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Prophylaxis of peritoneal carcinomatosis in experimental investigations

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Abstract *Background and aims:* We compared the effectiveness and side effects of various cytostatic agents for use in perioperative intraperitoneal irrigation to prevent peritoneal carcinomatosis. *Methods:* The adenocarcinoma cell line CC-531 was implanted during laparotomy at the mesenterial trunk of anesthetized male WAG rats. Direct perioperative intraperitoneal chemotherapy was performed after 5 min with either 5-fluorouracil, cisplatin, or mitomycin; controls received only tumor cells. The animals were inspected daily over 30 days for side effects. They were then killed, and the greater omentum and mesentery were resected, the tumor mass was examined for the presence of peritoneal carcinomatosis, and tumor nodules in the greater omentum and mesentery were counted. *Results:* All the animals in the control group developed histologically confirmed peritoneal carcinomatosis. Animals receiving cisplatin or mitomycin by direct intraperitoneal perioperative chemotherapy showed no macroscopic or histological evidence of tumor growth. Two animals in the fluorouracil group had macroscopically and histologically manifest tumor growth; another animal showed only histological evidence of malignancy. Substantial side effects were noted in the cisplatin group, with all animals experiencing bleeding in the peritoneum and toxic necrotic reactions of the colon; two animals died of these side effects. *Conclusion:* Direct intraperitoneal chemotherapy with cisplatin or mitomycin prevents peritoneal carcinomatosis in experimental investigations.

Keywords Peritoneal carcinomatosis · Intraperitoneal chemotherapy · 5-Fluorouracil · Cisplatin · Mitomycin

Introduction

Patients with malignant gastrointestinal or gynecological diseases often experience peritoneal carcinomatosis during their disease. The median survival time after the manifestation of peritoneal carcinomatosis is about 6 months, regardless of the primary source of the cancer cells [1, 2, 3, 4]. Several large studies of surgery for gastric cancer have documented the problems for surgeons. There is local or intra-abdominal recurrence in about one-half of cases after curative resection [5, 6, 7, 8, 9].

Palliative treatment concepts such as systemic chemotherapy and limited surgery have recently been used for peritoneal carcinomatosis [1, 10, 11, 12, 13, 14, 15]. Some trials have reported remarkable results in preventing and treating peritoneal carcinomatosis by combining surgery and intraperitoneal chemotherapy [3, 11, 14, 16]. Unfortunately, few of these trials were randomized, and the number of patients involved was too small. Other groups describe contrasting results using multimodal treatment concepts [10, 12, 17]. Because basic experimental trials are incomplete regarding perioperative intraperitoneal chemotherapy, no standard therapy proto-

Table 1 Definition of treatment groups

Group	Drug	*n*	Concentration (mg/m^2)	Concentration 1/10 of LD_{50} (mg in 4 ml sodium chloride)
5-Fluorouracil	Ribofluor Ribosepharm	6	425	16.5
Cisplatin	Cisplatin Medac	6	25	0.95
Mitomycin	Mito-meda Medac	6	10	0.3

cols are available for preventing or treating peritoneal carcinomatosis. Pross et al. [18] at our institution has carried out initial research in an animal tumor model that demonstrated intraperitoneal tumor growth.

Methods

Animal model

For our studies on the prevention of peritoneal carcinomatosis we used the animal experimental model developed by Pross et al. [18]. The animals used were adult male WAG/RIJ rats (Harlan, Borchen, Germany) weighing 240–250 g. They were kept under standard conditions (free access to pelleted chow and water, 24 C° room temperature, 12 day/night cycle) in the experimental animal laboratory. Prior to the experimental surgical intervention animals were fasted for 12 h. The planned animal experiment (prophylaxis of peritoneal carcinomatosis) was approved by the animal protection authority of Sachsen-Anhalt-Dessau (Chir/G/4-99).

