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Title and subtitle
Colonic healing: the effect of irradiation and chemotherapy
– an experimental study, resembling adjuvant therapy for colorectal carcinoma

Abstract
Adjuvant treatment of colon and rectal carcinoma is of major interest. Irradiation and chemotherapy are modalities used widely. Concern for the integrity of anastomoses performed when this treatment is used has been put forth. The purpose of this study was to evaluate the effect of preoperative irradiation and postoperative intraperitoneal 5-fluorouracil treatment on colonic healing.

In rats preoperative irradiation of the lower abdominal region by 10 + 10 Gy four days apart caused inflammatory reaction in the colon as evaluated by histology and determination of myeloperoxidase activity. The inflammatory reaction reached its peek within a week of the second irradiation. When standardised colonic resections and anastomes were performed within the irradiated part of the colon the anastomotic healing was not affected during the first week after operation as judged by complications and breaking strength. A lower breaking strength and an increase in myeloperoxidase activity two months after operation may indicate late changes within the intestinal wall.

Intraperitoneal 5-fluorouracil in rat given immediately after colonic resection and repeated as daily injections caused a weight loss and a marked reduction in breaking strength of the anastomosis as well as in the abdominal skin wound. The addition of intravenous leucovorin did not further impair wound healing. A reduction in 5-fluorouracil concentration did not alter the negative wound healing effect of the chemotherapy. In a group of rats subjected to nutritional depletion, mimicking the weight curve of 5-fluorouracil treated animals, anastomotic breaking strength was not compromised to the same extent as when 5-fluorouracil was given. This indicates a direct toxic effect rather than an effect of reduced food intake caused by 5-FU treatment. Collagen synthesis and the formation of new tissue in the wound gap was reduced in 5-fluorouracil treated animals compared to controls as judged by in vivo incorporation of ^{3}H-proline in the anastomotic segment and determination of anastomotic breaking strength after removal of sutures. When administration of 5-fluorouracil was postponed by three days the negative wound healing effects of 5-FU were eliminated.

The results indicate an early vulnerable phase in which 5-fluorouracil may have a detrimental effect on colonic healing.

Key words
Wound healing, colon, adjuvant, chemotherapy, irradiation

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COLONIC HEALING
THE EFFECT OF IRRADIATION AND CHEMOTHERAPY

An experimental study
resembling adjuvant therapy for colorectal carcinoma

Staffan Weiber

Akademisk avhandling

Som med vederbörligt tillstånd av Medicinska Fakulteten vid Lunds Universitet, för avläggande av doktorsexamen i medicinsk vetenskap, kommer att offentligen försvaras i kirurgiska klinikens föreläsningssal, Malmö Allmänna Sjukhus, fredagen den 24 september 1993, kl. 13.00

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Malmö 1993

COLONIC HEALING

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and Experimental Research
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Acta Oncologica, in press.

II. **Preoperative irradiation and colonic healing.**
S Weiber, H Jiborn, B Zederfeldt.
European Journal of Surgery, in press.

III. **Influence of 5-fluorouracil and folinic acid on colonic healing: an experimental study in the rat.**
W Graf, S Weiber, B Glimelius, H Jiborn, L Påhlman, B Zederfeldt.
British Journal of Surgery, 1992; 79: 825-828.

IV. **The role of nutrition depletion and drug concentration in 5-fluorouracil induced inhibition of colonic healing.**
W Graf, S Weiber, B Glimelius, H Jiborn, L Påhlman, B Zederfeldt.
Journal of Surgical Research, accepted.

V. **The effect of 5-fluorouracil on wound healing and collagen synthesis in left colon anastomoses.**
S Weiber, W Graf, B Glimelius, H Jiborn, L Påhlman, B Zederfeldt.
European Journal of Surgical Research, submitted .

VI. **Colonic healing in relation to timing of 5-fluorouracil therapy.**
S Weiber, W Graf, B Glimelius, H Jiborn, L Påhlman, B Zederfeldt.
British Journal of Surgery, submitted .

LIST OF ABBREVIATIONS

5-FU	5-fluorouracil
CRE	cumulative radiation effect
dpm	disintegration per minute
FdUMP	5-fluoro-deoxyuridine monophosphate
Gy	Grey
HVL	Half value layer
hypro	hydroxyproline
i.p.	intraperitoneal
i.v.	intravenous
LV	leucovorin
MPO	myeloperoxidase
TGFβ	transforming growth factor β

INTRODUCTION AND BACKGROUND

Colorectal carcinoma is the second most common malignancy in both sexes in Sweden. The incidence of colon and rectal carcinoma in Sweden is 35 and 20/100 000 respectively (Swedish Cancer Registry 1989). The primary treatment for colorectal carcinoma is surgery but in one third of the cases it is not possible to perform curative surgery due to local overgrowth or metastasic disease (Berge et al. 1973, Öhman 1981, Habib et al. 1983). In patients operated for cure one half will suffer from recurrent disease (Nilsson et al. 1984) and more than half of the patients with a primary diagnosis of colorectal carcinoma will die due to the disease (Alarcon and Greenwood 1978, Turunen and Peltokallio 1982, Davis et al. 1987). The postoperative mortality has decreased over the last decades but the long term survival rate has remained unchanged (Enblad 1988). Dukes' classification (Dukes 1932) as modified by Astler and Collins (1954) has been the most adopted for prognostics with crude 5 year survival rate of approximately 85% in Dukes A, 60% in Dukes B and 30% in Dukes C with prognosis being somewhat better in colon carcinoma than in rectal carcinoma (Berge et al. 1973).

In colon carcinoma hepatic metastases are more common than local recurrence. In rectal carcinoma local recurrence along with hepatic metastases are frequent (Lavin et al. 1980, Gilbert et al. 1984, Gunderson et al. 1985). Possible causes of recurrence was early demonstrated by Fisher (1955) to be through metastatic emboli via the portal vein during surgery and by spread through local implantation prior to or at surgery (Moore et al. 1961).

Various frequencies of recurrence have been reported and the surgical technique has been discussed as being of major importance in the outcome for the patient. "No touch" technique with primary ligation of the central vein and artery (Turnbull et al. 1967) or extensive surgery with lymphadenectomi along the iliacal vessels (Koyama et al. 1984) have been reported beneficial for the outcome in rectal carcinoma, however this has not been reproduced in other studies (Glass et al. 1985).

The rather poor results in patients operated for cure have lead to discussions about adjuvant therapy. Irradiation and chemotherapy are the two modalities that have been tried clinically.

Adjuvant radiotherapy

The recurrences of rectal carcinoma are confined to the pelvic region in approximately 50% in clinical series (Gilbert et al. 1984, Gunderson et al. 1985). In attempts to reduce the number of local recurrences and thereby possibly also to improve survival adjuvant pre- and postoperative radiotherapy has been suggested and tried. In evaluating different regimens of irradiation it is important to estimate the biological effect of irradiation. One such concept taking into account the dose of each fraction, the total dose and the duration of treatment is calculation of the cumulative radiation effect (CRE) (Kirk et al. 1971). **Preoperative** irradiation can reduce local recurrence provided the dose level is high (CRE value of above 14.0), but there is no substantial evidence that it can improve survival (Gerard et al. 1988, Påhlman et al. 1990, Stockholm Rectal Cancer Group 1990). If **postoperative** irradiation is used it is possible to exclude patients with Dukes A tumours and patients with disseminated disease from irradiation. In spite of patient selection postoperative irradiation has failed to reduce local recurrences (Gastrointestinal Tumor Study Group 1985, Balslev et al. 1986, Fisher et al. 1988, Påhlman and Glimelius 1990). Meta analysis of pre- as well as postoperative irradiation indicate however that there may be a reduced mortality with irradiation (Gray et al. 1991).

Adjuvant chemotherapy

In metastatic disease of colorectal carcinoma 5-FU alone or in combination with other drugs is most frequently used in treatment. Improved response rates and increased survival seen with the combination of 5-FU and leucovorin in advanced disease (Arbuck 1989, Poon et al. 1989, Doroshow et al. 1990, Köhne-Wömpner et al. 1992) has focused interest on this combination in adjuvant therapy for colorectal carcinoma.

Different forms of drug administration have been tried. Intravenous or oral systemic administration is widely used where as intraportal and intraperitoneal administration have gained increasing interest over the last decade.

