Annals of Surgical Oncology **14**(2):533–540
DOI: 10.1245/s10434-006-9247-x

Timing of Adjuvant Radioimmunotherapy after Cytoreductive Surgery in Experimental Peritoneal Carcinomatosis of Colorectal Origin

Frits Aarts, MD,[1] Manuel J. Koppe, MD,[1] Thijs Hendriks, PhD,[1] Julliëtte E. M. vanEerd, PhD,[2] Wim J. G. Oyen, MD, PhD,[2] Otto C. Boerman, PhD,[2] and Robert P. Bleichrodt, MD, PhD[1]

[1]410 Department of Surgery, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB, Nijmegen, The Netherlands
[2]444 Department of Nuclear Medicine, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB, Nijmegen, The Netherlands

Background: Treatment of patients with peritoneal carcinomatosis (PC) of colorectal cancer (CRC) includes cytoreductive surgery (CS) in combination with (hyperthermic) intraperitoneal chemotherapy (HIPEC), resulting in a limited survival benefit with high morbidity and mortality rates. Radioimmunotherapy (RIT) as adjuvant therapy after CS of CRC has been shown to prolong survival in preclinical studies. However, the optimal setting of RIT remains to be determined.

Methods: PC was induced by intraperitoneal inoculation of CC-531 colon carcinoma cells in Wag/Rij rats. Animals were subjected to exploratory laparotomy (Sham), CS only or CS + RIT at different time points after surgery. RIT consisted of 55 MBq lutetium-177-labelled anti-CC531 antibody MG1 (183 μg). The primary endpoint was survival.

Results: Cytoreductive surgery with or without RIT was well tolerated. Median survival of animals in the Sham and CS group was 29 days and 39 days, respectively ($P < 0.04$). Compared to CS alone, median survival of rats after adjuvant RIT was 77 days ($P < 0.0001$), 52 days ($P < 0.0001$) and 45 days ($P < 0.0001$) when given directly, 4 and 14 days after surgery, respectively.

Conclusion: The efficacy of adjuvant RIT after CS for the treatment of PC of colonic origin decreases when the administration of the radiolabelled MAbs is postponed. This study shows that adjuvant RIT should be given as early as possible after surgery.

Key Words: Radioimmunotherapy—Cytoreductive surgery—Peritoneal carcinomatosis—Colon cancer; adjuvant—Time optimization.

If untreated, peritoneal carcinomatosis (PC) of colorectal carcinomas (CRC) is one of the end stages of colorectal cancer, occurring either synchronous or metachronous in 5–50% of patients.[1] Efforts to improve survival in these patients include extensive surgical procedures in combination with (hyperthermic) intraperitoneal chemotherapy (HIPEC).[2,3] An analysis of the results of 16 clinical trials on the use of cytoreductive surgery (CS) + HIPEC in patients with PC of colorectal origin, indicated that the extent of carcinomatosis and completeness of resection were the factors most prominently related to survival.[4] Still, 5-year survival rates of the patients with the most favourable clinicopathological characteristics varies from only 20 to 53%, with most recurrences occurring intraperitoneally.[5] Therefore, more effective adjuvant treatments are necessary to improve the results of CS.

Received June 26, 2006; accepted September 18, 2006; published online November 23, 2006.

Address correspondence and reprint requests to: Frits Aarts, MD; E-mail: F.Aarts@chir.umcn.nl

In recent years, an increased interest in various experimental treatments developed to further improve this survival in both preclinical as well as in clinical studies.[6–8] Preclinical studies focussed on the efficacy of targeted therapies for the treatment of PC, some utilizing monoclonal antibodies (MAbs) directed against tumour-associated antigens and labelled with a radionuclide in order to selectively irradiate tumour cells (radioimmunotherapy, RIT).[9,10] Our previous RIT studies in Wag/Rij rats with intraperitoneal CC-531 tumours demonstrated the feasibility and efficacy of adjuvant intraperitoneal RIT after CS. RIT was given using ^{177}Lu-labelled anti-CC531 antibodies that were administered 3 days after CS. Administration of RIT at this time interval caused prolonged survival but did not influence the number of cures. Based on these promising results, we hypothesized that the efficacy of adjuvant RIT after CS could be further improved when the timing of postoperative administration of the radiolabelled Mabs would be optimized. To test this hypothesis, the efficacy of adjuvant RIT administered on various time points after CS was investigated.

MATERIALS AND METHODS

Experimental Design

Seven days after intraperitoneal tumour induction with 2.0×10^6 CC-531 tumour cells, 75 rats—15 per treatment group, were randomly assigned to undergo exploratory sham surgery (Sham), CS only (CS), CS + RIT administered immediately postoperatively (CS + RIT 0), CS + RIT administered 4 days postoperatively (CS + RIT 4), or CS + RIT administered 14 days postoperatively (CS + RIT 14).

