane na gatunkach lub grupach gatunków[6]: Mysz domowa (*Mus musculus*), szczep C57BL/6, 8 tygodni, samice - 30 szt. oraz samce - 30 szt.;
4. Doświadczenia będą przeprowadzane przez: mgr inż. Magdalena Klyta, mgr inż. Maja Lipińska, mgr inż. Kamila Czyszczoń, mgr Sebastian Makuch;
5. Doświadczenie będzie przeprowadzane w terminie[7] od 19.07.2023 do 31.12.2023;
6. Doświadczenie będzie przeprowadzone w ośrodku[8]: nie dotyczy;
7. Doświadczenie będzie przeprowadzone poza ośrodkiem: nie dotyczy;
8. Użyte do procedur zwierzęta dzikie zostaną odłowione przez: nie dotyczy, w sposób: nie dotyczy;
9. Doświadczenie ~~zostanie~~/nie zostanie[9] poddane ocenie retrospektywnej ~~w terminie do 3 miesięcy od dnia przekazania przez użytkownika dokumentacji, mającej stanowić podstawę dokonania oceny retrospektywnej. Użytkownik jest zobowiązany do przekazania ww.~~

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[1] Niewłaściwy zapis usunąć

[2] Imię i nazwisko oraz adres i miejsce zamieszkania albo nazwę oraz adres i siedzibę użytkownika, który przeprowadzi to doświadczenie, z tym że w przypadku gdy użytkownikiem jest osoba fizyczna wykonująca działalność gospodarczą, zamiast adresu i miejsca zamieszkania tej osoby – adres i miejsce wykonywania działalności, jeżeli są inne niż adres i miejsce zamieszkania tej osoby;

[3] Imię i nazwisko osoby, która zaplanowała i jest odpowiedzialna za przeprowadzenie doświadczenia

[4] Wypełnić w przypadku dopuszczenia do postępowania organizacji społecznej.

[5] Niewłaściwy zapis usunąć

[6] Podać liczbę, szczep/stado, wiek/stadium rozwoju

[7] Nie dłużej niż 5 lat

[8] Podać jeśli jest to inny ośrodek niż użytkownik

[9] Niewłaściwy zapis usunąć

~~dokumentacji niezwłocznie, tj. w terminie, o którym mowa w art. 52 ust. 2 ustawy, nie później niż ... .~~

§ 3

Uzasadnienie:

Referująca omówiła wniosek 035/2023/P2 oraz odpowiedzi wnioskodawcy. Po dyskusji członków Komisji stwierdzono, że wniosek nie zawiera błędów formalnych i merytorycznych.

Na skutek przeprowadzonego głosowania **zadecydowano o udzieleniu zgody na realizację badań objętych wnioskiem 035/2023/P2**. 7 osób głosowało za udzieleniem zgody na realizację badań, nikt nie był przeciwny, 1 osoba wstrzymała się od głosu. Wniosek nie został objęty oceną retrospektywną. 7 osób było przeciwnych objęciu badań oceną retrospektywną, nikt nie był za, jedna osoba wstrzymała się od głosu.

§ 4

Integralną część niniejszej uchwały stanowi kopia wniosku, o którym mowa w § 1.

(Pieczęć lokalnej komisji etycznej)

Podpis przewodniczącej komisji

Pouczenie:

Zgodnie z art. 33 ust. 3 i art. 40 ustawy w zw. z art. 127 § 1 i 2 oraz 129 § 2 ustawy z dnia z dnia 14 czerwca 1960 r. Kodeks postępowania administracyjnego (Dz. U. 2017, poz. 1257 – t.j.; dalej KPA) od uchwały Lokalnej Komisji Etycznej strona może wnieść, za jej pośrednictwem, odwołanie do Krajowej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach w terminie 14 od dnia doręczenia uchwały.

Na podstawie art. 127a KPA w trakcie biegu terminu do wniesienia odwołania strona może zrzec się prawa do jego wniesienia, co należy uczynić wobec Lokalnej Komisji Etycznej, która wydała uchwałę. Z dniem doręczenia Lokalnej Komisji Etycznej oświadczenia o zrzeczeniu się prawa do wniesienia odwołania przez ostatnią ze stron postępowenie, decyzja staje się ostateczna i prawomocna.