The effect of **systemic** 5-FU alone or in combination with other cytotoxic agents and/or radiotherapy as adjuvant treatment after curative surgery has been subject to investigations in a large number of trials. Few studies report minor benefit in disease-free survival (Higgins et al. 1984, Gastrointestinal Tumor Study Group 1985,

Wolmark et al. 1988). Meta-analysis of systemic chemotherapy indicates an improvement in survival (Buyse et al. 1988).

The combination of 5-FU and Levamisol has been reported by Windle (1987) to improve five year survival as compared to controls and 5-FU treatment alone. Laurie (1989) could not reproduce this overall survival improvement in a prolonged 5-FU/Levamisol combination study but by using subset analyses survival improvements reached statistical significance in patients with Dukes' stage C. These findings were followed by an inter group study reported in an interim analysis in which a survival benefit was found for patients with Dukes stage C after 5-FU/Levamisol treatment (Moertel et al. 1990). This lead to a consensus in the US. National Cancer Institute declaring 5-FU/Levamisol as the treatment of choice and that control arms with "no treatment" was not acceptable (NIH Consensus 1989). The interim analysis was confirmed for Dukes' stage C carcinoma in the final report (Moertel et al. 1992).

From the standpoint that **intraportal** treatment has the advantage of high hepatic concentration of cytotoxic agents as well as a systemic effect, trials with postoperative intraportal administration have been made. Taylor (1985) reported a reduction in hepatic recurrence as well as an overall survival advantage with 5-FU treatment during one week postoperatively. In similar trials these benefits of postoperative intraportal 5-FU treatment have not been reproduced (Beart et al. 1990, Metzger et al. 1990, Wolmark et al. 1990, Fielding et al. 1992) with the exception of reduced risk of hepatic recurrence in one study (Wereldsma et al. 1990). Meta analysis of all intraportal studies with 5-FU demonstrated an advantageous effect on survival (Gray et al. 1991).

As proposed by Sugerbaker (1989) and Cunliffe (1989) **intraperitoneal** administration of cytotoxic agents has the advantage of exposing tumour cells at the resection site as well as in the peritoneal cavity to chemotherapy. In addition to this "local effect" intraperitoneal administration would still have the advantages of intraportal administration of high hepatic drug concentration as well as systemic effects (Campora et al. 1987).

Irradiation and anastomotic healing

In the clinical situation with adjuvant preoperative irradiation for rectal carcinoma an increased rate of perineal wound complications has been reported. The frequency of

anastomotic complications has however not been higher in irradiated groups as compared to controls (Gerard et al. 1988, Påhlman et al. 1990, Stockholm Rectal Cancer Group 1990). In using preoperative irradiation the integrity of the anastomoses has been of major concern and in experimental models this has been evaluated.

In experimental studies of the small intestine Rohrer (1990) and Sacarides (1991) could not show any change in anastomotic strength with irradiation in a single dose of less than 20 Gy when given at the time of making the anastomosis. In testing fractionated irradiation (CRE 9.46) Crowely (1968) found no effect on anastomotic bursting strength. The incidence of intestinal wound complications in irradiated ileum is dose dependent and the number of complications increase with the interval between irradiation and wounding (Ormistone 1985).

In the colon 8.75Gy and 14.14 Gy in a single dose given to the whole abdomen one day prior to construction of an anastomosis reduces the anastomotic bursting strength (Murphy et al. 1981). In evaluating the time interval between irradiation (14.5 Gy, single dose, whole abdomen) and creation of the colon anastomosis a delay of five and fifteen days had negative influence on anastomotic bursting strength whereas no negative influence was seen when the time interval was ten days (Degges et al. 1983).

End-to-end colonic anastomoses have been shown to be relatively safe in dogs irradiated three weeks prior to operation when a single dose of 9.2 Gy (Schauer et al. 1982) or 13.6 Gy (Bubrick et al. 1982) was given regionally, but when it was administered as a total of 40.0 Gy in eight fractions over four weeks (CRE 16.83) (Morgenstern et al. 1984) it had a detrimental effect on healing with a high frequency of anastomotic leak and high mortality. Thus it could be assumed from experimental studies that irradiation corresponding to approximately 14.0 - 15.0 Gy single dose and a time interval between irradiation and surgery of approximately one week is optimal in ensuring a maximal effect of irradiation and yet little influence on anastomotic healing.

5-FU and wound healing

5-FU is a prodrug and exerts its effect through several of its metabolites. One of the more important mechanisms is FdUMP's ability to inhibit tymidylsynthetas (Santi et al. 1974). This leads to reduced intracellular tymidin and suppression of the DNA-synthesis and subsequently a reduced capacity for proliferation. In order for FdUMP

to form complex bindings with tymidylsynthetas folinic acid is necessary and the addition of folinic acid can thus enhance the effect of 5-FU (Bleyer 1989). Proliferation is also influenced by a direct incorporation of 5-FU metabolites leading to a defect DNA replication (Danenberg et al. 1981). Metabolites of 5-FU can also be incorporated in RNA resulting in defect m-RNA formation (Cory et al. 1979) and thus 5-FU has the potential ability to influence protein synthesis.

A high rate of morbidity and mortality was reported (Ferrara et al. 1982) in patients after surgery during treatment with various cytotoxic agents but in using 5-FU in adjuvant intraportal administration during the first postoperative week (Taylor et al. 1985, Beart et al. 1990, Metzger et al. 1990, Wolmark et al. 1990, Fielding et al. 1992) there are no reports on increased rate of anastomotic complications.

In most experimental studies anastomotic healing is compromised by 5-FU treatment as judged by bursting or breaking strength and anastomotic complications. Goldman (1969) treated rats preoperatively with 5-FU and had a marked increase in anastomotic complications (ileum) but examination of breaking strength carried out 12 days after operation (and 5-FU treatment) did not show any difference between treatment groups and controls. In immediate postoperative treatment Morris (1978) demonstrated adverse effects of 5-FU (i.v.) on abdominal wound healing, and Morris (1979) further reported a reduced anastomotic breaking strength in the colon after 5-FU (i.v.) treatment. Hillian (1988) concluded from his study of immediate postoperative 5-FU treatment that it did not have adverse effects on colonic healing when studied 14 days after surgery and 10 days after the last 5-FU treatment. Other antineoplastic agents (combination of bleomycin, 5-FU, cis-platinum, 5 days i.v.) reduced anastomotic bursting pressure when administered prior to or during surgery whereas a delay of two days did not influence anastomotic healing in the ileum (de Roy van Zuidewijn et al. 1986) and similar results were found in the colon (de Roy van Zuidewijn et al. 1987). This combination given intraperitoneally impairs collagen synthesis in the anastomoses when given during surgery (Martens et al. 1992).

AIM

The purpose of the present investigation

was to study

A. the effect of preoperative irradiation on left colon anastomotic healing in a setting resembling adjuvant treatment for rectal carcinoma

by setting up an experimental model.

by evaluating

- the effect of irradiation in the colonic wall as judged by histology, hydroxyproline and myeloperoxidase activity (I).
- the effect of preoperative irradiation on anastomotic healing in the left colon (II).

B. the effect of postoperative 5-FU treatment on left colon anastomotic healing in a setting resembling adjuvant treatment for colorectal carcinoma

by setting up an experimental model.

by evaluating

- the effect of intraperitoneal 5-FU with or without the addition of i.v. leucovorin on wound healing in left colon anastomoses and abdominal wall incisions (III).
- the effect of variations in i.p. 5-FU concentration on colonic healing (IV).
- the etiologic role of nutritional alterations in 5-FU-induced inhibition of large bowel healing (IV).
- the effect of 5-FU on formation of granulation tissue in the anastomotic gap of colon anastomoses and collagen synthesis in the anastomotic segment (V).
- the effect of a short postponement of 5-FU treatment on wound healing (VI).

MATERIAL AND METHODS

Experimental animals

A total of 676 Wistar male rats were used (Möllegaard Ltd., Skensved, Denmark) in the experiments. In studies I and II the animals were caged in groups of five to six throughout the study In studies III - VI the animals were kept in separate cages postoperatively.

Comments

Rats were used as experimental animals since they are suitable for studies of gastrointestinal healing. A substantial amount of knowledge in this field has been accumulated over the years in our laboratory making it possible to compare new findings with a pool of previously established data. Animals were kept in separate cages in study III to minimise the risk of displacement of the intravenous catheter and in study IV to make it possible to monitor food intake. In studies V and VI this setting was not changed to allow comparison.

