Hepatic and Peritoneal Colorectal Metastases

Aspects of Prognosis and Treatment

BY

HAILE MAHTEME
ABSTRACT


Although two-thirds of colorectal cancer patients are cured by surgery, approximately 50% of the patients with this disease develop locally recurrent or distant metastases during the course of their illness. The aim of this study was to identify metastatic sites associated with poor prognosis in rectal cancer and then to investigate methods that can prevent the development and growth of metastases and optimise uptake of drugs at these sites in animal models.

In a defined population, 151 patients with irresectable metastatic or local rectal cancer were identified. Bilateral liver involvement, abnormal liver function tests, peritoneal growth or abdominal lymph node metastases implied a poor prognosis.

In a study on Wistar rats with liver metastases from colorectal cancer, blocking of hyaluronan uptake and elimination by the liver enhanced the hyaluronan uptake in liver metastases. Hyaluronan may thus be used to promote uptake of drugs in specific hyaluronan receptor-positive tumour sites.

Adjuvant intravenous radioimmunotherapy delivered as a specific or unspecific monoclonal antibody prevented human colonic cancer cells inoculated into the portal vein of nude rats from developing into liver metastases. Furthermore, intraperitoneally administered radioimmunotherapy inhibited the growth of peritoneal metastases.

Blocking of 5-FU absorption with a vasoconstrictive agent enhanced the uptake of 5-FU in peritoneal metastases. In addition, the uptake of 5-FU in peritoneal metastases could be improved when these tumours were mechanically disintegrated by surgical tumour reduction and the drug was given intraperitoneally.

Key words: Liver metastases, peritoneal metastases, hyaluronan, radioimmunotherapy, cytoreductive surgery, intraperitoneal administration, 5-FU.

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To Charlotte

Josef

Christopher and

Salomon

And in memory of my mother
This thesis is based on the following papers, which will be referred to by their Romans numerals:


IV Mahteme H, Sundin A, Larsson BS, Khamis H, Arow K, Graf W. 5-FU Uptake in peritoneal metastases after pretreatment with radioimmunotherapy or norbormide: An autoradiographic study in the rat (Manuscript)

V Mahteme H, Larsson BS, Sundin A, Khamis H, Graf W. Uptake of 5-FU in peritoneal metastases in relation to mode of administration and surgical tumour reduction: An autoradiographic study in the rat (Manuscript)

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# ABBREVIATIONS

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CEA</td>
<td>Carcinoembryonic antigen</td>
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<td>CS</td>
<td>Chondroitin sulphate</td>
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<tr>
<td>EBRT</td>
<td>External beam irradiation therapy</td>
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<tr>
<td>5-FU</td>
<td>5-fluorouracil</td>
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<td>HA</td>
<td>Hyaluronan</td>
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<td>HAI</td>
<td>Hepatic artery infusion</td>
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<td>HIPEC</td>
<td>Hyperthermic intraperitoneal chemotherapy</td>
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<td>IFP</td>
<td>Interstitial fluid pressure</td>
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<td>IORT</td>
<td>Intra-operative irradiation therapy</td>
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<tr>
<td>i.p.</td>
<td>Intraperitoneal</td>
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<tr>
<td>IPO</td>
<td>Intraportal</td>
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<tr>
<td>i.v.</td>
<td>Intravenous</td>
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<tr>
<td>kDa</td>
<td>Kilo Dalton</td>
</tr>
<tr>
<td>MAb</td>
<td>Monoclonal antibody</td>
</tr>
<tr>
<td>Mw</td>
<td>Molecular weight</td>
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<tr>
<td>MVP</td>
<td>Microvascular pressure</td>
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<tr>
<td>RF</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>RIT</td>
<td>Radioimmunotherapy</td>
</tr>
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<td>TR</td>
<td>Tumour reduction</td>
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1. INTRODUCTION

1.1 Prognostic features in colorectal cancer

It is estimated that adenocarcinoma of the colon and rectum is currently diagnosed in about 678 000 new cases annually (Parkin et al 1993) and that 394 000 patients die of the disease each year (Pisani et al 1993) worldwide. It is one of the major health problems in the western world, and with about 5000 new cases of the disease per year in Sweden, it is the third commonest form of cancer in this country (Swedish Cancer Registry 1999). Although in the great majority - in about two-thirds - of these patients the diagnosis is made at a stage when all apparently diseased tissue can be surgically removed (Gordon et al 1993, Singh et al 1995, Klein et al 1996), metastases are present in about 25% of the patients at the time of diagnosis (McArdle 1990) and the disease will recur in about 40% at some point in time (Goldberg et al 1998, Laurie et al 1989, Moertel et al 1990). In a substantial proportion of the patients, recurrent disease is likely to be due to residual cancer already present at surgery, existing in an occult and microscopic stage. The overall 5-year survival rate has been reported to vary between 50 and 60% (McArdle 1990, Semmens et al 2000, Ries el al 2000). Postoperative adjuvant chemotherapy has been associated with improved prognosis in colonic cancer (Moertel et al 1995, O’Connel et al 1998), and preoperative radiotherapy in combination with total mesorectal excision (TME) has been shown to improve survival in rectal cancer (Dahlberg et al 1998). However, the prognosis in advanced colorectal cancer is poor, especially when metastasis to the liver or peritoneal layer has occurred (Graf et al 1991, Newland et al 1993, Yamamura et al 1997, Assersohn et al 1999, Sadeghi et al 2000), and treatment remains a challenging problem (Nordic gastrointestinal adjuvant therapy group 1992, Scheithauer et al 1993, Van Halteren et al 1999). Intravenous 5-
fluorouracil (5-FU) either alone or modulated with leucovorin, methotrexate or oxaliplatin is used with the aim of achieving regression of the tumour and thereby improved survival (Nordic gastrointestinal adjuvant therapy group 1992, Glimelius et al 1998, de Gramont et al 2000). However, the response rate has been limited (Glimelius et al 1992, Graf et al 1994, Szélenyi et al 2000, de Gramont et al 2000). This poor outlook underlines the importance of increasing the efficacy of the available treatments.
2. BACKGROUND

2.1 Mechanisms of metastatic dissemination

2.1.1 Direct spread

Direct spread of colorectal cancer may occur longitudinally, transversely or radially, but as adequate proximal and distal clearance by surgery is technically feasible, it is the radial spread that is of most importance. An intraperitoneal tumour may involve adjacent organs such as the small intestine, stomach, gallbladder, urinary bladder and anterior or lateral abdominal wall. In a retroperitoneal colonic cancer, radial spread may involve the ureter, duodenum, pancreas, large vessels and muscles of the posterior abdominal wall, and rectal cancer may involve seminal vesicles, the prostate, vagina or bladder, or pelvic bones.

2.1.2 Lymphatic spread

In general, the lymphatic spread of colonic cancer progresses from the paracolic nodes along the main colonic vessels and eventually reaches the para-aortic glands. This orderly process does not always occur, however, and in about 20% of the cases perineural or perivascular tumour deposits are found, which can easily be mistaken for lymph nodes (Goldstein et al 2000). In contrast to rectal disease, it is rare for colonic cancer that has not breached the muscle wall to give rise to lymph node metastases (Newland et al 1987).

2.1.3 Blood borne spread

The most common site for blood borne spread of colorectal cancer is the liver (Weiss et al 1986), presumably arriving by the portal venous system (Fisher et al 1955). About
25-30% of the patients may have detectable liver metastases at the time of operation (Bergmark et al 1969, Finlay 1982), and around 50% of patients may be expected to develop overt disease (Patanaphan et al 1993, Kjeldsen et al 1997). The lung is the next most common site of haematogenic spread, with around 10% of patients developing lung metastases at some stage (Patanaphan et al 1993, Kjeldsen et al 1997). Other reported sites include ovary-uterus, brain, bone, adrenals and skin (Patanaphan et al 1993, Kjeldsen et al 1997, Goldberg et al 1998).

