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ORIGINAL ARTICLE

Multimodal approach for treatment of peritoneal surface malignancies in a tumour-bearing rat model

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Abstract
Purpose Surgical cytoreduction of peritoneal surface malignancy of colorectal origin in combination with hyperthermic intraoperative peritoneal chemotherapy (HIPEC) has become an established treatment approach. Only a few of animal models for scientific research on various therapeutic strategies have been described yet. The feasibility of an established rat model with a peritoneal surface malignancy from colorectal origin for treatment investigation should be examined in this study.
Methods Peritoneal surface malignancy of colonic origin was induced in 90 male BD IX rats. Animals were randomised into six groups (15 animals per one control and five treatment groups). One treatment group underwent only surgical debulking. The animals of the other four treatment groups received additional interventions: hyperthermic intraperitoneal chemotherapy with mitomycin or gemcitabine, photodynamic therapy or taurolidine lavage. Twenty-one days after treatment, the intraperitoneal status was investigated. Tumour weight, count of tumour nodules and experimental Peritoneal Carcinosis Index (ePCI) were detected.
Results Extended surgical cytoreduction and additional treatments including HIPEC were feasible in this rat model. All treatment groups had a significant lower tumour weight, account of tumour nodes and ePCI if compared with the control group. Comparing the additional therapies only HIPEC with mitomycin lead to relevant tumour reduction after surgery.
Conclusion This rat model is suitable for research on the multimodal treatment of peritoneal malignancies. A persisting cytoreductive effect of surgical tumour debulking could be proven. Only additional HIPEC therapy with mitomycin showed a significant tumour reduction. This animal model provides the opportunity to investigate different therapeutic strategies.

Keywords Peritoneal surface malignancy · Hyperthermic intraperitoneal chemotherapy · HIPEC · Animal model

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Introduction

A manifest peritoneal carcinomatosis is the limiting factor for patient's long-term survival. The median survival time can be prognosticated as 6 months from the date of diagnosis [1]. The development of a multimodal treatment strategy based on empirical data by Sugarbaker nearly 20 years ago lead to a considerable improvement in outcome for selected populations and tumour entities [2–5]. This therapy concept combines complete surgical peritoneal tumour reduction and hyperthermic intraoperative intraperitoneal chemotherapy (HIPEC) with different cytostatic agents [3, 4].

Few multimodal concepts are currently under research in animal models [6–8]. There is no model comparing the

different steps of treatment as already used in humans. However, only such a complex animal model allows for the detection of any differences between effects of surgical treatment and the additionally used therapies. Especially research on effects, dosage, toxicity and side effects of different cytostatic agents used for HIPEC has to take into consideration the complexity of the whole procedure. For treatment of peritoneal carcinomatosis from colorectal origin, the HIPEC procedure with mitomycin is currently the first choice and should therefore be investigated in animal models [9, 10]. Other cytostatic agents like gemcitabine have proven their efficacy in treatment of solid tumours [11, 12]. Furthermore, several other therapeutic options like taurolidine lavage or photodynamic therapy (PDT) might have an additional positive effect and could represent new approaches [13–15].

The aim of this trial was to examine the feasibility of an established animal model for research in the treatment of peritoneal carcinomatosis. This model should provide the opportunity to evaluate different therapies and their efficacies. The complete surgical cytoreduction should be performed as well as additional therapies including HIPEC with different cytostatic agents.

Materials and methods

Animals and tumour induction

Ninety male BD IX/HansHsd rats weighing between 170 and 280 g were obtained from a single breeding colony (Harlan Winkelmann, Borchen, Germany). Animals were individually housed and allowed free access to standard laboratory food and water ad libitum and 12 h of light cycle per day. Maintenance and care were carried out according to the guidelines of the local Animal Protection Commission and UKCCCR. This protocol was approved by the local Animal Protection Committee.

For tumour induction, 2×10^5 syngeneic colonic adenocarcinoma cells DHD/K12/TRb (European Collection of Cell Cultures, Salisbury, England) were implanted subperitoneal in the right upper quadrant via a median laparotomy of 2 cm in rats under general intraperitoneal anaesthesia. Tumour cell viability was assessed with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT method as recommended by the manufacturer (Sigma-Aldrich, Munich, Germany) and as described elsewhere [16].

