Cytoreductive Surgery and Radioimmunotherapy to treat Peritoneal Carcinomatosis of Colorectal Cancer

Investigations towards Improvement of Outcome and Morbidity

Cytoreductive Surgery and Radioimmunotherapy to treat Peritoneal Carcinomatosis of Colorectal Cancer: Investigations towards Improvement of Outcome and Morbidity;

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Investigations towards Improvement of Outcome and Morbidity

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op het gebied van de Medische Wetenschappen

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The greatest thing you'll ever learn is to love and be loved in return.

Voor Diana en Mael

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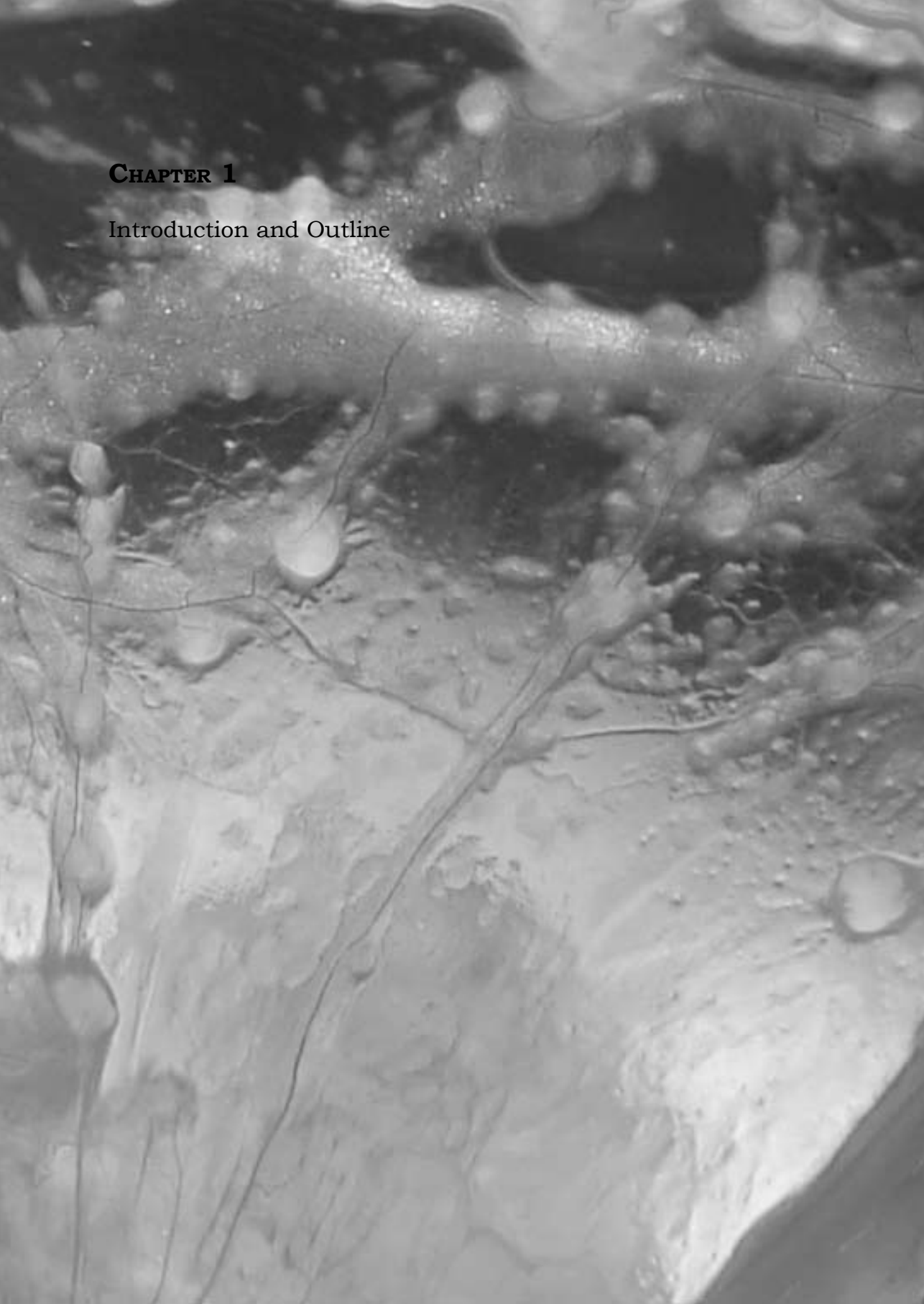


Chapter 1

Introduction and Outline

Introduction

Peritoneal Carcinomatosis

Colorectal cancer (CRC) is second in incidence to lung cancer in men and breast cancer in women as a cause of cancer-related deaths in Europe and the USA. More than 900,000 new cases of CRC are diagnosed worldwide each year, and nearly 500,000 patients die as a result of CRC.[1] In 19% of patients with CRC, distant metastases are found at the time of diagnosis.[2] One specific entity of the regional spread of CRC is peritoneal carcinomatosis (PC), which may develop in patients after resection of the primary tumor.[3] PC is present as synchronous disease at the time of resection of the primary tumor in 7% of patients with CRC and as metachronous carcinomatosis in 4.5%.[4] In approximately 25% of patients with recurrent CRC, the peritoneal cavity is the only site of metastatic disease.[3]

PC may result from spread of cells from the primary tumor into the peritoneal cavity or from tumor spillage during surgery of the primary tumor. Tumor cells can be isolated from the peritoneal cavity in as many as 30% of the patients during surgery.[5] These cells may cause peritoneal metastases. However, their role in causing PC and the significance for the prognosis of these patients are still ambigious[3].

The locations of peritoneal metastases—which are found mainly in the right paracolic gutter and in the subhepatic and right subdiaphragmatic spaces (a metastatic pattern that follows the intraabdominal fluid stream in the abdominal cavity)—support the hypothesis that free-floating tumor cells may cause PC. It is hypothesized that, after surgery, tumor cells are trapped in fibrin clots that place them beyond the reach of the immunologic defenses in the abdominal cavity. These tumor clots may be the nidus of peritoneal metastases. Peritoneal metastases may cause serious complications such as intestinal obstruction and the formation of fistulas and ascites. Therefore, it is worthwhile to develop methods to prevent and treat PC.

Treatment of Peritoneal Carcinomatosis

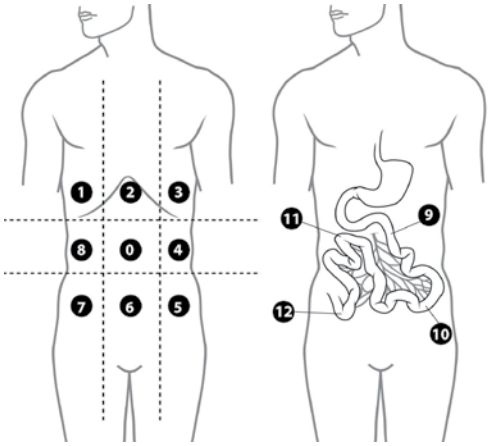
Treatment of PC has historically comprised palliative measures only, either surgically (bypass of the obstruction) or chemotherapeutically. Several treatment regimens have been developed to offer patients with PC of CRC a chance of survival.

Patients with PC have a median survival of six months, if untreated. Therefore, nonoperative intervention was proposed instead of palliative surgical intervention in case of intestinal obstruction.[6] Palliative chemotherapy alone did not improve survival in these patients.[4,7]

Sugarbaker explored the feasibility of radical debulking procedures, or cytoreductive surgery (CS), to treat PC.[8] CS comprises the removal of all peritoneal tumor deposits, including a greater omentectomy. In most cases a partial peritonectomy, cholecystectomy, splenectomy, and partial bowel resection are performed to remove all visible tumor in all regions of the abdomen.[8] The extent of PC is scored using the peritoneal cancer index (PCI) of Sugarbaker. See Figure 1. The radicality of the resections are graded R0–R3. See Table 1.[9] Median survival ranges from 18 months to 39 months after R0 resections but from 13 months to 24 months after R2 resections and only 5 months to 12 months after R3 resections.[3] Fortunately, most resections (80%–90% are R0–R1 resections, probably because of careful patient selection.[10,11] The results of treatment of patients with PC depend on the extent of the metastatic disease (PCI) and the type of resection performed (R0–R3).

R-stage	Radicality
0	No visible tumor
1	<2.5 mm
2	>2.5 mm to <5 mm
3	>5 mm

Table 1. Resection type



Regions	Anatomic structures
0 Central	Midline abdominal incision — entire great omentum — transverse colon
1 Right upper	Superior surface of the right lobe of the liver — undersurface of the right hemidiaphragm — right retrohepatic space
2 Epigastrium	Lleft lobe of the liver — lesser omentum — falciform ligament
3 Left upper	Undersurface of the left hemidiaphragm — spleen — tail of pancreas — anterior and posterior surfaces of stomach
4 Left flank	Descending colon — left abdominal gutter
5 Left lower	Pelvic sidewall lateral to the sigmoid colon — sigmoid colon
6 Pelvis	Female internal genitalia — bladder — Douglas pouch — rectosigmoid colon
7 Right lower	Right pelvic sidewall — cecum — appendix
8 Right flank	Ascending colon — right abdominal gutter
9 12 Jejunum/ ileum	Including mesentery

Figure 1. Peritoneal cancer index

Sugarbaker introduced adjuvant intraperitoneal chemotherapy to reduce recurrent intraperitoneal disease after CS.[12] Compared with intravenous administration, intraabdominal administration of direct cytotoxic (cell-cycle independent) chemotherapy agents results in higher local concentrations of chemotherapy in the abdominal cavity. Moreover, free-floating tumor cells are rinsed from the abdomen and killed by the cytotoxic agents. In addition, if administered at a later time, intraperitoneal chemotherapy may cause fibrous adhesions to form, preventing the chemotherapy from being distributed equally within the peritoneal cavity. The penetration depth of the used agents is 1 ± 3 mm.[13]

Efforts to improve the outcome of intraperitonally administered chemotherapy resulted in investigations on the use of hyperthermia. In 1993 it was found that the therapeutic efficacy of intraperitoneal carboplatin was improved when administered under hyperthermic conditions (41.5°C). In vitro studies showed also that the antitumor effect of mitomycin C and cisplatin on human colon cancer cells increased when these treatments were combined with hyperthermia (43°C).[14] See Table 2. Hyperthermia has a direct cytotoxic

effect (impaired DNA repair and denaturation of proteins), and the effect is dependent on temperature and exposure time. The penetration depth of hyperthermia, however, is limited to 1 mm; thus, the effect of direct cytotoxicity will be limited.[15] Clinically, hyperthermic intraperitoneal chemotherapy (HIPEC) was originally performed after CS with anastomotic construction. Later, the anastomoses were constructed after the perfusion in order to decrease tumor entrapment within them.

During the late 90s, phase I/II studies indicated that the use of HIPEC could achieve long-term survival (60% 2-year survival).[16] Later, it was shown that a 2-year survival of 74% could be reached for patients who underwent a complete resection.[17]

Chemotherapy Agent	Heat Synergysm
Mitomycin C	yes
Cisplatinum	yes
Melphalan	yes
Mitroxantrone	yes
Oxaliplatin	yes
Irinotecan	yes
5-fluorouracil	no

Table 2. Heat synergism of chemotherapy agents

During HIPEC, the peritoneal cavity is continuously perfused for 60–120 minutes with a hyperthermic dialysis fluid (41°C-43°C) containing the cytotoxic drug. The perfusion is generally performed using the closed-abdomen technique. See Figure 2.[18,19] The closed system has two disadvantages. First, some parts of the abdominal cavity may not be reached by the perfusate because of adhesions, which may affect the intraabdominal temperature distribution as well. Second, tumor cells may be entrapped within the constructed bowel anastomoses. Because of these disadvantages, an open technique was developed. During the perfusion, the abdomen is left open, with retraction of the skin using an expander, allowing for intraabdominal manipulation and resulting in a more equal distribution of the chemotherapy agent. Thereafter, continuity of the bowel can be restored.

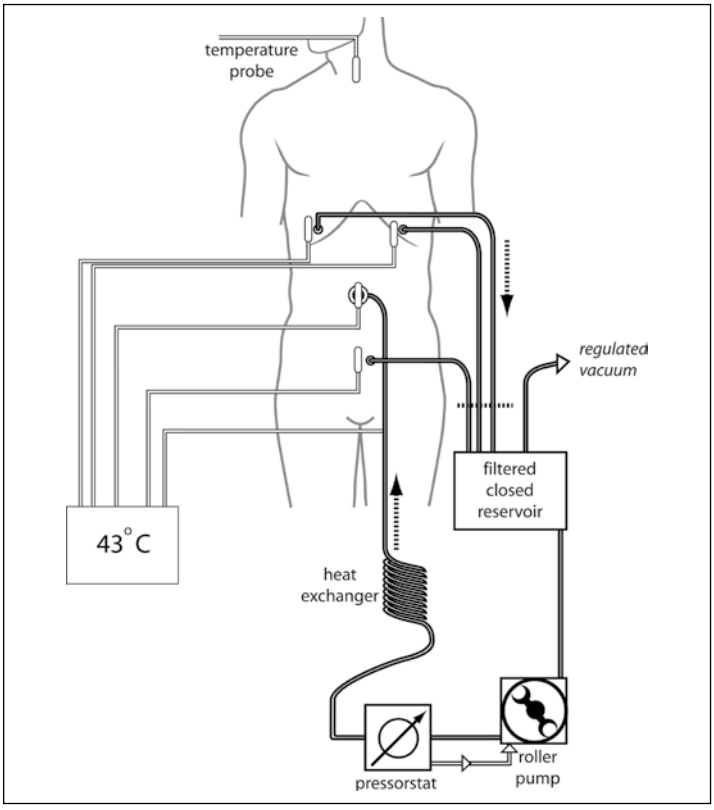


Figure 2. HIPEC procedure.

Although PC is relatively common, most data on survival and morbidity are derived from phase I/II studies. There has been only one phase III study, comparing HIPEC (using a 17 mg/m^2 dose of mitomycin C) to the standard treatment of PC (5-fluorouracil/leucovorin).[20] This study showed that the application of HIPEC after CS significantly improved survival. At a median follow-up of 21.6 months, patients treated with the standard treatment showed a median survival of 12.6 months, compared to 21.6 months in patients treated with CS and HIPEC in PC of colorectal origin. The gain in survival, however, was achieved at the cost of significant morbidity of up to 65%. Recurrent disease was found in 65% of patients.[21,22]

Radioimmunotherapy in Peritoneal Carcinomatosis

Based on the results of the combined treatment of CS and HIPEC, efforts to improve survival while decreasing morbidity warranted the development of new, more specific, i.e., targeted, treatment modalities for PC.

Koppe and colleagues therefore investigated the feasibility and therapeutic efficacy of the intraperitoneal administration of radiolabeled antibodies against colon cancer cells (radioimmunotherapy, or RIT) in an experimental setting.[23] In a rat model of PC, induced using the syngeneic colon carcinoma cell line CC531, CS was performed seven days after tumor induction. Subsequently, four days after surgery, the animals received an intraperitoneal injection of the anti-CC531 antibody MG1 labeled with the ß-radiation–emitting radionuclide ^{177}lutetium (^{177}Lu) at a dose of 1.5 mCi.[23] In this study, the median survival of the control rats that underwent an exploratory laparotomy only (without surgical removal of tumor) was 41 days. The median survival of the animals that had undergone CS only was 51 days. Median survival of animals that were treated with exploratory laparotomy followed by intraperitoneal RIT was 61.5 days. CS followed by RIT resulted in the highest median survival of 88 days.

The results of this experiment showed that animals treated with the combination of both CS and RIT survived longer than animals treated with either treatment modality alone. This experimental study thus provided the proof of principle that RIT as an adjuvant to CS could be an effective treatment for PC.

Outline of the Thesis

Based on the results of the studies performed by Koppe and colleagues, investigations were undertaken to improve the therapeutic effect and to decrease the treatment-related toxicity of RIT. Moreover, the effect on survival, treatment-related toxicity, and morbidity of adjuvant RIT after CS was compared to HIPEC, being the gold standard of care for the treatment of PC.
Within this thesis, the following questions have been answered:

- Is there clinical evidence of the treatment efficacy of locally or regionally administered RIT to treat solid cancer (**Chapter 2**)?
- Can survival of animals with induced PC of CRC be enhanced after CS and intraperitoneally administered RIT by altering the interval between the surgical procedure and the intraperitoneal administration of RIT, and if so, what would be the optimal interval (**Chapter 3**)?
- In the era of multimodality treatment of cancer, can the therapeutic effect of adjuvant RIT be improved by the concomitant application of other treatment modalities, such as fibrinolytic therapy or whole-body hyperthermia (**Chapter 4**)?
- Following the determination of the optimal setting of adjuvant RIT, what is the therapeutic efficacy of adjuvant RIT after CS, compared to that of adjuvant HIPEC (**Chapter 5**)?
- What is the effect of adjuvant RIT, compared to that of HIPEC, on wound healing of the small and large bowel and the abdominal wall after CS (**Chapter 6**)?
- Is it possible to enhance the treatment efficacy of intraperitoneally administered RIT using a sugar-modified antibody (**Chapter 7**)?
- Is imaging of patients with proven colorectal cancer using two-step pre-targeted radioimmunoscintigraphy feasible (**Chapter 8**)?

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Abstract

Radioimmunotherapy (RIT) potentially is an attractive treatment for radiosensitive early stage solid tumors and as an adjuvant to cytoreductive surgery. Topical administration of RIT may improve the efficacy because higher local concentrations are achieved. We reviewed the results of locally applied radiolabeled monoclonal antibodies for the treatment of solid tumors. Intracavitary RIT in patients with ovarian cancer and glioma showed improved targeting after local administration as compared to the intravenous administration. In addition, various studies showed the feasibility of locally applied RIT in these patients. Adjuvant RIT in ovarian cancer and glioma showed to be at least as effective as standard therapy. The information about RIT for peritoneal carcinomatosis of colorectal origin is scarce while results from pre-clinical data are promising.

Studies on the application of radiolabeled antibodies in early urothelial cell cancer showed that intracavitary RIT may hold a promise. In patients with malignant pleural mesothelioma or malignant pleural effusion, RIT may play a role in the palliative treatment.

The future of RIT may therefore not only be in the inclusion in contemporary multimodality treatment, but also in the expansion to palliative treatment.

Chapter 2

Intracavitary Radioimmunotherapy to treat solid tumors

This chapter is based on:
Intracavitary radioimmunotherapy to treat solid tumors. Aarts F, Bleichrodt RP, Oyen WJ, Boerman OC. Cancer Biother Radiopharm. 2008 Feb;23(1):92-107

Introduction

Biologicals play an increasingly important role in the treatment of cancer. Monoclonal antibodies (mAbs) against CD20 are standard therapy in non-Hodgkin's lymphoma (NHL), anti-vascular endothelial growth factor (VEGF) antibodies are used to treat colon and lung carcinoma and anti-HER2 antibodies play a role in breast cancer treatment. mAbs can also be used to direct anticancer drugs to tumor cells. Drugs, toxins, or radionuclides can be conjugated to mAbs for selective delivery of these agents to tumor tissues, thus sparing normal tissues in contrast to conventional systemic anticancer therapies.[1,2] In radioimmunotherapy (RIT), mAbs are labeled with radionuclides to selectively irradiate tumor cells. RIT has proven to be effective in hematologic malignancies but is less effective in solid cancers.[3–5] Large solid tumors have a limited blood supply, rendering these tumors less accessible for therapeutic agents being delivered via the blood. Impaired blood supply and concomitant ischemia make tumor cells less radiosensitive.[6,7] In addition, solid tumors have a high interstitial fluid pressure that limits the uptake and penetration of antibodies.[8] As a result, in large tumors, the uptake of mAbs from the blood is relatively low and heterogeneous, thus limiting the efficacy of RIT. In small tumor lesions, the uptake of mAbs is higher and the heterogeneity of antibody uptake within the tumor can be overcome by the penetration range of the radiation. Therefore, RIT seems to be an attractive adjuvant therapy in small-volume disease or microscopic residual tumor deposits. RIT may also be administered topically in order to reduce systemic activity and enhance tumor targeting. In addition, there are conditions that are particularly suitable for RIT owing to tumor localization (i.e., the development of cancer within a natural cavity) and its concomitant pharmacologic advantages. In both preclinical and clinical studies, the mAb levels in intraperitoneally growing tumors were found to be higher during the first 24 hours after intraperitoneal administration, as compared to systemic administration, thus favoring this route of administration.[9,10] In this paper, we reviewed the results of locally applied radiolabeled mAbs for the treatment of solid tumors.

Radioimmunotherapy

Antibodies

Since the development of the hybridoma technology, it is possible to generate antibodies specifically directed against tumor-associated antigens. [11] Murine antibodies were the first clinically applied antibodies. A major disadvantage was the production of human antimouse antibodies (HAMAs) that could induce humoral immune responses. In order to reduce immunogenicity, recombinant DNA techniques were applied to produce chimeric and humanized mAbs.[12] In chimeric mAbs, the variable regions of heavy and light chains are of murine origin, and these are then fused with the constant regions of human origin (Fig. 1). In humanized mAbs, only the Complimentarity Determining Regions (CDR) of the murine origin are grafted in a human antibody framework.[13,14] To improve *in vivo* targeting properties, new antibody constructs have been produced, using recombinant DNA techniques (e.g., scFv, minibodies, diabodies, and so forth).

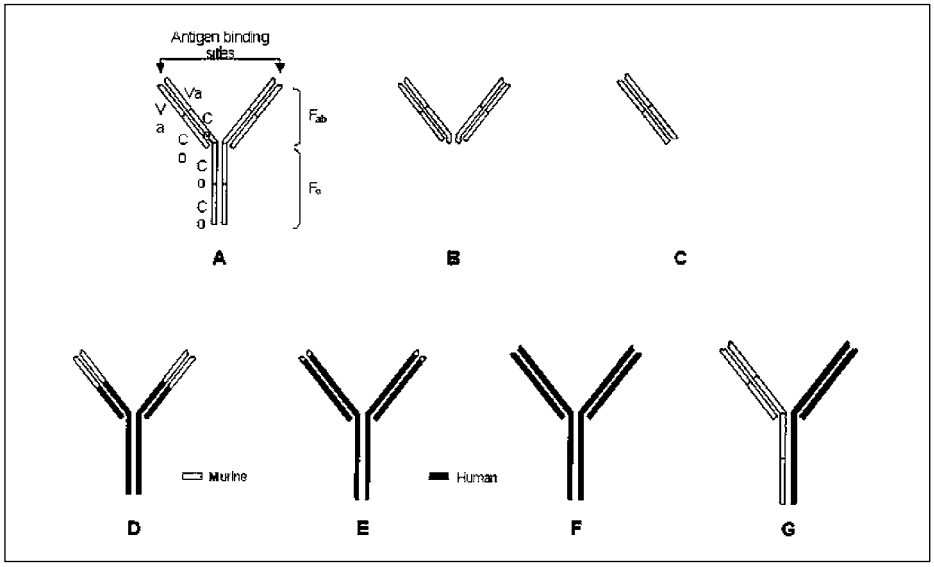

Figure 1.

Most important forms of monoclonal antibodies used in clinical radioimmunotherapy.

A, Whole (murine) IgG (MW 150 kDa). Va, variable region; Co, constant region; B, $F(ab')_2$ fragment (MW 100 kDa); C, Fab' fragment (MW 50 kDa); D, Chimeric IgG (67% human). The constant regions of the murine antibody have been replaced by their human analogues; E, Humanized IgG (90-95% human); F, Fully human IgG; G, Bispecific antibody. The antibody, with both arms originating from two separate antibodies, is reactive with two distinct antigens. Copyright British Journal of Surgery Society Limited. Reprinted by permission from M.J. Koppe et al. Radioimmunotherapy and colorectal cancer.

Radionuclides

The most commonly used radionuclides in RIT are beta-emitters. Beta-particles are electrons that are emitted from the nucleus of an unstable atom. In RIT, iodine-131 (^{131}I) and yttrium (^{90}Y) are the most commonly used beta-emitters. More recently, rhenium-186 (^{186}Re), copper-67 (^{67}Cu), and lutetium-177 (^{177}Lu) have also been applied (see Table 1) The differences in physical half-life, the presence or absence of gamma rays, the energy, and consequently the range of the beta-particles in tissue are important with respect to the radiation dose that can be delivered to the tumor. For example, ^{90}Y-labeled mAbs are theoretically not suitable for the treatment of minimal or residual disease with a diameter of only a few millimeters or less, since 70% of the radiation energy will be deposited outside small

tumors (diameter <5 mm) owing to the high energy of the ß-particles (mean, 0.9 MeV).[15] For this application, the use of ^{177}Lu-labeled antibodies with medium-energy electron emissions is better suited.[16] Internalization of the radiolabeled antibodies depends on various factors, including the antibody itself and the targeted antigen. All antibodies eventually are internalized by the target cell and subsequently catabolized.[17] When internalized, the radiolabeled mAb is degraded in the lysosomes. In case of radioiodinated mAbs, the radiolabeled metabolites are excreted from the cell. Labeling of mAbs with radiometals, such as ^{90}Y, ^{177}Lu, and ^{67}Cu, is performed by first linking chemical moieties that can complex the metal ions to the antibody (chelators such as DTPA, DOTA, or TETA). When catabolized, the radioactive metabolites of mAbs labeled with radiometals are trapped in the lysosomes, increasing the retention of these radiolabels in the tumor.[18,19]

Table1.
RadioNuclides

Radionuclide	Half life	ß-energy	γ-energy	Range ß
^{131}I	8 days	192 keV	362 keV	3 mm
^{90}Y	64 hr	935 keV	-	12 mm
^{188}Re	17 hr	795 keV	155 keV	27 mm
^{177}Lu	7 days	149 keV	208 keV	3 mm
^{67}Cu	62 hr	141 keV	185 keV	2 mm

Intraperitoneal RIT

The abdominal cavity is lined with mesothelial cells that not only play a role in the sliding of the peritoneal surfaces, but also play an active roll in the immunologic response, inflammation, and coagulation.[20] Peritoneal clearance is regulated via the peritoneum that behaves as a semipermeable membrane and the lymphatic lacunae underlining diaphragmatic stomata. This clearing process starts within minutes after contamination of the peritoneal cavity. Antibodies administered into the peritoneal cavity cross the peritoneal lining mainly by diffusion. The rate of diffusion depends on the volume

of the instilled agent and the concomitant increase in fluid pressure.[21,22] Moreover, antibodies will be cleared via the diaphragmatic stomata. For RIT of intraperitoneal lesions, the intraperitoneal (i.p.) administration is advantageous, as compared to the systemic administration, because higher concentrations of the antibody can be reached in the tumor.[23] When mAbs are administered into the peritoneal cavity, their pharmacokinetics are similar to that of other large-serum proteins. Apart from specific binding to i.p. tumors, there is transportation to thecirculation and subsequent uptake in normal tissues. [24,25] However, in both preclinical and in clinical studies, the concentration of mAbs in the tumor lesions is higher during the first 24 hours after an i.p. administration while blood levels are lower, as compared to a systemic administration. In addition, not only the tumor uptake is increased, but also the tumor:nontumor ratio. This favors this route of administration for i.p. growing tumors.[10,26]

Ovarian Cancer

Ovarian cancer mostly originates from the surface epithelium of the ovaries, and in most patients, metastases are confined to the peritoneal cavity without further distant metastases.[27] The overall survival rate is 35%. The majority of patients presents with advanced disease at the time of diagnosis. Radical debulking, in combination with platinum- or taxol-based chemotherapy, is the standard treatment, resulting in 50% local recurrencesand a median survival of 2 years.[28,29] Ovarian cancer seems to be well suited for i.p. administered adjuvant RIT.[30] The disease has been targeted by using antibodies directed against tumorassociated antigens as the mucin-1 (MUC1) antigen, CA-125, TAG-72, and gp38 (see Table 2). As mentioned above, several preclinical and clinical studies demonstrated that an i.p. administration of RIT resulted in a higher uptake of radiolabeled antibodies in tumor, as compared to systemic route administration.[10,26,31,32]

MUC-1

MUC-1 is expressed on glandular epithelium in the majority of adenocarcinomas, includingbreast, ovary, and pancreas, where it is both overexpressed as well as aberrantly glycosylated.[33] One of the first RIT trials in patients with ovarian cancer was carried out by Epenetos et al., treating 24 patients with stage III ovarian cancer in an adjuvant setting to cytoreductive surgery and platinum-based chemotherapy. In this study, ^{131}I-labeled anti-MUC1 mAbs, HMFG1-2, AUA 1, and H17E2, were used.[34] The best response was found in the 16 patients with minimal or small-volume (<2 cm diameter) disease. No response was seen in the 8 patients with largevolume disease (>2 cm diameter) who all died of recurrent disease within 9 months. In another trial, the efficacy of adjuvant ^{90}Y-labeled HMFG1 following surgery and chemotherapy was compared to a historic control group that was treated with surgery and chemotherapy alone. Five (5)-year survival in the RIT group was 80%, as compared to 55% in the control group.[35] Later, these investigators reported that after 12 years of follow-up, the median survival of a group with complete remission following surgery, chemotherapy, and adjuvant ^{90}Y-HMFG1 had not yet been reached. These results were better than the results in a historic control group that had a median survival of 42% survival after 5 years of follow- up.[36] Based on the encouraging results of these phase I/II studies, a phase III open-label, randomized, multicenter phase III trial was undertaken. This study included patients to be treated with ^{90}Y-HMFG1 RIT (maximum dose, 25 mCi; N = 224) and compared them to conventionally treated patients (N =223).[37] Patients were eligible after a complete clinical response to platinum-based chemotherapy following surgical cytoreduction. Intraperitoneally administered RIT was applied after a confirmed macroscopically negative laparoscopy. This study failed to show a benefit in overall survival or a prolonged disease-free survival after RIT. Although peritoneal recurrence was significantly delayed in the RIT group, extraperitoneal metastases, mainly located in the para-aortic lymph nodes, were found more frequently than in the control group (49% versus 14%).[38]

TAG-72

TAG-72 is a mucin-like antigen that is heterogeneously expressed in virtually all ovarian cancers. The pharmacology, metabolism, and tissue distribution of ^{90}Y-labeled anti-TAG-72 B72.3 was investigated in 9 patients with ovarian cancer.[39] Following this report, Meredith et al.[40] and Rosenblum et al.[41] performed i.p. RIT studies in patients with chemotherapy-resistant or refractory ovarian cancer limited to the peritoneal cavity. Rosenblum et al. included 58 patients treated with ^{90}Y-labeled B72.3, 1–25 mCi, resulting in 2 complete remissions and 30 patients with stable disease with a median of 6 months (range, 4–24). In a study using ^{177}Lu-labeled CC49, a secondgeneration anti-TAG-72 antibody with a higher affinity for TAG-72 than the B72.3 antibody (dose, 10–50 mCi/m2), Alvarez et al. included 27 patients. Fourteen (14) of these patients with small (<1 cm) or microscopic disease were stable for 6–35 months, whereas in patients with measurable disease (>1 mm), only 1 patient showed a partial response. The other patients showed progression within 3 months.[42] Meredith et al. published a report including 12 patients treated with ^{177}Lu-labeled CC49, 10–30 mCi/m2.[40] This resulted in complete remission in 3 patients and stable disease in 1. Complete responses were only found in patients with microscopic disease. Following the report by Roselli et al.,[43] who showed that interferon (IFN) enhances the expression of TAG-72, Meredith et al. Subsequently tested the feasibility and efficacy of a combination of subcutaneous (s.c.) injections of IFN, i.p. paclitaxel (100 mg/m2), and RIT consisting of 40–45 mCi/m2 ^{177}Lu-labeled CC49. This study included 44 patients with ovarian cancer, 27 of whom had only microscopic disease or disease not measurable on computed tomography (CT).[44] Four (4) of 17 patients with macroscopic disease on CT showed a partial response (decrease of >50% diameter), whereas 4 of 27 patients with microscopic disease showed progression- free time intervals of more than 18 months. Subsequently, the same research group treated 20 patients with persistent or recurrent ovarian cancer after conventional cytoreductive surgery and chemotherapy, with a combination of RIT using ^{90}Y-labeled CC49 and a single i.p. administered dose of paclitaxel (100 mg/m2) and IFN. In 3 of 11 patients with nonmeasurable disease, disease-free survival was more than 18 months. The researchers concluded that this combination of treatments was well tolerated and showed antitumor activity.

gp38

MOv18 is an antibody directed against the folate receptor gp38, which is expressed at high levels in 90% of serous ovarian carcinomas. The expression level on other epithelial tissues is low.[45] The murine MOv18 mAb is highly immunogenic, inducing human antimouse antibodies (HAMA) in most patients. MOv18 targeting of ovarian cancer was investigated by van Zanten-Przybysz et al.[26] by comparing the i.p. administration to the intravenous (i.v.) injection. *Ex vivo* measured tumor uptake after i.p. administration was 3.4%– 12.3%, whereas this was 3.6%–5.4% after the i.v. administration.[32] In addition, van Zanten-Przybysz et al. determined the pharmacokinetics and tumor accumulation of both i.v. and i.p. administered cMOv18 in the same patient in a dual-label $^{125/131}$I-study in 15 patients. They showed a tumor uptake of 4.9% and 2.4% injected dose per kilogram (ID/kg) after the i.v. and i.p administration after 2 days, respectively. After 6 days, there was no difference in tumor uptake between the routes of administration (3.8% and 3.9%ID/ kg for i.v. and i.p). Moreover, the mean AUC for the blood-activity versus time curve was 3.5 times higher after the i.v injection at 2 days and 2.1 times higher at 6 days, as compared to the i.p injection. The researchers, therefore, concluded that the i.p. route could be advantageous owing to the significantly lower bone marrow toxicity as a result of lower blood levels of the radiolabeled antibody when administered i.p.[26] Crippa et al. tested the efficacy of i.p. administered RIT, using ^{131}I-MOv18 (mean dose, 100 mCi) in 16 patients with minimal or small-volume ovarian cancer.[46] Clinical follow-up and/or third-look evaluation performed 90 days after the administration of RIT showed a complete response in 5, stable disease in 6, and progressive disease in 5 patients. Of the 5 patients that showed a complete response, 1 patient remained disease free after a follow-up of 34 months, whereas the remaining 4 patients relapsed after a mean period of 10.5 months.

