Evaluating the concept of gas-based intraperitoneal hyperthermia beyond 43˚C in the treatment of peritoneal metastasis: A pilot study

SIMON THELEN[1*], AGATA MIKOLAJCZYK-MARTINEZ[2*], AGATA DIAKUN[3], TANJA KHOSRAWIPOUR[4], KACPER ZIELINSKI[5], JAKUB NICPOŃ[6], ZDZISŁAW KIEŁBOWICZ[6], PRZEMYSŁAW PRZĄDKA[6], BARTŁOMIEJ LISZKA[6], PIOTR KUROPKA[7], SHIRI LI[8], HIEN LAU[9], WOJCIECH KIELAN[3] and VERIA KHOSRAWIPOUR[3,10]

[1]Department of Orthopedics and Trauma Surgery, Medical Faculty, Heinrich-Heine University Dusseldorf, D-40225 Duesseldorf, Germany; [2]Department of Biochemistry and Molecular Biology, Faculty of Veterinary Sciences, Wroclaw University of Environmental and Life Sciences, 50-375 Wroclaw; [3]2nd Department of General Surgery and Surgical Oncology, Wroclaw Medical University, 50-367 Wroclaw, Poland; [4]Department of Surgery (A), University-Hospital Düsseldorf, Medical Faculty, Heinrich-Heine University Dusseldorf, D-40225 Düsseldorf, Germany; [5]Department of Anesthesiology, Wroclaw Medical University, 50-367 Wroclaw; [6]Department of Surgery, Faculty of Veterinary Sciences, Wroclaw University of Environmental and Life Sciences, 50-366 Wroclaw; [7]Department of Biostructure and Animal Physiology, Wroclaw University of Environmental and Life Sciences, 50-375 Wroclaw, Poland; [8]Division of Colon and Rectal Surgery, Department of Surgery, New York Presbyterian Hospital-Weill Cornell College of Medicine, New York, NY 10065; [9]Department of Surgery, University of California, Irvine, CA 92868, USA; [10]Department of Surgery, Petrus-Hospital Wuppertal, D-42283 Wuppertal, Germany

Received June 6, 2022; Accepted September 8, 2022

DOI: 10.3892/etm.2022.11687

Abstract. While hyperthermic intraperitoneal applications have demonstrated high efficacy in treating peritoneal metastases (PM), these applications are limited to temperatures of 41-43˚C to prevent a harmful increase in core temperature. However, since gaseous substances display low specific heat capacities, gas-based hyperthermia could potentially increase surface temperatures without affecting the body's core temperature. To the best of our knowledge, the present study is the first to explore the *in vivo* feasibility of gas-based hyperthermia via spatial and time-based distribution. In the present study, a temperature-isolated, abdominal box model was created with fresh peritoneal tissue exposed to continuous high-volume airflow temperatures ranging between 47 and 69˚C. Heat conduction within the peritoneal tissues was measured using temperature microsensors. Temperature build-up at different time points during the procedure was calculated and the safest option to perform gas-based intraperitoneal hyperthermia beyond 43˚C was identified using an *in vivo* swine model. In subsequent experiments, viability and cytotoxicity of HT-29 colon cancer cells were measured following short-term hyperthermia. The present study demonstrated that the application of gas-based intraperitoneal hyperthermia with temperatures up to 50˚C is possible without increasing the core temperature to harmful levels. Gas-based intraperitoneal hyperthermia can induce a histological reaction on the peritoneal surface, and it can also result in decreased viability and increased cytotoxicity of HT-29 cells. The concept of extreme hyperthermia may be of great clinical importance as it could significantly increase local cytotoxicity in PM without increasing the body's core temperature. Further studies are required to investigate the benefits, as well as the restrictions, of this novel concept.

Correspondence to: Dr Tanja Khosrawipour, Department of Surgery (A), University-Hospital Düsseldorf, Medical Faculty, Heinrich-Heine University Düsseldorf, 5 Moorenstrasse, D-40225 Düsseldorf, Germany
E-mail: tkhosrawipour@gmail.com

*Contributed equally

Key words: hyperthermia, peritoneal metastasis, intraperitoneal, chemotherapy, colorectal cancer

Introduction

With many unsuccessful attempts in designing novel therapeutic approaches, advanced peritoneal metastasis (PM) remains one of the key challenges in current surgical oncology. Many known concepts have demonstrated only little improvement, especially regarding the outcome of advanced, unresectable PM (1-5). Hyperthermic intraperitoneal chemotherapy (HIPEC) in combination with cytoreductive surgery

(CRS) has raised hopes for a potentially curative treatment in patients with limited disease progression (6). Until today, effective hyperthermic intraperitoneal chemotherapy remains limited to temperatures of 42-43˚ Celsius (C). During HIPEC procedures, hyperthermic liquid chemotherapy is introduced into the abdominal cavity with the aim to cover organ surfaces, displaying only little distribution inhomogeneity (6). The inflow temperature, medium perfusate temperature and core body temperatures usually remain at around 40˚C (7). The relatively low temperature gradient between the HIPEC solution and core body temperature decreases the risk of overheating abdominal organs which could otherwise cause severe complications. A recent study by Goldenshluger *et al* (8) demonstrated, that increases in core body temperature were a positive predictor of postoperative complications in HIPEC procedures. To avoid complications associated with the application of heated fluid solutions, we believe that replacing a liquid-based heating system by an air-based heating system can be of great significance. The sensitivity of cancer cells to increasing hyperthermia has been extensively demonstrated (8-11), and hyperthermia has shown to increase the response rate of cancer cells to chemo- and radiotherapy (12-14). However, water-based solutions restrict any further temperature increase in hyperthermic solutions. H_2O has a heat-capacity of 4.186 kj/liter˚C which is the highest heat-capacity of any known substance. According to the rules of thermodynamics, high heat-capacities cause the transfer of significant heat-energy to any objects in close proximity. In contrast to water, air has a much lower heat capacity of around 0.718 kj/kg˚C, considering a density of 1.127 kg/m^3 at 40˚C and atmospheric pressure. Therefore, any close-range objects should retain their temperature for a longer time when surrounded by a medium with an over 5000-fold decreased heat capacity. In fact, we assume that there is a large temperature gradient between hyperthermic air and the superficial tissues. Thus, only the superficial layer is exposed to higher temperatures whereas deeper tissues remain unaffected. By means of this study, we aim to evaluate the feasibility of extreme hyperthermia for potential intraperitoneal treatment. To our knowledge, this was the first study to ever explore the hyperthermic signature, physical and structural effects on the peritoneal tissue of extreme hyperthermia as well as its feasibility in clinical applications. Our aim was to develop a reliable and sensitive model which incorporates important aspects such as regular heat transfer and heat conduction in an anatomical model (Fig. 1A). For research purposes, this model has been standardized for further analyses (Fig. 1B). After initial evaluation of this model, we also conducted the first *in vivo* experiment to evaluate the time and spatial heat signature in gas-based intraperitoneal hyperthermia beyond 43˚C.