Randomisation and treatment

All rats were randomised into six equal groups after implantation of tumour cells. Thereby group 1 (G1) served as control without any therapeutic intervention after tumour induction. All other groups (G2-6) underwent a surgical cytoreduction at day 21 (Fig. 1). Therefore, a standard median relaparotomy was performed in general intraperitoneal anaesthesia with xylazine (3.7 mg/kg body weight) and ketamine (66.5 mg/kg body weight). The complete resection of all visible tumour manifestations was aimed. Group 2 (G2) did not receive any further therapy. Following surgery, a HIPEC using 15 mg/m² body surface area mitomycin was performed in group 3 (G3) The body surface area of the animals was calculated according the formula BSA $(m^2)=0.007178\times\text{weight}\ (kg)^{0.425}\times\text{height}\ (cm)^{0.725}$.The HIPEC equipment consisted of a heat exchanger, a fluid reservoir and a roller pump with two synchronously running Masterflex® pumpheads on a single axis (Cole-Parmer Instrument Co., Vernon Hills, Illinois, USA) for inflow and outflow lines with a tube diameter of 6 mm. HIPEC was performed in an open 'coliseum' technique (Fig. 2). For temperature measurement, a 0.5 mm diameter thermoprobe was placed in the open abdominal cavity and a second one in the rectal cavity. The intraperitoneal temperature was maintained between 41.2°C and 42.3°C. Perfusion was performed over 60 min with a flow of about 60 ml/min. The mitomycin was applied in 250 ml saline solution. The dose was divided in halves. First dose was given at the start of the procedure and the second administration followed 30 min later. After accomplishing the HIPEC procedure, the perfusate was removed and abdominal cavity was rinsed with isotherme saline solution 0.9% for 5 min. For the treatment of group 5 (G5), the same procedure was performed with a single-dose gemcitabine (24 mg/kg body weight) in a 250 ml saline solution. Duration and temperature were kept as described above. The animals randomised into group 4 (G4) were treated after surgery with an intraperitoneal lavage of 250 ml Taurolin® 0.5% (Geistlich Pharma AG, Wolhusen, Switzerland). A continuous flow of 60 ml/min and a constant temperature of 37°C were ensured by the same test arrangement described above. The duration of lavage was set on 30 min. The animals of group 6 (G6) received 150 mg/kg body weight 5-aminolevulinic acid as a photosensitiser intravenously 6 h before relaparotomy. After completing the surgical tumour resection, a PDT was performed using the Visulas 630® diode-pumped continuous wave laser (Carl Zeiss, Jena, Germany) with a wavelength of 630 nm and maximum target power of 3.0 W. The duration of PDT duration was 2×10 min. An evenly distributed effective power in the illuminated field was ensured by the use of a microlensfibre (FDI-253®, Medlight SA, Switzerland). The laparotomy was closed in all animals after accomplishing surgery and additional intervention if applied with absorbable polyglactine sutures.

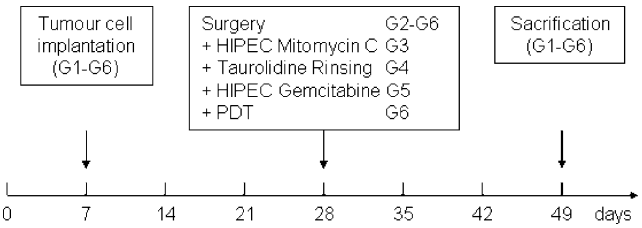

Fig. 1 Schedule of the trial

Assessment of therapy efficacy

Twenty-one days after the surgical procedure all animals were killed in a CO_2 chamber. After autopsy, tumour weight and spread classified with a modified experimental Peritoneal Cancer Index (ePCI) were assessed by two independent observers using magnification glasses. This ePCI was adapted to the modified classification by Steller et al. [17]. Therefore, the abdominal cavity was divided into four parts. In every quadrant, the score ranging from 0 to 5 was assessed. A score of 0 meant no visible tumour in a quadrant. A score of 1 indicated a tumour size from 0 to 0.5 cm, score of 2 from 0.5 to 1.0 cm, score of 3 indicated a tumour diameter from 1.0 to 2.0 cm, score of 4 indicated a tumour diameter from 2.0 to 3.0 cm and a tumour with a diameter that is larger than 3.0 cm was scored as 5. The results of all four quadrants were summarised. Therefore, the ePCI could range between 0 and 20. The tumourous tissue harvested during the autopsy was fixed for further histological investigation.

Statistical analysis

All data were collected prospectively in a relational database. Statistical analysis was performed using SPSS 15.0® for Windows®. Because of the small sample size, a normal distribution of continuous data could not be expected. Therefore, all continuous data are given as median and range. The two-sided non-parametric Mann–Whitney *U* test was used to detect differences between the groups. *P* values less than 0.05 were considered to be significant.

Results

All animals were treated according to the study protocol. No protocol violations took place. There were no problems in regards to the feasibility of tumour induction, surgical cytoreduction or the additional therapeutic regimen.

