Original article

# Intraoperative hyperthermic intraperitoneal chemotherapy after cytoreductive surgery for peritoneal carcinomatosis in an experimental model

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**Background:** **The combination of cytoreductive surgery (CS) and hyperthermic intraperitoneal chemotherapy (HIPEC) is the treatment of choice for selected patients with peritoneal carcinomatosis (PC) of colorectal origin. However, it remains to be proven whether the addition of HIPEC to CS is essential for the reported survival benefit.**

**Methods:** **Sixty WAG/Rij rats were inoculated intraperitoneally with the rat colonic carcinoma cell line CC-531. Animals were randomized into three treatment groups: CS alone, CS followed by HIPEC (mitomycin 15 mg/m$^2$) and CS followed by HIPEC (mitomycin 35 mg/m$^2$). Survival was the primary outcome parameter.**

**Results:** **The median survival of rats treated with CS alone was 43 days. Rats receiving HIPEC 15 mg/m$^2$ and HIPEC 35 mg/m$^2$ both had a significantly longer median survival of 75 days ($P = 0{\cdot}003$) and 97 days ($P < 0{\cdot}001$) respectively. Rats receiving HIPEC showed a significantly lower tumour load at autopsy compared with rats treated with CS alone.**

**Conclusion:** **A combination of CS and HIPEC results in longer survival than CS alone in rats with PC of colorectal origin.**

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## Introduction

Peritoneal carcinomatosis (PC) is an important cause of morbidity and mortality in patients with colorectal cancer. Synchronous peritoneal metastases are found in 7 per cent of patients[1] and during follow-up a further 4–19 per cent will develop PC[2]. Median survival in conservatively treated patients varies between 5·2 and 12·6 months[3–5]. PC has long been considered to be a manifestation of systemic metastasis with no curative treatment options. Recently, local treatment strategies have been developed combining cytoreductive surgery (CS) and hyperthermic intraperitoneal chemotherapy (HIPEC). The only phase III randomized trial comparing CS and HIPEC with standard palliative care found median survival in the CS + HIPEC group to be 22·4 months, compared with 12·6 months in patients treated with standard palliative care alone[6]. Although these results are certainly encouraging, it remains unclear whether the combination of CS and HIPEC is indeed required to achieve the survival benefit. Unfortunately, no experimental arm was included where patients were treated with CS alone. At this time, it cannot be ruled out that the gain in survival was mainly or entirely due to CS[7].

The addition of HIPEC to CS prolongs operating time and increases the risk of postoperative morbidity and mortality[8,9]. Therefore, the additional benefit of HIPEC should be demonstrated unequivocally before it is widely accepted as the standard of care.

Efforts to define the role of HIPEC in a randomized trial failed to attain the required number of patients, because of patient dissatisfaction with randomization[10]. Thus, it seems unlikely that this issue will be resolved shortly in randomized clinical trials. Therefore, an experimental study was performed in rats with PC of colorectal origin, aiming to establish the benefit of HIPEC as adjuvant therapy after CS for PC.

## Methods

### Animals

Sixty male WAG/Rij rats, 10–12 weeks old and median weight 269 (range 236–303) g, were obtained from Harlan, Horst, The Netherlands. The animals were allowed to accustom to laboratory conditions for at least 1 week before experimental use. Rats were housed in filter-topped cages (three rats per cage) under clean, non-sterile, standardized conditions (temperature 20–24°C, relative humidity 50–60 per cent, 12 h light/12 h dark cycle), with free access to food (ssniff®; Bio Services, Uden, The Netherlands) and water. All experiments were approved by the Animal Welfare Committee of Radboud University and carried out in accordance with the Dutch Animal Welfare Act 1997.

### Experimental design

PC was induced in all animals. Seven days after intraperitoneal tumour induction, animals were randomized into three groups of 20 each: exploration and CS alone (CS group); CS followed by HIPEC, total dose of mitomycin 0·5 mg (15 mg/m$^2$) (HIPEC-15 group); and CS followed by HIPEC, total dose of mitomycin 1·2 mg (35 mg/m$^2$) (HIPEC-35 group). Survival was the primary outcome parameter.