Material and methods

Abdominal model and cavitary heat exposure. Tissue experiments were performed in an *ex vivo* model using commercially available porcine tissue samples (local pork supplier, Zerniki Wielkie). Fresh postmortem swine parietal peritoneum samples (5x5x8 cm) were placed at the bottom of a sealed and heat-isolated box (Fig. 1B). Two trocars, one of 5 mm and one of 12 mm diameter (Kii Balloon Blunt Tip System; Applied Medical Resources Corporation, Rancho Santa Margarita, USA) were placed at the side and top of the box, respectively. The styropor box was additionally isolated with bubble wrap. The heat isolated box was placed in a warm water bath (Lighted Tissue Bath XH-1003, Gabe Court Manassas, USA). Sensitive miniature temperature probes (Digital thermometer, FisherbrandTM Tracebale, Pittsburgh, USA) were placed at multiple sites in the box (Fig. 1B). One was placed in the incoming tube (Probe 0), a second close to the peritoneal surface (Probe 1) and two further probes (Probe 2 at 2 mm and Probe 3 at 5 mm penetration) were placed within the peritoneum. The incoming airflow was kept constant at 15 liters per minute (l/min). Prior to entering the box, the air was directed through a separately heated water bath to regulate incoming air temperature at this flow rate. By means of an underlying heater, the temperature in the box was kept constant at an equilibrium of 37˚C. All temperature probes indicated a stable temperature for 5 min before experiments were conducted. Experiments were conducted three times for each temperature. The following temperatures were applied: 47˚C, 50˚C, 60˚C, 66˚ and 69˚C. Temperature increases at probes 1, 2 and 3 were measured for 1 h at an airflow of 15 l/min (Fig. 2A and B).

Close-range tissue heat exposure. Fresh postmortem small intestinal samples (12 cm length) were placed at the bottom of the box. The head of the temperature probe was placed inside the small intestinal lumen. Both sides of the lumen were closed. One group was treated with heated 0.9% saline at 68˚C, 70˚C and 72˚C by pouring the saline solution into the box. In the second group, the airstream was heated to 70˚C, directed through a tube and impacted the small intestinal samples at 1 cm distance and a flow rate of 15 l/min for a total of 50 sec. The temperature increase was measured using temperature probes (Fig. 2C).

In vivo swine model. The data used for this study is part of a larger *in vivo* study protocol on 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. For this study, data from three 65-day-old, ca. 50 kg swine were used. The swine received a diagnostic laparoscopy without surgical intervention, under a high-flow air stream at 15 l/min at 48˚C (Swine A), 49˚C (Swine B) and 50˚C (Swine C). Swine were premedicated prior to laparoscopy 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. Anesthesia was performed with Propofol at 1 mg/kg. Swine were intubated and further anesthesia was continued with isoflurane 1%. Additional analgesia was provided with fentanyl 2 μg/kg and crystalloid fluid at 0.2-0.3 μg/kg/min. Swine were placed in supine position. An infra-umbilical mini laparotomy was performed and another 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 (Fig. 2)

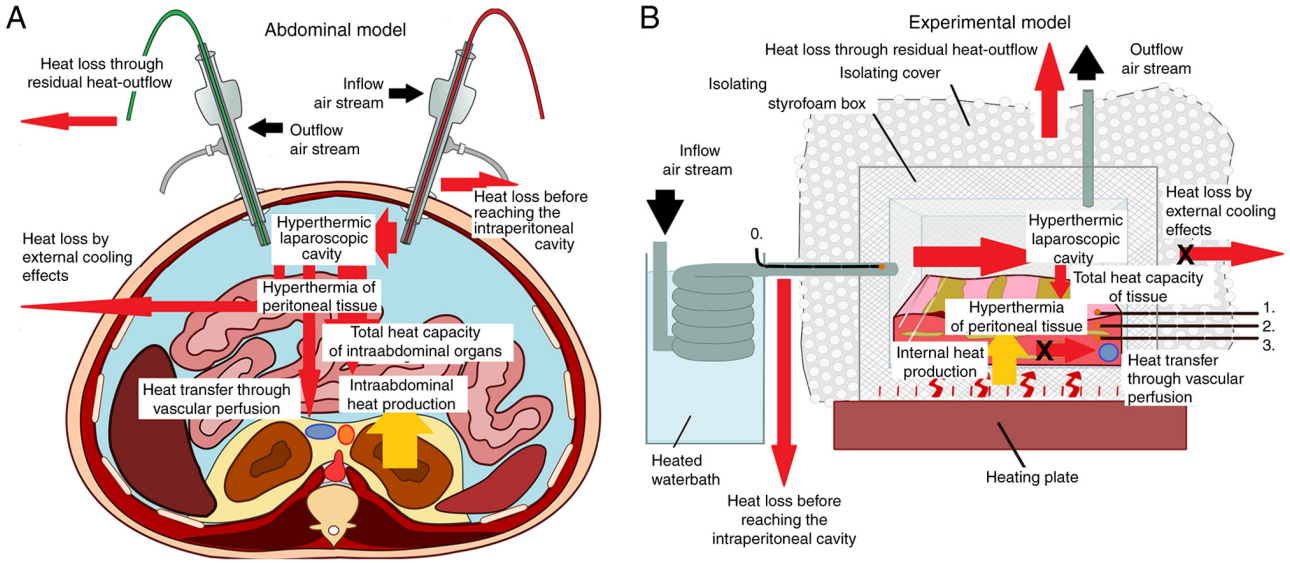

Figure 1. (A) Abdominal model of suspected heat transfer and conduction in a gas-based hyperthermic intervention. The intervention is based on a continuous stream of hyperthermic gas from two trocars with an in- and outflow. Expected heat loss and conduction is indicated by the predominantly red arrows. The yellow arrow indicates the continuous internal heat-production of the body regardless of the intervention. The heat conduction pathway is practically identical to the fluid model pathway. (B) Experimental model of suspected heat transfer and conduction in a gas-based hyperthermic intervention. Similarly to the previous abdominal model, this system is based on a continuous stream of hyperthermic gas with two trocars allowing in- and outflow. Expected heat loss and conduction is indicated by red arrows. Two red arrows with an X-mark demonstrate the heat transfer which is absent in the box model but present in the abdominal model (first conduction through blood stream and second external cooling and convection). The yellow arrow indicates the internal body heat production represented by the water bath which continues regardless of the intervention.