CA-125

Mahe et al. performed a phase I study comprising of i.p. administered 120 mCi of the ^{131}I-labeled anti-CA-125 antibody OC125 to 6 patients with residual macroscopic (<5 mm) or microscopic disease, resulting in stable disease in 2 patients.[47] In addition, there was HAMA formation in all 6 patients. The reported lack of efficacy might be owing to the nature of CA-125, being a antigen that is shed from the cell membrane.[48,49]

In conclusion, the data available on the use of adjuvant i.p. RIT in patients with ovarian cancer show improved targeting after the i.p. administration, as compared to the i.v. administration. Despite the lack of survival benefit, the phase III study with ^{90}Y-HMFG1 showed an improved local control of i.p. disease in patients treated with RIT.

Table 2. Ovarian Cancer Author	No. of patients	TAA	Antibody	Stable disease	Remission	Partial remission	Nuclide	Dose
Epenetos et al.[34]	24	MUC1	HMFG1-2, AUA 1, H17E2	5/24	4/24		^{131}I	140 mCi
Nicholoson S [35]	25	MUC1	HMFG1				^{90}Y	18 mCi/m^2
Epenetos et al.[36]	52	MUC1	HMFG1		21/54		^{90}Y	
Verheijen et al.[37]	224	MUC1	HMFG1				^{90}Y	18 mCi/m^2
Meredith et al.[40]	12	TAG-72	CC-49		3/12	1/12	^{177}Lu	10-30 mCi/m^2
Rosenblum et al.[41]	58	TAG-72	B72.3	30	2/52	2/57	^{90}Y	1-25 mCi
Alvarez et al.[42]	27	TAG-72	CC-49	2	3/27		^{177}Lu	10-50 mCi/m^2
Meredith et al.[44]	44	TAG-72	CC-49	4/44		4/44	^{177}Lu	40-45 mCi/m^2
Crippa et al.[46]	16	GP38	MOv-18		5/16		^{131}I	100 mCi
Mahe et al.[47]	6	CA-125	OC-125	3			^{131}I	120 mCi

Colorectal Cancer

Colorectal cancer (CRC) may spread intraperitoneally, causing peritoneal carcinomatosis (PC). In 25% of the patients with recurrent CRC, this is the only site of metastasis.[50] If untreated, patients suffering from PC have a median survival of only 6 months.[51] Today's gold standard for the treatment of PC is cytoreductive surgery, followed by heated i.p. chemotherapy or HIPEC.[50] This highly specialized, extensive treatment is associated with a high morbidity (14%–55%) and high mortality (19%).[50] Although the results of this treatment are promising, the median survival is 13–34 months[52,53] and the 5-year survival rate is 19%–27%.[54,55] Owing to the high complication rates, there is a need for new treatment modalities. RIT for CRC has been under clinical investigation since 1992, mainly with anti-carcinoembryonic antigen (CEA) antibodies.[56] The results of RIT in CRC were recently reviewed by our group.[57] So far, only one trial gave the results of i.p. administered RIT in patients with advanced CRC. Patients with local as well as regional and systemic spread were included.[58] Thirty-one (31) patients with large peritoneal metastases were treated. Patients received 5 cycles of i.p. administered ^{131}I-labeled anti-CEA/TAG-72/MUC-1. This was done either as monotherapy (one antibody) or in combination with multiple antibodies. The average activity dose per cycle was 98 mCi. The researchers reported a median survival of 41 months, complete remission lasting 23 months in 10 patients, stable disease that lasted for 12 months in 8 patients, and a partial remission in 10 patients.

Preclinical studies in mice with colon cancer xenografts were performed by Keenan et al. As early as 1984, suggesting RIT might be successful for the treatment of colon cancer in humans.[59] The preclinical as well as the clinical investigations of this group focussed on the i.v. route of administration, despite a more efficient tumor targeting in patients with PC after a i.p. administration than after an i.v. administration, as shown in a dual-label study of concomitant i.v.- and i.p.- administered ^{131}I-labeled B72.3.[10] In nude mice with i.p. growing human colon carcinoma, the i.p. route of administration of radiolabeled MN14, an anti-CEA antibody, showed a higher tumor uptake during the first 48 hours (58.5% ± 6.8%ID/g for i.p. vs. 34.9% ± 4.7% ID/g for i.v.) after administration. Thereafter, the uptake

was similar.[60] In addition, an increased therapeutic efficacy of ^{177}Lu-(median survival, 136 days) and ^{131}I-labeled MN14 (median, 100 days) was reported, when compared to equitoxic doses (8.33 MBq) of ^{90}Y-(median, 82 days) and ^{186}Re-labeled MN14 (median, 72 days) in this model.[61] The feasibility of i.p. RIT in an adjuvant setting to cytoreductive surgery, leaving only microscopic disease, was also investigated by Koppe et al. The researchers used ^{177}Lu-labeled MG1, a radiolabeled anti-CC531 (rat colon carcinoma) antibody administered after cytoreductive surgery (CS). In this model, adjuvant RIT resulted in a significantly improved survival of rats treated with the combination of CS and RIT, as compared to either CS or RIT alone.[62] Despite these favorable preclinical studies, there are no clinical trials investigating the efficacy of RIT as an adjuvant treatment to cytoreductive surgery for peritoneal carcinomatosis of colorectal origin.

In conclusion, RIT is an effective treatment for i.p. metastases and an effective adjuvant to cytoreductive surgery in preclinical studies. The limited experience in phase I clinical trials warrants clinical studies.

RIT in brain tumors

An estimated 41,000 cases of primary central nervous system (CNS) tumors occur annually in the United States, 42% of which are glial tumors that are, in most cases, malignant.[63] Glioblastoma multiforme, the most malignant form of CNS tumors, offers patients a very poor overall survival of only 3.3% after 2 years, despite the combination of surgery, radiotherapy, and chemotherapy. [64] More than 90% of all recurrences are adjacent to the site of origin, indicating a failure of local tumor control. Conventional radiotherapy plays a primary role in brain cancer treatment. However, its lack of tumor specificity is a significant limitation of this form of therapy. Owing to its nonspecific nature, toxicity to normal brain limits the radiation dose that can be delivered to tumor cells and compromises the quality of life of the few longer term survivors. There are three factors responsible for treatment failure: (1) the delivery of therapeutic agents is limited by the blood-brain barrier (BBB), despite the fact that it is disrupted in regions of macroscopic tumor[65]; (2) dysfunctional tumor vasculature, as is the case in most solid tumors, leads to local hypoxia and a reduced responsiveness to chemo and radiotherapy in combination with an elevated intratumoral interstitial pressure[66–68]; (3) inter- and intratumoral cellular and genetic heterogeneity, leading to heterogeneous antigen expression. [69,70]

In general, adjuvant treatment consists of the use of local radiation by means of stereotactic radiotherapy or brachytherapy with implanted ^{125}I rods. However, these approaches did not show additional effects on survival.[71,72] Recent applications of combinations of chemotherapy (temozolomide) with radiotherapy showed a survival benefit. The majority of patients, however, died within 1–2 years from progressive disease, underlining the need for new treatment strategies.[73]

The BBB regulates the exchange of substances between the vasculature and the CNS. This physiologic barrier is composed of tight junctions between the capillary endothelial cells and is supported by a microenvironment of astrocytes, pericytes, and microglial cells.[74] This almost impenetrable barrier prohibits the delivery of potentially effective therapeutic agents, thereby limitating the treatment of CNS diseases. In the case of malignant disease, the deterioration of the BBB may occur, which may improve the delivery of

therapeutic agents to the CNS. Brain tumors can cause a complete breakdown of the BBB, eventually leading to peritumoral, vasogenic edema.[74] Imaging of CNS malignancies, using radiolabeled antibodies, showed intratumoral accumulation following the i.v. administration, allowing for scintigraphic imaging. This indicates damage to the BBB. However, in general, there is insufficient targeting for therapeutic studies.[75] To overcome the problem of limited penetration, the locoregional application of therapeutic agents in patients with malignant glioma has been investigated.[76] To investigate the application of RIT, candidates for therapy, or tumor-associated antigens (TAAs), needed to be identified.

Tenascin-c

In the majority of clinical RIT studies in glioma patients, antitenascin mAbs were used. m81C6, a murine IgG2b with affinity for tenascin-c (TN), has been administrated through a s.c. implanted reservoir connected to an intracavitary-placed drain. TN is an extracellular matrix protein modulating cell-matrix interactions,[77] which is overexpressed in malignant glioma tissue, but not expressed in the normal brain. The distribution and intensity of TN expression correlates well with tumor neovasculature and shows evident expression in aggressive histotypes as well as in those tumors with high proliferation indices.[78–80] This distribution of TN makes it a suitable target in RIT in glioma (see Table 3). A series of phase I–III studies has been conducted at Duke University. In the first phase I study, 42 patients with recurrent glioma were included and the maximal tolerable dose (MTD) was assessed in a dose-escalation study after intracavitary (into the surgically created cavity; SCC) administration. This study showed that the MTD was 100 mCi for intracavitary-administered ^{131}I-labeled 81C6, with neurotoxicity being the dose-limiting factor.[81] The results of this study suggested that there was a potential survival benefit, as compared to patients treated with stereotactic radiotherapy and high-dose brachytherapy (a median survival of 60 weeks in the present study, as compared to 41 and 46 weeks, respectively). In the second study, 42 patients with newly diagnosed glioma were included in order to investigate dosimetry and doseresponse relationships.[82,83] In these patients, the MTD was 120 mCi, with neurotoxicity being the dose-limiting

factor. The median survival of these patients was 79 weeks, as compared to 46 weeks of historic controls, when patients were treated with surgery, chemotherapy, and radiotherapy. Based on these encouraging results, a phase II trial was performed in 33 patients with newly diagnosed, previously untreated patients. The median survival after treatment with 120 mCi of ^{131}I-labeled 81C6 in this study was 79–85 weeks, depending on the pathologic type of glioma (patients with astrocytic oligodendroglioma showed a better response than those with glioblastoma multiforme).[84] When 100 mCi of radiolabeled antibody was administered to 43 patients with recurrent glioma, survival was still 69 weeks.[85] The results of these trials warranted a phase III trial, which is currently ongoing. Recently, a human/mouse chimeric mAb, originating from 81C6, has been developed, showing better tumor targeting in animal studies. The targeting capabilities of the antibody were subsequently tested in a phase I study that included 47 patients with recurrent disease.[86] This chimeric antibody showed a prolonged retention time within the SCC, as compared to the antibody of murine origin. Owing to the enhanced circulatory half-life of the chimeric antibody, a MTD of 80 mCi was found, as compared to 120 mCi found in previous studies with the murine antibody. In this phase I dose-escalation study, the median survival was 87 weeks for patients with newly diagnosed glioma and 65 weeks for those patients with recurrent disease.

Riva et al. used another anti-tenascin antibody labeled with ^{131}I, BC-2/4, initially injected directly into the tumor.[87] Thereafter, the antibody was, in some patients repeatedly, administered into the SCC in 23 patients with recurrent glioblastoma. In their first report, no toxicity and an objective response in 11 patients, in whom were 3 complete remissions and a median survival of 16 months, were reported.[88] The follow-up study compared the results of antibody administration in recurrent and newly diagnosed patients.[89] The reported total median survival was 20 months. When bulky (median, 17 months) versus minimal disease (median, 26 months) were compared, the results supported the concept that RIT is most suitable in patients with small-volume disease. Later, combined data of 111 patients that were included in phase I and II studies showed a median survival of 20 months.[90] The researchers concluded that the application of RIT is

most suitable in small-volume disease. In a following phase I study,[91] the researchers switched to the application of an other radionuclide, ^{90}Y, in 20 patients and determined the MTD to be 25 mCi with neurotoxicity as the dose-limiting toxicity. No diffusion of the radiolabeled antibody to normal tissue was reported. However, this study failed to show clinical responses, presumably owing to the inclusion of patients with advanced disease.

Paganelli et al.[92,93] investigated the use of the pretargeting technique consisting of the biotinylated antitenascin MAb BC4, avidin and ^{90}Y-biotin, both i.v. administered as well as directly into the SCC, following surgical debulking. In this pretargeting approach, the large IgG-avidin conjugate was administered first, followed by a ^{90}Y-labeled biotin injection. These studies showed no hematologic toxicity following the RIT when applied into the SCC, as compared to almost all the other studies that used mAbs, including the study where pretargeting was used and administered i.v. This latter study supports the local administration technique by showing a total body distribution of the biotinylated mAb and fast blood clearance within hours after the i.v. administration.

To summarize, the phase I–II studies from Duke University on the use of adjuvant RIT after debulking surgery in patients with malignant glioma indicate an improved survival, as compared to historic control groups. In addition, there was a reduction in treatment-related toxicity, compared to stereotactic radiotherapy or brachy therapy. This resulted in a phase III trial that is currently ongoing. The results from Riva et al., despite the more heterogeneous group of patients, support this conclusion.

Table 3. Intra-SCC RIT Author	No. of patients	Antibody	Stable disease	Remission	Partial remission	Nuclide	Dose
Bigner et al.[81]	42	81C6				^{131}I	100 mCi
Cokgor et al.[83]	42	81C6	4/42			^{131}I	20-180 mCi
Reardon et al.[84]	33	81C6	0	nr	nr	^{131}I	120 mCi
Reardon et al.[85]	43	81C6	0	0	0	^{131}I	120 mCi
Reardon et al.[86]	47	ch81C6				^{131}I	< 80-100 mCi
Riva et al.[88]	10	BC-2/4	3/10	1/10	2/10	^{131}I	15 mCi
Riva et al.[90]	50	BC-2/4	11/50	3/50	6/50	^{131}I	
Riva et al.[91]	111	BC-2/4	10	1	9	^{131}I	70 mCi
Paganelli et al.[93]	24	BC-4	12/24		6/24	^{90}Y	15-30 mCi

Intravesical RIT

Bladder cancer is the second most common cancer of the genito-urinary system, mainly occurring in men. Two distinct types of urothelial cancer exist, the majority of which are superficial and noninvasive. However, the remaining 20% are solid, highly aggressive bladder cancers that are invasive and metastasize in an early phase of the disease.[94] Cystectomy is the treatment of choice for a tumor stage of T2–T4 (muscle invasive), leaving local resection to CIS and T1 disease. In T1 urothelial carcinoma recurrence rates are as high as 80% after resection.[95] When intravesical chemotherapy is added to surgery, recurrence rates are reduced by 50% at 2 years. Part of the contemporary bladder-sparing treatment is the use of immunotherapy by means of an intravesical Bacille Calmette-Guérin (BCG) instillation, the results of which are comparable to those achieved with chemotherapy.[96] Since the standard therapy in invasive bladder cancer is radical cystectomy, the role of RIT is limited to CIS and T1 disease. Despite several preclinical experiments, reports on the clinical use of radiolabeled antibodies in patients with bladder cancer are limited to investigations on radioimmunoscintigraphy studies rather than RIT (see Table 4). In these studies, antibodies directed against the target antigen MUC-1 are used. MUC-1 mucin is a high-molecular-weight cell-surface glycoprotein that is found on normal urothelium and that is both unregulated as well as abnormally glycosylated in bladder cancer.[97] One of the first studies in 20 patients was performed by Bamias et al., who intravesically administered the ^{111}In-labeled anti-MUC-1 antibody HMFG2.[98] Autoradiography of the resected specimen showed selective tumor targeting. Murray et al. used an intravesically administered ^{188}Re-labeled C595 antibody in 3 patients with transitional cell carcinoma.[99] In addition, this group investigated the intravesical administration of ^{67}Cu-labeled C595 in 16 patients with bladder cancer and studied the systemic absorption of the radiolabeled antibody.[100] The results of this study showed no detectable activity above the background, meaning there was no systemic radiolabel present after the local application, while 80% of the tumors were successfully visualized.

Malamitsi et al. investigated the intravesical application of ^{99m}Tc-labeled MUC-1 antibody HMFG1 in 14 patients and concluded that, despite the

excellent imaging of tumors, future attempts to administer RIT using HMFG1 should not be undertaken owing to a low and heteroge-neous uptake in the 6 patients with positive imaging (0%–9% injected dose) caused by heterogeneous antigen expression.[101]

In conclusion, intravesically administered RIS in bladder cancer may hold a promise for RIT, using anti-MUC1 antibodies, in early urothelial cell cancer owing to selective targeting and low systemic concentrations of the targeting agent.

Table 4. Intravesical RIS Author	Indication	TAA	Antibody	Nuclide	Targeting
Bamias et al.[98]	SUC	MUC1	HMFG2	^{111}In	+
Murray et al.[99]	TCC	MUC1	C595	^{188}Re	4/5
Hughes et al.[100]	SUC-T2	MUC1	C595	^{67}Cu	12/16
Malamitsi et al.[101]	SUC	MUC1	HMFG1	^{99m}Tc/ ^{131}I	+/-
Bamias et al.[98]	SUC	MUC1	HMFG2	^{111}In	+

Intrathoracic RIT

The prognosis of patients with malignant pleural mesothelioma (MPM) is poor. In general, median survival is 6–16 months. The malignant form can be classified in two categories: diffuse or localized. Both are essentially insensitive to any treatment.[102] Different agents for intracavitary chemotherapy to treat mesothelioma have been utilized. The response rate varied between 15% and 37%, without effect on survival, even when used in combination with paclitaxel and docetaxel. The clinical data regarding studies that used intrathoracic chemotherapy adjuvant to surgical debulking were disappointing.[103,104]

Therefore, the intrathoracic application of chemotherapeutic agents is mainly used to treat malignant pleural effusion.[105,106] Another obstacle for RIT of mesothelioma is the low tumor antigen (mesothelin, tenascin-c) expression.[107] Enhancing antigen expression with proinflammatory cytokines, however, do not improve survival after immunotherapy.[108]

Mesothelin is a 40-kD cell-surface glycosylated phosphatidylinositol (GPI)-anchored glycoprotein, with functions in cell-to-cell adhesion expressed by normal mesothelial cells. It is highly overexpressed in cancers as malignant mesothelioma, pancreatic or ovarian carcinoma sarcomas, and in some gastrointestinal or pulmonary carcinomas.[109] In preclinical studies, Hassan et al. and Fan et al. have used antimesothelin antibodies and antibody fragments linked to exotoxins to treat mesothelin-expressing tumors in nude mice. The development of experimental metastases was inhibited, and even a complete regression of the tumor was observed in some cases. [110,111]

In conclusion, this antigen may be an attractive target for the intrathoracic application of RIT, but no clinical RIT studies have been performed. Currently, a clinical trial is being conducted, using a chimeric mAb (MORAb-009) directed against a cell-surface glycoprotein, GP-9, that is overexpressed in epithelial type cancers as mesothelioma, ovarian, and pancreatic cancer.(www.clinicaltrials.gov,trailnr.NCT00325494) GP-9 may, therefore, be a potentially suitable target for radiolabeled MORAb-009 in future RIT of MPM.

Malignant pleural effusion (MPE)

MPE is thought to arise from tumor emboli detaching from visceral tumor nodules and concomitant attachment to the parietal pleura. Also, direct tumor invasion (in lung cancers, chest wall neoplasms, and breast carcinoma), hematogenous spread to the parietal pleura, and lymphatic involvement may be a mechanism for development of MPE. The effusion is composed of extracellular matrix proteins, cytokines, and growth factors, thereby promoting cell proliferation and invasion.[112]

In women, the most common causes of these effusions are breast and ovarian cancer, whereas in men, these are lung cancer and malignant pleural mesothelioma. Treatment of this specific entity can be done by either

therapeutical pleural aspiration (in case of a very short life expectancy), talc pleurodesis, or indwelling catheters. MPM with MPE is also an indication for intrapleural therapy, as is the case with MPE arising from ovarian cancer. Schmidt et al. described the successful intrapleural application of rituximab, an anti-CD20 mAb, in a patient with NHL who was free of symptoms for 8 months after this treatment.[113] The researchers described a case report regarding treatment failure of repeated percutaneous drainage and bilateral continuous chest tube drainage. This result may be promising, in particular when considering the possibilities of the effects of the application of radiolabeled antibodies. This is the case with ^{90}Y-labeled ibritumomab tiuxetan for the treatment of NHL, where the RIT produces significantly better responses than the mAb alone.[114]
Awaiting the results of the trial using MORAb- 009 in the case of MPM and the future development of TAAs directed against MPM, there may be a role for RIT in the treatment of MPM. In addition, RIT may play a role in the palliative treatment in patients with MPE, regardless of the origin of the primary tumor.

Discussion

The intracavitary application of radiolabeled antibodies combines the advantage of high tumor doses and low systemic toxicity. Therefore, higher doses of RIT can be applied than with systemic administration.
Currently, RIT with radiolabeled anti-CD20 antibodies is an accepted treatment for patients with NHL. For other indications, only a limited number of clinical phase I/II and one phase III RIT trials have been performed, using different antibodies and radionuclides in patients with different types of cancer. The results various studies on the therapeutic efficacy and toxicity of RIT in patients with ovarian cancer (^{90}Y-HMFG-1, ^{177}Lu-B72.3) and malignant glioma (^{131}I-81C6) indicate that the adjuvant application of RIT within a confined area limits toxicity and improves tumor targeting. In addition, RIT should be applied as a treatment of patients with minimal residual or microscopic disease of solid cancers in order to gain from its maximal potential.

For the treatment of ovarian cancer, the application of RIT was studied in a randomized, phase III trial. Unfortunately, the results of the latter study showed no survival benefit. The lack of efficacy of adjuvant RIT in this trial could be owing to several factors. First, the selection of the high-energy beta-emitter ^{90}Y, with a maximum tissue penetration of 12 mm, is not appropriate, since most of the energy will be deposited outside small tumor deposits in small-volume or microscopic disease. Second, the protein dose was augmented with 20 mg of unlabeled antibody to a total of 25 mg ^{90}Y-HMFG1, with the intent to provoke a human antimouse antibody response. This high antibody dose might have had a negative effect on the uptake of the radiolabel in the tumor lesions owing to the saturation of MUC-1 epitopes on the tumor cells.[115] In the future, however, optimizing this treatment, using nuclides such as ^{177}Lu that are more suitable for minimal residual disease than ^{90}Y in order to enhance the antitumor effect, may result in survival benefit.

The same can be true for the treatment of peritoneal carcinomatosis of colorectal origin when trials will be undertaken to treat this entity. Progress has been made in the treatment of recurrent glioma by the application of RIT and this treatment may, therefore, become an additional adjuvant treatment modality in neuro-oncology.

Conclusion

In the case of superficial bladder cancer, promising results of preclinical and radioimmunoscintigraphy studies may precede phase I and II trials. When therapeutic options are no longer available, RIT may offer patients a chance of minimally invasive palliation in patients with MPM or MPE. The future of RIT may, therefore, not only be in the inclusion in contemporary multimodality treatment, but also in the expansion of its indication to palliative treatment as a supplement or even a substitution for present-day treatments.

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Abstract

The purpose of this study was to investigate the effect of postoperative timing of radioimmunotherapy when administered as adjuvant treatment to cytoreductive surgery. PC was induced by intraperitoneal inoculation of CC-531 colon carcinoma cells in Wag/Rij rats. Subsequently, seven days after tumor induction, animals were subjected to exploratory laparotomy (C), CS only or CS + RIT at different time points after surgery. RIT consisted of 55 MBq ^{177}lutetium- labelled anti-CC531 antibody MG1. The primary endpoint in this study was survival. CS with or without RIT was well-tolerated. Median survival of animals in the C 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, four days and 14 days after surgery, respectively. From this study we concluded that the efficacy of adjuvant RIT after CS for the treatment of PC of colonic origin decreases when the administration of the radiolabeled MAbs is postponed. Therefore, the results of this study indicate that adjuvant RIT should be given as early after surgery as possible.

Chapter 3

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

This chapter is based on:
Timing of Adjuvant Radioimmunotherapy after Cytoreductive Surgery in Experimental Peritoneal Carcinomatosis of Colorectal Origin. Aarts F, Koppe MJ, Hendriks T, van Eerd-Vismale J, Oyen W. J.G, Boerman O.C, Bleichrodt R.P. Ann Surg Oncol. 2007 Feb;14(2):533-40.

Introduction

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 + 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, five-year survival rates of the patients with the most favorable 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.

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 tumor-associated antigens and labeled with a radionuclide in order to selectively irradiate tumor cells (radioimmunotherapy (RIT)).[9,10] Our previous RIT studies in Wag/Rij rats with intraperitoneal CC-531 tumors demonstrated the feasibility and efficacy of adjuvant intraperitoneal RIT after CS. RIT was given using ^{177}Lu-labeled 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 radiolabeled 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 tumor induction with 2.0 x 10^6 CC-531 tumor cells, 75 rats, fifteen 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 four days postoperatively (CS+RIT 4), or CS+RIT administered fourteen 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, tumor cells were washed with 0.9% sodium chloride, disaggregated with 0.25% trypsin and resuspended in RPMI-1640 medium to a concentration of 1x10^6 cells/ ml. Two mL 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 (2 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 one 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 labeling of the antibody with ^{177}Lu, the MAb was conjugated with 2-(4-isothiocyanatobenzyl)-diethylenetriaminepentaacetic acid (ITC-DTPA, Macrocyclics, Dallas, Texas). 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 labeled 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.), 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 radiolabeled 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 labeling procedures using ^{177}Lu were performed under strict metal-free conditions. The immunoreactivity of the radio labeled MG1 preparations was determined on freshly trypsinized CC531 cells essentially as described by Lindmo et al.[14] with minor modifications. [15]

RIT (185 µg MG1 per rat, radiolabeled 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 tumor growth. In all experimental treatment groups CS was performed, consisting of a midline laparotomy and careful inspection of the abdominal contents for tumor growth. Tumor growth at each of the intra-abdominal sites was scored 0 (no macroscopic tumor growth), 1 (limited tumor growth), 2 (moderate tumor growth), or 3 (abundant tumor growth). The sum of the tumor scores of all sites represented the peritoneal cancer index (PCI)[16]. Subsequently, CS was performed, removing macroscopic tumor deposits as radical as possible. Irresectable tumors 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 one 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 tumor 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 tumor 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).

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 cecum was opened while removing a cecal tumor, without further adverse events. At laparotomy, all animals had extensive tumor growth. Multiple tumor deposits of 1-3 mm were found in the omentum. Other sites of tumor 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 7 rats, equally distributed over the 4 experimental groups (p=0.6).