Overall, seven rats died before the date of planned death (mortality 8%). One death in group 4 was strictly related to the narcosis. Postoperative bleeding (n=2, G3+G5), postoperative mesenteric ischaemia (n=2, both G2) and ileus (n=2, G1+G3) were responsible for the remaining deaths. Overall, the therapeutic effects of the different treatment regimen could be investigated in 83 animals.

Tumour was successfully inducted in 88 animals. In only two rats, peritoneal carcinomatosis was not macroscopically visible. However, these animals (G4+G5) were treated as randomised. The mean peritoneal tumour weight was 1.4 g (0–23.5). The highest tumour load with 6.4 g (0.6–23.5) was detected in the control group. (Table 1) This value was significantly higher than the measured tumour weight in each treatment group. Within the treatment groups, there were no differences. The additional treatment in groups 3–6 did not lead to a reduction of tumour weight when compared with surgery alone (G2). In G3, the result tended to be lesser than in all other treatment groups with a narrow range, but the differences did not reach statistical significance (P=0.09; Fig. 3).

For all groups, the mean ePCI was 9 (0–20). In the control group, it was significantly higher than in all treatment groups (Table 1). There were no differences between G2 and G4–G6. G3 (HIPEC with mitomycin) had 4 (0–14), the lowest ePCI count. This result was significantly different from all other treatment groups (P=0.03; Fig. 4).

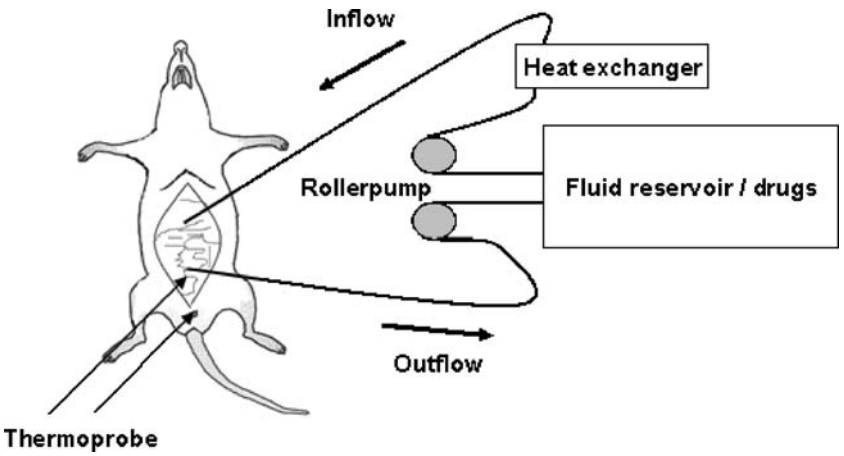

Fig. 2 Experimental setup for HIPEC

Table 1 Results of tumour weight and ePCI according to the treatment groups

Groups Treatment	G1 Control group –	G2 Surgery –	G3 Surgery HIPEC mitomycin	G4 Surgery Taurolidine	G5 Surgery HIPEC gemcitabine	G6 Surgery PDT
Number	15	14	13	12	14	15
Tumour weight (g)	6.4 (0.6–23.5)	1.4 (0–21.5)	0.4 (0–10.8)	1.3 (0–15.4)	1.5 (0–13.3)	0.3 (0–20)
ePCI	18 (8–20)	9 (0–20)	4 (0–14)	7 (0–20)	8 (0–20)	6 (0–20)

Data are given as median (range)

Discussion

Despite the increasing importance of surgical cytoreduction and additional HIPEC as a multimodal treatment of peritoneal carcinomatosis in clinical routine, there are only a few appropriate animal models for fundamental research described in the existing literature [6–8]. The present trial introduces a feasible experimental model of peritoneal carcinomatosis in a small animal model. Thereby it is for the first time possible to evaluate current clinical therapeutic strategies as well as novel interventional approaches in comparison with one another. The induction of peritoneal carcinomatosis in a rat model is well established [15, 18]. For the realisation of the study, we could use experiences in rat models for investigations on peritoneal malignancies [13, 19, 20]. The achieved tumour induction rate of 98% is high in comparison to other investigations [21, 22]. The perioperative mortality (8%, $n=7$) was not related to the postsurgical therapy. One rat died because of anaesthesiological complications. All other deaths were caused by intra- or postoperative surgical complications. As there is no other comparable data published yet, we consider this data to be acceptable. In clinical trials, the mortality differs between 2% and 10% if comparable treatment concepts are used [22–24].

The clinical treatment strategies introduced by Sugarbaker follow merely empirical grounds and theoretical considerations. In fact, several prospective clinical trials could verify the improvement of survival time when comparing aggressive multimodal strategies and previous palliative therapies [2, 5].