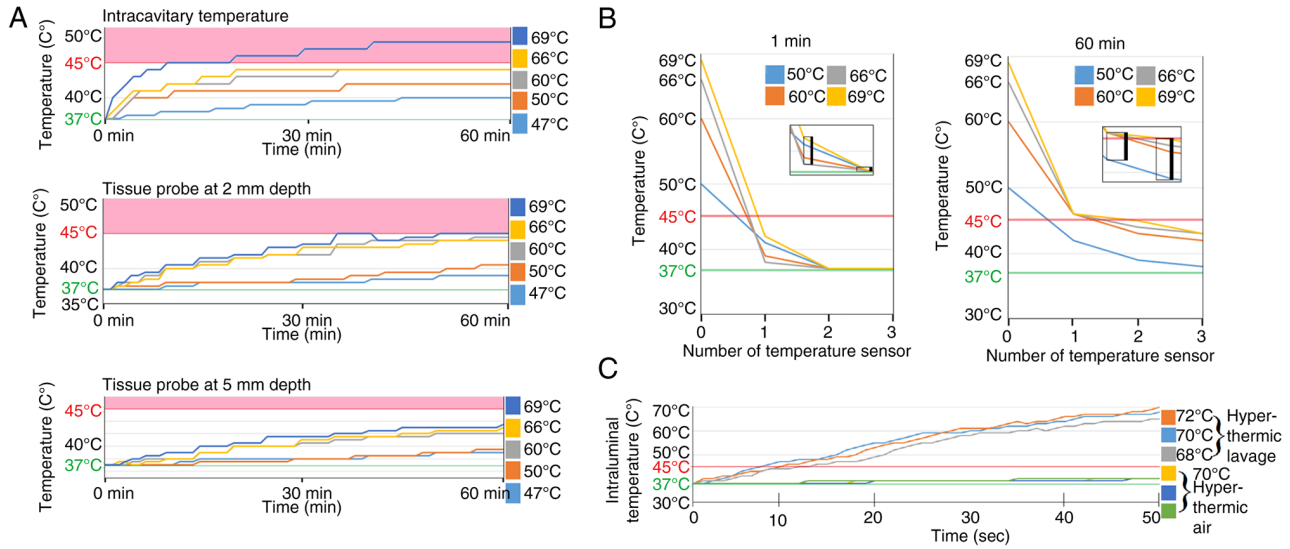

Figure 2. (A) Temperature development in the box model. Mean temperature from three independent measurements. Upper figure: Medium intracavitary temperature was measured three different times for the following listed inflow temperatures: 47˚C, 50˚C, 60˚C, 66˚C and 69˚C. The presented colored tissue curves represent the mean temperature of these three measurements for each listed temperature. Data shows the temperature increase during hyperthermic air insufflation in the experimental model. The temperature increase is constantly recorded over a period of 60 min. Middle figure: Mean temperature at 2 mm intraperitoneal tissue depth. Temperature increase during hyperthermic air insufflation in the experimental model is recorded at this location. Bottom figure: Mean intraperitoneal tissue temperature at 5 mm depth. Temperature increase is recorded during hyperthermic air insufflation at this location. (B) Mean temperature measurements at three different locations for the listed inflow temperatures: 50˚C, 60˚C, 66˚C and 69˚C. The colored tissue curves represent the mean temperature of these three measurements for each listed temperature. The mean temperature after starting hyperthermic insufflation at 1 and 60 min. The graphs demonstrate different insufflation temperatures of the incoming tube. Temperatures were measured at the insufflation point (0), cavity temperature 5 mm close to the peritoneum (1), temperature at 2 mm depth in the peritoneum (2) and at 5 mm depth into the peritoneum (3). (C) Development of the intraluminal temperature of small intestine following direct exposure to hyperthermic lavage or air at 70˚C for 50 sec. The temperature probe is in direct contact with the inner wall and about 2 mm of tissue separates the probe from direct exposure to the hyperthermic medium.

after insufflation. The abdominal cavity was insufflated with filtered room air through a tube entering the central 10 mm trocar. An initial diagnostic check-up was made via laparoscopic imaging via a 5 mm camera system (Karl Storz 5 mm/30˚ Laparoscope/Tuttlingen, Germany) through a 5 mm trocar. After visual confirmation, and placement of

multiple temperature sensors within the abdominal cavity the high-flow air stream was started at 15 l/min for a total of 45 min. Several temperature sensors were also placed outside the abdominal cavity. The temperature development was monitored continuously throughout the laparoscopic procedure. A total number of 9 temperature sensors were placed for the experiment. The location of these sensors was: (1) the inside of the inflow tube, (2) the inside of the outflow tube. One sensor was placed in the upper right quadrant (3), one in the upper left quadrant (4), and one in the lower abdomen (5). One sensor was placed directly on the peritoneum in the lower left quadrant (6). One sensor (7) was placed in the cystohepatic triangle, another was placed and taped on the skin of the abdomen/periumbilical (8) and a final one was placed in the esophageal area by the anesthesiologist.

Cell cultures. Human colorectal cancer cell line HT-29 was obtained from CLS (Cell Lines Service GmbH, Eppelheim, Germany). HT-29 cells were grown in Dulbecco's modified Eagle's medium (DMEM-high glucose, Sigma-Aldrich, Poznan, Poland) and supplemented with 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) in a humidified 5% CO_2 incubator (NuAire CO_2 Incubator, Biogenet, Warszawa, Poland) at 37˚C. Cells ($1.4x10^5$/well) were seeded in 24-well plates (TC Plate 24 Well, Standard, F, Sarstedt AG & Co. KG, Germany) and incubated for 48 h.