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. (Figure 1). Maximum body weight loss after Sham or CS only was similar (5.6 ± 1.4% vs. 6.5 ± 2.0% four 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 four days postoperatively was 7.7 ± 2.5% five days postoperatively. At 21 days after surgery, body weight loss of the rats that received RIT fourteen days postoperatively, was significantly lower as compared to that of the rats that received adjuvant RIT immediately or four days postoperatively (P=0.004).

Table 1. Pathological characteristics	Median (range)				
Group	Sham	CS	RIT 0	RIT 4	RIT 14
Tumor score per site					
Sucutaneous	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

Table 1. 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

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.

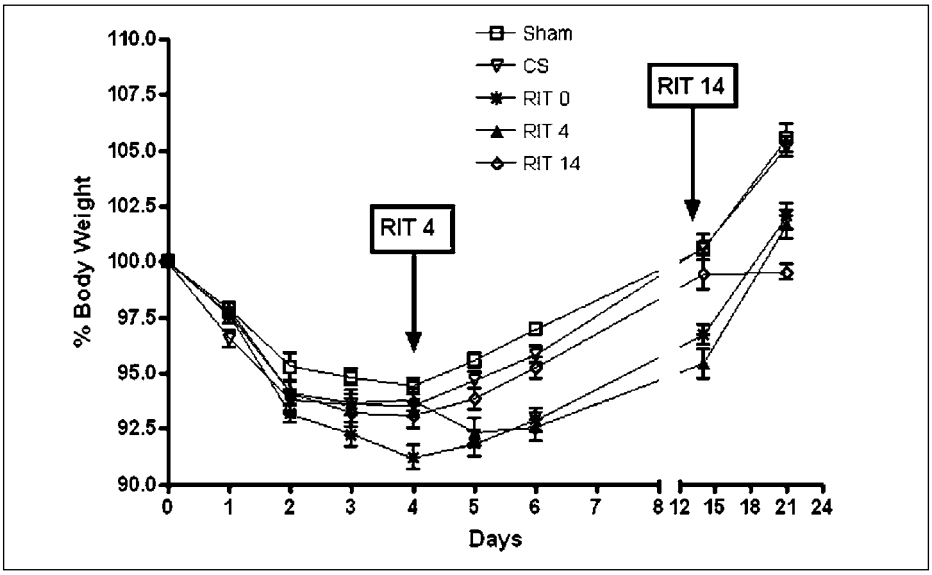

Figure 1. he absolute body weight of rats with peritoneal metastases during 15 weeks of follow- up after sham surgery (Sham), cytoreductive surgery (CS), or CS + RIT at day 0 (RIT 0), CS + RIT at day 4 (RIT 4) or CS + RIT at day 14 (RIT 14) Data represent means ± standard error of the mean (SEM).

Survival

During the follow-up of 16 weeks, 64 rats died as a result of intraperitoneal tumor growth. In most cases, this was accompanied by the formation of ascites. The median amount of ascites was 33 ml (range 0-62) when rats were taken out of the experiment and did not differ significantly between the groups. (Figure 2) At dissection, all of the animals in the Sham group showed adhesions due to the massive amount of tumor 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 tumor related cause. These two rats had only small tumor 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 obvious cause. For the other rat no explanation was found as cause for the intercurrent death.

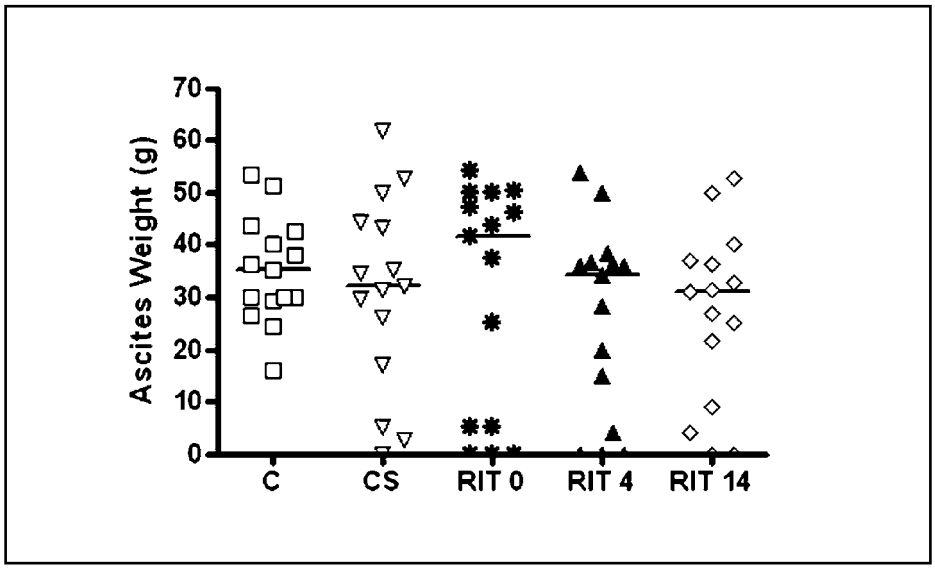

Figure 2. Ascites weight in rats with peritoneal metastases at the time of death after sham surgery (Sham), cytoreductive surgery (CS), or CS + RIT at day 0 (RIT 0), CS + RIT at day 4 (RIT 4) or CS + RIT at day 14 (RIT 14). The horizontal lines in side the graph depict the median.

After 16 weeks, the experiment was terminated. At that time 9 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 tumor deposits. Microscopic investigation showed tumor cells in one rat in RIT 0 group while the other two rats in this group were free of tumor. The one rat in the RIT 4 group without macroscopic evidence was also free of tumor after microscopic investigation. At the time of death, median PCI of those rats with tumors 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. (Figure 3)

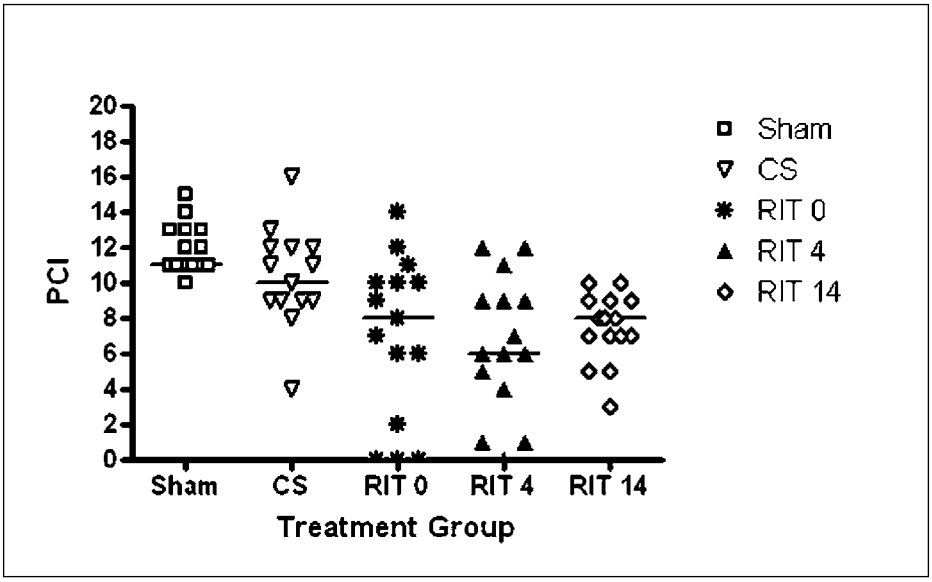

Figure 3. PCI in rats with peritoneal metastases at the time of death after sham surgery (Sham), cytoreductive surgery (CS), or CS + RIT at day 0 (RIT 0), CS + RIT at day 4 (RIT 4) or CS + RIT at day 14 (RIT 14). The horizontal lines in side the graph depict the median.

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. (Figure 4) 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 fourteen days after surgery (P<0.02), whereas RIT 0 compared to RIT 4 did not (P=0.17).

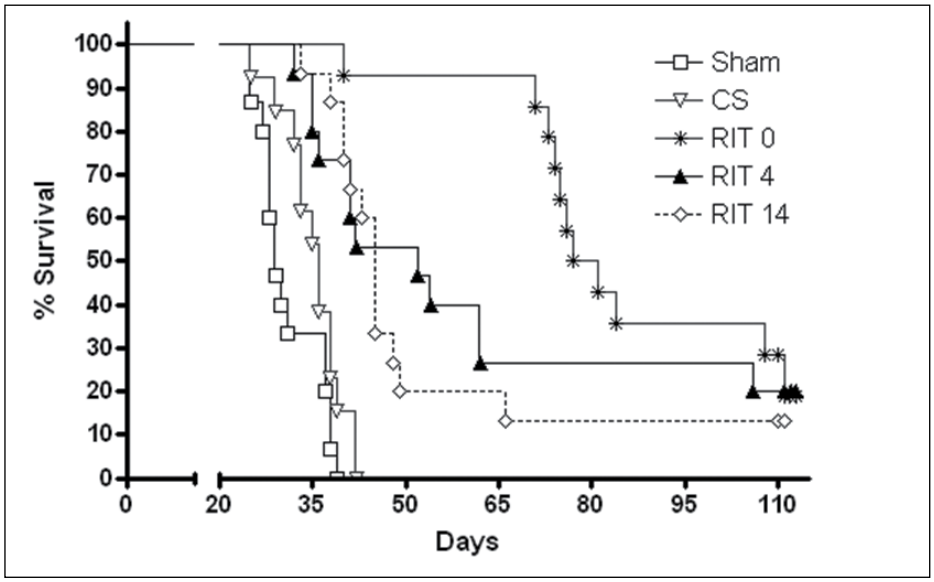

Figure 4. Survival curves for rats with peritoneal metastases after sham surgery (Sham), cytoreductive surgery (CS), or CS + RIT at day 0 (RIT 0), CS + RIT at day 4 (RIT 4) or CS + RIT at day 14 (RIT 14).

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 tumors 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 targeting of the CC531 tumors in this model[16]. The antibody preferentially localizes in the CC-531 tumors, 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 tumor uptake and retention and adequate physical properties for treatment of minimal residual disease (medium-energy ß-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 four or fourteen 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 tumor cells can be encapsulated in the fibrin network and as such become less accessible to local therapy, such as chemotherapy or antibodies (tumor cell entrapment theory) [19] and may even increase tumor growth.[20] In the present study, the formation of fibrin might have hampered tumor targeting of the radiolabeled MG1 antibodies and consequently might have impaired the therapeutic efficacy of RIT at four and fourteen 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 radiolabeled MAbs over the peritoneal surfaces in the rats that received adjuvant RIT four or fourteen 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 cecal 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 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

tumor in WAG/Rij rats, microscopic residual disease might have grown to macroscopic disease, especially in the rats that received adjuvant RIT fourteen days postoperatively.[12] The uptake and consequently therapeutic efficacy of radiolabeled antibodies is inversely correlated with tumor size.[24] The growth of minimal residual disease into larger tumors in excess of 3 mm might therefore have had a negative effect on therapeutic efficacy of the radiolabeled 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 considered. 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 pre-clinical 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-labeled 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 radiolabeled 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 tumor deposits. Furthermore, the protein dose was augmented with 20 mg of unlabeled 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 tumor lesions. The mechanism of antigen saturation due to excessive amounts of antibody may have interfered with intratumoral antibody uptake as described previously. [31,32] Thirdly, in view of the results of the present study, the time interval between CS and the administration of at least two 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 ^{131}I-labeled 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 ^{131}I-labeled 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 five-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 radiolabeled 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.

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Abstract

The purpose of this study was to improve survival by combining CS and RIT with the application of whole body hyperthermia (WBH) and fibrinolytic therapy in the rat model of induced PC.

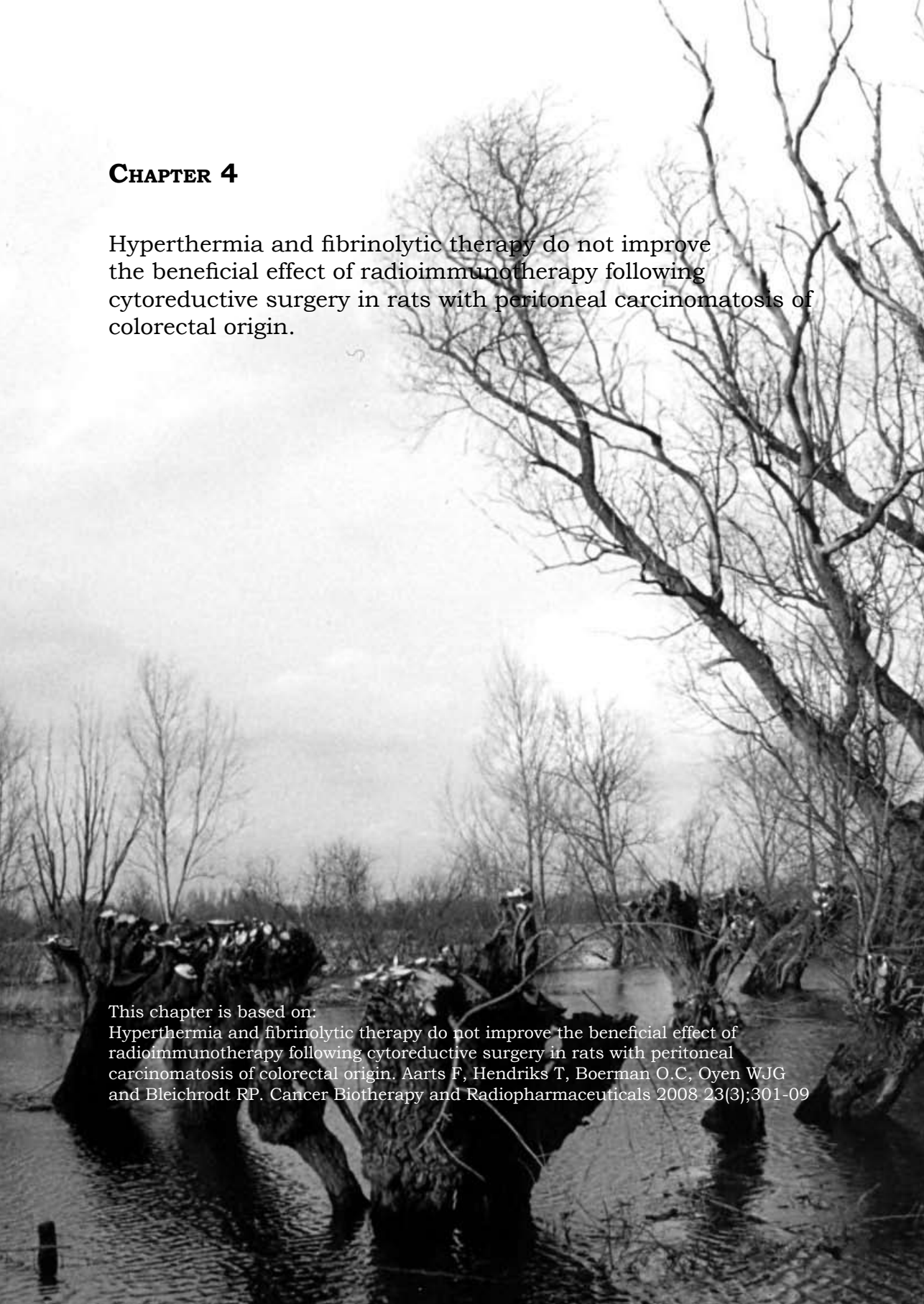
As in our previous experiment, PC was induced by intraperitoneal inoculation of CC-531 colon carcinoma cells in Wag/Rij rats. Animals were subjected to CS only, CS+WBH, CS+RIT at normothermia and CS+WBH+RIT in experiment 1. WBH was induced by housing the rats at a temperature of 40^0C for 3 hours. In experiment 2, rats were subjected to CS, CS followed by the intraperitoneal administration of recombinant tissue plasminogen activator (rtPA) twice daily for 3 days, CS followed by RIT (CS+RIT) and CS followed by RIT+rtPA. RIT consisted of 74 MBq ^{177}Lu- labelled anti-CC531 antibody MG1. The endpoint was survival.

Median survival of animals in the CS and CS+WBH group was 34 days and 37 days respectively (P=0.28). Median survival of rats that were treated with adjuvant NRIT or HRIT was 63 days and 86 days ((P<0.0003, P<0.0006 compared to CSH), respectively. No difference was found between CS+RIT and CS+WBH+RIT. In experiment 2, median survival after CS and CS+rtPA was 50 days and 42 days respectively (P=0.1). Median survival of CS+RIT was 106 days and 103 days for CS+RIT+rtPA (P<0.0001 compared to CS+rtPA). No difference was found between CS+RIT and CS+RIT+rtPA (P= 0.83).

From these studies we concluded that the application of HT or rtPA in combination with adjuvant RIT after CS for the treatment of PC of colonic was feasible but did not potentiate the efficacy of RIT.

Chapter 4

Hyperthermia and fibrinolytic therapy do not improve the beneficial effect of radioimmunotherapy following cytoreductive surgery in rats with peritoneal carcinomatosis of colorectal origin.



This chapter is based on:
Hyperthermia and fibrinolytic therapy do not improve the beneficial effect of radioimmunotherapy following cytoreductive surgery in rats with peritoneal carcinomatosis of colorectal origin. Aarts F, Hendriks T, Boerman O.C, Oyen WJG and Bleichrodt RP. Cancer Biotherapy and Radiopharmaceuticals 2008 23(3);301-09

Introduction

Cytoreductive surgery followed by hyperthermic intraperitoneal chemotherapy or HIPEC is considered one of the most promising treatments of peritoneal carcinomatosis (PC) of colorectal carcinoma (CRC).[1] In HIPEC, hyperthermia is used to enhance the chemosensitivity of the tumor.[2] Median survival after these radical surgical debulking procedures (cytoreduction) is 13-34 months with a 5-year survival of 19–27 %, which constitutes a significant improvement to palliative treatment consisting of chemotherapy and palliative surgery.[3-7] However, the improved survival is reached at the cost of a considerable morbidity of 23% and a mortality of 4%.[4,8] Thus, the search for alternative treatment strategies is warranted.

Radioimmunotherapy (RIT) using radiolabeled monoclonal antibodies directed against tumor-associated antigens may be an attractive anti-cancer therapy in patients with small volume disease.[9] In previous studies we have demonstrated the efficacy of RIT as an adjuvant to surgery in an animal model of PC. Survival in Wag/Rij rats with intraperitoneal CC-531 (colon carcinoma) tumors was significantly improved if cytoreductive surgery (CS) was followed by adjuvant RIT using the ^{177}Lutetium-labeled anti-CC531 monoclonal antibody (MAb) MG1.[10] The effect was most explicit when RIT treatment was administered immediately following surgery, as compared to 4 or 14 days after surgery.[11] In rats with intraperitoneal CC531 tumors, RIT was found to be at least as effective as HIPEC, in terms of survival after cytoreductive surgery, while RIT induced less side effects than the adjuvant treatment with HIPEC.[12] These experiments suggest that RIT is a promising therapeutic approach to ameliorate treatment of PC. The question now arises whether the efficacy of adjuvant RIT can be further improved. It has been shown in preclinical studies with subcutaneously growing xenografts that the efficacy of RIT can be enhanced by locally applied hyperthermia.[13] Hyperthermia increases tumor blood flow and interstitial pO_2, reducing tumor hypoxia and disturbed cellular repair mechanisms.[14-16]

During surgical procedures, tissue damage causes deposition of fibrin, which may result in the formation of fibrinous adhesions. This phenomenon occurs as the result of a shift in the equilibrium between coagulation and

fibrinolysis, in favor of the coagulation system.[17-19] Tumor cells may be trapped in these fibrin clots and become inaccessible to therapeutic agents, such as radiolabeled antibodies, which may promote tumor growth. [20-22] Post-operative fibrinolytic therapy might prevent these events from occuring. Here, we report on two separate animal studies where we investigated if either whole body hyperthermia or fibrinolytic therapy (with tissue-type plasminogen activator, rtPA) could improve the efficacy of adjuvant RIT, as applied after cytoreductive surgery for peritoneal carcinomatosis of colonic origin.

Material and Methods

Experimental Design

Two experiments were performed to study the effects of hyperthermia and recombinant tPA (rtPA), respectively. In both experiments, intraperitoneal tumor growth was induced by the inoculation of tumor cells. After seven days, cytoreductive surgery was performed. In the hyperthermia experiment, 60 rats, 15 in each treatment group, were randomly assigned to undergo either CS alone, CS followed by whole body hyperthermia (CS+H), CS followed by RIT (CS+RIT) or CS followed by whole body hyperthermia and RIT (CS+HRIT). In the fibrinolysis experiment, 58 rats were randomly assigned to undergo cytoreductive surgery alone (CS, n=15), CS followed by rtPA treatment (CS+rtPA, n=15), CS followed by RIT (CS+RIT, n=13) or a combination of both adjuvant treatments (CS+RIT+rtPA, n=15). In both experiments, survival was determined and at autopsy the extent of tumor growth was scored.

Cell line

The syngeneic rat colon carcinoma cell line CC531, originally induced in Wag/Rij rats by intravenous injection of 1,2- dimethylhydrazine [23], 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, tumor cells were washed with 0.9% sodium

chloride, disaggregated with 0.25% trypsin and resuspended in phosphate buffered NaCl solution, 0.9% (PBS) to a concentration of $1x10^6$ cells/ml. Two mL of this cell suspension was injected intraperitoneally, as previously described.[10]

Animals

Male Wag/Rij rats, ten to twelve weeks old (body weight 240-285 g, Harlan Horst, The Netherlands) were accustomed to laboratory conditions for at least 1 week before experimental use and housed under non-sterile standard conditions (temperature, 20–24°C; relative humidity, 50-60%; 12 h light/dark cycle) in filter-topped cages (2 rats per cage), with free access to food (Ssniff, Bio Services Uden, The Netherlands) and water. Physical condition was examined daily and body-weight was recorded daily during the first 14 days after surgery by a biotechnician, who was blinded to the therapeutic regimen. Thereafter, body-weight was recorded weekly. 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.

Cytoreductive Surgery

Surgical procedures were performed under general anaesthesia using isoflurane 3%, O_2 and N_2O 1:1. All rats underwent a midline laparotomy, followed by careful inspection of the abdominal contents for the presence of tumor. Tumor growth at each of the intra-abdominal quadrants was then scored as 0 (no macroscopic tumor growth), 1 (limited), 2 (moderate) or 3 (abundant). The peritoneal cancer index (PCI) was then the sum of the score for the for quadrants as described previously.[10]

In all treatment groups, cytoreductive surgery was performed, including a routine omentectomy, and all macroscopic tumor deposits were removed surgically. Irresectable tumors were cauterized. 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 wound. After completion of the procedure 10 mL of warmed normal NaCl solution 0.9% was given subcutaneously, for rehydration.

Thirty minutes prior to surgery and once daily until the third postoperative day, rats were given buprenorphine (5 µg, 0.1 mL/rat/day) subcutaneously for analgesia.

Radioimmunotherapy

The murine MG1 monoclonal antibody (MAb), an anti-CC531 IgG2a monoclonal antibody (Antibodies for Research Applications BV, Gouda, The Netherlands) that recognizes an 80 kDa cell surface antigen on CC531 cells, was used in these studies. The MG1 MAb localizes preferentially in tumors when injected in rats bearing CC-531 tumors [24]. Labelling of the antibody with ^{177}Lu (halflife 7 days, ß-energy 149 keV, γ- energy 208 keV, penetration depth 3 mm) was carried out as previously described.[10] In brief, the MAb was conjugated with 2- (4-isothiocyanatobenzyl) diethylenetriamine pentaacetic acid (ITC-DTPA) (Macrocyclics, Dallas, TX), subsequently labelled with ^{177}Lutetium (IDB Holland, Baarle Nassau, The Netherlands) and purified by gel filtration on a PD10 column (Amersham, Pharmacia Biotech, Maarsen, The Netherlands). The purified ^{177}Lu-MG1 preparation was diluted in PBS with 0.5% BSA for injection. The specific activity of the administered ^{177}Lu-MG1 preparation was 0.4 MBq/µg. The ^{177}Lu labelling procedure was performed under strict metal-free conditions.

^{177}Lu-labeled MG1 was used as the therapeutic agents in these experiments based on the favourable tumor-to-blood ratios obtained with ^{111}In-labeled MG1 in this model as compared to ^{125}I-labeled MG1 (9.2 ± 5.3 vs. 2.4 ± 1.4 with $P < 0.040$) reported by Koppe et al.[10] In addition, it was shown that intraperitoneal RIT using^{177}Lu-labeled antibodies to treat small peritoneal metastases resulted in the highest percentage survival as compared to treatment with ^{131}I-, ^{186}Re- or ^{90}Y-labeled antibodies.[25]

RIT (185 µg MG1/rat, radiolabeled with 74 MBq ^{177}Lu in 3.0 ml) was intraperitoneally injected immediately following surgery, as this was determined to be the most optimal time point for adjuvant administration in relation to CS.[11] In addition, this was presumed to be the most optimal time point in relation to the application of hyperthermia.[26]

Hyperthermia

Immediately upon recovery from general anaesthesia, animals in the CS+H group and the CS+HRIT group were placed in an incubator preset at a temperature of 39^0C. After 30 minutes, the temperature was raised to 40 ^{0}C. Inside the incubator, conditions were similar to those within the animal facility housing: a relative humidity between 20% and 40%. General whole body hyperthermia was maintained for three hours. The rectal temperature was monitored every 30 minutes.

Fibrinolytic Therapy

Immediately after the surgical procedure and after closure of the abdominal wall, the fibrinolytic therapy was administrated. Fibrinolytic therapy comprised of the intraperitoneal administration of 1.25 mg human rtPA (Actilyse, Boehringer Ingelheim, Germany) twice daily during the first three days after surgery. This dose was chosen because it was shown to be effective in reducing intraperitoneal abscess formation in a model of induced peritonitis in rats.[27]

Follow up

The primary endpoint in both experiments was survival. Animals were observed daily by an experienced biotechnician, blinded to the therapeutic regimen, who determined the humane endpoint. When this was reached (signs of massive hemorrhagic ascites, physical inactivity or signs of intra-abdominal tumor growth with invalidating consequences), rats were killed by O_2/CO_2 asphyxiation and immediately dissected. At dissection, the intraperitoneal tumor growth was scored as described above. At 16 weeks postoperatively, the study was terminated and the remaining rats were killed and dissected. In case of absence of macroscopic tumor, all relevant organs, including the greater momentum, the mesentery and the diaphragm were removed for histology in order to examine tumor presence microscopically. Tissues were fixed in 1% formaldehyde, dehydrated and embedded in paraffin. Sections were stained using haematoxylin & eosin (H&E) and immunohistochemical stained using the murine MG1 antibody in combination with a peroxidase conjugated horse-anti-mouse IgG antibody (Vector Laboratories Inc., Burlingame, CA, USA).

Results

Hyperthermia

Surgery and adjuvant treatment

Preoperative clinical condition and body weight did not differ between groups. At laparotomy, tumor nodules were often present in the omentum, liver hilum, the mesentery and gonadal fat pads (1-3 mm diameter). Median PCI score at time of surgery was 8 (range 0-12, Table 1) and did not differ between groups (P=0.59). After CS, 2 of the 15 animals in the CS group had residual disease after surgery. During surgery, a bowel perforation occurred in one rat. No animals died during or immediately after surgery. Mean rectal temperatures during hyperthermia were equal in both hyperthermia groups: 39.4 ± 0.8 ^{0}C in the CS+H group and 39.5 ± 1.2 ^{0}C in the CS+HRIT group, respectively. Two animals were euthanized directly after hyperthermia, one in the CS+H group and one in the CS+HRIT group. Two animals showed massive unexplained weight loss (one each in the CS and CS+HRIT groups) and were euthanized four days after surgery. All animals lost weight after surgery (Figure 1A). Maximum weight loss was 7.6 ± 1.3 (mean and SD) % and 8.5 ± 0.8 % for the CS and CS+H groups and 10.3 ± 0.4 % and 10.7 ± 0.7 % for the CS+RIT and CS+HRIT groups, respectively. At day 4 and day 5 after surgery, weight loss was significantly higher in the CS+RIT group than in the CS group (P<0.05). In addition, from day 5 until day 9, animals that were treated with CS+HRIT lost significantly more body weight as compared to animals treated with CS alone (P<0.05).

Table 1a.		Hyperthermia		
Disease characteristics	Median (range) CS	CS+H	CS+RIT	CS+HRIT
Body weight	264 (246-281)	265 (251-278)	265 (249-282)	268 (252-285)
Tumor score per site				
Greater omentum	2 (1-3)	2 (0-3)	2 (1-3)	2 (2-3)
Liver hilum	2 (1-3)	2 (0-2)	2 (0-3)	2 (1-2)
Perisplenic	1 (0-2)	1 (0-2)	1 (0-2)	1 (0-1)
Mesentery	1 (0-2)	1 (0-2)	1 (0-2)	2 (1-3)
Gonadal fatpads	1 (0-2)	1 (0-2)	1 (0-2)	1 (0-2)
Diaphragm	0 (0-1)	0 (0-1)	0	0
Parietal peritoneum	1 (0-2)	1 (0-2)	1 (0-2)	1 (1-2)
Total	8 (4-11)	8 (0-12)	9 (3-10)	9 (7-11)
Complete Resection				
Yes	13	15	15	15
No	2	0	0	0

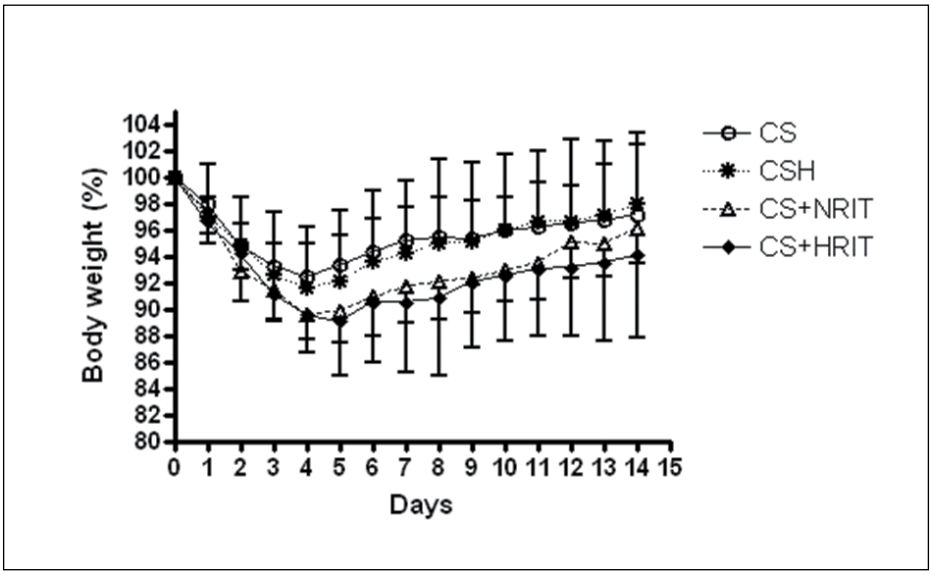


Figure 1. Course of body weight during the first two weeks after cytoreductive surgery. Body weight (mean + SD) is given relative to weight at operation. Effect of hyperthermia.