Tumor cell implantation and cytostatic treatment

The adenocarcinoma cell line CC-531 (Cell-Lines Service, Heidelberg, Germany) was used to induce peritoneal carcinomatosis. These immunocompetent tumor cells stemming from a moderately differentiated colon carcinoma induced by 1,2-dimethylhydrazine induce tumor growth in WAG rats. The CC-531 cells were cultured under standardized conditions in RPMI-1649 (Gibco, Eckstein, Germany) to which were added 10% heat-inactivated fetal bovine serum (Gibcoo) and antibiotics/antimycotics (Life-Technologies, Karlsruhe, Germany) at 37C° in a CO_2 incubator. Cells were counted in a Coulter Counter Z II (Coulter Immunotec, Marseille, France). Cell viability was confirmed by trypan blue exclusion test using a Neubauer chamber.

In 24 animals the tumor cells were implanted via laparotomy performed under general anesthesia. The adenocarcinoma cells CC-531 were implanted at a concentration of 5×10^6 cells directly into the region of the mesentery trunk. The body surface for the animals was 0.03–0.04 m^2 [calculated as $A(m^2)=m_k^{0.425}\times l_K^{0.725}/139.315$]. The following concentrations of the cytostatic agents were used 5 min after tumor-cell implantation into the peritoneal cavity: 5-fluorouracil (5-FU) 16.5mg=425 mg/m^2, cisplatin 0.95 mg=25 mg/m^2, mitomycin 0.3 mg=10 mg/m^2. The clinical situation of surgically spilled cancer cells was simulated in these experimental groups. Six animals served as controls (receiving only tumor cells; Table 1). The surgical intervention was completed by closing the wound in layers.

Postoperative examination of tumor growth and statistical analysis

All animals were kept in individual cages and the presence of side effects (loss of appetite, lethargy, fatigue syndrome, wound infection) of the operation/chemotherapy was assessed twice daily. The animals were killed under general anesthesia on the 30th postoperative day. They were autopsied to identify peritoneal carcinomatosis, with qualitative and quantitative determination of metastases. The liver, lungs, small bowel together with the trunk of the mesentery (parietal peritoneum), and the greater omentum were removed and isolated. Organ weight was determined on a high-tech digital balance (sartorious research balance). Using a counter field, the tumor nodules were counted macroscopically on an area measuring 1×1 cm in the region of the greater omentum and the parietal peritoneum. All organs and specimens were investigated for evidence of metastatic disease by a conventional histological preparation and hematoxylin and eosin staining. At least eight histological sections were obtained for each animal. Ascites was collected after 30 days by a 17-gauge needle near the right and left kidney.

Results

All control animals developed massive intraperitoneal tumor growth (Figs. 1, 2). The median weights of the greater omentum and mesentery were 4.585 and 5.216 g. Median macroscopic tumor nodules per 1 cm^2 were 10.5 in the greater omentum and 9 in the mesentery. All animals showed histological tumor growth (Figs. 3, 4). In the 5-FU group two animals revealed macroscopic evidence of intraperitoneal malignancy; the median weight of the greater omentum and mesentery were 1.845 and 2.543 g, respectively. There were a median of 1.333 tumor nodules per 1 cm^2 in the greater omentum and 0.833 in the mesentery. We found histological evidence of tumor growth in the greater omentum and mesentery in three animals of the 5-FU group, but there was no macroscopic or histological evidence of tumor growth in the cisplatin or mitomycin C groups. The median weight of the greater omentum was 0.893 g in the former and 0.713 g in the latter, and that of the mesentery was 2.132 and 2.369 g, respectively (Table 2). The median amount of ascites in the control group animals was 3.250 ml, while ascites was not detected in any the animals in the three treatment groups (Table 2).