In vitro short-interval hyperthermia. Cells were seeded in 24-well plates at a concentration of $2x10^5$ cells/well in 1 ml of medium. After 48 h of incubation, medium was changed for 10 sec and replaced with 2 ml of heated medium at the following temperatures: 37˚C (control), 42˚C, 45˚C, 50˚C, 55˚C, 60˚C, 65˚C, 70˚C. After 10 sec medium was again replaced with 1 ml of medium heated to 37˚C and cells were incubated for another 24 h. For positive control, Oxaliplatin was used at a concentration of 1.2 mg/ml and added to the well for 1 h, then standard medium was applied and incubated for a further 23 h. Next, cytotoxicity and viability testing were performed. Before these experiments were conducted, a previous experiment was performed to ensure that heated medium maintained its temperature when placed in a 24 well plate for 10 sec (data not shown).

Analysis of in vitro effects of short-term hyperthermia using viability testing and cytotoxicity assay. An MTS test (colorimetric CellTiter 96® AQueous One Solution assay, Promega, Poland) was used to measure cell viability following heat or oxaliplatin treatment. The test was performed according to the manufacturer's instruction. Medium was removed from each well and replaced by 0.3 ml of fresh DMEM. Next, after 1 h of incubation at 37˚C and 5% CO_2, an MTS-based reagent was added to each well and absorbance was detected at 490 nanometer (nm) using a microplate reader (Tecan, Basel, Switzerland). Cells treated with medium heated to 37˚C were used as control. The percentage of viability was referenced to control for all groups. The extent of cytotoxicity caused by heat or oxaliplatin, respectively, was measured by release of lactate dehydrogenase (LDH) into the supernatants using Pierce LDH Cytotoxicity Assay Kit (Thermo Scientific). 50 μl of medium was taken from each well. The test was performed according to the manufacturer's protocol. Cytotoxicity levels were calculated as the percentage of LDH released from test samples cells compared to LDH released by lysis buffer treated cells and normalized to the spontaneous release from control cells. As reference, color reaction was measured spectrophotometrically on a microplate reader (Tecan, Basel, Switzerland) at 490 and 680 nm.

Statistical analysis. Tissue experiments have been performed three different times at the following inflow temperatures: 47˚C, 50˚C, 60˚C, 66˚C and 69˚C. The presented colored tissue curves represent the mean of these three temperature measurements for each exposed temperature. Cell experiments were repeated three different times. Each well was considered a single value, corresponding to the subgroups, meaning six wells were exposed to the same conditions in each experiment. A one-way ANOVA was used to compare independent groups. A post-hoc (Bonferroni) test was performed to confirm significance levels. $P<0.05$ was considered to indicate a statistically significant difference. Data are presented as the mean standard deviation unless otherwise indicated.

Results

Temperature in the experimental cavity. Data from the cavity probe showed a slow mean temperature increase when insufflation was performed at lower (47˚C) vs. higher temperatures (69˚C). After about 30 min, the temperature increase reached a plateau, or barely increased any further. This plateau is assumed to be the maximum achievable cavitary temperature. Our reference temperature of 45˚C is surpassed by the highest medium insufflation temperature of 69˚C. Data from the superficial tissue samples showed a slow medium temperature increase when insufflation was performed compared to data from the intracavitary sample. The mean temperature continuously increases and does not seem to reach a clear plateau within the observed timeframe. Our reference temperature at 45˚C is achieved but not surpassed by the highest insufflation temperature of 69˚C. Data from the deepest cavity sample (3) showed an even slower mean temperature increase compared to the previous two probes (1 and 2). Here again, the temperature increased to a plateau after about 30 min. This indicated that the maximum cavitary temperature was achieved. Our temperature reference of 45˚C was surpassed by the highest insufflation temperature of 69˚C.

Temperature measured at various time points and locations in the experimental model. A major temperature difference is detected when comparing air temperature of the incoming tube (0) with the cavity temperature. This difference decreases after one hour of hyperthermic insufflation. A further temperature decrease is noted when comparing sensors at the position 0 and 1 with those located at points 2 and 3. The temperature jump from point 0 to 1 is quite drastic while the jump from point 1 to 2 is less intense. Furthermore, the temperature at the furthest point 3 seems

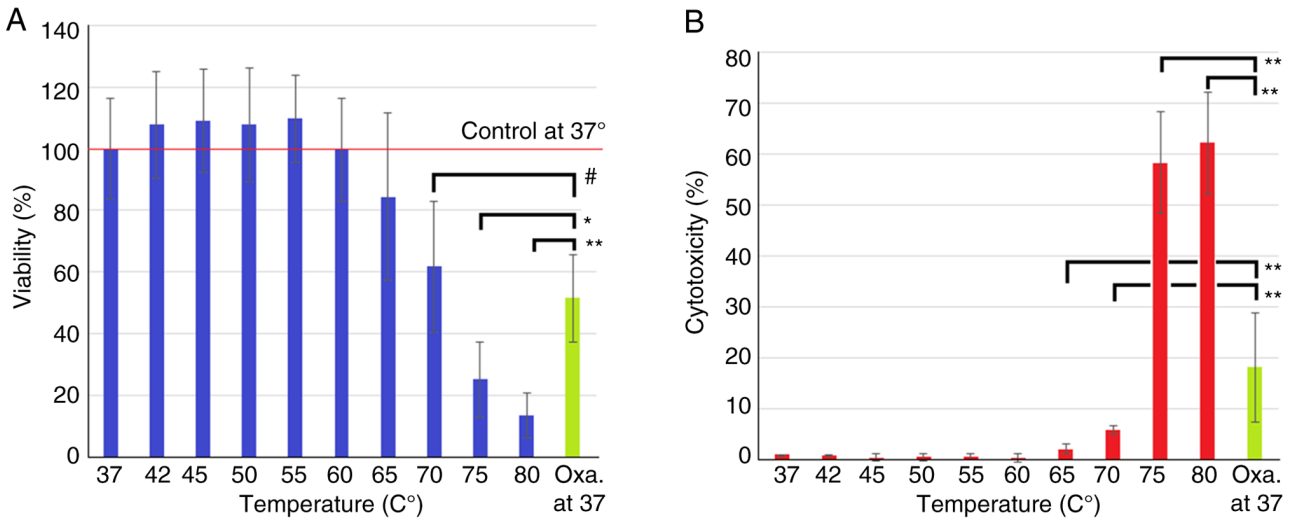

Figure 3. (A) Measuring cell viability following short-term hyperthermia. *In vitro* exposure of colorectal cancer cells (HT-29) to short-term hyperthermia (10 sec). Exposure to temperature levels from 37˚C (control) in 5˚C step increments until 80˚C. An additional control with Oxa. At 37˚C was conducted to compare vitality levels with chemotherapy. (B) Measuring cytotoxicity following hyperthermia: *In vitro* exposure of colorectal cancer (HT-29) cells to short-term hyperthermia (10 sec). Exposure to temperature levels of 37˚C (Control) in 5˚C step increments until 80˚C. An additional control with Oxa. At 37˚C was conducted to help compare vitality levels with chemotherapy. $^{\#}$P>0.05, *P<0.05, **P<0.01. Oxa., oxaliplatin; LDH, lactate dehydrogenase.

to stabilize within a temperature range above 37˚C but still below 45˚C. At 60 min, it seems as if a temperature equilibrium is reached within the tissue despite the large temperature difference at the incoming tube 0 (Fig. 3).