Otrzymuje:

1) Użytkownik,
2) Organizacja społeczna dopuszczona do udziału w postępowaniu (jeśli dotyczy)
3) a/a

Użytkownik kopie przekazuje:

- Osoba planująca doświadczenie
- Zespół ds. dobrostanu

8. Curriculum Vitae

mgr Sebastian Makuch

Data urodzenia: 23.02.1995

Adres zamieszkania: Zielna 53/15, 51-313, Wrocław

Email: s.makuch@umw.edu.pl

Tel.: 737 901 267

Wykształcenie

Studia licencjackie: Uniwersytet Wrocławski (2014-2017)

Kierunek: Biologia Eksperymentalna

Praca licencjacka w zakładzie Genetyki i Fizjologii Komórki pt. „Metody molekularne w diagnostyce
dziedzicznych predyspozycji do nowotworów"

Studia magisterskie: Uniwersytet Wrocławski (2017-2019)

Kierunek: Biotechnologia Medyczna

Praca magisterska w zakładzie Cytobiochemii pt. „Identyfikacja białka EFR3A w tratwach błonowychoraz jego interakcji z flotyllną-2 w komórkach raka prostaty PC-3"

Szkoła doktorska: Uniwersytet Medyczny we Wrocławiu (2020-obecnie)

Katedra Patologii Klinicznej i Doświadczalnej

Temat pracy doktorskiej: „Cukrowa pochodna cynkowej ftalocyjaniny jako innowacyjne narzędzieterapii fotodynamicznej w leczeniu łuszczycy"

Doświadczenie naukowe

- Wykonawca projektu pt. „**Wykorzystanie insuliny i koniugatów glukozy w celowanej terapii raka piersi**" - finansowanego przez NCN – konkurs Preludium (2015/19/N/NZ5/00001) **(2019-2020)**
- Wykonawca projektu pt. „**Zastosowanie innowacyjnego glikokoniugatu w celowanym leczeniu chorób onkologicznych i autoimmunologicznych" –** finansowanego przez NCBiR – konkurs TANGO (426098 – IGTT) **(2020-2021)**
- Główny wykonawca projektu pt. „**Cukrowa pochodna cynkowej ftalocyjaniny jako innowacyjne narzędzie terapii fotodynamicznej w leczeniu łuszczycy**" - finansowanego przez NCN – konkurs Preludium BIS (2019/35/O/NZ4/01463) **(2020-2024)**
- Wykonawca projektu pt. „**Zastosowanie osocza ozdrowieńców w terapii chorych na COVID-19 wraz z metabolomiczną i laboratoryjną oceną postępu terapii osoczem**" – finansowanego przez ABM – grant (2020.ABM.COVID19.0005) – badanie kliniczne
- Praca w projekcie NCBiR w firmie **LABPLUS (2021-2023)** – jako młodszy specjalista ds. systemu ekspertowego - **gromadzenie, analizowanie i wybieranie istotnych informacji na podstawie literatury, w celu utworzenia baz danych używanych do uczenia modelu maszynowego,** umożliwiających pacjentowi interpretację wyników badań laboratoryjnych bez angażowania lekarza (umowa nr. POIR.01.01.01-00-0297/19-00)

Publikacje

Współautor kilkudziesięciu artykułów naukowych opublikowanych w renomowanych i indeksowanych w bazie Scopus i JCR czasopismach naukowych w dziedzinie celowanej terapii fotodynamicznej nowotworów litych i hematologicznych, chorób autoimmunologicznych, chorób zakaźnych, oraz szeroko pojętej biologii medycznej

37 opublikowanych artykuły naukowych w latach 2019-2024:
sumaryczny Impact Factor = **139,7** / Punkty KBN/MNiSW = **3830,** Index Hirsch = **12**, liczba cytacji = **475.** Najważniejsze publikacje:

Makuch S., *et al*. Glycoconjugation as a promising treatment strategy for psoriasis. *J.Pharmacol.Exp.Ther.* (2020): Vol.373 no.2; s.204-212.

Makuch S., *et al*. "The Impact of Proinflammatory Cytokines and Imiquimod on GLUT1 in HaCaT Keratinocytes–a Potential Anti-Psoriatic Therapeutic Target?." *Cell Physiol Biochem* 57(2023): 54-62.

Makuch S., *et al*. In Vitro and In Vivo Antipsoriatic Efficacy of Protected and Unprotected Sugar–Zinc Phthalocyanine Conjugates. *Pharmaceutics 2024, 16(6), 838*.