2.1.4 Transcoelomic spread

Colonic cancer may spread throughout the peritoneum, either via subperitoneal lymphatics or by virtue of viable cells being shed from the serosal surface of a tumour, giving rise to malignant tumour deposition (Moore et al 1961, Hase et al 1998). Colorectal cancer is the most common origin of peritoneal metastases (about 45%), next to gynaecological malignancy (Chu et al 1989).

2.2 Treatment of metastases

2.2.1 Surgery

Surgery is the fundamental treatment of primary colorectal cancer and, when possible, also of metastases. In a study by Scheele et al (1990) of patients with resectable solitary hepatic metastases who did not undergo resection, there were no 5-year survivors. However, a small proportion (about 25%) of patients with localised hepatic (Scheele et al 1995) and lung metastases can possibly be cured by surgery. In selected patients without extra-hepatic disease in whom liver resection has been performed, 5-year survival of about 30% has been achieved (Harms et al 1999, Seifert et al 2000,
Minagawa et al 2000). Furthermore, resection of pulmonary metastases has proved to be curative in about 30-60% (Goldberg et al 1998).

The main mode of presentation in advanced locoregional disease is intestinal obstruction (Glass et al 1973, Annest et al 1979). Moreover, in the past this disease has been regarded as a terminal condition, and most patients have been left to a palliative procedure or no surgery at all. However, in locoregional metastases of ovarian cancer, cytoreductive surgery has been applied since the 1970s (Griffiths 1975) and is still being used (Scarabelli 2000) with claimed survival benefit. Perhaps by reason of these results and the fact that in rectal cancer combined preoperative radiotherapy and surgery has been found to improve survival and reduce local recurrence rates (Swedish Rectal Cancer Trial 1997), there has been renewed interest in treating locoregional colorectal cancer by an aggressive surgical approach (Esquivel et al 1993, Sugarbaker 1995, Elias et al 1997, Cavaliere et al 1999, Loggie et al 2000) in combination with other therapy modalities with the aim of improving survival.

2.2.2 Chemotherapy

The effect of adjuvant chemotherapy after curative resection of colorectal carcinoma has been investigated in a large number of trials (Wolmark et al 1988, Moertel et al 1995). At a consensus conference in April 1990 the national cancer institute recommended 5-FU plus levamisole as the standard treatment after resection of stage III colonic cancer. However, colorectal cancer is usually resistant to chemotherapy (Houghton et al 1981). In order to exert its effect on the target organ, an anti-cancer drug must gain entrance into the tumour. Insufficiency of drug levels at target organs may be due to pharmacokinetic factors, such as poor vascular supply (Jain 1988), elevated interstitial fluid pressure (IFP), (Less 1992), a microvascular pressure (MVP),
which is low in relation to the raised IFP (Boucher et al 1992), a higher onotic pressure (Stohrer et al 2000), altered extracellular matrix, e.g. anomalous assembly of the collagen network (Netti et al 2000), and high drug clearance (Los et al 1991). Further reasons for resistance of the tumours to currently available anti-cancer drugs, which may be at least partly responsible for the generally dismal outcome of therapy may be related to cellular mechanisms. Examples of these are over-expression of transport molecules such as P-glycoprotein, a so-called “primary active” drug pump and multidrug resistance-associated protein (MRP), a glutathione (GSH)-dependent increase in the activity of cellular detoxification systems, altered function of nuclear target enzymes such as topoisomerase II, and a change in tubulin binding or function (Germann 2000, Keppler et al 2000, Wright et al 1998, Cole et al 1992, Jedlitschky et al 1996).

As a possible means of obtaining a maximum drug concentration in the target organ, administration routes other than intravenous (i.v) have been suggested (hepatic artery infusion [HAI], intra-portal [IPO] injection, intraperitoneal [i.p]). Different therapy modalities based on radiation, e.g. radio-labelled chemotherapy, external beam irradiation therapy (EBRT) and intra-operative irradiation therapy (IORT) have been used in colorectal cancer. This is discussed separately later.

Another possible way to promote drug penetration into tumours would be to use a carrier substance that specifically targets the tumour. Hyaluronan (HA) is an endogenous macromolecule that is cleared from the circulation via receptor-mediated uptake in liver endothelial cells. The HA receptors in these cells are not down-regulated after ligand binding and also recognise chondroitin sulphate (Gustafson et al 1997). In addition to binding to these “normal” receptors, HA has the ability to accumulate in tumours (Gustafson et al 1995) and an in vitro study of rat colorectal adenocarcinoma
has shown that the cells carry saturable HA binding sites that do not bind chondroitin sulphate (Samuelsson et al 1998).

2.2.2.1 Intravenous chemotherapy

Intravenously administered 5-FU has a central role in the treatment of advanced colorectal cancer (Glimelius et al 1992, Graf et al 1994, Van Halteren el tal 1999). To exert its anti-neoplastic activity, 5-FU has to be anabolised into its active forms. 5-FU may be converted along the deoxyribonucleotide pathway to the active metabolite, 5-fluoro-deoxyuridine monophosphate (Pinedo et al, 1988), which is a potent inhibitor of the target enzyme thymidylate synthase, leading to inhibition of DNA synthesis. 5-fluoro-uridine diphosphate and 5-fluoro-uridine triphosphate are also produced along the ribonucleotide pathway and these compounds are incorporated into RNA, leading to fraudulent RNA and causing cell death (El Hag et al 1990). However, when 5-FU has been used as a single agent, the response rate is usually low (Carter et al 1976,). The limited activity of 5-FU alone in advanced colorectal cancer has prompted efforts to improve its effect either by altering the rate of administration (by using bolus or pulse therapy) (Conti et al 1995, Leichman et al, 1995, Glimelius et al 1998) or by combination with other cytostatics or addition of agents that will increase its effectiveness, i.e. biochemical modulation (Nobile et al, 1992, Glimelius et al 1993, Leichman et al, 1995, Blijham et al 1996).

2.2.2.2 Hepatic arterial infusion

In non-resectable liver metastases, regional chemotherapy has been advocated. Chemotherapy administered by HAI is based principally on the following rationale: 1) liver metastases larger than a few millimeters derive most of their blood supply from the
hepatic arterial circulation, while the normal liver is supplied mainly from the portal circulation (Ridge et al 1987); and 2) clearance of chemotherapeutic agents by first-pass through the liver can increase the drug level in the liver and reduce systemic toxicity (Collins 1984). Lorenz et al (2000) found that HAI therapy was associated with prolonged time to tumour progression and longer survival than i.v. chemotherapy. Besides the marginal effect of HAI on survival, the number of patients who switched to i.v. administration and the problems related to the HAI catheter in the study by Lorenz et al, the problem of hepatotoxicity causing sclerosing cholangitis (Hohn et al 1988) warrants further investigation of HAI before it can be recommended as a routine therapeutic measure.