### Induction of peritoneal carcinomatosis

The tumour cell line used was the syngeneic rat colonic carcinoma cell line CC-531, originally induced in WAG/Rij rats by intravenous injection of 1,2-dimethylhydrazine[11]. The cell line was cultured and maintained as described previously[12] and 2 ml of a cell suspension (10$^6$ cells/ml) was injected intraperitoneally.

### Surgery

One week after tumour cell inoculation, CS was performed under general anaesthesia using isoflurane 3 per cent, and 1 : 1 oxygen and nitrous oxide. For analgesia, rats were given carprofen (Rimadyl®; Pfizer Animal Health, Capelle aan de Ijssel, The Netherlands) 5 mg per kg per day 30 min before surgery and once daily until the third day after the operation. During surgery, rats were placed on a warmed mattress to limit body heat loss.

After laparotomy, the abdomen was carefully inspected for tumour growth at ten different sites, as shown in *Table 1*. The tumour load at each site was scored semiquantitatively: 0, no macroscopic tumour; 1, limited tumour growth (diameter 1–2 mm); 2, moderate tumour growth (diameter 2–4 mm); or 3, abundant (diameter more than 4 mm). The sum of scores from all sites represented the Peritoneal Cancer Index (PCI) for that animal.

Subsequently, CS including standard omentectomy was performed in all animals, aiming at complete removal of the macroscopic tumour deposits. Unresectable tumour deposits were cauterized using an electrocoagulation device. After CS the amount of residual tumour was scored using a system currently employed in clinical practice. Absence of residual tumour was recorded as R1, a residual tumour of 2·5 mm or less was scored as R2a, and a tumour larger than 2·5 mm as R2b.

**Table 1** Tumour score before cytoreduction, and results of cytoreductive surgery

| | CS ($n = 20$) | HIPEC-15 ($n = 20$) | HIPEC-35 ($n = 20$) |
|---|---|---|---|
| Bodyweight (g)* | 270(15) | 269(17) | 271(16) |
| Tumour score per site† | | | |
| Subcutaneous | 0 (0–3) | 0 (0–2) | 0 (0–2) |
| Injection site | 1 (0–2) | 1 (0–2) | 1 (0–2) |
| Greater omentum | 1 (1–1) | 1 (1–1) | 1 (0–1) |
| Liver hilum | 1 (0–1) | 1 (0–2) | 1 (0–2) |
| Liver | 0 (0–1) | 0 (0–1) | 0 (0–1) |
| Perisplenic | 1 (0–2) | 1 (0–1) | 1 (0–1) |
| Mesentery | 1 (1–1) | 1 (0–2) | 1 (0–2) |
| Gonadal fatpads | 0 (0–3) | 0 (0–2) | 0 (0–2) |
| Diaphragm | 0 (0–1) | 0 (0–1) | 0 (0–1) |
| Parietal peritoneum | 0 (0–2) | 0 (0–2) | 0 (0–3) |
| Overall PCI* | 6·1(1·9) | 6·1(1·8) | 6·0(2·9) |
| Splenectomy | | | |
| Yes | 5 | 5 | 6 |
| No | 15 | 15 | 14 |
| Completeness of resection | | | |
| R1 | 14 | 16 | 16 |
| R2a | 6 | 3 | 4 |
| R2b | 0 | 1 | 0 |

Values are *mean(s.d.) and †median (range). CS, cytoreductive surgery; HIPEC-15, CS + hyperthermic intraperitoneal chemotherapy (HIPEC) with 15 mg/m$^2$ mitomycin C; HIPEC-35, CS + HIPEC with 35 mg/m$^2$ mitomycin C; PCI, Peritoneal Cancer Index; R1, no macroscopic residual tumour after CS; R2a, residual tumour 2·5 mm or less after CS; R2b, residual tumour greater than 2·5 mm after CS.