Patenty: Glikokoniugatowa pochodna metotreksatu i glukozy w leczeniu chorób onkologicznych i autoimmunologicznych" numery patentów: Pat.242529, Pat.243572, Pat.243573

Umiejętności, szkolenia, wyróżnienia

- 5-letnie doświadczenie w stosowaniu podstawowych metod biologii molekularnej, takich jak izolacja DNA/RNA oraz białek, qPCR, Western blot, mikroskopia fluorescencyjna, testy cytotoksyczności, cytometria przepływowa oraz barwienia immunocytochemiczne**.** Ukierunkowane doświadczenie w dziedzinie **immunologii nowotworów, hematoonkologii, chorób autoimmunologicznych, terapii celowanej nowotworów, terapii fotodynamicznej.**
- Duże doświadczenie w analizie danych naukowych z użyciem programów statystycznych i graficznych takich jak **GraphPad, Statistica, FlowJo**.
- Ukończenie **Kursu prowadzenia i zarządzania zespołem badawczym** – prowadzonego przezfirmę KnowHub sp. o.o. w ramach projektu pt. „Dolnośląscy liderzy Medycyny UniwersytetuMedycznego we Wrocławiu (umowa nr POWR.03.05.00-00-Z085/17-00) (Wrocław 25- 27.03.2022)
- Ukończenie **Kursu skutecznego planowania i zarządzania projektami naukowymi, oraz pozyskiwania środków na badania naukowe** – prowadzonego przez firmę KnowHub sp. o.o. w ramach projektu pt. „Dolnośląscy liderzy Medycyny Uniwersytetu Medycznego we Wrocławiu (umowa nr POWR.03.05.00-00-Z085/17-00) (Wrocław 15-16.01.2022)
- Ukończenie **Szkolenia GCP** Polskiego Związku Pracodawców Firm Prowadzących Badania Kliniczne na Zlecenie (POLCRO) - Dobra Praktyka Kliniczna
- Ukończenie **Kursu PolLASA** dla osób wykonujących czynności związane z wykorzystywaniem zwierząt do celów naukowych w celu realizacji badań przedklinicznych - **certyfikat i wyznaczenia do wykonywania oraz planowania badań na zwierzętach**
- Stypendysta konkursu PRELUDIUM BIS, finansowanego przez NCN (2020-2024)
- Stypendysta NAWA – stypendium na zagraniczny wyjazd do University of Udine, Włochy (05-08 2022)
- 3-krotne stypendium rektora za wybitną publikację naukową (2021-2023)

Życiorys

Sebastian Makuch jest absolwentem Biotechnologii Medycznej Uniwersytetu Wrocławskiego oraz doktorantem IV roku w Katedrze Patologii Klinicznej i Doświadczalnej Uniwersytetu Medycznego we Wrocławiu, gdzie realizuje liczne projekty badawcze pod opieką merytoryczną Prof. dr hab. Piotra Ziółkowskiego. Doktorant swoją przygodę z działalnością naukową rozpoczął w Laboratorium Hodowli Komórkowej jako wykonawca projektu, testując działanie innowacyjnego koniugatu glukozy z metotreksatem w celowanym leczeniu nowotworów w ramach projektu Preludium NCN „Wykorzystanie insuliny i koniugatów glukozy w celowanej terapii raka piersi" w 2018 roku. Obiecujące wyniki jego badań pozwoliły na dalsze zgłębianie skuteczności i selektywności związku w projekcie Tango NCBiR pt. „Zastosowanie innowacyjnego glikokoniugatu metotreksatu w celowanym leczeniu chorób onkologicznych i autoimmunologicznych" w 2019 roku. Odkrycia doktoranta odnośnie koniugatów glukozy i cytostatyków zostały opisane w kilkunastu artykułach opublikowanych w renomowanych czasopismach oraz przedstawione na licznych krajowych oraz międzynarodowych konferencjach. Ponadto, innowacyjność badanych przez doktoranta związków została potwierdzona licznymi krajowymi patentami: „Glikokoniugatowa pochodnametotreksatu i glukozy w leczeniu chorób onkologicznych i autoimmunologicznych" numery patentów: Pat.242529, Pat.243572, Pat.243573). Sebastian Makuch bierze również czynny udział w komercjalizacji wyników prowadzonych badań. Podczas szkoły doktorskiej, doktorant równocześnie pełnił funkcję specjalisty ds. uczenia maszynowego w firmie Labplus, gdzie zajmował się gromadzeniem danych medycznych w celu opracowywania innowacyjnych algorytmów. Aktualnie mgr Sebastian Makuch rozwija swoją wiedzę oraz prowadzi badania *in vitro* oraz *in vivo* jako doktorant-stypendysta w ramach projektu PRELUDIUM BIS NCN pt. „Cukrowa pochodna cynkowej ftalocyjaniny jako innowacyjne narzędzie terapii fotodynamicznej w leczeniu łuszczycy", pod kierownictwem prof. dr. hab. Piotra Ziółkowskiego. Doktorant jest laureatem stypendium NAWA, dzięki któremu miał możliwość wyjazdu na 3 miesiące w 2022 roku do University of Udine, Department of Medicine we Włoszech. Podczas swojego pobytu w tym instytucie, testował działanie innowacyjnych pochodnychcynkowej ftalocyjaniny w terapii fotodynamicznej. Jako członek interdyscyplinarnego zespołu badawczego, zdobył nie tylko cenne doświadczenie laboratoryjne, ale również miał okazję współpracować z uznanymiekspertami w dziedzinie terapii fotodynamicznej, takich jak Prof. Valentina Rapozzi oraz Prof. Luigi Xodo. Ponadto, jest laureatem piątej edycji konkursu Młode Talenty organizowanego przez Dolnośląski Klub Kapitału w kategorii sukces w zakresie innowacji. Doktorant jest współautorem 37 publikacji w renomowanych czasopismach naukowych z bazy JCR, o łącznym IF 139,7 oraz 3830 pkt MNiSW, index Hirsch = 12.