2.2.2.3 Intraportal chemotherapy

The blood supply to liver metastases up to a size of a few mm predominantly comes from the portal vein (Ackermann 1974) and initial promising clinical results of adjuvant IPO treatment have been reported (Taylor et al 1985), but other studies have shown conflicting results (Liver infusion meta-analysis group 1997, Rougier et al 1998). Among patients with non-resectable liver metastases, Taylor (1978) found a mean survival of 4 months in those who received only IPO 5-FU, whereas patients who had both hepatic artery ligation and IPO infusion of 5-FU survived for a mean of 10 months. However, because of the small number of patients in that study (n=24), no firm conclusions can be drawn about the value of IPO infusion. In a randomised study by Hafstrom et al (1994) in 60 patients with hepatic metastases, a survival benefit was found in those who underwent hepatic artery occlusion and IPO infusion of 5-FU compared with patients given systemic treatment. However, the high mortality rate in
the study by Gerard et al (1991), who compared hepatic artery occlusion with and without IPO needs to be considered.

2.2.2.4 Intraperitoneal (i.p.) chemotherapy

The rationale of i.p. chemotherapy is the lower drug clearance from the peritoneal cavity compared with that from the plasma, higher peritoneal drug concentrations, and to some extent avoidance of the vascular barrier associated with i.v. drug administration (Dedrick et al 1978). Furthermore, studies by Speyer et al (1981) of 5-FU showed a uniform distribution within the peritoneal cavity and a high drug concentration in the portal blood and hepatic parenchyma when 5-FU was delivered in a large volume of fluid. An experimental study (Mahteme et al 1998) on the uptake of 5-FU in rat liver metastases after i.v., IPO and i.p. administration and different times of sacrifice showed that early (both 20 min and 2 h) tumour 5-FU uptake was inferior after i.p. and IPO administration as compared with the i.v. route. However, after 24 hours, i.p. administration gave a better result than i.v. injection.

Early initiation of i.p. treatment has been considered essential for avoiding adhesive processes, which can limit the drug distribution in the peritoneal cavity (Schellinx et al 1996). However, one of the concerns of i.p. chemotherapy is anastomotic dehiscence. In an experimental study, Graf et al (1992) compared the influence of early i.p. 5-FU and i.v. folinic acid on colonic anastomotic healing. They found impaired healing after i.p. 5-FU, but when folinic acid was added, no further deterioration occurred. However, this problem and other chemotherapy-related toxicities have been investigated in several clinical studies and this form of administration has not been associated with an increased complication rate (Graf et al 1994, Kelsen et al 1994, Scheithauer et al 1998, Vaillant et al 2000). Furthermore, in selected patients with peritoneal metastases, i.p.
treatment following complete cytoreduction has shown promising results (Portilla et al 1999, Horsell et al 1999). In our own non-randomised study (unpublished data) in 28 patients with peritoneal carcinomatosis, who underwent a cytoreduction procedure and received i.p. 5-FU and i.v. leucovorin, 18 survived 1.5 to 9 years.

2.2.4.1. **Hyperthermic intraperitoneal chemotherapy (HIPEC)**

This method was first applied clinically in 1980 by Spratt et al (1980), in a patient with pseudomyxoma peritonei. The anti-tumoural effect of chemotherapy is believed to be enhanced by hyperthermia (41-42°C), possibly through an increase in cell membrane permeability, alteration of active drug transport, a change in cell metabolism and a decrease in IFP (Hahn 1979, Hahn et al 1983, Leunig et al 1992, Kong et al 2000). Recent clinical studies have shown promising results (Stephens et al 1999, Cavaliere et al 2000, Beaujard et al 2000). However, the lack of consensus about the optimal target temperature, a report on an increased anastomotic dehiscence rate when HIPEC is combined with preoperative radiotherapy in a rat experimental study (Biert et al 1996), and a finding of increased morbidity and mortality when a cytoreduction procedure has been followed by HIPEC (Jacquet et al 1996), all warrant further studies.

2.2.3 **Radio-labelled chemotherapy**

By combining a short-range beta-emitting radionuclide with chemotherapy (111In bleomycin) in an in vitro study by Hou et al (1989), apoptosis was achieved in human lung cancer cells. In colorectal cancer patients with liver metastases, F-18 labelled 5-FU has been used in a combination with positron emission tomography (PET), for pharmacokinetic and diagnostic studies (Kissel et al 1999).
2.2.4 Radioimmunotherapy

Theoretically radioimmunotherapy (RIT) appears promising, as the irradiation can be directed against antigen-expressing tumour cells, whereas the normal host tissue is less exposed (Welt et al 1994). Furthermore, the inverse relationship between tumour size and uptake of RIT is of importance (Sigel et al 1990, Blumenthal et al 1994). An experimental study in mice (comparing conventional chemotherapy with RIT) and a Phase I study in patients with small-volume liver metastases (<2.5 cm) receiving RIT were performed by Behr et al (1999). In mice, RIT led to an 80% permanent cure rate when this therapy was initiated 10 days after tumour inoculation, and in the phase I study 2 out of 11 patients had partial remissions, while 5 patients showed a minor/mixed response. This outlines the potential importance of RIT as an adjuvant tool, since the anti-tumour effects of RIT are most impressive in minimal residual disease. One of the major limitations of the clinical usefulness of RIT is the small percentage of injected MAb that is selectively delivered to the tumour (Buchegger et al 1995), since bone marrow depletion is a major factor for dose-limiting toxicity (Ychou et al 1998).

2.2.5 Radiofrequency (RF)

This technique involves percutaneous or intraoperative insertion of an RF electrode into the centre of a metastasis under ultrasonic or CT guidance. RF energy is emitted from the electrode and absorbed by the surrounding tissue. This process generates extreme heat, leading to coagulative necrosis of treated tissue (Goldberg et al 1995). A study of RF treatment in patients with hepatic metastases was carried out recently by Goldberg et al (2000) who found that this method induced irreversible cellular damage. However, at
present this treatment is in an experimental phase, and further studies are needed in order to define its role in the therapeutical arsenal.

2.2.6 *External beam irradiation therapy (EBRT) and intra-operative irradiation therapy (IORT)*

At the less favourable end of the clinical spectrum of rectal cancer, i.e. locally advanced tumours that are adherent to adjoining structures such as the sacrum, lateral pelvic walls, prostate or bladder, pre-operative EBRT has been used to promote tumour regression (Ahmad et al 1997). Furthermore, an attempt to increase the dose within a restricted area without introducing significant toxicity to the small bowel and ureter, IORT has been suggested (Wiig et al 2000).
3. AIMS OF THE INVESTIGATION

The general aims of this investigation were to identify clinical factors with influence on survival in advanced rectal cancer and then use experimental models to study methods that might optimise treatment. The specific aims were to answer the following questions:

* What is the prognosis in advanced rectal cancer and what clinical factors influence survival? What is the outcome after surgical and non-surgical treatment of non-curable rectal tumours in a defined population? (Paper I).

* Is the uptake of hyaluronan in hepatic metastases influenced by blocking of liver endothelial cell receptors? (Paper II)

* What is the effect of adjuvant radioimmunotherapy on the development of liver metastases? (Paper III)

* Is 5-FU uptake in peritoneal metastases influenced by pre-treatment with radioimmunotherapy or a locally vasoconstrictive agent? (Paper IV)

* Can cytoreductive surgery optimise 5-FU uptake in peritoneal metastases? (Paper V)

* Is intraperitoneal 5-FU administration superior to intravenous infusion with respect to 5-FU uptake in peritoneal metastases? (Paper V)
4. MATERIALS AND METHODS

4.1 Animals and tumour cells

Rats were used for the experimental studies (II-V). All animals were treated in compliance with the “Principles of Laboratory Animal Care” and the “Guide for the Care and Use of Laboratory Animals” (National Institute of Health Publication No. 80-23, revised 1985). In all experiments, a 10-day acclimatisation period preceded the first surgical procedure. All animals had free access to standard laboratory diet and water throughout the experiments. Approval of the study was obtained from the local Ethics Committee for animal research.

