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IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

Agata Diakun

„Analysis of an intraperitoneal gas-based hyperthermia beyond 43°C appliance in-vivo study”

DISSERTATION

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Parents for „fishing rods".

CONTENTS

1. INFORMATION NOTE

This doctoral dissertation consists of a monothematic cycle of three original scientific papers published in a peer-reviewed international medical journal. The periodical in which the above-mentioned articles are included is on the List of Scientific Journals of the Minister of Science and Education and on the Philadelphia List.

The total number of points of the Minister of Science and Education obtained by the above-mentioned publications are 300, and the total Impact Factor (IF) is 17,218 points.

The PhD student is the first author in all papers. She made a significant contribution to the creation of all works, consisting of:

1. creating scientific hypotheses,
2. conducting in vitro tests,
3. verification and interpretation of the collected data,
4. statistical analysis,
5. elaboration of results,
6. development and presentation of applications,
7. preparation of the publication.

The research project was approved by the Local Ethical Committee for Animal Experiments in Wrocław, Resolution No. 029/2021/P1 of May 19, 2021 (attached).

2. LIST OF ABBREVIATIONS

BMI - Body Mass Index,

CRS - cytoreductive surgery,

EU - European Union,

HIPEC - Hyperthermic Intraperitoneal Chemotherapy,

MPM - malignant peritoneal mesothelioma,

PCI - Peritoneal Cancer Index,

PIPAC - Pressurized Intra Peritoneal Aerosol Chemotherapy,

PM - peritoneal metastases,

PMP - peritoneal pseudomyxoma.

3. INTRODUCTION

Despite the dynamic development of oncology, there is still a group of cancers that pose therapeutic difficulties. It includes malignant tumors of the peritoneum. In many cases, the methods of treatment used so far do not bring satisfactory results in the form of recovery or a significant extension of the survival of patients affected by this disease. The prognosis of patients with this type of cancer is poor. The average survival time of patients with a neoplastic process involving the peritoneum is about 6 months [1, 2]. Moreover, the presence of metastases within the peritoneum leads to disturbances in the physiological functioning of the abdominal organs, pain in the abdominal cavity caused by gastrointestinal motility dysfunction, and often symptoms of subileus or ileus. This significantly affects patients' quality of life.

These tumors may have primary or metastatic character. The first group includes rare primary tumors of the peritoneum, i.e., peritoneal mesothelioma, peritoneal pseudomyxoma, primary peritoneal carcinoma, mesothelial cysts, fibromatosis, or desmoplastic round cell carcinoma. Cancers that metastasize to the peritoneum include among others, malignant tumors of the appendix, colon, stomach and ovary. It should be emphasized that in the population of patients suffering from tumors located within the abdominal cavity, peritoneal metastases occur in about 5-15% of patients with colorectal cancer [3, 4], about 40% of patients with gastric cancer [5, 6] and as much as about 50% of the population patients with ovarian cancer.

Conventional methods of oncological treatment have limited effectiveness in the case of peritoneal cancer involvement. Systemic treatment in the form of intravenous chemotherapy is characterized by low effectiveness due to poor penetration of cytostatics into the neoplastic peritoneum, while the use of radiotherapy is associated with many side effects.

Nowadays, the involvement of the peritoneal cavity is treated as a local dissemination. The recommended treatment is cytoreductive surgery (CRS) supplemented with hyperthermic intraperitoneal chemotherapy (HIPEC)[3,5]. This treatment carries a high risk of peri- and postoperative complications and can be used only in a strictly selected group of patients.

The temperature used in the HIPEC procedure does not exceed 43°C, which is related to the high heat capacity of the liquid introduced into the peritoneal cavity. Heating the chemotherapeutic solution above this temperature would threaten the life and health of patients with an increase in the internal body temperature.

Scientific research confirms the ability of hyperthermia to induce cell death. It is known that the cytotoxic effect is enhanced in an oxygen-poor environment where the pH is reduced. Hyperthermia leading to a decreased pH environment increases the cytotoxic effect. Due to the presence of pathological vascularity within tumors, these tissues are characterized by a lower thermoregulatory capacity compared to healthy tissues (e.g., lack of possibility to dilate the vascular bed), which leads to an intensification of the thermal effect within the tumor.

Hyperthermia also enhances the effects of radio- and chemotherapy - the phenomenon of additivity of radio- or chemotherapy and hyperthermia [10].

Direct cell death caused by hyperthermia is primarily influenced by protein denaturation, which leads to instability of cell membranes and impaired cytoskeletal function 8. Moreover, the temperature above 40°C promotes the formation of clots and emboli in the lumen of abnormal vessels, which may additionally intensify hemorrhagic necrosis within the tumor, which may potentially influence the inhibition of distant metastases. Some studies suggest that the beneficial clinical effects of hyperthermia may result from the occlusion of pathological blood vessels of tumor structures. The beneficial effect of local hyperthermia on the treatment of metastatic peritoneal neoplastic lesions has been well documented in the literature [1, 34, 35, 36].

A phenomenon coexisting with gaseous hyperthermia is dehydration. Limited dehydration of the superficial layers of the peritoneum may significantly disturb the conditions in the peritoneal cavity that are conducive to the spread of cancer. Thus, the dehydration process may potentially limit or prevent the spread of metastatic changes and be an alternative method of treating peritoneal cancers.

Due to the limitations described above related to the currently used treatment of peritoneal cancer, modern medicine is faced with the challenge of searching for new forms of therapy that would lead to recovery, improvement of the quality of life or a significant extension of patients' survival. Among the methods used so far in the treatment of the neoplastic process disseminated to the peritoneum, the most effective method is the cytoreductive procedure combined with HIPEC [5, 11.] In the presented dissertation, the possibility of safely producing hyperthermia at a temperature exceeding 43 °C was investigated, avoiding the side effects of this phenomenon observed when using solution water. For this purpose, a significant difference in heat capacity between liquid and gas was used. The heat capacity of the gas, which is much lower than that of the liquid, allowed to reduce the risk associated with raising the temperature of the patient's body, which would pose a threat to the patient's health and life.

In the research presented in the above dissertation, a heated gas mixture, i.e., atmospheric air, was used to produce hyperthermia exceeding 43 °C.

The conducted research confirmed the possibility of safely producing hyperthermia exceeding 43°C using gas as a temperature carrier. The phenomenon observed during this process was dehydration. The dehydration process involving the superficial layers of the peritoneum may adversely affect the environment in the peritoneal cavity, which is conducive to the spread of cancer. Thus, gas hyperthermia combined with the dehydration process may potentially limit or prevent the spread of metastatic changes and be an alternative method of treating peritoneal cancer.

3.1. PRIMARY PERITONEAL CANCERS

3.1.1. Peritoneal mesothelioma

Malignant peritoneal mesothelioma (MPM) is an extremely rare cancer. It accounts for 10-15% of all mesotheliomas [12]. Based on the data of the National Cancer Registry in Poland, in 2012, 299 patients were diagnosed with peritoneal mesothelioma, and 222 died from this cancer. Peritoneal mesothelioma develops from serosa cells. In most cases, it is a malignant tumor. MPM occurs most often in people over the age of 60, 2 to 5 times more often in men than in women. Exposure to asbestos may be a predisposing factor for disease. Due to the lack of specific symptoms, this cancer is often diagnosed at an advanced stage [12].

3.1.2. Pseudoperitoneal myxoma

Peritoneal pseudomyxoma (PMP) is a very rare disease with a frequency of 3-4 cases/per one million people/a year. It is more common in women than in men. It causes symptoms called , jello belly', which is the result of the accumulation of mucus content produced by the tumour. It is almost always associated with perforation of the mucinous tumor of the appendix. We then observe the passage of mucus into the peritoneal cavity and its redistribution. The clinical picture of this disease includes mucous ascites, neoplastic implants involving the peritoneum, massive infiltration of the greater omentum (omental cake) and infiltration of the ovaries [13]. Due to the lack of specific symptoms, like MPM, this cancer is often diagnosed at an advanced stage.

3.2. SECONDARY PERITONEAL CANCER

3.2.1. Mechanism of metastatation to the peritoneal cavity:

In order to create effective methods of treatment and prevention of disseminated neoplastic disease affecting the peritoneal cavity, it is necessary to know the process in which it occurs.

The presence of metastases in the peritoneal cavity (peritoneal metastasis, PM) is evidence of the progression of the primary malignancy. This process is based on the exfoliation of cancer cells and their entry into the peritoneal cavity. Several mechanisms of cell detachment from the primary focus are known. One of the typical tumors occurring within the abdominal cavity is the spontaneous desquamation of tumor cells infiltrating the serosa of organs. This mechanism has been observed in for colorectal, gastric, and ovarian cancers. The presence of free tumor cells in the peritoneal cavity may also be caused by intraoperative manipulations, tumor damage during surgery, or result from its perforation. Another mechanism leading to the appearance of neoplastic cells in the peritoneal cavity is damage to the lymphatic and blood vessels during the resection procedure[14]. Moreover, the ability of peritoneal mesothelial cells to transform into mesenchymal cells has been observed, which is a significant morphological change creating a favorable environment for the spread of cancer cells [15]. This facilitates implantation of metastatic changes within the peritoneum. Published studies have

shown that due to this transformation, peritoneal mesothelial cells gain the ability to produce inflammatory factors and factors that enhance angiogenesis and thus promote the growth of cancer cells. This process underlies the early presence of metastases, which often occur at the stage of disease diagnosis [6,14,16.] Cancer cells, after being detached from the primary tumor and then entering the peritoneal cavity, undergo distribution characteristic of peritoneal fluid circulation. This process is the result of phenomena occurring within the abdominal cavity, i.e., peristaltic movements, changes in intraperitoneal pressure resulting from movements of the diaphragm and gravity [9,13].

The fluid in the peritoneal cavity moves upwards along the right gutter, which is a consequence of the negative pressure caused by diaphragm movement and the peristaltic. Most of the fluid gathers on the right side, which is caused by the greater depth of the right sulcus and the presence of a specific barrier, which is the falciform ligament. The fluid is then directed to the pelvis below the mesentery of the small intestine. Cancer cell deposits are localized in the places of peritoneal fluid stagnation, e.g.

3.2.2 Colon cancer

Colorectal cancer (CRC) is the third most frequently diagnosed cancer and the second leading cause of cancer-related death in the world [17]. Early diagnosed patients have a chance for resection, potentially leading to a complete cure (70-80%), with a 5-year recovery period survival rate of 72-93% for stage I-II. Approximately 20% of patients have metastases at the time of diagnosis. Among them, about 8% of patients have synchronous (occurring at the same time, diagnosed at the same time or earlier) peritoneal metastases, and 20% have metastatic changes in the liver [14]. Cancer dissemination takes place via the lymphatic and hematopoietic routes and by infiltrating the surrounding tissues. The risk factors favoring the occurrence of metastases to the peritoneum are tumor perforation, tumor stage [T4], low grade, mucinous type of tumor, and presence of signet ring cells [13]. Colorectal cancer cells are invasive. Peritoneal implants often occur in the mesentery and serosa of the small intestine, which significantly worsens the prognosis. A significant proportion of metastases are metachronous, which means that the lesions appear within 12 months of the primary lesion and may result in recurrence of the disease in previously operated areas [13].

3.2.3. Gastric cancer

Gastric cancer is one of the most frequently diagnosed gastrointestinal cancers 2. Although the incidence has been decreasing in recent years, in the European Union (EU) it remains at the level of 18.9 new diagnoses/per 100,000 people/a year, while the mortality rate is 14.7 deaths/per 100,000 people/a year and is approximately 5 times higher among men [18]. The dissemination of gastric cancer, as in the case of colorectal cancer, occurs through the

lymphatic, hematopoietic and intraperitoneal routes. In the group of patients diagnosed de novo with gastric cancer, 15-40% will have metastases to the peritoneum [13]. After surgery, 35-45% of patients will develop metastases within the peritoneum [14]. This is the most common location of tumor recurrence after surgical treatment. 5-year survival rates in stages II and III, in the case of resectable lesions, are 20-40%. Most patients with serous-invasive gastric cancer will develop peritoneal metastases despite surgical treatment [20]. Median survival of patients with gastric cancer undergoing surgery systemic therapy is 9.5-11.6 months [14].

3.2.4 Ovarian cancer

Ovarian cancer is the leading cause of death among cancers of the female reproductive organs in developed countries. The vast majority of cancers are diagnosed at an advanced stage 16, 21. Cytoreductive treatment with cis- or carbo-platinum chemotherapy and a taxane enables remission in 75% of patients with disseminated cancer, and the 5-year survival rate in such advanced disease after treatment is about 50% [22, 23]. Ovarian cancer dissemination to the peritoneum takes place through the lymphatic route and intraperitoneally through the circulation of the peritoneal fluid. In many cases, even in the early stages of the disease, cancer cells are present in the peritoneal fluid.

3.3. TREATMENT

3.3.1 HIPEC

Patients who have failed standard methods of cancer treatment, i.e., chemotherapy or radiotherapy, may, in the absence of contraindications, be qualified for the procedure of cytoreductive surgery (CRS) with HIPEC, i.e., hyperthermic intraperitoneal perfusion chemotherapy (HIPEC). Unfortunately, only a limited group of patients with peritoneal metastases are eligible for this procedure. The procedure is associated with a, therefore careful qualification of patients for the above-mentioned treatment is of great importance. The frequency of peritoneal metastases in colorectal cancer is 5-15% in the case of synchronous metastases and as much as 40% in the case of local recurrence. The best results of treatment of a neoplastic process disseminated to the peritoneum are obtained by combining a cytoreductive procedure with intraperitoneal hyperthermic perfusion chemotherapy (HIPEC). The results of such treatment are better and allow 5-year survival at the level of 30-50%. These procedures require extensive experience in abdominal surgery, are time-consuming (average surgical procedure time is 6-8 hours) and are burdened with complications related to the surgical procedure, as well as the administration of a cytostatic at an elevated temperature (41.5 - 43°C) into the peritoneal cavity [3].

HIPEC is reserved for patients with an advanced, potentially resectable, neoplastic process limited to the peritoneum. The exception to this rule is the presence of a single lung metastasis or no more than three liver metastases that can be surgically removed. The extent of changes

within the peritoneal cavity cannot score more than 20 points on the Sugebaker scale Peritoneal Cancer Index (PCI). Patients with peritoneal myxoma. An important factor limiting the possibility of using the method is the involvement of the neoplastic process in the small intestine.

Intraperitoneal chemotherapy penetrates the tumor tissue to a depth of 1mm to 3mm. In order to obtain a favorable prognosis, it is necessary to perform a cytoreductive surgery (CRS) CC-0 [24]. CRS is an extensive, time-consuming, and often technically difficult procedure with a high risk of complications and should be performed by experienced operators in centers specialized in this field. Despite complications risk in a selected group of patients, the cytoreductive procedure with HIPEC significantly affects the length of survival and the improvement of the quality of life of patients with peritoneal cancer [3, 23, 24, 25].

Patients in poor general condition, cachectic and significantly burdened with comorbidities will will not be good candidates to the procedure. A contraindication to performing cytoreductive procedure with HIPEC is the age above 70 years old, which results from the potential systemic comorbidities of patients in this age group. Relative contraindications include Body Mass Index exceeding 40 (BMI), radiotherapy of the pelvis, numerous interventions in the abdominal cavity, obstruction of the gastrointestinal tract, obstruction of the bile ducts, obstruction of the urinary tract, as well as progression of cancer after neoadjuvant chemotherapy. However, it should be emphasized that each time the qualification of patients for the procedure should be considered individually [5].

3.3.2 PIPAC

An alternative method of treating patients with disseminated neoplastic processes involving the peritoneum is pressurized intraperitoneal aerosol PIPAC (Pressurized IntraPeritoneal Aerosol Chemotherapy). This procedure consists in the laparoscopic administration of the chemotherapeutic agent in the form of an aerosol into the peritoneal cavity [26], while maintaining the room temperature of the drugs. It may be a therapeutic option for patients who are not eligible for cytoreductive surgery.

3.4. SUMMARY

Restrictive eligibility criteria for cytoreductive surgery in combination with HIPEC disqualify a large group of patients with metastatic changes within the peritoneal cavity. Modern medicine can only offer them palliative treatment. Therefore, it is necessary to search for methods that could be an alternative form of treatment and limit the intraperitoneal spread of cancer for patients who are not eligible for cytoreductive surgery or cytoreductive surgery combined with HIPEC.

In order to search for an alternative to the method known so far, the following research was carried out and presented in this dissertation.

- In vitro study to assess the viability of the HT-29 human colorectal cancer cell line exposed to hyperthermia and dehydration, using the colorimetric method (CellTiter 96® AQueous One Solution Cell Proliferation Assay MTS) and evaluation in an electron microscope.
- An in vitro study to assess the cytotoxic effect of hyperthermia and dehydration on the HT-29 human colorectal cancer cell line, based on the measurement of LDH concentration, using a colorimetric method and evaluation in an electron microscope.
- In vivo study, i.e., laparoscopic generation of intraperitoneal gaseous hyperthermia above 43° C in an animal model, assessment of the feasibility and safety of this process - the study was carried out on three pigs.
- Observation of thermodynamic processes occurring during the creation of hyperthermia above 43° C in the peritoneal cavity of pigs.
- 7-day observation of animals with morphological and biochemical blood tests.
- Autopsy examination with macroscopic evaluation of tissues subjected to gaseous hyperthermia above 43°C and dehydration.
- Histological examination of tissues collected post mortem.

The results of the studies listed above are presented in the form of a series of publications that make up this dissertation.

4. SUMMARY IN POLISH

Wprowadzenie

Zastosowanie chemioterapii dootrzewnowej w hipertermii jest coraz ważniejszym aspektem leczenia choroby nowotworowej rozsianej do jamy otrzewnej. W dostępnej literaturze specjalistycznej nadal brakuje informacji dotyczących hipertermii dootrzewnowej wytworzonej gazem oraz możliwości jej zastosowania. Bazując na właściwościach fizycznych gazu, zbadano nieopisywane dotąd wytworzenie wewnątrzotrzewnowej, gazowej hipertermii przekraczającej 43 °C, przeprowadzono ocenę jej bezpieczeństwa i ograniczeń. Niewiadomą wymagającą zbadania jest, czy tak osiągnięta hipertermia oraz związane z nią zjawiska biologiczne i termodynamiczne, mogą mieć potencjał terapeutyczny w leczeniu choroby nowotworowej ze zmianami metastatycznymi w obrębie otrzewnej.

Cel badania

Celem pracy jest zbadanie wpływu hipertermii gazowej i dehydratacji na linię komórkową ludzkiego nowotworu jelita grubego HT-29 oraz ocena możliwości bezpiecznego wytworzenia wewnątrzotrzewnowej hipertermii gazowej powyżej 43 °C w organizmie zwierzęcym.

Co więcej, przedmiotem badań jest ocena wpływu gazowej hipertermii i dehydratacji

na organizm zwierzęcy oraz poznanie ww. metody i jej ograniczeń. W przeprowadzonych badaniach zwrócono również uwagę na zjawiska termodynamiczne zachodzące podczas wewnątrzotrzewnowej gazowej hipertermii. Dzięki przeprowadzeniu eksperymentów *in vitro* i in vivo uzyskane zostały dane niezbędne do wstępnej ewaluacji opisywanej metody.

Wyniki i ich przewidywane zastosowanie:

Badanie przeprowadzone na liniach komórkowych nowotworu jelita grubego HT-29 wykazało wzrost cytotoksyczności na skutek oddziaływania hipertermii na poziomie 45°C względem 37°C o niemal 60%. Co więcej, ustalono, że pod wpływem dehydratacji cytotoksyczność względem komórek linii nowotworu złośliwego jelita grubego HT-29 była podwyższona w temperaturze 37°C. Dehydratacja współistniejąca z hipertermią 45°C i 48°C wykazały podobny poziom cytotoksycznego wpływu na komórki nowotworowe. Oprócz cytotoksycznego wpływu dehydratacji i hipertermii przekraczającej 43°C, oceniano również żywotność komórek nowotworowych poddanych ww. zjawiskom. Żywotność komórek nowotworowych eksponowanych na temperatury na poziomie 45°C i 48°C nie zmieniła się w porównaniu do komórek nowotworowych wystawionych na działanie temperatury 37°C. Zjawisko dehydratacji natomiast, w istotny sposób ją zmniejszało nawet przy 37°C. Efekt ten wydawała się potęgować jednoczasowa ekspozycja na temperatury 45°C i 48°C.