9. Dorobek naukowy

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10. Oświadczenia współautorów

mgr Sebastian Makuch
Katedra Patologii Klinicznej i Doświadczalnej
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 31.07.2024

OŚWIADCZENIE

1) Oświadczam, że w pracy Makuch Sebastian, Woźniak Marta, Krawczyk Monika, Pastuch-Gawołek Gabriela, Szeja Wiesław, Agrawal Siddarth: Glycoconjugation as a promising treatment strategy for psoriasis, Journal of Pharmacology and Experimental Therapeutics, 2020, vol. 373, nr 2, s. 204-212, mój udział polegał na konceptualizacji problemu badawczego, przeprowadzeniu przeglądu literatury oraz napisaniu manuskryptu.

2) Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Makarec Alicja, Chodaczek Grzegorz, Ziółkowski Piotr, Woźniak Marta: The impact of proinflammatory cytokines and imiquimod on GLUT1 in HaCaT keratinocytes - a potential anti-psoriatic therapeutic target?, Cellular Physiology and Biochemistry, 2023, vol. 57, nr 2, s. 54-62, mój udział polegał na konceptualizacji problemu badawczego, przeprowadzeniu eksperymentów *in vitro*, analizy statystycznej części wyników, oraz napisaniu manuskryptu.

3) Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Woźniak Marta, Makarec Alicja, Lipińska Maja, Klyta Magdalena, Sulecka-Zadka Joanna, Szeja Wiesław, Gani Mariachiara, Rapozzi Valentina, Ziółkowski Piotr, Smoleński Piotr: In vitro and in vivo antipsoriatic efficacy of protected and unprotected sugar-zinc phthalocyanine conjugates, Pharmaceutics, 2024, vol. 16, nr 6, art.838 [20 s.], mój udział polegał na konceptualizacji problemu badawczego, przeprowadzeniu eksperymentów *in vitro* oraz *in vivo*, analizy statystycznej części wyników oraz napisaniu manuskryptu.

4) Oświadczam, że w pracy Makuch Sebastian, Dróżdż Mateusz, Makarec Alicja, Ziółkowski Piotr, Woźniak Marta: An update on photodynamic therapy of psoriasis - current strategies and nanotechnology as a future perspective, International Journal of Molecular Sciences, 2022, vol. 23, nr 17, art.9845 [18 s.], mój udział polegał na konceptualizacji problemu badawczego, przeprowadzeniu przeglądu literatury oraz napisaniu manuskryptu.