Results of short-term hyperthermia on small intestine via air and lavage. The performed experiments show that there is a significant difference in heat conduction between different media. While hyperthermic lavage rapidly heats up the entire tissue, exposure to hyperthermic air only slowly heats up deeper tissues. In the observed timeframe of 50 sec, the hyperthermic air curve appeared nearly flat despite exposure to high temperatures of 70˚C from the hot applied air stream (Fig. 2C). The direct hyperthermic temperature of 70˚C corresponds to the outer wall temperature of the small intestine and the inner wall temperature as recorded in Fig. 2C. A large temperature gradient can be created and maintained which leads to high surface temperatures while deeper tissues retain their original temperature.

Analyzing short-term in vitro hyperthermia on colon cancer cells using viability and cytotoxicity assays. The performed viability test shows that in a short-term exposure of 10 sec, significant effects on viability can be observed with temperatures of 70˚C and higher (Fig. 3A). Temperatures below 60˚C have no statistically significant effect. Viability decreases with temperatures of 65˚C and higher. Observed effects on viability increase with each temperature jump. Temperatures at 70˚C have similar effects on viability as oxaliplatin treatment. Temperatures beyond that, namely at 75˚ and 80˚C, outweigh the effects of Oxaliplatin treatment by far. The performed cytotoxicity results are similar to results in the viability tests. While temperatures below 60˚C do not seem to affect cytotoxicity (Fig. 3B), with temperatures of 65˚C and higher rapidly increasing signs of cytotoxicity were observed. At temperatures between 65˚ and 70˚C, cytotoxicity is significantly lower compared to oxaliplatin treatment. Temperatures between 75˚ and 80˚C have proven to be more toxic than oxaliplatin application.

Intraoperative temperature development during laparoscopy. From a technical point of view, the application of a high-flow constant airstream in the peritoneal cavity was possible (Fig. 4). No intraoperative or postoperative complications were observed. The total duration of 45 min under high flow was well tolerated. The mean intraoperative temperatures did not exceed 40˚C except at the inflow trocar (Fig. 5A). The highest measured mean temperature was at the cystohepatic triangle. No significant difference was observable within the applied temperatures in swine A, B and C at the measured sites. The mean intracavitary temperatures remained at around 35.9±0.95˚C (A), 36.2±0.99˚C (B), 36.5±1.3˚C (C) (Fig. 5B). No indications of critical intracavitary peaks were observed (Fig. 5C). The temperature fluctuations measured within the cavity remained within 3˚C. There were no postoperative problems within the observed time frame of 7 days post-surgery after which an autopsy was performed.

Histopathological examination of peritoneal tissue after high temperature exposure. After autopsy of the swine tissue samples were removed from multiple location of the peritoneum, areas that were exposed to the hyperthermic laparoscopic space were compared to unexposed peritoneal samples in the same swine (Fig. 6). Hematoxylin and eosin staining shows changes after one week after intraperitoneal hyperthermia. Peritoneal edema and an increase of white blood cell infiltration in the peritoneum was detected in the peritoneal tissue exposed to the laparoscopic cavity. Peritoneal tissue samples which were not exposed to the cavity did not show any specific changes, nor did they present signs of edema or infiltration by white blood cells.

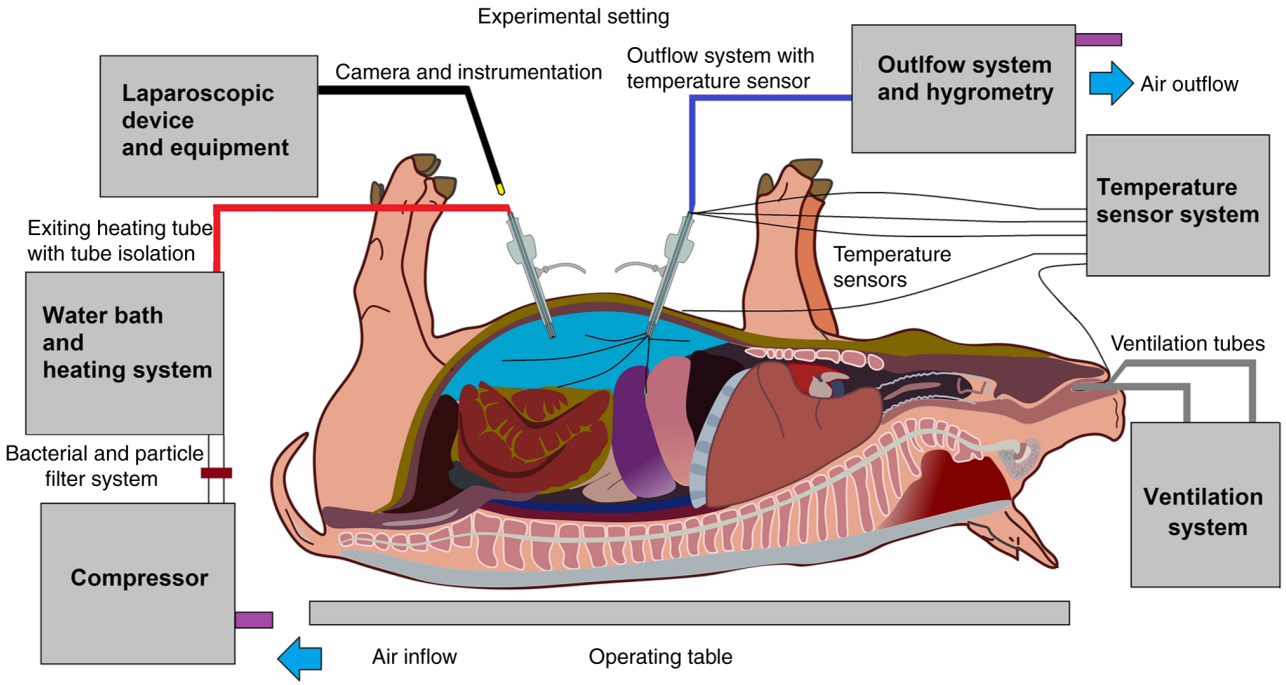

Figure 4. Experimental model of high-flow air-based hyperthermia in an *in vivo* laparoscopic setting. Similarly to the previous box-model, this system is based on a continuous stream of hyperthermic gas with at least two trocars allowing in- and outflow. Furter trocars are inserted to be able to place several temperature sensors at different locations within the abdominal cavity. All sensors and detection systems are monitored throughout the procedure.