Dowiedziono ponadto, iż bezpieczne wytworzenie gazowej hipertermii powyżej 43°C jest możliwe i bezpieczne w organizmie zwierzęcym. Po wykonaniu procedury nie obserwowano około- i pooperacyjnych powikłań. W badaniach morfologicznych i biochemicznych krwi świń nie odnotowano istotnych odchyleń od prawidłowych wartości ocenianych parametrów. Nie obserwowano również nieprawidłowości w zachowaniu zwierząt.

Po wykonaniu badania autopsyjnego, pobrano tkanki do dalszych badań. W badaniu histopatologicznym otrzewnej oraz wątroby eksponowanych na działanie dehydratacji i hipertermii gazowej przekraczającej 43° C obserwowano obecność mikrowylewów, petechii, zakrzepów, nacieku leukocytarnego oraz obrzęk otrzewnej.

Powyższe wyniki wskazują, iż hipertermia gazowa przekraczająca 43° C potencjalnie może stać się podstawą do powstania metody ograniczającej rozsiew choroby nowotworowej w obrębie jamy otrzewnej. Jednak, aby to potwierdzić, konieczne jest przeprowadzenie obszernych, szczegółowych badań.

5. SUMMARY IN ENGLISH

Introduction

The use of intraperitoneal chemotherapy in hyperthermia has shown its increasingly important aspect of the treatment of neoplastic disease disseminated to the peritoneal cavity. The available specialist literature still lacks information on gas-based intraperitoneal hyperthermia and its possible use. Based on the physical properties of the gas, the previously undescribed generation of intraperitoneal gaseous hyperthermia exceeding 43 °C was investigated, its safety and limitations assessed. The unknown that needs to be investigated is whether the hyperthermia achieved in this way and the related biological and thermodynamic phenomena may have therapeutic potential in the treatment of cancer with metastatic changes within the peritoneum.

Purpose of the study:

The aim of the work is to investigate the effect of gas hyperthermia and dehydration on the HT-29 human colorectal cancer cell line and to assess the possibility of safe generation of intraperitoneal gas hyperthermia exceeding 43 °C in an animal organism. Moreover, the subject of the research is the assessment of the impact of gaseous hyperthermia and dehydration on the animal organism and the recognition of the above-mentioned factors method and its limitations. In the conducted research, attention was also paid to thermodynamic phenomena occurring during intraperitoneal gaseous hyperthermia. Thanks to the in vitro and in vivo experiments, the data necessary for the initial evaluation of the described method were obtained.

Results:

The study conducted on the HT-29 colorectal cancer cell line showed an increased cytotoxicity effect of hyperthermia at 45°C versus 37°C by almost 60%. Moreover, it was found that under the influence of dehydration, the cytotoxicity of the HT-29 colorectal cancer cell line was increased at 37°C. Dehydration coexisting with 45°C and 48°C hyperthermia showed similar levels of cytotoxic effects on tumor cells. In addition to the cytotoxic effect of dehydration and hyperthermia exceeding 43°C, the viability of tumor cells subjected to the above-mentioned phenomena was also assessed. The viability of tumor cells exposed to temperatures of 45°C and 48°C did not change compared to tumor cells exposed to 37°C. However, the phenomenon of dehydration significantly reduced it even at 37°C. This effect seemed to be enhanced by simultaneous exposure to 45°C and 48°C.It has also been proven that the safe generation of gaseous hyperthermia above 43°C is possible and safe in the animal body. No peri- and post-operative complications were observed after the procedure. In the morphological and

biochemical tests of swine blood, no significant deviations from the normal values of the evaluated parameters were noted. Neither no abnormal behavior of the animals was observed.

After the autopsy was performed, tissues were collected for further examination. In the histopathological examination of the peritoneum and liver tissue exposed to dehydration and gas hyperthermia exceeding 43°C, the presence of microhemorrhages, petechiae, blood clots, leukocyte infiltration and peritoneal edema were observed.

The above results indicate that gas hyperthermia exceeding 43°C can potentially become the basis for creating a method limiting the dissemination of neoplastic disease within the peritoneal cavity. However, extensive, detailed research is required to confirm this.

6. ARTICLES CONSTITUTING A MONOTHEMIC CYCLE OF DISERTTATION

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In-vivo thermodynamic exploration of gas-based intraperitoneal hyperthermia

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Background: While hyperthermic intraperitoneal (i.p) applications are highly efficient in treating peritoneal metastases (PM), they are currently limited to temperatures of 41 – 43° Celsius (C). First data on gas-based i.p. hyperthermia is promising, as this novel method allows a significant temperature rise in superficial peritoneal layers without increasing core temperatures. Until now, key mechanisms of this novel tool, e.g. thermodynamic energy transfer, have not been investigated. This study aims to explore the volume of thermodynamic energy transfer during gas-based i.p. hyperthermia at 48-50°C and its peritoneal effects.

Methods: For this study, three swine were subjected to gas-based i.p. hyperthermia at varying temperatures (48°, 49° and 50°C) in a diagnostic laparoscopy setting with a high-flow air stream. Temperatures of the i.p. cavity, in- and outflow airstream at the trocar were measured and the thermodynamic energy transfer was calculated. Tissue samples were collected on postoperative day 7 for histopathologic analyses.

Results: According to our data, temperatures within the intraabdominal cavity and at the outflow site remain relatively stable at < 40°C. An increase in thermodynamic energy transfer is observed with increasing applied temperatures. Gas-based i.p. hyperthermia induced capillary coagulation and white blood cell infiltration within peritoneal layers.

Conclusions: Gas-based i.p. hyperthermia is an innovative approach which enables the i.p. delivery of specific amounts of thermodynamic energy. Following this procedure, our data indicate remarkable histologic changes on

ARTICLE 1

the superficial peritoneal layer most likely attributable to the applied thermodynamic energy. Further studies are required to investigate how these findings can be applied in PM management.

KEYWORDS

Hyperthermia, thermodynamics, peritoneal metastases, intraperitoneal, chemotherapy, colorectal cancer

Introduction

Advanced peritoneal metastasis (PM) remains one of the key challenges in current surgical oncology. New concepts and approaches have been designed to improve the outcome of advanced, unresectable PM (1–5). With the introduction of Hyperthermic intraperitoneal chemotherapy (HIPEC) in combination with cytoreductive surgery (CRS) as a potentially curative treatment, hopes have been raised for some patients (6, 7). During HIPEC procedures, heated liquid chemotherapy is introduced into the abdominal cavity to eliminate remaining microscopic tumor cells after CRS (8). This effect is achieved by the combination of hyperthermia and chemotherapy. In the HIPEC setting, the medium perfusate temperature and core body temperatures usually remain at around 40°Celsius (C) (9). The increase of central body or total organ temperature is not desirable. A recent study by Goldenshluger et al. (10) demonstrated that increases in core body temperature served as a positive predictor for postoperative complications following HIPEC procedures. In fact, the relatively low temperature gradient between the applied HIPEC solution and core body temperature not only reduce the risk of overheating of single organs, but also the entire body. The beneficial effects of local hyperthermia in PM management have been well documented (11–13). While the sensitivity of cancer cells to increasing hyperthermia has been extensively demonstrated (14, 15), and hyperthermia has shown to increase the response rate of cancer cells to chemo- and radiotherapy (16–19), some studies suggest that the observed clinical effects of hyperthermia might be related to the occlusion of neo-vascular tumor structures (20–22). Currently effective hyperthermic intraperitoneal (i.p.) chemotherapy is limited to temperatures of 42 - 43°C. Yet, a previous study has demonstrated that far higher temperatures can be applied when using a gaseous medium as a mean for heat transportation (23). In contrast to water, air has a much lower heat capacity of around 0.718 kj/liter °C, when considering a density of 1.127 kg/m^3 at 40°C and atmospheric pressure. Close-range objects retain their temperature for a longer time when surrounded by a medium with an over 5000-fold decreased heat capacity. This concept has also been demonstrated in a biological setting (23). As a result, superficial tissue layers can be exposed to higher temperatures while deeper tissues basically remain unaffected (23). The *in-vivo* feasibility of gas-based i.p. hyperthermia with temperatures extending beyond 43°C has been recently demonstrated and further studies in this field are currently conducted. Until now, there has been no data on how much thermodynamic energy is transferred during a gas-based i.p. hyperthermic procedure with temperatures extending beyond 43° C. By means of this study, we intend to explore the extent of thermodynamic energy transfer during gas-based i.p. hyperthermia at 48-50°C as well as its potential heating impact on peritoneal tissues. Furthermore, we aim to analyze the structural effects of gas-based i.p. hyperthermia on the peritoneal surface from a pathohistological perspective.

Material and methods

In-vivo swine model

Three 65-day-old swine (Polish white flod) received gas-based i.p. hyperthermia at 48°, 49° and 50°C, respectively, in a diagnostic laparoscopy setting under a high-flow air stream of 15 liters per minute (l/min). The swine were part of a multicenter and multinational research study on peritoneal hyperthermia and dehydration. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals as published by the National Institutes of Health.

Gas-based i.p. hyperthermia in the laparoscopic setting

For the procedure, swine were placed under general anesthesia. Premedication was conducted using an intramuscular (i.m) injection of midazolam (0.3 mg/kg, WZF Polfa S.A., Poland), medetomidine (0.02 mg/kg, Cepetor 1 mg/ml, CP-Pharma Handelsgesellschaft, Germany) and ketamine (9 mg/kg, Ketamina 100 mg/ml, Biowet Puławy sp. z o.o., Poland) mixture. Analgesia was performed with Propofol at 1mg/kg. Swine were intubated and anesthesia was continued with isoflurane 1%. Additional analgesia was provided with fentanyl 2μg/kg and crystalloid fluid at 0.2-0.3

µg/kg/min. For the surgical procedure, swine were placed in a supine position. An infra-umbilical mini-laparotomy was performed and another one at about 8 cm distance to the first one. A 10 mm trocar (Kii®Balloon Blunt Tip System, Applied Medical, Rancho Santa Margarita, CA, USA) was inserted *via* the infra-umbilical trocar while several 5 mm trocars were placed at the other sites after insufflation (Figure 1A). The abdominal cavity was insufflated with filtered room air using a tube entering the central 10 mm trocar. An initial diagnostic check-up was conducted *via* laparoscopic imaging using a 5 mm camera system (Karl Storz 5mm/30° Laparoscope/Tuttlingen, Germany) and a 5 mm trocar. After visual confirmation and placement of multiple temperature sensors (Figure 1B), the high-flow air stream was turned on at 15 l/min for a total of 45 minutes. Intracavitary temperatures were monitored to detect the onset of critical intraabdominal heating. A total of four temperature sensors were placed in the abdominal cavity. One was placed in the lower quadrant, one in the right upper and one in the left upper quadrant, an additional probe was placed at close contact to the small intestine in the right central area but still within the cavity. In- and outflow temperatures were measured at the trocar site. Postoperatively, swine were monitored for seven days, and blood samples were drawn to detect possible postoperative complications.

Analyzing the operative model for laparoscopic dehydration of the abdominal cavity

Based on mean temperatures at the in- and outflow site, we calculated the temperature difference. With a given flow rate of 15 l/min, the thermodynamic energy transfer was calculated based on the first law of thermodynamics (conversation of energy) adapted for the thermodynamic process. The calculation was based on the specific heat capacity of air at 1 bar (atmospheric pressure), which ranges between 0.718 - 0.7206 KiloJoule (kJ) per Kilogram (kg) air at 27 - 67° C, and air density at atmospheric pressure (1 bar) given with 1.271 Kg per m^3 at 40° C. Based on the model of laparoscopic surface area quantification by Khosrawipour et al. (24), we used 16 dm^2 as the interacting peritoneal surface to estimate the extent of heat buildup on the peritoneal layer.

Euthanization

Seven days after the procedure, the swine were subjected to euthanization and autopsy. For this purpose, swine were premedicated with an i.m. injection of midazolam (0.1 mg/kg, Midanium 5 mg/ml, WZF Polfa S.A., Poland), medetomidine (0.02 mg/kg, Cepetor 1 mg/ml, CP-Pharma Handelsgesellschaft, Germany) and ketamine (8 mg/kg, Ketamina 100 mg/ml, Biowet Puławy sp. z o.o., Poland) mixture. After that, swine were euthanized with an intravenous injection by Sodium Pentobarbital with Pentobarbital (50mg/kg with 12 mg/kg, Morbital 133.3 mg/ml + 26.7 mg/ml, Biowet Pulawy Sp. z o.o., Poland) according to current recommendations (18). Post-mortem swine cadavers were placed in supine position and a median laparotomy was performed. Peritoneal tissue samples were removed from distinct locations within the abdomen for further histological analyzes. Additionally, hepatic samples were collected.

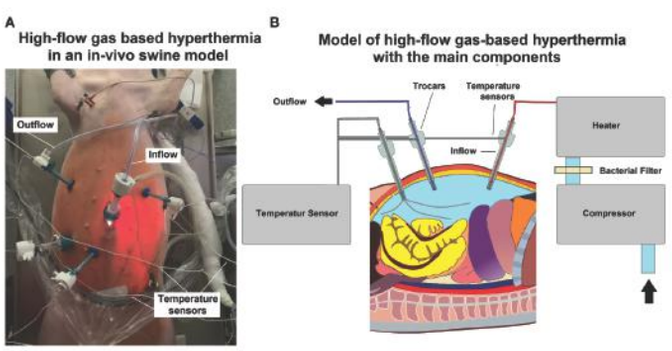

FIGURE 1
(A) Laparoscopy in the *in vivo* swine during high-flow gas-based hyperthermia. Multiple placements of temperature-sensors for intraoperative monitoring **(B)** Technical model of high-flow gas-based hyperthermia.

Histopathological examination of peritoneal tissue and liver samples

Samples were fixed for 48 hours in 10% neutral buffered formalin (cat# 22-026-354, Fisher Scientific). Formalin-fixed samples were prepared for paraffin processing by serial dehydration in increasing concentrations of ethanol solutions using a tissue processor (Leica TP1020, Leica Microsystems). After preparation, tissues were embedded in paraffin wax using a tissue embedder (Leica EG 1150C, Leica Microsystems). Paraffin-embedded tissue blocks were sectioned into 5µm sections on a microtome (Leica RM 2255, Leica Microsystems). 5µm sections were stained with HE. All slides were imaged using an inverted microscope (Nikon Ti-E Widefield microscope, Nikon Instruments Inc.).

Graphic design

For the graphics provided, multiple graphic programs were used. These programs included Inkscape 1.0.1,2020, GNU, USA as well as programs provided by Windows office 2019, Microsoft.

Statistical analysis

Three swine were exposed to mean temperature levels of 48°C, 49°C and 50°C.

Data are presented as the mean and the standard deviation unless otherwise indicated. A boxplot was used to visualize heat distribution. Mean heat-values were used to effectively calculate the thermodynamic gradient and energy transfer. Liver-samples for each swine were removed at 3 different, exposed sites within the laparoscopic cavity. Further peritoneal samples were taken at a total of 8 different sites per swine.

The student t-test was used to compare independent groups. Probability (p) values were considered as follows: *=$p<0.01$, and #=$p>0.05$, with p-value <0.05 considered to be statistically significant.

Results

Thermodynamics of gas-based i.p. hyperthermia

The experimental protocol was successfully executed. No intra- or postoperative complications were observed. All swine were extubated without problems. No distress or signs of abnormal behavior were detected. Intraoperative temperature measurements during the 45-minute procedure revealed that inflow, outflow and cavity temperature remained constant (Figures 2A–C). While the inflow temperature varies around the targeted 48°C, 49°C, and 50°C, the outflow temperature remained constant in each case within a narrow margin of 33.2 ± .1.3°C (48°C), 33.4± 1.9 C° (49°C) and 33.6 ± 2°C (50°C). The temperature difference (inflow vs. outflow) therefore increases with higher inflow temperature (Figure 2D). The mean difference is 15.3°C (48°C), 15.4°C (49°C) and 17.9°C (50°C) (Figure 3). For the presented 45-minute procedure, the thermodynamic energy transfer was calculated to be 6.4 kJ for the 48°C, 6.5 kJ for 49°C and 7.6 kJ for 50°C application, respectively (Figure 3). The mean thermodynamic energy transfer was approximately 0.42 kJ/°C during the 45-minute application. Based on these calculations, the presumed temperature effects on tissue were estimated. The temperature effect of 7.6 kJ, which corresponds to the energy transfer during a 45-minute gas-based hyperthermia procedure at 50°C on a 1 kg tissue sample, corresponds to 2.2°C (Figure 3). Assuming the effect of the thermodynamic energy of 7.6 kJ would have been limited on the most superficial 0.2 cm vs. 1 cm of the peritoneal surface (16 dm^2), the estimated temperature effect would have been 1.51°C vs. 7.56°C (Figure 3).

Histopathological examination, liver tissue and serum parameters

Following autopsy, swine tissue samples were removed from multiple locations within the peritoneum. Areas exposed to the hyperthermic laparoscopic space were compared to unexposed peritoneal samples of the same swine. Specific changes in exposed areas were analyzed and documented (Figure 4A). Hematoxylin and eosin (HE) staining depicted changes 7 days after i.p. hyperthermia. Spots of micropetechial bleeding were detected within the superficial peritoneal layer (Figure 4A1). Additionally, within the first few hundred millimeter of the peritoneal surface, the capillary system (Figures 4B, C) showed signs of occlusion by clotting (Figures 4A1, 2). In the peritoneal tissue exposed to the laparoscopic cavity, peritoneal edema, and an increase in white blood cell infiltration were detected (Figure 4A). In only a few cases, damage to muscle membrane cells of the intestine were observed. Unexposed peritoneal tissue samples did not show any specific changes, nor did they present signs of edema or white blood cell infiltration. The phenomenon of capillary clotting was also observed in liver tissues a few hundred millimeter close to the liver capsule (Figure 5A1 vs A2). With respect to serum related liver parameters alanine aminotransferase (ALT) and alkaline phosphatase (ALKP), only an ALT increase was noticed on postoperative days 1, 3 and 7. The peak ALT elevation was on day 3 (Figures 5B1, B2).

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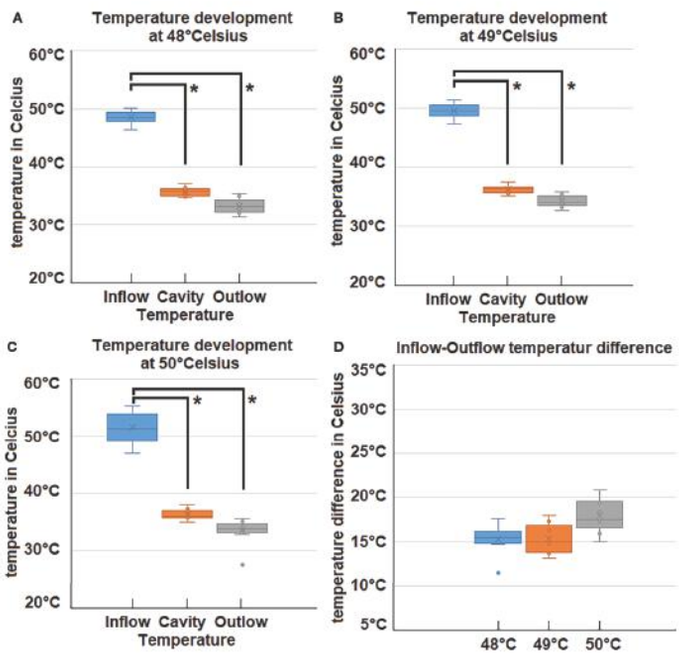

FIGURE 2
(A–C) Measured temperatures during laparoscopy at 48°C, 49°C and 50°C inflow, cavity- and outflow temperatures. **(D)** Difference (Delta) of Inflow and Outlfow temperatures for high-flow hyperthermia at 48°C, 49°C and 50°C. *p < 0.05.