Side effects

Loss of appetite, fatigue syndrome, conjunctivitis and lethargy were the main side effects observed in each of the treatment groups (Table 3). These were particularly pronounced in the cisplatin group. Wound infections were not found in any group. After 30 days all animals in the cisplatin group had minor bleeding in the peritoneum and necrotic toxic reactions involving kidneys, small in-

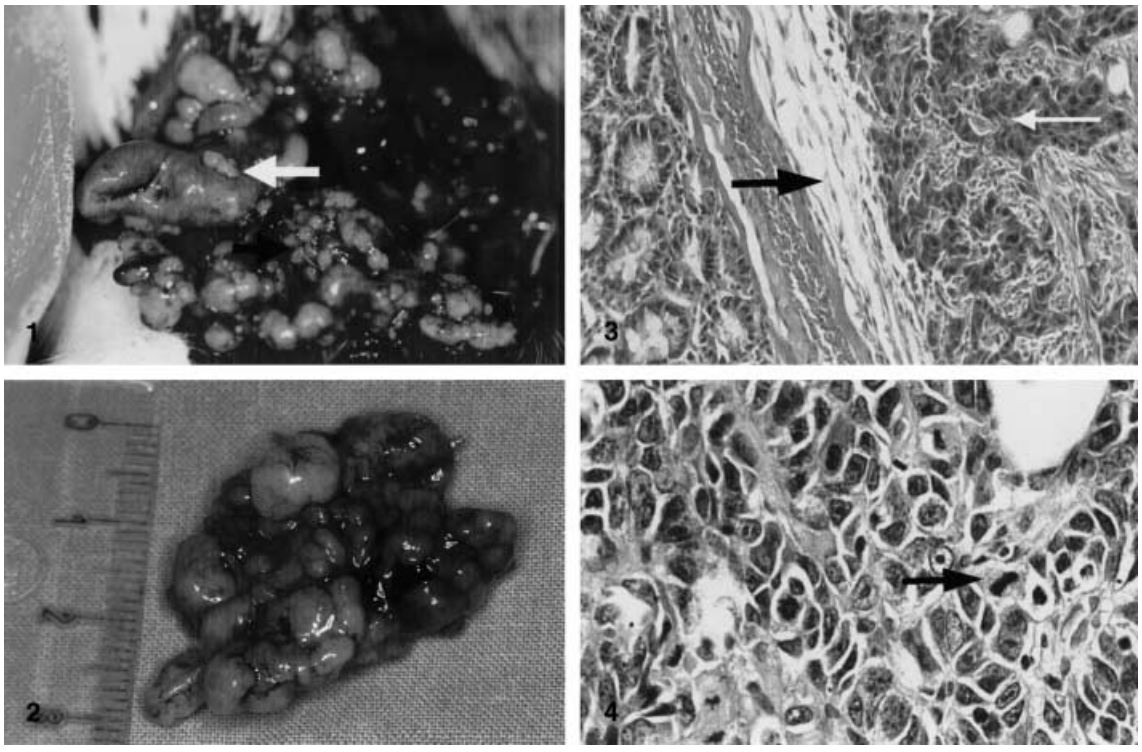

Fig. 1 Diffuse intraperitoneal tumor spread in the control group 30 days after tumor cell implantation

Fig. 2 Greater omentum with complete invaded tumor nodules 30 days after tumor cell implantation in the control group

Fig. 3 Carcinomatosis of the colonic serosa. Border between tumor cells (*white arrow*) and clonic serosa (*black arrow*). *On the left* Normally structured colon; *on the right* solid and tubular pattern of transplanted adenocarcinoma cells. ×100

Fig. 4 Transplantated pleomorphic tumor cells resembles the picture of a badly differentiated adenocarcinomoa. Several atypical mitoses are shown (*black arrow*). ×300

Table 2 Results

	Control	5-Fluorouracil	Cisplatin	Mitomycin
Weight				
Greater omentum (g)	4.58±0.55*	1.84±1.19	0.89±0.79	0.71±0.20
Mesentery (g)	5.05±0.72*	2.54±0.73	2.13±0.18	2.36±0.23*
Tumor nodules				
Greater ometum	10.50±3.728*	1.33±2.422	0.00	0.00
Mesentery	9.00±2.89*[a]	0.83±1.32[a]	0.00	0.00
Histological tumor evidence				
Greater omentum	+++ in all	++ in 2, + in 1	None	None
Mesentery	+++ in all	++ in 3	None	None
Ascites (ml)	3.25*	0	0	0

*$P<0.05$ vs. treatment groups (*t* test)
+++ high-grade tumor growth,
++ medium tumor growth,
+ low tumor growth
[a] Only in two animals

testine, and colon. Two animals died from these toxic reactions after 5 days. Animals in the 5-FU group had no side effects in the gastrointestinal tract. Low-toxic reactions of the colon and small intestine were found in two animals in the mitomycin group.