In the CS group, the abdomen was closed after surgery. In HIPEC-15 and HIPEC-35 groups, surgery was followed immediately by HIPEC.

### Hyperthermic intraperitoneal chemotherapy

Two multiperforated catheters were introduced into the abdominal cavity through the flanks, as described previously[13]. The catheters were connected to a closed perfusion system containing 250 ml 0·9 per cent sodium chloride. The peritoneal perfusate was warmed in a tube coil using a thermostatically regulated water bath. Perfusion of the peritoneal cavity was performed for 90 min at 10 ml/min. Mitomycin C (Nycomed Christiaens, Breda, The Netherlands) was dissolved in 0·9 per cent sodium chloride to the appropriate concentration and added to the perfusate in three separate doses at 30-min intervals, each containing 50, 25 and 25 per cent respectively of the total dose. During HIPEC, the abdomen was massaged gently to achieve a uniform heat distribution.

After completion of the perfusion, the abdominal cavity was irrigated with warmed (42°C) saline for 5 min. The catheters were removed and the abdominal wall was closed in two layers using continuous 3/0 polyglactin 910 (Vicryl™; Ethicon, Edinburgh, UK) sutures for the muscular layer and wound clips for the skin. All rats were given 10 ml 0·9 per cent sodium chloride subcutaneously for rehydration.

### Follow-up

The primary endpoint of the experiment was survival. Rats were observed and weighed daily for the first 7 days after surgery, and three times a week thereafter. Bodyweight was expressed as relative bodyweight compared with the bodyweight on the day of operation, and taken to reflect the toxicity of the treatment.

When the humane endpoint was reached (physical inactivity, signs of intra-abdominal tumour growth with disabling consequences or signs of massive haemorrhagic ascites), rats were killed by administration of carbon dioxide, and subjected to autopsy. Ultimately, the decision regarding the humane endpoint was made by an experienced biotechnician who was unaware of the experimental group to which the animal belonged.

After autopsy, the intraperitoneal tumour load was scored as described above. In addition, the weight of ascites was determined. The study was terminated 140 days after surgery. Remaining rats were killed and subjected to autopsy.

### Statistical analysis

Statistical analysis was performed using GraphPad® Prism (GraphPad Software, San Diego, California, USA) and SPSS® version 16.0 (SPSS, Chicago, Illinois, USA) software. The primary objective of the study was to demonstrate an improvement in median survival from 48 days in the CS group to 90 days in the HIPEC groups. Sample size was calculated by lifespan analysis assuming exponential survival from day 35 onwards and using a power of 0·90, $\alpha = 0{\cdot}05/2 = 0{\cdot}025$ and an one-sided test. The various assumptions were based on data from previous studies using the same animal model and surgical cytoreductive procedures[13–15]. For comparison of dichotomous values, $\chi^2$ or Fisher's exact tests were used. One-way ANOVA or Kruskal–Wallis testing was used for comparison of continuous values. Survival outcomes were analysed and expressed using Kaplan–Meier curves, and compared with the log rank test. Cox survival regression analysis was applied to correct for confounding factors. $P < 0{\cdot}050$ was considered statistically significant.

## Results

### Surgical procedures

Sixty animals were randomized. Preoperative clinical condition and bodyweight did not differ between the groups. Findings at laparotomy and results of CS are shown in *Table 1*. Peritoneal tumour deposits (*Fig. 1*) were present in 59 animals. In the rat with no macroscopic tumour growth, omentectomy and exploration were followed by HIPEC as determined by randomization. The mean PCI score was similar in the three groups. There were no differences between groups regarding residual disease *in situ* after resection. The mean time taken for the CS procedures, without HIPEC, was 45 min and did not differ between groups.

One rat in the HIPEC-15 group died from respiratory failure immediately after the procedure, and one rat in the CS group died shortly after the surgical procedure as a result of excessive blood loss. On postoperative day 11, one rat in the HIPEC-35 group died with signs of peritonitis and sepsis.