Applied treatment temperature per swine	Temperature difference in Celsius	Joule per liter	Joule per minute (15 liter)	Kilo-Joule per procedure (45 minutes)	Presumed temperature effect (in C°) on 1 Kg body tissue over 45 minutes	Presumed temperature effect (in C°) in the laparoscopic cavity over 45 minutes (1 cm depth)	Presumed temperature effect (in C°) in the laparoscopic cavity over 45 minutes (0,2 cm depth)
	1° C	0,6	9,4	0,42	0,12	0,08	0,41
48°C	15,3°C	9	143	6,4	1,8	1,24	6,19
49°C	15,4°C	9	144	6,5	1,9	1,31	6,53
50°C	17,9°C	11	168	7,6	2,2	1,51	7,56

FIGURE 3
Calculated energy transfer during high-flow gas-based hyperthermia at 48°C, 49°C and 50°C based on delta (inflow and outflow temperature difference) and flow-rate).

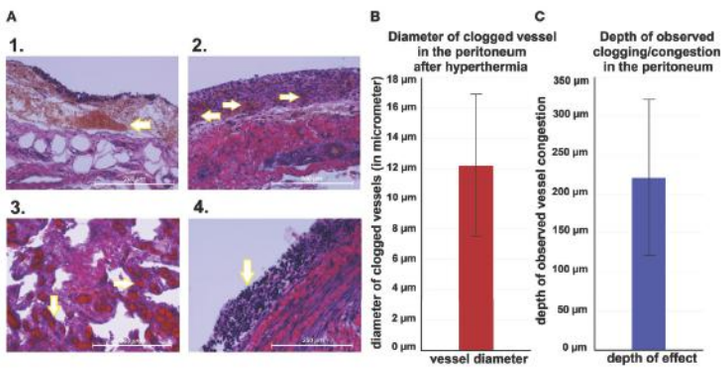

FIGURE 4
(A) Microscopic peritoneal changes following high-flow gas-based hyperthermia. 1. small „hyperthermic" petechia, 2. Micropetechia on the peritoneal surface. 3. Clotting of capillary vessels on the peritoneal surface. 4. Peritoneal white blood cell infiltration. **(B)** Measured diameter of clotted vessels on the peritoneal surface. **(C)** Depth land extent of vessel clotting.

Discussion

Continuous efforts are made to improve the management and therapeutic options for PM patients. These efforts include attempts to apply new physical principles to the already existing pharmacological options to enhance local antitumoral effects (25–29). Some of these concepts include irradiation (30–32), high-intensity ultrasound (33–35), nanoparticles (36) as well as the application of new substances (37, 38). Hyperthermia notably impacts PM management as it demonstrates great efficacy in enhancing antitumoral effects when combined with chemotherapy or radiation, without causing disproportionate additional side effects (39–41). Until now, hyperthermia is usually applied using water-based fluid solutions, which

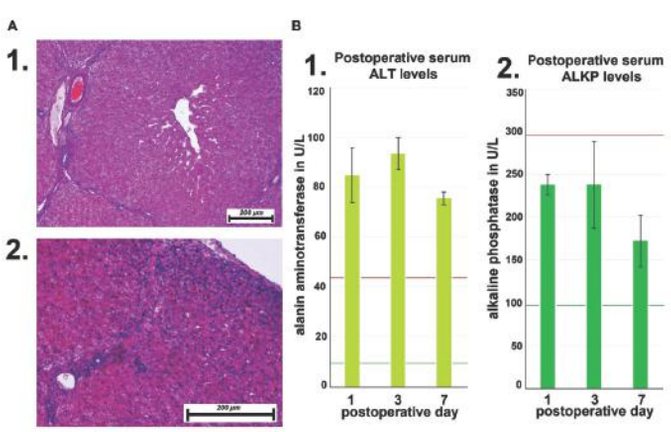

FIGURE 5
(A) Microscopic changes of liver tissue following high-flow gas-based hyperthermia. 1. Deep tissue samples from the liver without pathologies. 2. Vascular clotting in capillaries close to the liver capsule. **(B)** Mean postoperative serum levels of alanin aminotransferase (ALT) at day 1, 3 and 7. Mean postoperative serum levels of alkaline phosphatase (ALT).

display their own set of limitations due to the unique physical properties of water. However, based on the different physical properties of air, new therapeutic constellations might be available. The presented data indicates that the abdominal cavity can be exposed to gas-based hyperthermia exceeding temperatures of 43°C with a defined thermodynamic energy dose. Although we do not observe any relevant general temperature increase within the peritoneal cavity or systemically in the treated patient, we do observe the effect of the applied temperature dose on the upper superficial peritoneal layer.

There are a variety of alternative explanations for this phenomenon. Of course, the most obvious explanation is that the measured intraperitoneal temperatures are as presented and the observed changes on the peritoneum are merely related to dehydration effects of the peritoneum. Another possibility is that the temperature effect is limited to the first few hundred millimeters of the peritoneal layer, thus only affecting the superficial peritoneal layer. In this scenario, the thermodynamic energy may be insufficient to heat up and cause a measurable increase in the heat sensor due to its own heat-capacity. Another explanation is that low air velocity around the intracavitary temperature probes and low thermodynamic air conductivity does not heat up the temperature probes. The temperature sensors at the high-velocity sites at the outflow and inflow are affected in the same way. The last possible explanation is that the thermodynamic energy applied on the peritoneal surface is wasted in chemical processes which change the peritoneal tissue in the area. When we look at alternative gas-mediums and their relationship with the applied regular air in our model, we can estimate the changes in energy transfer assuming the same inflow-outflow temperatures as set values. Based on the available data on the isobaric volumetric heat capacity of the listed gases compared to room air, the heat carrying capacity of the medium at the same volume would increase or decrease approximately in the following manner: CO_2: +28%; N_2: -2%; O_2: +7%, Helium: -24%. To determine to what extent the flow rate influences the temperature difference between the outflow and inflow, further separate studies are required. However, as surface velocity is a factor in the heat transfer from one medium to another, we must assume that this will be a relevant factor. In fact, the contribution of dehydration to the observed effects should be separately analyzed and studied. This requires a separate study on normothermic dehydration on a high-flow laparoscopic model. We believe that with this presented study, we have a strong argument to plan and argue for future and more comprehensive *in-vivo* studies.

However, the extent to which this novel tool is beneficial and or relevant for PM treatment must be further studied. In fact, capillary occlusion of neovascular structures could be a highly relevant mechanism to halt invasive cell growth.

Yet, it has been recognized that there is still no experience and only little understanding of the effects of a hyperthermic capnoperitoneum due to the physical challenges created by air as a carrier medium with an extremely low-heat capacity and unique physical qualities. More basic research must be conducted to improve the understanding and management of gas-based hyperthermia. Further studies are required to investigate whether gas-based i.p. hyperthermia can serve as an independent therapeutic option for PM treatment or if its use as an add-on therapy is more favorable (41–44). Gas-based i.p. hyperthermia with temperatures extending beyond 43°C is feasible and might serve as a new therapeutic option for advanced PM. This novel tool has the potential to create a temperature gradient along the peritoneal surface to apply hyperthermia, stop neo-vascularization and further dehydrate metastatic nodules along the peritoneum (24) to decelerate overall disease progression. However, applicational, biological and technical aspects of this novel approach must be further analyzed.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The experiments were approved (Nr 030/2021/P2) by the local Ethics Committee of Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland as well as the local Board on Animal Welfare.

Author contributions

AD: Study design, laboratory analysis, data acquisition, manuscript drafting. TK: manuscript drafting and critical revision for important intellectual content of the manuscript. AM-M: Study design, laboratory analysis, data acquisition. PK: laboratory analysis, data analyses. JN: Study design, drafting and critical revision for important intellectual content of the manuscript. ZK: laboratory analysis, data acquisition. PP: Study design, laboratory analysis, data acquisition. BL: Study design, laboratory analysis, data acquisition. SL: data acquisition and manuscript drafting. HL: data analyses and intellectual content of the manuscript. WK: manuscript drafting and critical revision for important intellectual content of the manuscript. VK: Supervision on study design, laboratory analyses, conception of the study and manuscript drafting. All

authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Aoyagi T, Terracina KP, Raza A, Takabe K. Current treatment options for colon cancer peritoneal carcinomatosis. *World J Gastroenterol* (2014) 20 (35):12493–500. doi: 10.3748/wjg.v20.i35.12493

2. Glehen O, Kwiatkowski F, Sugarbaker PH, Elias D, Levine EA, De Simone M, et al. Cytoreductive surgery combined with perioperative intraperitoneal chemotherapy for the management of peritoneal carcinomatosis from colorectal cancer: A multi-institutional study. *J Clin Oncol* (2004) 22:3284–92. doi: 10.1200/JCO.2004.10.012

3. Chu DZ, Lang NP, Thompson C, Osteen PK, Westbrook KC. Peritoneal carcinomatosis in nongynecologic malignancy. *A prospective study prognostic factors Cancer* (1989) 63:364–7. doi: 1097-0142(19890115)63:2/1097-0142(19890115)63:2<364::aid-cncr2820630228>3.0.co;2-v

4. Köhne CH, Cunningham D, Di Costanzo F, Glimelius B, Blijham G, Aranda E, et al. Clinical determinants of survival in patients with 5-fluorouracil-based treatment for metastatic colorectal cancer: Results of a multivariate analysis of 3825 patients. *Ann Oncol* (2002) 13:308–17. doi: 10.1093/annonc/mdf034

5. Verwaal VJ, van Ruth S, de Bree E, van Sloothen GW, van Tinteren H, Boot H, et al. Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. *J Clin Oncol* (2003) 21:3737–43. doi: 10.1200/JCO.2003.04.187

6. van Driel WJ, Koole SN, Sikorska K, Schagen van Leeuwen JH, Schreuder HWR, Hermans RHM, et al. Hyperthermic intraperitoneal chemotherapy in ovarian cancer. *N Engl J Med* (2018) 378(3):230–40. doi: 10.1056/NEJMoa1708618

7. Spiliotis J, Halkia E, Lianos E, Kalantzi N, Grivas A, Efstathiou E, et al. Cytoreductive surgery and HIPEC in recurrent epithelial ovarian cancer: A prospective randomized phase III study. *Ann Surg Oncol* (2015) 22(5):1570–5. doi: 10.1245/s10434-014-4157-9

8. Sugarbaker PH. Peritoneal metastases from gastrointestinal cancer. *Review Curr Oncol Rep* (2018) 20(8):62. doi: 10.1007/s11912-018-0703-0

9. Rettenmaier MA, Mendivil AA, Gray CM, Chapman AP, Stone MK, Tinnerman EJ, et al. Intra-abdominal temperature distribution during consolidation hyperthermic intraperitoneal chemotherapy with carboplatin in the treatment of advanced stage ovarian carcinoma. *Int J Hyperthermia* (2015) 31(4):396–402. doi: 10.3109/02656736.2015.1007399

10. Goldenshluger M, Zippel D, Ben-Yaacov A, Dux J, Yalon T, Zendel A, et al. Core body temperature but not intraabdominal pressure predicts postoperative complications following closed-system hyperthermic intraperitoneal chemotherapy (HIPEC) administration. *Ann Surg Oncol* (2018) 25(3):660–6. doi: 10.1245/s10434-017-6279-3

11. Hynynen K, Lulu BA. Hyperthermia in cancer treatment. *Invest Radiol* (1990) 25(7):824–34. doi: 10.1097/00004424-199007000-00014

12. Stewart JR, Gibbs FAJr. Hyperthermia in the treatment of cancer. *Perspect its promise its problems Cancer* (1984) 54(11 Suppl):2823–30. doi: 10.1002/1097-0142(19841201)54

13. Bergs JW, Franken NA, Haveman J, Geijsen ED, Crezee J, van Bree C. Hyperthermia, cisplatin and radiation trimodality treatment: a promising cancer treatment? a review from preclinical studies to clinical application. *Int J Hyperthermia* (2007) 23(4):329 –341. doi: 10.1080/02656730701378684

14. de Andrade Mello P, Bian S, Savio LEB, Zhang H, Zhang J, Junger W, et al. Hyperthermia and associated changes in membrane fluidity potentiate P2X7 activation to promote tumor cell death. *Oncotarget* (2017) 8(40):67254–68. doi: 10.18632/oncotarget.18595

15. Li DY, Tang YP, Zhao LY, Geng CY, Tang JT. Antitumor effect and immune response induced by local hyperthermia in B16 murine melanoma: Effect of thermal dose. *Oncol Lett* (2012) 4(4):711–8. doi: 10.3892/ol.2012.804

16. Zivanovic O, Chi DS, Filippova O, Randall LM, Bristow RE, O'Cearbhaill RE. It's time to warm up to hyperthermic intraperitoneal chemotherapy for patients with ovarian cancer. *Gynecol Oncol* (2018) 151(3):555–61. doi: 10.1016/j.ygyno.2018.09.007

17. Muckle DS, Dickson JA. The selective inhibitory effect of hyperthermia on the metabolism and growth of malignant cells. *Br J Cancer* (1971) 25(4):771–8. doi: 10.1038/bjc.1971.91

18. Seifert G, Budach V, Keilholz U, Wust P, Eggert A, Ghadjar P. Regional hyperthermia combined with chemotherapy in paediatric, adolescent and young adult patients: current and future perspectives. *Radiat Oncol* (2016) 11:65. doi: 10.1186/s13014-016-0639-1

19. Sugarbaker PH. Laboratory and clinical basis for hyperthermia as a component of intracavitary chemotherapy. *Int J Hyperthermia* (2007) 23(5):431–42. doi: 10.1080/02656730701455318

20. Emami B, Song CW. Physiological mechanisms in hyperthermia: a review. *Int J Radiat Oncol Biol Phys* (1984) 10(2):289–95. doi: 10.1016/0360-3016(84)90015-4

21. Eddy HA. Alterations in tumor microvasculature during hyperthermia. *Radiology* (1980) 137(2):515–21. doi: 10.1148/radiology.137.2.7433685

22. Kang MS, Song CW, Levitt SH. Role of vascular function in response of tumors *in vivo* to hyperthermia. *Cancer Res* (1980) 40(4):1130–5.

23. Diakun A, Khosrawipour T, Mikolajczyk-Martinez A, Nicpoń J, Kiełbowicz Z, Prządka P, et al. The onset of *in-vivo* dehydration in gas -based Intraperitoneal Hyperthermia and Its Cytotoxic Effects on Colon Cancer Cells. *Front Oncol*(2022) 12:927714. doi: 10.3389/fonc.2022.927714

24. Zieliński KW. Postępowanie doktorskie zakończone. rada dyscypliny nauki medyczne. Biuletyn informacji publicznej uniwersytetu medycznego we wrocławiu. Available at: https://bip.umw.edu.pl/artykul/222/4000/kacper-wojciech-zielinski-postepowanie-doktorskie-zakonczone. (Accessed 2022-07-04)

25. Khosrawipour T, Schubert J, Kulas J, Pawel Migdal P, Arafkas M, Bania J, et al. Creating nanocrystallized chemotherapy: The differences in pressurized aerosol chemotherapy (PAC) *via* intracavitary (IAG) and extracavitary aerosol

generation (EAG) regarding particle generation, morphology and structure. *J Cancer* (2020) 11(6):1308–14. doi: 10.7150/jca.39097

26. Khosrawipour V, Reinhard S, Martino A, Khosrawipour T, Arafkas M, Mikolajczyk A. Increased tissue penetration of doxorubicin in pressurized intraperitoneal aerosol chemotherapy (PIPAC) after high-intensity ultrasound (HIUS). *Int J Surg Oncol* (2019) 2019:6185313. doi: 10.1155/2019/6185313

27. Schubert J, Khosrawipour T, Pigazzi A, Kulas J, Bania J, Migdal P, et al. Evaluation of cell-detaching effect of EDTA in combination with oxaliplatin for a possible application in HIPEC after cytoreductive surgery: A preliminary *in-vitro* study. *Curr Pharm Des* (2019) 25(45):4813–9. doi: 10.2174/1381612825666191106153623

28. Mikolajczyk A, Khosrawipour V, Schubert J, Plociennik M, Nowak K, Fahr C, et al. Feasibility and characteristics of pressurized aerosol chemotherapy (PAC) in the bladder as a therapeutical option in early-stage urinary bladder cancer. *In Vivo* (2018) 32(6):1369–72. doi: 10.21873/invivo.11388

29. Khosrawipour V, Mikolajczyk A, Paslawski R, Plociennik M, Nowak K, Kulas J, et al. Intrathoracic aerosol chemotherapy *via* spray-catheter. *Mole Clin Oncol* (2020) 14(4):350–4. doi: 10.3892/mco.2020.1999

30. Khosrawipour V, Bellendorf A, Khosrawipour C, Hedayat-Pour Y, Diaz-Carballo D, Förster E, et al. Irradiation does not increase the penetration depth of doxorubicin in normal tissue after pressurized intra-peritoneal aerosol chemotherapy (PIPAC) in an *ex vivo* model. *In Vivo* (2016) 30(5):593–7.

31. Khosrawipour V, Giger-Pabst U, Khosrawipour T, Pour YH, Diaz-Carballo D, Förster E, et al. Effect of irradiation on tissue penetration depth of doxorubicin after pressurized intra-peritoneal aerosol chemotherapy (PIPAC) in a novel *ex-vivo* model. *J Cancer* (2016) 7(8):910–4. doi: 10.7150/jca.14714

32. Khosrawipour V, Khosrawipour T, Hedayat-Pour Y, Diaz-Carballo D, Bellendorf A, Böse-Ribeiro H, et al. Effect of whole-abdominal irradiation on penetration depth of doxorubicin in normal tissue after pressurized intraperitoneal aerosol chemotherapy (PIPAC) in a post-mortem swine model. *Anticancer Res* (2017) 37(4):1677–80. doi: 10.21873/anticanres.11498

33. Mikolajczyk A, Khosrawipour V, Schubert J, Grzesiak J, Chaudhry H, Pigazzi A, et al. Effect of liposomal doxorubicin in pressurized intra-peritoneal aerosol chemotherapy (PIPAC). *J Cancer* (2018) 9(23):4301–5. doi: 10.7150/jca.26860

34. Mikolajczyk A, Khosrawipour T, Kulas J, Migdal P, Arafkas M, Nicpon J, et al. The structural effect of high intensity ultrasound on peritoneal tissue: a potential vehicle for targeting peritoneal metastases. *BMC Cancer* (2020) 20(1):481. doi: 10.1186/s12885-020-06981-4

35. Mikolajczyk A, Khosrawipour T, Martino A, Kulas J, Pieczka M, Zacharski M, et al. Enabling microparticle imprinting to achieve penetration and local endurance in the peritoneum *via* high-intensity ultrasound (HIUS) for the treatment of peritoneal metastasis. *Int J Surg Oncol* (2020) 2020:9679385. doi: 10.1155/2020/9679385

36. Mikolajczyk A, Khosrawipour V, Kulas J, Kocielek K, Migdal P, Arafkas M, et al. Release of doxorubicin from its liposomal coating *via* high intensity ultrasound. *Mol Clin Oncol* (2019) 11(5):483–7. doi: 10.3892/mco.2019.1917

37. Mikolajczyk A, Khosrawipour V, Lau H, Li S, Migdal P, Labbe MK, et al. Exploring the potential of taurolidine in inducing mobilisation and detachment of colon cancer cells: A preliminary *in-vitro* study. *BMC Pharmacol Toxicol* (2022) 23 (1):38. doi: 10.1186/s40360-022-00572-8

38. Schubert J, Khosrawipour V, Chaudhry H, Arafkas M, Knoefel WT, Pigazzi A, et al. Comparing the cytotoxicity of taurolidine, mitomycin c, and oxaliplatin on the proliferation of *in vitro* colon carcinoma cells following pressurized intra-peritoneal aerosol chemotherapy (PIPAC). *World J Surg Oncol* (2019) 17(1):93. doi: 10.1186/s12957-019-1633-5

39. Wust P, Hildebrandt B, Sreenivasa G, Rau B, Gellermann J, Riess H, et al. Hyperthermia in combined treatment of cancer. *Lancet Oncol* (2002) 3:487–97. doi: 10.1016/S1470-2045(02)00818-5

40. Cihoric N, Tsikkinis A, van Rhoon G, Crezee H, Aebersold DM, Bodis S, et al. Hyperthermia-related clinical trials on cancer treatment within the ClinicalTrials.gov registry. *Int J Hyperthermia* (2015) 31:609–14. doi: 10.3109/02656736.2015.1040471

41. Kok HP, Wust P, Stauffer PR, Bardati F, van Rhoon GC, Crezee J. Current state of the art of regional hyperthermia treatment planning: a review. *Radiat Oncol* (2015) 10:196. doi: 10.1186/s13014-015-0503-8

42. Khosrawipour T, Schubert J, Khosrawipour V, Chaudhry H, Grzesiak J, Arafkas M, et al. Particle stability and structure on the peritoneal surface in pressurized intra-peritoneal aerosol chemotherapy (PIPAC) analysed by electron microscopy: First evidence of a new physical concept for PIPAC. *Oncol Lett* (2019) 17(6):4921–7. doi: 10.3892/ol.2019.10162

43. Schubert J, Khosrawipour T, Reinhard S, Arafkas M, Martino A, Bania J, et al. The concept of foam as a drug carrier for intraperitoneal chemotherapy, feasibility, cytotoxicity and characteristics. *Sci Rep* (2020) 10(1):10341. doi: 10.1038/s41598-020-67236-7

44. Mikolajczyk A, Khosrawipour V, Schubert J, Chaudhry H, Pigazzi A, Khosrawipour T. Particle stability during pressurized intra-peritoneal aerosol chemotherapy (PIPAC). *Anticancer Res* (2018) 38(8):4645–9. doi: 10.21873/anticanres.12769

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The Onset of In-Vivo Dehydration in Gas -Based Intraperitoneal Hyperthermia and Its Cytotoxic Effects on Colon Cancer Cells

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Background: Peritoneal metastasis (PM) is an ongoing challenge in surgical oncology. Current therapeutic options, including intravenous and intraperitoneal (i.p.) chemotherapies display limited clinical efficacy, resulting in an overall poor prognosis in affected patients. Combined hyperthermia and dehydration induced by a high-flow, gas-based i.p. hyperthermic procedure could be a novel approach in PM treatment. Our study is the first to evaluate the therapeutic potential of i.p. dehydration, hyperthermia, as well as the combination of both mechanisms in an *in-vivo* setting.