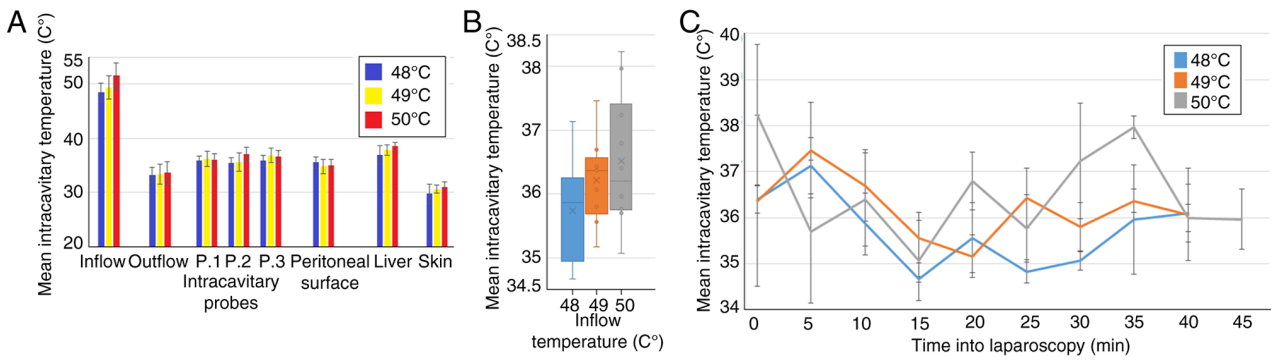

Figure 5. Temperature development during laparoscopy. (A) Mean measured temperatures at various location throughout the 45-min laparoscopy for each swine. (B) Mean intracavitary temperature for each swine throughout the 45 min laparoscopy (Including the locations P1(right upper abdomen), P2 (left upper abdomen) and P3 (lower abdomen). (C) Medium intracavitary temperature development during the 45-min laparoscopy for each swine.

Discussion

The concept of applying new physical principles and incorporating them into treatments for peritoneal metastases (PM) (15,16) and other surface malignancies (17-19) has been promising. Many concepts including irradiation (20-22), high-intensity ultrasound (23-25) and nanoparticles (26) or new substances (27,28) have been previously investigated for potential clinical use. Beside radiation, hyperthermia is probably the second most widely applied physical principle which is added to chemotherapeutic procedures. In fact, hyperthermia has demonstrated great efficacy in enhancing antitumoral effects when combined with chemotherapy or radiation, without causing disproportionate additional side effects (29-31). Until now, hyperthermia was usually applied via water-based fluid solutions, which display their own set of limitations due to the unique physical properties of water. However, based on the different physical properties of air, the same limitations do not apply when changing the carrier medium from water to air. The presented data indicates that a hyperthermic medium e.g. air could probably be introduced into a body cavity at temperatures exceeding far beyond 43˚C, without significantly heating up the abdominal cavity itself. Also, the core body temperature remains stable even at a high flow at 15 l/min with temperatures ranging between 48˚ to 50˚C. By means of our cell-model, we could demonstrate that hyperthermia is indeed cytotoxic. However, during the short period of exposure, HT-29 cells seemed to display high-resistance to temperatures below 60˚C. The changes observed on the peritoneal tissue are an indication that gas-based hyperthermia affects the peritoneal surface even if no major temperature peaks can be detected. The possible impact of this observation in PM treatment must

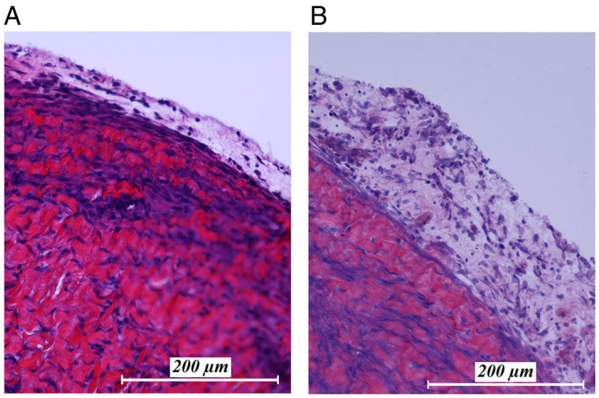

Figure 6. Histology of parietal peritoneal tissue; magnification, x100 (Eosin/Hematoxylin staining). (A) Untreated peritoneum and subperitoneal tissue. (B) Edematous peritoneum with infiltration on white blood cells.