Operative procedures

The animals were anaesthetised with an intraperitoneal (i.p.) injection of chloral hydrate; 0.25 g/kg (I, II) or fentanyl/midazolam/aq. sterile, 1:1:2 (3.3 ml/kg)(III-VI). A 5 mm left colonic resection one (II) or two (III-VI) cm above the peritoneal reflection was then performed through a standardised midline incision. Bowel continuity was restored with an end to end anastomosis of nine (II-IV, VI) or seven (V) interrupted, inverting sutures (Surgilene® 7-0; Davis and Geck, New York, USA). The abdominal muscle layer and the skin incision were closed separately with running sutures (Ti-Cron®, 5-0; Davis and Geck). In experiment III the right groin was explored through a separate incision and a catheter (Clay Adams PE50; Becton-Dickson, New Jersey, USA) was introduced into the right femoral vein and tunnelled subcutaneously to the neck of the rat.

Comments

The anaesthesia was changed after study II since there were observations in the laboratory of mild peritoneal reactions after i.p. injections of chloral hydrate. The

reason for these findings are unknown. Monofilament synthetic suture material was used in making the anastomoses as this causes a minimum of tissue reaction and could be removed from the tissue (V) with a minimum of trauma. Seven sutures were used in study V in order to avoid damage to the anastomoses when sutures were removed prior to breaking strength test. Single layer interrupted suture technique was used since this is easier to standardise than a continuous suture. To insure firm adaptation and a good knot stability a running braided polyester suture was used in closing fascia and skin .

Randomisation

Prior to irradiation in studies I and II, and after surgery but prior to drug administration in studies III-VI all animals were randomised to treatment groups. In study V animals were again randomised at sacrifice to determine if anastomotic breaking strength was to be tested with or without sutures.

Technique of irradiation

The irradiation was given on two separate days, four days apart. Sham-irradiated animals did not receive irradiation but were else handled as irradiated animals. In study II irradiation was given eight and four days prior to surgery.

Under general anaesthesia (chloral hydrate; 0.25 g/kg intraperitoneally) the rat was placed in a plastic shell with lead shields on both the anterior and posterior side. In both shields field openings of three by four cm (width x length) gave access to irradiation of the pelvic and lower abdominal region. The location of the radiation field was previously checked by X-ray films with barium enema and the rat positioned in the shell. Through the two openings irradiation was given using two opposing fields with an anterior - posterior technique. Irradiation was given with 240 kV X-rays, 15mA, total filtration HVL 1.1 mm Cu. The focus to skin distance was 42 cm. The dose rate was 1.135 Gy/min and the total dose given was 10 Gy on each day of irradiation. The dose was homogenous within the irradiated volume. The dosage was checked by a Farmer electrometer and an ion chamber placed in a rat phantom made of wax.

Comments

In creating a model of irradiation the dose is most important. In an effort to resemble a clinical schedule in an experimental model it is advantageous to use the CRE value in calculating the effect of irradiation (Kirk et al. 1971). In Scandinavia a fractionated dose of 5 Gy given daily for five consecutive days (CRE = 14.59) has been used in clinical trials of adjuvant preoperative irradiation for rectal carcinoma (Påhlman et al. 1990, Stockholm Rectal Cancer Group 1990). In resembling this biological effect, local irradiation in a two dose scheme with 10 Gy each day of irradiation four days apart was given (CRE = 14.54).

In using animal models it is further important to protect the rest of the animal to reduce general effects of irradiation which causes high mortality. This was done by lead shields and carefully focusing on the lower left colon and rectum.

The timing of irradiation prior to surgery in study II was chosen in order to mimic the clinical situation where operation is performed within a short interval after irradiation, usually within a week.

Drug administration

5-fluorouracil (Hoffmann-La Roche, Basel, Switzerland) was given as intraperitoneal (i.p.) injections in a daily dose of 20mg/kg. Two different concentrations (5 mg/ml and 1 mg/ml) were used in experiment IV.

Leucovorin (5 mg/ml) (Cyanamid Nordiska, Sundbyberg, Sweden) was given as daily intravenous bolus injections at a dose of 2mg/kg/day or 10 mg/kg/day through the femoral catheter 15 - 30 minutes after the 5-FU injection (III). After administration of leucovorin the catheter was heparinised.

In studies III-V chemotherapy started immediately after operation and continued until the day before sacrifice. In study VI animals were randomised to **early treatment** or **delayed treatment**. Early treatment started immediately after operation as in studies III-V and was given once daily for seven days. Delayed treatment started on the third postoperative day and was continued until the day before sacrifice.

Comments

A 5-FU dose of 20mg/kg in the rat corresponds to 550 mg/m^2 in the human according to Freireich (1966). This dose was chosen as it is within range of previously reported and ongoing clinical studies of adjuvant treatment for colorectal carcinoma with intraportal or intraperitoneal administration (Taylor et al. 1985, Beart 1990 et al, Metzer et al. 1990, Wereldsma 1990, Fielding et al. 1992). Intraperitoneal administration has been of increasing interest as this could give the benefit not only of high drug concentrations in the liver (Speyer et al. 1980, Speyer et al. 1981, Campora et al. 1987) but also the exposition of local and peritoneal tumour remnants to the drug. Two different concentrations of 5-FU were used in order to study the effect of local drug concentration on anastomotic healing. Different doses of leucovorin have been suggested in the clinical situation and thus one moderate and one high dose of leucovorin were tested in study III.

Diet

All animals had free access to a standard laboratory diet and tap water except in experiment IV. Prior to experiment IV the average food consumption in three rats was recorded for seven days after colon resection in a pilot study. Three groups of four animals in each were given 75, 50 or 25 % of this amount of food respectively. The group given 25 % of "normal" postoperative food consumption induced a similar weight loss as found in 5-FU treated animals and was thus chosen for study IV.

Comments

In study IV the intention was to adjust for the nutritional deprivation caused by 5-FU treatment after a bowel resection. The loss in body weight was found to be due to reduced food intake and thus food restriction where all nutritional components were reduced equally was thought to mimic the "malnutrition" caused by postoperative 5-FU treatment.

Blood tests

Blood samples were taken through a tailcut. Analysis of haemoglobin and albumin were made according to hospital routines and white blood cell count was performed in a Bürger chamber.

Sacrifice

All animals were sacrificed in a CO_2 box. Animals which died before intended sacrifice underwent autopsy to determine cause of death.

Anastomotic complications

At sacrifice the abdomen was explored and macroscopic abscesses and dehiscences recorded (II-VI). The outer diameter of the bowel was determined (II) by a calliper one cm above and one cm below the anastomosis enabling an objective evaluation of obstruction. A ratio between the diameter proximal and distal to the anastomosis was calculated. If the ratio was more than two it was considered a significant stenosis.

Comments

Abscesses and dehiscences causes an increased inflammatory reaction and this could influence anastomotic breaking strength and collagen metabolism (Jiborn et al. 1980). Faecal loading as present in stenosis could also alter healing parameters (Smith et al. 1983, Törnqvist et al. 1989). Thus all animals with anastomotic complications were excluded from further evaluation.

Sampling technique

For studies of collagen and myeloperoxidase the left colon was carefully freed of adhesions and dissected at the mesenteric border. In study I six standardised 5 mm colonic segments were taken from colon within as well as outside the field of irradiation (for details see study I). In studies II-VI marking sutures were placed 5 mm from the anastomosis on each side at sacrifice. After breaking strength test the colon was cut at the suture markings. The segments were further divided longitudinally at the mesenteric and the anti mesenteric line. One part was analysed for hydroxyproline as a marker of collagen and the other part was analysed for myeloperoxidase activity as a marker of neutrophile leukocyte accumulation.

For histological studies in study I two standardised one cm colonic segments were taken, from the left colon 1 cm above the peritoneal reflection (within the irradiated area) and from the transverse colon (outside the field of irradiation).

Comments

In determining biochemical parameters in a biopsy special care must be taken in standardising the segment taken for analysis. Marking sutures must be placed prior to determining breaking strength as this procedure else could influence the size of biopsies. It would be desirable to come as close to the wound gap as possible but in doing so the standardisation may be hampered and as a compromise 5 mm segments on each side of the anastomosis were chosen.

Mechanical strength determination

In studies II, III, IV and VI the anastomotic breaking strength was determined with sutures left in place. In study V animals were randomised into two groups for breaking strength test with or without sutures. Careful removal of sutures under a magnifying lens was made prior to testing anastomotic breaking strength without sutures.