Cell Line

The syngeneic rat colon carcinoma cell line CC531, originally induced in Wag/Rij rats by intravenous injection of 1,2-dimethylhydrazine,[11] was cultured and maintained as monolayer in RPMI-1640 medium (GIBCO, BRL Life Sciences Technologies, The Netherlands) supplemented with 10% fetal calf serum (GIBCO), 2 mM L-glutamine, penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37°C in a humidified atmosphere with 5% CO_2. Before inoculation, tumour cells were washed with 0.9% sodium chloride, disaggregated with 0.25% trypsin and resuspended in RPMI-1640 medium to a concentration of 1×10^6 cells/mL. Two millilitres of this cell suspension was injected intraperitoneally, as previously described.[12]

Animals

Male WAG/Rij rats (10–12 weeks old, body weight 240–260 g, Harlan, Horst, The Netherlands) were and housed under non-sterile standard conditions (temperature, 20–24°C; relative humidity, 50–60%; 12 h light/dark cycle) in filter-topped cages (two rats per cage), with free access to food (Ssniff, Bio Services, Uden, The Netherlands) and water. The rats were allowed to accustome to laboratory conditions for at least 1 week before experimental use. Physical condition was examined daily and total body weight was recorded twice a week by a biotechnician, who was blinded to the therapeutic regimen. All experiments were approved by the local Animal Welfare Committee of the Radboud University Nijmegen and were carried out in accordance with the Dutch Animal Welfare Act of 1997.

Reagents

The murine MG1 MAb, an anti-CC531 IgG2a monoclonal antibody, was purchased from Antibodies for Research Applications BV (Gouda, The Netherlands). To allow labelling of the antibody with ^{177}Lu, the MAb was conjugated with 2-(4-isothiocyanatobenzyl)-diethylenetriaminepentaacetic acid (ITC-DTPA, Macrocyclics, Dallas, TX, USA). Conjugation of the MAb was carried out in 0.1 mol/L $NaHCO_3$ buffer, pH 9.5 using a 50-fold molar excess of DTPA, as described by Ruegg et al.[13] with minor modifications (1 h conjugation at room temperature). The DTPA-MG1 conjugate (185 μg) was labelled with 55MBq ^{177}Lutetium (IDB Holland, Baarle Nassau, The Netherlands) in a 0.25 M ammonium acetate buffer, pH 5.4 for 30 min at room temperature. The non-MAb-bound radiolabel was determined by instant thin-layer chromatography (ITLC) using ITLC silica gel strips (Gelman Sciences, Inc., Ann Arbor, MI, USA), using 0.1 mol/L citrate buffer (pH 6.0) as the mobile phase (Rf = 0 for MAb associated ^{177}Lu, and Rf = 0.8–1 for unbound ^{177}Lu). All radiolabelled MG1 preparations were purified by gelfiltration on a PD10 column (Amersham, Pharmacia Biotech, Maarsen, The Netherlands) and eluted with PBS supplemented with 0.5% BSA, 1 mM EDTA. After PD10 elution, the radiochemical purity was checked by ITLC. The purified ^{177}Lu-MG1 was diluted in PBS with 0.5% BSA, 1 mM EDTA for injection. The specific activity of the

administered ^{177}Lu-MG1 preparation was 0.3 Mbq/μg.

All conjugation and labelling procedures using ^{177}Lu were performed under strict metal-free conditions. The immunoreactivity of the radiolabelled MG1 preparations was determined on freshly trypsinized CC531 cells essentially as described by Lindmo et al.[14] with minor modifications.[15]

Radioimmunotherapy (185 μg MG1 per rat, radiolabelled with 55 MBq ^{177}Lu in 3.0 mL) was intraperitonally injected immediately after surgery (RIT 0 group) or four (RIT 4 group) or 14 days (RIT 14 group) after surgery to evaluate the optimal timing of RIT.

Surgery

Surgical procedures were performed under general anaesthesia using isoflurane 3%, O_2 and N_2O 1:1. Thirty minutes prior to and once daily until the third day postoperatively, rats were given buprenorphine (5 μg, 0.1 mL/rat/day) for analgesia. All rats underwent a complete midline laparotomy. Rats in the control group C underwent exploratory laparotomy only in order to score intraperitoneal tumour growth. In all experimental treatment groups CS was performed, consisting of a midline laparotomy and careful inspection of the abdominal contents for tumour growth. Tumour growth at each of the intra-abdominal sites scored 0 (no macroscopic tumour growth), 1 (limited tumour growth), 2 (moderate tumour growth), or 3 (abundant tumour growth). The sum of the tumour scores of all sites represented the peritoneal cancer index (PCI).[16]

Subsequently, CS was performed, removing macroscopic tumour deposits as radical as possible. Irresectable tumours were cauterized using an electrocautery device. CS was followed by RIT at different time intervals. After completion of the surgical cytoreduction, the abdominal wall was closed in two layers using continuous Vicryl 3/0 sutures for the muscular component and iron wound clips for the skin. At the end of the procedure, a 10 mL of warmed normal saline was given subcutaneously, for rehydration.