Statistical analysis

The differences between treatment groups and controls were statistically significant, but not the differences between the various treatment groups ($P<0.05$, *t* test using SPSS 9.0 for Windows; Table 1).

Table 3 Results: side effects by WHO grade

	Control	5-Fluorouracil	Cisplatin	Mitomycin
Loss of appetite	All animals grade II 24 days after tumor implantation	Grade I immediately after i.p. administration over 2 days	Grade II/III immediately after i.p administration for 4–6 days (all animals)	Grade II immediately after i.p administration for 2 days (all animals)
Fatigue syndrome	0	0	Grade II immediately after i.p administration for 4 days (all animals)	Grade II immediately after i.p administration for 2–3 days (4 animals)
Conjunctivitis	0	0	Grade II/III immediately after i.p administration for 4–6 days (all animals)	0
Lethargy	All animals grade II 26–28 days after tumor implantation	0	II° immediately after i.p administration for 4–8 days (all animals)	Grade I/II immediately after i.p administration for 2 days (4 animals)
Death	0	0	2 animals 5 days after i.p. administration	0
Wound infection	0	0	0	0
Gastrointestinal side effects				
Peritoneal bleeding	0	0	6	0
Toxic necrotic reactions colon, small intestine	0	0	6	2
Toxic reactions: kidneys	0	0	6	0

Discussion

Peritoneal carcinomatosis is a common form of tumor recurrence in solid gastrointestinal tumors. When peritoneal carcinomatosis occurs, the prognosis is extremely poor [2, 3, 16], regardless of whether the primary tumor is a gastric, pancreatic, or colorectal carcinoma. The major problem for the surgeon is the fact that peritoneal carcinomatosis often occurs shortly after successful Ro resection of a malignant gastrointestinal tumor. Theoretical considerations suggest two main reasons: (a) perioperative tumor cell dissemination resulting in seeding of the tumor cells in the peritoneum and (b) advanced carcinomas that invade the serosa and spread beyond the boundaries of the organ involved. In such cases local peritoneal carcinomatosis in the former bed of the tumor is common. At particular risk for this type of tumor recurrence are tumors in thin-walled organs, for example, a carcinoma of the appendix.

A number of studies involving various gastrointestinal tumor entities have attempted to use perioperative or early postoperative intraperitoneal chemotherapy to prevent peritoneal carcinomatosis or to treat existing peritoneal carcinomatosis in advanced carcinomas [3, 10, 14, 19]. There is already a certain tradition of using intraperitoneal chemotherapy in the case of gastric cancer, and a number of Japanese studies have reported remarkable results in terms of survival time in cases of advanced gastric carcinoma [15, 19, 20, 21], while European investigations have obtained very contrasting results [10, 12, 17]. As a result, despite the "traditional chemotherapy" applied in gastric cancer, no standard protocol now exists for the prophylaxis or treatment of peritoneal carcinomatosis. Studies using multimodal treatment concepts have investigated intraperitoneal chemotherapy for preventing locoregional recurrence and peritoneal carcinomatosis in carcinomas of the colon and appendix [3, 11, 13, 14, 22, 23]. Unfortunately, most of these studies involved only small numbers of patients and were not randomized. The randomized study by Scheithauer et al. [23] in 241 patients with carcinoma of the colon in stage III or high-risk stage II (T4, N0, M0) who received combined postoperative 5-FU and leucovorin found significantly less local recurrence in those receiving intraperitoneal/intravenous chemotherapy than in those receiving only intravenous chemotherapy. In a study of 385 patients with peritoneal spread of appendix carcinoma, Sugarbaker [14] found a 5-year survival rate of 86% in patients receiving perioperative/intraperitoneal chemotherapy followed by cytoreductive surgery. These studies are among the few carried out in randomized fashion and containing a reasonably large number of patients.