Follow-up

Forty-nine (out of 56) animals reached the humane endpoint within 16 weeks after surgery. At time of death, mean PCI in the CS, CS+H, CS+RIT and CS+HRIT groups was 20 (range 16-21), 20 (range 14-21), 17 (range 12-20) and 15 (range 2-21), respectively (Figure 2). The PCI was significantly lower in the CS+HRIT group than in both the CS ($P<0.01$) and CS+H ($P<0.05$) groups. All these animals showed hemorrhagic ascites. At dissection, the volume of ascites was equal in all four groups (mean 19.9 g, $P=0.35$). Median survival after CS was 34 days (range 25-112, End Of Study) and after CS+H 37 days (range 15-83, Figure 3). The groups that were treated with adjuvant RIT survived significantly longer; after CS+RIT median survival was 63 days (range 39-112, $P<0.0003$ vs. CS+H) and after CS+HRIT 86 days (range 35-112, $P<0.0006$ vs. CS+H). However, the apparent difference between the latter groups remained non-significant ($P=0.72$). Seven rats (1 in the CS group, 4 in the CS+RIT group and 2 in the CS+HRIT group) survived until 16 weeks after surgery and were killed subsequently.

Three of these animals had macroscopic tumor (2 in the CS+RIT group and 1 in the CS+HRIT group). Microscopic investigation of the remaining four animals did not reveal any tumor.

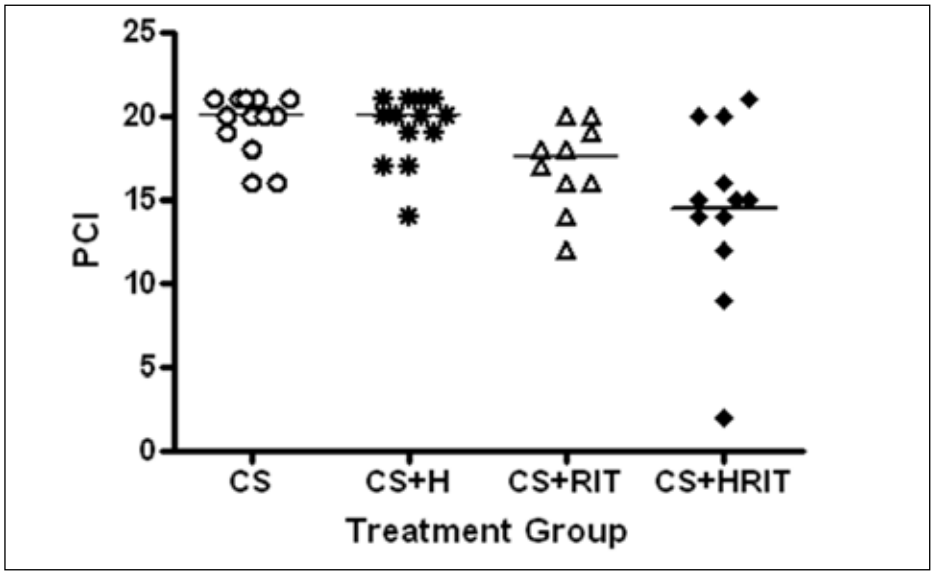


Figure 2. Peritoneal cancer index at time of death. Individual values are given together with the median (horizontal bar). In the hyperthermia experiment (A), the PCI was significantly lower in the CS+HRIT group than in both the CS ($P<0.01$) and CS+H ($P<0.05$) groups.

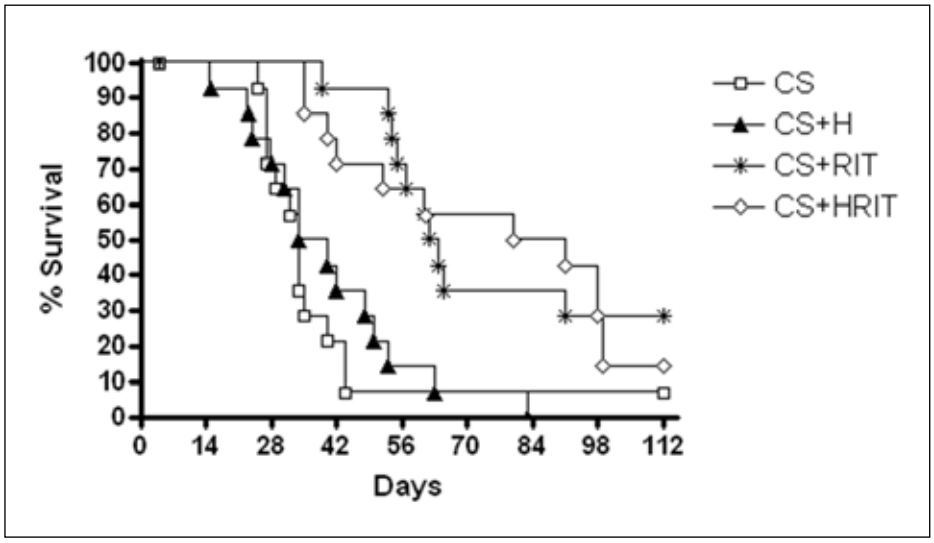


Figure 3. Survival in days. In the hyperthermia experiment, the groups that were treated with adjuvant RIT survived significantly longer.

Fibrinolytic therapy

Surgery and adjuvant treatment

Preoperative clinical condition and body weight did not differ between groups. At laparotomy, tumor nodules were present in the omentum, liver hilum, the mesentery and gonadal fat pads (1-3 mm diameter). The median PCI score at time of surgery was 5 (range 3-10) and was similar in all groups (P=0.28, Table 1b). No animals died during or immediately after surgery. One animal in the CS+RIT group was killed two days after surgery due to a burst abdomen. All animals lost weight after surgery (Figure 4). Maximum weight loss was 6.6 ± 2.6 % and 7.9 ± 3.8 % in the CS and CS+rtPA groups and 10.1 ± 2.6 % and 9.5 ± 5.8 in the CS+RIT and CS+RIT+rtPA groups, respectively. There was a significant difference in weight loss between the CS and CS+rtPA groups from day 2 until day 4 ($P<0.05$). In addition, from day 3 onwards up until day 13, animals treated with RIT or RIT+rtPA lost significantly more weight than animals treated with CS alone ($P<0.01$ and $P<0.05$ respectively).

Table 1b. Disease characteristics	Median (range)	Fybrinolytic Therapy		
	CS	CS+rtPA	CS+RIT	CS+RIT+rtPA
Body weight	274 (257-290)	274 (260-288)	275 (253-292)	274 (263-287)
Tumor score per site				
Greater omentum	1 (1)	1 (1-3)	1 (1)	1 (1)
Liver hilum	1 (0-2)	1 (1)	1 (0-1)	1 (1-2)
Perisplenic	1 (0-1)	1 (1-0)	0 (0-1)	1 (0-1)
Mesentery	1 (0-1)	1 (0-3)	1 (0-1)	1 (0-1)
Gonadal fatpads	1 (0-1)	0 (0-1)	1 (0-1)	0 (0-1)
Diaphragm	0 (0)	0 (0)	0 (0)	0 (0)
Parietal peritoneum	1 (0-1)	0 (0-1)	1 (0-1)	0 (0-3)
Total	5 (4-6)	5 (4-10)	5 (3-8)	5 (4-6)
Complete Resection				
Yes	15	15	13	15
No	0	0	0	0

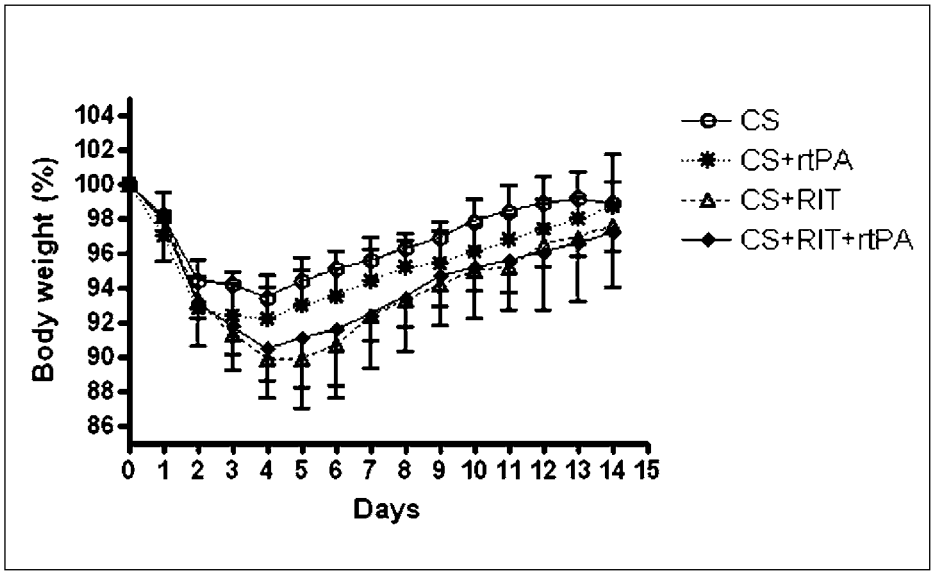


Figure 4. Course of body weight during the first two weeks after cytoreductive surgery. Body weight (mean + SD) is given relative to weight at operation. Effect of rtPA.

Follow up

Forty-seven (out of 57) animals reached the humane endpoint before the termination of the experiment 16 weeks after surgery. The mean PCI at the time of death in the CS, CS+rtPA, CS+RIT and CS+RIT+rtPA groups was 17 (range 14-21), 20 (range 13-21), 15 (range 0-19) and 17 (range 7-21), respectively (Figure 5). The PCI was significantly lower in the CS+RIT group than in both the CS alone ($P<0.01$) and CS+RIT+rtPA ($P<0.05$ and $P<0.01$, respectively) groups. No difference in the volume of ascites was found between the groups at the time of sacrifiction. Median survival after CS alone and CS+rtPA was 50 days (range 30-112) and 42 days (range 23-67) ($P=0.09$), respectively. The groups that were treated with adjuvant RIT survived significantly longer (Figure 6). The median survival of animals that were treated with CS+RIT was 106 days (range 54-112), while those treated with CS+RIT+rtPA survived for 103 days (range 46-112): $P<0.0001$ when compared to CS+rtPA for both these treatment groups. There was, however, no difference in survival between both RIT-treated groups ($P=0.52$).

Of the 10 animals that were still alive at the end of the experiment (1 in the CS group, 5 in the CS+RIT group and 4 in the CS+RIT+rtPA group), 3 animals (2 in the CS+RIT group and 1 in the CS+RIT+rtPA group) showed macroscopic and one (in the CS+RIT group) showed microscopic tumor at dissection.

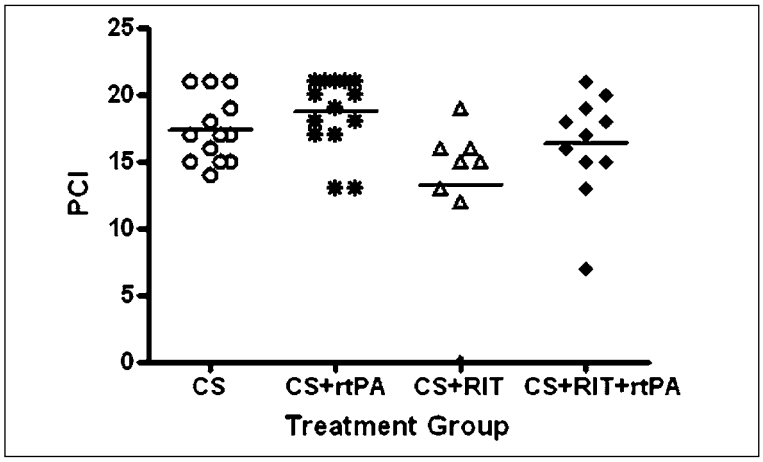


Figure 5. Peritoneal cancer index at time of death. Individual values are given together with the median (horizontal bar). The PCI was significantly lower in the CS+RIT group than in both the CS ($P<0.01$) and CS+RIT+rTPA ($P<0.05$ and $P<0.01$, respectively) groups.

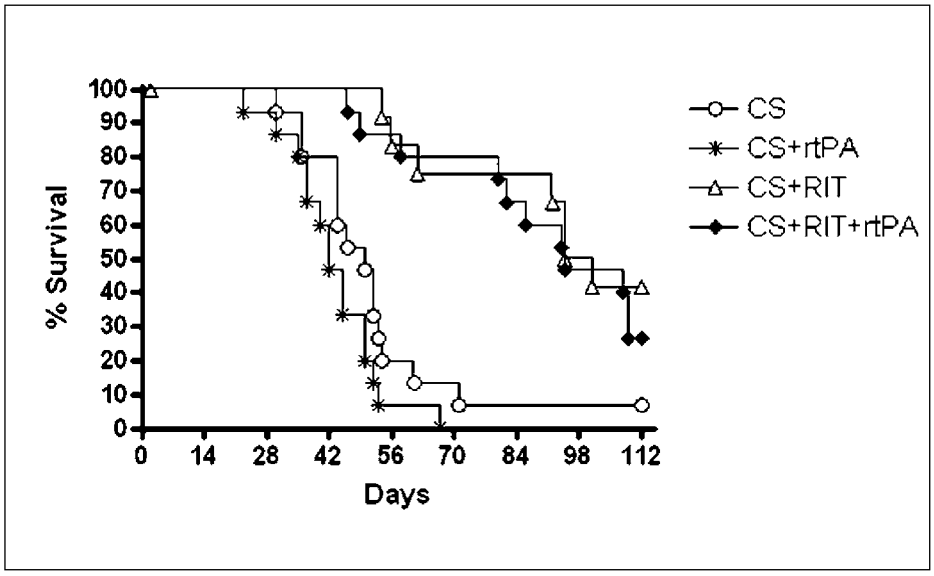


Figure 6. Survival in days. In the fibrinolytic therapy experiment, the groups that were treated with adjuvant RIT survived significantly longer.

Discussion

The present data demonstrate that application of RIT immediately after surgery (more than) doubles survival time. However, the results of both experiments demonstrate that the efficacy of RIT as an adjuvant treatment after cytoreductive surgery for peritoneal carcinomatosis is not significantly improved by hyperthermic treatment or the use of fibrinolytic drugs in an established model of PC in the rat.

In Wag/Rij rats, the intraperitoneally growing syngeneic rat colon carcinoma cell line CC531 has been shown to present a highly reproducible model of PC that is similar to the human entity of PC.[28] We have shown before that RIT with ^{177}Lu-labelled MG1, administered after cytoreductive surgery, is a promising adjuvant treatment. [10,11]

In the clinical situation, hyperthermia may enhance the effect of radiotherapy during 1 hour and combined with a single high dose of radiotherapy. [29] The application of prolonged (3-6 hours) mild whole body hyperthermia

(40 ^{0}C) in combination with low-dose RIT (^{131}I-labeled anti-CEA antibody F-33-104) delayed the growth of subcutaneously growing human colon cancer xenografts.[30] The 6 hour course of hyperthermia led to a mortality of 23%. Probably, a shorter time of hyperthermia is effective as well. It has been shown that the effect of hyperthermia, i.e. increased blood flow and oxygenation, even when applied for 60 min only, lasted for 24 h.[31] Kinuya et al.[32] demonstrated an increased (2.4 fold) accumulation of the radiolabeled antibody in subcutaneously growing human colon cancer xenografts in nude mice resulting in improved therapeutic efficacy when hyperthermia was applied directly after Ab injection. If hyperthermia was applied either two days prior or two days after the injection of the radiolabeled MAb, the opposite effect was observed. In the present study, we therefore applied whole body hyperthermia of 40 ^{0}C immediately after the intraperitoneal injection of the radiolabeled antibody. This procedure resulted in a hyperthermia-related mortality of 7%. In contrast to the above-mentioned studies, the present study failed to show a significantly enhanced therapeutic efficacy of RIT after hyperthermia, although the median survival increased by 36 %. The lack of a significant effect of the adjuvant application of mild hyperthermia in our model may be due to various reasons. The micro-environmental factors influenced by hyperthermia are 1. perfusion, 2. vascular permeability, 3. pO_2, 4. pH and 5. interstitial fluid pressure in the tumor.[31]

An increase in tumor perfusion reduces hypoxia, which may enhance radiosensitivity. This has been reported by Mittal and colleagues [33], who used an ^{131}I-labeled anti-CEA antibody in combination with temperatures between 41 ^{0}C and 43 ^{0}C for the duration of 45 min to treat human colon cancer xenografts. The authors concluded that hyperthermia increased the effectiveness of RIT but without an increase uptake of the antibody in the tumor. Most likely, intraperitoneally growing small tumor nodules (<1 mm) originating from CC531 cells only have a small hypoxic fraction. This may explain the lack of increased efficacy in our model.

Increased vascular permeability is reported to occur at temperatures exceeding 41.5^{0}C. Under the present experimental conditions these high temperatures would lead to an unacceptably high morbidity and thus is not applicable in this model.

Abdominal surgery inevitably results in peritoneal trauma followed by the deposition of fibrin.[34] Tumor cells can be encapsulated within this fibrin network and as such would become less accessible to intraperitoneal RIT[35]. Administration of rtPA will lyse fibrin and may counteract this effect. In the present study the administration of rtPA did not result in increased survival. This may be due to ineffective fibrinolysis. However, using a similar dose, Buyne et al. reported that two intraperitoneal injections with 1.25 mg rtPA reduced abscess formation with 30% in an intra-abdominal sepsis model.[27] Jacquet and colleagues found that intra-operative administration of rtPA (5mg/kg) resulted in a decreased number of tumor implants when colon carcinoma cells were administered intraperitoneally after creating peritoneal wounds.[36] The applied dose of 1.25 mg twice daily for three days, however, did not result in an improved survival in the present study. Apparently, in the presently used model, fibrin deposition is not a factor limiting the effectiveness of RIT. The use of an alpha-emitter in this model may have been more appropriate than the use of ^{177}Lu. One might argue that the penetration range of the alpha particles (<70 μm) is more suited for treating tumor cell clusters and very small tumor nodules, than the penetration range of the beta particles emitted by ^{177}Lu (<3.3 mm).[37]

In summary, we showed that the administration of RIT adjuvant to CS in a model of induced PC of colonic origin improves survival.

In addition, we demonstrated that the application of mild WBH or rtPA as adjunct to RIT and CS is feasible but did not show a significant additive effect to the combined treatment of RIT and CS in our model of PC of CRC.

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Abstract

Survival in patients with peritoneal carcinomatosis (PC) of colorectal cancer is improved by radical surgical debulking procedures in combination with intraperitoneal chemotherapy, either in combination with normo- or hyperthermia (HIPEC). These procedures result in considerable morbidity and mortality rate of up to 23% and 4%, respectively. We compared the efficacy and toxicity of combined cytoreductive surgery (CS) and radioimmunotherapy (RIT) for treatment of peritoneal carcinomatosis of colonic origin to the standard of care in clinical practice, HIPEC. Regarding treatment related toxicity, the results were in favour of RIT. Survival of rats treated with CS+RIT was significantly longer than after CS alone, whereas survival after CS+HIPEC or CS alone were not significantly different.

From this study we concluded that survival after CS is significantly improved by RIT. In contrast, adjuvant HIPEC did not and proved to be more toxic than RIT.

Chapter 5

A comparison between radioimmunotherapy and hyperthermic intraperitoneal chemotherapy for the treatment of peritoneal carcinomatosis of colonic origin in rats.

This chapter is based on:
A Comparison Between Radioimmunotherapy and Hyperthermic Intraperitoneal Chemotherapy for the Treatment of Peritoneal Carcinomatosis of Colonic Origin in Rats. Aarts F, Hendriks T, Boerman O.C., van Eerd-Vismale J, Oyen W.J.G., Bleichrodt R.P. Ann Surg Oncol. 2007 Nov;14(11):3274-82.

Introduction

Peritoneal carcinomatosis (PC) of colorectal cancer (CRC) frequently is an end stage of colorectal cancer, occurring in 5-50% of the patients, either synchronous or metachronous[1]. If untreated, patients suffering from PC have a median survival of only six months[2]. Survival is significantly improved by radical surgical debulking procedures (cytoreduction) in combination with intraperitoneal chemotherapy, either in combination with normo- or hyperthermia (HIPEC)[3-7]. The median survival after cytoreductive surgery and HIPEC is 13-34 months[4,8] and the 5-year survival rate is 19%-27% at the cost of considerable morbidity and mortality rates of up to 23% and 4%, respectively.[6,9]

In the latest reported clinical trial on adjuvant RIT in the setting of colon cancer, Liersch et al. reported results of a Phase II trial with ^{131}I-labeled anti-CEA antibody Labetuzumab administered to patients after complete resection of colorectal liver metastases. This study, where RIT was applied in an adjuvant setting to complete resection, resulted in a promising 5-year survival rate of 51.5%.[10] Radioimmunotherapy using radiolabelled monoclonal antibodies directed against tumour-associated antigens may be therefore be an attractive anti-cancer therapy in patients with small volume disease.

We therefore have studied the application of RIT as adjuvant therapy following cytoreductive surgery (CS) in the setting of PC. In previous studies regarding PC of CRC in a rat model we showed that RIT could be an effective adjuvant treatment after CS. The efficacy of adjuvant RIT in combination with CS was investigated and compared to no treatment, CS only and RIT only. The results of this study showed a significantly improved survival of animals treated with CS followed by RIT (median 88 days) compared to those treated with CS only (median 51 days) and RIT only (median 61.1 days).[11] Based on the encouraging results, showing the observed increase in survival that was achieved with low dose RIT and concomitant low toxicity, we now aimed to compare the efficacy of this treatment to that of today's standard of care, HIPEC[12,13], in a preclinical setting.

Materials and Methodes

Animal model of peritoneal carcinomatosis

WAG/Rij rats (10-12 weeks old, body weight 240-290 g, Harlan Horst, The Netherlands) were housed under non-sterile standard conditions (temperature, 20–24°C; relative humidity, 50-60%; 12 h light/dark cycle) in filter-topped cages (2 rats per cage), with free access to food (Ssniff, Bio Services Uden, The Netherlands) and water. Rats were accustomed to laboratory conditions for at least one week before experimental use. Peritoneal carcinomatosis was induced by intraperitoneal inoculation of 2.0×10^6 CC-531 colon cancer cells, as described previously.[14] 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.

Operative procedure

Prior to the laparotomy, all rats were given 10 mL of saline in order to prevent hypovolemia. 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. During the operation, rats were placed on a warmed mattress to limit body heat loss. All rats underwent a midline laparotomy. After opening the abdomen the extent of intraperitoneal tumour growth was scored semi quantitatively, 0 (no macroscopic tumour), 1 (little; located at 1-2 sites with a diameter of 1-2 mm), 2 (moderate; located at 1-2 sites and a diameter 2-5 mm), or 3 (abundant; located at multiple sites and/or diameter >5 mm) in all four quadrants of the abdomen. The sum of the tumour scores of all sites represented the peritoneal cancer index (PCI)[11]. Subsequently, CS, including a routine omentectomy, was performed in all rats. Irresectable tumour deposits were cauterized using an electrocoagulation device. 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 in animal treated with CS only or CS+RIT.

Monoclonal antibody, radiolabeling and RIT

The murine MG1 monocolonal antibody (MAb), an anti-CC531 IgG2a monoclonal antibody that recognizes a 80 kDa cell surface antigen and localizes preferentially in tumours when injected in rats bearing CC-531 tumours[15], was purchased from Antibodies for Research Applications BV (Gouda, The Netherlands). Labeling of the antibody with ^{177}Lu was carried out as previously described.[11] In brief, the MAb was conjugated with 2-(4-isothiocyanatobenzyl)-diethylenetriaminepentaacetic acid (ITC-DTPA) (Macrocyclics, Dallas, TX) and subsequently labelled with ^{177}Lutetium (IDB Holland, Baarle Nassau The Netherlands) and purified by gel filtration on a PD10 column (Amersham, Pharmacia Biotech, Maarsen, The Netherlands). The purified ^{177}Lu-MG1 preparation was diluted in PBS with 0.5% BSA for injection, the specific activity of the administered ^{177}Lu-MG1 preparation was 0.4 MBq/µg. The labeling procedure using ^{177}Lu was performed under strict metal-free conditions.

RIT (185 µg MG1/ rat, radiolabeled with 74 MBq ^{177}Lu in 3.0 ml) was intraperitoneally injected immediately after surgery, as this was determined to be the most optimal time point for adjuvant administration.[16]

Mitomycin-C

Mitomycin-C (MMC) was obtained from Nycomed Christiaens BV (Breda, The Netherlands) as a powder in glass vial (40mg/vial). Immediately before use, MMC was dissolved in 0.9% sodium chloride to the appropriate concentrations.

HIPEC procedure

Following CS, while the abdomen was still exposed, two multiperforated catheters (Argyle, Sherwood Medical, Ireland) were inserted laterally through the abdominal wall and subsequently fixed in the abdominal cavity. The inflow drain was placed in the right paracolic gutter, the outflow drain in the left subdiaphragmatic space. The intraperitoneal temperature was monitored with an intraabdominal thermometer, (PTFE Insulated thermocouple, VWR International, Amsterdam, The Netherlands), at the site with generally the highest tumour load (omentum). In addition, a thermometer was placed

inside the rectum. After placement of the catheters, the abdominal wall was closed using a continuous suture (Ethilon 3.0, Johnson & Johnson, Ethicon). (Figure 1) During the HIPEC procedure, rats were removed from the warmed mattress to prevent general hyperthermia. The perfusion system was filled with 250 ml saline, containing 4 mg MMC (Mitomycin-C Kyowa, Christiaens). The perfusate was heated in a tube coil using a thermostatically regulated water bath set to a temperature of 48°C and infused into the peritoneal cavity by a roller pump (Ismatec IPS-8, Ismatec SA, Glattbrugg, Switzerland.) for the duration of 60 minutes at 10 ml/min. Abdominal inflow temperature was set at 44°C. In order to achieve a uniform heat distribution, gentle massage of the abdomen was applied throughout the duration of the HIPEC procedure. After completion of the perfusion, the abdominal cavity was flushed with warmed (37°C) saline for a period of ten minutes. The abdomen was opened again to remove the catheters. Subsequently, the abdomen was closed as described above.

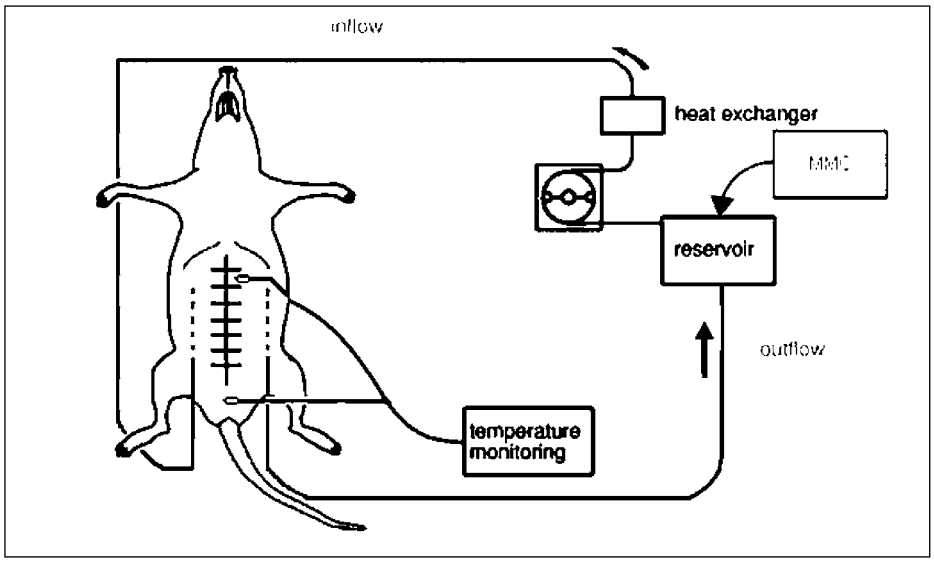

Figure 1. HIPEC Perfusion System; MMC Mitomycin C Kyowa 16 mg/l perfusate. Adapted from: A new survival model for hyperthermic intraperitoneal chemotherapy (HIPEC) in tumor-bearing rats in the treatment of peritoneal carcinomatosis. Pelz JO, Doerfer J, Hohenberger W, Meyer T. BMC Cancer. 2005 30;5(1):56 Copyright BMC Cancer. Reproduced with permission.

Intraperitoneal distribution of MMC and dose determination

Prior to the therapy experiment with HIPEC, we investigated the intraperitoneal distribution of the perfusion fluid using a Methylene Blue stained perfusate. The perfusate was administered in the same fashion as in the therapeutic experiment. After completion, the abdominal cavity was inspected for the presence of blue dye in all quadrants on both parietal as well as visceral peritoneum of the intra-abdominal organs and the diaphragm. Subsequently, a study to determine the dose of MMC that resulted in acceptable toxicity was performed in 9 animals (3 animals per group). Animals underwent a laparotomy including an omentectomy and complete bowel inspection followed by heated perfusion of the abdominal cavity with MMC at 4 mg/L or 16 mg/L. Control rats underwent laparotomy and an omentectomy only. Body weight and physical condition were monitored during 6 days following the procedure in order to assess treatment related toxicity.

Treatment efficacy

Seven days after intraperitoneal tumour induction with 2.0 x 10^6 CC-531 tumour cells, 45 rats, fifteen per treatment group, were randomly assigned to undergo either CS only, CS+RIT or CS+HIPEC. The operative procedures and application of the adjuvant therapies were performed as described above. Toxicity of the treatment was determined clinically and by weighing the rats. Body weight was expressed as relative body weight compared to the body weight on the day of surgery. Survival was scored and at autopsy the extent of tumour growth was determined.