Methods: For this study, three swine were subjected to diagnostic laparoscopy under a high-flow air stream at 48°, 49° and 50°Celsius (C). Hygrometry of the in- and outflow airstream was measured to calculate surface evaporation and i.p. dehydration. To analyze the effects of this concept, *in vitro* colon cancer cells (HT-29) were treated with hyperthermia and dehydration. Cytotoxicity and cell viability were measured at different time intervals. Additionally, structural changes of dehydrated cells were analyzed using scanning electron microscopy.

Results: According to our results, both dehydration and hyperthermia were cytotoxic to HT-29 cells. However, while dehydration reduced cell viability, hyperthermia did not. However, dehydration effects on cell viability were significantly increased when combined with hyperthermia ($p<0.01$).

Conclusions: Changes to the physiological milieu of the peritoneal cavity could significantly reduce PM. Therefore, limited dehydration of the abdominal cavity might be a feasible, additional tool in PM treatment. Further studies are required to investigate dehydration effects and their applicability in PM management.

Keywords: dehydration, colorectal cancer, peritoneal metastasis (PM), hyperthermia, electron microscopy

INTRODUCTION

While peritoneal metastasis (PM) is a common manifestation of advanced gastrointestinal and gynecological cancers, it remains a challenge to surgical oncology with an overall poor prognosis. Statistically, affected patients do not survive the first year after diagnosis due to rapid tumor progression in the abdominal cavity and subsequent complications (1, 2). In recent years, various attempts have been made to develop new strategies for PM management. Despite these efforts, there have not been any significant scientific developments that ensure long-term clinical improvement.

In fact, one of the main shortcomings observed with current therapies include limited therapeutic effects observed with systemic chemotherapy. This can be attributed to systemic drug loss and subtherapeutic drug concentrations in the peritoneum (3, 4). As a result, locoregional concepts e.g. intraperitoneal (i.p.) chemotherapy have been introduced to further improve local drug availability. However, later studies uncovered that i.p. chemotherapies also display limited clinical efficacy (5). Currently, the combination of cytoreductive surgery (CRS) with fluid based hyperthermic intraperitoneal chemotherapy (HIPEC), offers hope for a curative outcome in patients with limited disease. CRS and HIPEC is gaining more popularity in the field of surgery, as clinical trials describe advantageous results in highly selective patients (6, 7). Despite this encouraging data, it is important to note that only patients suffering from limited diseases and without visible peritoneal seeding following CRS are considered to benefit from this therapeutic option (8, 9). For patients with extensive PM who do not qualify for CRS and HIPEC, the option of pressurized intraperitoneal aerosol chemotherapy (PIPAC) has been introduced to the clinical setting (10, 11). In PIPAC, a fluid chemosolution is aerosolized and injected into the abdominal cavity covering the abdominal compartment. In fact, PIPAC has shown some positive results (12, 13) and several attempts have been made to improve (14, 15) and extend (16) its application. Nevertheless, the use of PIPAC in the clinical setting does not seem to fundamentally change patient outcome.

Many patients without distant metastasis often display the clinical picture of metastatic seeding in the peritoneal cavity. PM cells are often either confined to the peritoneum or minimally infiltrate local and peritoneal tissues (17, 18). It is suggested that peritoneal seeding is enhanced due to facilitated movement of floating cancer cells within the peritoneum (19, 20). The presence of disseminated cancer cells has been associated with a higher recurrence rate and a poorer survival (21). Using the peritoneal milieu as a favorable habitat for growth, adherent cancer cells can further infiltrate and permeate into tissues (22, 23). Additionally, the glucose and protein-rich cavitary fluid can further enhance this effect. In fact, the abdominal cavity offers optimal growth potential for local malignant tumor cells. Consequently, we assume that changing the biology of this compartment could lead the way to optimized therapies. Thus, it is necessary to investigate whether changing the biological properties of the peritoneum could be a feasible option to delay or even halt PM progression.

The application of dehydration combined with hyperthermia could be an innovative approach to alter the biological properties of this compartment. Malignant tumor cell progression could be terminated in a less hospitable environment. Our study is in strong contrast to previous attempts, which all included the use of chemotherapeutic applications. By means of this study, we aim to calculate the extent of evaporation and dehydration that may occur during laparoscopy. Finally, using an *in-vivo* swine model, we examine whether dehydration can be achieved by the proposed laparoscopic system as well as analyze the sensitivity of colon cancer cells to hyperthermia and dehydration at different levels of exposure.

MATERIAL AND METHODS

In-Vivo Swine Model

For the *in-vivo* part of this study, three 65-day-old swine (Polish white flod) were used. The swine received diagnostic laparoscopy without any additionally surgical intervention. Laparoscopy was performed under a high-flow air stream of 15 liters per minute (l/min). The swine used were part of a larger study on hyperthermia and dehydration. The inflowing air temperature for the swine were 48°, 49 and 50°Celsius (C), respectively. Intracavitary temperatures were monitored to detect the onset of critical intraabdominal heating. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals as published by the National Institutes of Health. The swine were premedicated with an intramuscular injection of midazolam (0.3 mg/kg, WZF Polfa S.A., Poland), medetomidine (0.02 mg/kg, Cepetor 1 mg/ml, CP-Pharma Handelsgesellschaft, Germany) and ketamine (9 mg/kg, Ketamina 100 mg/ml, Biowet Puławy sp. z o.o., Poland) mixture. Analgesia was performed with Propofol at 1mg/kg. Swine were intubated and further anesthesia was continued with isoflurane 1%. Additional analgesia was provided with fentanyl 2µg/kg and crystalloid fluid at 0.2 - 0.3 µg/kg/min. The swine were placed in a supine position. An infra-umbilical mini laparotomy was performed and another one at about 8 cm

distance to the first one. A 10 mm trocar (Kii®Balloon Blunt Tip System, Applied Medical, Rancho Santa Margarita, CA, USA) was inserted through the infra-umbilical trocar while multiple 5 mm trocars were placed at the other sites (**Figure 1A**) after insufflation. The abdominal cavity was insufflated with filtered room air *via* a tube entering the central 10 mm trocar. An initial diagnostic check-up was conducted *via* laparoscopic imaging using a 5 mm camera system (Karl Storz 5mm/30° Laparoscope/ Tuttlingen, Germany) through a 5 mm trocar. After visual confirmation and placement of multiple temperature sensors (**Figure 1B**), the high-flow air stream was turned on at 15 l/min for a total of 45 minutes. Humidity levels at the in- and outflow was measured.

Creating an Operative Model for Laparoscopic Dehydration of the Abdominal Cavity

The operative model for dehydration assumes a 4 Liter ($V_B = 4$ L) abdominal capnoperitoneum, which corresponds to an average sized capnoperitoneum observed during laparoscopy. Humidity levels of in- and outflowing air were measured. Based on the mean humidity difference, the amount of removed water from the abdominal cavity was calculated. The saturation pressure of room air is 7297 Pascal and the maximum density of water vapor at 40°C is 0.051 kg/m³. At a constant flow of 15 l/min, a model for a time dependent removal rate was calculated.

Cell Cultures

The human colorectal cancer cell lines HT-29 were purchased from the CLS (Cell Lines Service GmbH, Eppelheim, Germany). Cells were then grown in Dulbecco's modified Eagle's medium (DMEM - high glucose, Sigma-Aldrich, Poznan, Poland). The following supplements were added to the medium: 10% heat-inactivated fetal bovine serum (FBS, Gibco, Thermo Fisher Scientific, Poland), 2 mmol/L glutamine, 100 IU/mL penicillin, and 100 µg/mL streptomycin (Sigma-Aldrich). Cells were then incubated in a humidified 5% CO_2 incubator (NuAire CO_2 Incubator, Biogenet, Warszawa, Poland) at 37°C. Afterwards, 1.4 x 10^5 cells per well were seeded in 24-well plates (TC Plate 24 Well, Standard, F, Sarstedt AG & Co. KG, Germany) and incubated for 48 hours. Cells subjected to electron microscopy (EM) analysis were seeded on sterile glasscovers.

In Vitro Cell Dehydration

Cells were divided into three main groups and exposed to 37°, 45° and 48°C for 30 minutes. Each group was separately exposed to different durations of dehydration (0, 5, 10, 20, 25 and 30 minutes) (at 85% percent humidity) in the cell incubator. After 0 and 20 minutes of dehydration, cells that were seeded on sterile glasscovers were subjected to cellular analyses using scanning electron microscopy (SEM).

Cytotoxicity Assay

To obtain maximum LDH activity, cell lysis reagent was added into each well following exposure to hyperthermia and dehydration and incubated at 37°C for 45 minutes. Spontaneous LDH activity was assessed for each group. Wells were incubated in darkness and at room temperature for 30 minutes, then a stop reagent solution was added and absorbance was measured at 490nm on a microplate reader (Tecan, Basel, Switzerland). Percentage of cytotoxicity was calculated using the following formula:

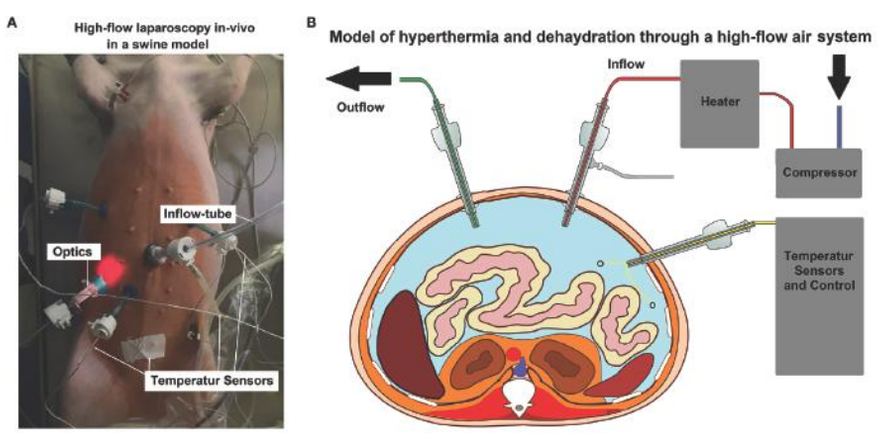

FIGURE 1 | **(A)** *In-vivo* high-flow laparoscopy model to evaluate dehydration and hyperthermic airflow effects. **(B)** Model of the laparoscopic setting. Two trocars are used as in- and outflow while other trocars are placed for optics and temperature sensors.

% cytotoxicity = (compound-treated LDH activity – spontaneous LDH activity) ÷ (Maximum LDH activity – Spontaneous LDH activity) x 100%.

MTS-*Testing* on *Cell Viability*

Cell proliferation was determined *via* colorimetric CellTiter 96® AQueous One Solution assay (Promega, Poland) 24 hours after hyperthermia and dehydration exposure. The test was performed with our modifications according to the manufacturer's instructions. Briefly, medium was removed from each well and replaced by 0.3 mL of fresh DMEM. Next, following one hour of incubation at 37°C at 5% CO_2, an MTS-based reagent was added to each well and absorbance at 490nm was measured using a microplate reader (Tecan, Basel, Switzerland). The percentage of proliferation was regulated for all groups using untreated cells as control.

Electron Microscopy to Determine Cell Structure and Single Cell Dehydration

The HT-29 control groups and dehydrated cells (20min) were subjected to cryogenic scanning electron microscopy (cryoSEM) analysis. The probes were washed with Dulbecco's phosphate buffered saline (DPBS, Sigma-Aldrich) and fixated in 2.5% glutaraldehyde solution (Sigma-Aldrich). After fixing the samples, they were washed in PBS, rinsed in ultrapure (sterilized through 0.1µm filter) deionized water, and finally mounted on cryo-shuttle using OT/colloid graphite mixture and plunged in liquid nitrogen. Then, frozen specimen were quickly transferred to a cryo-preparation chamber (Cryo Quorum PP3010T) and sputtered with a conductive layer of platinum at 140°C. Next, specimen were transferred to the microscopy chamber. At each point of transfer, the same temperature of -140°C (Auriga60, Zeiss) was maintained. Samples were ultimately observed at 2kV of acceleration voltage using In Lens and SE2 secondary electron detector.

Graphic *D*esign

For the graphics provided, multiple graphic programs were used. These programs included Inkscape 1.0.1,2020, GNU, USA and programs provided by Windows office 2019, Microsoft.

Statistical Analysis

All experiments were executed at least three times. Each well was considered as a single value, according to the classified subgroups. The student t-test was used to compare independent groups. Probability (p) values were considered as follows: *=$p<0.05$, **=$p<0.01$, and #=$p>0.05$, with p-value <0.05 considered to be statistically significant. Data are presented as the mean standard deviation unless otherwise indicated.

RESULTS

Extent of Laparoscopic Intraabdominal Dehydration and Transport Capacity

Under the presented constellation at 15 l/min flow, outflow humidity levels reached > 95% within the first 5 minutes and stabilized at around 99%. The mean inflow humidity level was 51%. The intraperitoneal temperature remained stable and did not exceed 40°C. Macroscopically visible dehydration set in after 25, 28 and 33 minutes within the procedure (**Figures 2A, B**). The calculation of the maximum transport capacity uncovered that under the presented constellation of high flow *via* the laparoscopic approach, a mean amount of 0.382 grams per minute of water was removed from the peritoneum (**Figure 3A**). This approximated to a total of 17.2 grams of water in 45 minutes (**Figure 3B**). Thus, dehydration set in after the removal of circa 10.7 ± 1.6 g of water from the abdominal cavity.

In-Vitro Cytotoxicity of Dehydration and Hyperthermia

Cytotoxicity levels were significantly increased following hyperthermia at 45°C ($p<0.05$) and 48°C ($p<0.001$) compared to controls at 37°C (**Figure 4A**). Moreover, the combination of hyperthermia and dehydration showed similarly high cytotoxicity levels (**Figure 4B**). After 10 minutes of dehydration, cytotoxicity levels were high for all temperature groups and remained at this elevated level. However, in cells kept at 37°C and only exposed to dehydration, cytotoxicity increased to elevated levels after 10 minutes (**Figure 4C**).

Viability of HT-29 Cells Following Dehydration and Hyperthermia

The viability of HT-29 cells was not affected by hyperthermia at 45°C and 48°C compared to controls at 37°C (**Figure 5A**). However, the combination of hyperthermia at 48°C and dehydration of 30 minutes resulted in significantly decreased viability levels ($p<0.01$) (**Figure 5B**). Dehydration of cells at 37°C resulted in declined viability with time (0 - 30 minutes). However, this effect seemed to be further enhanced when hyperthermia was added at 45°C and 48°C (**Figure 5C**).

Electron Microscopic Analysis on Dehydrated HT-29 Cells

Cryo-SEM was used to study cell morphology following partial dehydration. Structural analyses of the extracellular cell surface revealed morphological changes which progressed with increased extent of dehydration. Control cells were more distinguishable and separable from each other. Some had a round shape and podocytes were visible for most cells (**Figure 6A**). Their dehydrated counterparts had blurred margins, and the cells appeared hollow and flattened (**Figure 6B**). Cell boundaries were more difficult to identify. Podocyte structures of cells had become visually unidentifiable. EM analyses concluded that dehydration significantly affected the structural integrity of HT-29 cells.

DISCUSSION

The concept of a therapeutic pneumoperitoneum was inspired by Italian physician Carlo Forlanini (1847 - 1918), whose pioneering innovations helped to manage extensive pulmonary tuberculosis following decades of stagnating therapeutic options.

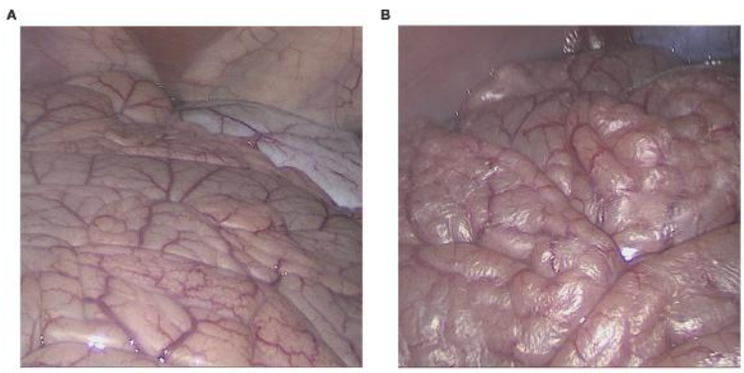

FIGURE 2 | Laparoscopic view of the intraabdominal cavity during high-flow air-based hyperthermia. **(A)** Start of the laparoscopy. The figure displays normal small intestinal tissue as is expected during laparoscopy. **(B)** Laparoscopic view of the intraabdominal cavity after 30 minutes into the procedure. Peritoneal dehydration "drying" is visible in the exposed area. The peritoneal surface appears to "peel-off" and light reflection on the peritoneal surface increases.

In the early 20th century, he developed the idea of an artificial pneumothorax for patients suffering from pulmonary tuberculosis. This innovative concept benefited severely ill patients, and Forlanini was later nominated for the Nobel prize for medicine for his discovery (24). Based on the consideration that biophysical changes of the thorax and a less hospitable environment for tuberculosis bacteria might have been the reason for the success of this unconventional approach, we theorize that our model of cell dehydration could have similar promising effects on PM. In patients with cancer diseases, the metastatic process in the peritoneal cavity is a critical factor for survival (25). Using physical principles in innovative treatments of PM and other surface malignancies seems promising and has been attempted several times (26–29). Some of these novel treatment options have been extensively tested, including the application of irradiation (30–32), high-intensity ultrasound (33, 34) and nanoparticles (16). The current management of PM displays significant shortcomings due to the aggressive growth behavior displayed by PM, which further limits effects of i.p. chemotherapy even when locally applied during HIPEC or PIPAC.