The entire anastomotic segment was mounted in a tensiometer between two clamps one cm apart with the anastomosis perpendicular to the pulling force and in the centre of the gap. The tensiometer provided an increasing force of 0.03-0.05 N/s, and the maximum force at rupture of the anastomosis was recorded as the anastomotic breaking strength.

In study II skin flaps, five mm wide, perpendicular to the incision line were prepared from the abdominal wound after removal of the running suture for determining the skin breaking strength as above.

Comments

The mechanical strength of an anastomosis can be tested by determining either the bursting pressure or the breaking strength. Bursting pressure in the early postoperative phase gives information about the point of lowest strength of the anastomotic circumference. In the healing process the anastomosis becomes more ridged restricting it from widening as much as the intact intestinal wall outside the anastomosis. In testing bursting pressure from the seventh day the rupture usually occurs outside the anastomosis, according to Laplace law. This gives no or only limited information about the healing process of the anastomosis (Nelsen and Anders 1966, Hendriks et al. 1990).

Breaking strength represents the wound healing properties of the entire anastomosis and can also be performed at a later stage in the healing process. When an anastomosis is just completed the breaking strength is almost entirely dependent upon the mechanical properties of the tissue under the sutures (suture holding capacity). During the first three days the suture holding capacity decreases gradually due to lysis of tissue (Blomquist et al. 1984, Högström et al. 1985, Jönsson et al. 1985). As the anastomosis heals new tissue forms in the anastomotic gap and old tissue remodels in the zone adjacent to the wound. Thus determination of breaking strength with sutures in place becomes dependent upon the suture holding capacity plus the newly formed tissue while determination of breaking strength tested without sutures offers information merely about the mechanical properties of newly formed granulation tissue in the wound gap of the anastomosis.

Collagen studies

Specimens were dried to a constant weight and the dry weight of each colonic sample was determined. The samples were hydrolysed in 6 N HCl for 6 hours in an oven at 130^0. Collagen was studied by determining hydroxyproline (hypro) according to a method described by Stegemann and Stalder (1967) as modified by Pikkarainen (1968). The collagen content (hydroxyproline/biopsy) of each segment was calculated.

Collagen synthesis was determined by in vivo labelling with 100 µCi L-2,3,4,5-^{3}H-proline (New England Nuclear, Boston, USA - 142.1 Ci/mmol) injected into the tail vein of the conscious rat 24 hours prior to sacrifice. At sacrifice a 10 mm segment of colon was taken from the anastomosis (5mm on each side). Hydroxyproline in each segment was determined as above. ^{3}H-hydroxyproline of the anastomotic segment was determined according to Juva and Prockop (1966) and radioactivity was measured in a scintillation counter (Wallac 1410, Wallac OY, Turku, Finland). Total collagen synthesis was calculated as dpm-^{3}H-hydroxyproline of the entire segment (dpm-hypro/biopsy). Specific activity of hydroxyproline was calculated as dpm-^{3}H-hydroxyproline in relation to the total amount of hydroxyproline in the segment (dpm-hypro/µg hypro).

Comments

The submucosa of the intestine is dominated by collagen types I and III. These fibrous types of collagen are synthesised by the fibroblast and form copolymers in a fibrillar

structure (Miller 1992). To form a more stable structure intermolecular crosslinkings are formed and the collagen fibers are arranged in a regular cross-ply manner (Komuro 1988). Type IV collagen is also present in the intestine but localised closer to the basement membranes beneath the mucosa (Miller 1992). In synthesis of the collagen, proline is incorporated in one of the first steps. Part of the incorporated proline is hydroxylated thus forming hydroxyproline which constitutes between 8 and 14 % of the collagen molecule (Neumann and Logan 1950, Eastoe 1967). Hydroxyproline is in small amounts found in elastin, acetylcholinesteras and complement factors but the overwhelming amount is found in collagen molecules. Thus the determination of hydroxyproline is considered proportional to the amount of collagen. Collagen can be determined as collagen concentration or collagen content. Collagen concentration determined as total amount of collagen per gram tissue is an evaluation of collagen in relation to other components in the biopsy also liable to undergo alterations during the healing process. Collagen content reflects the total amount of collagen irrespective of other alterations in the standardised biopsy and was thus used in the present study.

In evaluation of the collagen metabolism it is desirable to determine lysis and synthesis of collagen along with the total amount of collagen. In vitro studies have indicated that 5-FU has an influence on the fibroblasts ability to proliferate and synthesise collagen (Hendriks et al. 1993). To further evaluate these findings in an in vivo model we choose to study collagen synthesis using a pulse labelling technique in study V (Jiborn et al. 1980).

Myeloperoxidase assay

Myeloperoxidase activity (MPO) was determined in the colonic specimen by a method described by Bradley (1982). Each biopsy was homogenised in 1.5 ml 0.5 % hexadecyltrimethylammonium bromide (Sigma Chemicals, St. Louis, Mo. USA). MPO was extracted and purified. The change in absorbance at 460 nm was measured in a spectrophotometer at room temperature. One unit of MPO activity was defined as the amount of enzyme reducing 1 µmole peroxide per minute.

Comments

MPO assay correlates well with the number of neutrophils in tissue samples as shown by Lundberg (1983) using ^{51}Cr-labelled cells and by Krawisz (1984) using histological methods. Other sources for peroxidase activity are the mononuclear

inflammatory cells which could be present in the chronic inflammatory reaction. Haemoglobin has a small peroxidase activity not taken into account in the present study. MPO was thus chosen as a marker for inflammatory reaction and in specific the neutrophile accumulation.

Histology

Specimen were fixed in formaldehyde for 24 hours and stained with heamatoxylin and eosin. Examination was performed by one pathologist not informed about treatment group, time of sacrifice or location of biopsy. Each specimen was evaluated for inflammatory reaction, atypia and dysplasia. Each parameter was judged by an arbitrary scale from 0 to 3 (normal, mild, moderate and severe reaction). Inflammatory reaction was judged according to Borgström (1982). Atypia was judged according to degree of degenerative changes in the epithelium and gland crypts (Jeremy 1987). Dysplasia was judged by nuclear changes of preneoplastic nature (Jeremy 1987).

Statistical methods

The mean (m) and standard deviation (SD) were calculated. Comparisons of the means were carried out by Student's t-test for unpaired observations. one-way analysis of variance and when indicated Duncan's multiple range test was used. Differences between proportions were evaluated with X^2 test or Fisher's exact test.

RESULTS

The effect of irradiation (I, II)

Body weight

Irradiated as compared to sham-irradiated animals had a pronounced weight loss initiated at the time of irradiation. Animals in the control group which underwent surgery had a small drop in weight postoperatively where as irradiated animals had an additional more pronounced weight loss. Delayed weight gain was seen in irradiated animals throughout the experiment.

Haemoglobin, albumin and white blood cell count

No differences were seen in Hb and albumin between irradiated and control groups. WBC was significantly reduced in irradiated animals on the day of the second irradiation and four days after completion of irradiation.

Anastomotic complications

Four animals in the irradiated group suffered from complications, two anastomotic abscesses and two stenoses. In sham-irradiated animals there were no complications but there was no statistical difference between groups.

Anastomotic breaking strength

The anastomotic breaking strength was similar in irradiated and sham-irradiated groups in the early postoperative period. On the 56th postoperative day the irradiated animals had significantly lower breaking strength.

Myeloperoxidase activity

During the first eleven days after irradiation myeloperoxidase activity increased in irradiated segments of the left colon with a maximum (a 20 fold increase) eleven days after completion of irradiation. Up to the eleventh day this increase was not seen in segments outside the irradiation field but sixty days after irradiation myeloperoxidase was elevated in irradiated as well as in protected parts of colon.

In the anastomotic segment of operated animals there was a nine fold increase in myeloperoxidase activity in the anastomotic segments compared with the day of operation. This increase in myeloperoxidase activity was seen during the first postoperative week in both the irradiated and sham-irradiated group without difference between the groups.

Histology

In the left colon, within the irradiation field, a moderate to severe inflammatory reaction and atypia was seen, three as well as ten days after irradiation. In addition dysplasia was observed in 6 out of 9 specimens 10 days after irradiation. A mild inflammatory reaction was noted in sham irradiated animals. After 30 days there were no signs of inflammation, atypia or dysplasia with the exception of one animal in the irradiated group at 240 days with moderate inflammation and dysplasia. In the protected transverse colon histological changes were not observed throughout the study period.