The rats used in study II were anesthetised with fentanyl:midazolam:sterile water (1:1:2), 3.3 ml kg\(^{-1}\) i.p., and in the rats in studies III-V the anaesthetic agent was chloral hydrate 36 mg/ml in a mixture of sodium pentobarbital 0.97 mg/ml and manganese oxide 2.1 mg/ml in distilled water, given i.p. In studies II and V, Wistar FU rats were inoculated with a chemically induced rat colon carcinoma (NGW cell line, Steele et al 1974), and in studies III and IV nude rats were inoculated with a CEA-producing human colonic adenocarcinoma, LS 174T (Tom et al 1976). Both tumour cell lines were grown as a monolayer culture in a nutrient mixture composed of Ham’s F10 medium (Flow Laboratories Swedish AB, Stockholm, Sweden) supplemented with 10 % fetal calf serum, 2 mM L-glutamine, penicillin (20 U/ml) and streptomycin (20 \(\mu\)g/ml). Tumour cell aggregates were achieved mechanically with a scraper (Cell Lifter, Costar Corporation, Cambridge, MA, USA). Previously it has been shown that this preparation comprises 40% single cells and 60% tumour cluster, up to 200 \(\mu\)m in diameter, each containing an average of 200 cells (Graf et al 1992).
4.2 Hyaluronan and chondroitin sulphate (CS)

The HA used for labelling and turnover studies was of bacterial origin (Hyal Pharmaceutical Corporation, Toronto, Canada). The mean molecular weight (Mw) was 400 kDa (range 100 – 2000 kDa), as determined by size exclusion chromatography (Lebel et al 1989). The HA was labelled with $^{125}$I-tyrosine, which does not change the Mw of HA or its binding to cell surface receptors (Gustafson et al 1994). CS extracted from a bovine trachea (Sigma Chemical Company, St Louis, MO, USA) was used. The Mw was approximately 30 kDa as determined by gel filtration chromatography on Sephacryl S-1000 and S-300 calibrated with hyaluronan standards under conditions previously described (Gustafson 1997).

4.3 Monoclonal antibodies and radiolabelling

In studies III and IV, the previously investigated (Ahlström et al 1987, Sundin et al 1991, 1993) anti-CEA MAb 38S1 was used. In addition, the anti-TSH MAb 79C was used in study III. These mouse-derived monoclonal antibodies of the IgG1 kappa isotype were a gift from Pharmacia AB (Uppsala, Sweden). The $^{131}$I-38S1 direct labelling of the MAbs was achieved by the chloramine-T method (Primus et al 1973). Briefly, 3.5 mg MAb 38S1 and anti-TSH MAb 79C were separately mixed with 1.5 GBq of a highly concentrated $^{131}$I preparation (Amersham International, Little Chalfont, Buckinghamshire, England), and 150 mg chloramine-T in 750 µl phosphate buffer, pH 7.4, was added. After 10 min of incubation at 0°C, the reaction was terminated by adding 300 mg sodium metabisulphite in 50 µl of 50 mM phosphate buffer, pH 7.4. Gel filtration was used to separate labelled MAb from unreacted $^{131}$I.
4.4 $^{14}$C-labelled 5-fluorouracil

In studies IV and V tracer amounts of labelled 5-FU were used. Gerard et al (1990) reported that the drug uptake did not differ after injection of tracer amounts of 5-FU compared with therapeutic doses of labelled 5-FU. The major catabolites of 5-FU are fluoro-ureidopropionic acid and fluoro-$\beta$-alanine. Further, Chadwick et al (1972) showed that the radioactivity represents 5-FU as well as drug anabolites and catabolites.

In study IV, for each animal a dose of 17 $\mu$Ci 5-fluoro[2-$^{14}$C]uracil, (Amersham, Buckinghamshire, England), and in study V, a dose of 23 $\mu$Ci 5-fluoro[2-$^{14}$C]uracil, (ICN, Pharmaceuticals, Irvine, Calif, USA) was dissolved in 2 ml 0.9% NaCl (37°C). The injected volume, 2000 $\mu$l (i.p.) or 300 $\mu$l (i.v.), was administered in 30 seconds.

4.5 Autoradiography and phosphoimaging

In studies IV and V, a whole body autoradiography procedure was carried out to determine the radioactivity concentration in tumours and various organs. In brief, immediately after the rats were sacrificed, they were frozen in hexane (paper II) or in ethanol (papers IV and V) that was cooled with dry ice to $-78^{0}$C in 10 min. The frozen rats were then mounted in an aqueous gel of carboxymethyl cellulose, which was rapidly frozen around the animals. Sagittal whole-body sections 10 or 20 $\mu$m thick were attached onto a tape (No. 810, Minnesota Mining & Manufacturing Co., USA). The sectioning was performed at $-20^{0}$C with a cryomicrotome (PMV Co, Stockholm, Sweden) as previously detailed by Ullberg et al (1982) and Dencker et al (1991). The sections were freeze-dried and apposed to Bio-Rad phosphoimager screen and exposed for 10 days (paper II), or exposed to X-ray film (Agfa Structurix D7, Agfa-Geavert, Belgium) for 8 weeks (paper IV) or 12 weeks (paper V). In study II, the developed
image was analysed on a Bio-Rad GS-525 Molecular Imager and the average radioactivity was expressed in phosphoimaging density units. In studies IV and V, for subsequent quantification of autoradiograms, carbon-14 standard staircases (Autoradiographic $^{14}$C-Micro-Scales, Amersham, UK) were co-exposed with the sections, and to evaluate the sections objectively, computer-based image analysis was performed as previously described (d’Argy et al 1990).

**Paper I**

Between 1973 and 1992, 927 patients in the province of Uppsala were identified in the Swedish Cancer Registry as having been given a diagnosis of rectal cancer. A total of 290 patients were excluded for various reasons (histopathological diagnosis of polyp or carcinoma in situ (144), other tumours than a rectal adenocarcinoma (72), not living in the province of Uppsala at the time of diagnosis (5), diagnosis made at autopsy (37), or their records could not be found (32)), leaving 637 patients for the analysis. Of these, 151 patients (24%) had surgically non-curable disease (unresectable metastatic or locally advanced disease (Fig 1, Table 1).

**Figure 1.** Patients with rectal cancer and surgical treatment. APR = abdominoperineal resection. AR = anterior resection

637 study patients

- Incurable rectal cancer, resection of primary tumour (n=81) (Group I)
  - APR = 36
  - AR = 37
  - Hartmann’s = 8

- Incurable rectal cancer, no primary tumour resection (n=70) (Group II)

- Curatively resected (n=444) (Group III)
  - Stoma = 31
  - Laparotomy = 6
  - No surgery = 33

- Resectable primary tumour, no metastases, no primary tumour resection (n=42) (Group IV)
Table 1. Characteristics of patients with surgically incurable rectal adenocarcinoma.