Uniwersytet Medyczny we Wrocławiu
Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik
prof. dr hab. Piotr Ziółkowski

Sebastian Makuch
Podpis

Prof. dr hab. n. med Piotr Ziółkowski
Katedra Patologii Klinicznej i Doświadczalnej
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 31.07.2024

OŚWIADCZENIE

1) Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Makarec Alicja, Chodaczek Grzegorz, Ziółkowski Piotr, Woźniak Marta: The impact of proinflammatory cytokines and imiquimod on GLUT1 in HaCaT keratinocytes - a potential anti-psoriatic therapeutic target?, Cellular Physiology and Biochemistry, 2023, vol. 57, nr 2, s. 54-62, mój udział polegał na kierowaniu projektem oraz nadzorowaniu badań.

2) Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Woźniak Marta, Makarec Alicja, Lipińska Maja, Klyta Magdalena, Sulecka-Zadka Joanna, Szeja Wiesław, Gani Mariachiara, Rapozzi Valentina, Ziółkowski Piotr, Smoleński Piotr: In vitro and in vivo antipsoriatic efficacy of protected and unprotected sugar-zinc phthalocyanine conjugates, Pharmaceutics, 2024, vol. 16, nr 6, art.838 [20 s.], mój udział polegał na kierowaniu projektem oraz nadzorowaniu badań.

3) Oświadczam, że w pracy Makuch Sebastian, Dróżdż Mateusz, Makarec Alicja, Ziółkowski Piotr, Woźniak Marta: An update on photodynamic therapy of psoriasis - current strategies and nanotechnology as a future perspective, International Journal of Molecular Sciences, 2022, vol. 23, nr 17, art.9845 [18 s.], mój udział polegał na kierowaniu projektem oraz nadzorowaniu prac nad manuskryptem.

Podpis

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Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik

prof. dr hab. Piotr Ziółkowski

Uniwersytet Medyczny we Wrocławiu
Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik

prof. dr hab. Piotr Ziółkowski

Dr n. med Marta Woźniak
Katedra Patologii Klinicznej i Doświadczalnej
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 31.07.2024

OŚWIADCZENIE

1) Oświadczam, że w pracy Makuch Sebastian, Woźniak Marta, Krawczyk Monika, Pastuch-Gawołek Gabriela, Szeja Wiesław, Agrawal Siddarth: Glycoconjugation as a promising treatment strategy for psoriasis, Journal of Pharmacology and Experimental Therapeutics, 2020, vol. 373, nr 2, s. 204-212, mój udział polegał na redakcji manuskryptu.

2) Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Makarec Alicja, Chodaczek Grzegorz, Ziółkowski Piotr, Woźniak Marta: The impact of proinflammatory cytokines and imiquimod on GLUT1 in HaCaT keratinocytes - a potential anti-psoriatic therapeutic target?, Cellular Physiology and Biochemistry, 2023, vol. 57, nr 2, s. 54-62, mój udział polegał na nadzorowaniu badań.

3) Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Woźniak Marta, Makarec Alicja, Lipińska Maja, Klyta Magdalena, Sulecka-Zadka Joanna, Szeja Wiesław, Gani Mariachiara, Rapozzi Valentina, Ziółkowski Piotr, Smoleński Piotr: In vitro and in vivo antipsoriatic efficacy of protected and unprotected sugar-zinc phthalocyanine conjugates, Pharmaceutics, 2024, vol. 16, nr 6, art.838 [20 s.], mój udział polegał na nadzorowaniu badań.

4) Oświadczam, że w pracy Makuch Sebastian, Dróżdż Mateusz, Makarec Alicja, Ziółkowski Piotr, Woźniak Marta: An update on photodynamic therapy of psoriasis - current strategies and nanotechnology as a future perspective, International Journal of Molecular Sciences, 2022, vol. 23, nr 17, art.9845 [18 s.], mój udział polegał na redakcji i nadzorowaniu prac nad manuskryptem.