Hydroxyproline

There were no differences in hydroxyproline content between irradiated and control animals in neither left nor transverse colon irrespective of time after irradiation or operation with one exception on the second day after operation when the sham-irradiated animals had lower hydroxyproline content in the anastomotic segment than irradiated animals.

The effect of chemotherapy (III, IV, V, VI)

Body weight

All animals had a body weight loss during the initial two postoperative days. 5-FU treated animals continued to loose weight whereas animals given NaCl regained body weight after two to three days. Animals receiving 25 % of "normal" postoperative food intake had an even more pronounced body weight drop than 5-FU treated animals. Animals treated with leucovorin alone did not differ from controls.

Haematological findings

Hb did not differ between groups at any time during the experimental period. WBC was reduced after seven days of 5-FU treatment. In one of the two experiments in study III albumin was lower in animals treated with 5-FU for seven days.

Anastomotic complications

Abscesses or dehiscences were registered in about 25 % of the animals in groups with 5-FU treatment from the day of operation (early treatment) irrespective of 5-FU concentration or if leucovorin was added (III=29%, IV=19%, V=29% and VI=27%). Corresponding rate for NaCl treated animals was about 5% (III=8%, IV=13%, V=6% and VI=0%). In animals with food restriction (IV) no complications were noted. When 5-FU treatment started on the third postoperative day (delayed treatment) no complications were registered.

Breaking strength

Colonic anastomoses

On the seventh postoperative day, anastomotic breaking strength tested **with sutures** (III-VI) was reduced by 40-60% in 5-FU treated animals as compared to controls. When breaking strength was tested **without sutures** a reduction of the same magnitude was found (V). Further on the seventh postoperative day the anastomotic breaking strength of 5-FU treated animals was significantly lower when tested **with sutures** as compared with NaCl treated animals when tested **without sutures** (V).

A restriction to 25 % of normal food intake did not reduce the anastomotic breaking strength (IV). Different intraperitoneal 5-FU concentrations did not influence breaking strength of the anastomosis (IV).

When chemotherapy was postponed for three days after surgery anastomotic breaking strength was similar in 5-FU and NaCl treated animals seven as well as ten days postoperatively (VI).

Abdominal skin wound

Breaking strength of the skin wound tested without sutures was reduced in 5-FU treated animals by 35-55% (III, IV) on the seventh postoperative day as compared to controls. No significant reduction was seen after restriction of food intake.

Myeloperoxidase

There were no differences in MPO activity in the anastomotic segment between 5-FU treated and control animals with the exception of an elevation on the tenth postoperative day in the delayed 5-FU treatment group (VI). In study III MPO was elevated in the anastomotic segment on the seventh postoperative day in groups of animals treated with leucovorin alone or in combination with 5-FU.

Hydroxyproline

The hydroxyproline content of anastomotic segments was not influenced by chemotherapy or restriction in food intake except in study III where hydroxyproline was reduced on the third and seventh postoperative day in the 5-FU group.

Collagen synthesis

During the third postoperative day total collagen synthesis in the anastomotic segment was similar in controls and 5-FU treated animals but during the seventh postoperative day the collagen synthesis was reduced to 50 % in 5-FU treated animals. In control animals an increase of total collagen synthesis was observed from day three to day seven. This increase was not found when 5-FU was given. When calculating specific activity the results where similar to the total collagen synthesis.

GENERAL DISCUSSION

Wound healing is a fundamental process in all species. When special demands are put forth on a healing process as after colorectal surgery with primary anastomosis it is important to minimise all factors, general and local, known to negatively influence the healing process, i.e. to use atraumatic technique, ensure optimal blood supply, use good suture technique and create an anastomosis without tension. When irradiation and chemotherapy as adjuvant treatment for colorectal carcinoma, is superimposed the surgical trauma, it is important to clarify if the treatment affects the healing process and thus the outcome of the operative procedure.

Preoperative irradiation

The difficulties in mimicking the clinical situation in experimental studies is substantial. In every animal model much consideration has to be given to the model used. In creating an experimental model with irradiation the dose is most important. Using the CRE value in calculating the biological effect of irradiation offers an opportunity to come as close as possible to the clinical situation (Kirk et al. 1971). In Scandinavian clinical trials a fractionated dose of 5 Gy given daily for five consecutive days (CRE = 14.59) has been used (Påhlman 1989 et al, Stockholm Rectal Cancer Group 1990). In the rat irradiation requires anaesthesia. In pilot studies daily anaesthesia/irradiation for five days was accompanied by a high mortality. Local irradiation in a two dose scheme 10 + 10 Gy with four days interval was however well tolerated by the animals. This dose (CRE = 14.54) corresponds to the biological effect in the clinical setting described above and was thus chosen for this experimental study.

In using animal models it is also important to protect the rest of the animal to avoid general effects of irradiation with high mortality rate. This could be performed using lead shields with defined openings. By carefully focusing on the lower left colon and rectum general effects could be reduced.

In this study general effects of irradiation were observed as an early decrease in WBC, a more pronounced postoperative body weight loss and a delayed weight development throughout the study period. There was however no reduction in albumin and morbidity was low. In animals receiving irradiation there was a reduction in WBC already at the time of operation. There were however no general infections related to leukopenia in any of the animals.

Irradiation effect varies considerably in different tissues (Withers 1988). The effect of irradiation in the colon of experimental animals has previously been described in terms of histological findings (Hubmann 1981, Breiter and Trott 1986, Dewit et al. 1987, Hauer-Jensen et al. 1990). In the present study we correlated the histological finding to biochemical markers for neutrophile leukocytes i.e. myeloperoxidase, which enabled us to quantify the inflammatory reaction in the bowel wall. This method has not previously been used in this context but is well defined in experimental models of inflammatory bowel disease in the rat (Wallace and Keenan 1990, Yamada et al. 1991).

A gradual increase in myeloperoxidase activity was seen in the left colon of irradiated animals with a maximum on the eleventh day after irradiation indicating a successive accumulation of leukocytes in the early phase. This increase of myeloperoxidase was confined to the irradiated part of the bowel up to eleven days. The non irradiated parts of colon showed no signs of inflammatory reaction neither histologically nor in myeloperoxidase activity at this time. It is notable that the histological findings of inflammatory reaction is 2-4 days earlier than the myeloperoxidase reaction. This could be explained by the fact that neutrophile leukocyte migration is secondary to the cell damage caused by irradiation and detected early by histology.

After two months there was an overall increase in myeloperoxidase activity not confined to the irradiated parts but more general throughout the colon. One explanation for this increase in myeloperoxidase activity might be that it originated from monocytes as being part of a chronic reaction throughout the colon not detected by histology.

The timing of operation in relation to the radiation may be of importance for the effect of irradiation on the anastomotic healing (Degges et al. 1983, Ormistone 1985). In the clinical trials patients usually have surgery within a week after completion of irradiation (Påhlman et al. 1989, Stockholm Rectal Cancer Group 1990). In mimicking this setting the time of operation in our experimental study was set to four days after the completion of irradiation. In our experimental study without operation (I) there were clear histological changes in the bowel wall at the time of intended operation but the inflammatory reaction, as judged by the MPO activity had not at this time reached its peak. The rapid increase in MPO activity in the anastomotic segment elicited by the surgical trauma widely exceeded that caused by irradiation alone. MPO activity within

the anastomotic segment was thus found to be similar in sham-irradiated and irradiated animals.

Anastomotic complications after irradiation were few and did not significantly differ from controls. It is however notable that complications were seen only in the irradiated group. Three out of four complications were observed in the early postoperative phase. Rats have a good capacity to cope with and heal complications. This ability could explain the low number of complications seen at sacrifice in the late postoperative phase.

In sham-irradiated animals the collagen content of the anastomotic segment was reduced on the second postoperative day. This decrease was not found in irradiated animals. One explanation might be that the healing process was already initiated by the trauma and energy brought to the bowel wall by irradiation. An activation of the fibroblasts and collagen synthesis already at the time of surgery could enable them to compensate for the initial collagenolysis found in sham-irradiated animals two days after operation. In spite of this difference in collagen content there was no difference in anastomotic breaking strength in the early postoperative period.

Two months after operation the breaking strength was approximately 20% lower in the irradiated group. At this time breaking strength represents a measure of the intestinal wall rather than the anastomosis. The collagen content in the anastomotic segment at this time was however similar to sham-irradiated animals. A large standard deviation was due to two animals with very high values of collagen content. This could imply an individual variability of reaction to irradiation and that some animals react with fibrosis. Even if these two animals were excluded the collagen content of the anastomotic segment was similar to sham-irradiated animals and anastomotic breaking strength reduced.