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<th>Group 1 (n=81)</th>
<th>Group 2 (n=70)</th>
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<tbody>
<tr>
<td>Sex ratio (M : F)</td>
<td>42 : 39</td>
<td>36 : 34</td>
</tr>
<tr>
<td>Mean age (range), years</td>
<td>68 (42-91)</td>
<td>73 (50-94)</td>
</tr>
<tr>
<td>Tumour site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distant*</td>
<td>48</td>
<td>33</td>
</tr>
<tr>
<td>Local†</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Abdominal‡</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Distant and local</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Distant and abdominal</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Abdominal and local</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Distant, abdominal and local</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

*Liver, lung, extra-abdominal lymph nodes, skeleton and brain
†Lateral pelvic wall, sacrum, urinary bladder, vagina, uterus and prostate
‡Peritoneal surfaces or para-aortic lymph nodes

Paper II

In 36 inbred female Wistar FU rats (mean weight 193 g, range 170-210 g, Möllegaards Ltd., Denmark), a peripheral branch of the superior mesenteric vein was cannulated with a plastic catheter and a tumour suspension (5 x 10⁶ viable tumour NGW cells) was injected as previously described (Graf et al 1992). After 3 weeks, 17 rats with hepatic metastases (19 had no hepatic metastases and were excluded from further analysis) were randomly allocated to three groups and given the following treatment i.v. in the caudal vein or i.p. In group I, the animals received 25 µg ¹²⁵I-labelled HA by i.v. injection. The animals in group II received 2.5 mg CS i.v. 3 min prior to i.v. labelled HA. The animals of group III received treatment similar to that in group II, with the addition of 2.5 mg CS i.p., 30 min after i.v. injection of labelled HA. Seven animals were sacrificed after 30 min, and nine after 5 hours. One animal was killed 1-2 min after injection of a high dose of unlabelled HA followed by a tracer dose of labelled HA to observe the
distribution of circulating HA. The animals were sacrificed in a CO₂ chamber and were frozen immediately for subsequent evaluation with phosphoimaging.

**Paper III**

Thirty-three male nude rats (mean weight 249 g, range 180 – 318, Rowett nu/nu, Wallenberg Laboratory, Lund, Sweden) were inoculated with a LS 174T tumour cell suspensions (5 x 10⁶), using the same procedure as mentioned above (paper II). The right femoral vein was catheterised (for i.v. administration purposes) and animals were randomly allocated to five different groups. Within half an hour, 20 MBq (n=2), 75 MBq (n=5) or 150 MBq (n=10) of the ¹³¹I-labelled anti-CEA MAb (38S1) was injected i.v., and control groups received either i.v. saline injections (n=12) or 150 MBq of the irrelevant ¹³¹I-labelled Mab 79C (n=4). Body weight was recorded every 5 days and immediately before sacrifice. Whole-body and blood clearance (blood samples taken from a tail vein) of ¹³¹I activity was monitored every 2-4 days. Whole-body radioactivity was measured by means of a solid-state Ge-detector and blood clearance in a well-type NaI-detector. After a period of 5-7 weeks, all rats were sacrificed in a CO₂ chamber, and blood was obtained by cardiac puncture for determination of haemoglobin, haematocrit, leucocytes and platelets. The abdomen was inspected for the presence and extent of hepatic metastases and for extrahepatic tumour growth.
Paper IV

A total of 32 nude rats (17 females and 15 males, mean weight 205 g, range 151-341 g, Rowett nu/nu, Wallenberg Laboratory, Lund, Sweden) underwent abrasion of the right lateral abdominal peritoneum (1 cm²), using a scalpel, and were inoculated with 1.0 x 10⁷ LS 174T. Two weeks later the animals were randomly allocated to five groups. Group I (n=5) and group IV (n=4) received 3 ml NaCl/rat i.p. and served as control groups, and the animals in groups II (n=6) and V (n=8) received ¹³¹I-Mab 38S1 i.p. in the same volume. Six days (groups I, II and III) or 2 days (groups IV and V) later, a second laparatomy was performed and verified that all animals had a macroscopic tumour in the peritoneal cavity. One rat in group I died immediately after the second laparatomy and was excluded. Two rats, one from group IV and one from group V, underwent tumour dry-weight analysis. In the remaining 29 animals, a plastic catheter (Polyethylene Tubing, PP 380, Swevet AB, Stockholm, Sweden) was inserted i.p. and ¹⁴C-labelled 5-FU was injected. The animals in group III (n=6) received 2.5 mg /kg U/V Norbormide (rat specific, vasoconstrictive agent) i.p. 10 minutes prior to injection of labelled 5-FU. In all groups, the animals were killed in a CO₂ chamber 2 hours after 5-FU injection, for subsequent evaluation with autoradiography.

Paper V

Forty-six inbred female Wistar rats (mean weight 201 g, range 150 – 240 g, Möllegaards Ltd., Denmark) were used in study V. A suspension of an adenocarcinoma cell line (NGW), containing 1.0 x 10⁷ viable tumour cells, was applied by injection either into non-abraded (n=6) or into the abraded (1 cm²) (n=6) right lateral abdominal peritoneum. Since abrasion was associated with more reproducible tumour growth, this method was used in the remaining animals. Three weeks after tumour inoculation, a
second laparotomy was performed. Totally 34 animals had macroscopic tumours scattered in the abdomen. They were randomly allocated into eight groups and injected with $^{14}$C-labelled 5-FU either by the i.v. or the i.p. route, with or without an immediately preceding tumour debulking procedure, and sacrificed after 2 or 8 hours. In the i.v. group the right femoral vein was cannulated, and in the i.p. group a catheter was introduced as outlined above (papers II and IV respectively). The debulking procedure encompassed tumours that were located in the right half of the abdomen. These tumours were divided in the midline and half of the tumour was excised. To minimise blood loss, tumours in the left half of the abdomen were left in situ. The animals were killed in a CO$_2$ chamber and subjected to autoradiography.
5. STATISTICAL METHODS

In study I, differences in proportions were evaluated with the $X^2$ test and numerical differences with Student’s $t$-test. The log rank test and the Cox’s proportional hazards model (Cox 1972) were used to evaluate the influence of categorical and of continuous clinical variables, on survival in a univariate analyses.

To evaluate differences between groups in study II, non-parametric analyses were performed using the Mann-Whitney U test.

In study III, the proportions of animals with metastases in the groups were compared by Fisher’s exact test. A one-way analysis of variance was used to evaluate weight development and blood data differences between groups.

In studies IV and V, a two-factor repeated measure analyses of variance was performed (with or without stratification by tumour area) to determine whether the drug uptake in tumours differed between the groups. In these analyses, group and rat are treated as the “between” factors and tumour as the “within” factor. In study IV, Dunnett’s test was used to compare treated groups with the control group, and in study V Tukey’s studentized range test was used to conduct multiple comparisons among the groups. In both tests, a Bonferroni correction was used to control the overall type I error rate of 0.05 (Fleiss 1986). In all studies, statistical significance was accepted at $P < 0.05$. 
6. RESULTS

Paper I

The median survival was 7.5 months in group I, and 3.5 and 1.9 months in surgically and non-surgically treated patients, respectively, in group II. The incidence of postoperative ileus was higher in group I than in the patients treated with a curative resection ($P=0.02$). Analysis of patients with surgically non-curable disease (groups I and II combined) showed that bilateral hepatic, lymph node and peritoneal metastases as well as abnormal liver function tests correlated with poor survival (Table 2 and 3). Furthermore, a shorter survival was found in patients with abdominal metastases than in those with distant metastases ($P=0.01$, Table 2).

| Table 2 | Association between clinical variables and survival at univariate analysis in patients with surgically non-curable rectal cancer (n=151). |
|-----------------|-----------------|-----------------|
| Bilat hepatic   | Groups I and II | $P^*$           |
| Yes             | 75              | 4.3             | 0.04 |
| No              | 52              | 5.7             |
| Lymph nodes     |                 |                 |
| Yes             | 15              | 2.4             | 0.01 |
| No              | 136             | 5.7             |
| Peritoneal      |                 |                 |
| Yes             | 18              | 3.6             | 0.02 |
| No              | 133             | 5.5             |
| Abdominal†      |                 |                 |
| Yes             | 70              | 4.1             | 0.01 |
| No              | 81              | 5.7             |

Survival (median survival in months)
†Metastatic growth confined to peritoneal surfaces, local sites or para-aortic lymph nodes.
* Log rank test.
Table 3  Relationship between liver function tests and survival at univariate analysis in patients with surgically non-curable rectal cancer (n=151).