Follow up

The primary endpoint was 16-week survival. As part of monitoring physical condition during the immediate postoperative period, general condition was monitored and body weight was measured daily during the first two weeks. When the humane endpoint (HEP) was reached (signs of massive hemorrhagic ascites, physical inactivity or signs of intra-abdominal tumour growth with invalidating consequences), rats were killed by O_2/CO_2-administration and immediately dissected. The HEP was determined by an experienced biotechnician who was blinded to the therapeutic regimen. At the time of

the HEP rats were generally lethargic, showing signs of advanced PC as the presence of ascites. At dissection, the intraperitoneal tumour growth was scored as described above. At 16 weeks postoperatively, the study was terminated and the remaining rats were euthanized and dissected. In case of absence of macroscopic tumour, all relevant organs, including the greater momentum, the mesentery and the diaphragm were removed for histopathological staining in order to determine tumour presence microscopically. Sections were stained using haematoxylin & 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).

Results

Intraperitoneal distribution of HIPEC and dose determination

The intraperitoneal distribution of the perfusate administered according to the above described procedure, showed a distribution pattern amongst all quadrants, including the diaphragm bilaterally and at the mesenterial root. Figure 2.

The applied dose of 16 mg MMC/L resulted in a maximum mean weight loss of 13.7% ± 2.9% at 4 days postoperatively. In addition, the first three days following the heated perfusion, animals were lethargic and suffered from diarrhoea from day two until day four postoperatively. In contrast, the maximum weight loss in the 4 mg/L was 8.3% ± 2.9% at day 3 and 7.5 % ± 2.3% at day 3 in the control group. Figure 3. None of the animals died during the immediate postoperative period. Based on these observations, HIPEC, when administered at a dose of 16 mg/L for the duration of 60 minutes at the given temperature, was considered to be the maximal tolerable dose to be used for the HIPEC procedure.

Operative procedure

Preoperative body weight did not differ between groups, P=0.52. (Table 1) At laparotomy, tumour nodules were present in the omentum, liver hilum, the mesentery and gonadal fatpads (1-3 mm diameter). Median PCI score at

time of surgery was 5 (range 4-8) and was similar in all experimental groups. After surgical cytoreduction, residual disease remained in situ in 7 rats after cauterization and was equally distributed among the groups (P=0.84). The surgical procedures without adjuvant therapy took 20-30 minutes per animal.

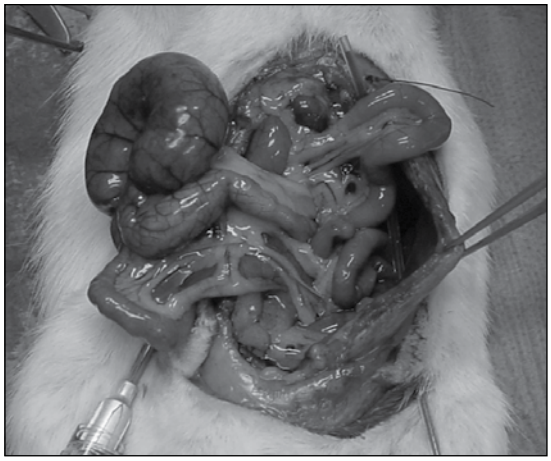

Figure 2. Intraperitoneal distribution of Methylene Blue stained perfusate.

Table 1. Disease characteristics	Median (range) CS	CS+HIPEC	CS+RIT
Pre-operative Body Weight (g)	266 (251-287)	264 (245-285)	262 (244-276)
Tumor score per site			
Greater omentum	2 (2-3)	2 (1-2)	2 (1-2)
Liver hilum	1 (0-1)	1 (0-1)	1 (0-1)
Perisplenic	0 (0-1)	0 (0)	0 (0)
Mesentery	1 (0-2)	1 (0-2)	1 (0-2)
Gonadal fatpads	0 (0-2)	0 (0-2)	1 (0-2)
Diaphragm	0 (0-1)	0 (1)	0 (0-1)
Parietal peritoneum	1 (0-1)	1 (0-1)	1 (0-1)
Total	5 (4-8)	5 (4-6)	5 (4-8)
Resection macroscopically complete			
Yes	12	13	13
No	3	2	2

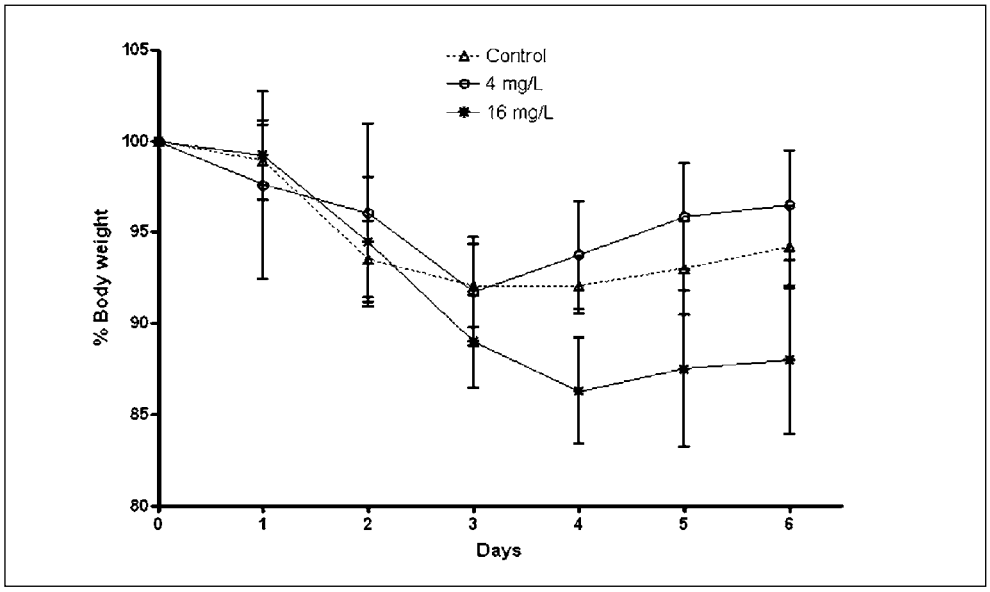

Figure 3. The relative body weight of Wag/Rij rats after exploratory laparotomy (Control) and heated intraperitoneal chemotherapy (HIPEC) given immediately postoperatively in different doses. Data represent means ± standard error of the mean (SEM).

There was no intraoperative mortality. However, one rat in the CS+HIPEC and one rat in the CS+RIT group were euthanized after 2 and 9 days respectively. The animal in the CS+HIPEC group showed massive weight loss as a result of bowel necrosis and subsequent perforation, the cause of death of the animal in the CS+RIT group remained unclear.

The median intra-abdominal temperature during the HIPEC procedure, measured at the site where the greater omentum was removed, was 41.0 °C (range 40.4-41.6°C). In contrast, the median rectal temperature was 34.6°C (range 34.1-34.8 °C). (Figure 4).

CS and CS+RIT were well tolerated whereas animals in the CS+HIPEC groups showed signs of physical discomfort; animals of the latter group were generally lethargic and showed pilo erection two days following the procedure. In addition, these animals all suffered from diarrhoea up to 4 days after the HIPEC procedure. The relative body weight after of the various treatment groups is depicted in Figure 5. Maximum body weight loss after CS or CS+RIT was similar (7.3 ± 2.6% vs. 9.3 ± 1.8% four days postoperatively, $P>0.05$). Rats that received adjuvant HIPEC had a maximum body weight loss of 12.3 ± 1.7%, which was significantly higher than that after CS alone ($P<0.001$) or CS+RIT ($P<0.001$). Rats generally gained weight from the fifth postoperative day onwards. In the HIPEC group, however, postoperative mean body weight remained significantly lower than that of the animals in the CS group, until five weeks postoperatively.

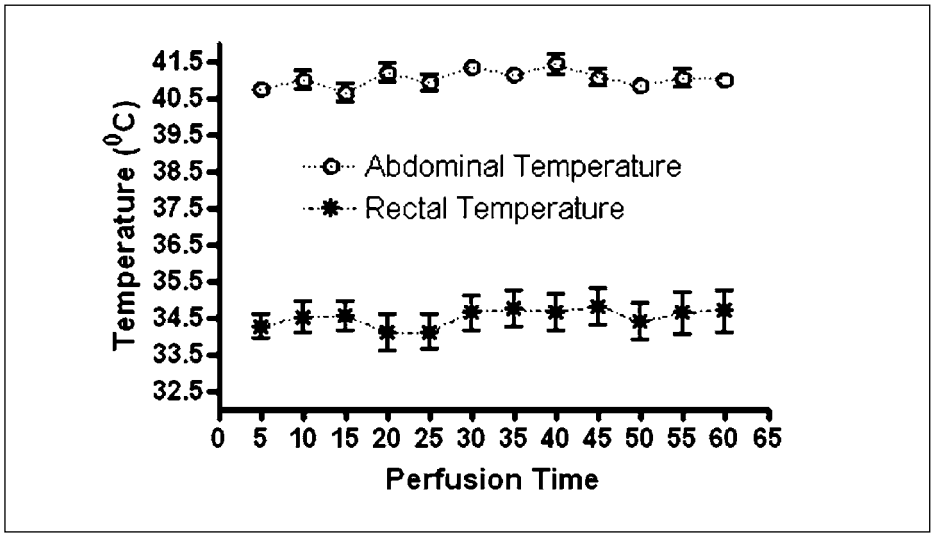

Figure 4. The recorded intra-abdominal and rectal temperature during the HIPEC procedure. Data represent means ± standard error of the mean (SEM).

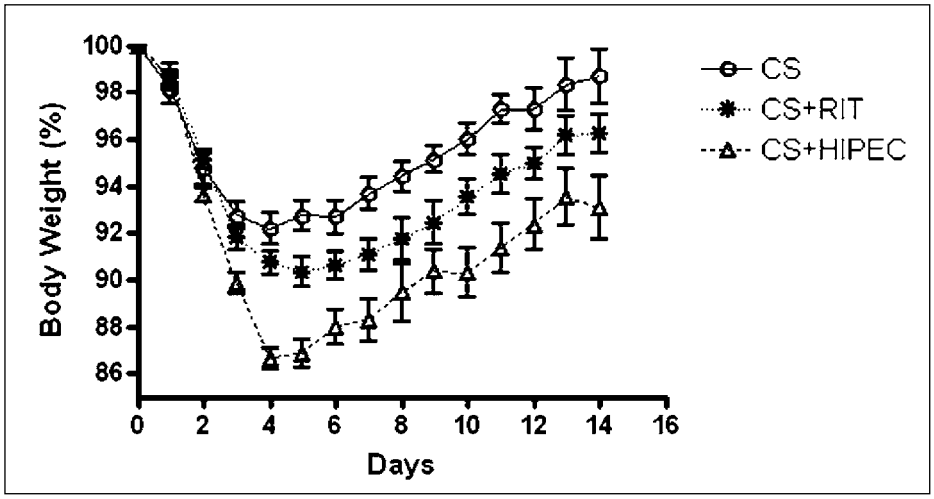

Figure 5. The relative body weight of Wag/Rij rats with small peritoneal CC-531 tumours in the first 14 days after cytoreductive surgery (CS) only, CS + radioimmunotherapy given immediately postoperatively (RIT) or heated intraperitoneal chemotherapy (HIPEC) given immediately postoperatively. Data represent means ± standard error of the mean (SEM).

Treatment efficacy

During the experiment, 29 animals were euthanized due massive amounts of ascites that resulted from intraperitoneal tumour growth. The mean amount of ascites at the humane endpoint was 31 ml ± 22.6, 26.5 ml ± 23.8 and 26.4 ml ± 22.6 ml in the CS, RIT and HIPEC groups, respectively (P=0.82). At the time of death, mean PCI in the CS, CS+HIPEC and CS+RIT groups was 18 (range 9-22), 12 (range 5-15) and 18 (range 16-19), respectively, with significant differences between the CS+HIPEC and both other treatment groups (P<0.001 for both comparisons).

The survival curves of the various treatment groups are depicted in Figure 6. Median survival of the rats that were treated with CS only was 57 days (range 36-112). Adjuvant HIPEC resulted in a median

survival of 76 days (range 33-112), P=0.17, when compared to CS only. Median survival of the rats that were treated with CS followed by the adjuvant administration of RIT was improved to a median survival of 97 days (range 49-112), P<0.001 compared to CS only. When compared to CS followed by adjuvant HIPEC, the adjuvant administration of RIT to surgery did not result in an improved survival, P=0.33).

At the endpoint of the study, 16 weeks after CS, 14 animals (two animals in the CS group, five animals in the CS+HIPEC group and seven animals in the CS+RIT group) were still alive, without any physical signs of intraperitoneal tumour growth. Of these 14 animals that were still alive 16 weeks after surgery, one animal in the CS+HIPEC group and three animals in the CS+RIT group showed macroscopic evidence of tumour at dissection. In the remaining 10 animals (2 in the CS alone group, 4 in the HIPEC group and 4 in the RIT group) not even microscopic tumour presence was found.

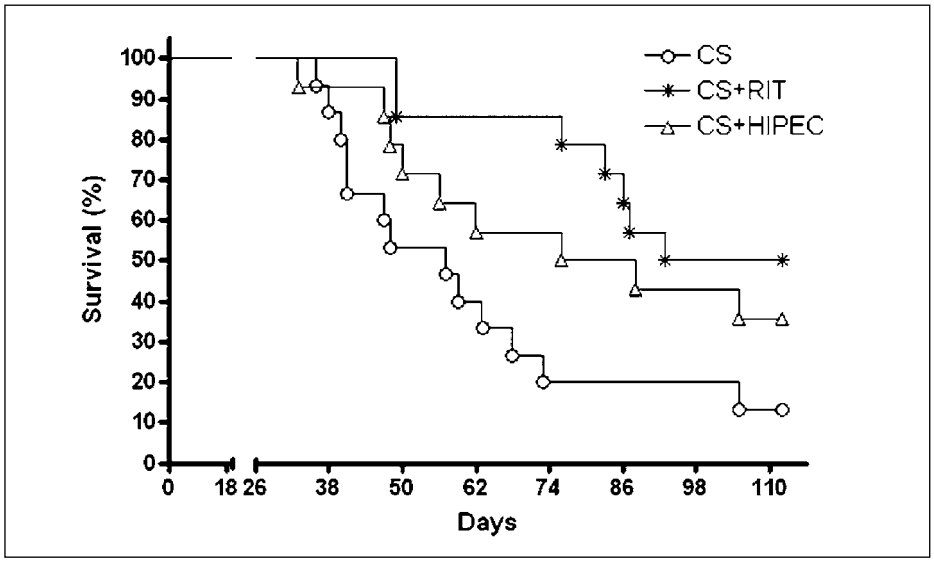

Figure 6. Kaplan-Meier survival curves for Wag/Rij rats with small peritoneal CC-531 tumours after cytoreductive surgery (CS), CS + RIT (RIT) or CS + HIPEC (HIPEC).

Discussion

In the present study, adjuvant radioimmunotherapy after cytoreductive surgery for peritoneal carcinomatosis of colorectal origin in rats significantly improved survival, whereas HIPEC did not. In addition, the application of HIPEC was associated with considerably more toxicity as compared to RIT.

The treatment of peritoneal carcinomatosis was studied with CC-531 syngeneic tumours that grew intraperitoneally in Wag/Rij rats. This model is highly reproducible and the growth and distribution pattern throughout the abdominal cavity are similar to the human entity of PC.[14] Cytoreduction performed at 7 days after tumour reduction resulted in minimal residual disease (<1 mm). In this setting, both HIPEC and RIT result in maximum therapeutic efficacy.[12,13,17]

The MG1 MAb preferentially localizes in the CC-531 tumours [11], with only minor localization in thymus, lymph node, salivary gland tissue and skin[15]. ^{177}Lu was selected as the radionuclide for RIT of minimal residual disease because of its high tumour uptake and adequate physical properties including

a medium-energy ß-emission with a maximum penetration range in tissue of 2.5 mm. In our previous studies we have used the combination of ^{177}Lu-MG1 radionuclide-antibody. These studies demonstrated the combination to be highly effective for the improvement of survival in the model of PC as described above.[11,18] Moreover, in previous experiments we have shown that radioimmunotherapy with a radiolabeled irrelevant antibody is less effective by far as compared to therapy with a radiolabeled specific antibody.[19] HIPEC has been studied in only a few preclinical studies. [20-23] These studies showed that its use was associated with a decreased tumour load as compared to control groups[20]. However, in these studies, HIPEC was associated with a considerable toxicity, indicated by lethargy, marked body weight loss and bacterial translocation[24]. These results on toxicity are in corroboration with the results of our study and mimic the clinical effects of HIPEC. Clinical studies with postoperative intraperitoneal chemotherapy are associated with a high mortality and morbidity[12].

Of sixteen published reports on the use of HIPEC in the clinical setting, thirteen reports described the administration of MMC[12]. In vitro, MMC has also shown to inhibit growth of CC531 cells in a concentration a thousand fold lower than the concentration used in the present experiment.[25] The applied dose of 16 mg/l MMC in our study is within the range of doses applied in clinical practice (5 mg/l - 20 mg/l [26]) and is higher than described in other preclinical studies (2.25 mg/l [20] and 4 mg/l [23]). In addition, in the present study HIPEC was applied as an adjuvant treatment to cytoreduction whereas Pelz et al. applied HIPEC as monotherapy with subsequent killing of the animals after only 10 days.

The intraperitoneal distribution of MMC during the perfusion was studied before the start of the actual experiment and showed equal distribution amongst all quadrants. The effect of the perfusion technique on distribution differences and their associated differences in survival have never been studied clinically. Glehen and colleagues performed a large clinical study in 506 patients that were treated with both the open and closed abdomen perfusion technique. The authors reported no differences in survival between both perfusion techniques.[6] This observation was confirmed by the study of Sugarbaker and colleagues[27].

RIT with 74 MBq of the ^{177}Lu-MG1 radionuclide-antibody in 3 mL has previously been shown to be an effective treatment for experimentally induced PC of colonic origin when administered intraperitoneally.[11,16] The biodistribution of intraperitoneally injected ^{111}In-labeled MG1 was studied by Koppe et. al. and showed a preferential uptake of the radiolabeled antibody in the tumour.[11]

The extraperitoneal temperature of 48°C necessary in order to obtain inflow temperatures of 44°C, would not have had a negative influence on the cytotoxicity of MMC, since Ahrar et al.[28] showed that only temperatures exceeding 60 ^{0}C decreased its cytotoxic effect. On the other hand, one has to bear in mind the fact that the additive effect of hyperthermia in HIPEC in the clinical setting has not yet been proven in a randomized trial. Elias et al. and Glehen et al. both reported the use of early postoperative chemotherapy (EPIC), without hyperthermia, ranging from day one to day five after surgery, and HIPEC. Elias et al. found no significant difference in survival.

Similarly, in the multicentre study in 506 patients of whom 53.3% and 24.3 % underwent HIPEC and EPIC alone, no significant difference was found in survival between the two treatment groups. [6] The duration of the heated perfusion, 60 min. in this experiment, is in concordance with the recent consensus statement regarding Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy in the Management of Peritoneal Surface Malignancies of Colonic Origin, stating that the perfusion should last 60-120 minutes.[13] These data on the dose of MMC, the used perfusion time and –temperature, together with our results that showed anti-tumour effect in the model of induced PC from CC531 cells (significantly lower PCI at HEP in favour of CS+HIPEC), we can conclude that the HIPEC model used in our study was able to induce regression of PC of colonic origin.

In the latest reported clinical trial on adjuvant RIT in the setting of colon cancer, Liersch et al. reported results of a Phase II trial with ^{131}I-labeled anti-CEA antibody Labetuzumab administered to patients after complete resection of colorectal liver metastases. This study, where RIT was applied in an adjuvant setting to complete resection, resulted in a promising 5-year survival rate of 51.5%.[10] The results of this study is in accordance with the conclusion of a recent review on the use of RIT to treat colon cancer.[17]

In this review, the authors state that the time may have come for clinical trials in which RIT is added to standard regimens, in order to establish the place of this treatment modality as an adjuvant treatment after CS.

To our knowledge, the present study is the first study comparing the use of RIT and HIPEC in an adjuvant setting to CS for the treatment of PC in colorectal cancer. Our preclinical studies indicate that the application of RIT immediately following CS can improve survival in rats with PC of CRC. Moreover, from the present study we conclude that the use of adjuvant RIT is an effective treatment with low toxicity. When comparing to today's standard of care, HIPEC, RIT was at least as effective. RIT consisted of an activity dose of 74 MBq of ^{177}Lu-labeled MG1per rat, resulting in only minor toxicity, whereas the theoretical MTD of ^{177}Lu-labeled antibodies in 250 g rats could be approximately 150 MBq.[11]

There are, however, some challenges to the clinical applications of adjuvant RIT. E.g. after cytoredective surgery, patients are transferred to the intensive care unit. Optimal patient care has to be balanced with radiation safety issues for the medical staff. Previously, we reported on the optimal time interval between RIT and CS.[16] In that study we showed that RIT should be administered as soon as possible after CS, with a window of opportunity for RIT administration up to 4 days after surgery. It can therefore be envisioned that for radiation safety reasons the therapy should be given not before discharge of the patient from ICU and removal of the abdominal drains.

Our study therefore justifies the consideration of intraperitoneal radioimmunotherapy after cytoreductive surgery in case of peritoneal carcinomatosis of colorectal cancer. In clinical studies this approach should be compared to HIPEC.

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Abstract

The purpose of this study was to investigate the effect of adjuvant radioimmunotherapy on anastomitic healing following cytoreductive surgery. In addition we compared the effects of adjuvant RIT to the effects of HIPEC with regard to anastomitic strength.
In order to do this, PC was induced by intraperitoneal inoculation of CC-531 colon carcinoma cells in Wag/Rij rats. Subsequently, seven days after tumour induction, animals were subjected to exploratory laparotomy (C). This was then followed by the construction of both a small bowel anastomosis as well as a colonic anastomisis. Immediately after the construction of these anastomoses, 74 MBq ^{177}lutetium-labelled anti-CC531 antibody MG1 was intraperitoneally administered, HIPEC was performed by the closed abdomen perfusion technique described in the previous chapter, or PBS was intraperitoneally injected. Anastomotic strength measurement was peformed by means of bursting pressure and breaking strength 3 and 5 days after anastomotic construction.
From this study we concluded that adjuvant RIT after CS for the treatment of PC of colonic origin showed no detrimental effects on anastomotic healing and –strength.

Chapter 6

The Effects of Adjuvant Experimental Radioimmunotherapy and Hyperthermic Intraperitoneal Chemotherapy on Intestinal and Abdominal Healing after Cytoreductive Surgery for Peritoneal Carcinomatosis in the Rat.

This chapter is based on:
F. Aarts, R.P Bleichrodt, B. de Man, R. Lomme, O.C. Boerman and T. Hendriks. The effects of adjuvant experimental radioimmunotherapy and hyperthermic intraperitoneal chemotherapy on intestinal and abdominal healing after cytoreductive surgery for peritoneal carcinomatosis in the rat. Annals of Surgical Oncology, Accepted for publication

Introduction

Extensive surgical debulking procedures (cytoreductive surgery, CS) in combination with hyperthermic intraperitoneal chemotherapy (HIPEC) are considered as today's first choice for the treatment of peritoneal carcinomatosis (PC).[1] The extensive nature of the surgical procedure, where all macroscopic tumour is removed, often requires the construction of multiple bowel anastomoses.[2,3] The concomitant application of cytotoxic temperatures and high local concentrations of chemotherapeutic agents result in a median survival of 13-34 months. This way, a 5-year survival rate of 19%-27% can be obtained.[4-7] However, patients suffer from high morbidity and mortality rates of up to 50% and 8%, respectively. A substantial proportion of the morbidity consists of complications related to anastomotic repair such as anastomotic dehiscence and intra-abdominal abscess formation. In addition, enteral fistulae may develop.[2,3]

Previously, we have demonstrated in rats with PC that CS followed by adjuvant radioimmunotherapy (RIT) effectively prolongs survival [8,9], and could be even more effective than HIPEC in this respect.[10] Moreover, adjuvant intraperitoneal RIT resulted in significantly less treatment-related toxicity, as reflected in loss of body weight and the occurrence of diarrhoea and clinical discomfort.

Very recently, it has been reported that HIPEC reduces anastomotic strength in the colon of healthy rats [11]. Our hypothesis is that RIT may be less detrimental in this respect. Here, we report on an experiment in rats with peritoneal carcinomatosis of colonic origin which were treated by CS and subsequent anastomotic construction in both ileum and colon. The effects of adjuvant HIPEC and RIT on the development of early wound strength in the intestine and the abdominal wall were determined.

Materials and Methods

Study Design

Seven days after intraperitoneal tumour induction with CC531 tumour cells, 72 rats (24 per treatment group) underwent CS, followed by the construction

of anastomoses in both ileum and colon. Thereafter, animals were randomly assigned to immediately receive either phosphate buffered saline (CS group) or adjuvant HIPEC (CS+HIPEC group) or RIT (CS+RIT group). Within each treatment group, half of the animals were sacrificed three and five days after operation for the measurement of mechanical and biochemical parameters for repair. A further nine animals, three for each experimental group, were used for histological examination of the anastomoses five days after surgery.

Model of peritoneal carcinomatosis

Male WAG/Rij rats (10-12 weeks old, body weight 240-260 g, Harlan Horst, The Netherlands) were housed under non-sterile standard conditions (temperature, 20–24°C; relative humidity, 50-60%; 12 h light/dark cycle) in filter-topped cages (2 rats per cage), with free access to food (Ssniff, Bio Services Uden, The Netherlands) and water. Rats were allowed to accustom to laboratory conditions for at least one week before experimental use. Physical condition was examined daily and total body weight was recorded daily by a biotechnician, who remained 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. The syngeneic rat colon carcinoma cell line CC531, originally induced in Wag/Rij rats by intravenous injection of 1,2- dimethylhydrazine[12], was injected intraperitoneally, as previously described.[8]

Surgical procedures

Surgery was performed under general anaesthesia using isofluorane 3%, O_2 and N_2O 1:1. All rats underwent a complete midline laparotomy, followed by careful inspection of the abdominal contents for tumour growth. Tumour growth at the various intra-abdominal locations was scored as 0 (no tumour), 1 (little), 2 (moderate), or 3 (abundant). Within each animal, the sum of the tumour scores represented the peritoneal cancer index (PCI)[8]. Cytoreductive surgery, including an omentectomy, was performed by radically removing all macroscopic tumour deposits. Irresectable tumours were cauterized. Subsequently, 5 mm of the distal ileum was

resected, approximately 15 cm proximal to the cecum, and continuity was restored by constructing an end-to-end anastomosis with 8 single-layer, inverting, interrupted sutures (Ethilon 8-0, Ethicon, Germany). A similar procedure was then performed in the descending colon, approximately 3 cm proximal to the peritoneal reflection. Following the construction of this anastomosis, saline (3 ml) was administered intraperitoneally or a RIT or HIPEC procedure were performed. After anastomotic construction, 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. When followed by HIPEC, the laparotomy was closed in one layer after installing the catheters. Prior to and at the end of the procedure 10 mL of saline was given subcutaneously, for pre- and rehydration. Thirty minutes prior to surgery and twice daily until the third postoperative day, rats were given buprenorphine (5 μg, 0.1 mL/rat/day) for analgesia.

HIPEC

The HIPEC procedure was performed as previously described.[10] Briefly, two multiperforated catheters were placed through the lateral abdominal wall bilaterally and the abdomen was closed, thus creating a closed perfusion system. The perfusion system was filled with 250 ml 0.9% NaCl, containing 4 mg MMC (Mitomycin-C Kyowa, Christiaens). This perfusate was heated (inflow temperature was set at 44°C) and infused into the peritoneal cavity for the duration of 60 min. Gentle massage of the abdomen was used throughout the HIPEC procedure to achieve a uniform heat distribution within the peritoneal cavity. Following the heated perfusion, the abdominal cavity was flushed with warm (37 ^{0}C) 0.9% NaCl for a period of ten minutes. Thereafter, the continuous suture was opened and the perforated catheters were removed. Subsequently, the abdomen was closed in two layers.

Radioimmunotherapy

The murine MG1 monoclonal antibody (MAb), an anti-CC531 IgG2a monoclonal antibody that recognizes a 80 kDa cell surface antigen and localizes preferentially in tumours when injected in rats bearing CC531 tumours[13], was purchased from Antibodies for Research Applications BV

(Gouda, The Netherlands). Labelling of the antibody with ^{177}Lu was carried out as previously described.[8] The purified ^{177}Lu-MG1 preparation was diluted in PBS with 0.5% BSA to 0.4 MBq/µg (185 µg MG1/ rat, radiolabeled with 74 MBq ^{177}Lu in 3.0 ml) prior to injection. RIT was applied intraperitoneally immediately after surgery.

Measurement of anastomotic and abdominal wall strength

Rats were killed by CO/CO_2 asphyxiation at either the third or the fifth postoperative day. The abdomen was opened and, if necessary, adhesions were dissected carefully without manipulation of the anastomosis. Presence of adhesions, abscesses or anastomotic dehiscence was recorded. The segments containing the anastomoses were then resected. Anastomotic bursting pressure was measured in succession as described previously.[14] The abdominal wall breaking strength was measured in two isolated strips (2-5 mm x 10 mm) from the sutured midline incision using a tensiometer.

Biochemical analysis and histology

After measuring wound strength, tissue samples were cleaned from adhering tissue and debris, and 5-mm samples containing the anastomosis or the abdominal suture line in the middle were frozen in liquid nitrogen and stored at -80 °C until further processing. After lyophilization, samples were weighed, pulverized, and lyophilized again. The hydroxyproline content, as a measure of the collagen content, was measured by high-performance liquid chromatography after hydrolysis with 6 M HCl and derivatization with dabsyl-chloride. Preparation of tissue extracts and procedures for quantitative gelatin zymography to measure the activity of matrix metalloproteinases (MMP) 2 and 9 have been described previously.[15]

Sections of anastomoses and abdominal wall, originating from animals that had not been subjected to strength measurements were stained using haematoxylin & eosin (H&E) and/or used for immunohistochemistry using the murine MG1 antibody in combination with a HRP conjugated horse-anti-mouse IgG antibody, (Vector Laboratories Inc., Burlingame, CA, USA). For analysis of collagen fibres, sections were stained with picrosirius red.