Uniwersytet Medyczny we Wrocławiu
Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik
prof. dr hab. Piotr Ziółkowski

Podpis

Uniwersytet Medyczny we Wrocławiu
KATEDRA PATOLOGII KLINICZNEJ I DOŚWIADCZALNEJ
Zakład Patologii Ogólnej i Doświadczalnej
adiunkt
dr n. med. Marta Woźniak

mgr Alicja Makarec
Katedra Patologii Klinicznej i Doświadczalnej
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 31.07.2024

OŚWIADCZENIE

1) Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Makarec Alicja, Chodaczek Grzegorz, Ziółkowski Piotr, Woźniak Marta: The impact of proinflammatory cytokines and imiquimod on GLUT1 in HaCaT keratinocytes - a potential anti-psoriatic therapeutic target?, Cellular Physiology and Biochemistry, 2023, vol. 57, nr 2, s. 54-62, mój udział polegał na przeprowadzeniu części eksperymentów dotyczących metody glucose uptake assay. AM

2) Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Woźniak Marta, Makarec Alicja, Lipińska Maja, Klyta Magdalena, Sulecka-Zadka Joanna, Szeja Wiesław, Gani Mariachiara, Rapozzi Valentina, Ziółkowski Piotr, Smoleński Piotr: In vitro and in vivo antipsoriatic efficacy of protected and unprotected sugar-zinc phthalocyanine conjugates, Pharmaceutics, 2024, vol. 16, nr 6, art.838 [20 s.], mój udział polegał na przeprowadzeniu części eksperymentów dotyczących fotocytotoksyczności związków badanych oraz redakcji manuskryptu. AM

3) Oświadczam, że w pracy Makuch Sebastian, Dróżdż Mateusz, Makarec Alicja, Ziółkowski Piotr, Woźniak Marta: An update on photodynamic therapy of psoriasis - current strategies and nanotechnology as a future perspective, International Journal of Molecular Sciences, 2022, vol. 23, nr 17, art.9845 [18 s.], mój udział polegał na napisaniu wstępu do publikacji. AM

Podpis

Alicja Makarec

Uniwersytet Medyczny we Wrocławiu
Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik
prof. dr hab. Piotr Ziółkowski

dr n. biol. Piotr Kupczyk
Katedra Patologii Klinicznej i Doświadczalnej
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 31.07.2024

OŚWIADCZENIE

1) Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Makarec Alicja, Chodaczek Grzegorz, Ziółkowski Piotr, Woźniak Marta: The impact of proinflammatory cytokines and imiquimod on GLUT1 in HaCaT keratinocytes - a potential anti-psoriatic therapeutic target?, Cellular Physiology and Biochemistry, 2023, vol. 57, nr 2, s. 54-62, mój udział polegał na przeprowadzeniu części eksperymentów związanych z mikroskopią oraz redakcji manuskryptu.

2) Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Woźniak Marta, Makarec Alicja, Lipińska Maja, Klyta Magdalena, Sulecka-Zadka Joanna, Szeja Wiesław, Gani Mariachiara, Rapozzi Valentina, Ziółkowski Piotr, Smoleński Piotr: In vitro and in vivo antipsoriatic efficacy of protected and unprotected sugar-zinc phthalocyanine conjugates, Pharmaceutics, 2024, vol. 16, nr 6, art.838 [20 s.], mój udział polegał na przeprowadzeniu części eksperymentów związanych z histologią in vivo oraz redakcji manuskryptu.

Podpis

Uniwersytet Medyczny we Wrocławiu
Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik
prof. dr hab. Piotr Ziółkowski

Prof. dr hab. Wiesław Szeja
Katedra Chemii Organicznej, Bioorganicznej i Biotechnologii
Politechnika Śląska

Gliwice, 31.07.2024

OŚWIADCZENIE

1) Oświadczam, że w pracy Makuch Sebastian, Woźniak Marta, Krawczyk Monika, Pastuch-Gawołek Gabriela, Szeja Wiesław, Agrawal Siddarth: Glycoconjugation as a promising treatment strategy for psoriasis, Journal of Pharmacology and Experimental Therapeutics, 2020, vol. 373, nr 2, s. 204-212, mój udział polegał na redakcji manuskryptu.

2) Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Woźniak Marta, Makarec Alicja, Lipińska Maja, Klyta Magdalena, Sulecka-Zadka Joanna, Szeja Wiesław, Gani Mariachiara, Rapozzi Valentina, Ziółkowski Piotr, Smoleński Piotr: In vitro and in vivo antipsoriatic efficacy of protected and unprotected sugar-zinc phthalocyanine conjugates, Pharmaceutics, 2024, vol. 16, nr 6, art.838 [20 s.], mój udział polegał na projektowaniu syntezy, nadzorowaniu procesu syntezy chemicznej oraz redakcji manuskryptu.