Cancer cells within the abdominal cavity usually originate from either gastrointestinal or gynecological cells. Therefore, they are habituated to a fluid-surrounding environment. This aspect remains unchanged, regardless of mutations in the cancer

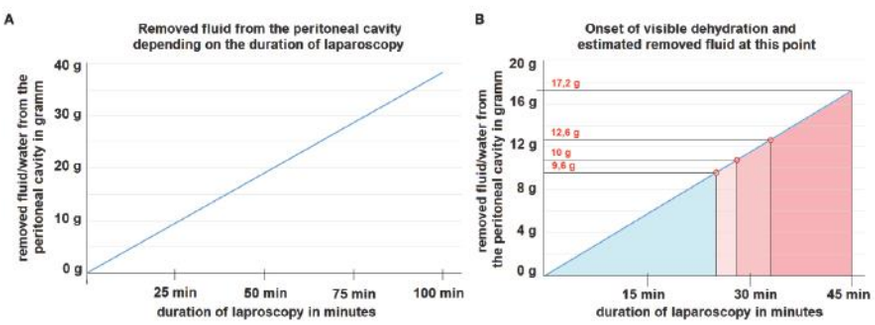

FIGURE 3 | **(A)** Mathematical model for the removal of fluid/water from the abdominal cavity at 15 liter/minute flow rate assuming an inflow air humidity of 50%. The correlation is linear. **(B)** Mathematical model of dehydration at 15 liter/minute flow rate assuming an inflow air humidity of 50%. At the marked red points, dehydration of tissue is visible during laparoscopy. The estimated amount of removed water per time is indicated at each time point.

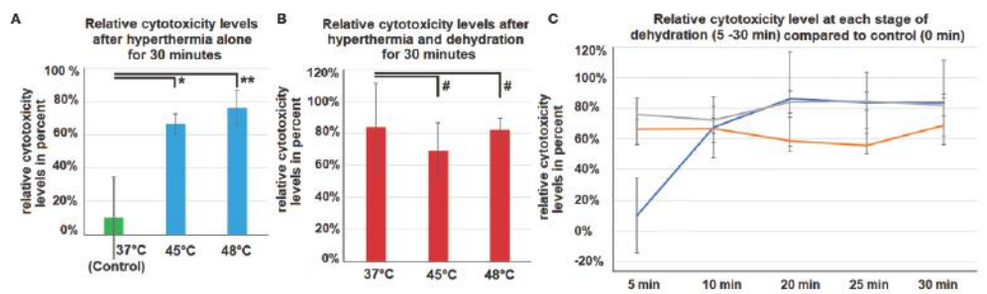

FIGURE 4 | **(A)** *In vitro* cytotoxicity on colon cancer cells after 30 minutes of hyperthermia exposure (without dehydration) at 45°C and 48°C. Control cells were only exposed to the physiological temperature of 37°C. **(B)** *In vitro* cytotoxicity on colon cancer cells after 30 minutes of hyperthermia exposure and dehydration at 37°, 45°C and 48°C. **(C)** Cytotoxicity compared to control cells. Levels of cytotoxicity were measured at different exposure times to dehydration and hyperthermia (5, 10, 20, 25 and 30 minutes). Significance levels: #=p>0.05, *=p<0.05, **= p<0.001.

cell genome (35). Consequently, changing the basic biology of the abdominal cavity might interfere with any cancerous progression in that compartment. Dehydrating the internal cavity accounts for one of these basic biological changes in the human body. With this study, we were able to demonstrate that HT-29 cells subjected to dehydration show reduced viability rates, especially when combined with hyperthermia. Our model has thoroughly demonstrated that dehydration can be implemented in a laparoscopic model established within a reasonable timeframe of less than 45 minutes. With our work, we have outlined a basic conceptual comprehension of the technical and physical aspects as well as applicational limitations of dehydration. This approach needs to be further investigated to evaluate the potential of i.p. dehydration in the management of advanced PM. Dehydration with hyperthermia as a combined application or possibly in addition to chemotherapy, could be a realistic tool for changing the i.p. environment to slow down or even terminate PM progression. Significant reduction of HT-29 cell viability and significantly increased cytotoxicity has been observed following dehydration combined with hyperthermia. Applicational, biological and technical features of dehydration within the peritoneal cavity have been demonstrated and must be further examined and documented. While this new concept operates without any pharmacological agents, it can possibly be combined with some of the current concepts of i.p. chemotherapy (36, 37).

With regards to limitations in this study, we must point out that PM can originate from different cancer entities, including a variety of gastrointestinal or gynecological tumors, and thus may show differing reactions to dehydration. Furthermore, there is still a

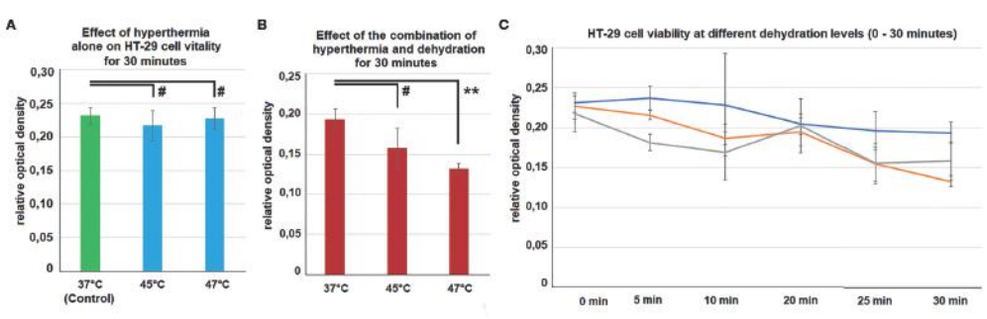

FIGURE 5 | **(A)** *In vitro* cell-viability of colon cancer cells after 30 minutes of hyperthermia (without dehydration) at 45°C and 48°C. Control cells were only exposed to the physiological temperature of 37°C. **(B)** *In vitro* cell-viability of colon cancer cells after 30 minutes of hyperthermia and dehydration at 37°, 45°C and 48°C. **(C)** Course of cell viability following different exposure times of dehydration and hyperthermia 0 (Control), 5, 10, 20, 25 and 30 minutes. Significance levels: #=p>0.05,**= p<0.01.

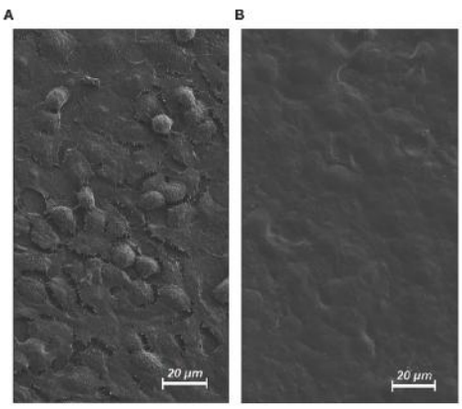

FIGURE 6 | Electron microscopy (EM) of HT-29 cells. **(A)** Untreated HT-29 Cells in EM (control/magnification level 1000X). **(B)** HT-29 Cells in EM following dehydration and hyperthermia for 20 minutes (magnification level 1000X).

limited understanding and knowledge on potential local and systemic effects of this novel concept. Also, the potential hemodynamic effects of dehydration and hyperthermia must be further evaluated. In the future, clinical studies in selected and qualified research centers are required to further assess this concept and allow for a more in-depth evaluation of its applicability.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Wroclaw university of life and environmental sciences.

AUTHOR CONTRIBUTIONS

AD: Study design, laboratory analysis, data acquisition, editing. TK: Study design, conception of the study and manuscript drafting. AM-M: Study design, laboratory analysis, data acquisition. JN: Study design, laboratory analysis, data acquisition. ZK: Manuscript drafting and critical revision for important intellectual content of the manuscript. PP: laboratory analysis, data acquisition. BL: Study design, laboratory analysis. WK: Drafting and critical revision for important intellectual content of the manuscript. KZ: laboratory analysis, data acquisition, PM: laboratory analysis, data acquisition. HL: Drafting and critical revision for important intellectual content of the manuscript. SL: Drafting and critical revision for important intellectual content of the manuscript. VK: Supervision on study design, laboratory analyses, conception of the study and manuscript drafting. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Paul Olson TJ, Pinkerton C, Brasel KJ, Schwarze ML. Palliative Surgery for Malignant Bowel Obstruction From Carcinomatosis: A Systematic Review. *JAMA Surg* (2014) 149(4):383–92. doi: 10.1001/jamasurg.2013.4059
2. Heaney RM, Shields C, Mulsow J. Outcome Following Incomplete Surgical Cytoreduction Combined With Intraperitoneal Chemotherapy for Colorectal Peritoneal Metastases. *World J Gastrointest Oncol* (2015) 7(12):445–54. doi: 10.4251/wjgo.v7.i12.445
3. Walker JL, Brady MF, Wenzel L, Fleming GF, Huang HQ, DiSilvestro PA, et al. Randomized Trial of Intravenous Versus Intraperitoneal Chemotherapy Plus Bevacizumab in Advanced Ovarian Carcinoma: An NRG Oncology/Gynecologic Oncology Group Study. *J Clin Oncol* (2019) 37(16):1380–90. doi: 10.1200/JCO.18.01568

4. Verwaal VJ, van Ruth S, de Bree E, van Sloothen GW, van Tinteren H, Boot H, et al. Randomized Trial of Cytoreduction and Hyperthermic Intraperitoneal Chemotherapy Versus Systemic Chemotherapy and Palliative Surgery in Patients With Peritoneal Carcinomatosis of Colorectal Cancer. *J Clin Oncol* (2003) 21(20):3737–43. doi: 10.1200/JCO.2003.04.187
5. Oseledchyk A, Zivanovic O. Intraoperative Hyperthermic Intraperitoneal Chemotherapy in Patients With Advanced Ovarian Cancer. *Oncol (Williston Park)* (2015) 29(9):695–701.
6. Rosa F, Galiandro F, Ricci R, Di Miceli D, Quero G, Fiorillo C, et al. Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy (HIPEC) for Colorectal Peritoneal Metastases: Analysis of Short- and Long-Term Outcomes. *Langenbecks Arch Surg* (2021) 406(8):2797–805. doi: 10.1007/s00423-021-02353-z
7. Chen WC, Huang HJ, Yang LY, Pan YB, Huang KG, Lin CT, et al. Hyperthermic Intraperitoneal Chemotherapy for Recurrent Epithelial Ovarian Cancer. *BioMed J* (2021) 137(21):2319–4170. doi: 10.1016/j.bj.2021.10.003
8. Parikh MS, Johnson P, Romanes JP, Freitag HE, Spring ME, Garcia-Henriquez N, et al. Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy for Colorectal Peritoneal Metastases: A Systematic Review. *Dis Colon Rectum* (2022) 65(1):16–26. doi: 10.1097/DCR.0000000000002315
9. Carboni F, Federici O, Zazza S, Corona F, Massimi F, Sperduti I, et al. Cytoreductive Surgery With Hyperthermic Intraperitoneal Chemotherapy for Peritoneal Metastasis of non-Primary Origin. *Langenbecks Arch Surg* (2021) 406(8):2817–25. doi: 10.1007/s00423-021-02354-y
10. Khosrawipour T, Schubert J, Khosrawipour V, Chaudhry H, Grzesiak J, Arafkas M, et al. Particle Stability and Structure on the Peritoneal Surface in Pressurized Intra-Peritoneal Aerosol Chemotherapy (PIPAC) Analysed by Electron Microscopy: First Evidence of a New Physical Concept for PIPAC. *Oncol Lett* (2019) 17(6):4921–7. doi: 10.3892/ol.2019.10162
11. Mikolajczyk A, Khosrawipour V, Schubert J, Chaudhry H, Pigazzi A, Khosrawipour T. Particle Stability During Pressurized Intra-Peritoneal Aerosol Chemotherapy (PIPAC). *Anticancer Res* (2018) 38(8):4645–9. doi: 10.21873/anticanres.12769
12. Khosrawipour T, Khosrawipour V, Giger-Pabst U. Pressurized Intra Peritoneal Aerosol Chemotherapy in Patients Suffering From Peritoneal Carcinomatosis of Pancreatic Adenocarcinoma. *PLoS One* (2017) 12(10):e0186709. doi: 10.1371/journal.pone.0186709
13. Schubert J, Khosrawipour V, Chaudhry H, Arafkas M, Knoefel WT, Pigazzi A, et al. Comparing the Cytotoxicity of Taurolidine, Mitomycin C, and Oxaliplatin on the Proliferation of *In Vitro* Colon Carcinoma Cells Following Pressurized Intra-Peritoneal Aerosol Chemotherapy (PIPAC). *World J Surg Oncol* (2019) 17(1):93. doi: 10.1186/s12957-019-1633-5
14. Khosrawipour V, Reinhard S, Martino A, Khosrawipour T, Arafkas M, Mikolajczyk A. Increased Tissue Penetration of Doxorubicin in Pressurized Intraperitoneal Aerosol Chemotherapy (PIPAC) After High-Intensity Ultrasound (HIUS). *Int J Surg Oncol* (2019) 2019:6185313. doi: 10.1155/2019/6185313
15. Mikolajczyk A, Khosrawipour V, Kulas J, Kocielek K, Migdal P, Arafkas M, et al. Release of Doxorubicin From its Liposomal Coating *via* High Intensity Ultrasound. *Mol Clin Oncol* (2019) 11(5):483–7. doi: 10.3892/mco.2019.1917
16. Khosrawipour T, Schubert J, Kulas J, Migdal P, Arafkas M, Bania J, et al. Creating Nanocrystallized Chemotherapy: The Differences in Pressurized Aerosol Chemotherapy (PAC) *via* Intracavitary (IAG) and Extracavitary Aerosol Generation (EAG) Regarding Particle Generation, Morphology and Structure. *J Cancer* (2020) 11(6):1308–14. doi: 10.7150/jca.39097
17. van Baal JOAM, van Noorden CJF, Nieuwland R, Van de Vijver KK, Sturk A, van Driel WJ, et al. Development of Peritoneal Carcinomatosis in Epithelial Ovarian Cancer: A Review. *J Histochem Cytochem* (2018) 66(2):67–83. doi: 10.1369/0022155417742897
18. Lemoine L, Sugarbaker P, van der Speeten K. Pathophysiology of Colorectal Peritoneal Carcinomatosis: Role of the Peritoneum. *World J Gastroenterol* (2016) 22(34):7692–707. doi: 10.3748/wjg.v22.i34.7692
19. Kristensen VN, Lingjærde OC, Russnes HG, Vollan HK, Frigessi A, Børresen-Dale AL. Principles and Methods of Integrative Genomic Analyses in Cancer. *Nat Rev Cancer* (2014) 14(5):299–313. doi: 10.1038/nrc3721
20. Chin L, Andersen JN, Futreal PA. Cancer Genomics: From Discovery Science to Personalized Medicine. *Nat Med* (2011) 17(3):297–303. doi: 10.1038/nm.2323
21. Tran B, Dancey JE, Kamel-Reid S, McPherson JD, Bedard PL, Brown AM, et al. Cancer Genomics: Technology, Discovery, and Translation. *J Clin Oncol* (2012) 30(6):647–60. doi: 10.1200/JCO.2011.39.2316
22. Jayne D. Molecular Biology of Peritoneal Carcinomatosis. *Cancer Treat Res* (2007) 134:21–33. doi: 10.1007/978-0-387-48993-3_2
23. Sodek KL, Murphy KJ, Brown TJ, Ringuette MJ. Cell-Cell and Cell-Matrix Dynamics in Intraperitoneal Cancer Metastasis. *Cancer Metastasis Rev* (2012) 31(1-2):397–3414. doi: 10.1007/s10555-012-9351-2
24. Hansson N, Polianski IJ. Therapeutic Pneumothorax and the Nobel Prize. *Ann Thorac Surg* (2015) 100(2):761–5. doi: 10.1016/j.athoracsur.2015.03.100
25. Kim YJ, Kim CH. Treatment for Peritoneal Metastasis of Patients With Colorectal Cancer. *Ann Coloproctol* (2021) 37(6):425–33. doi: 10.3393/ac.2021.00920.0131
26. Göhler D, Große S, Bellendorf A, Falkenstein TA, Ouaissi M, Zieren J, et al. Hyperthermic Intracavitary Nanoaerosol Therapy (HINAT) as an Improved Approach for Pressurised Intraperitoneal Aerosol Chemotherapy (PIPAC): Technical Description, Experimental Validation and First Proof of Concept. *Beilstein J Nanotechnol* (2017) 8:2729–40. doi: 10.3762/bjnano.8.272
27. Schubert J, Khosrawipour T, Reinhard S, Arafkas M, Martino A, Bania J, et al. The Concept of Foam as a Drug Carrier for Intraperitoneal Chemotherapy, Feasibility, Cytotoxicity and Characteristics. *Sci Rep* (2020) 10(1):10341. doi: 10.1038/s41598-020-67236-7
28. Mikolajczyk A, Khosrawipour V, Schubert J, Plociennik M, Nowak K, Fahr C, et al. Feasibility and Characteristics of Pressurized Aerosol Chemotherapy (PAC) in the Bladder as a Therapeutical Option in Early-Stage Urinary Bladder Cancer. *In Vivo* (2018) 32(6):1369–72. doi: 10.21873/invivo.11388
29. Khosrawipour V, Mikolajczyk A, Paslawski R, Plociennik M, Nowas K, Kulas J, et al. Intrathoracic Aerosol Chemotherapy *via* Spray-Catheter. *Mol Clin Oncol* (2020) 12(4):350–4. doi: 10.3892/mco.2020.1999
30. Khosrawipour V, Khosrawipour T, Hedayat-Pour Y, Diaz-Carballo D, Bellendorf A, Böse-Ribeiro H, et al. Effect of Whole-Abdominal Irradiation on Penetration Depth of Doxorubicin in Normal Tissue After Pressurized Intraperitoneal Aerosol Chemotherapy (PIPAC) in a Post-Mortem Swine Model. *Anticancer Res* (2017) 37(4):1677–80. doi: 10.21873/anticanres.11498
31. Khosrawipour V, Giger-Pabst U, Khosrawipour T, Pour YH, Diaz-Carballo D, Förster E, et al. Effect of Irradiation on Tissue Penetration Depth of Doxorubicin After Pressurized Intra-Peritoneal Aerosol Chemotherapy (PIPAC) in a Novel *Ex-Vivo* Model. *J Cancer* (2016) 7(8):910–4. doi: 10.7150/jca.14714
32. Khosrawipour V, Bellendorf A, Khosrawipour C, Hedayat-Pour Y, Diaz-Carballo D, Förster E, et al. Irradiation Does Not Increase the Penetration Depth of Doxorubicin in Normal Tissue After Pressurized Intra-Peritoneal Aerosol Chemotherapy (PIPAC) in an Ex Vivo Model. In Vivo (2016) 30(5):593–7.
33. Lau H, Khosrawipour T, Mikolajczyk A, Frelkiewicz P, Nicpon J, Arafkas M, et al. Intraperitoneal Chemotherapy of the Peritoneal Surface Using High-Intensity Ultrasound (HIUS): Investigation of Technical Feasibility, Safety and Possible Limitations. *J Cancer* (2020) 11(24):7209–15. doi: 10.7150/jca.48519
34. Mikolajczyk A, Khosrawipour T, Kulas J, Migdal P, Arafkas M, Nicpon J, et al. The Structural Effect of High Intensity Ultrasound on Peritoneal Tissue: A Potential Vehicle for Targeting Peritoneal Metastases. *BMC Cancer* (2020) 20(1):481. doi: 10.1186/s12885-020-06981-4
35. Karunasena E, Sham J, McMahon KW, Ahuja N. Genomics of Peritoneal Malignancies. *Surg Oncol Clin N Am* (2018) 27(3):463–75. doi: 10.1016/j.soc.2018.02.004
36. Mikolajczyk A, Khosrawipour V, Schubert J, Grzesiak J, Chaudhry H, Pigazzi A, et al. Effect of Liposomal Doxorubicin in Pressurized Intra-Peritoneal Aerosol Chemotherapy (PIPAC). *J Cancer* (2018) 9(23):4301–5. doi: 10.7150/jca.26860
37. Khosrawipour V, Mikolajczyk A, Schubert J, Khosrawipour T. Pressurized Intra-Peritoneal Aerosol Chemotherapy (PIPAC) *via* Endoscopical

Microcatheter System. *Anticancer Res* (2018) 38(6):3447–52. doi: 10.21873/anticanres.12613

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Safety, feasibility, and application of intraperitoneal gas-based hyperthermia beyond 43°C in the treatment of peritoneal metastasis: An *in-vivo* pilot study

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Background: 43°Celsius (C) is currently the highest temperature used in the treatment of peritoneal metastasis (PM). Despite sufficient data on water-based hyperthermic solutions in PM treatment, there is currently no information on gas-based hyperthermia extending beyond 43°C. This study is the first to provide *in-vivo* data on different organ systems during and after intraperitoneal gas-based hyperthermia beyond 43°C. The aim of this study is to explore *in-vivo* feasibility, safety, and efficacy of this novel concept from a biological perspective.