### Perfusion characteristics

During the HIPEC procedure, the intra-abdominal temperature was similar in both groups, with a mean(s.d.) of 42·0(0·9)°C. Mean(s.d.) rectal temperature at the start of the procedure was 32·9(0·7)°C in the HIPEC-15 group and 32·7(0·7)°C in the HIPEC-35 group ($P = 0{\cdot}443$). This

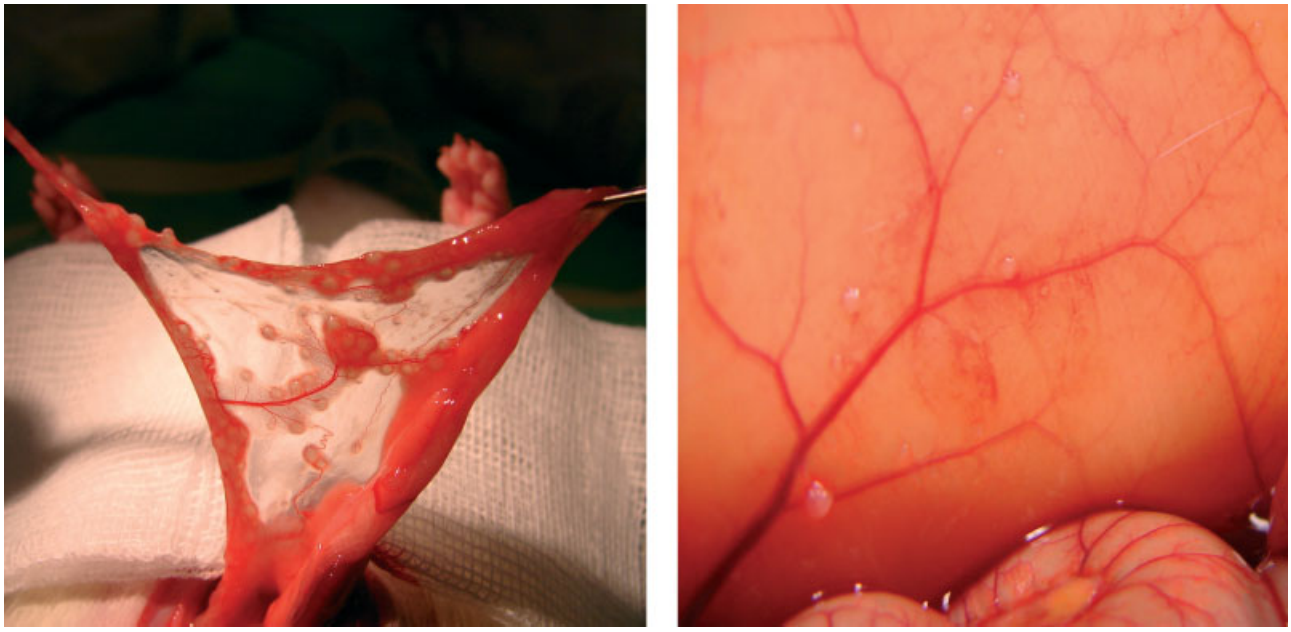


**Fig. 1** Macroscopic aspect of peritoneal metastases found at laparotomy. **a** Greater omentum; **b** abdominal wall

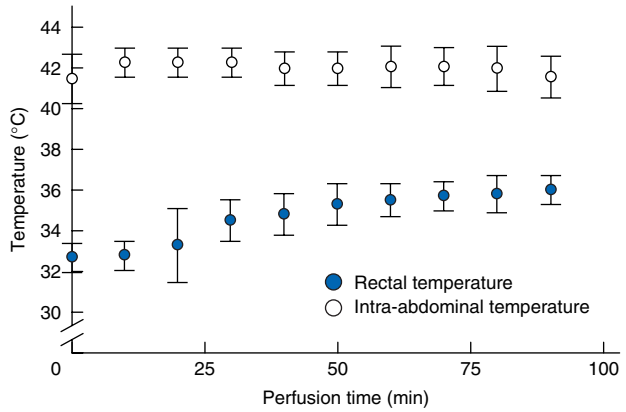


**Fig. 2** Rectal and intra-abdominal temperatures during the hyperthermic intraperitoneal chemotherapy procedure. Values are mean(s.d.) of all 40 procedures