Follow-up

The primary endpoint was survival. As part of the assessment of physical well-being during the immediate postoperative period, general condition and body weight were measured daily in the first 7 days. After 1 week, rats were monitored daily and total body weight was monitored twice weekly until the humane endpoint had been reached, as determined by an experienced biotechnician who was blinded for the experimental procedures. At the time of the humane endpoint, rats showed signs of advanced PC, such as the presence of ascites, and were killed by O_2/CO_2-asphyxiation and dissected. At dissection, the tumour deposits were scored as described above. The experiment was terminated at 16 weeks after CS by killing and dissecting the remaining rats. In case macroscopic tumour was absent, all relevant organs, including the greater omentum, the mesentery and the diaphragm were removed for histopathological analysis. Slices were stained using hemotoxilin & eosin (H&E) and/or immunohistochemical staining using the murine MG1 antibody in combination with a horse-anti-mouse IgG antibody, HRP conjugated (Vector Laboratories Inc., Burlingame, CA, USA).

Statistical Analysis

Statistical analysis was performed using SPSS (Chicago, IL, USA) software and Graphpad Prism (Graphpad Software Inc., San Diego, CA, USA) for analysis. For comparison of dichotomous values, Chi-square or Fisher's exact test and post hoc testing with homogeneity of variance correction using Games–Howell was used. Survival portions were analysed using Kaplan–Meier Curves, with posttesting using Bonferroni for multiple group comparison. All tests were two-sided; the level of statistical significance was set at a P value of < 0.05.

RESULTS

Surgery

No animals died during or immediately after surgery. Complications occurred in two rats during surgery. One rat needed resuscitation twice and in one rat the caecum was opened while removing a caecal tumour, without further adverse events.

At laparotomy, all animals had extensive tumour growth. Multiple tumour deposits of 1–3 mm were found in the omentum. Other sites of tumour involvement were the liver hilum and the mesentery. Median PCI score at time of surgery (5, range 3–8) was similar in all experimental groups ($P = 0.2$), indicating the treatment groups were well balanced. (Table 1) Omentectomy was routinely performed in all groups but the sham group. Residual disease remained in situ in seven rats, equally distributed over the four experimental groups ($P = 0.6$).

TABLE 1. *Pathological characteristics*

	Median (range)				
	Sham	CS	RIT 0	RIT 4	RIT 14
Tumour score per site					
Subcutaneous	0 (0–3)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
Laparotomy scar	1 (0–3)	1 (0–3)	0 (0–2)	0 (0–2)	0 (0–2)
Greater omentum	2 (2)	2 (2)	2 (2–3)	2 (2–3)	2 (2–3)
Liver hilum	1 (0–1)	1 (0–1)	1 (0–1)	1 (0–1)	1 (0–1)
Perisplenic	0 (0)	0 (0–1)	0 (0)	0 (0)	0 (0–1)
Mesentery	1 (0–1)	1 (0–3)	1 (0–2)	1 (0–2)	1 (0–2)
Gonadal fatpads	0 (0–1)	0 (0–2)	1 (0–2)	1 (0–1)	1 (0–2)
Diaphragm	0 (0)	0 (0)	0 (0–1)	0 (0–1)	0 (0–1)
Parietal peritoneum	1 (0–1)	1 (0–1)	1 (0–1)	1 (0–2)	1 (0–1)
Total	5 (3–6)	5 (3–7)	5 (3–8)	5 (3–7)	6 (3–7)
Resection macroscopically complete					
Yes	NA	14	14	13	12
No	NA	1	1	2	3

Pathological characteristics: CS, cytoreductive surgery; RIT 0, CS + RIT at day 0; RIT 4, CS + RIT at day 4; RIT 14, CS + RIT at day 14; NA, not applicable.

As a marker for treatment-related toxicity, body weight was measured and expressed as relative body weight compared to the body weight on the day of surgery (Fig. 1). Maximum body weight loss after Sham or CS only was similar (5.6 ± 1.4% vs. 6.5 ± 2.0% 4 days postoperatively, $P = 0.272$). Rats that were given adjuvant RIT immediately postoperatively had a maximum body weight loss of 8.8 ± 2.1%, which was significantly higher than that after Sham surgery ($P = 0.0001$) or CS only ($P = 0.003$). Maximum body weight loss of those rats that received adjuvant RIT 4 days postoperatively was 7.7 ± 2.5% 5 days postoperatively. At 21 days after surgery, body weight loss of the rats that received RIT 14 days postoperatively, i.e. 7 days after RIT, was significantly lower as compared to that of the rats that received adjuvant RIT immediately or 14 days postoperatively ($P = 0.004$).