Methods: For this study, three swine were subjected to laparoscopy and subsequent gas-based intraperitoneal hyperthermia at 48°, 49° and 50°C under a high-flow air stream. Intraoperative data from multiple temperature sensors were analysed. Additionally, intraoperative anaesthesiologic and gasometrical data was analysed. Postoperatively, swine were monitored for one week and laboratory work-up was performed on postoperative days 1, 3 and 7.

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Results: During gas-based intraperitoneal hyperthermia, anesthesiologic parameters did not exhibit critical values. No intra- or postoperative complications were observed. Distinct temperature measurements on the skin, cystohepatic triangle and esophagus did not display any temperature increase. Postoperative laboratory workup did not show any changes in hemoglobin, white blood cell count, platelets, or kidney function.

Discussion: Based on our data, there are no safety concerns for the application of gas-based hyperthermia between 48 - 50°C. In fact, no critical systemic temperature increase was observed. With respect to possible limitations, further *in-vivo* studies are required to evaluate whether gas-based intraperitoneal hyperthermia may be a therapeutic option for PM patients.

KEYWORDS

intraperitoneal, heat, hyperthermia, peritoneal metastases, gas based

Introduction

The management of disseminated peritoneal metastasis (PM) remains a tremendous challenge in surgical oncology. PM is an aggressive disease with a poor overall prognosis, mostly originating from gastrointestinal tract or gynaecological tumour cells. Depending on the extent of PM, median survival is estimated at 3.7 - 9.8 months after diagnosis (1, 2). Systemic treatments such as intravenous chemotherapy have displayed limitations in changing the overall PM outcome. This is attributed to subtherapeutic chemotherapeutic concentrations in the peritoneal tissue due to systemic drug loss (3, 4). Due to these shortcomings, locoregional concepts have been considered as a more promising alternative to overcome current limitations in PM management. The most effective of these concepts, cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (HIPEC), has demonstrated efficacy in a selected group of patients (5, 6). Many studies have described the beneficial effects of local hyperthermia in PM management (7–9), and hyperthermia has shown to increase the response rate of cancer cells to chemo- and radiotherapy (10–13). However, liquid-based hyperthermia also exhibits limitations. In the HIPEC setting, medium perfusate and core body temperatures usually remain at around 40°Celsius (C) (14). An increase in central body or total organ temperature is not desirable and should be avoided. In fact, a recent study by Goldenshluger et al. demonstrated that increases in core body temperature served as a positive predictor for postoperative complications following HIPEC procedures (15). Thus, further increasing applied heat to the peritoneal surface is limited by this countervailing factor. A recent study by Diakun et al. demonstrated that intraperitoneal (i.p.) hyperthermia could be a feasible option to increase applied temperatures far beyond 43°C using a gas-based approach (16). In the presented model, the aim was to investigate whether gas-based intraperitoneal hyperthermia could be a feasible and safe option. Currently, further attempts have been made to analyze this approach. By means of an *in-vivo* swine model, we aim to closely investigate if gas-based hyperthermia exceeding 43°C can be safely applied *in-vivo*, and whether there is an indication of systemic or postoperative complications with respect to organ functions.

Materials and methods

In-vivo swine model

Using a diagnostic laparoscopy setting with a high-flow air stream of 15 liters per minute (l/min), three 65-day-old swine (Polish white flod) were subjected to gas-based i.p. hyperthermia at 48°, 49° and 50°C, respectively. The swine were part of a multicenter and multinational research study on peritoneal hyperthermia and dehydration called "Intraperitoneal gas-based Hyperthermia and Dehydration". All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals as published by the National Institutes of Health.

Pre-laparoscopic setting

Gas-based i.p. hyperthermia was delivered *via* a laparoscopic approach using a high-airflow system with compressed and filtered room air. No further additions were made to the composition of applied air.

Following compression, air flowed through a bacterial filter system (Cytiva Whatman™ HEPA-Vent Filter, Thermo Fisher Scientific, Walthman, USA) and was redirected through tubing *via* a heated water bath system which heated the passing air to the desired temperature level. At the tube's exit site, the temperature was again monitored to ensure exact air temperature calibration. The tube's exit site was placed into a 10 mm trocar. The subsequent surgical procedure was performed under general anaesthesia. All swine were premedicated with an intramuscular injection of midazolam (0.3 mg/kg, WZF Polfa S.A., Poland), medetomidine (0.02 mg/kg, Cepetor 1 mg/ml, CP-Pharma Handelsgesellschaft, Germany) and ketamine (9 mg/kg, Ketamina 100 mg/ml, Biowet Puławy sp. z o.o., Poland) mixture. Analgesia was performed with Propofol at 1mg/kg. Swines were intubated and further anaesthesia was continued with isoflurane 1%. Additional analgesia was provided with fentanyl 2µg/kg and crystalloid fluid at 0,2-0,3 µg/kg/min.

Surgical setting

Swines were placed in a supine position. An infra-umbilical mini-laparotomy was performed and another one at about 8 cm distance to the first one. A 10 mm trocar (Kii®Balloon Blunt Tip System, Applied Medical, Rancho Santa Margarita, CA, USA) was inserted while multiple 5 mm trocars were placed at the other sites following insufflation (Figure 1A). The abdominal cavity was insufflated with filtered room air through a tube entering the central 10 mm trocar (Figure 1B). An initial diagnostic check-up was made *via* laparoscopic imaging using a 5 mm camera system (Karl Storz 5mm/30° Laparoscope/Tuttlingen, Germany) *via* a 10 mm trocar. After visual confirmation and placement of multiple temperature sensors, the high-flow air stream was turned on at 15 l/min for a total of 45 minutes. A wide range of temperature and hygrometric sensors (Digital thermometer, FisherbrandTM Tracebale, Pittsburgh, USA) were placed. These allowed for temperature measurements during the procedures. Data from four of these temperature sensors were included in this study. One sensor was directly placed on the peritoneum of the lower left quadrant. One sensor was placed in the cystohepatic triangle, another one was placed and taped onto the skin of the abdomen in the periumbilical region and a final one was placed in the oesophageal area by the anaesthesiologist. During each procedure, a total of three arterial gasometric measurements were performed at the start of the procedure, 20 minutes into the procedure and shortly before extubating.

Postoperative management

Postoperatively, swines were observed for 7 days before they were euthanized. During the observation period, daily clinical evaluations were conducted with regards to changes in behavior, eating habits, indications of pain or discomfort and evaluation of the trocar wounds. Furthermore, a blood work-up was performed on postoperative days 1, 3 and 7. Red blood count, hemoglobin, white blood cell count and platelets were quantified. Levels of creatinine, blood urea nitrogen, total protein and albumin were also collected. Furthermore, electrolytes such as sodium, potassium, chloride, as well as

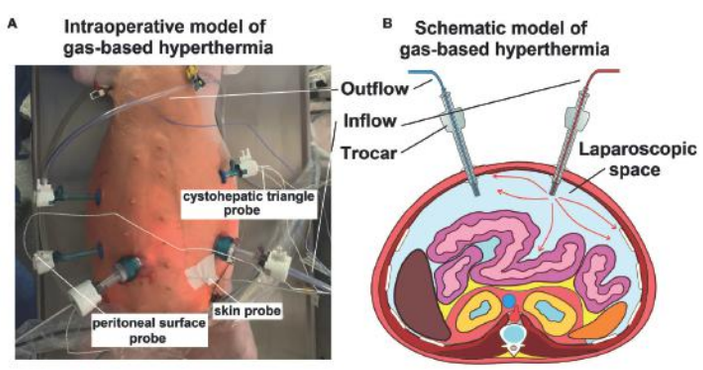

FIGURE 1
(A) Intraoperative model of gas-based i.p. hypertermia in an *in-vivo* swine model. Multiple trocars are placed for visual control, including at the in- and outflow and the entrance point for the temperature sensors. **(B)** Schematic model (transverse section) of the abdominal cavity including the in- and outflow trocar system.

C-reactive protein (CRP) levels were measured. After euthanization, laparotomy was performed to evaluate possible signs of macroscopical changes, perforation and or postoperative adhesions.

Results

Intraoperative parameters and temperature measurements

Ph., sodium, potassium, and chloride serum levels were measured. These parameters remained mostly constant for each swine within a narrow range (Figures 2A1–4). Additionally, both heart rate and blood pressure remained mostly constant despite some fluctuation during the procedure. However, while blood pressure remained constant, an initial drop in heart rate was observed within the first few minutes after initiating the operative procedure (Figures 2B1, B2). Oxygen saturation mostly remained constant with only a short intraoperative drop observed in one swine (49°C/Figure 2A5). Peritoneal surface temperature was measured during the entire procedure (Figures 3A1, A2). While the temperature fluctuated within 5°C, it always remained below 38°C (Figure 3A2). Among the swine, the abdominal temperature showed a higher degree of fluctuation (Figure 3B2). The skin temperature of one of the swine (48°C) dropped from an initial 33°C to below 28°C during the procedure (Figure 3B1). The "liver" temperature measured in the cystohepatic triangle showed a similar behaviour. In two swine, temperatures dropped during the procedure (from 37°C to 34° C° and 39°C to ca. 36°C, respectively, Figures 3C1, C2). As for the liver and core body temperature (oesophageal temperature), a slight temperature decrease was noticed from the beginning until the end of the procedure (Figure 3D).

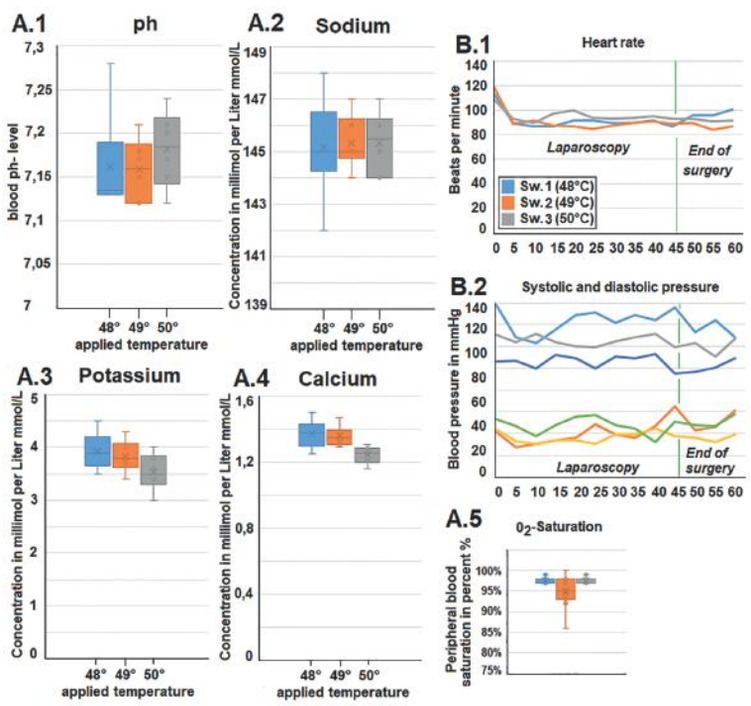

FIGURE 2
(A). Intraoperative gasometric data. 1) pH-data, 2) plasma sodium level, 3) Plasma potassium level and 4) Plasma calcium level. 5) Intraoperative blood oxygen (0_2) saturation. **(B.1)** Intraoperative heart rate and **(B.2)** systolic and diastolic blood pressure (via invasive measurement).

Postoperative parameters and laboratory test analysis

Postoperatively, swine were observed for a total of 7 days before euthanization. No intraoperative complications were detected. Daily clinical postoperative evaluations did not show any changes in behaviour, eating habits, indication of pain or discomfort. All trocar wounds displayed appropriate wound healing. Blood work-up was performed on postoperative day 1, 3 and 7 and did not reveal any concerning pathological signs. Red blood cell count, hemoglobin, platelet count, creatinine, total protein and albumin, as well as sodium chloride and CRP remained within the physiological range and did not show any fluctuation during the study period (Figure 4). Potassium levels slightly increased up to day 7, and blood urea nitrogen peaked at day 3 while still remaining within physiological levels. As previously mentioned, CRP always remained below 0.5 mg/dl while a slight elevation in white blood cell count was noted. Again, white blood cell count reached the upper level and was still considered within the physiological range. Autopsy did not show any signs of organ perforation, adhesions, or ascites (Figure 5). Small superficial petechia were visible at a few distinct spots.

Discussion

Cancer cells in the peritoneal cavity usually originate from either gastrointestinal or gynecological cells. Thus, they are used to a stable fluid and temperature environment. This aspect remains unchanged, regardless of mutations in the cancer cell genome (17). Consequently, changing the basic biology of the abdominal cavity might halt tumor progression to a significant degree. Heating and potentially even dehydrating the peritoneal cavity *via* application of continuous gas-based i.p. hyperthermia may serve as a tool to alter basic biology in the human body (16).

The idea of using new physical principles in the treatment of PM and other surface malignancies is promising and has been attempted several times (18–20). Some of these attempts, including irradiation (21–23), high-intensity ultrasound (24, 25) and nanoparticles (26), have been created and extensively tested. Limitations in these approaches are related to the aggressive behavior of PM as well as limited efficacy of i.p chemotherapy even in local applications. However, hyperthermia combined with chemotherapy and CRS has already demonstrated its clinical relevance and impact on the overall outcome in PM management (6–8). How an additional temperature increase beyond 43°C may impact PM management must be further evaluated. Although the inflow temperature exceeds 43°C, we did not detect temperature levels extending beyond 43°C in the organ or at different points within the abdominal cavity. Therefore, the question remains as to whether the occurring thermodynamic energy transfer has an actual effect on the peritoneal surface or if no critical temperature increase is detectable at all. The sensitivity of cancer cells to hyperthermia has already been well demonstrated (27, 28). Moreover, hyperthermia has shown to

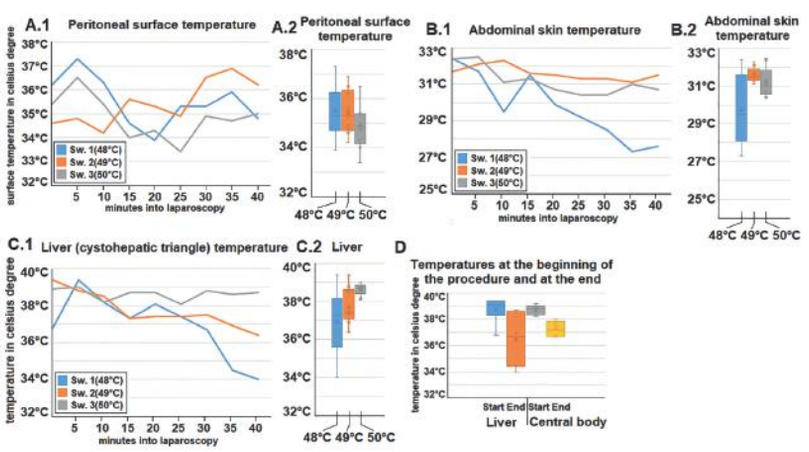

FIGURE 3
Intraoperative temperature measurements during gas-based i.p. hyperthermia at distinct sites presented for each swine (**A.1, 2**) at the peritoneal surface (**B1, 2**) Abdominal skin, (**C1, 2**) temperature of the "liver" (cystohepatic triangle). (**D**) Measured temperatures at the beginning and end of gas-based i.p. hyperthermia in all swine of the "liver" (cystohepatic triangle) and core body temperature (measured in the oesephageal probe).

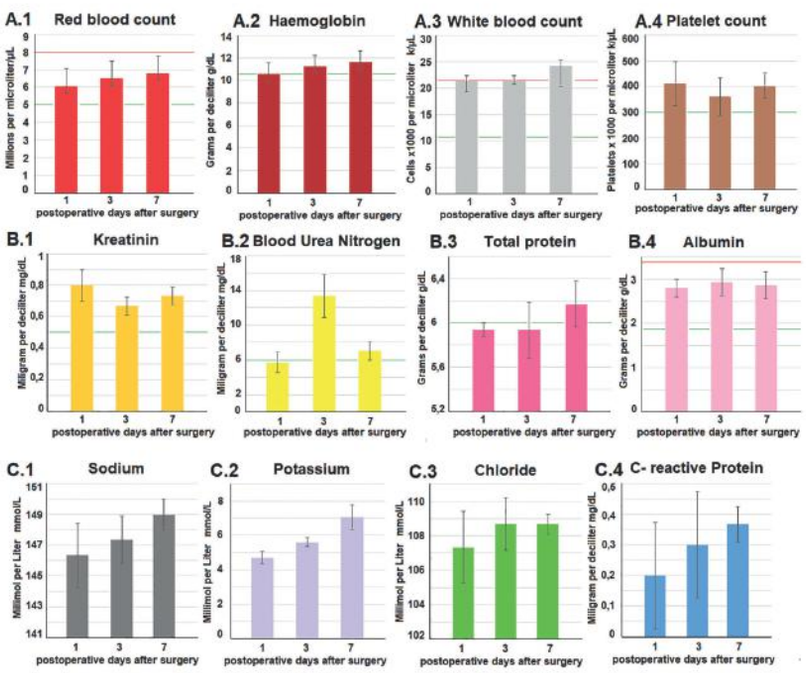

FIGURE 4
(A–C) Blood work-up on postoperative days 1, 3 and 7. Testing included a wide range of parameters. The red (upper) and green lines (lower) in each diagramm demonstrate physiological reference values of the analyzed parameters.

increase the response rate of cancer cells to chemo- and radiotherapy (10–13). Recently, an enhanced cytotoxic effect of dehydration on colon cancer cells combined with hyperthermia has been described (16). In fact, there are some indications that some of the effects of hyperthermia in the management of local progressive cancer diseases might also be related to the occlusion of neo-vascular tumor structures (29–31)".

Our data on gas-based hyperthermia beyond 43°C does not indicate any signs of intraoperative or postoperative complications. Blood parameters did not display any indications for possible internal bleeding, platelet depletion, or infections. Additionally, no postoperative complications or organ failure were observed. Moreover, there are no indications for renal issues or protein disbalances in the porcine serum. The blood workup reflects the clinical picture which was void of any complications. Intraoperative measurements did not detect any critical temperature increase. A slight cooling effect during the procedure is attributed to anaesthesiology and supine positioning on a metal-based surgical table.

At this point, it is safe to assume that the application of gas-based i.p. hyperthermia is feasible. While the number of investigated swine does not warrant any conclusive evaluation on potential side effects or complications associated with this concept, this study offers insight into important *in-vivo* aspects of basic feasibility, safety as well as specific characteristics of this novel method. This approach could be combined with the application of chemotherapy, e.g. pressurized intraperitoneal aerosol chemotherapy (PIPAC). However, there are key differences in these applications. During the conventional PIPAC approach, aerosolized chemotherapy is defacto applied in a closed cavity whereas in the presented approach, chemotherapy is applied in a continuous flow system. At the end of gas-based hyperthermia, subsequent PIPAC could be applied. A parallel application is technically challenging since

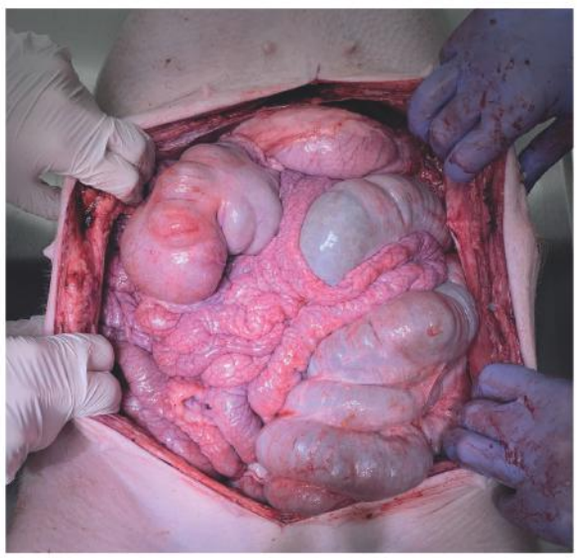

FIGURE 5
Macroscopic view during postoperative autopsy (day 7). No indication of perforation, adhesions or ascites within the abdominal cavity.

gas turbulence would interfere with chemoaerosol sedimentation. Additionally, management of a high flow, chemoaerosol loaded gas outflow would be demanding on many levels. Further research on this innovative concept is required to evaluate possible *in-vivo* complications and assess its antitumoral effects and benefits for PM management.