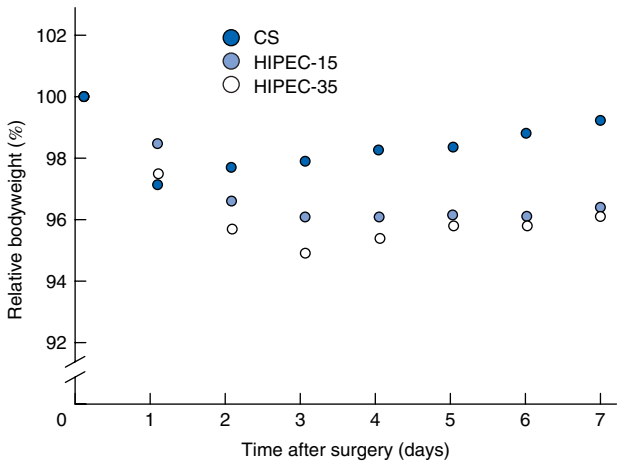


**Fig. 3** Course of mean bodyweight, relative to weight at operation, during the first week after surgery. CS, cytoreductive surgery; HIPEC-15, CS + hyperthermic intraperitoneal chemotherapy (HIPEC) with 15 mg/m$^2$ mitomycin C; HIPEC-35, CS + HIPEC with 35 mg/m$^2$ mitomycin C

increased to 36·7(1·9)°C in the HIPEC-15 group and 36·2(1·4)°C in the HIPEC-35 group by the end of perfusion ($P = 0·372$). The course of rectal and intra-abdominal temperatures during the HIPEC procedures is shown in *Fig. 2*.

## Clinical appearance

*Fig. 3* shows the course of mean bodyweight in the three groups during the first weeks after surgery. Rats in the CS group generally gained weight from the second postoperative day onwards. In the HIPEC groups, the lowest mean(s.d.) bodyweight was recorded on the third day after surgery: reduction of 4·1(0·6) *versus* 5·2(0·6) per cent in HIPEC-15 and HIPEC-35 groups respectively. In the HIPEC-35 group, mean(s.d.) maximum weight loss

was significantly higher than that in the CS group (5·2(0·6) *versus* 3·0(0·2) per cent respectively; $P = 0·042$).

## Survival

Survival curves are shown in *Fig. 4*. During follow-up two rats died from non-tumour-related causes. Median survival was 43 (95 per cent confidence interval (c.i.) 39 to 64) days in the CS group and 75 (67 to 99) days in the HIPEC-15 group. The highest median survival of 97 (76 to 113) days was achieved in the HIPEC-35 group. This difference in survival outcome was significant for both HIPEC groups in comparison with the CS group: $P = 0·003$ *versus* HIPEC-15, hazard ratio for dying 0·42 (95 per cent c.i. 0·22 to 0·78); $P < 0·001$ *versus* HIPEC-35, hazard ratio 0·31 (0·16 to 0·59). Survival in the HIPEC-35 group tended to be higher than that in the HIPEC-15 group ($P = 0·197$). After 20 weeks, 11 rats were still alive (CS, 1; HIPEC-15, 3; HIPEC-35, 7). Three of these animals showed no macroscopic evidence of tumour growth (CS, 1; HIPEC-35, 2).

At autopsy, rats in the CS group were found to have a higher mean PCI than rats in the HIPEC-35 group ($P < 0·001$). One rat in the CS group had extensive lung metastases. Post-mortem findings are shown in *Table 2*.