Autoradiography

Tissue sections containing the anastomosis and part of the abdominal wall containing the suture line were exposed to a storage phosphor imager screen for 20 min immediately after strength measurement at day 3. The screen was scanned in a phosphor imager system (Molecular Imager GS363, BioRad Laboratories, Hercules, CA) at a pixel size of 100x100 μm. Images were processed with Quantity One software (version 4.5.2, BioRad Laboratories, Hercules, CA).

Results

Surgical procedures

No animals died and no complications occurred during the various treatment procedures. At dissection three animals, one in the control and two in the RIT group, showed abscess formation at the site of the anastomosis, without statistical differences between groups (P=0.4).

At the time of surgery, tumour nodules were present in the omentum, liver hilum, the mesentery and gonadal fat pads (1-3 mm diameter). Median PCI score at time of surgery was 5 (range 1-9) and was the same in all experimental groups (Table 1). Intra-abdominal temperature during the HIPEC procedure ranged between 39.0 ^{0}C and 43.2 ^{0}C, with a median of 42.3 ^{0}C. The rectal temperature ranged between 34.1 ^{0}C and 34.8 ^{0}C, with a median of 34.6 ^{0}C.

Pre-operative weight was similar in all groups. All animals lost weight and after five days weight loss after CS, CS+HIPEC or CS+RIT was 10.1 ± 4.2%, 14 ± 6.6% and 12.2 ± 5.2%, respectively (P<0.01 for CS vs. CS+HIPEC). Also, animals treated with CS+HIPEC were lethargic and showed signs of physical discomfort during the first days. In addition, these animals suffered from diarrhoea.

Table 1.

Disease characteristics	Median (range) CS + PBS		CS +HIPEC		CS + RIT	
	Day 3	Day 5	Day 3	Day 5	Day 3	Day 5
Body weight Mean (range)	252 (232-274)	254 (242-266)	258 (246-270)	259 (244-270)	257 (248-277)	258 (243-269)
Tumor score per site						
Greater omentum	2 (1-3)	2 (2)	2 (1-2)	2 (2)	2 (1-2)	2 (2-3)
Liver hilum	1 (1)	1 (0-1)	1 (1)	1 (1)	1 (0-1)	1 (1)
Perisplenic	0 (0)	0 (0-1)	1 (0-2)	0 (0)	0 (0)	0 (0)
Mesentery	1 (0-2)	1 (0-1)	1 (1)	1 (0-2)	0 (0-1)	1 (1-2)
Gonadal fatpads	1 (0-2)	0 (0-2)	1 (0-1)	1 (0-2)	0 (0-2)	1 (0-2)
Diaphragm	0 (0)	0 (0)	0 (0)	0 (0-1)	0 (0-1)	0 (0-1)
Parietal peritoneum	1 (1)	1 (1)	1 (0-1)	1 (0-1)	1 (0-1)	1 (1)
Total	5 (1-7)	6 (2-8)	5 (2-8)	6 (2-9)	5 (4-9)	5 (2-8)

Table 1. Treatment group characteristics (peritoneal cancer index; PCI) found during laparotomy before the administration of the adjuvant therapy.

Wound strength

The bursting pressure at day 3 showed no differences between the three groups with respect to both absolute value or bursting site (always within the anastomotic area). The average bursting pressure of the ileal anastomoses increased significantly between day 3 and day 5 in all groups ($P<0.0001$, $P<0.04$ and $P<0.03$ for CS, CS+RIT and CS+HIPEC, respectively), although the relative increase was lowest in the CS+HIPEC group (Figure 1). In the colon anastomoses, bursting pressures significantly increased in the CS and CS+RIT groups ($P<0.0001$ and $P<0.02$, respectively) but not in the CS+HIPEC group. As a result, marked differences between groups were seen at day 5 (Figure 2). The lowest median bursting pressure in both ileal and colonic anastomoses was found in the CS+HIPEC group and the highest in the CS group ($P<0.01$). Although median values in the CS+RIT group were lower than in the CS group, these differences were non-significant. Loss of strength in the CS+HIPEC group was further illustrated by a shift

in bursting site, particularly in the colon. Here, rupture occurred within the suture line in 4 out of 12 cases in the CS group and in 11 out of 12 ($P<0.05$) cases in the CS+HIPEC group, while the CS+RIT group showed an intermediate value (7/12).

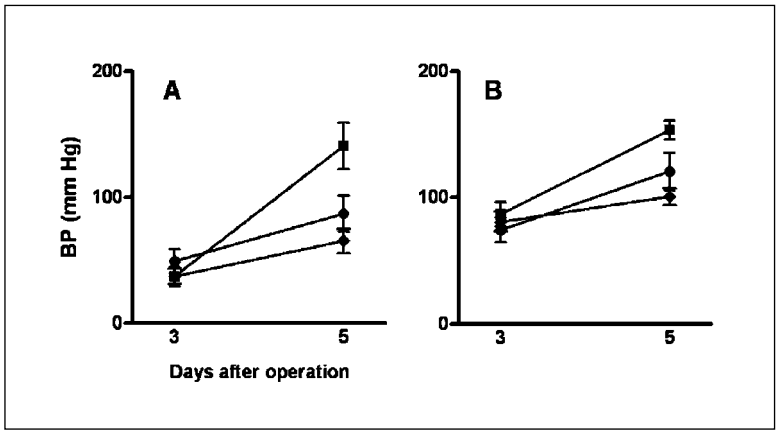

Figure 1. Gain of wound strength in intestinal anastomoses. Mean (± SEM) of the bursting pressure in ileal (A) and colonic (B) anastomoses at 3 and 5 days after operation, for the CS (■), CS+HIPEC (■) and CS=RIT (■) groups.

For the abdominal wall strength, significant differences were seen in breaking strength at both postoperative days (Figure 3). From day 3 to day 5 the abdominal breaking strength increased approximately 6-fold in all groups. Again, strength was highest in the CS group and lowest in the CS+HIPEC group: $P<0.01$ at both day 3 and day 5. Also, abdominal wound strength was significantly ($P<0.01$) lower in the CS+HIPEC group than in the CS+RIT group. The average wound strength was similar in the CS and CS+RIT groups.

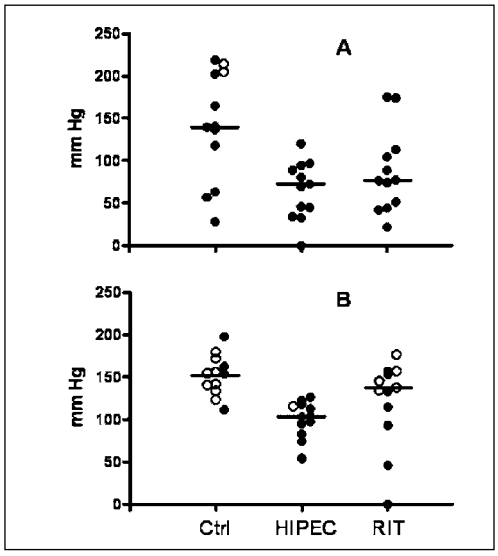

Figure 2. Anastomotic bursting pressure 5 days after operation. Data represent individual measurements in ileum (A) and colon (B). Closed symbols denote anastomoses rupturing within the suture line and open symbols those rupturing outside the wound area. Horizontal lines give the median values.

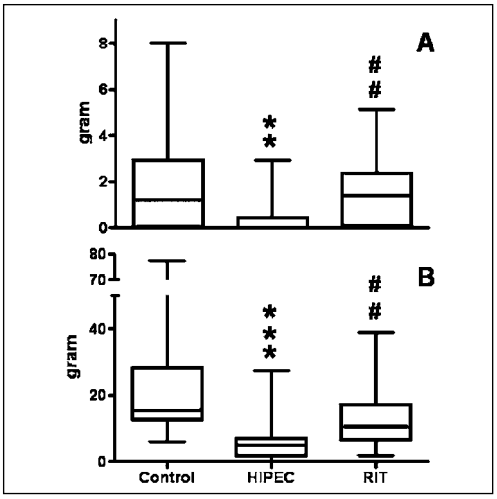

Figure 3. Fascial wound strength. The breaking strength is given for day 3 (A) and day 5 (B) postoperatively. Horizontal bars represent medians and vertical bars range while boxes give 25-75 percentiles. ***: P<0.001 vs CS; **: P<0.01 vs CS; ##: P<0.01 vs CS+HIPEC.

Hydroxyproline content and gelatinase activity

The wound hydroxyproline content showed no differences between groups at day 3 after surgery. At day 5, the average hydroxyproline content at all three sites was lowest in the CS+HIPEC group and highest in the CS+RIT group (Figure 4). However, these differences remained non-significant in the colonic

anastomoses. In the ileum, values in the CS+RIT group were significantly higher than in both the CS (P<0.01) and the CS+HIPEC (P<0.001) group, while in the abdominal wound this was only the case if compared to the CS+HIPEC group (P<0.05).

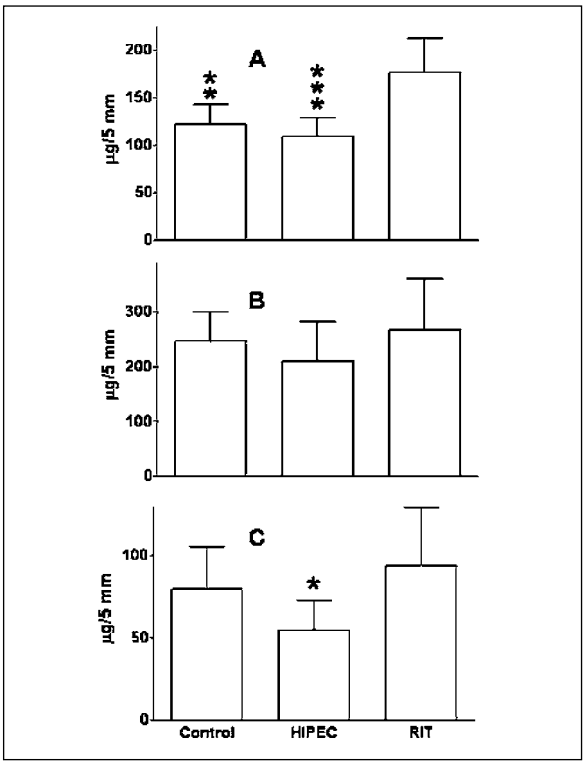

Figure 4. Wound hydroxyproline content 5 days after operation. Bars represent mean values (+SD) for ileal (A) and colonic (B) anastomoses and fascial wounds (C). ***, ** and *: P<0.001, 0.01 and 0.05, respectively, vs CS+RIT group.

There were no differences in proMMP-9 activity between groups. Active MMP-9 remained absent. On average, pro-MMP-2 activities were highest in the CS+RIT groups, most clearly in the intestinal wounds. More explicit differences were observed for active MMP-2 (Figure 5). With the exception of colonic anastomoses at day 3, median values were significantly higher in the CS+RIT group than in the other groups, in some cases by far more than 100%. As a consequence, the ratio between active and inactive MMP-2 was also highest in the CS+RIT group. For instance, median values for this ratio

at day 3 in ileal anastomoses were 0.19 in both CS and CS+HIPEC groups and 0.37 ($p<0.001$ vs both other groups) in the CS+RIT group.

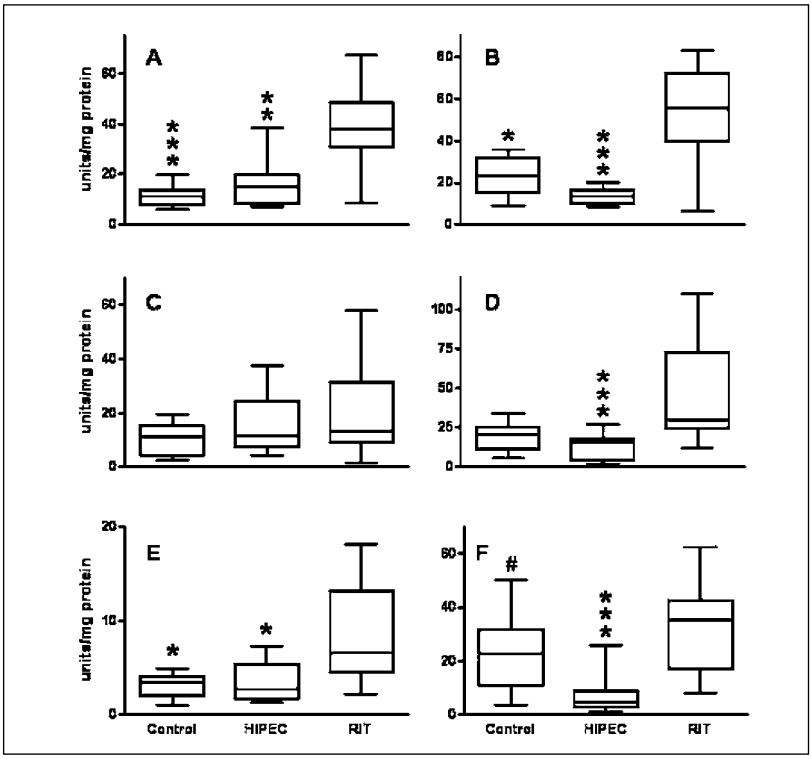

Figure 5. Active MMP-2 in wounds. Specific activity is given at both 3 (A,C,E) and 5 (B,D,F) days after operation in ileum (A,b), colon (C,D) and fascia (E,F). Horizontal bars represent medians and vertical bars range while boxes give 25-75 percentiles. ***, ** and *: $P<0.001$, 0.01 and 0.05, respectively, vs CS+RIT group.# $P<0.05$ vs CS+HIPEC group.

Histology

H&E staining of sections of the ileum and colon showed an increased number of polymorphonuclear neutrophils at the anastomoses as compared to the adjacent bowel wall, indicating an inflammatory response in all groups, but no architectural differences as a result of adjuvant treatment (Figure 6).

Picrosirius red staining of the ileum sections revealed few collagen fibres in the true wound area in all treatment groups. In contrast, in the colon anastomoses there was significant collagen formation in the CS and CS+RIT groups, whereas this appeared less pronounced in the CS+HIPEC group. Sections of the abdominal wall showed collagen formation in all treatment groups.

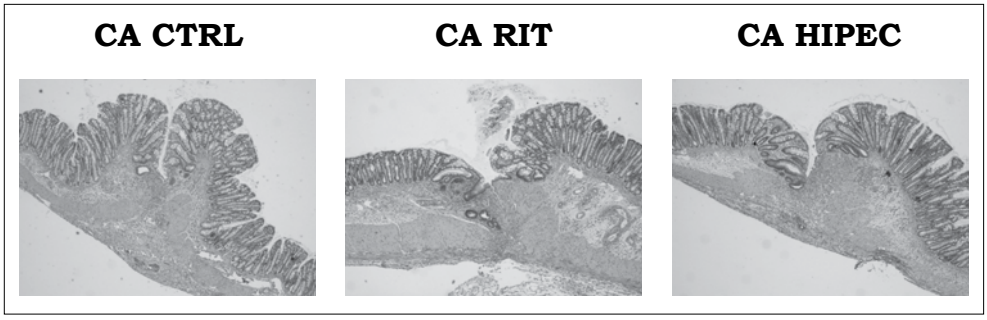

Figure 6. Representative H&E stained slices of colon anastomoses (CA) 5 days after surgery for the respective treatment groups. Arrows indicating the anastomoses.

Autoradiography

Autoradiographic analysis of the bowel anastomosis and abdominal wall sections are shown in figure 7. Clearly, non-specific binding of the radiolabeled antibody to the surgical sites occurred, both at the anastomosis and the laparotomy wound.

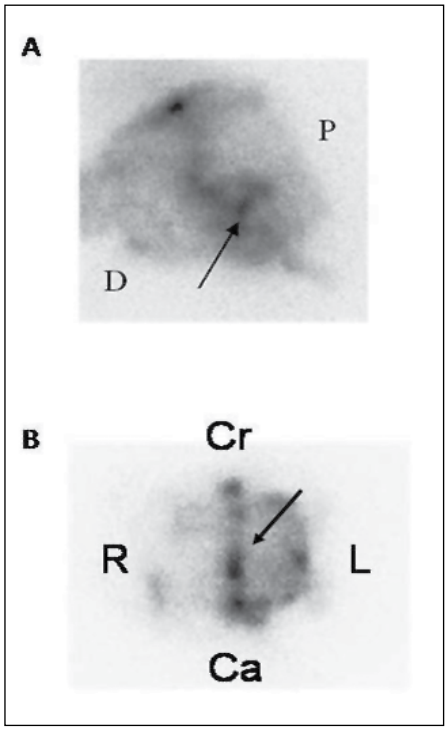

Figure 7. Autoradiography of surgical wounds. A longitudinal cross section of an ileal anastomosis (A) showing uptake of the radiolabeled antibody at the surgical site (arrow). Autoradiographic images of an abdominal wall (b) containing the laparotomy wound, showing uptake of the radiolabeled antibody at the surgical site (arrow). CR indicating the cranial part, CA indicating the caudal part of the wound, R indicates the right side, L indicates the left side of the animal.

Discussion

This study demonstrates that the use of adjuvant RIT after CS did not significantly affect anastomotic strength. In contrast, the adjuvant application of HIPEC was associated with a significantly decreased anastomotic strength. In addition, HIPEC as an adjuvant after CS resulted in significantly lower abdominal wall strength than adjuvant RIT.

Morbidity after cytoreductive surgery followed by heated intraperitoneal chemotherapy is related to surgical / anastomotic related complications in 35%-54% of the cases. [2,16,17] Moreover, the number of suture lines constructed is an independent variable linked to morbidity, suggesting that an increased number of anastomoses is associated with a growing chance for anastomotic leakage.[18] A substantial part of anastomotic related complications stems from small bowel perforation or anastomotic leakage.[2,3,17] Also, not only morbidity, but also mortality is linked to anastomotic related complications.

Patients may succumb to peritoneal sepsis due to intra-abdominal abscess formation related to anastomotic leakage and, as a consequence, succumb to sepsis.[19] Therefore, the focus of new adjuvant treatment modalities should not be aimed at improving disease-free survival alone but also at reducing per-operative morbidity and mortality.

Few preclinical studies on the application of HIPEC or the intraperitoneal administration of MMC have been reported. In these reports, HIPEC and MMC were associated with marked toxicity in the form of lethargy, marked weight loss and bacterial translocation[20]. It is been well known that intraperitoneal cytostatics can severely affect anastomotic strength.[21] This has also been suggested to be the case for MMC, although in the most recent report anastomoses were analysed at the 10th postoperative day, when the bursting pressure does no longer reflect actual wound strength.[22] Makrin and colleagues performed a HIPEC procedure in rats, using MMC in a concentration of 0.02 mg/mL in 200 ml of perfusate (total dose 4 mg), circulating for 20 minutes at a temperature of 40 ^{0}C. [23] The anastomosis consisted of a subtotal dissection (70%-80% of the circumference) of the cecum. Thus, the experimental setup deviated significantly from the clinical situation (incomplete anastomoses, short perfusion time), the investigators found a decreased bursting pressure in the MMC group as compared to controls. The most recent study on anastomotic strength and HIPEC was performed by Pelz and colleagues.[11] Here, HIPEC was also performed with MMC (20 mg/m^2, intraperitoneal temperature 40.5 ^{0}C-41 ^{0}C) either before or after anastomotic construction in the colon. In both cases, HIPEC significantly reduced the anastomotic bursting pressure as measured both 4 and 10 days after surgery. However, so far all studies on the effects of HIPEC on wound repair in a preclinical setting have been performed in healthy rats lacking the preceding tumor growth and cytoreductive surgery applied in the present study. Moreover, in our study anastomoses were constructed in those parts of the bowel that in the clinical setting are prone to be resected during surgical debulking procedures (ileum and sigmoid colon). Thus, the present experiment more closely resembles the clinical situation than any of the experiments reported so far.

In our previous report on the comparison of the adjuvant use of RIT versus the adjuvant application of HIPEC, we showed that adjuvant RIT was capable of achieving a significant improvement in survival as compared to CS alone in a model of peritoneal carcinomatosis of colonic origin in rats. In contrast, the increased survival after adjuvant HIPEC remained not significant.[10] In addition, the treatment related toxicity was highest in the CS+HIPEC group. So far, there have been no reports about complications in healing of wounds in the intestine or abdominal wall after intraperitoneal RIT. The reason for this might be the fact that in the clinical trials adjuvant RIT is administered weeks after any surgical procedure. At that time, anastomoses (and laparotomy wounds) are already strong and less prone to disruption. However, we also demonstrated that the optimal moment, in terms of maximal effect on survival, for administration of adjuvant RIT is immediately after cytoreductive surgery.[9] The only preclinical data available on wound healing and irradiation regard the results of intra-operative external beam radiation therapy. In this setting, even moderate radiation doses delayed the healing of colonic anastomoses.[24] There have been, as yet, no reported data on the potential effects of low dose radioimmunotherapy on (intestinal) wound healing.

Although the bursting pressure of both anastomoses at day five after surgery was lowest in the HIPEC group, there was no significant difference in hydroxyproline content with the CS group. The same was true for the abdominal wall at both days after surgery. Thus, the hydroxyproline content in the segments containg the wound does not correlate with wound strength. This might be due to the fact that those segments contain an excess of uninjured tissue and thus are not very specific for the true wound area. On the other hand, it may also mean that a defect in collagen quality rather than quantity leads to the loss of wound strength in the CS+HIPEC group. Active MMP-2 levels were highest in the CS+RIT group but this did not lead to reduced wound strength. Apparently, the presence of enhanced MMP-2 activity does not lead to a degree of matrix degradation which would be sufficient to affect wound strength. A rise in active MMP-2 and MMP-9 after radiation was also reported by Strup-Perrot after external beam radiation of the colon in rats.[25]

A possible explanation for the presence of antibody at the site of the anastomosis, as observed by autoradiographic imaging, could be non-specific binding. Non-specific antibodies such as IgG have been used in infection imaging. The nonspecific localization of antibodies in inflamed tissues is due to the enhanced vascular permeability in these tisssues.[26] This would then result in antibody accumulation at sites of vasodilation (surgical injury) as can be seen by autoradiography. However, the suggested non-specific binding was not associated with decreased wound strength in the present study.

From the present data we conclude that the use of RIT as an adjuvant to cytoreductive surgery is a treatment with low toxicity which does not significantly impair wound healing. In contrast, today's first choice of adjuvant treatment, HIPEC, is associated with a marked reduction in anastomotic and abdominal wall strength. These data therefore provide further justification for clinical studies utilizing intraperitoneal RIT postoperatively in case of peritoneal carcinomatosis of colorectal cancer.

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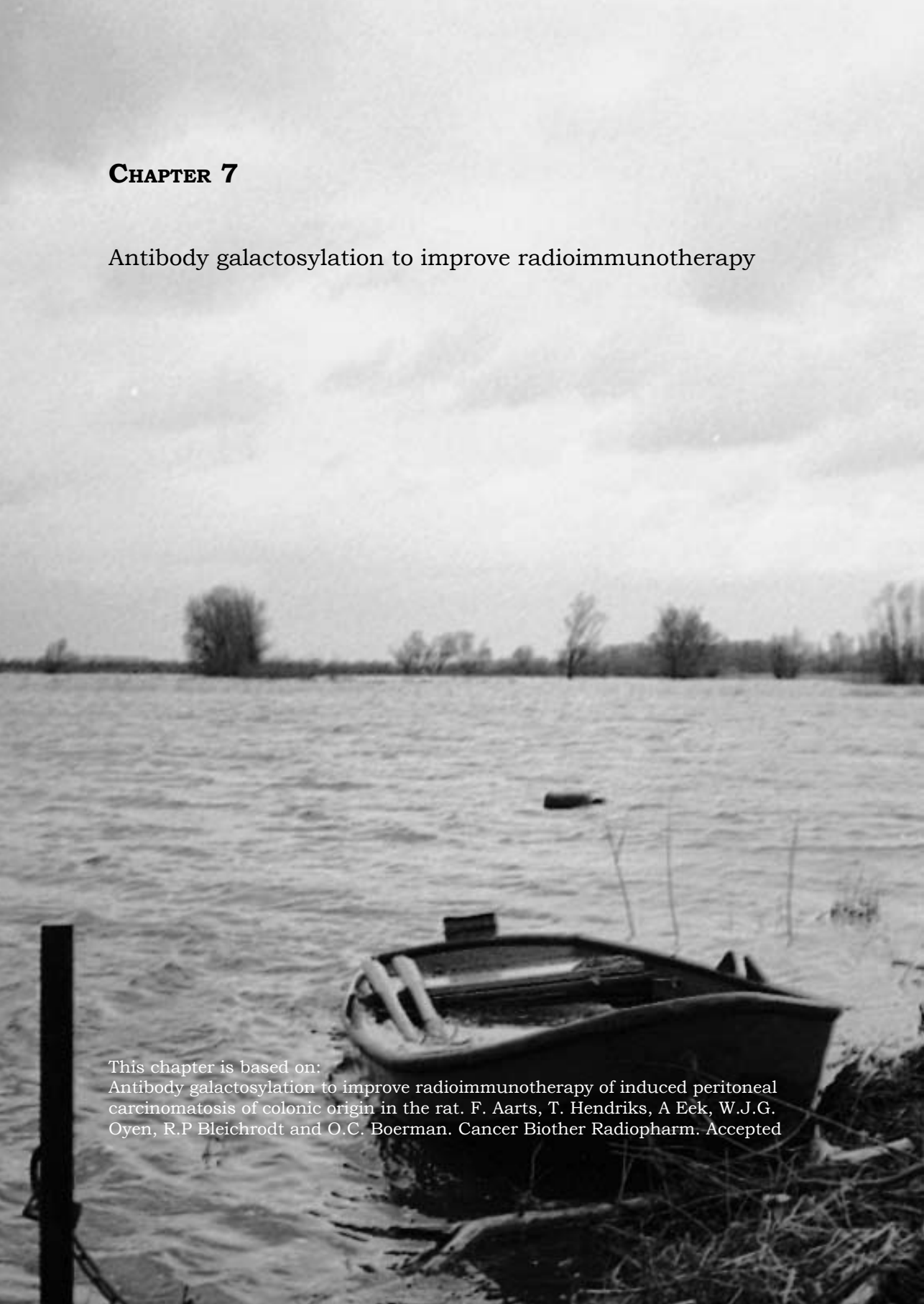
Abstract

In radioimmunotherapy (RIT), hematological toxicity is the dose limiting toxicity due to the long circulatory half-life of the antibody. Carbohydrate modification of antibodies could induce accelerated clearance of the antibody via the hepatic asialoglycoprotein receptor, thereby reducing exposure to normal tissues. In this study, we investigated whether galactosylation of an antibody in a model of peritoneal carcinomatosis (PC) of colonic origin could be used to improve targeting of i.p. growing tumors. Therefore, the biodistribution of the galactosylated and non-galactosylated anti-CC531 antibody MG1 after intraperitoneal injection was determined in a model of peritoneal carcinomatosis of CC-531 colon tumors in Wag/Rij rats. Uptake of the radiolabeled antibodies in the tumor and relevant organs was determined at 2, 4, 24 and 48 hours after injection. Galactosylation of the antibody did not affect the binding affinity of MG1. The uptake of Gal-MG1 in tumors was higher than that of MG1 at 2 and 4hr after injection. After 24h and 48h, uptake of Gal-MG1 in tumor tissue was lower than that of MG1. Gal-MG1 cleared from the blood within hours after administration. At 2hr-24 hr after administration, tumor-to-blood ratios obtained with Gal-MG1 were significantly higher than those obtained with unmodified MG1, resulting in improved tumor-non-tumor ratios.

This could improve the efficiency of RIT, especially in combination with short-lived non-residualizing radionuclides.

Chapter 7

Antibody galactosylation to improve radioimmunotherapy



This chapter is based on:
Antibody galactosylation to improve radioimmunotherapy of induced peritoneal carcinomatosis of colonic origin in the rat. F. Aarts, T. Hendriks, A Eek, W.J.G. Oyen, R.P Bleichrodt and O.C. Boerman. Cancer Biother Radiopharm. Accepted

Introduction

Radioimmunotherapy (RIT) comprises selective irradiation of tumor cells with radiolabeled anti-tumor antibodies (Abs). RIT has been shown to be an effective treatment in hematological malignancies.[1] However, in solid cancers, RIT is less effective, which is partly due to their intrinsic radioresistance and a limited uptake and penetration of antibodies in solid tumors.[2] An inverse relation has been shown to exist between the size of the lesion and the uptake of the radiolabeled antibody.[3] Therefore, RIT seems to be an attractive adjuvant therapy after surgical debulking procedures leaving only microscopic residual tumor.

We have shown previously that adjuvant intraperitoneal administration of RIT after cytoreductive surgery (CS) is an effective treatment for experimental peritoneal carcinomatosis (PC) of colonic origin[4,5] Survival in Wag/Rij rats with intraperitoneal CC531 (colon carcinoma) tumors improved significantly when CS was followed by the intraperitoneal administration of RIT with 2 mCi of the ^{177}Lu-labeled anti-CC531 antibody MG1. The effect was most explicit when RIT was administered immediately after surgery. In addition, RIT was found to be at least as effective as hyperthermic intraperitoneal chemotherapy (HIPEC), which is the current standard of care, while it resulted in significantly less treatment related toxicity.[6]

In order to enhance the therapeutic efficacy of radiolabeled antibodies (Ab) different strategies have been pursued, ranging from locoregional administration of these Abs, as described above, to the application of Ab fragments and pretargeting systems.[7] Here we studied galactosylation of the anti-tumor antibody as a new method to enhance the efficacy of intraperitoneally applied RIT for the treatment of intraperitoneal tumors. After direct tumor targeting following intraperitoneal administration, this modification induces very rapid blood clearance via the hepatic asialoglycoprotein receptor (AGPR). This could result in high tumor-to-blood and tumor-to-non-tumor ratios.[8,9] As a consequence of rapid clearance, the radiation dose that is delivered to the bone marrow will be reduced, thus lowering hematological toxicity. Ultimately, this effect would allow administration of higher doses of radioactivity and potentially increase the

efficacy of RIT. To test this hypothesis, the tumor targeting and uptake in non-target tissues of intraperitoneally injected Gal-MG1 was compared with that of non-galactosylated MG1 in Wag/Rij rats with small volume peritoneal CC-531 carcinomatosis.