Rats generally gained weight from the fifth day post-RIT onwards. In the RIT 0 and RIT 14 groups, post-RIT mean weight appeared lower than that of the other treatment groups, but these differences were not statistically significant.

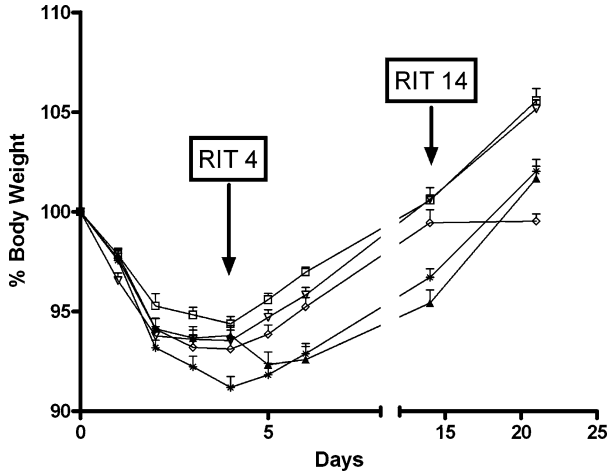

FIG. 1. The absolute body weight of rats with peritoneal metastases during 15 weeks of follow-up after sham surgery (□ Sham), cytoreductive surgery (▽ CS), or CS + RIT at day 0 (✳ RIT 0), CS + RIT at day 4 (▲ RIT 4) or CS + RIT at day 14 (◇ RIT 14) Data represent means ± standard error of the mean (SEM).

Survival

During the follow-up of 16 weeks, 64 rats died as a result of intraperitoneal tumour growth. In most cases, this was accompanied by the formation of ascites. The median amount of ascites was 33 mL (range 0–62 mL) when rats were taken out of the experiment and did not differ significantly between the groups (Fig. 2). At dissection, all of the animals in the Sham group showed adhesions due to the massive amount of tumour growth at the site of the omentum and underlying small bowel. All the other treatment groups, except for two animals in the CS group, did not show signs of extensive or dense adhesions. Two rats, one in the CS group (57 days after surgery) and one in the RIT 0 group (26 days after surgery), were taken out of the experiment early because of intercurrent death without evidence of tumour-related cause. These two rats had only small tumour deposits in the liver hilum, without obstruction of the biliary tree or vasculature, which could not explain deterioration. One animal in the RIT 0 group died most likely due to a small bowel obstruction (ileus) without

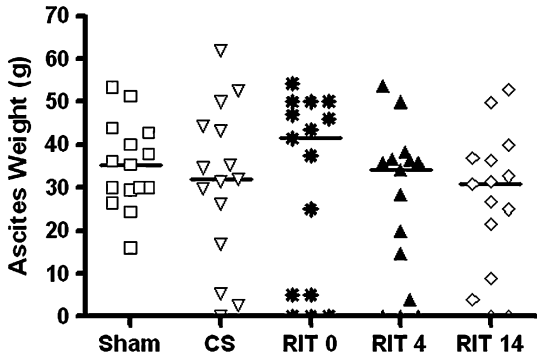

FIG. 2. Ascites weight in rats with peritoneal metastases at the time of death after sham surgery (□ Sham), (▽ CS), or CS + IT at day 0 (✳ RIT 0), CS + IT at day 4 (▲ RIT 4) or CS + RIT at day 14 (◇ RIT 14). The horizontal lines in side the graph depict the median.

obvious cause. For the other rat no explanation was found as cause for the intercurrent death. After 16 weeks, the experiment was terminated. At that time nine rats (four in group RIT 0, three in group RIT 4 and two in group RIT 14) were still alive. Of those, one out of four rats in group RIT 0, two out of three rats in group RIT 4 and both rats in group RIT 14 showed macroscopic tumour deposits. Microscopic investigation showed tumour cells in one rat in RIT 0 group while the other two rats in this group were free of tumour. (Fig. 3A, B) The one rat in the RIT 4 group without macroscopic evidence was also free of tumour after microscopic investigation. At the time of death, median PCI of those rats with tumours was 9, range 0–16, with significant differences between the groups, (Sham vs. RIT 0 $P = 0.008$, Sham vs. RIT 4 $P = 0.001$ and Sham vs. RIT 14 $P < 0.001$). No significant differences were found between the Sham and CS group ($P = 0.25$) and between the RIT groups (Fig. 4).

Rats treated with RIT in combination with CS and those treated with CS alone had an improved survival as compared to the animals in the control group (Fig. 5). Median survival of animals in the Sham and CS group was 29 days (range 25–39) and 39 days (range 25–57), respectively ($P = 0.04$).

Compared to CS alone, animals treated with adjuvant RIT had a median survival of 77 days (range 26–113, $P < 0.0001$), 52 days (range 32–65, $P < 0.0001$) and 45 days (range 33–111, $P < 0.0001$) for the RIT 0, RIT 4 and RIT 14 groups, respectively. Moreover, the median survival proved to be significantly longer when RIT was administered directly postoperatively when compared to 14 days after surgery ($P < 0.02$), whereas RIT 0 compared to RIT 4 did not ($P = 0.17$).