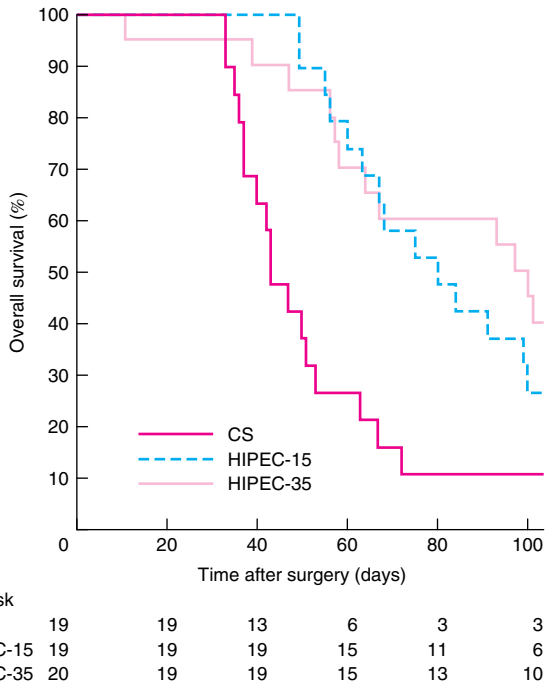


**Fig. 4** Kaplan–Meier survival curves for the three treatment groups. CS, cytoreductive surgery; HIPEC-15, CS + hyperthermic intraperitoneal chemotherapy (HIPEC) with 15 mg/m$^2$ mitomycin C; HIPEC-35, CS + HIPEC with 35 mg/m$^2$ mitomycin C. $P = 0·003$ for CS *versus* HIPEC-15, $P < 0·001$ for CS *versus* HIPEC-35 (log rank test)

**Table 2** Tumour score and ascites weight at autopsy

| | CS | HIPEC-15 | HIPEC-35 |
|---|---|---|---|
| Tumour score per site | | | |
| Scar | 2 (0–3) | 1 (0–3) | 0 (0–2) |
| Injection site | 0 (0–3) | 0 (0–3) | 0 (0–0) |
| Greater omentum | 3 (0–3) | 3 (0–3) | 3 (0–3) |
| Liver hilum | 3 (0–3) | 3 (0–3) | 3 (0–3) |
| Liver | 2 (0–3) | 1 (0–3) | 0 (0–3) |
| Perisplenic | 3 (0–3) | 3 (1–3) | 1 (0–3) |
| Mesentery | 3 (0–3) | 3 (1–3) | 2 (0–3) |
| Fatpad 1 | 3 (0–3) | 3 (0–3) | 3 (0–3) |
| Fatpad 2 | 3 (0–3) | 2 (0–3) | 2 (0–3) |
| Diaphragm | 3 (0–3) | 3 (0–3) | 3 (2–3) |
| Parietal peritoneum | 3 (0–3) | 2 (0–3) | 1 (0–3) |
| Overall PCI* | 24(6) | 23(5) | 17(6) |
| Ascites weight (g)* | 24(18) | 38(19) | 37(20) |

Values are median (range), except *mean(s.d.). CS, cytoreductive surgery; HIPEC-15, CS + hyperthermic intraperitoneal chemotherapy (HIPEC) with 15 mg/m$^2$ mitomycin C; HIPEC-35, CS + HIPEC with 35 mg/m$^2$ mitomycin C; PCI, Peritoneal Cancer Index.

In rats treated with HIPEC, haemorrhagic ascites was the most common reason for reaching the humane endpoint. Rats in the CS group were most often removed from the experiment because of significant weight loss and palpable tumours in the abdomen. Differences in the mean ascites weight were observed between both HIPEC groups and the CS group, but, owing to massive variation, remained non-significant ($P = 0·355$).

In multivariable analysis, the only other independent factor influencing survival was completeness of resection. Rats with a complete (R1) resection had a longer survival (median 75 days) than animals in which tumour had been left behind (R2a or R2b, median 49 days), independent of treatment (hazard ratio 2·5, 95 per cent c.i. 1·3 to 4·9; $P = 0·008$).