Materials and Methods

Reagents

Antibody

The murine MG1 monoclonal antibody (MAb), an anti-CC531 IgG2a monoclonal antibody (Antibodies for Research Applications BV, Gouda, The Netherlands) specifically directed against an 80 kDa cell surface antigen expressed on CC531 cells, was used in these studies. The MG1 MAb localizes preferentially in tumors when injected in rats bearing CC531 tumors [10].

Galactosylation

To galactosylate MG1, cyanomethyl-2,3,4,6-tetra-O-acetyl-1-thio-beta-D-galactopyranoside (CAGP, C-4141 Sigma) was dissolved in methanol at a concentration of 34 mg/mL and mixed with 0.1 volume of 0.1 M sodium methoxide, also in methanol. After 48 hours at room temperature, the methanol was evaporated (Argon flow, 35 °C) to dryness and the residue was dissolved in 1 ml 0.025 M sodium borate buffer, pH 8.5, containing 5 mg MG1. After 2 hours at room temperature, the reaction mixture was dialyzed against phosphate buffered saline (PBS).

To determine the number of galactosyl groups that were conjugated per MG1 molecule, the method described by Dubois et al. was used with minor modifications.[11] To 0.1 mL of galactose solution (0,8-20 μg), 0.1 mL of 5% phenol solution was added and mixed. Then 0.5 mL concentrated H_2SO_4, was added to the solution. The mixture was vortexed, and allowed to stand for 30 min at room temperature. The galactosylated antibody solution (0.1 mL) was similarly treated. Absorbance at 490 nm was measured with a Pharmacia BioTech Ultrospec 2000 spectrophotometer. It was determined that 25 molecules of galactose were conjugated per MG1 molecule.

Radioiodination

Galactosylated MG1 was radioiodinated with ^{125}I (Amersham, Den Bosch, The Netherlands) while the ungalactosylated MG1 was radioiodinated with ^{131}I using the IODOGEN-method as described previously.[12] Briefly, the antibody (1 mg) and the radioiodide (600 µCi) were incubated at room temperature in phosphate buffer (pH 7.4) in an eppendorf tube, coated with 50 µg iodogen. After ten minutes, the reaction was stopped by adding 100 µl of a saturated tyrosine solution. The radioiodinated antibodies were purified on a PD-10 column (Amersham Biosciences, Uppsala, Sweden), eluted with PBS, 0.5% bovine serum albumin (BSA). This resulted in a specific activity of 0.56 µCi/µg for MG1 and 0.48 µCi/µg for galactosylated MG1.

Competitive binding assay

To determine the effect of galactosylation on the affinity of the MG1 antibody, the IC_{50} value of Gal-MG1 and MG1 was determined in a competitive binding assay. Binding of the ^{125}I-labeled MG1 was competed by unlabeled non-galactosylated MG1 or Gal-MG1 and in a concentration dependent manner . ^{125}I-labeled MG1 was used as the tracer in this assay. 6-well Costar culture plates (Corning Inc. USA) were seeded with CC531 cells and cultured until confluency. The plates were washed twice with PBS. Then 3 mL binding buffer containing 50,000 cpm ^{125}I-MG1 with a serial dilution (0 mg/ml - $1.5x10^{-7}$ mg/ml) of non-labeled MG1 or galactosylated MG1 in binding buffer was incubated in the wells at 37 °C for 1 h. After incubation, the plates were washed three times with PBS. Radioactivity in each well was determined in a γ-counter (1480 Wizard, Wallac, Turku, Finland). IC_{50} values of MG1 and Gal-MG1 were calculated by non-linear regression using GraphPad Prism (GraphPad Prism 4.0 Software, San Diego, CA, USA).

Model of peritoneal carcinomatosis

The syngeneic rat colon carcinoma cell line CC531, originally induced in Wag/Rij rats by intravenous injection of 1,2- dimethylhydrazine[13], 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. Tumor cells were harvested from culture flasks with 0.25% trypsin and resuspended in RPMI-1640 medium to a concentration of $1x10^6$ cells/ ml. Two mL of this cell suspension was injected intraperitoneally, as previously described.[14] Male WAG/Rij rats (10-12 weeks old, body weight 240-260 g, Harlan Horst, The Netherlands), were housed under non-sterile standard conditions (temperature, 20–24°C; relative humidity, 50-60%; 12 h light/ dark cycle) in filter-topped cages (2-3 rats per cage), with free access to food (Ssniff, Bio Services Uden, The Netherlands) and water. Rats were accustomed to laboratory conditions for at least one week before experimental use. 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.

Biodistribution

The biodistribution of galactosylated MG1 labeled with ^{125}I and non-galactosylated MG1 labeled with ^{131}I in Wag/Rij rats with intraperitoneally growing CC531 tumor nodules was determined at 2 h, 4 h , 24 h and 24 h (n=5 /group) after intraperitoneal injection. Both radiolabeled Abs (1 ml/rat, 8 µCi ^{131}I-MG1/rat, 9 µCi ^{125}I-Gal-MG1/rat) were administered simultaneously. At dissection, samples of the tumor, blood, liver, spleen, kidneys, intestine, lung, and muscle were removed and immediately weighed. Radioactivity was measured in a well-type γ-counter (Wizard; Pharmacia-LKB). To correct for physical decay and to calculate the uptake of the radiolabeled antibody in each sample as a fraction of the injected dose, aliquots of the injected dose were counted simultaneously. The uptake was expressed as the percentage of the injected dose per gram tissue (%ID/g).

Results

Reagents

Affinity

The affinity of the galactosylated MG1 was determined in a competitive binding assay. The results are shown in Figure 1. Binding of ^{125}I-MG1 to CC531 cells was competed by both Gal-MG1 and MG1 in a concentration dependent manner. Both IC_{50} values were in the nanomolar range with 1.6 nM for MG1 and 1.2 nM for Gal-MG1.

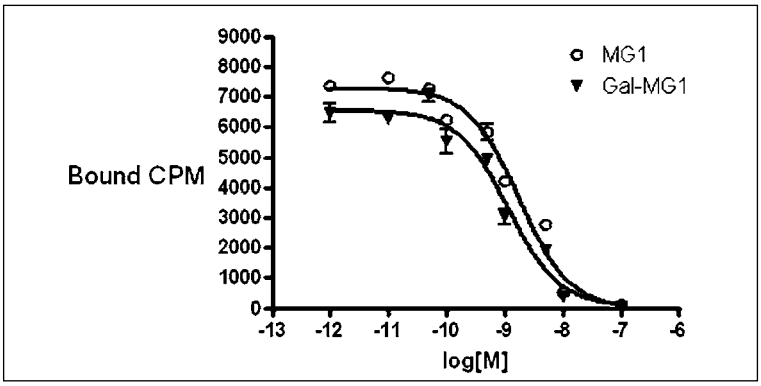


Figure 1. Competitive binding assay showing the affinity of both galactosylated and ungalactosylated MG1 for CC531 cells.

Biodistribution

The results of the biodistribution studies are summarized in Figure 2a-d. There was preferential uptake of both radiolabeled antibody preparations in the intraperitoneal tumors. At 2 and 4 hr post injection, the uptake of Gal-MG1 in tumor tissue (14.0 ± 7.5 % ID/g and 9.9 ± 3.0 % ID/g, respectively) was significantly higher than that of the ungalactosylated MG1 (5.4 ± 1.9 % ID/g and 4.3 ± 1.0 % ID/g, $P<0.04$). Tumor-to-blood ratios at 2 and 4 hr after injection for Gal-MG1 were 10-fold higher than those obtained with ungalactosylated MG1 (32.4 ± 18.9 vs 3.2 ± 1.48 at 2 hr and 18.7 ± 5.51 vs 1.9 ± 0.59 at 4 hr, $P<0.0001$). At 24 h and 48 h after administration, the uptake of Gal-MG1 in tumor tissue (0.7 ± 0.2 % ID/g at 24 hr and 0.2

± 0.1 % ID/g at 48 hr) tended to be lower than that of ungalactosylated MG1 (2.6 ± 1.0 % ID/g, P=0.06, and 1.5 ± 1.3 % ID/g, P=0.10, respectively). Although the tumor uptake of the galactosylated Ab was lower at 24 hr and 48 hr after administration, the tumor-to-blood ratios for Gal-MG1 remained significantly higher at 24 hr (2.70 ± 0.81) than those obtained with ungalactosylated MG1 (1.04 ± 0.39, P<0.04. After 48 hr there was no significant difference in tumor to blood ratios. In addition, uptake of Gal-MG1 in all normal tissues except the liver was lower than that of MG1 at 2 and 4 hr after injection. One and two days after administration, liver uptake was lower for Gal-MG1 as compared to MG1. (Figure 2c/d) The uptake in non-target organs like muscle, lung, spleen and kidneys was low for both Abs, albeit lower for Gal-MG1 after 24 hr and 48 hr.

The total area under the curve (AUC) for Gal-MG1 for the tumor was 502 % ID/g·h as compared to 201 % ID/g·h for ungalactosylated MG1. For the blood, the total AUC for the galactosylated Ab was 7.9 % ID/g·h as compared to 24.5 % ID/g·h for MG1. The ratio of total AUC of Gal-MG1 in the tumor/ AUC for blood was 197 as compared to 8 for the ungalactosylated MG1.

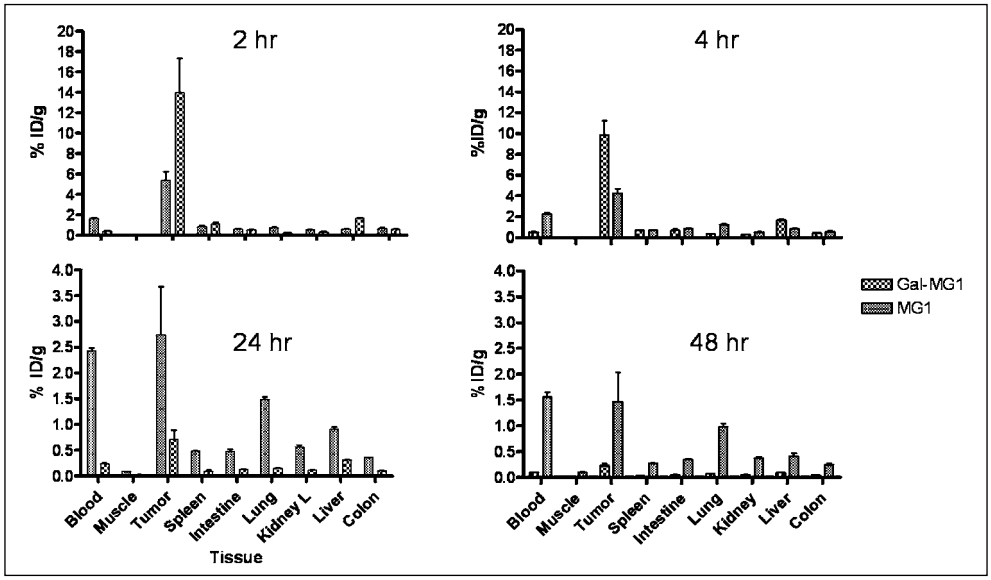


Figure 2a-d. Biodistribution results of Gal-MG1 and MG1 at 2, 4, 24 and 48 hr after intraperitoneal administration,expressed as mean % ID/g tissue. Bars indicate standard error of the mean (SEM)

Discussion

The aim of this study was to investigate whether galactosylation of anti-tumor antibody MG1 could improve the preferential targeting of intraperitoneal tumors in a rat model. The rationale for using a carbohydrated anti-tumor antibody is that galactosylated antibodies clear very rapidly from the blood via the hepatic asialoglycoprotein receptor (AGPR), resulting in low blood levels.[8,9] In cases of intraperitoneal tumors, this could result in optimal tumor targeting after intraperitoneal administration with concomitant rapid clearance when the antibody enters the circulation.

Indeed, at all the time points after intraperitoneal injection blood levels of Gal-MG1 were significantly lower than the blood levels of MG1. Ong and colleagues investigated the administration of galactosylated Abs in a model of an intraperitoneal ovarian cancer cell-line.[15] The authors described a tuoruptake of 4.3% ID/g after 28 hr after injection with a peritoneal

retention of the administered Ab of 10% after 24 hr after administration. In these experiments, however, peritoneal clearance was disturbed by the application by Freund's adjuvant causing a major inflammatory respons, thus not resembling normal physiology. Sharma and colleagues used a model of subcutaneously growing human colon cancer xenografts in mice. [16] The authors used blocking agents to obtain prolonged circulation of the galactosylated antibodies for the duration of 8 hrs. This resulted in tumor-to-blood ratios of 45:1

Galactosylation of MG1 did not affect the affinity of the Ab for the MG1 antigen. Remarkably, within the first 24 hr after administration tumor uptake of the Gal-Ab was significantly higher, 2.6 fold at 2 hr and 2.3 fold at 4 hr after administration, than that of the non-galactosylated antibody. The higher tumor uptake could be due to a longer intraperitoneal retention time of the Gal-Ab. The more negative charge of the Gal-MG1 could result in a slower transit from the intraperitoneal cavity to the circulation. The lower tumor uptake of the galactosylated MG1 after 24 hr and 48 hr is due to the reduced blood levels of Gal-MG1. We assume that antibody uptake in intraperitoneal tumors after intraperitoneal injection is the result of delivery both directly from the peritoneal cavity as well as via the intravenous route. After 24 hr most of the galactosylated antibodies have cleared from the peritoneal cavity. As a consequence of galactosylation, the antibody will have cleared from the circulation, whereas the ungalactoslated antibody still circulates at relatively high levels in the blood. [17-19]

Despite the significantly lower tumor uptake of Gal-MG1 after 24 hr and 48 hr, the total AUC of the tumor was higher for the galactosylated antibody. Moreover, the ratio of $AUC_{(tumor)}/AUC_{(blood)}$ for Gal-MG1 was 24 times higher than the ratio for ungalactosylated MG1. Thus, when normalized for the AUC of the blood, the AUC_{tumor} of the galactosylated Ab is 24 times higher. Bone marrow toxicity is related to the AUC_{blood}. The AUC_{tumor} can be normalized for AUC_{blood} and thus the comparison for both AUC for tumor at an equitoxic bone marrow dose can be determined. This would indicate that the radiation dose of ^{131}I-Gal-MG1 to the tumor is approximately 24-times higher than that of ^{131}I-MG1 at an equitoxic dose. Considering the high initial tumor uptake, the high tumor-to-non-tumor ratios after intraperitoneal injection of Gal-Ab

and the favourable normalized AUC_{tumor}, RIT using these modified Abs seems feasible. In this model, tumor uptake peaks within 24 hr after intraperitoneal administration. As a result, the favourable effect of the galactosylated Ab could be more pronounced when other radionuclides with relatively short half-lives and non-radiometals were used. In addition, since clearance of galactosylated antibodies is via the hepatic AGPR, the use of residualizing radiometals like ^{177}Lu and ^{90}Ycould result in increased radiation dose to the liver. Almqvist et al. showed excellent tumor targeting of subcutaneous colon tumors in a mouse model with a low liver uptake of the ^{211}At-labeled antibody A33. [20] ^{211}At is a non-residualizing radionuclide with a relatively short half-life (7.2 hr) and has exquisite characters for RIT of ip. tumors with galactosylated antibodies. Therefore, the intraperitoneal application of RIT for the treatment of peritoneal carcinomatosis using galactosylated antibodies need further exploration.

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Abstract

Pre-targeting, unlike directly radiolabeled antibodies, separate the radionuclide from the antibody, thus allowing time for tumour accumulation before the radionuclide-carrier is given, thereby enhancing imaging contrast due to lower background ratios.

An open-label, non-randomized, single-arm trial to assess a pretargeting procedure that utilizes a bispecific antibody (bsAb) and an ^{111}In-labeled peptide for diagnostic imaging of carcino-embryonic antigen (CEA)-producing cancers focusing on colorectal cancer was performed. The study focused on the assessment of safety, pharmacokinetics (Pk), and targeting ability of the ^{111}In-peptide alone (3 patients) and in a pretargeting procedure with the bsAb (11 patients). Patients received 5 mCi ^{111}In-peptide in combination with the pretargeting procedure to assess the appropriate interval to be used with 5.0 mg of bsAb for the selection of the optimal time (3-, 4-, and 5-days) to allow the bsAb to clear from the blood and tissues before the administration of the radiolabeled peptide. The image quality at these 3 intervals were studied.

Peptide pharmacokinetics showed increased circulating levels of ^{111}In-labeled peptide in patients in the 3-day interval cohort as compared to the other cohorts. Tumor to background ratios were 4.5:1-6.5-1 in the 3-day interval group, 6.0:1-16.6:1 in the 4-day interval group and 3.9:1-8.3:1 in the 5-day interval group. The best images were acquired with a 4-day interval, at 24 h after injection of the radiolabeled peptide.

Chapter 8

Radioimmunoscintigraphy of colorectal cancer using a two-step pre-targeting method

This chapter is based on:
Radioimmunoscintigraphy of colorectal cancer using a two-step pre-targeting method. F. Aarts, R.P Bleichrodt, E. Visser, T. Hendriks, W.J.G Oyen, D.M Goldenberg, R. Shakey, O.C Boerman. In preparation

Introduction

The concept of targeting radionuclides to tumors using radiolabeled antibodies against tumor-associated antigens was proposed more than a century ago. Targeting antibodies to tumors is a slow and inefficient process. Upon intravenous injection, antibodies accumulate in tumors relatively slowly, and several days after injection maximally a few percent of the injected dose accumulates in the tumor.

Clinically, radiolabeled anti-CEA antibodies have been used for the detection and therapy of colorectal, pancreatic, breast, lung, medullary thyroid and ovarian cancers.[1-5] The anti-CEA mAb MN-14 is a high affinity anti-CEA antibody (Ka = 109 M-1).4 To reduce the immunogenicity, a humanized version of MN-14 antibody was produced (hMN-14). Radiolabeled hMN-14 has been studied primarily for radioimmunotherapy (RIT) in patients with colorectal cancer.[5-7]

RIS with radiolabeled (intact) antibodies resulted in moderate contrast several days after injection.3 Thus, there is a need for a more effective targeting strategy allowing to guide higher activity doses to the tumor. The driving force of accumulation of radiolabeled antibodies in tumor tissue is their sustained high level in the circulation. On the other hand, the long residence time of these radiolabeled mAbs in the blood enhances background activity, resulting in low contrast images and leads to long bone marrow exposure, the dose-limiting organ in RIT. Therefore, antibody fragments (Fab, F(ab)2, scFv) have been developed that clear faster from the blood. This, however, at the cost of reduced accumulation in the tumor. In fact, this is the central dilemma in antibody targeting: on one hand one aims to optimize the accumulation of the antibody in the tumor; on the other hand, the uptake in normal tissues, thus, the residence time in the circulation should be minimized.

In pretargeting, the tumor is pretargeted with an unlabeled antibody construct. When this antibody construct has accumulated in the tumor and has cleared from the blood, the radioactivity is injected. The radionuclide is administered linked to a relatively small molecule in general, a peptide with affinity for the antibody construct, which is cleared rapidly from the blood.

This small molecule should distribute rapidly throughout the body and should bind to the pre-localized antibody construct in the tumor, whereas the unbound radiolabeled molecule should clear rapidly from the body.
Two antibody-based pretargeting approaches can be distinguished: 1. procedures exploiting the avid interaction between (strept)avidin and biotin, and 2. procedures using bispecific antibodies (bsAb) with affinity for the tumor and for the radiolabeled small molecule.
The group of Chatal and Barbet and colleagues have developed a pre-targeting system based on an anti-CEA × anti-DTPA bispecific F(ab')2 antibody construct. In combination with high activity doses of a ^{131}I-labeled di-DTPA peptide, this system was tested for pre-targeted radioimmunotherapy of CEA-expressing tumors. In patients with medullary tyroid carcinoma (MTC), this approach was shown to be very efficacious.[8]
These studies aimed to develop pretargeted RIT in patients with CEA expressing tumors. Therefore, relatively high peptide doses (labeled with high activity doses) are guided to the tumor. For RIS purposes, lower doses of the radiolabeled peptide need to be administered. Consequently, much lower doses of the bsAb are required.
In the present study, the imaging ability of a bsAb (anti-CEA × anti-DTPA) pre-targeting method using an ^{111}In-labeled peptide for the use of RIS in patients with CEA expressing primary colorectal cancer was investigated. The optimal time interval between injection of a low bsAb dose and the radiolabeled peptide was determined.

Patients and Methods

Study design

This study is an open-label, non-randomized, single-arm trial intended to assess the feasibility of a pretargeting procedure that utilizes an anti-CEA × anti-DTPA-In bsMAb and an ^{111}In-labeled peptide for diagnostic imaging of carcinoembryonic antigen (CEA)-expressing colorectal cancer. The first three patients enrolled in this study underwent an imaging study using the 111In-labeled di-DTPA peptide alone, thus providing information on the organ distribution, tumor uptake and clearance of the radiolabeled peptide

alone. Thereafter, the remaining patients received the bsAb in combination with the ^{111}In-di-DTPA peptide. Three consecutive cohorts were studied after the administration of 5.0 mg of bsAb to assess the optimal interval (3-, 4-, and 5-days) for optimal imaging. The dose of the di-DTPA peptide was kept constant at 40 nanomoles. This peptide dose was labeled with 185 MBq ^{111}In.

Patient eligibility

The entry study protocol was approved by the institutional ethics committee. The inclusion criteria were: age over 18 years, a diagnosis of primary CRC, plasma CEA ≥10 ng/mL or immunohistology positive CRC. Patients should not have been treated with chemotherapy within four weeks prior to the start of the study or at least 2 weeks in cases of external radiation. In addition, patients should not have been injected for prior diagnostic or therapeutical procedures with murine or other antibodies. Women had to practice contraception during the procedure and for 3 months after imaging. A minimal life expectancy of more than 3 months; a Karnofsky performance score of more than 70; normal serum chemistries, normal peripheral CBC counts were required prior to inclusion in the protocol. Written informed consent was obtained from all patients.

Study agents and Administration

The anti-CEA × anti-DTPA-In bsAb hMN-14 × m734 F(ab')2 (vial of 6 mg/ 5ml) was provided by Immunomedics Inc. (Morris Plains NJ). This antibody was obtained by coupling an equimolar amount of the Fab' fragment of the humanized anti-CEA monoclonal antibody (hMN-14) to the Fab' fragment of the murine anti-DTPA-indium monoclonal antibody (m734) activated by o-phenylenebismaleimide. The infusion of the bsAb was divided into two parts; first, 10% of the injected volume (6 mg bsAb dissolved in 30 ml NaCl 0.9%) was infused over 10 min, followed by the remaining volume in 10 min. Vital signs were monitored before, during and at the end of the infusion.

IMP-205 is a tyrosine-lysine dipeptide in which both primary amino groups are substituted with DTPA. Vials containing 0.5 ml, 0.25 M ammoniumacetate buffer, pH 5.5 (5 μg/ vial) were stored at -20 °C until use. The peptide was

labeled with 185 MBq ^{111}In (Covidien, Petten, The Netherlands). Subsequently, the DTPA moieties were saturated with stable indium by adding a three-fold molar excess (15 nmol) of $InCl_3$. Thereafter, the volume of the preparation was adjusted to 30 ml with 0.9% NaCl and the ^{111}In-labeled IMP-205 peptide was infused intravenously. Radiochemical purity of the peptide was checked by RP-HPLC and always exceeded 95%. The ^{111}In-labeled peptide was injected 3-, 4-, and 5-days after the bsAb infusion at a rate of 3 ml/min.

Pharmacokinetic data

Serum was collected from all patients to assess the pharmacokinetics of the ^{111}In-peptide. The peptide clearance was determined by radioactivity measurements of collected blood samples. For measurement of peptide clearance, samples were taken at 5, 15 and 30 minutes after the peptide infusion, as well as after 1, 2, 4 and 24 hours. Blood samples were taken during the infusion, at the end of the infusion 1 hr and 2 hr after the infusion of the antibody in order to determine bsAb pharmacokinetics.

Imaging protocol, Follow-Up and Toxicity

Scintigraphic images were acquired at 30 min after injection of the ^{111}In-peptide and 4 and 24 hr later. Whole-body scans were acquired using a Siemens dual-head gamma camera (Ecam, Hoffmann Estates, IL) with medium-energy collimators. Both anterior and posterior whole-body scans were performed 30 min., three hours and 24 hrs after the ^{111}In-labeled peptide administration. Symmetric 15% windows were used over both the 172 KeV and 246 KeV energy peaks. The data were stored digitally in a 256 × 1024 matrix. Tumor-to-non-tumor ratios were determined using planar whole body images using the para-iliacal region as standard background.

According to the study protocol, routine blood chemistry and blood cell counts with differential counts were obtained before, on the day of bsAb injection, 24 hr, 1 and 2 weeks and 1 month after antibody administration. All adverse effects were documented according to the National Cancer Institute Common Toxicity Criteria.

Dosimetry

To estimate the radiation-absorbed dose to the relevant organs, the scintigraphic images acquired at 0, 4 and 24 h after injection were analyzed quantitatively using the conjugated views counting technique with partial background subtraction and correction for attenuation and physical decay as described previously.[9] The time activity curves for each organ were calculated using the SPRIND software package in combination with the OLINDA software (Vanderbilt University, Nashville Tennessee USA).[10] This software calculates residence times based on whole-body planar scintigraphic images using first-order exponential decay.

Results

Patient characteristics

In the 14 patients enrolled in the study, the primary disease site was the colon in nine patients, and the rectum in five patients (Table 1). The classification of rectal or colon cancer was based on the TNM/UICC classification. At time of surgery, one patient with rectal cancer had lymph node involvement. During the thirty three months follow-up, three patients (nr. 3, 5 and 9) died, of whom two due to advanced colorectal cancer.

Antibody, Radiolabeling, and Administration

The infusion of both the bsAb and the radiolabeled peptide were well tolerated. During and immediately after the administration of both the bsAb and the radiolabeled peptide no adverse events occurred. However, in two patients, adverse events were seen at seven and nine days after the administration of the bsAb. This consisted of erythema confined to the hand and of erythema confined to the inguinal area. In both patients the erythema declined without the need for medical intervention and was graded as intensity grade 1. Serum chemistry and peripheral CBC counts remained normal during follow up.

Pharmacokinetics

Peptide pharmacokinetics showed highest blood levels (mean % ID 0.007 ± 0.002 % ID) 20 minutes after infusion of the peptide in the peptide alone

group. In contrast, in the patients that received the bsAb, peak % ID was observed 30 min after infusion. The highest blood levels were observed in the 3-day interval group (mean % ID 0.024 ± 0.007 at 30 min after injection), as compared to the 4-day interval and 5-day interval group. Figure 1.

Table 1.
Demographic data

	Sex	Age (Y)	Disease site	Stage (AJCC)	CEA (μgl/L)	Operative procedure
Cohort 1 *Peptide alone*						
1	M	59	Rectum	IV	31.7	APR
2	M	75	Transverse colon	III	<1	HC
3	F	57	Cecum	II	7.3	HC
Cohort 2 *3-day interval*						
4	F	63	Cecum	II	2.4	HC
5	M	86	Descending colon	II	5.4	HC
6	F	59	Transverse colon	III	1.1	SC
13	F	65	Rectum	I	2.3	TEM
Cohort 3 *4-day interval*						
7	M	46	Transverse colon	II	1.2	SC
8	F	58	Cecum	I	<1	SC
9	F	60	Sigmoid colon	III	3.4	TE
14	F	75	Rectum	III	4.7	TME
Cohort 4 *5-day interval*						
10	M	61	Rectum	II	16.2	APR
11	M	67	Rectum	III	3.1	APR
12	F	73	Ascending colon	IV	1.3	HC

Table 1. APR: abdominoperineal resection; HC:hemicolectomy; SC: subtotal colectomy; TE: total excenteration, TME: total mesocolon excision.

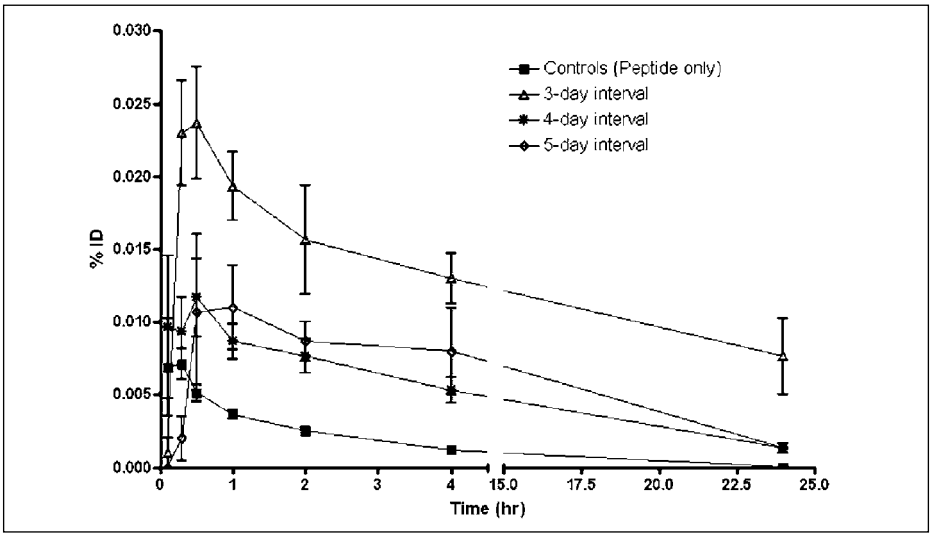

Figure 1. Peptide pharmacokinetics during 24 hr after intravenous administration in the 4 patient groups, expressed as mean percentage injected dose (% ID). Vertical bars indicate standard error of the mean (SEM).

Imaging

Wholebody scintigraphy of the patients in cohort 1 revealed tumor visualization in one patient 24 hrs after injection with only low uptake. The other two patients in this cohort showed no tumor uptake. In the pretargeting cohorts, adequate tumor visualization was observed in 9 out of 11 patients (sensitivity 82%). In cohort 2, mean tumor-to-background ratio (determined from the images acquired 24 h after peptide infusion) was 5.7 ± 1.5, whereas this was 14.1 ± 5.4 and 6.1 ± 3.1 in cohort 3 and 4 respectively. Thus, best image quality was acquired in the 4-day interval group. In one patient (nr.11) inguinal- and para-aortic lymph node involvement was clearly visualized. These lymph nodes were confirmed by FDG- PET imaging. Figure 2.