be further studied. Until today, very limited *in vivo* data were available on hyperthermic insufflation beyond 43˚C during laparoscopy. In fact, the clinical research community has just developed some awareness on how the insufflation temperature may be a potentially relevant factor during laparoscopic procedures. Therefore, multiple studies in the last 10 years have tried to analyze the effect of normothermic, humidified CO_2 vs. cold dry CO_2 which is currently the standard in laparoscopic procedures. For instance, the use of normothermic, humidified CO_2 for pneumoperitoneum in laparoscopic procedures seems to be associated with reduced postoperative pain, lower risk of postoperative hypothermia, and lower analgesic requirements (32,33). A meta-analysis of current data performed by Dean *et al* (33) indicated that heated, humidified CO_2 insufflation during laparoscopic abdominal surgery can potentially improve intraoperative maintenance of normothermia when compared with cold dry CO_2. For decades, the applied, relatively mild hypothermia induced by CO_2 gas at room temperature (31) has been regarded as unproblematic. In fact, dry cold CO_2 will probably remain the worldwide standard in laparoscopy for the foreseeable future. With the core body temperature at around 38˚C, laparoscopy causes a temperature difference of 10-11˚C for procedures lasting up to hours. This de-facto hypothermic insufflation can be used as an example that an intracavitary temperature deviation of 10-11˚C and beyond may be well tolerated, possibly even in a hyperthermic setting. However, it has been recognized that there is still no experience and only little understanding of the effects of a hypo- or hyperthermic capnoperitoneum due to the physical challenges created by air as a carrier medium with an extremely low-heat capacity and unique physical qualities (30). However, more basic research should be conducted to improve the understanding and management of air-based hyperthermia. Furthermore, this novel concept should be studied further and evaluated. Possibly some skepticism and prejudice will have to be overcome to adapt to the thought that large volumes of air heated beyond 43˚C can be applied within the abdominal cavity without causing any measurable systemic side effects. Additionally, further studies are required to investigate if extreme hyperthermia can serve as an independent therapeutic option for PM treatment or whether it is rather more favorably applied as an add-on therapy in a setting with novel concepts of intraperitoneal chemotherapies (34-37). Intraperitoneal hyperthermia beyond temperatures of 43˚C is possible and might serve as a tool to revolutionize PM treatment by creating and temperature gradient along the peritoneal surface to reduce PM progression (38,39). However, applicational, biological and technical aspects of this novel approach must be further analyzed.

Acknowledgements

Not applicable.

Funding

This study was funded by institutional funds from the participating departments. No specific funds or grants were applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ST designed the study and drafted the manuscript. AMM designed the study, performed lab analysis and data acquisition. AD designed the study, performed lab analysis and data acquisition. TK made substantial contributions to conception and design, acquisition of data, drafted the manuscript and critically revised it for important intellectual content. JN designed the study, drafted the manuscript and critically revised it for important intellectual content. KZ designed the study, performed lab analysis and data acquisition. ZK performed lab analysis and data acquisition. PP designed the study, performed lab analysis and data acquisition. BL designed the study, performed lab analysis and data acquisition. PK performed histology, lab analysis and data acquisition. SL performed data acquisition and drafted the manuscript. HL performed data analyses and critically revised it for important intellectual content. WK performed data analyses and critically revised the manuscript for important intellectual content. VK supervised the study, performed lab analyses, conceptualized the study and drafted the manuscript. ST, TK, VK and AMM confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Experiments were approved (approval no. 030/2021/P2) by the local Board on Animal Welfare at Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Aoyagi T, Terracina KP, Raza A and Takabe K: Current treatment options for colon cancer peritoneal carcinomatosis. World J Gastroenterol 20: 12493-12500, 2014.
2. Glehen O, Kwiatkowski F, Sugarbaker PH, Elias D, Levine EA, De Simone M, Barone R, Yonemura Y, Cavaliere F, Quenet F, *et al*: Cytoreductive surgery combined with perioperative intraperitoneal chemotherapy for the management of peritoneal carcinomatosis from colorectal cancer: A multi-institutional study. J Oncol 22: 3284-3292, 2004.
3. Chu DZ, Lang NP, Thompson C, Osteen PK and Westbrook KC: Peritoneal carcinomatosis in nongynecologic malignancy. A prospective study of prognostic factors. Cancer 63: 364-367, 1989.
4. Köhne CH, Cunningham D, Di Costanzo F, Glimelius B, Blijham G, Aranda E, Scheithauer W, Rougier P, Palmer M, Wils J, *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 13: 308-317, 2002.
5. Verwaal VJ, van Ruth S, de Bree E, van Sloothen GW, van Tinteren H, Boot H and Zoetmulder FA: 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 21: 3737-3743, 2003.
6. Sugarbaker PH: Peritoneal metastases from gastrointestinal cancer. Curr Oncol Rep 20: 62, 2018.
7. Rettenmaier MA, Mendivil AA, Gray CM, Chapman AP, Stone MK, Tinnerman EJ and Goldstein BH: Intra-abdominal temperature distribution during consolidation hyperthermic intraperitoneal chemotherapy with carboplatin in the treatment of advanced stage ovarian carcinoma. Int J Hyperthermia 31: 396-402, 2015.
8. Goldenshluger M, Zippel D, Ben-Yaacov A, Dux J, Yalon T, Zendel A, Rayman S, Mor E, Berkenstadt H, Fogel-Grinvald H, *et al*: Core body temperature but not intraabdominal pressure predicts postoperative complications following closed-system hyperthermic intraperitoneal chemotherapy (HIPEC) administration. Ann Surg Oncol 25: 660-666, 2018.
9. de Andrade Mello P, Bian S, Savio LEB, Zhang H, Zhang J, Junger W, Wink MR, Lenz G, Buffon A, Wu Y and Robson SC: Hyperthermia and associated changes in membrane fluidity potentiate P2X7 activation to promote tumor cell death. Oncotarget 8: 67254-67268, 2017.
10. Li DY, Tang YP, Zhao LY, Geng CY and Tang JT: Antitumor effect and immune response induced by local hyperthermia in B16 murine melanoma: Effect of thermal dose. Oncol Lett 4: 711-718, 2012.
11. Zivanovic O, Chi DS, Filippova O, Randall LM, Bristow RE and O'Cearbhaill RE: It's time to warm up to hyperthermic intraperitoneal chemotherapy for patients with ovarian cancer. Gynecol Oncol 151: 555-561, 2018.
12. Muckle DS and Dickson JA: The selective inhibitory effect of hyperthermia on the metabolism and growth of malignant cells. Br J Cancer 25: 771-778, 1971.
13. Seifert G, Budach V, Keilholz U, Wust P, Eggert A and Ghadjar P: Regional hyperthermia combined with chemotherapy in paediatric, adolescent and young adult patients: Current and future perspectives. Radiat Oncol 11: 65, 2016.
14. Sugarbaker PH: Laboratory and clinical basis for hyperthermia as a component of intracavitary chemotherapy. Int J Hyperthermia 23: 431-442, 2007.
15. Khosrawipour T, Schubert J, Kulas J, Migdal P, Arafkas M, Bania J and Khosrawipour V: 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 11: 1308-1314, 2020.
16. Khosrawipour V, Reinhard S, Martino A, Khosrawipour T, Arafkas M and Mikolajczyk A: Increased tissue penetration of doxorubicin in pressurized intraperitoneal aerosol chemotherapy (PIPAC) after High-Intensity Ultrasound (HIUS). Int J Surg Oncol 2019: 6185313, 2019.
17. Schubert J, Khosrawipour T, Pigazzi A, Kulas J, Bania J, Migdal P, Arafkas M and Khosrawipour V: 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 25: 4813-4819, 2019.
18. Mikolajczyk A, Khosrawipour V, Schubert J, Plociennik M, Nowak K, Fahr C, Chaudhry H and Khosrawipour T: Feasibility and characteristics of pressurized aerosol chemotherapy (PAC) in the bladder as a therapeutical option in early-stage urinary bladder cancer. In Vivo 32: 1369-1372, 2018.
19. Khosrawipour V, Mikolajczyk A, Paslawski R, Plociennik M, Nowak K, Kulas J, Arafkas M and Khosrawipour T: Intrathoracic aerosol chemotherapy via spray-catheter. Mol Clin Oncol 12: 350-354, 2020.
20. Khosrawipour V, Bellendorf A, Khosrawipour C, Hedayat-Pour Y, Diaz-Carballo D, Förster E, Mücke R, Kabakci B, Adamietz IA and Fakhrian K: 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 30: 593-597, 2016.
21. Khosrawipour V, Giger-Pabst U, Khosrawipour T, Pour YH, Diaz-Carballo D, Förster E, Böse-Ribeiro H, Adamietz IA, Zieren J and Fakhrian K: Effect of irradiation on tissue penetration depth of doxorubicin after pressurized intra-peritoneal aerosol chemotherapy (PIPAC) in a novel ex-vivo model. J Cancer 7: 910-914, 2016.
22. Khosrawipour V, Khosrawipour T, Hedayat-Pour Y, Diaz-Carballo D, Bellendorf A, Böse-Ribeiro H, Mücke R, Mohanaraja N, Adamietz IA and Fakhrian K: 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 37: 1677-1680, 2017.
23. Mikolajczyk A, Khosrawipour V, Schubert J, Grzesiak J, Chaudhry H, Pigazzi A and Khosrawipour T: Effect of liposomal doxorubicin in pressurized intra-peritoneal aerosol chemotherapy (PIPAC). J Cancer 9: 4301-4305, 2018.
24. Mikolajczyk A, Khosrawipour T, Kulas J, Migdal P, Arafkas M, Nicpon J and Khosrawipour V: The structural effect of high intensity ultrasound on peritoneal tissue: A potential vehicle for targeting peritoneal metastases. BMC Cancer 20: 481, 2020.
25. Mikolajczyk A, Khosrawipour T, Martino A, Kulas J, Pieczka M, Zacharski M, Nicpon J and Khosrawipour V: 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: 9679385, 2020.
26. Mikolajczyk A, Khosrawipour V, Kulas J, Kocielek K, Migdal P, Arafkas M and Khosrawipour T: Release of doxorubicin from its liposomal coating via high intensity ultrasound. Mol Clin Oncol 11: 483-487, 2019.
27. Mikolajczyk A, Khosrawipour V, Lau H, Li S, Migdal P, Labbe MK, Kielan W, Nicpon J, Stieglitz S and Khosrawipour T: Exploring the potential of taurolidine in inducing mobilisation and detachment of colon cancer cells: A preliminary in-vitro study. BMC Pharmacol Toxicol 23: 38, 2022.
28. Schubert J, Khosrawipour V, Chaudhry H, Arafkas M, Knoefel WT, Pigazzi A and Khosrawipour T: 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 17: 93, 2019.
29. Wust P, Hildebrandt B, Sreenivasa G, Rau B, Gellermann J, Riess H, Felix R and Schlag PM: Hyperthermia in combined treatment of cancer. Lancet Oncol 3: 487-497, 2002.
30. Cihoric N, Tsikkinis A, van Rhoon G, Crezee H, Aebersold DM, Bodis S, Beck M, Nadobny J, Budach V, Wust P and Ghadjar P: Hyperthermia-related clinical trials on cancer treatment within the ClinicalTrials.gov registry. Int J Hyperthermia 31: 609-614, 2015.
31. Kok HP, Wust P, Stauffer PR, Bardati F, van Rhoon GC and Crezee J: Current state of the art of regional hyperthermia treatment planning: A review. Radiat Oncol 10: 196, 2015.
32. Sajid MS, Mallick AS, Rimpel J, Bokari SA, Cheek E and Baig MK: Effect of heated and humidified carbon dioxide on patients after laparoscopic procedures: A meta-analysis. Surg Laparosc Endosc Percutan Tech 18: 539-546, 2008.
33. Dean M, Ramsay R, Heriot A, Mackay J, Hiscock R and Lynch AC: Warmed, humidified CO_2 insufflation benefits intraoperative core temperature during laparoscopic surgery: A meta-analysis. Asian J Endosc Surg 10: 128-136, 2017.