When interpreting our results one has to consider that the anastomosis in the rat was localised intraperitoneally, while in the clinical situation after resection of rectal carcinoma the anastomosis is situated below the peritoneal reflection which is known to increase the risk of anastomotic complications. The reason for not choosing a low anterior resection in this model is that rectum in the rat is short and a standardised anastomosis is technically difficult to perform. Another difference from the clinical situation is that in the experimental model we used young and healthy animals whereas patients with rectal carcinoma are mainly elderly people. However in clinical

studies (Gerard 1988, Påhlman et al. 1990, Stockholm Rectal Cancer Group 1990) there were no increase in morbidity caused by anastomotic complications after preoperative irradiation. In the Stockholm study there was an increase in mortality in patients above 70 years, but this was due to cardio-pulmonary complications.

Our experimental findings support that it is safe to perform an anastomosis in the colon within a week after preoperative irradiation in a dose corresponding to a CRE value of 14.0. Late changes in the intestinal wall with impairment of the mechanical properties of collagen might occur and there is probably a marked variability in the individual reaction to irradiation.

Postoperative chemotherapy

This part of the study was designed to resemble an adjuvant treatment of colorectal carcinoma where 5-FU is given intraperitoneally to obtain high drug concentrations locally (Gyves 1985) as well as in the portal vein (Speyer et al. 1981). Folinic acid was given intravenously to achieve schedule dependent synergism (Bleyer 1989).

In previous investigations conflicting results concerning the effect of 5-FU on wound healing have been reported. This is likely to be due to variations in cumulative 5-FU dose and experimental design (Staley et al. 1961, Morris 1979, Hillian et al. 1988). We choose to evaluate 5-FU's effect on wound healing in a setting as close to the adjuvant treatment of colorectal carcinoma as possible. In designing and testing an experimental model we found a five fold increase of anastomotic complications (macroscopic abscesses and dehiscences) after 5-FU treatment and a reduction in anastomotic and skin wound breaking strength by approximately 40% seven days after operation. In looking at the first phase of wound healing, on the third postoperative day, there were no reductions in breaking strength.

Folinic acid is suggested to be used in order to enhance the effect of 5-FU (Bleyer 1989). When folinic acid (leucovorin) was added the negative effect of 5-FU on wound healing was not aggravated nor did folinic acid have a negative effect in itself. This result was independent of the dose of folinic acid used. One explanation to this might be that wound healing after 5-FU treatment is not affected by the synergism of folinic acid. Another possible explanation is that in our experimental model wound healing was already suppressed maximally by the 5-FU dose given.

In the rat considerable weight loss occurs during 5-FU treatment (Staley et al. 1961, Morris 1979, Hillian et al. 1988). Similar weight loss was found in our study. The relevance of this postoperative poor nutritional state for the impairment of wound healing was important to address. Previous experimental studies on malnutrition and wound healing have dealt with preoperative malnutrition and negative influence on wound healing was only found if the malnutrition is substantial and kept for a long period (Daley et al. 1972, Irvin 1974, Ruberg 1984). Weight loss during 5-FU treatment is mainly due to reduced food consumption and hence it was thought relevant to use the method of food restriction in order to mimic the "malnutrition" of 5-FU. In a pilot study with different regimens of food restriction we found that a reduction to 25% of normal postoperative food intake could mimic the weight curve found in 5-FU treated animals. In animals with this highly reduced postoperative food intake anastomotic healing was not compromised suggesting that the negative influence of 5-FU on wound healing is mainly independent of nutritional factors.

In man a relative large volume of intraperitoneal infusion is needed to achieve a homogeneous drug distribution (Rosenhein et al. 1978). A high concentration of 5-FU might negatively influence anastomotic healing by a local - direct toxic effect of 5-FU. However after giving the same dose of 5-FU in a five fold volume of saline the anastomotic healing was still reduced to the same extent as previously found. Further skin wound healing was hampered to the same extent as anastomotic healing suggesting that the negative effect on wound healing is systemic rather than local.

Animals receiving 5-FU treatment had a lower white blood cell count in peripheral blood but there were no general infections observed. One explanation for the impaired anastomotic healing and the large number of anastomotic complications after 5-FU treatment could be an impaired local defence. As 5-FU reduces WBC in the peripheral blood it was important to address the question if this was accompanied by reduced local accumulation of leukocytes in the anastomotic segment. In order to study the local inflammatory reaction myeloperoxidase activity was evaluated in the anastomotic segment as a marker for neutrophile leukocytes. There was no sign of local leukocyte depletion in any of the 5-FU treated groups. This could be explained by an early leukocyte migration to the wound prior to the onset of leukopenia. However, the amount of leukocytes does not necessarily reflect their activity in local defence since the function of the leukocyte might be altered by 5-FU treatment.

Breaking strength of the anastomoses was tested in two ways, with sutures in place and after removal of sutures. Breaking strength tested **with sutures** in place is a measure of the suture holding capacity of the tissue adjacent to the wound plus the breaking strength of newly formed tissue in the wound gap. Testing **without sutures** reflects the breaking strength of the wound gap it self. Seven days after operation the strength is mainly dependent on newly formed collagen in the granulation tissue (Blomquist et al. 1984). 5-FU treatment initiated at the day of operation reduced the anastomotic breaking strength with 40% when tested seven days after operation both **with** and **without** sutures (V). In fact the anastomotic breaking strength in 5-FU treated animals was lower when tested with sutures than in control animals tested without sutures. This implies an inability in 5-FU treated animals to overcome the lysis under the sutures by formation of new collagen as well as a marked reduction in formation of new tissue in the wound gap. These findings could be due either to an increase in the lysis and/or to a reduction in the synthesis of collagen. Three days postoperatively we found anastomotic breaking strength tested with sutures to be similar between 5-FU treatment and controls (III). During this inflammatory phase most of the lysis take place and it is therefore likely that there is no difference in lysis caused by 5-FU.

Collagen content in the anastomotic segment was reduced in study III after 5-FU treatment but this was not a consistent finding. In order to evaluate the newly synthesised collagen the incorporation and hydroxylation of ^{3}H-proline was determined in the anastomotic segment by an in vivo method (V). In the early healing course on the third postoperative day there was no difference found in total collagen synthesis between treatment groups. During the seventh postoperative day total collagen synthesis was reduced in 5-FU treated animals as compared with controls. In the control group collagen synthesis increased in the anastomotic segment up to the seventh postoperative day, which is in consistency with earlier studies (Jiborn et al. 1980). In animals receiving 5-FU treatment however collagen synthesis remained low on the seventh postoperative day. Thus our results suggest that 5-FU has a negative influence on the synthesis of collagen in the anastomosis.

In cell cultures 5-FU has a negative effect on skin fibroblasts (but not colon fibroblast) ability to respond to stimulation by TGFß (Hendriks et al. 1993). The fibroblasts fail to respond in proliferation as well as in their ability to synthesise collagen. When not stimulated they keep their basal synthesis of collagen intact. A most potent environment for stimulating the fibroblast is the healing wound. In man collagen

accumulation was reduced in polytetrafluoroetylen grafts implanted subcutaneously after adjuvant treatment with intraperitoneal 5-FU and intravenous leucovorin for six days (Graf et al. 1992).

An increase in mRNA for procollagen can be observed during the first days after experimental wound healing studies (Miller and Gay 1992) and synthesis of collagen gradually increases from day two to day seven (Jiborn et al. 1980). The early stimulation of the fibroblasts is essential for both proliferation and synthesis. If cortisone is administered in the early postoperative phase it exerts a negative effect on anastomotic healing. This could be avoided by postponing treatment until the third postoperative day (Sandberg 1964). Cortisone affects collagen synthesis by decreasing procollagen mRNA gene expression (Fuller and Cutroneo 1992). Hence it is likely that once mRNA is formed, and synthesis initiated, collagen (and other proteins) will continue to be synthesised by the fibroblasts. Thus it might be important not to use substances known to interfere with this mechanism in the very early phase of wound healing in order to avoid complications. In an attempt to evaluate this theory a postponement by three days of the 5-FU treatment was tested in study VI. With such postponement all the observed negative effects of 5-FU on wound healing were avoided. Our study thus supports the hypothesis that once the initiation of proliferation and synthesis is established the healing course does not seem to be hampered by 5-FU.