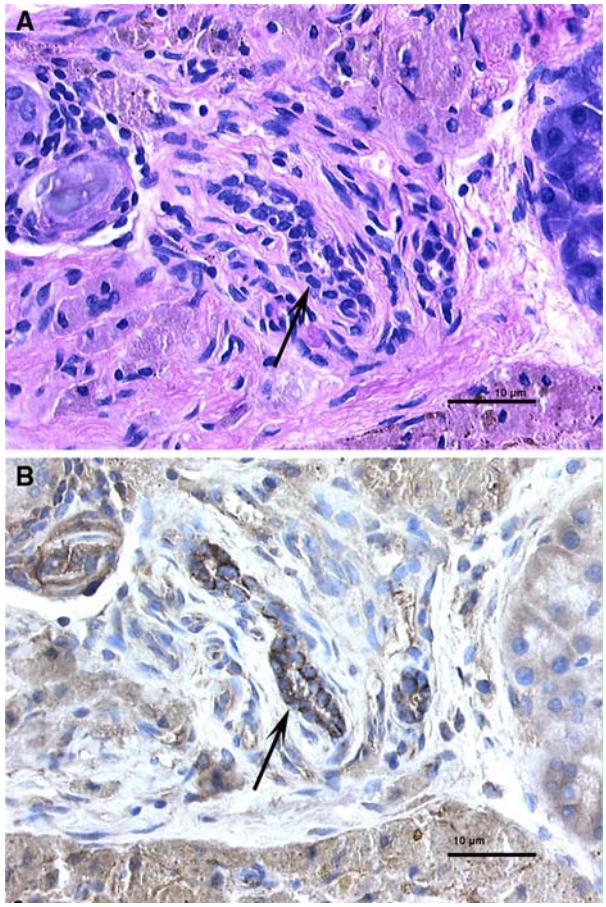

FIG. 3. (**A**, **B**) Section of WAG/Rij omentum with with CC531 tumour depostits (arrow). (**A**) H&E staining; (**B**) immunohistochemical staining. Magnification factor ×400.

DISCUSSION

This study demonstrates that early application of RIT after CS has a pronounced effect on the efficacy of this combined treatment, as application of RIT directly after surgery was more effective than the application of RIT 14 days after surgery. The positive effects on survival of ^{177}Lu-labelled MG1 are in line with previous studies in this experimental model.[16] Our combined data therefore support the reproducibility of the model, methods and outcome.

The present model of WAG/Rij rats with the intraperitoneally growing syngeneic rat colon carcinoma CC-531 was used, because of the reproducible growth pattern of these tumours in Wag/Rij rats and its similarity to the human entity of PC,[12] regarding growth and distribution pattern throughout the abdominal cavity. The MG1 MAb showed selective

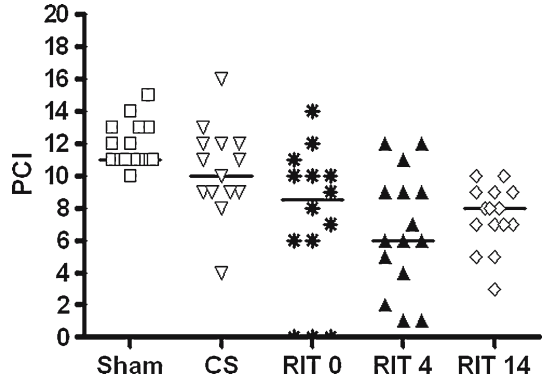

FIG. 4. Peritoneal cancer index in rats with peritoneal metastases at the time of death after sham surgery (□ Sham), (▽ CS), or CS + RIT at day 0 (✳ RIT 0), CS + RIT at day 4 (▲ RIT 4) or CS + RIT at day 14 (◇ RIT 14). The horizontal lines inside the graph depict the median.

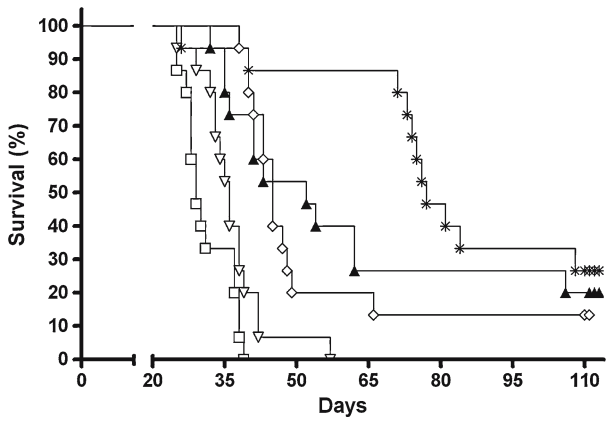

FIG. 5. Survival curves for rats with peritoneal metastases after sham surgery (□ Sham), (▽ CS), or CS + RIT at day 0 (✳ RIT 0), CS + RIT at day 4 (▲ RIT 4) or CS + RIT at day 14 (◇ RIT 14).