Limitations

Due to the incalculable potential side effects related to the novelty of this pilot study the initial number of swine was kept small to meet ethical and safety concerns. Therefore, the statistical power of this temperature escalation study is limited. The swine model can only partially reflect the conditions and effects on a human. Additionally, the observation period does not fully cover potential complications which might set-in after one week, e.g. adhesions or late perforations. It is important to remember that an additional evaluation of cytoreductive surgery and chemotherapy combined with the presented procedure might also change safety evaluations and could cause concerns that are currently not observed in our model. Hyperthermia's cellular effects on cancer cells and peritoneal tissue should be further investigated using *in-vitro* and *in-vivo* studies, respectively.

Data availability statement

The original contributions presented in the study are included in the article/supplementary materials. Further inquiries can be directed to the corresponding author.

Ethics statement

Experiments were approved (Nr 030/2021/P2) by the Ethics Committee of Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland as well as the local Board on Animal Welfare.

Author contributions

AD: Study design, laboratory analysis, data acquisition, editing. TK: Study design, conception of the study and manuscript drafting. AM-M and JN: Study design, laboratory analysis, data acquisition. ST, ZK: Manuscript drafting and critical revision for important intellectual content of the manuscript. BL: Study design, laboratory analysis. PP, JK, and KZ: laboratory analysis, data acquisition. SL, HL, and WK:

Drafting and critical revision for important intellectual content of the manuscript. VK: Supervision on study design, laboratory analyses, conception of the study and manuscript drafting. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Paul Olson TJ, Pinkerton C, Brasel KJ, Schwarze ML. Palliative surgery for malignant bowel obstruction from carcinomatosis: a systematic review. *JAMA Surg* (2014) 149(4):383–92. doi: 10.1001/jamasurg.2013.4059

2. Heaney RM, Shields C, Mulsow J. Outcome following incomplete surgical cytoreduction combined with intraperitoneal chemotherapy for colorectal peritoneal metastases. *World J Gastrointest Oncol* (2015) 7(12):445–54. doi: 10.4251/wjgo.v7.i12.445

3. Walker JL, Brady MF, Wenzel L, Fleming GF, Huang HQ, DiSilvestro PA, et al. Randomized trial of intravenous versus intraperitoneal chemotherapy plus bevacizumab in advanced ovarian carcinoma: An NRG Oncology/Gynecologic oncology group study. *J Clin Oncol* (2019) 37(16):1380–90. doi: 10.1200/JCO.18.01568

4. Verwaal VJ, van Ruth S, de Bree E, van Sloothen GW, van Tinteren H, Boot H, et al. Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. *J Clin Oncol* (2003) 21 (20):3737–43. doi: 10.1200/JCO.2003.04.187

5. Chen WC, Huang HJ, Yang LY, Pan YB, Huang KG, Lin CT, et al. Hyperthermic intraperitoneal chemotherapy for recurrent epithelial ovarian cancer. *BioMed J* (2021) S2319–4170(21)00137-2. doi: 10.1016/j.bj.2021.10.003

6. Van Driel WJ, Koole SN, Sikorska K, Schagen van Leeuwen JH, Schreuder HWR, Hermans RHM, et al. Hyperthermic intraperitoneal chemotherapy in ovarian cancer. *N Engl J Med* (2018) 378(3):230–40. doi: 10.1056/NEJMoa1708618

7. Hynynen K, Lulu BA. Hyperthermia in cancer treatment. *Invest Radiol* (1990) 25(7):824–34. doi: 10.1097/00004424-199007000-00014

8. Stewart JR, Gibbs FAJr. Hyperthermia in the treatment of cancer. perspectives on its promise and its problems. *Cancer* (1984) 54(11 Suppl):2823–30. doi: 10.1002/1097-0142(19841201)54:2+<2823:aid-cncr2820541430>3.0.co;2-

9. Bergs JW, Franken NA, Haveman J, Geijsen ED, Crezee J, van Bree C. Hyperthermia, cisplatin and radiation trimodality treatment: a promising cancer treatment? a review from preclinical studies to clinical application. *Int J Hyperthermia* (2007) 23(4):329 –41. doi: 10.1080/02656730701378684

10. Zivanovic O, Chi DS, Filippova O, Randall LM, Bristow RE, O'Cearbhaill RE. It's time to warm up to hyperthermic intraperitoneal chemotherapy for patients with ovarian cancer. *Gynecol Oncol* (2018) 151(3):555–61. doi: 10.1016/j.ygyno.2018.09.007

11. Muckle DS, Dickson JA. The selective inhibitory effect of hyperthermia on the metabolism and growth of malignant cells. *Br J Cancer* (1971) 25(4):771–8. doi: 10.1038/bjc.1971.91

12. Seifert G, Budach V, Keilholz U, Wust P, Eggert A, Ghadjar P. Regional hyperthermia combined with chemotherapy in paediatric, adolescent and young adult patients: Current and future perspectives. *Radiat Oncol* (2016) 11:65. doi: 10.1186/s13014-016-0639-1

13. Sugarbaker PH. Laboratory and clinical basis for hyperthermia as a component of intracavitary chemotherapy. *Int J Hyperthermia* (2007) 23(5):431–42. doi: 10.1080/02656730701455318

14. Rettenmaier MA, Mendivil AA, Gray CM, Chapman AP, Stone MK, Tinnerman EJ, et al. Intra-abdominal temperature distribution during consolidation hyperthermic intraperitoneal chemotherapy with carboplatin in the treatment of advanced stage ovarian carcinoma. *Int J Hyperthermia* (2015) 31(4):396–402. doi: 10.3109/02656736.2015.1007399

15. Goldenshluger M, Zippel D, Ben-Yaacov A, Dux J, Yalon T, Zendel A, et al. Core body temperature but not intraabdominal pressure predicts postoperative complications following closed-system hyperthermic intraperitoneal chemotherapy (HIPEC) administration. *Ann Surg Oncol* (2018) 25(3):660–6. doi: 10.1245/s10434-017-6279-3

16. Diakun A, Khosrawipour T, Mikolajczyk-Martinez A, Nicpoń J, Kiełbowicz Z, Prządka P, et al. The onset of *in-vivo* dehydration in gas - based intraperitoneal hyperthermia and its cytotoxic effects on colon cancer cells. *Front Oncol* (2022) 12:925724. accepted for publication. doi: 10.3389/fonc.2022.927714

17. Karunasena E, Sham J, McMahon KW, Ahuja N. Genomics of peritoneal malignancies. *Surg Oncol Clin N Am* (2018) 27(3):463–75. doi: 10.1016/j.soc.2018.02.004

18. Schubert J, Khosrawipour T, Reinhard S, Arafkas M, Martino A, Bania J, et al. The concept of foam as a drug carrier for intraperitoneal chemotherapy, feasibility, cytotoxicity and characteristics. *Sci Rep* (2020) 10(1):10341. doi: 10.1038/s41598-020-67236-7

19. Mikolajczyk A, Khosrawipour V, Schubert J, Plociennik M, Nowak K, Fahr C, et al. Feasibility and characteristics of pressurized aerosol chemotherapy (PAC) in the bladder as a therapeutical option in early-stage urinary bladder cancer. *In Vivo* (2018) 32(6):1369–72. doi: 10.21873/invivo.11388

20. Khosrawipour V, Mikolajczyk A, Paslawski R, Plociennik M, Nowas K, Kulas J, et al. Intrathoracic aerosol chemotherapy *via* spray-catheter. *Mol Clin Oncol* (2020) 12(4):350–4. doi: 10.3892/mco.2020.1999

21. Khosrawipour V, Khosrawipour T, Hedayat-Pour Y, Diaz-Carballo D, Bellendorf A, Bôse-Ribeiro H, et al. Effect of whole-abdominal irradiation on penetration depth of doxorubicin in normal tissue after pressurized intraperitoneal aerosol chemotherapy (PIPAC) in a post-mortem swine model. *Anticancer Res* (2017) 37(4):1677–80. doi: 10.21873/anticanres.11498

22. Khosrawipour V, Giger-Pabst U, Khosrawipour T, Pour YH, Diaz-Carballo D, Förster E, et al. Effect of irradiation on tissue penetration depth of doxorubicin after pressurized intra-peritoneal aerosol chemotherapy (PIPAC) in a novel *ex-vivo* model. *J Cancer* (2016) 7(8):910–4. doi: 10.7150/jca.14714

23. Khosrawipour V, Bellendorf A, Khosrawipour C, Hedayat-Pour Y, Diaz-Carballo D, Förster E, et al. Irradiation does not increase the penetration depth of doxorubicin in normal tissue after pressurized intra-peritoneal aerosol chemotherapy (PIPAC) in an ex vivo model. *In Vivo* (2016) 30(5):593–7.

24. Lau H, Khosrawipour T, Mikolajczyk A, Frelkiewicz P, Nicpon J, Arafkas M, et al. Intraperitoneal chemotherapy of the peritoneal surface using high-intensity ultrasound (HIUS): Investigation of technical feasibility, safety and possible limitations. *J Cancer* (2020) 11(24):7209–15. doi: 10.7150/jca.48519

25. Mikolajczyk A, Khosrawipour T, Kulas J, Migdal P, Arafkas M, Nicpon J, et al. The structural effect of high intensity ultrasound on peritoneal tissue: A potential vehicle for targeting peritoneal metastases. *BMC Cancer* (2020) 20(1):481. doi: 10.1186/s12885-020-06981-4

26. Khosrawipour T, Schubert J, Kulas J, Migdal P, Arafkas M, Bania J, et al. Creating nanocrystallized chemotherapy: The differences in pressurized aerosol chemotherapy (PAC) *via* intracavitary (IAG) and extracavitary aerosol generation (EAG) regarding particle generation, morphology and structure. *J Cancer* (2020) 11(6):1308–14. doi: 10.7150/jca.39097

27. de Andrade Mello P, Bian S, Savio LEB, Zhang H, Zhang J, Junger W, et al. Hyperthermia and associated changes in membrane fluidity potentiate P2X7 activation to promote tumor cell death. *Oncotarget* (2017) 8(40):67254–68. doi: 10.18632/oncotarget.18595

28. Li DY, Tang YP, Zhao LY, Geng CY, Tang JT. Antitumor effect and immune response induced by local hyperthermia in B16 murine melanoma: Effect of thermal dose. *Oncol Lett* (2012) 4(4):711–8. doi: 10.3892/ol.2012.804

29. Emami B, Song CW. Physiological mechanisms in hyperthermia: a review. *Int J Radiat Oncol Biol Phys* (1984) 10(2):289–95. doi: 10.1016/0360-3016(84)90015-4

30. Eddy HA. Alterations in tumor microvasculature during hyperthermia. *Radiology* (1980) 137(2):515–21. doi: 10.1148/radiology.137.2.7433685

31. Diakun A, Khosrawipour T, Mikolajczyk-Martinez A, Kuropka P, Nicpoń J, Kiełbowicz Z, et al. *In-vivo* thermodynamic exploration of gas-based intraperitoneal hyperthermia. *Front Oncol* (2022) 29(12):925724M. doi: 10.3389/fonc.2022.925724

8. CONCLUSIONS

- Gas hyperthermia exceeding 43°C and dehydration in vitro have cytotoxic effects on colorectal cancer cells from the HT-29 colorectal cancer cell line and reduce their viability.
- Safe generation of intraperitoneal gaseous hyperthermia exceeding 43°C is possible in an animal organism.
- The gas used in the experiment as a temperature transmitter, due to its heat capacity, allows the safe use of higher temperatures inside the peritoneal cavity than in the case of liquid-produced hyperthermia.
- Intraperitoneal gas hyperthermia exceeding the temperature of 43°C and accompanying dehydration cause macro- and microscopic changes on the surface of the peritoneum. This can potentially make it more difficult for cancer cells to spread inside the peritoneal cavity.
- Obtaining intraperitoneal dehydration is technically possible during laparoscopy. The mentioned phenomenon may potentially contribute to the development of a new therapeutic method and find application in the treatment of patients with cancer disseminated to the peritoneal cavity. However, this method requires further detailed research.

7. LITERATURE

1. *Yang X, Gao M, Xu R, et al. Hyperthermia combined with immune checkpoint inhibitor therapy in the treatment of primary and metastatic tumors. Front Immunol. 2022;13:969447. doi:10.3389/fimmu.2022.969447*

2. *Yarema R, Ohorchak M, Hyrya P, et al. Gastric cancer with peritoneal metastases: Efficiency of standard treatment methods. World J Gastrointest Oncol. 2020;12(5):569-581. doi:10.4251/wjgo.v12.i5.569*

3. *Jastrzębski T, Zegarski W. Przerzuty do otrzewnej raka jelita grubego. Pol J Surg. 2017;89(5):34-42. doi:10.5604/01.3001.0010.5605*

4. *Chicago Consensus Working Group. The Chicago Consensus on Peritoneal Surface Malignancies: Management of Colorectal Metastases. Ann Surg Oncol. 2020;27(6):1761-1767. doi:10.1245/s10434-020-08315-x*

5. *Polish clinical practice guidelines on Hyperthermic Intraperitoneal Chemotherapy (HIPEC) with Cytoreductice Surgery (CRS) in peritoneal malignancy treatment | Rutkowski | Nowotwory. Journal of Oncology. Accessed January 21, 2023. https://journals.viamedica.pl/nowotwory_journal_of_oncology/article/view/NJO.2014.0089/34071*

6. *Rijken A, Lurvink RJ, Luyer MDP, et al. The Burden of Peritoneal Metastases from Gastric Cancer: A Systematic Review on the Incidence, Risk Factors and Survival. J Clin Med. 2021;10(21):4882. doi:10.3390/jcm10214882*

7. *Krishnan R, Kurnit KC, Kim JS. Ovarian cancer peritoneal carcinomatosis: a narrative review. Dig Med Res. 2022;5:43-43. doi:10.21037/dmr-22-13*

8. *J.Skowronek. Hipertermia w leczeniu nowotworów złośliwych. Wielkopolska Izba Lekarska - Biuletyn Informacyjny. 2006;(3). Wielkop Izba Lek Biul Inf. (2006;(3)).*

9. *Lemoine L, Sugarbaker P, Van der Speeten K. Pathophysiology of colorectal peritoneal carcinomatosis: Role of the peritoneum. World J Gastroenterol. 2016;22(34):7692. doi:10.3748/wjg.v22.i34.7692*

10. *Oei AL, Kok HP, Oei SB, et al. Molecular and biological rationale of hyperthermia as radio- and chemosensitizer. Adv Drug Deliv Rev. 2020;163-164:84-97. doi:10.1016/j.addr.2020.01.003*

11. *Gamboa AC, Lee RM, Turgeon MK, et al. Implications of Postoperative Complications for Survival After Cytoreductive Surgery and HIPEC: A Multi-Institutional Analysis of the US HIPEC Collaborative. Ann Surg Oncol. 2020;27(13):4980-4995. doi:10.1245/s10434-020-08843-6*

12. *Li CY, Alexander HR. Peritoneal Metastases from Malignant Mesothelioma. Surg Oncol Clin N Am. 2018;27(3):539-549. doi:10.1016/j.soc.2018.02.010*

13. *Bębenek M, Ashraf-Kashani N. Pierwotne i wtórne nowotwory otrzewnej: diagnostyka, leczenie. Wydanie I. PZWL Wydawnictwo Lekarskie; 2021.*

14. *Sánchez-Hidalgo JM, Rodríguez-Ortiz L, Arjona-Sánchez Á, et al. Colorectal peritoneal metastases: Optimal management review. World J Gastroenterol. 2019;25(27):3484-3502. doi:10.3748/wjg.v25.i27.3484*

15. *Rafał Sapierzyński. Przerzuty nowotworowe – drogi szerzenia się nowotworów. Życie Weterynaryjne. 2012;87(10):821-827.*

16. *Purbadi S, Anggraeni TD, Vitria A. Early stage epithelial ovarian cancer metastasis through peritoneal fluid circulation. J Ovarian Res. 2021;14(1):44. doi:10.1186/s13048-021-00795-z*

17. *Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Gastroenterol Rev. 2019;14(2):89-103. doi:10.5114/pg.2018.81072*

18. *Fuchs R, Galm O, Polkowski W, Kosikowski W. Nowotwory przewodu pokarmowego: diagnostyka i leczenie. Wyd. 1. pol. Wydawnictwo Czelej; 2012.*

19. *Rak żołądka. Wydanie 1. PZWL Wydawnictwo Lekarskie; 2020.*

20. *Chen D, Liu Z, Liu W, et al. Predicting postoperative peritoneal metastasis in gastric cancer with serosal invasion using a collagen nomogram. Nat Commun. 2021;12(1):179. doi:10.1038/s41467-020-20429-0*

21. *van Baal JOAM, van Noorden CJF, Nieuwland R, et al. Development of Peritoneal Carcinomatosis in Epithelial Ovarian Cancer: A Review. J Histochem Cytochem. 2018;66(2):67-83. doi:10.1369/0022155417742897*

22. *Halkia E, Spiliotis J, Sugarbaker P. Diagnosis and Management of Peritoneal Metastases from Ovarian Cancer. Gastroenterol Res Pract. 2012;2012:1-12. doi:10.1155/2012/541842*

23. *Tang Q, Huang M, Zhang J, et al. Comparative Survival Outcomes of Hyperthermic Intraperitoneal Chemotherapy, Intraperitoneal Chemotherapy and Intravenous Chemotherapy for Primary Advanced Ovarian Cancer: A Network Meta-Analysis. J Clin Med. 2023;12(3):1111. doi:10.3390/jcm12031111*

24. *Piotr Rutkowski1, Beata Śpiewankiewicz2, Krzysztof Herman, Tomasz Jastrzębski, Józef Kładny, Zbigniew Kojs, Maciej Krzakowski, Wojciech Polkowski, Lucjan Wyrwicz, Piotr Wysocki, Marcin Zdzienicki, Wojciech Zegarski. Zasady stosowania dootrzewnowej chemioterapii w hipertermii (HIPEC) w leczeniu nowotworów złośliwych powierzchni otrzewnej w połączeniu z zabiegami cytoredukcyjnymi: zalecenia krajowe. 2014;Vol 64(6). https://journals.viamedica.pl/nowotwory_journal_of_oncology/article/view/NJO.2014.0089/34071*

25. *Levine EA, Stewart JH, Shen P, Russell GB, Loggie BL, Votanopoulos KI. Intraperitoneal Chemotherapy for Peritoneal Surface Malignancy: Experience with 1,000 Patients. J Am Coll Surg. 2014;218(4):573-585. doi:10.1016/j.jamcollsurg.2013.12.013*

26. *Göhler D, Khosrawipour V, Khosrawipour T, et al. Technical description of the microinjection pump (MIP®) and granulometric characterization of the aerosol applied for pressurized intraperitoneal aerosol chemotherapy (PIPAC). Surg Endosc. 2017;31(4):1778-1784. doi:10.1007/s00464-016-5174-5*

27. *Zivanovic O, Chi DS, Filippova O, Randall LM, Bristow RE, O'Cearbhaill RE. It's time to warm up to hyperthermic intraperitoneal chemotherapy for patients with ovarian cancer. Gynecol Oncol. 2018;151(3):555-561. doi:10.1016/j.ygyno.2018.09.007*

28. *Muckle DS, Dickson JA. The Selective Inhibitory Effect of Hyperthermia on the Metabolism and Growth of Malignant Cells. Br J Cancer. 1971;25(4):771-778. doi:10.1038/bjc.1971.91*

29. *Seifert G, Budach V, Keilholz U, Wust P, Eggert A, Ghadjar P. Regional hyperthermia combined with chemotherapy in paediatric, adolescent and young adult patients: current and future perspectives. Radiat Oncol. 2016;11(1):65. doi:10.1186/s13014-016-0639-1*

30. *Sugarbaker PH. Laboratory and clinical basis for hyperthermia as a component of intracavitary chemotherapy. Int J Hyperthermia. 2007;23(5):431-442. doi:10.1080/02656730701455318*

31. *Emami B, Song CW. Physiological mechanisms in hyperthermia: A review. Int J Radiat Oncol. 1984;10(2):289-295. doi:10.1016/0360-3016(84)90015-4*

32. *Eddy HA. Alterations in tumor microvasculature during hyperthermia. Radiology. 1980;137(2):515-521. doi:10.1148/radiology.137.2.7433685*

33. *Kang MS, Song CW, Levitt SH. Role of vascular function in response of tumors in vivo to hyperthermia. Cancer Res. 1980;40(4):1130-1135.*

34. *Hynynen K, Lulu BA. Hyperthermia in Cancer Treatment: Invest Radiol. 1990;25(7):824-834 doi:10.1097/00004424-199007000-00014*

35. *Stewart JR, Gibbs FA. Hyperthermia in the treatment of cancer: Perspectives on its promise and its problems. Cancer. 1984;54(S2):2823-2830. doi:10.1002/1097-0142(19841201)54:2+<2823::AID CNCR2820541430>3.0.CO;2-7*

36. *Bergs JWJ, Franken NAP, Haveman J, Geijsen ED, Crezee J, van Bree C. Hyperthermia, cisplatin and radiation trimodality treatment: A promising cancer treatment? A review from preclinical studies to clinical application. Int J Hyperthermia. 2007;23(4):329-341. doi:10.1080/02656730701378684*

9. OPINION OF THE BIOETHICAL COMMISSION

UCHWAŁA NR 029/2021/P1

z dnia 19.05.2021

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. *„Testowanie nowoczesnych technik wewnątrzotrzewnowej chemioterapii bezpośredniej w oparciu o koncepcję: Foam – based intraperitoneal chemiotherapy (FBIC)."*z dnia 10.05.2021, złożonego przez Uniwersytet Przyrodniczy we Wrocławiu Wydział Medycyny Weterynaryjnej, adres: ul. Norwida 31, 50-375 Wrocław,[2] zaplanowanego przez lek. wet. Agatę Mikołajczyk[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 § , Lokalna Komisja Etyczna ustaliła, że:

1. Wniosek należy przypisać do kategorii: [PT21] badania translacyjne - nowotwór człowieka
2. Najwyższy stopień dotkliwości proponowanych procedur to: umiarkowany.
3. Doświadczenia będą przeprowadzane na gatunkach lub grupach gatunków[6]: świnia WBP/PBZ oraz ich mieszańce, warchlaki, 8-12 tygodni, 5 szt.
4. Doświadczenia będą przeprowadzane przez: lek. wet. Agata Mikołajczyk, dr hab. lek. med. Veria Khosrawipour, dr hab. lek. med.Tanja Khosrawipour, lek. wet. Piotr Frelkiewicz, prof. dr hab. Zdzisław Kiełbowicz, dr hab. lek. wet. Jakub Nicpoń, dr n. wet. Przemysław Prządka, dr n. wet. Bartłomiej Liszka, lek. med. Kacper Zieliński, lek. stom. Maya Labbe
5. Doświadczenie będzie przeprowadzane w terminie[7] od 01.07.2021 do 30.06.2022
6. Doświadczenie będzie przeprowadzone w ośrodku[8]: nie dotyczy
7. Doświadczenie będzie przeprowadzone poza ośrodkiem w : nie dotyczy.