The rationale for early postoperative adjuvant treatment is that the probability for cure is inversely related to the number of malignant cells. Further in experimental studies surgery promotes the growth of residual tumour cells thus making them more susceptible to cytotoxic agents (Fisher et al. 1983). A short delay is not likely to be of major disadvantage in adjuvant therapy from these standpoints.

In the clinical situation of adjuvant therapy for colorectal carcinoma it could be of major importance to postpone treatment for two reasons. Firstly complications from defect anastomotic healing could be minimised and secondly it would be possible to determine the histopathological diagnosis for selection of patients prior to the intended treatment. So far the subgroup shown to benefit from adjuvant therapy is Dukes' group C.

CONCLUSIONS

Preoperative irradiation in a setting resembling adjuvant treatment for rectal carcinoma:

- caused acute localised inflammatory reaction in the colonic wall as judged by histology and myeloperoxidase activity.
- did not negatively influence healing in the left colon anastomosis performed four days after completion of irradiation.
- may lead to late changes of chronic inflammation and fibrosis within the colonic wall.

Immediate postoperative intraperitoneal 5-FU treatment in a setting resembling adjuvant treatment for colorectal carcinoma:

- caused an increase in anastomotic and abdominal wound complications.
- had a detrimental effect on anastomotic and skin wound healing. The negative effect of 5-FU on wound healing was largely independent of nutritional factors and drug concentration and was not altered by the addition of folinic acid.
- negatively influenced collagen synthesis in the anastomotic segment and reduced the strength of granulation tissue in the anastomotic gap of colon anastomoses.

Postoperative intraperitoneal 5-FU treatment postponed by three days:

- did not cause an increase in anastomotic complication.
- did not negatively influence anastomotic healing.

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REFERENCES

Alarcon J, Greenwood GR. Adenocarcinoma of the colon and rectum: a review of surgical treatment in 302 patients. Dis Colon Rectum 1979; 22: 35-39.

Arbuck SG. Overview of clinical trials using 5-fluorouracil and leucovorin for treatment of colorectal cancer. Cancer 1989; 63: 1036-1044.

Astler J, Coller FA. The prognostic significans of direct extension of carcinoma of the colon. Ann Surg 1954; 139: 846-851.

Balslev I, Pedersen M, Teglbjaerg PS, et al. Postoperative radiotherapy in Dukes'B and C carcinoma of the rectum and rectosigmoid. A randomized multicenter study. Cancer 1986; 58: 22-28.

Beart RW, Moertel CG, Wieand HS, et al. Adjuvant therapy for resectable colorectal carcinoma with fluorouracil administered by portal vein infusion. Arch Surgery 1990; 125: 897-901.

Berge T, Ekelund G, Mellner C, Pihl B, Wenchert A. Carcinoma of the colon and rectum in a defined population. Acta Chir Scand 1973, suppl. 438.

Bleyer WA. New vistas for leucovorin in cancer chemotherapy. Cancer 1989; 63: 995-1007.

Borgström S, Aronsen KF, Dougan P, Jacobsson L, Lindström C, Nylander G A. Study of the radioprotective effect of vasopressin induced ischaemia on the small intestine of the dog and its relation to circulatory parameters. Br J Radiol 1982; 55: 568-573.

Bradley PP, Priebat DA, Christiansen RD, Rothstein G. Measurements of cutaneous inflammation: estimation of neutrophile content with an enzyme marker. J Invest Dermatol 1982; 78. 206-209.

Breiter N, Trott KR. Chronic radiation damage in the rectum of the rat after protracted fractionated irradiation. Radiother Oncol 1986; 7: 155-163.

Bubrick MP, Rolfsmeyer ES, Schauer RM, Feeny DA, Johnston GR, Strom RL, Hitchkock CR. Effects of high-dose and low-dose preoperative irradiation on low anterior anastomosis in dog. Dis Col & Rect 1982; 25: 406-415.

Buyse M, Zeleniuch-Jacquotte A, Chalmers TC. Adjuvant therapy of colorectal cancer. Why we still don't know. JAMA 1988; 259: 3571-3578.

Campora E, Esposito M, Civalleri D, et al. Serum, urine an peritoneal fluid levels of 5-FU following intraperitoneal administration. Anticancer Res 1987; 7: 829-832.

Cory JG, Breland JC, Carter GL. Effect of 5-fluorouracil on RNA metabolism in Novikoff hepatoma cells. Cancer Res 1979; 39: 4905-4913.

Crowley LG, Anders CJ, Nelsen T, Bagshaw M. Effect of radiation on canine intestinal anastomoses. Arch Surg 1968; 96: 423-428.

Cunliffe WJ, Sugerbaker PH. Gastrointestinal malignancy: rationale for adjuvant therapy using early postoperative intraperitoneal chemotherapy. Br J Surg 1989; 76: 1082-1090.

Daly JM, Vars HM, Dudrick SJ. Effects of protein depletion on strength of colonic anastomoses. Surg Gynecol Obstet 1972; 134: 15-21.

Danenberg PV, Heidelberger C, Mulkins MA, Peterson AR. The incorporation of 5-fluoro-2'-deoxyuridine into DNA of mammalian tumour cells. Biochem Biofys Res Commun 1981; 102: 654-658.

Davis NC, Evans EB, Cohen JR, Theile DE, Job DM. Colorectal cancer: a large unselected Australian series. Aust N Z J Surg 1987; 57: 153-159.

Degges RD, Cannon DJ, Land NP. The effects of preoperative radiation on healing of rat colonic anastomosis. Dis Col & Rect 1983; 26: 598-600.

Dewit L, Oussoren H, Bartelink H. Early and late damage in the mouse rectum after irradiation and cis-diamminedichloroplatinum(II). Radiother Oncol 1987; 8: 57-69.

Doroshow JH, Multhauf P, Leong L, et al. Prospective randomized comparison of fluorouracil versus fluorouracil and high-dose continuous infusion leucovorin calcium for the treatment of advanced measurable colorectal cancer in patients previously unexposed to chemotherapy. J Clin Oncol 1990; 8: 491-501.

Dukes CE. The classification of cancer of the rectum. J Pathol 1932; 35: 323-332.

Eastoe JE. Composition of collagen and allied proteins in: Treaties in collagen. Edited by Ramachandran GN. Academ Press, London. 1967; Chapter 1:1-72.

Enblad P, Adami HO, Bergström R, Glimelius B, Krusemo UB, Påhlman L. Improved survival of patients with cancer of the colon and rectum?. J Natl Cancer Inst 1988; 80: 586-591.

Ferrara JJ, Martin EW, Carey LC. Morbidity of emergency surgery in patients with metastatic cancer receiving chemotherapy. Surgery 1982; 92: 605-609.

Fielding LP, Hittinger R, Grace RH, Fry JS. Randomised controlled trial of adjuvant chemotherapy by portal-vein perfusion after curative resection for colorectal adenocarcinoma. Lancet 1992; 340. 502-506.

Fisher ER, Turnbull RB. The cytologic demonstration and significance of tumour cells in the mesenteric venous blood in patients with colorectal carcinoma. Surg Gynecol Obstet 1955; 100: 102-108.

Fisher B, Gunduz N, Saffer EA. Influence of the interval between primary tumour removal and chemotherapy on kinetics and growth of metastases. Cancer Res 1983; 43: 1488-1492.

Fisher B, Wolmark N, Rockette H, et al. Postoperative adjuvant chemotherapy or radiation therapy for rectal cancer: results from NSABP Protocol R-01. J Natl Cancer Inst 1988; 80: 21-29.

Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, and man. Cancer Chemother Rep 1966; 50: 219-244.

Fuller G, Cutroneo K. Pharmacological interventions in Wound Healing, Biochemical and Clinical Aspects. Edited by Cohen, Diegelmann, Lindblad. Saunders, 1992:Chapter 19; 305-315

Gastrointestinal Tumor Study Group. Prolongation of the disease-free interval in surgically treated rectal carcinoma. N Engl J Med 1985;312:1465-1472.

Gerard A, Buyse M, et al. Preoperative radiotherapy as adjuvant treatment in rectal cancer. Ann Surg 1988; 208: 606-614.

Gilbert JM, Jeffrey I, Evans M, Kark AE. Sites of recurrent tumour after 'curative' colorectal surgery: implications for adjuvant therapy. Br J Surg 1984; 71: 203-205.

Glass RE, Ritchie JK, Thompson HR, Mann CV. The results of surgical treatment of cancer of the rectum by radical resection and extended abdominal-iliac lymphadenectomy. Br J Surg 1985; 72: 599-601.