targeting of the CC531 tumours in this model.[16] The antibody preferentially localizes in the CC-531 tumours, with only minor cross-reactivity to other organs (thymus, lymph node, salivary gland tissue and skin).[17] ^{177}Lu was selected as the radionuclide for RIT because its high tumour uptake and retention and adequate physical properties for treatment of minimal residual disease (medium-energy β-emission with a maximum penetration range in tissue of 2.5 mm, half-life of 6.7 days). The ^{177}Lu-MG1 radionuclide-antibody combination has been shown to be effective in the model of PC as described above.[16]

The marked differences in survival between those rats that received adjuvant RIT immediately postoperatively and the rats that received RIT 4 or 14 days later might be related to several factors. First, abdominal surgery inevitably results in peritoneal trauma, which may elicit an inflammatory response and the production of fibrinogen-rich peritoneal exudate.[18] Activation of the coagulation cascade subsequently results in the formation of a fibrin network. It has been hypothesized that tumour cells can be encapsulated in the fibrin network and as such become less accessible to local therapy, such as chemotherapy or antibodies (tumour cell entrapment theory) [19] and may even increase tumour growth.[20] In the present study, the formation of fibrin might have hampered tumour targeting of the radiolabelled MG1 antibodies and consequently might have impaired the therapeutic efficacy of RIT at 4 and 14 days postoperatively. Second, the production of fibrin is a common pathway for the development of adhesions, which can be formed after abdominal surgery and develop within a week after surgery.[21,22] Intra-abdominal adhesions may have hampered the distribution of the radiolabelled MAbs over the peritoneal surfaces in the rats that received adjuvant RIT 4 or 14 days postoperatively. This hypothesis can be corroborated by the results reported by Dwivedi and colleagues.[23] The authors reported adhesion formation 21 days following a comparable surgical technique with a 6 cm midline laparotomy, subsequent caecal abrasion and inspection of the entire small bowel. This resulted in thin and easily separable adhesions only 21 days after the surgical procedure. We can, however, not make an estimation of the influences of thin and easy separable adhesions on the intraperitoneal distribution of RIT during the early and late time period after surgery since these thin adhesions were not an endpoint of this study and concomitantly were not recorded during this phase of the experiment. Third, since CC-531 is a rapidly growing tumour in WAG/Rij rats, microscopic residual disease might have grown to macroscopic disease, especially in the rats that received adjuvant RIT 14 days postoperatively.[12] The uptake and consequently therapeutic efficacy of radiolabelled antibodies is inversely correlated with tumour size.[24] The growth of minimal residual disease into larger tumours in excess of 3 mm might therefore have had a negative effect on therapeutic efficacy of the radiolabelled MAbs.

Only a few preclinical studies addressing the relevance of timing of postoperative adjuvant therapy have been undertaken.[25–28] These studies showed that administration of adjuvant chemotherapy immediately after surgery impaired outcome when factors as (intestinal) wound healing and recurrence are con-

sidered. Data of clinical studies with postoperative intraperitoneal chemotherapy support these preclinical data with regard to high mortality and morbidity rates.[29] To our knowledge, the present study is the first preclinical study investigating the issue of postoperative timing of RIT.

To date, one randomized phase III clinical trial has been published investigating the efficacy of adjuvant RIT for the treatment of minimal PC residual disease. Verheijen et al. compared the efficacy of a single intraperitoneal administration of the ^{90}Y-labelled murine anti-MUC1 MAb HMFG1 plus standard treatment to standard treatment alone in patients with stage Ic to IV ovarian cancer.[30] Patients were randomized after they had attained a laparoscopically confirmed complete remission after CS and platinum-based chemotherapy. The radiolabelled antibodies were administered intraperitoneally via a CAPD catheter after scintigraphic confirmation of equal intra-abdominal distribution. RIT using ^{90}Y-HMFG1 did not prolong disease-free nor overall survival. The lack of efficacy of adjuvant RIT in this trial could be due to several factors. Firstly, the selection of the high-energy beta-emitter ^{90}Y with a maximum tissue penetration of 12 mm does not seem appropriate in this particular setting, since most of the energy will be deposited outside the small tumour deposits. Furthermore, the protein dose was augmented with 20 mg of unlabelled antibody to a total of 25 mg ^{90}Y-HMFG1, with the intent to provoke a human-anti-mouse-antibody response. However, the high antibody dose might have had a negative effect on the uptake of the radiolabel in the tumour lesions. The mechanism of antigen saturation due to excessive amounts of antibody may have interfered with intratumoural antibody uptake as described previously (Kranenborg et al. and Koppe et al.).[31,32] Thirdly, in view of the results of the present study, the time interval between CS and the administration of at least 2 months might have had a negative impact on the efficacy of RIT.