<table>
<thead>
<tr>
<th></th>
<th>Group I and II</th>
<th>Relative hazard</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum bilirubin</td>
<td>114</td>
<td>1.02</td>
<td>0.002</td>
</tr>
<tr>
<td>Serum alkaline phosphate</td>
<td>115</td>
<td>1.07</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum ASAT</td>
<td>116</td>
<td>2.32</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum ALAT</td>
<td>116</td>
<td>3.17</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>121</td>
<td>0.95</td>
<td>0.004</td>
</tr>
</tbody>
</table>

ASAT: aspartate aminotransferase.
ALAT: alanine aminotransferase.
* Cox proportional hazards model.

Paper II

Sixteen rats had a total of 50 hepatic metastases (Table 4). At 5 hours, the radioactivity in hepatic metastases was higher in group II than in group I (P=0.01, Table 5, Fig 2).

The liver/tumour ratio was higher in group I than in groups II and III (P=0.01).

Table 4  Number of rats (and hepatic metastases) in the three groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4(9)</td>
<td>8(34)</td>
<td>4(7)</td>
</tr>
</tbody>
</table>

Group I= Not inhibited by chondroitin sulphate
Group II= Inhibited by i.v. chondroitin sulphate
Group III= Inhibited by i.v. and i.p. chondroitin sulphate
### Table 5
Concentration of radioactivity in hepatic metastases and skeletal muscle after 5 hours. Figures are mean (SD) in the experimental groups and individual animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumour</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.8 (0.6)</td>
<td>0.1 (0.1)</td>
</tr>
<tr>
<td>Animal 1</td>
<td>1.4 (-)</td>
<td>0.3 (-)</td>
</tr>
<tr>
<td>Animal 2</td>
<td>1.9 (0.7)</td>
<td>0.1 (-)</td>
</tr>
<tr>
<td>Group II</td>
<td>4.8 (1.8)</td>
<td>0.5 (0.2)</td>
</tr>
<tr>
<td>Animal 1</td>
<td>5.7 (2.7)</td>
<td>0.7 (-)</td>
</tr>
<tr>
<td>Animal 2</td>
<td>4.3 (1.8)</td>
<td>0.5 (-)</td>
</tr>
<tr>
<td>Animal 3</td>
<td>4.5 (-)</td>
<td>0.2 (-)</td>
</tr>
<tr>
<td>Group III</td>
<td>3.6 (1.1)</td>
<td>0.7 (0.4)</td>
</tr>
<tr>
<td>Animal 1</td>
<td>2.8 (0.4)</td>
<td>0.4 (-)</td>
</tr>
<tr>
<td>Animal 2</td>
<td>3.9 (0.8)</td>
<td>0.5 (-)</td>
</tr>
<tr>
<td>Animal 3</td>
<td>4.6 (1.3)</td>
<td>1.2 (-)</td>
</tr>
<tr>
<td>Animal 4</td>
<td>2.4 (-)</td>
<td>0.4 (-)</td>
</tr>
</tbody>
</table>

For definition of groups, see Table 4

**Figure 2.** Autoradiographs of whole body sections of two rats. The left autoradiograph is from a rat which received chondroitin sulphate prior to labelled HA (group II), and the right one is from a rat which was given labelled HA alone (group I). The arrows point to liver metastases.
Paper III

In the 20 MBq, 75 MBq and 150 MBq $^{131}$I-38S1 groups, 2/2, 3/5 and 0/10 rats, respectively, developed liver metastases, compared with 6/12 animals in the group injected with saline only and 0/4 animals in the 150 MBq $^{131}$I-79C group (Table 6). The difference in tumour induction between the saline group and the groups injected with the 150 MBq dose of MAb (either specific or non-specific) was significant ($P=0.03$). Furthermore, a mean weight loss was found in both 150 MBq groups, whereas a mean weight gain occurred in the other groups ($P=0.01$, Table 6). The highest doses of $^{131}$I-38S1 and $^{131}$I-79C were associated with depletion of B-haemoglobin, B-erythrocyte volume fraction and B-white blood cell count ($P=0.001$).

<table>
<thead>
<tr>
<th>Group</th>
<th>Rats with metastases</th>
<th>Hb (g/L)</th>
<th>B-EVF (%)</th>
<th>B-TRC x10$^9$/L</th>
<th>B-WBC x10$^9$/L</th>
<th>Weight Change (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>6/12</td>
<td>160±16</td>
<td>47±4</td>
<td>653±342</td>
<td>6.1±3.3</td>
<td>80±113</td>
</tr>
<tr>
<td>20 MBq</td>
<td>2/2</td>
<td>151±4</td>
<td>--</td>
<td>575±6</td>
<td>5.0±0.4</td>
<td>139±30</td>
</tr>
<tr>
<td>75 MBq</td>
<td>3/5</td>
<td>148±14</td>
<td>44±1</td>
<td>570±81</td>
<td>4.5±1.7</td>
<td>61±5</td>
</tr>
<tr>
<td>150 MBq</td>
<td>0/10</td>
<td>139±19</td>
<td>42±7</td>
<td>560±297</td>
<td>3.8±2.0</td>
<td>-7±1-0</td>
</tr>
<tr>
<td>150 MBq‡</td>
<td>0/4</td>
<td>126±15</td>
<td>38±4</td>
<td>623±272</td>
<td>3.2±2.0</td>
<td>-35±25</td>
</tr>
</tbody>
</table>

20 MBq, 75 MBq and 150 MBq refer to labelling of $^{131}$I-38S1 MAb
‡150 MBq $^{131}$I-79C (unspecific) MAb
EVF= erythrocyte volume fraction
TRC= thrombocyte count
WBC= white blood cell count
Paper IV

Totally 243 peritoneal metastases were observed (Table 7). A high uptake of $^{131}$I in peritoneal tumours and skin was found in group II after 1 week of exposure (Fig. 3). After 8 weeks of exposure, the tumour size was reduced in group II compared with group I ($P=0.01$), whereas the size did not differ between groups IV and V. The tumour uptake of 5-FU was higher in group III than in group I (Table 8, $P=0.04$). When the tumours were divided into small tumours (<627 pixels) and large tumours ≥627), where 627 was the median tumour area, large tumours in group III were found to have a higher radioactivity concentration than group I (Table 8, $P=0.002$), whereas the difference was less pronounced in small tumours. There was no difference in the tumour fluid content between group IV (79%) and group V (77%).

Table 7  Number of rats (and peritoneal metastases) in the experimental groups. The numbers of tumours larger or smaller than 627 pixels (median tumour area) are shown separately.