[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

1

8. Użyte do procedur zwierzęta dzikie zostaną odłowione przez ..., w sposób: nie dotyczy.
9. Doświadczenie ~~zostanie~~/nie zostanie[9] poddane ocenie retrospektywnej ~~w terminie do 3 (trzech) miesięcy od dnia przekazania przez użytkownika dokumentacji, mającej stanowić podstawę dokonania oceny retrospektywnej. Użytkownik jest zobowiązany do przekazania ww. dokumentacji niezwłocznie, tj. w terminie, o którym mowa w art. 52 ust. 2 ustawy~~.

§ 3

Uzasadnienie:

Wniosek został uzupełniony o wszystkie uwagi LKE z dnia 21.04.2021 r. W obecnej wersji streszczenia nietechnicznego wniosku, wnioskodawca podaje odniesienia literaturowe (w postaci cyfr) jednak nie podaje odniesień (ze względu na ograniczenia liczby znaków we wzorze NTSW). Lke zadecydowała o przeprowadzeniu głosowania nad wyrażeniem zgody na realizację doświadczenia objętego wnioskiem oraz zobligowanie użytkownika do usunięcia odniesień literaturowych z NTSW przed rozpoczęciem realizacji wniosku i przesłanie poprawionej wersji na adres lke@hirszfeld.pl. Uwagi lke do NST wniosku 029/2021/P1 nie stanowią błędów formalnych oraz merytorycznych mających wpływ na ocenę wniosku przez lke.

Na skutek przeprowadzonego głosowania **zadecydowano o udzieleniu zgody na realizację badań objętych wnioskiem 029/2021/P1.** 7 osób głosowało za, 1 osoba wstrzymała się od głosu, nikt nie głosował przeciw. **Wniosek nie podlega ocenie retrospektywnej.** 6 osób głosowało przeciw, nikt nie wstrzymał się od głosu, dwie osoby były za oceną retrospektywną wniosku.

§ 4

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

LOKALNA KOMISJA ETYCZNA
DS. DOŚWIADCZEŃ NA ZWIERZĘTACH WE WROCŁAWIU
Instytut Immunologii i Terapii Doświadczalnej
im. L. Hirszfelda
53-114 Wrocław, ul. Rudolfa Weigla 12
Tel. (71) 337 11 72 wew. 112, 113, 155 wew. 181

prof. dr hab. Joanna Wietrzyk
Przewodnicząca Lokalnej Komisji Etycznej
ds. Doświadczeń na Zwierzętach we Wrocławiu

(Pieczęć lokalnej komisji etycznej)

Podpis przewodniczącego 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 postępowania, 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

[9] Niewłaściwy zapis usunąć

10. DECLARATION OF CO-AUTHORS

Wrocław, 24.01.2023

Dr Bartłomiej Liszka
Katedra i Klinika Chirurgii
Wydział Weterynarii
Uniwersytet Przyrodniczy we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy:

"Safety, feasibility, and application of intraperitoneal gas-based hyperthermia beyond 43°C in the treatment of peritoneal metastasis: An in-vivo pilot study"

Front Oncol. 2022 Oct 11;12:953920. doi: 10.3389/fonc.2022.953920. eCollection2022.

mój udział polegał na opiece anestezjologicznej w trakcie wykonywania eksperymentów na świniach, pobieraniu krwi od zwierząt oraz nadzorze nad wykonywanymi badaniami laboratoryjnymi.

Bartłomiej Liszka

Podpis

Wrocław, 24.01.2023

Dr Bartłomiej Liszka
Katedra i Klinika Chirurgii
Wydział Weterynarii
Uniwersytet Przyrodniczy we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy:

„In-vivo thermodynamic exploration of gas-based intraperitoneal hyperthermia"

Front Oncol. 2022 Aug 29;12:925724. doi: 10.3389/fonc.2022.925724. eCollection 2022.

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Bartłomiej Liszka

Podpis

Wrocław, 24.01.2023

Dr Bartłomiej Liszka
Katedra i Klinika Chirurgii
Wydział Weterynarii
Uniwersytet Przyrodniczy we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy:

„The Onset of In-Vivo Dehydration in Gas -Based Intraperitoneal Hyperthermia and Its Cytotoxic Effects on Colon Cancer Cells"

Front Oncol. 2022 Jun 29;12:927714. doi: 10.3389/fonc.2022.927714. eCollection 2022.

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Bartłomiej Liszka

Podpis

Wrocław, 24.01.2023

Dr hab. Przemysław Prządka
Katedra i Klinika Chirurgii
Wydział Weterynarii
Uniwersytet Przyrodniczy we Wrocławiu

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mój udział polegał na wykonaniu zabiegu laparoskopii na świniach oraz istotnym wkładzie intelektualnym w powstawanie manuskryptu.

Podpis

Wrocław, 24.01.2023

Dr hab. Przemysław Prządka
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Uniwersytet Przyrodniczy we Wrocławiu

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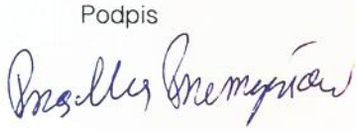
Podpis



Wrocław, 24.01.2023

Dr hab. n. med. Przemysław Prządka
Katedra i Klinika Chirurgii
Wydział Weterynarii
Uniwersytet Przyrodniczy we Wrocławiu

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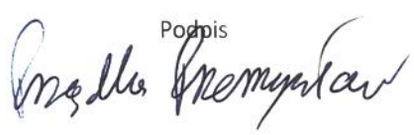

Wrocław, 24.01.2023

Prof. dr hab. Jakub Nicpoń
Katedra i Klinika Chirurgii
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Podpis

Prof. dr hab. Jakub Nicpoń
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Specjalista chirurg
tel. 601 927 239
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Wrocław, 24.01.2023

Prof. dr hab. Jakub Nicpoń
Katedra i Klinika Chirurgii
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Wrocław, 24.01.2023

Dr wet. Agata Mikołajczyk-Martinez
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Uniwersytetu Przyrodniczy we Wrocławiu

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A. Mikołajczyk-Martinez

Podpis

Wrocław, 24.01.2023

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A. Mikołajczyk-Martinez
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Wrocław, 24.01.2023

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Zakład Histologii i Embriologii
Wydział Weterynarii
Uniwersytet Przyrodniczy we Wrocławiu

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mój udział polegał na pobraniu materiału do badań histologicznych, wykonaniu preparatów histologicznych oraz ich analizie.

KIEROWNIK ZAKŁADU HISTOLOGII I EMBRIOLOGII
dr hab. Piotr Kuropka prof. nadzw.
Podpis

Wrocław, 24.01.2023

Prof. dr hab. Zdzisław Kiełbowicz
Katedra i Klinika Chirurgii
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KIEROWNIK
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Uniwersytetu Przyrodniczego we Wrocławiu
Prof. dr hab. Zdzisław Kiełbowicz

Podpis

Wrocław, 24.01.2023

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KIEROWNIK
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Uniwersytetu Przyrodniczego we Wrocławiu
Prof. dr hab. Zdzisław Kiełbowicz

Podpis

Wrocław, 24.01.2023

Prof. dr hab. n. med. Wojciech Kielan
Klinika i Katedra
Chirurgii Ogólnej i Onkologicznej
Uniwersytetu Medycznego we Wrocławiu

OŚWIADCZENIE

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mój udział polegał na istotnym wkładzie intelektualnym w powstanie manukryptu.

Podpis

Uniwersytet Medyczny we Wrocławiu
II KATEDRA I KLINIKA
CHIRURGII OGÓLNEJ
I CHIRURGII ONKOLOGICZNEJ
Kierownik
prof. dr hab. Wojciech Kielan

Wrocław, 24.01.2023

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Podpis

Uniwersytet Medyczny we Wrocławiu
I KATEDRA I KLINIKA
CHIRURGII OGÓLNEJ
I CHIRURGII ONKOLOGICZNEJ
kierownik
prof. dr hab. Wojciech [illegible]

Wrocław, 21.03.2023

Prof. dr hab. n. med. Wojciech Kielan
Klinika i Katedra
Chirurgii Ogólnej i Onkologicznej
Uniwersytetu Medycznego we Wrocławiu

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Uniwersytet Medyczny we Wrocławiu
II KATEDRA I KLINIKA
CHIRURGII OGÓLNEJ
I CHIRURGII ONKOLOGICZNEJ
kierownik
prof. dr hab. Wojciech Kielan

Wrocław, 21.03.2023

Dr n.med. Kacper Zieliński
Klinika Anestezjologii I intensywnej Terapii
Uniwersytecki Szpital Kliniczny we Wrocławiu

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na mój udział polegał na stworzeniu modelu teoretycznego badania oraz uczestnictwie w przeprowadzonym eksperymencie.

Kacper Zieliński
dr n. med.
3846195

Podpis

Wrocław, 21.03.2023

Dr n.med. Kacper Zieliński
Klinika Anestezjologii I intensywnej Terapii
Uniwersytecki Szpital Kliniczny we Wrocławiu

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mój udział polegał na współprzygotowaniu artykułu i wkładzie intelektualnym w powstanie artykułu.

Kacper Zieliński
dr n. med.
3846195

Podpis

Wrocław, 21.03.2023

Dr n.med. Kacper Zieliński
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Podpis Zieliński dr n. med. 3846195

Wrocław, 21.03.2023

Joanna Kulas
Katedra Biochemii i Biologii Molekularnej
Wydział Weterynarii
Uniwersytet Przyrodniczy we Wrocławiu

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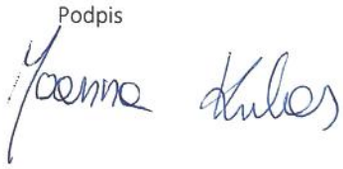
mój udział polegał na przygotowaniu projektu graficznego.

Podpis



Wrocław, 26.04.2023

Dr inż. Paweł Migdał

Katedra Higieny Środowiska

i Dobrostanu Zwierząt

Uniwersytet Przyrodniczy we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy:

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Front Oncol. 2022; 12: 927714. Published online 2022 Jun 29. doi: 0.3389/fonc.2022.927714 PMCID: PMC9278806 PMID: 35847916

mój udział polegał na analizie laboratoryjnej i pozyskiwaniu danych.

Podpis

11. SCIENTIFIC ACHIEVEMENTS

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asystent
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ORCID: 0000-0001-9765-7508

h-index: 1	liczba cytowań*: 2	Źródło: Web of Science
h-index: 0	liczba cytowań*: 0	Źródło: Google Scholar
h-index: 1	liczba cytowań*: 2	Źródło: Scopus

Σ IF* 19,965

Biogram

Publikacje

2022

Artykuły z czasopism

Diakun Agata, Khosrawipour Tanja, Mikolajczyk-Martinez Agata *[i in.]* : In-vivo thermodynamic exploration of gas-based intraperitoneal hyperthermia, Frontiers in Oncology, 2022, vol. 12, art.925724 [9 s.]. DOI:10.3389/fonc.2022.925724

Diakun Agata, Khosrawipour Tanja, Mikolajczyk-Martinez Agata *[i in.]* : Safety, feasibility, and application of intraperitoneal gas-based hyperthermia beyond 43°C in the treatment of peritoneal metastasis: an in-vivo pilot study, Frontiers in Oncology, 2022, vol. 12, art.953920 [9 s.]. DOI:10.3389/fonc.2022.953920

Diakun Agata, Khosrawipour Tanja, Mikolajczyk-Martinez Agata *[i in.]* : The onset of in-vivo dehydration in gas -based intraperitoneal hyperthermia and its cytotoxic effects on colon cancer cells, Frontiers in Oncology, 2022, vol. 12, art. 927714 [9 s.]. DOI:10.3389/fonc.2022.927714

Thelen Simon, MikolajczykMartinez Agata, Diakun Agata *[i in.]* : Evaluating the concept of gasbased intraperitoneal hyperthermia beyond 43°C in the treatment of peritoneal metastasis: a pilot study, Experimental and Therapeutic Medicine, 2022, vol. 24, nr 6, art.752 [9 s.]. DOI:10.3892/etm.2022.11687

Rozdziały z monografii

Kielan Wojciech, Marek Grzegorz, Diakun Agata *[i in.]* : Zakażenia chirurgiczne, W: Przegląd piśmiennictwa chirurgicznego 2021 / Dziki Adam (*red.*), 2022, Łódź, Stowarzyszenie Popierania Rozwoju Proktologii, s.198-203, ISBN 978-83-941338-3-2

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2021

Rozdziały z monografii

Kielan Wojciech, Marek Grzegorz, Frejlich Ewelina *[i in.]* : Zakażenia chirurgiczne, W: Przegląd piśmiennictwa chirurgicznego 2020 / Dziki Adam (*red.*), 2021, Łódź, Stowarzyszenie Popierania Rozwoju Proktologii, s.207-211, ISBN 978-83-941338-3-2

Abstrakt (praca oryginalna opublikowana w streszczeniach)

Hap Wojciech, Zawadzki Marcin, Diakun Agata *[i in.]* : Czynniki ryzyka zakażeń rany pooperacyjnej w chirurgii raka jelita grubego, W: 70. Jubileuszowy Kongres Towarzystwa Chirurgów Polskich. Toruń, 15-18.09.2021. Streszczenia, 2021, s.200

Hap Wojciech, Zawadzki Marcin, Diakun Agata *[i in.]* : Protokół ERAS w laparoskopowych resekcjach jelita grubego z powodu raka jest bezpieczny i skraca czas hospitalizacji, W: 70. Jubileuszowy Kongres Towarzystwa Chirurgów Polskich. Toruń, 15-18.09.2021. Streszczenia, 2021, s.56

2020

Artykuły z czasopism

Dzierżek Przemysław, Kurnol Krzysztof, Hap Wojciech *[i in.]* : Assessment of changes in body composition measured with bioelectrical impedance in patients operated for pancreatic, gastric and colorectal cancer, Polski Przegląd Chirurgiczny, 2020, vol. 92, nr 2, s.8-11. **DOI:10.5604/01.3001.0013.7951**

Rozdziały z monografii

Grzebieniak Zygmunt, Diakun Agata, Frejlich Ewelina *[i in.]* : Zakażenia chirurgiczne, W: Przegląd piśmiennictwa chirurgicznego 2019 / Dziki Adam (*red.*), 2020, Łódź, Stowarzyszenie Popierania Rozwoju Proktologii, s.205-216, ISBN 978-83-941338-3-2

2019

Rozdziały z monografii

Grzebieniak Zygmunt, Zawadzki Marcin, Diakun Agata *[i in.]* : Zakażenia chirurgiczne, W: Przegląd piśmiennictwa chirurgicznego 2018 / Dziki Adam (*red.*), 2019, Stowarzyszenie Popierania Rozwoju Proktologii, s.211-217, ISBN 978-83-941338-3-2

Abstrakt (praca oryginalna opublikowana w streszczeniach)

Hap Wojciech, Rudno-Rudzińska Julia, Frejlich Ewelina *[i in.]* : ERAS and non-ERAS protocols in colorectal cancer surgery comparison of complications with Clavien-Dindo classification, W: 48th World Congress of Surgery - WCS 2019 incorporating the 69th Congress of the Association of Polish Surgeons (APS). Krakow, Poland, 11-15 August 2019. Abstract book, 2019, s.poz.124.01

Hap Wojciech, Rudno-Rudzińska Julia, Frejlich Ewelina *[i in.]* : ERAS complications may be falsified by technical errors caused by insufficient experience of surgeons in laparoscopy, W: 48th World Congress of Surgery - WCS 2019 incorporating the 69th Congress of the Association of Polish Surgeons (APS). Krakow, Poland, 11-15 August 2019. Abstract book, 2019, s.poz.124.02

Hap Wojciech, Frejlich Ewelina, Kurnol Krzysztof *[i in.]* : Specific ERAS problems in gastric cancer based on the observation of colorectal patients, W: 13th International Gastric Cancer Congress "Building bridges between the medical communities involved in gastric cancer treatment worldwide". Prague, Czech Republic, 8-11 May 2019. Abstracts [online], 2019, s.poz.P.07-338-Fri

Kurnol Krzysztof, Diakun Agata, Rudno-Rudzińska Julia *[i in.]* : Prognostic value of tesmin expression in gastric cancer, W: 13th International Gastric Cancer Congress "Building bridges between the medical communities involved in gastric cancer treatment worldwide". Prague, Czech Republic, 8-11 May 2019. Abstracts [online], 2019, s.poz.P.02-071-Thu

Żebrowska P., Karpiński Paweł, Hap Wojciech *[i in.]* : Analysis of presence of viral DNA in sporadic gastric tumors, W: 13th International Gastric Cancer Congress "Building bridges between the medical communities involved in gastric cancer treatment worldwide". Prague, Czech Republic, 8-11 May 2019. Abstracts [online], 2019, s.poz.P.03-149-Thu

2018

Rozdziały z monografii

Abstrakt (praca oryginalna opublikowana w streszczeniach)

Rudno-Rudzińska Julia, Frejlich Ewelina, Zawadzki Marcin *[i in.]* : Irreversible electroporation - sophisticated palliative method, W: Third Triangle Scientific Meeting of the Japan-Hungary-Poland Surgical Society - Semmelweis Symposium "Clean hands save life". Budapest, Hungary, June 3-5, 2018. Program & abstracts, 2018, s.140 poz.P5-7