Podpis

Wiesław Szeja

Uniwersytet Medyczny we Wrocławiu
Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik
prof. dr hab. Piotr Ziółkowski

Dr Monika Domińska (Krawczyk)
Katedra Chemii Organicznej, Bioorganicznej i Biotechnologii
Politechnika Śląska

Gliwice, 31.07.2024

OŚWIADCZENIE

1) Oświadczam, że w pracy Makuch Sebastian, Woźniak Marta, Krawczyk Monika, Pastuch-Gawołek Gabriela, Szeja Wiesław, Agrawal Siddarth: Glycoconjugation as a promising treatment strategy for psoriasis, Journal of Pharmacology and Experimental Therapeutics, 2020, vol. 373, nr 2, s. 204-212, mój udział polegał na redakcji manuskryptu.

Podpis

Monika Domińska

Uniwersytet Medyczny we Wrocławiu
Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik

prof. dr hab. Piotr Ziółkowski

mgr inż. Magdalena Klyta
Zwierzętarnia Doświadczalna
Centrum Badań Przedklinicznych

Wrocław, 31.07.2024

OŚWIADCZENIE

1) Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Woźniak Marta, Makarec Alicja, Lipińska Maja, Klyta Magdalena, Sulecka-Zadka Joanna, Szeja Wiesław, Gani Mariachiara, Rapozzi Valentina, Ziółkowski Piotr, Smoleński Piotr: In vitro and in vivo antipsoriatic efficacy of protected and unprotected sugar-zinc phthalocyanine conjugates, Pharmaceutics, 2024, vol. 16, nr 6, art.838 [20 s.], mój udział polegał na opiece nad zwierzętami do badań in vivo, wykonywaniu procedur oraz eutanazji.

Podpis

Magdalena Klyta

Uniwersytet Medyczny we Wrocławiu
Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik

prof. dr hab. Piotr Ziółkowski

mgr Mateusz Dróżdż
Department of Biology, Chemistry, and Pharmacy
Freie Universität Berlin

Berlin, 31.07.2024

OŚWIADCZENIE

1) Oświadczam, że w pracy Makuch Sebastian, Dróżdż Mateusz, Makarec Alicja, Ziółkowski Piotr, Woźniak Marta: An update on photodynamic therapy of psoriasis - current strategies and nanotechnology as a future perspective, International Journal of Molecular Sciences, 2022, vol. 23, nr 17, art.9845 [18 s.], mój udział polegał na napisaniu rozdziału o nanotechnologii i redakcji manuskryptu.

Dróżdż Mateusz
Podpis

Uniwersytet Medyczny we Wrocławiu
Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik
prof. dr hab. Piotr Ziółkowski

dr hab. Grzegorz Chodaczek
Sieć Badawcza Łukasiewicz –
PORT Polski Ośrodek Rozwoju Technologii
Grupa Badawcza Immunoterapii

Wrocław, 31.07.2024

OŚWIADCZENIE

Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Makarec Alicja, Chodaczek Grzegorz, Ziółkowski Piotr, Woźniak Marta: The impact of proinflammatory cytokines and imiquimod on GLUT1 in HaCaT keratinocytes - a potential anti-psoriatic therapeutic target?, Cellular Physiology and Biochemistry, 2023, vol. 57, nr 2, s. 54-62, mój udział polegał na przeprowadzeniu analizy statystycznej metody qPCR i mikroskopii konfokalnej oraz redakcji manuskryptu.

Grzegorz Chodaczek

Uniwersytet Medyczny we Wrocławiu
Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik
prof. dr hab. Piotr Ziółkowski

Valentina Rapozzi, Associate Professor
Department of Medicine, Laboratory of Biochemistry
P.le Kolbe 4, 33100 Udine, Italy

Udine, 22.08.2024

Co-Author Declaration

1) I hereby declare that in the work by Makuch Sebastian, Kupczyk Piotr, Woźniak Marta, Makarec Alicja, Lipińska Maja, Klyta Magdalena, Sulecka-Zadka Joanna, Szeja Wiesław, Gani Mariachiara, Rapozzi Valentina, Ziółkowski Piotr, Smoleński Piotr: "In vitro and in vivo antipsoriatic efficacy of protected and unprotected sugar-zinc phthalocyanine conjugates," Pharmaceutics, 2024, vol. 16, no. 6, art.838 [20 pages], my contribution involved validation of the experiments and editing the manuscript.