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5(58)</td>
<td>6(70)</td>
<td>6(50)</td>
<td>4(19)</td>
<td>8(46)</td>
</tr>
<tr>
<td>Small tumours</td>
<td>28</td>
<td>54</td>
<td>15</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Large tumours</td>
<td>30</td>
<td>16</td>
<td>35</td>
<td>11</td>
<td>31</td>
</tr>
</tbody>
</table>

Group I  = Treated with NaCl, 6 days prior to $^{14}$C-5-FU injection.
Group II  = Treated with $^{131}$I-MAb 38S1, 6 days prior to $^{14}$C-5-FU injection.
Group III = Treated with Norbormide, 10 min prior to $^{14}$C-5-FU injection.
Group IV  = Treated with NaCl, 2 days prior to $^{14}$C-5-FU injection.
Group V   = Treated with $^{131}$I-MAb 38S1, 2 days prior to $^{14}$C-5-FU injection.
Table 8 Concentration of $^{14}$C-originated radioactivity in peritoneal metastases in all tumours, in small and large tumours separately, and in the liver and intestine (kBq/g tissue), values are mean (SD).

<table>
<thead>
<tr>
<th></th>
<th>All tumours</th>
<th>Small</th>
<th>Large</th>
<th>Liver</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Gr I</td>
<td>11.8 (11)</td>
<td>30</td>
<td>28</td>
<td>21.3 (27)</td>
<td>9.1 (5)</td>
</tr>
<tr>
<td>Gr II</td>
<td>9.8 (11)</td>
<td>54</td>
<td>16</td>
<td>7.1 (4)</td>
<td>9.7 (10)</td>
</tr>
<tr>
<td>Gr III</td>
<td>21.4 (17)</td>
<td>15</td>
<td>35</td>
<td>17.3 (15)</td>
<td>34.4 (33)</td>
</tr>
<tr>
<td>Gr IV</td>
<td>12.1 (3)</td>
<td>8</td>
<td>11</td>
<td>15.6 (9)</td>
<td>11.4 (8)</td>
</tr>
<tr>
<td>Gr V</td>
<td>10.1 (10)</td>
<td>15</td>
<td>31</td>
<td>16.2 (11)</td>
<td>11.6 (5)</td>
</tr>
</tbody>
</table>

For definition of groups, see Table 7

Figure 3. Autoradiographs of whole body section of a rat after one week of exposure showing radioactivity originating from $^{131}$I in peritoneal metastases (arrow).
Paper V

A total of 360 peritoneal metastases were eligible for this study (V) of the uptake of 5-FU in relation to the mode of administration and surgical tumour reduction (Table 9). After 8 hours, tumours treated by i.p. injection after a debulking procedure (i.p._TR) had a higher uptake than those treated by i.p. injection without such a procedure or tumours treated by i.v. injection with such a procedure (i.v._TR), ($P=0.002$, Table 10, Fig. 4). The 95% confidence intervals for the mean differences i.p._TR-i.p. and i.p._TR-i.v._TR were [10.6 – 50.2] and [21.0 – 68.3], respectively. After 8 hours, small tumours (< 571 pixels, where 571 was the median tumour area) subjected to i.p._TR had a higher uptake than i.p. or i.v._TR tumours ($P=0.004$ Table 11). Among larger tumours ($\geq$ 571 pixels), after 2 hours i.p._TR tumours had higher radioactivity than i.p. or i.v._TR tumours ($P=0.03$, Table 11), and after 8 hours i.p._TR tumours showed higher uptake than i.p. or i.v._TR tumours ($P=0.001$, Table 11).

Table 9 Number of rats (and peritoneal metastases) studied 2 and 8 hours after injection of labelled $^{14}$C-5-FU, by route of administration and tumour debulking.

<table>
<thead>
<tr>
<th></th>
<th>2 hours</th>
<th>8 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.v._TR</td>
<td>4 (43)</td>
<td>4 (68)</td>
</tr>
<tr>
<td>i.p._TR</td>
<td>6 (98)</td>
<td>4 (62)</td>
</tr>
<tr>
<td>i.v._TR</td>
<td>4 (26)</td>
<td>4 (22)</td>
</tr>
<tr>
<td>i.p._TR</td>
<td>4 (22)</td>
<td>4 (19)</td>
</tr>
</tbody>
</table>

* i.v. = Intravenous injection
* i.p. = Intraperitoneal injection
* i.v._TR = Intravenous injection after debulking procedure
* i.p._TR = Intraperitoneal injection after debulking procedure
Table 10  Radioactivity concentration in peritoneal metastases (kBq/g tissue) 2 and 8 hours after injection of labelled $^{14}$C-5-FU in relation to route of administration and tumour debulking. Values are mean and (SD).

<table>
<thead>
<tr>
<th></th>
<th>2 hours</th>
<th>8 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.v.</td>
<td>73.2 (94)</td>
<td>22.1 (13)</td>
</tr>
<tr>
<td>i.p.</td>
<td>82.2 (113)</td>
<td>32.8 (14)</td>
</tr>
<tr>
<td>i.v.TR</td>
<td>26.1 (20)</td>
<td>18.5 (18)</td>
</tr>
<tr>
<td>i.p.TR</td>
<td>157.8 (81)</td>
<td>63.2 (28)</td>
</tr>
</tbody>
</table>

For definition of groups, see Table 9

Figure 4. Autoradiographs of whole body sections of rats, showing uptake of $^{14}$C-labelled 5-FU in peritoneal metastases (arrows). A rat treated with i.p. 5-FU and debulking (i.p.TR) is seen to the left and a rat treated with i.v. 5-FU and debulking (i.v.TR) to the right.
Table 11 Radioactivity (kBq/g tissue) in peritoneal metastases, stratified according to tumour area (small and large tumours; median tumour area = 571 pixels), 2 and 8 hours after injection of labelled $^{14}$C-5-FU, by route of administration and tumour debulking. Values are mean (and SD).

<table>
<thead>
<tr>
<th></th>
<th>Small tumours</th>
<th></th>
<th>Large tumours</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 hours</td>
<td>8 hours</td>
<td>2 hours</td>
<td>8 hours</td>
</tr>
<tr>
<td>i.v.</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>30</td>
<td>86 (107)</td>
<td>34</td>
<td>25 (17)</td>
<td>13</td>
</tr>
<tr>
<td>49</td>
<td>116 (141)</td>
<td>29</td>
<td>35 (17)</td>
<td>49</td>
</tr>
<tr>
<td>i.v.TR</td>
<td>15</td>
<td>8</td>
<td>23 (17)</td>
<td>11</td>
</tr>
<tr>
<td>i.p.</td>
<td>6</td>
<td>9</td>
<td>77 (26)</td>
<td>16</td>
</tr>
<tr>
<td>i.p.TR</td>
<td></td>
<td></td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

For definition of groups, see Table 9
7. DISCUSSION

Many factors influence survival after the development of metastases from colorectal cancer. In this investigation (study I), the clinical factors most strongly associated with a poor prognosis were found to be massive involvement of the liver and abdominal tumour growth, supporting previous findings (Graf et al.1991, Assersohn et al 1999, Sadeghi 2000). This result indicates that at the time of diagnosis there is a need to select a “subgroup” of patients, who may not benefit from surgical treatment. The median survival in patients who underwent resection was reduced by one-third when abdominal growth was present compared with those without abdominal involvement. On the other hand, patients with peritoneal metastases may benefit from therapeutic strategies other than conventional surgery or chemotherapy. To control local symptoms (bleeding, intestinal obstruction), palliative resection has been recommended (Tacke et al 1993, Fitzgerald et al 1993). However, in our study, about 15% of the patients who did not undergo either resection or a non-resectional procedure developed symptoms from the primary tumour that necessitated surgery. Moreover, the need for late surgery was not lower in patients in whom the primary tumour was resected than in those in whom it was left in situ, and it is therefore doubtful whether the risk of intestinal obstruction is a good argument for a palliative resection.