The first step of Sugarbaker's concept is the surgical cytoreduction aiming a complete resection of all visible tumour [25]. It has been shown earlier that this part of the procedure is reliably feasible in a rat model [21]. The second step is the intraperitoneal administration of hyperthermic cytostatic agents (HIPEC). Mitomycin C has become the standard chemotherapeutic drug for intraoperative intraperitoneal treatment of peritoneal carcinomatosis of colorectal [24]. The dosage of 15 mg/m² and mode of

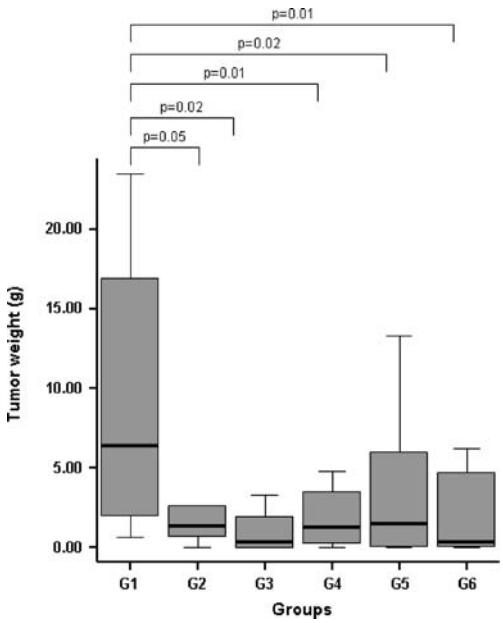

Fig. 3 Tumour weight 21 days after therapeutic intervention

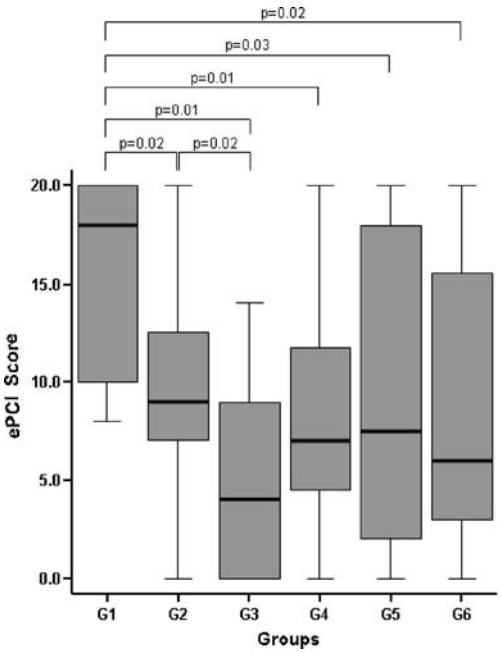

Fig. 4 ePCI 21 days after therapeutic intervention

administration with an aimed temperature of 42°C for 60 min comply with several clinical trials [24]. Because of its large molecule diameter, mitomycin is locally effective with simultaneously low systemic side effects. The effectiveness of the intraperitoneal administration has also been proven in animal models [9, 22].

The use of gemcitabine is not yet reported in multimodal treatment concepts. On the other hand, Ridwelski et al. could show relevant effects on solid intraperitoneal malignancies [12]. Taurolidine as another cytostatic agent has proven its capability in vitro and under clinical conditions [13, 14]. Therefore, we used in our study gemcitabine and taurolidine as drugs to be compared with the current standard. A further attempt for non-surgical peritoneal cytoreduction was made by Veenhuizen et al. They successfully used the photodynamic therapy for treatment of peritoneal malignancies [15]. This novel approach should also be compared with the standard mitomycin therapy in the current study. In all treatment groups, a reduced tumour mass was found when compared with the control group.

The investigated additional therapies achieved no further advantage than surgery alone. Also the ePCI was decreased in the treatment groups. Remarkable is the fact that only the additional HIPEC with mitomycin lead to a lower score than surgery alone. All other investigated additional HIPEC with mitomycin showed no advantage. Of course these results of the current study are limited by the small sample sizes. For the same reason, the influence of completeness of the surgical cytoreduction on the tumour mass and ePCI 21 days later could not be proven. In clinical studies on the long-term survival after surgery and HIPEC, this completeness has been realised as the main influencing factor for patients' survival [25, 26].

Conclusion

The current study shows that this rat model is feasible to investigate complex multimodal therapeutic regimen for treatment of peritoneal carcinomatosis. Only additional HIPEC with mitomycin had a further influence on tumour reduction after surgery. Therefore, further investigations should be compared with this treatment protocol. Nevertheless, this animal model provides the opportunity for further research on effectiveness and toxicity of new cytostatic agents or treatment protocols in this context for the first time. Also, questions on tissue penetration and systemic effects of the used drugs, on immunological reactions and on the impact of this therapy on circulation status and volume shifts are conceivable. Thus, this animal model could constitute a building block for further research.

Declaration of interest The authors declare that they have no conflict of interest.

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