In addition, Behr et al. studied the application of a high and low affinity 131I-labelled anti-CEA antibody MN-14 in a hepatic metastasis model colorectal carcinoma and compared this treatment to contemporary 5-fluorouracil/leucovorin and irinotecan at equitoxic doses, showing that RIT cured 20% of the animals with minimal disease. This preclinical study was followed by a phase II trial investigating the safety and efficacy of adjuvant RIT using the 131I-labelled humanized anti-CEA MAb Labetuzumab (MN-14) in 23 patients who had undergone R0 liver resection for metastatic colorectal cancer, i.e. were surgically cured. Median disease-free and overall survival was 18 months (95% CI 11–31) and 68 months (95% CI 41-infinity), with a 5-year survival rate of 51%. The authors concluded, that since these results seemed to be better than those obtained in historical controls, a phase III randomized controlled trial is justified. Adjuvant RIT was also performed in 33 patients with glioma at Dukes University. The median survival after treatment with 120 mCi of radiolabelled anti-tenascin antibody in this study was 79–85 weeks as compared to 46 weeks of historical controls. The results of these trials warranted a phase III trial, which is currently ongoing.

The present study indicated that early administration of RIT is a highly effective treatment. Clinical studies utilizing intraperitoneal radioimmunotherapy should therefore be focused on immediate postoperative or intraoperative administration of RIT.

CONCLUSION

This study showed that proper timing of RIT adjuvant to CS is crucial and can significantly improve the efficacy of the combined treatment in a model of PC of CRC. Therefore, adjuvant radioimmunotherapy should be given early after surgery in order to maximize its efficacy after CS of PC of colorectal origin.

ACKNOWLEDGMENTS

The authors wish to thank Ben de Man and Roger Lomme (Radboud University Nijmegen Medical Center, Surgical Research Laboratory) for their excellent assistence during the operative procedures, Gerry Grutters and Henny Eikholt (Radboud University Nijmegen Medical Center, Central Animal Laboratory) for their assistance in the animal experiments. Part of this study was supported by a grant from the Netherlands Organization for Health Research and Development (ZonMw); Grant number: 920–03-220

REFERENCES

1. Sugarbaker PH, Cunliffe WJ, Belliveau J, et al. Rationale for integrating early postoperative intraperitoneal chemotherapy into the surgical treatment of gastrointestinal cancer. *Semin Oncol* 1989; 16:83–97.
2. Sugarbaker PH. Colorectal carcinomatosis: a new oncologic frontier. *Curr Opin Oncol* 2005; 17:397–9.