In the light of the above finding, there is a need to find a way to optimise the uptake of currently available drugs in liver metastases. A previous in vitro study by Samuelsson et al (1998) had shown that NGW cell lines had the ability to bind HA with high affinity. Furthermore, in our in vivo study we found that HA targeted liver metastases and that
pretreatment with CS enhanced the tumour uptake of the labelled HA. The differences in specificity between HA binding sites can be utilised in attempts to block uptake of exogenous HA in healthy liver without reducing targeting to metastases. It is possible that targeting of HA and HA/drug complexes to HA binding sites can result in more effective drug treatment, with probably enhanced uptake and reduced side-effects. Klein et al (1993) compared the effects of 5-FU alone with those of 5-FU + HA on liver metastases from rat mammary carcinoma. They found an increased tumour drug uptake in rats treated with 5-FU + HA. However, the affinity between HA and 5-FU is believed to be low and the possibility of drug-targeting using HA needs to be investigated further. Another study indicating the potentiality of HA as a drug carrier was reported by Coradini et al (1999) who found that when sodium butyrate was linked to HA, the availability of the sodium butyrate increased in a breast cancer cell line.

The superior penetration of MAb into smaller lesions compared to larger ones has been demonstrated previously (Pervez et al 1988, Sato et al 1999). By contrast, Sundin et al (1993) reported that there was no relationship between MAb uptake by liver metastases and their size. However, the ability of MAb to prevent further progression of small metastases has been observed in both clinical and experimental studies (Juweid et al 1996, Behr et al 1999). In the study by Behr et al (1999), in which mice with liver metastases were treated with MAb 10 or 20 days after tumour inoculation, a definite cure was observed in the 10-day group but not in the 20-day. In our study III, in which RIT was given as adjuvant treatment, in experimental colonic cancer the development of liver metastases could be totally prevented by administration of 150 MBq of $^{131}$I-38S1 or $^{131}$I-79C, while in the control group (NaCl) and the groups that received 20 MBq or 75 MBq, liver metastases developed. The unexpected tumour-preventing effect
of the unspecific MAb (150 MBq) in our study might have been due to non-specific radiation, contributing to the inactivation of tumour cells. In a previous experimental study in mice, unspecific MAb labelled with a lower of $^{131}$I (14.8 MBq) exerted a slight inhibitory effect on the growth of inoculated human ductal mammary carcinoma (Senekowitsch et al 1989). These differences may be due to the total radiation dose.

The relation between peritoneal metastases from rectal cancer and a poor prognosis (paper I) raises the question as to how we can optimise the uptake of cytotoxic drugs in such metastases. It was found in study IV that RIT treatment given 6 days prior to sacrifice induced a decrease in tumour size but did not affect or perhaps negatively affected the uptake of 5-FU. The effect on tumour size is most likely due to the $\beta$-radiation, which reduces the capacity of the tumour cells to proliferate, as reported from previous experimental models (Blumenthal et al 1994). Theoretically, oedema can be initiated by the radiation, leading to an increase in intratumoral pressure. However, when we compared the water content in tumours from groups IV and V (see Table 7 for definition of groups), no difference was found. Clinically, especially in ovarian cancer, intraperitoneal RIT has been used in combination with debulking surgery or chemotherapy, but the results from these studies are not yet convincing (Meredith et al 1996, Mahe et al 1999).

The uptake of 5-FU in the peritoneal metastases, especially in “large tumours”, was improved after pretreatment with a vasoconstrictive agent (Norbormide). This drug, which is rodent-specific, produces an irreversible local vasoconstriction through an $\alpha$-adrenergic effect (Roszkowski 1965). The higher uptake in peritoneal metastases observed after administration of Norbormide may probably be due to reduced peritoneal absorption of 5-FU, leading to prolonged exposure of the tumours to 5-FU and thereby
promoting local diffusion of 5-FU. One possible reason for failure of intraperitoneally administered drugs to cure larger tumour masses is the poor penetration into tumour tissue. For example, Collins et al (1982) found that the concentration of 5-FU measured at a depth of 0.6 mm from the peritoneal surface was only 5% of that in the peritoneal fluid. Our results are consistent with those of Duvillard et al (1999), who reported that the anti-tumoral effect of i.p. chemotherapy was enhanced by a combination with i.p. epinephrine.

In paper V, the debulked, i.p. treated tumours overall showed a higher uptake at 8 hours than the native tumours treated i.p., and the uptake was also higher than in debulked tumours treated by the i.v. route. It is reasonable to conclude that 5-FU uptake is improved by breaking biological barriers and thereby possibly by decreasing the interstitial pressure to promote regional drug delivery. A previous study on the uptake of Cisplatin with a molecular weight (Mw) of 300.6 kDa and of Carboplatin with a Mw of 317.3 kDa, in rat peritoneal tumours, showed a favourable uptake of Cisplatin (Los et al 1991). 5-FU, which has a lower Mw (130.1 kDa) could be ideal to use intraperitoneally. However, the influence of the Mw of drugs on their tissue diffusion has been found to be limited (Flessner et al 1985). The enhanced uptake of 5-FU in the peritoneal tumours in the present study may have been due to mechanically disintegrating membranes, with different solubility for drugs (Kerr et al 1988), which the drug has to cross. An increased drug accumulation due to local diffusion was also indicated by the lower radioactivity concentration in the centre of the tumour compared to its periphery (data not shown), which is consistent with the results of Collins et al (1982).
The present study has thus confirmed that patients with bilateral liver metastases, peritoneal growth or abdominal lymph node metastases have a short survival. The experimental finding of adjuvant RIT in preventing liver metastases is promising, and this may be clinically applicable in the future. The observation of enhanced uptake of HA in liver metastases may be applied clinically, with use of HA as a drug-delivery molecule. In selected patients with peritoneal carcinomatosis, cytoreductive surgery and i.p. chemotherapy may be associated with a prolonged survival (Gough et al 1994, Schellinx et al 1996, Horsell et al 1999). Our finding of a higher drug uptake in debulked, i.p. treated tumours is encouraging. Moreover, the enhanced uptake of 5-FU observed in peritoneal metastases after pretreatment with a local vasoconstrictive agent is promising. Clinically, epinephrine injected intratumorally together with Cisplatin in patients with solid tumours located in subcutaneous tissue has yielded favourable results (Burris et al 1998). However, the risk of systemic (mainly cardiovascular) and local (intestinal infarction) effects of epinephrine limits intraperitoneal application of this drug in patients with peritoneal carcinomatosis, and the concept of peritoneal vasoconstriction merits further investigation.
8. SUMMARY AND CONCLUSIONS

The aims of this work were to identify metastatic sites associated with poor prognosis in rectal cancer patients and to investigate methods that might prevent the development of metastases and optimise the uptake of drugs at these sites in animal models. The main results were:

♦ Bilateral hepatic involvement, peritoneal growth or abdominal lymph node metastases implies a poor prognosis.

♦ Blocking of uptake and elimination of hyaluronic acid by the liver enhances the hyaluronan uptake in liver metastases. Hyaluronan may thus be of future value as a delivery-molecule for chemotherapy, targeting specific hyaluronan receptor-positive tumour sites.

♦ Radioimmunotherapy may prevent the development of liver metastases from colorectal carcinoma in an “adjuvant” setting, although the unspecifically delivered radiation dose is most likely the major contributing factor. Furthermore, radioimmunotherapy can inhibit the growth of peritoneal metastases.

♦ The uptake of intraperitoneally administered 5-FU in peritoneal metastases can be potentiated by blocking the 5-FU absorption with a vasoconstrictive agent. Moreover, uptake of 5-FU in peritoneal metastases can be improved if the tumours are surgically reduced and the drug is given intraperitoneally.
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