34. Binda MM: Humidification during laparoscopic surgery: Overview of the clinical benefits of using humidified gas during laparoscopic surgery. Arch Gynecol Obstet 292: 955-971, 2015.
35. Khosrawipour T, Schubert J, Khosrawipour V, Chaudhry H, Grzesiak J, Arafkas M and Mikolajczyk A: 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 17: 4921-4927, 2019.
36. Schubert J, Khosrawipour T, Reinhard S, Arafkas M, Martino A, Bania J, Pieczka M, Pigazzi A and Khosrawipour V: The concept of foam as a drug carrier for intraperitoneal chemotherapy, feasibility, cytotoxicity and characteristics. Sci Rep 10: 10341, 2020.
37. Mikolajczyk A, Khosrawipour V, Schubert J, Chaudhry H, Pigazzi A and Khosrawipour T: Particle stability during pressurized intra-peritoneal aerosol chemotherapy (PIPAC). Anticancer Res 38: 4645-4649, 2018.
38. Diakun A, Khosrawipour T, Mikolajczyk-Martinez A, Nicpoń J, Kiełbowicz Z, Prządka P, Liszka B, Kielan W, Zielinski K, Migdal P, *et al*: The onset of in-vivo dehydration in gas-based intraperitoneal hyperthermia and its cytotoxic effects on colon cancer cells. Front Oncol 12: 927714, 2022.
39. Diakun A, Khosrawipour T, Mikolajczyk-Martinez A, Kuropka P, Nicpoń J, Kiełbowicz Z, Prządka P, Liszka B, Li S, Lau H, *et al*: In-vivo thermodynamic exploration of gas-based intraperitoneal hyperthermia. Front Oncol 12: 92572, 2022.