3. Culliford AT, Brooks AD, Sharma S, et al. Surgical debulking and intraperitoneal chemotherapy for established peritoneal metastases from colon and appendix cancer. *Ann Surg Oncol* 2001; 8:787–95.
4. Koppe MJ, Boerman OC, Oyen WJ, et al. Peritoneal carcinomatosis of colorectal origin: incidence and current treatment strategies. *Ann Surg* 2006; 243:212–22.
5. Verwaal VJ, Boot H, Aleman BM, et al. Recurrences after peritoneal carcinomatosis of colorectal origin treated by cytoreduction and hyperthermic intraperitoneal chemotherapy: location, treatment, and outcome. *Ann Surg Oncol* 2004; 11:375–9.
6. Monneuse O, Mestrallet JP, Quash G, et al. Intraperitoneal treatment with dimethylthioampal (DIMATE) combined with surgical debulking is effective for experimental peritoneal carcinomatosis in a rat model. *J Gastrointest Surg* 2005; 9:769–74.
7. Mahteme H, Sundin A, Larsson B, et al. 5-FU uptake in peritoneal metastases after pretreatment with radioimmunotherapy or vasoconstriction: an autoradiographic study in the rat. *Anticancer Res* 2005; 25:917–22.
8. Koppe MJ, Soede AC, Pels W, et al. Experimental radioimmunotherapy of small peritoneal metastases of colorectal origin. *Int J Cancer* 2003; 106:965–72.
9. Kinuya S, Yokoyama K, Izumo M, et al. Locoreginal radioimmunotherapy with 186Re-labeled monoclonal antibody in treating small peritoneal carcinomatosis of colon cancer in mice in comparison with 131I-counterpart. *Cancer Lett* 2005; 219:41–8.
10. Kinuya S, Li XF, Yokoyama K, et al. Intraperitoneal radioimmunotherapy in treating peritoneal carcinomatosis of colon cancer in mice compared with systemic radioimmunotherapy. *Cancer Sci* 2003; 94:650–4.
11. Zedeck MS. A model system for studies of colon carcinogenesis: tumor induction by a single injection of methylazoxymethanol acetate. *J Natl Cancer Inst* 1974; 53:1419–21.
12. Lopes Cardozo AM, Gupta A, Koppe MJ, et al. Metastatic pattern of CC531 colon carcinoma cells in the abdominal cavity: an experimental model of peritoneal carcinomatosis in rats. *Eur J Surg Oncol* 2001; 27:359–63.
13. Ruegg CL, nderson-Berg WT, Brechbiel MW, et al. Improved in vivo stability and tumor targeting of bismuth-labeled antibody. *Cancer Res* 1990; 50:4221–6.
14. Lindmo T, Boven E, Cuttitta F, et al. Determination of the immunoreactive fraction of radiolabeled monoclonal antibodies by linear extrapolation to binding at infinite antigen excess. *J Immunol Methods* 1984; 72:77–89.
15. Steffens MG, Boerman OC, Oosterwijk-Wakka JC, et al. Targeting of renal cell carcinoma with iodine-131-labeled chimeric monoclonal antibody G250. *J Clin Oncol* 1997; 15:1529—37.
16. Koppe MJ, Hendriks T, Boerman OC, Oyen WJ, Bleichrodt RP. Radioimmunotherapy is an effective adjuvant treatment modality after cytoreductive surgery of peritoneal carcinomatosis of colonic origin. J Nucl Med 2006, in press.
17. Hagenaars M, Koelemij R, Ensink NG, et al. The development of novel mouse monoclonal antibodies against the CC531 rat colon adenocarcinoma. *Clin Exp Metastasis* 2000; 18:281–9.
18. Reijnen MM, Bleichrodt RP, Van Goor H. Pathophysiology of intra-abdominal adhesion and abscess formation, and the effect of hyaluronan. *Br J Surg* 2003; 90:533–41.
19. Nagy JA, Meyers MS, Masse EM, et al. Pathogenesis of ascites tumor growth: fibrinogen influx and fibrin accumulation in tissues lining the peritoneal cavity. *Cancer Res* 1995; 55:369–75.
20. Biggerstaff JP, Seth N, Amirkhosravi A, et al. Soluble fibrin augments platelet/tumor cell adherence in vitro and in vivo, and enhances experimental metastasis. *Clin Exp Metastasis* 1999; 17:723—30.
21. Dijkstra FR, Nieuwenhuijzen M, Reijnen MM, et al. Recent clinical developments in pathophysiology, epidemiology, diagnosis and treatment of intra-abdominal adhesions. *Scand J Gastroenterol Suppl* 2000; 52–9.
22. Herrick SE, Mutsaers SE, Ozua P, et al. Human peritoneal adhesions are highly cellular, innervated, and vascularized. *J Pathol* 2000; 192:67–72.
23. Dwivedi AJ, Kuwajerwala NK, Silva YJ, et al. Effects of surgical gloves on postoperative peritoneal adhesions and cytokine expression in a rat model. *Am J Surg* 2004; 188:491–4.
24. Koppe MJ, Postema EJ, Aarts F, et al. Antibody-guided radiation therapy of cancer. *Cancer Metastasis Rev* 2005; 24:539–67.
25. Uzunkoy A, Bolukbas C, Horoz M, et al. The optimal starting time of postoperative intraperitoneal mitomycin-C therapy with preserved intestinal wound healing. *BMC Cancer* 2005; 5:31.
26. Jacquet P, Stuart OA, Dalton R, et al. Effect of intraperitoneal chemotherapy and fibrinolytic therapy on tumor implantation in wound sites. *J Surg Oncol* 1996; 62:128–34.
27. Weiber S, Graf W, Glimelius B, et al. Experimental colonic healing in relation to timing of 5-fluorouracil therapy. *Br J Surg* 1994; 81:1677–80.
28. van der Kolk BM, de Man BM, Wobbes T, et al. Is early postoperative treatment with 5-fluorouracil possible without affecting anastomotic strength in the intestine?. *Br J Cancer* 1999; 79:545–50.
29. Koppe MJ, Boerman OC, Oyen WJ, et al. Peritoneal carcinomatosis of colorectal origin: incidence and current treatment strategies. *Ann Surg* 2006; 243:212–22.
30. Verheijen RH, Massuger LF, Benigno BB, et al. Phase III trial of intraperitoneal therapy with yttrium-90-labeled HMFG1 murine monoclonal antibody in patients with epithelial ovarian cancer after a surgically defined complete remission. *J Clin Oncol* 2006; 24:571–8.
31. Kranenborg MH, Boerman OC, de Weijert MC, et al. The effect of antibody protein dose of anti-renal cell carcinoma monoclonal antibodies in nude mice with renal cell carcinoma xenografts. *Cancer* 1997; 80:2390–7.
32. Koppe MJ, Soede AC, Pels W, et al. Experimental radioimmunotherapy of small peritoneal metastases of colorectal origin. *Int J Cancer* 2003; 106:965–72.