Valentina Rapozzi

Uniwersytet Medyczny we Wrocławiu
Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik

prof. dr hab. Piotr Ziółkowski

Mariachiara Gani, PhD
Department of Medicine, Laboratory of Biochemistry
P.le Kolbe 4, 33100 Udine, Italy

Udine, 22.08.2024

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Signature

Uniwersytet Medyczny we Wrocławiu
Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik
prof. dr hab. Piotr Ziółkowski

Mgr Joanna Sulecka-Zadka
Katedra Farmakologii i Toksykologii
Uniwersytet Przyrodniczy we Wrocławiu

Wrocław, 31.07.2024

OŚWIADCZENIE

1) Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Woźniak Marta, Makarec Alicja, Lipińska Maja, Klyta Magdalena, Sulecka-Zadka Joanna, Szeja Wiesław, Gani Mariachiara, Rapozzi Valentina, Ziółkowski Piotr, Smoleński Piotr: In vitro and in vivo antipsoriatic efficacy of protected and unprotected sugar-zinc phthalocyanine conjugates, Pharmaceutics, 2024, vol. 16, nr 6, art.838 [20 s.], mój udział polegał na analizie wychwytu komórkowego z użyciem mikroskopii fluorescencyjnej.

Podpis
Joanna Sulecka-Zadka

Uniwersytet Medyczny we Wrocławiu
Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik
prof. dr hab. Piotr Ziółkowski

mgr inż. Maja Lipińska
Zwierzętarnia Doświadczalna
Centrum Badań Przedklinicznych

Wrocław, 31.07.2024

OŚWIADCZENIE

1) Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Woźniak Marta, Makarec Alicja, Lipińska Maja, Klyta Magdalena, Sulecka-Zadka Joanna, Szeja Wiesław, Gani Mariachiara, Rapozzi Valentina, Ziółkowski Piotr, Smoleński Piotr: In vitro and in vivo antipsoriatic efficacy of protected and unprotected sugar-zinc phthalocyanine conjugates, Pharmaceutics, 2024, vol. 16, nr 6, art.838 [20 s.], mój udział polegał na opiece nad zwierzętami do badań in vivo, wykonywaniu procedur oraz eutanazji.

Uniwersytet Medyczny we Wrocławiu
Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik
prof. dr hab. Piotr Ziółkowski

Podpis

Dr inż. Gabriela Pastuch-Gawołek
Katedra Chemii Organicznej, Bioorganicznej i Biotechnologii
Politechnika Śląska

Gliwice, 31.07.2024

OŚWIADCZENIE

Oświadczam, że w pracy Makuch Sebastian, Woźniak Marta, Krawczyk Monika, Pastuch-Gawołek Gabriela, Szeja Wiesław, Agrawal Siddarth: Glycoconjugation as a promising treatment strategy for psoriasis, Journal of Pharmacology and Experimental Therapeutics, 2020, vol. 373, nr 2, s. 204-212, mój udział polegał na redakcji manuskryptu.

Podpis

Gabriela Pastuch

Uniwersytet Medyczny we Wrocławiu
Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik
prof. dr hab. Piotr Ziółkowski

Prof. dr hab. Piotr Smoleński
Wydział Chemii
Uniwersytet Wrocławski

Wrocław, 11.09.2024

OŚWIADCZENIE

1) Oświadczam, że w pracy Makuch Sebastian, Kupczyk Piotr, Woźniak Marta, Makarec Alicja, Lipińska Maja, Klyta Magdalena, Sulecka-Zadka Joanna, Szeja Wiesław, Gani Mariachiara, Rapozzi Valentina, Ziółkowski Piotr, Smoleński Piotr: In vitro and in vivo antipsoriatic efficacy of protected and unprotected sugar-zinc phthalocyanine conjugates, Pharmaceutics, 2024, vol. 16, nr 6, art.838 [20 s.], mój udział polegał na przeprowadzeniu syntez chemicznych koniugatów cynkowej ftalocyjaniny, opisaniu części chemicznej syntez oraz redakcji manuskryptu.

Podpis

Signed by / Podpisano przez:
Piotr Marek Smoleński
Date / Data: 2024-09-11 22:29

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Katedra Patologii Klinicznej i Doświadczalnej
ZAKŁAD PATOLOGII OGÓLNEJ I DOŚWIADCZALNEJ
kierownik
prof. dr hab. Piotr Ziółkowski