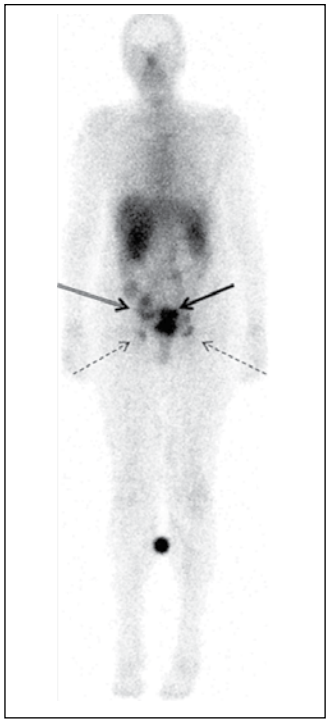

Figure 2. Wholebody scintigraphic image of patient nr.11. Image acquired 24 hr. after administration of peptide. Shown are the primary rectum tumor (closed bold arrow) inguinal lymphnodes (dotted arrows) and para-aortic lymph nodes (double-lined arrow).

Dosimetry

Whole body residence time of the radiolabeled peptide was 5.5 h ± 2.3 h in the peptide alone group, 18.6 h ± 9.4 h in the 3-day interval group, 7.6 h ± 0.6 h in the 4-day interval group and 9.3 h ± 4.6 h in the 5-day interval group. Mean effective dose was 0.029 ± 0.017 mSv/MBq for the peptide alone group, 0.047 ±0.022 mSv/MBq for the 3-day interval group, 0.039 ± 0.001 mSv/MBq for the 4-day interval group and 0.035 ± 0.01 mSv/MBq for the 5-day interval group. The bladder wall was the tissue that received the highest radiation dose with a mean bladder wall dose of 0.29 ± 0.09 mSv/MBq in cohort 1 (peptide alone) and 0.27 ± 0.06 mSv/MBq, 0.32 ± 0.06 mSv/MBq and 0.27 ± 0.05 mSv/MBq for the other respective cohorts. Mean renal dose, was 0.12 ± 0.03 mSv/MBq, whereas mean renal dose was 0.21 ± 0.03 mSv/MBq, 0.25 ± 0.09 mSv/MBq and 0.13 ± 0.04 mSv/MBq for the other cohorts, respectively. Radiation doses for other relevant organs were substantially lower. Table 2.

Table 2.	Mean ± SD			
	Cohort 1 (Peptide alone)	Cohort 2 (3-day)	Cohort 3 (4-day)	Cohort 4 (5-day)
Kidney	0.12 ± 0.03	0.21 ± 0.02	0.25 ± 0.09	0.13 ± 0.04
Liver	0.01 ± 0.003	0.1 ± 0.1	0.08 ± 0.04	0.05 ± 0.02
Spleen	0.02 ± 0.002	0.04 ± 0.01	0.03 ± 0.004	0.02 ± 0.01
Brain	0.006 ± 0.003	0.02 ± 0.008	0.007 ± 0.003	0.01 ± 0.008
Heart	0.009 ±0.004	0.04 ± 0.02	0.02 ± 0.002	0.02 ± 0.01
Lungs	0.008 ± 0.003	0.04 ± 0.02	0.01 ± 0.002	0.02 ± 0.01
Red Marrow	0.01 ± 0.003	0.03 ± 0.01	0.02 ± 0.002	0.02 ± 0.008
Bladder wall	0.29 ± 0.09	0.27 ± 0.06	0.32 ± 0.06	0.27 ± 0.05

Table 2. Organ dose (mSv/MBq)

Discussion

In the present study we showed that pretargeted radioimmunoscintigraphy of CRC is feasible. In twelve patients with primary CRC, this method resulted in excellent tumor imaging with only minor, grade 1, adverse events. Best images were acquired at 24 h after injection of the ^{111}In-labeled peptide using an interval of 4 days between the administration of the bsAb and the radiolabeled peptide.

The application of the murine version of the anti-CEA × anti-DTPA bsAb for pretargeted RIS of primary colorectal cancer was first described by le Doussal and colleagues in 11 patients.[3] In this study, the mean interval between the administration of the bsAb (0.9 - 9 mg/patient) and the radiolabeled peptide (^{111}In-di-DTPA-TL, 5-8 mCi) was 3.3 days (range 2-8 days). Imaging allowed

detection of 11 out of 13 lesions and best images were acquired at the longer time intervals between the administration of the agents. In addition, imaging quality of the planar scintigraphic images was low, requiring SPECT imaging in almost all patients for adequate delineation of the tumor. Seven out of the 11 patients developed human anti mouse antibodies (HAMA). Chetanneau and colleagues also described imaging of known locations of recurrent colorectal cancer using a bispecific antibody and an ^{111}In-labelled bivalent hapten conjugate.[6] First, the unlabelled bispecific antibody (0.1 mg/kg) was administered followed 4 to 5 days later by the administration of the divalent DTPA peptide labelled with 5 to 8 mCi ^{111}In. Images were obtained after 4.5 and 24 h after injection of the radiolabeled peptide. Mean tumor-to-background ratios were 6.4. Imaging sensitivity was 91%, which is comparable to the imaging sensitivity found in the present study.

Barbet and colleagues administered 5 mg/patient of a murine anti-CEA × anti DTPA bsAb in 44 patients with relapsed medullary thyroid cancer, followed four to five days later by the ^{111}In-labeled hapten (100-200 MBq).[11] In this study, images were recorded 2, 4 and 24 hr after injection of the hapten, resulting in a true-positive rate of 74%. Images recorded two h after administration of the radiolabeled peptide showed blood-pool distribution. Images recorded after five and 24 h showed tumor uptake. Due to the use of a murine Ab, HAMA developed in 61% of the patients. In the present study, ^{111}In was used to radiolabel the peptide for imaging purposes. ^{111}In is more suited for imaging than ^{131}I and will result in a lower radiation dose than ^{131}I due to the shorter physical half life (2.8 days vs. 8 days), the lower energy of the γ-emission (245 keV and 171 keV) and the fact that ^{131}I also emits ß-radiation (192 KeV). In addition, labelling procedures are less time consuming and technically less challenging. Also, peptides that can be used in combination with ^{99}Tc have been developed, making the pretargeting approach more flexible.[12]

The present study showed that pretargeting RIS in patients with primary CRC is feasible using a low dose bsAb (5 mg, 50 nmol) in combination with a low peptide dose (5 nmol) with an optimal time interval of four days between the administration of the two agents, 24 h after injection of the radiolabeled peptide.

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Chapter 9

Summary & Future Directions

Summary

The investigations presented in this thesis were conceived based on the hypothesis that the combined treatment of cytoreductive surgery and radioimmunotherapy could be improved and its therapeutic efficacy would compare favorable to the gold standard for the treatment of peritoneal carcinomatosis.

The clinical treatment of first choice for peritoneal carcinomatosis remains to be HIPEC. Numerous retrospective studies and a randomized trial confirmed that the adjuvant use of heated intraperitoneal perfusion with chemotherapy after cytoreductive surgery resulted in improved survival. Efforts to improve outcome and reduce morbidity resulted in the application of relatively new chemotherapeutical agents as oxaliplatin during the procedure.

In an effort to improve outcome and reduce morbidity, new treatment strategies haven been developed, one of which is antibody guided radiotherapy or radioimmunotherapy.

The results of clinical trials investigating the potential of radioimmunotherapy in the setting of regional administration or intracavitary use have been described in ***Chapter 2***. Intracavitary RIT in patients with ovarian cancer and glioma showed improved targeting after local administration as compared to the intravenous administration. In addition, various studies showed not only the feasibility of locally applied RIT in these patients but also showed adjuvant RIT to be at least as effective as the standard therapy. Clinical results about RIT for the treatment of peritoneal carcinomatosis of colorectal origin is scarce while results from pre-clinical data are promising. Studies on the application of radiolabeled antibodies in early urothelial cell cancer showed that intracavitary RIT may hold a promise. In patients with malignant pleural mesothelioma or malignant pleural effusion, RIT may play a role in the palliative treatment.

In ***Chapter 3***, it was investigated whether survival after intraperitoneally administered RIT can be enhanced by altering the time interval between the surgical procedure and the intraperitoneal administration of RIT, and if so, what the optimal time interval between these two treatment modalities would be. In this experiment, PC was induced by intraperitoneal inoculation of CC-

531 colon carcinoma cells in Wag/Rij rats. Fourteen days later, animals were subjected to exploratory laparotomy (Sham), cytoreductive surgery (CS) only or CS + RIT at different time points after surgery. RIT consisted of 55 MBq ^{177}lutetium labeled anti-CC531 antibody MG1 (183 µg). The primary endpoint was survival. The results showed that CS 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 days and 14 days after surgery, respectively. From this study we concluded that the efficacy of adjuvant RIT after CS for the treatment of PC of colonic origin decreases when the administration of the radiolabeled MAbs is postponed.

The background for the found differences in survival in chapter 3 was thought to be due to tumor cell entrapment within fibrin clots or adhesions that are formed during and immediately after the surgical procedures. The tumor cells within these clots would thus be unsusceptible for the adjuvant therapy, RIT, and consequently would not be killed. Due to the success of immediately administered RIT, we hypothesized that the administration of fibrinolytic therapy, consisting of two daily doses of recombinant tissue plasminogen activator (rtPA) for three consecutive days, would enhance the therapeutic efficacy of RIT after extensive surgery.

It is known that the application of hyperthermia can improve survival in the treatment of cancer when applied locally for the treatment of cervical cancer in women. In animal studies this has also been investigated in combination with RIT. We therefore investigated In ***Chapter 4***, using the same model of induced PC in rats, the former two treatments in combination with adjuvant RIT after surgery in an effort to improve treatment outcome. Animals underwent CS, CS+whole body hyperthermia (WBH) at a temperature of 40^0C for the duration of 3 hours), CS+RIT (74 MBq ^{177}Lu-labelled MG1) or CS+WBH+RIT. Median survival after CS and CS+WBH was 34 and 37 days. The adjuvant administration of RIT significantly improved survival. Median survival after CS+RIT or CS+RIT+WBH was 63 and 86 days ($P<0.0003$, $P<0.0006$ compared to CS+WBH). However, the concomitant application of WBH did not have a significant additive effect on survival, both after CS alone or after CS+RIT.

In the second experiment, rats underwent CS, CS+RIT, CS plus recombinant tissue plasminogen activator (rtPA, twice daily, 3 days) or CS+RIT+rtPA. Median survival after CS and CS+rtPA was 50 and 42 days (P=0.1). Median survival was 106 days after CS+RIT and 103 days after CS+RIT+rtPA (P<0.0001 compared to CS+rtPA). No difference was found between CS+RIT and CS+RIT+rtPA (P= 0.83).

We thus concluded that the application of WBH or rtPA in combination with adjuvant RIT after CS for the treatment of PC of colonic was feasible, but did not significantly potentiate the efficacy of RIT.

The survival of the standard of care treatment in our studies, CS immediately followed by intraperitoneal RIT, was than compared to the standard of care in clinical practice, HIPEC, in ***Chapter 5***.

Treatment, after using the same model of induced PC described in the former studies, comprised CS only, CS+RIT or CS+HIPEC, immediately after surgery. RIT consisted of intraperitoneal administration of 74 MBq Lutetium-177 labelled MG1. HIPEC was performed by a closed abdomen perfusion technique using mitomycin C (16 mg/l during 60 minutes). Results showed that rats receiving CS+HIPEC were lethargic, suffered from diarrhea and lost significantly more weight in the first postoperative week. Median survival of rats treated with CS+RIT was significantly longer than after CS alone (97 and 57 days, respectively, P<0.004), whereas survival after CS+HIPEC or CS alone were not significantly different (76 and 57 days, respectively, P=0.17). Thus, survival after CS was significantly improved by RIT whereas adjuvant HIPEC did not improve survival and was more toxic than adjuvant RIT.

The treatment related toxicity and –morbidity found in Chapter 5 are also present in clinical use of HIPEC. The main complications and morbidity seen in patient studies are derived from intestinal and anastomotic dehiscence related factors.

In ***Chapter 6*** we therefore aimed to investigate the effects of adjuvant RIT or HIPEC after cytoreductive surgery on the healing of small and large bowel anastomoses and the abdominal wall.

After the induction of PC, animals were subjected to CS and anastomotic construction only or followed by RIT or HIPEC. RIT consisted of 74 MBq ^{177}lutetium-labelled anti-CC531 antibody MG1. HIPEC was performed as

described above. Anastomotic and abdominal wall strength measurements were performed 3 and 5 days after surgery. At day 5, bursting pressure in ileum and colon anastomoses was lowest in the HIPEC group, CS vs. HIPEC $P<0.05$ and $P<0.001$, respectively. In the CS group, the bursting site was more common outside the true anastomotic area (6/12 animals) than in the HIPEC (1/12) and RIT (4/12) groups. Abdominal wall strength was highest in the CS group at both days after surgery (CS vs. HIPEC $P<0.05$ at day 3 and CS vs. HIPEC $P<0.001$ at day 5). In addition, there was a significantly lower abdominal wall strength after HIPEC than after RIT ($P<0.05$ at day 5).

We concluded that RIT, when administered as adjuvant treatment to CS, is superior to HIPEC regarding anastomotic and abdominal wall wound strength in a model of PC of CRC.

Unlike HIPEC, most of the toxicity of clinically applied RIT is derived from its effect on the hematological system due to the long circulatory half-life of the antibody. Although intraperitoneal RIT results in high uptake of intraperitoneally growing tumors, the radiolabeled antibody enters the circulation, resulting in bone marrow toxicity. Carbohydrate modification of antibodies could induce accelerated clearance of the antibody via the hepatic asialoglycoprotein receptor, thereby reducing exposure to normal tissues. In ***Chapter 7*** we therefore investigated whether carbohydrate modifications of the antibody could achieve the effects described above. The biodistribution of the galactosylated and non-galactosylated anti-CC531 antibody MG1 after intraperitoneal injection was determined in a model of peritoneal carcinomatosis of CC-531 colon tumors in Wag/Rij rats. Uptake of the radiolabeled antibodies in the tumor and relevant organs was determined at 2, 4, 24 and 48 hours after injection. Galactosylation of the antibody did not affect the binding affinity of MG1. Remarkably, the uptake of Gal-MG1 in tumors was higher than that of MG1 at 2 and 4hr after injection. After 24h and 48h, uptake of Gal-MG1 in tumor tissue was lower than that of MG1. Gal-MG1 cleared from the blood within hours after administration. At 2hr-24 hr after administration, tumor-to-blood ratios obtained with Gal-MG1 were significantly higher than those obtained with unmodified MG1. Antibody galactosylation resulted in improved tumor-non-tumor ratios after intraperitoneal injection in a model of PC.

This could improve the efficiency of RIT, especially in combination with short-lived non-residualizing radionuclides as alpha-emitters.
The preliminary data of a patient study where a two-step pretargeting radioimmunoscintigraphy technique was used, are presented in the final chapter, **Chapter 8**. In this study the optimal time interval between the administration of the antibody and the administration of the radiolabeled peptide was investigated in patients with colorectal cancer. These data show excellent tumor targeting where the best time interval was shown to be three or four days after Ab injection.

Future Directions

In the majority of patients with peritoneal carcinomatosis (PC) that are eligible for cytoreductive surgery (CS), a R0 resection can be performed. With the use of pre-operative diagnostic imaging it is difficult to adequately predict the extent of PC.[1] Final staging is therefore performed per-operatively. During these extensive surgical procedures however, small tumor nodules may be missed and left in situ. New molecular imaging techniques that can sensitively detect small lesions may preclude this problem. Alencar and colleagues investigated the use of near-infrared (NIR) fluorescent probes in mice with orthotopically implanted colon cancer.[2] They were able to detect lesions of 0.3 mm-1.4 mm). (Pre-) Clinical application of NIR during CS might further improve the results of surgery.

The recent incorporation of bevacizumab (anti-VEGF antibody) to the standard systemic chemotherapy for the treatment of metastatic colon carcinoma, resulted in an increase in overall survival of only three months.[3] The adoption of relatively new chemotherapy agents in HIPEC procedures resulted in the application of platinum-based compounds. This, however resulted in increased morbidity as compared to the use of Mitomycin-C whereas the effects on survival are not yet reported.[4] Thus, the scope for the development of new treatment strategies for the treatment of PC of colorectal origin might not be based on treatment with either intravenously or intraperitoneally administered chemotherapy.

Excellent response rates have been obtained with radioimmunotherapy (RIT) in patients with B-cell lymphomas (response rates of 60-70%) with radiolabeled anti-CD20 antibodies (^{90}Y, Zevalin® and ^{131}I, Bexxar®).[5,6]

The results of RIT for the treatment of solid tumor are less encouraging. Studies on the therapeutic efficacy and toxicity of intracavitary application of RIT in patients with ovarian cancer (^{90}Y-HMFG-1, ^{177}Lu-B72.3) and malignant glioma (^{131}I-81C6), indicated that the adjuvant application of RIT within a confined area limits toxicity and may improve tumor targeting.[7] In smaller lesions (e.g. in patients with minimal residual disease or in microscopic disease), improved/enhanced tumor uptake of the radiolabeled antibody was observed.[8] The studies described in this thesis investigated the

tumor targeting and efficacy after administration of ^{177}Lu-labeled antibodies in animals with microscopic disease after cytoreductive surgery. In this described setting of minimal residual disease (lesion size 0.5 mm.- 3mm.), the application of ^{177}Lu-labeled Abs resulted in an increased suvivival as compared to other radionuclides as ^{90}Y- or ^{188}Re-labeled Abs.[9] In patients who undergo R0 resections, ^{177}Lu may be an inappropriate radionuclide due to its physical properties (range of ß–particles in tissue: 3 mm). In these cases, alpha emitting radionuclides may be more suitable. The results of a phase I trial on the regional administration of ^{211}At-labeled 81C6 (an anti-tenascin antibody) after a total or near total resection in 18 patients with recurrent glioma showed the clinical feasibility of radioimmunotherapy with alpha-emitters.[10] In this study median survival (57 weeks for glioblastoma multiforme) was similar to the median survival obtained with administration of the ^{131}I-labeled Ab, while no dose-limiting toxicity occurred. Thus patients were treated at a suboptimal dose level, whereas the latter Ab construct was administered at the maximum tolerable dose. The use of ^{211}At-labeled antibodies seems particularly suitable for intraperitoneal administration in view of direct tumor targeting and the physical half-life of 7.2 hrs in combination with the peritoneal clearence time of antibodies that is within 24 hrs (≈ 10% is still present after 24 hrs, Thus, systemic toxicity would be limited when the short-lived radiolabeled antibodies enter systemic circulation.

One of the limitations of RIT in solid tumors is slow tumor uptake and low tumor-to-non-tumor ratios. It has been shown that solid tumors can be targeted more effectively using a pretargeting system. Pre-targeting techniques that utilize bispecific antibodies have been shown to achieve increased tumor-to-non-tumor ratios which could results in enhanced therapeutic efficacy when used in a therapeutic setting. Until now, the production of sufficient amounts of bispecific antibodies has been a major limitation to test this approach clinically. The recently developed DNL method allows the production of fully humanized trivalent bispecific monoclonal antibodies in high yields. Studies performed with these new pre-targeting constructs resulted in excellent tumor targeting and high tumor-to-non-tumor ratios with additional rapid blood clearance in mice bearing human colonic tumor

xenografts.[11] In general, higher radiation doses will be delivered to the tumor by means of pre-targeting as compared to the use of directly labeled antibodies.[12]

These recent advances in new imaging modalities and molecular science may preclude the clinical application of local or regionally applied RIT to treat microscopic tumor residue after CS for PC of colorectal origin.

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Appendices

Samenvatting

Dikkedarmkanker (coloncarcinoom) is na longkanker bij mannen en borstkanker bij vrouwen de meest voorkomende oorzaak van aan kanker gerelateerde sterfte in Europa en de Verenigde Staten. In ongeveer 1/5 van de patiënten bij wie deze diagnose gesteld wordt is de ziekte reeds uitgezaaid. Een specifiek patroon van deze uitzaaiingen, buiten de lever en de longen, is regionale uitbreiding in de buikholte, ofwel carcinosis peritonei (CP). Dit kan optreden in 1/3 van de patiënten na verwijdering van de darmkanker terwijl bij 25% van hen die na verwijdering van de tumor hernieuwde tumorgroei hebben, dit de enige plaats is waar de uitzaaiing voorkomen. Bij CP worden de tumoren meestal gevonden langs de rechter dikkedarm, het vetschort, rondom de lever en onder het middenrif. Indien onbehandeld, zullen deze tumoren aanleiding geven tot klachten van darmafsluiting en uiteindelijk tot overlijden. De overleving van deze categorie patiënten was slecht, gemiddeld bedroeg deze zes maanden, waarbij de behandeling bestond uit het opheffen van de afsluiting of slechts chemotherapie.
In de hoop deze mensen een beter uitzicht op overleving te bieden is in de loop der jaren uitgebreid onderzoek gedaan t.b.v. de verbetering van de behandeling. Dit heeft uiteindelijk geresulteerd in uitgebreide chirurgische verwijdering van alle zichtbare tumor, gevolgd door het spoelen van de buikholte met verwarmde chemotherapie (HIPEC). Dit zijn echter zeer langdurige en uitgebreide procedures waarbij het complicatierisico hoog is, nl 65%. Dit leidt echter wel tot een verbetering van overleving naar gemiddeld drie jaar. Vanwege de ernstige complicaties is het nodig dat er nieuwe strategieën ontwikkeld worden om deze naar de buikholte uitgezaaide dikkedarmkanker te behandelen. Een van deze nieuwe methoden is het gebruik van selectieve bestraling van tumorcellen door middel van het koppelen van radioactieve stoffen aan tumorspecifieke antilichamen, ofwel radio-immuuntherapie (RIT).
De resultaten van klinisch onderzoek, waarbij gebruik wordt gemaakt van RIT worden besproken in ***Hoofdstuk 2***. In dit hoofdstuk ligt de nadruk op de regionale toepassing ervan in een afgesloten holte (intracavitair). Uit de resultaten van dit onderzoek is geconcludeerd dat de toepassing van RIT

effectief is indien er een minimale hoeveelheid tumor aanwezig is (minimaal residuale of microscopische ziekte). Tevens blijkt dat er een groter anti-tumor effect verwacht kan worden indien men deze therapie ter plaatse van de tumor (lokaal of regionaal) aanbiedt in vergelijking met toediening via de bloedbaan.

In ***Hoofdstuk 3*** is in een proefdiermodel van geinduceerde CP het optimale tijdsinterval tussen de chirurgische ingreep en het toedienen van het radioactieve antilichaam onderzocht. De achterliggende hypothese is dat tumorcellen die tijdens de operatie achterblijven wellicht in bloedstolsels achterblijven. Hierdoor zouden deze tumorcellen onbereikbaar zijn voor het antilichaam en uit kunnen groeien tot nieuwe tumoren. Uit de resultaten van dit experiment bleek dat RIT, wanneer toegediend direct aansluitend aan de operatie, het beste overlevingsresultaat geeft wanneer dit wordt vergeleken met toediening na 4 of 14 dagen na operatie.

Vervolgens werd in ***Hoofdstuk 4*** , in hetzelfde proefdiermodel onderzocht of het toedienen van fibrinolytische therapie met behulp van rtPA, een stof die bloedstolsels oplost, zou kunnen zorgen voor een verbeterde beschikbaarheid van RIT ter plaatse van tumorcellen. Het uiteindelijke doel in dit experiment was verbetering van overleving. In dit hoofdstuk werd eveneens onderzocht of een gecombineerde behandeling met RIT en verhoging van de lichaamswarmte (hyperthermie) zou leiden tot een verbeterde overleving. Deze combinatie van bestraling met hyperthermie wordt reeds toegepast bij vrouwen met baarmoederhalskanker. Uit de resultaten van deze experimenten bleek dat zowel de gecombineerde behandeling van RIT met rtPA als de combinatie van RIT met hyperthermie niet zorgde voor een significant betere overleving in vergelijking met de behandeling met RIT alleen. Hieruit werd geconcludeerd dat beide combinaties niet zinvol werden geacht als aanvullende therapie en dat enkel het gebruik van RIT de optimale aanvullende therapie is na CS in het gebruikte model.

Nadat uit de voorgaande experimenten herhaaldelijk was gebleken dat de toediening van RIT na chirurgie zorgde voor een langere overleving dan de toepassing van chirurgie alleen, werd deze behandeling vergeleken met de huidige standaardbehandeling die toegepast wordt bij mensen, HIPEC. De resultaten hiervan zijn beschreven in ***Hoofdstuk 5***. Wederom bleek dat

het toedienen van RIT na chirurgie zorgde voor een langere overleving dan het gebruik van chirurgie alleen. HIPEC echter bleek in dit model niet in staat te zorgen voor een langere overleving. Bovendien traden er na HIPEC meer bijwerkingen op en waren de proefdieren gedurende de eerste week na toediening zieker dan wanneer RIT werd toegediend.

Deze bevindingen leidden tot het experiment uitgevoerd zoals beschreven in ***Hoofdstuk 6***. In dit hoofdstuk werd onderzocht of de toepassing van RIT na chirurgie leidde tot een verminderde darm- en buikwandgenezing. In de huidige klinische toepassing van HIPEC geeft deze verminderde genezing aanleiding tot het ontstaan van abcessen en verbindingen tussen de darm en de buikwand. Uit de resultaten van dit experiment bleek dat zowel de genezing van darmanastomosen als ook die van de buikwand significant slechter was na toedienen van verwarmde chemotherapie dan wanneer alleen chirurgie werd uitgevoerd. Het toedienen van RIT na chirurgie zorgde niet voor een significante verslechtering van deze genezing.

De dosisbeperkende factor van RIT is de toxiciteit die optreedt door beenmergdepressie. Dit is het gevolg van het langer in het bloed circulerende radioactieve antilichaam. In ***Hoofdstuk 7*** worden de resultaten van een experiment beschreven waarin met behulp van modificaties aan het antilichaam getracht is de hoeveelheid in het bloed circulerende antilichaam te verminderen. Deze aanpassing betreft het koppelen van suikergroepen (galactose) aan het antilichaam. Deze suikergroepen worden vervolgens herkend door receptoren in de lever (asialoglycoprotein receptor of ASGP) en binnen een aantal uren uit het lichaam geklaard. Uit dit experiment bleek dat in de eerste 24 uur na injectie van het gemodificeerde antilichaam in de buikholte er een hogere opname van dit antilichaam in de tumor bestond dan het niet gemodificeerde antilichaam. Tevens was er een significante lagere bloedspiegel van dit antilichaam gedurende de eerste 48 uur na injectie. Uit deze studie werd geconcludeerd dat het theoretisch mogelijk zou moeten zijn een hogere dosis radioactief antilichaam toe te dienen met daarbij een gelijkblijvende toxiciteit.

In ***Hoofdstuk 8*** worden de resultaten beschreven van een pre-targetingstudie in patiënten met een colorectaal carcinoom. In pre-targeting wordt gebruik gemaakt van een anti-tumor antilichaam die los wordt toegediend van de

radioactiviteit. Dit heeft als voordeel dat het antilichaam zich kan ophopen in de tumor, hetgeen enkele dagen kan duren, alvorens radioactiviteit toegediend wordt. In de hier beschreven studie werd eerst het niet-radioactieve antilichaam toegediend, na drie tot vijf dagen gevolgd door het toedienen van ^{111}Indium. In deze studie is onderzocht welk tijdsinterval, van drie tot vijf dagen, het beste resultaat geeft met betrekking tot het afbeelden van de tumor. Uit deze studie bleek dat een interval van vier dagen tussen het toedienen van het antilichaam en radioactief gelabelde peptide de beste afbeelding van de tumoren en uitzaaiingen toonde.

Op basis van de dierexperimentele studies beschreven in dit proefschrift concluderen wij dat het gerechtvaardigd is de toepassing van RIT na chirurgische debulking in de klinische praktijk te gaan vergelijken met HIPEC. Eveneens zou de toepassing van RIT kunnen liggen bij patiënten in de palliatieve setting indien er geen curatie meer mogelijk is.

De toekomst van RIT voor de behandeling van carcinosis peritonei van colorectale tumoren in de klinische praktijk zou dan ook een onderdeel van de huidige multidisciplinaire behandeling kunnen zijn alsmede een minimaal invasieve palliatieve therapie.

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Mael, dank je voor jouw zijn en de glimlach waarmee jij mijn dag maakt.

Frits was born november 15th 1975 in Breda but spent his childhood in the former Republic of West Germany. He graduated from secondary education (HAVO) at the Nassau Scholengemeenschap in Breda in 1994 with a curriculum that was very different from the application requirements for entering Medical School today. Thereafter, he started the study Medical Imaging and Radiation Therapy at the Fontys Hogescholen in Eindhoven from which he graduated with a Bachelor degree of Health in 1998. Finally, in 1998, he commenced his medical education at the Katholic University in Nijmegen from which he obtained his Medical Degree in 2004.

His surgical career began in 2004, at the Slingeland Hospital in Doetinchem. Only a few months later, he transferred to the University Medical Centre Nijmegen. During his employement as a surgical intern, he started his research. First in the field of vascular surgery, later, in 2005, he started the reseach in the field of gastro-intestinal surgery that led to this thesis. Frits presented the results of his studies at both national as well as international congresses.

In 2007 he started his surgical residency at the Radboud University Nijmegen Medical Centre (Prof. Dr. RP Bleichrodt).

Frits lives with Diana in Nijmegen. Together they are the proud parents of their son Mael.

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^{177}Lu	Lutetium 177, radionuclide
^{111}In	Indium-111, radionuclide
Bio	Biotinylated
BSA	bovine serum albumine
bsAb	bispecific antibody
CRC	colorectal cancer
CS	cytoreductive surgery
CP	Carcinosis peritonei
DTPA	diethylenetriaminepentaacetic acid
Gal	Galactose
HAMA	Human Anti Murine Antibody
HACA	Human Anti Chimeric Antibody
HIPEC	heated intraperitoneal chemotherapy
ID	injected dose
ITLC	instant thin layer chromatography
i.p	intraperitoneal
i.v	intravenous
MAb	monoclonal antibody
MBq	megabequerel
mCi	millicurie (1 mCi = 37 Mbq)
MG1	cell surface antibody raised against CC531 cells
PBS	phosphate buffered saline
PC	peritoneal carcinomatosis
RIT	radioimmunotherapy
r-tPA	recombinant tissue plasminogen activator
SA	Streptavidin
TAA	tumour associated antigen
WBH	Whole Body Hyperthermia