## Discussion

This is the first experimental study to demonstrate that HIPEC is an effective adjuvant intraoperative therapy after CS for PC of colorectal origin. Complete macroscopic removal of the tumour from the peritoneal cavity is a second independent factor that improves outcome.

The experiments were performed using a validated and reproducible model of PC of colorectal origin that resembles the clinical situation. After inoculation of CC-531 syngeneic colonic carcinoma cells, PC without distant metastasis develops within 3–5 days[12,16]. As this cell line has been shown to be sensitive to mitomycin, the

experimental model is attractive to determine the effect of HIPEC with this particular anticancer drug[17]. The model is also suitable for performing CS[15] and the feasibility of performing HIPEC after CS has been demonstrated[13,18].

The present experimental HIPEC procedure was designed to mimic the procedure used in the only previously reported randomized clinical trial[6] as closely as possible; the perfusion time was 90 min and mitomycin was introduced into the flow system in three sequential doses. The total dose of 1·2 mg, equivalent to 35 mg/m$^2$ body surface area, was similar to dosages used to treat patients with PC in the Netherlands. A second dose of 15 mg/m$^2$ mitomycin was chosen as it has been reported to reduce intraperitoneal tumour growth in rats[19]. Increasing doses appeared to be increasingly effective when used in the HIPEC procedures carried out in the present model.

HIPEC proved to be highly effective in prolonging survival and in delaying intra-abdominal recurrence. A previous study carried out in the authors' laboratory failed to demonstrate a significant gain in survival after HIPEC, although median survival time increased from 57 days following CS alone to 76 days with CS and HIPEC[13]. It is entirely conceivable that the toxicity resulting from the very high dose (120 mg/m$^2$) of mitomycin used, delivered in a single administration, may have neutralized its potential benefits. Adaptation of the dosage regimen resulted in a much lower toxicity in the present experiment, as indicated by a less severe loss of bodyweight, and a much improved outcome.

Rats that were treated by CS alone showed weight loss, palpable tumours in the abdomen and signs of bowel obstruction, most likely caused by rapid tumour growth. This was reflected by a significantly higher tumour load at autopsy than in either HIPEC group. In the latter groups the humane endpoint was based mainly on respiratory failure as a consequence of ascites. Most likely, tumour growth in these rats was inhibited and a fatal volume of ascites developed before tumour growth caused clinical symptoms. The beneficial effect of HIPEC on survival is therefore believed to be a result of tumour growth inhibition.

An interesting observation from this study is that a macroscopically complete removal of tumour is an independent favourable prognostic factor determining outcome. This is in accordance with observations in clinical practice[2,20,21].

Effects of intraperitoneal treatment in addition to CS for PC in rats have been reported previously[22–24]. Postoperative intraperitoneal administration of cisplatin and adrenaline (epinephrine) improved survival, whereas CS alone did not[22]. In addition, intraperitoneal apoptogenic agents administered after CS diminished tumour growth and ascites after 20 days[23]. Although these results support the rationale for performing HIPEC, the models used were not entirely relevant for dealing with questions concerning HIPEC, because hyperthermia is not a component of treatment and chemotherapy is not applied during surgery. Recently, Raue and colleagues[24] showed that HIPEC with 15 mg/m$^2$ mitomycin after CS can indeed lead to reduced tumour weight 21 days after surgery. However, the effect of treatment on survival was not evaluated in this study.

Although an experimental model, the results of the present study have provided an answer to the question whether the application of HIPEC is mandatory after CS to improve survival. This question has been under debate ever since publication of the only randomized trial currently available[6]. Although data on side-effects remain limited, the available studies[9,25] have reported acceptable morbidity and mortality rates. Most common complications appear to be associated with CS rather than the addition of HIPEC[9,25]. These data, together with the results of the present study, show that HIPEC is a safe and effective intraoperative adjuvant to CS as therapy for PC of colorectal origin.

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