Basic research data on this topic from animal experiments are virtually nonexistent. In view of the poor prognosis of peritoneal carcinomatosis, the above studies are justified; the rule that experimental data from animals are

needed should provide a basis for clinical studies continues to apply [24]. This dearth of definitive information led to the animal experimental study report here. Our principal goal was to compare the various cytostatic agents with regard to their intraperitoneal effect in an animal tumor model, considering also side effects and complications. It must of course be noted that such data cannot be transferred simply from rodents to humans.

A number of interesting differences were found in the effects of the various intraperitoneal administered cytostatic agents. Mitomycin C and cisplatin proved highly potent in the experimental setting and completely prevented intraperitoneal tumor growth. There were no macroscopic signs of tumor at the end of the 30-day experiment. Despite meticulous work-up and multiple histological evaluations (eight to ten histological sections per animal) of the greater omentum, mesentery, liver, kidneys, and lungs, no intraperitoneal tumor growth was detected. Differences were found between these two cytostatic agents in terms of side effects. In the cisplatin group two animals died 5 days after the operation, of toxic reactions involving small bowel, colon, and kidneys. In general, the animals showed better toleration of intraperitoneal administration of mitomycin than that of cisplatin. With the former the animals developed appreciably fewer side effects – fatigue syndrome and loss of appetite. In the mitomycin group no cases of conjunctivitis were observed. The intraperitoneal effectiveness of cisplatin has also been reported in a French animal experimental study on the treatment of existing peritoneal carcinomatosis [25]. Peritoneal carcinomatosis was experimentally induced in BDXI rats, and peritoneal and omental tumors were resected after 20 days. The survival rate of animals receiving 3 mg/kg cisplatin combined with 2 mg/kg epinephrine intraperitoneally was considerably higher than that of animals undergoing the surgical procedure only.

In our experimental trial two animals in the 5-FU group showed macroscopically suspected intraperitoneal tumor growth, although this was considerably less marked than in the untreated control group. Interestingly, in an animal with macroscopic suspicion the histological work-up revealed tiny micrometastases in the greater omentum and mesentery. This demonstrates the considerable value of the meticulous histopathological examination. Although the difference was not statistically significant, 5-FU was less successful than cisplatin and mitomycin in preventing peritoneal carcinomatosis in this tumor model. In terms of side effects, 5-FU was the best tolerated of the three cytostatic agents administered intraperitoneally.

The value of 5-FU in combination with other cytostatic agents in intraperitoneal chemotherapy was demonstrated in the experimental study by Maruyama et al. [26], in which nude mice were inoculated with MKN-45 tumor cells, resulting in severe peritoneal carcinomatosis. Combination therapy of methotrexate plus 5-FU administered on postoperative days 7, 14, and 21 led to a significant reduction in omental tumor weight.

Under experimental conditions the immediately perioperative intraperitoneal administration of cisplatin or mitomycin prevented peritoneal carcinomatosis in one 100% of animals. Although 5-FU was less potent in completely preventing peritoneal carcinomatosis, in combination therapy it nevertheless has a role to play in intraperitoneal chemotherapy. With regard to side effects 5-FU was better tolerated than either cisplatin or mitomycin, although the side effects cannot be directly extrapolated from the rodent to humans. However, the pronounced side effects of cisplatin cannot be completely ignored; this of course also applies to all the other obtained results. The results of this study must be seen as a contribution to the basic research into a very promising therapeutic approach. Most studies in on intraperitoneal chemotherapy colorectal carcinoma have used 5-FU as standard cytostatic agent. The results of this trial show that cisplatin or mitomycin should also be considered in prevention or treatment of peritoneal carcinomatosis in colorectal carcinoma.

More research is required if existing gaps in this area are to be filled. Combination treatments with the various medications that have proven potently capable of preventing peritoneal carcinomatosis should be tested. As has already been done in a few studies, further substances and new medications must be investigated for their effectiveness against intraperitoneal tumor growth [18, 27, 28].

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