Goldman LI, Lowe S, Al-Saleem T. Effect of fluorouracil on intestinal anastomoses in the rat. Arch Surg 1969; 98: 303-304.

Graf W, Ivarsson M, Gerdin B, Hellsing K, Påhlman L, Glimelius B. The influence of early postoperative intraperitoneal chemotherapy on human wound healing. In Thesis, Uppsala 1992.

Gray R, James R, Mossman J, Stenning S. AXIS - A suitable case for treatment. Br J Cancer 1991; 63: 841-845.

Gunderson LL, Sosin H, Levitt S. Extrapelvic colon-areas of failure in a reoperation series: implication for adjuvant therapy. Int J Radiation Oncology Biol Phys 1985; 11: 731-741

Gyves J. Pharmacology of intraperitoneal infusion 5-fluorouracil and mitomycin-C. Sem in Oncol 1985; 12: 29-32.

Habib NA, Peck MA, Sawyer CN, Blaxland JW, Luck RJ. An analysis of the outcome of 301 malignant colorectal tumours. Dis Colon Rectum 1983; 26: 601-605.

Hauer-Jensen M, Poulakos L, Osborne JW. Intestinal complications following accelerated fractionated X-irradiation. An experimental study in the rat. Acta Oncol 1990; 29: 229-234.

Heald RJ, Leicester RJ. The low stapled anastomosis. Dis Colon Rectum 1981; 24: 437-444.

Hendriks Th, Martens MFWC, Huyben CMLC, Wobbes Th. Inhibition of basal and TGFß-induced fibroblast collagen synthesis by antineoplastic agents. Br J Cancer 1993;67(3):545-550.

Hendriks Th, Mastbo· n WJB. Healing of experimental intestinal anastomoses, parameters for repair. Dis. Col. & Rect. 1990; : 891-901.

Higgins GA, Amadeo JH, McElhinney J, McCaughan JJ, Keehn RJ. Efficacy of prolonged intermittent therapy with combined 5-fluorouracil and methyl-CCNU following resection for carcinoma of the large bowel. Cancer 1984; 53: 1-8.

Hillan K, Nordlinger B, Ballet F, Puts JP, Infante R. The healing of colonic anastomoses after early intraperitoneal chemotherapy: an experimental study in rats J Surg Res 1988; 44: 166-171.

Hubmann FH. Effect of X irradiation on the rectum of the rat. Br J Radiol 1981; 54: 250-254.

Högström H, Haglund H. Postoperative decrease in suture holding capacity in laparotomy wounds and anastomoses. Acta Chir Scand 1985; 151: 533-535.

Irvin TT. Effects of malnutrition and hyperalimentation on wound healing. Surg Gynecol Obstet 1978; 146: 33-37.

Irvin TT, Hunt TK. Effect of malnutrition on colonic healing. Ann Surg 1974; 180: 765-772.

Jeremy RJ. The large intestine in: Systemic Pathology/Third edition, Alimentary tract. Edited by Morson BC. Churchill Livingstone 1987;Chapter 8:313-391.

Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomoses. Collagen metabolism in the colon after left colon resection. Am J Surg 1980; 139: 398-405.

Kirk J, Gray WM, Watson ER. Cumulative radiation effect. Part I. Fractionated treatment regimes. Clin Radiol 1971; 22: 145-155.

Komuro T. The lattice arrangement of collagen fibres of the submucosa of the rat small intestine: scanning electron microscopy Cell Tissue Res 1988; 251: 117-121.

Koyama Y, Moriya Y, Hojo K. Effects of extended systemic lymphadenectomy for adenocarcinoma of the rectum - significant improvement of survival rate and decrease of local recurrence. Jpn J Clin Oncol 1984; 14: 623-632.

Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Gastroenterology 1984; 87: 1344-1350.

Köhne-Wömpner CH, Schmoll HJ, Harstrick A, Rustum YM. Chemotherapeutic strategies in metastatic colorectal cancer: an overview of current clinical trials. Semin Oncol 1992; 19: 105-125.

Laurie JA, Moertel CG, Flemming TR, et al. Surgical adjuvant therapy of large-bowel carcinoma: an evaluation of levamisol and the combination of levamisol and fluorouracil. J Clin Oncol 1989; 7: 1447-1456.

Lavin P, Mittelman A, Douglass H, Engström P, Klaassen D. Survival and response to chemotherapy for advanced colorectal adenocarcinoma. Cancer 1980; 46: 1536-1543.

Lundberg C, Arlfors K-E. Polymorphonuclear leucocyte accumulation in inflammatory dermal sites as measured by 51Cr-labeled cells and myeloperoxidase. Inflammation 1983; 7: 247-255.

Martens MFWC, Hendriks T, Wobbes T, De Pont JM. Intraperitoneal cytostatics impair early post-operative collagen synthesis in experimental intestinal anastomoses. Br J Cancer 1992; 65: 649-654.

Metzger U, Laffer U, Aeberhard P, et al. Randomized multicenter trial of adjuvant intraportal chemotherapy for colorectal cancer SAKK 40/81. Acta Chir Scand 1990; 156: 467-474.

Miller E, Gay S. Collagen structure and function in Wound Healing, Biochemical and Clinical Aspects. Edited by Cohen, Diegelmann, Lindblad. Saunders, 1992:Chapter 8; 130-151.

Moertel CG, Flemming TR, Macdonald JS, et al. Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. N Engl J Med 1990; 322: 352-358.

Moertel CG, Flemming TR, Macdonald JS, Haller O, Laurie J. The intergroup study of fluorouracil (5-FU) plus levamisol (LEV) as an adjuvant therapy for stage C colon cancer. A final report. (Abstract). American Society of Clinical Oncology 1992; 11: 457.

Moore GE, Sako K, Kondo T, Badillo J, Burke E. Assessment of the exfoliation of tumour cells into the body cavities. Surg Gynecol Obstet 1961; 112: 469-474.

Morgenstern L, Sanders G, Wahlstrom E, Yadegar J, Amodeo P. Effect of preoperative irradiation on healing of low colorectal anastomoses. Am J Surgery 1984; 147: 246-249.

Morris T, Lincoln F, Lee A. The effect of 5-fluorouracil on abdominal wound healing in rats. Aust N Z J Surg 1978; 48: 219-221.

Morris T. Retardation of healing of large-bowel anastomoses by 5-fluorouracil. Aust N Z J Surg 1979; 49: 743-745.

Murphy K, Frith C, Lang N, Westbrook KC. Effect of radiotherapy on healing of colonic anastomoses. Surg. Forum 1981; : 222-223.

Nelsen TS, Anders CJ. Dynamic aspects of small intestinal rupture with special consideration of anastomotic strength. Arch Surg 1966; 93: 309-314.

Neumann RE, Logan MA. The determination of collagen and elastine in tissues. J Biol Chem 1950;186:549-566.

NIH Consensus Conference. Adjuvant therapy for patients with colon and rectal cancer. J A M A 1990; 264: 1444-1450.

Nilsson E, Gregersen NP, Hartvig B, Sjödahl R. Carcinoma of the colon and rectum. Results of treatment in 284 cases. Acta Chir Scand 1984; 150: 177-182.

Ormiston MCE. A study of rat intestinal wound healing in the presence of radiation injury. Br J Surg 1985; 72: 56-58.

Poon MA, O'Connel MJ, Moertel CG, et al. Biochemical modulation of fluorouracil: evidence of significant improvement of survival and quality of life in patients with advanced colorectal carcinoma. J Clin Oncol 1989; 7: 1407-1418.

Påhlman L, Glimelius B. Pre- and postoperative radiotherapy in rectal and rectosigmoid carcinoma: report from a randomized multicenter trial. Ann Surg 1990; 211: 187-195.

Påhlman L, Glimelius B. Radiotherapy additional to surgery in the management of primary rectal carcinoma. Acta Chir Scand 1990; 156: 475-485.

Rohrer DA, Saclarides T, Bhattacharyya A, Bapna MS. Effect of intraoperative irradiation on tensile strength of small bowel anastomoses. Dis Colon Rectum 1990; 33: 27.

Rosenhein N, Blake D, Mcintyre PA, et al. The effect af volume on distribution of substances instilled in the peritoneal cavity. Gynecol Oncol 1978; 6: 106-110.

de Roy van Zuidewijn DBW, de van, Hendriks T, Wobbes T, Klompmakers AA, de Boer HHM. Healing of experimental colonic anastomoses: effect of antineoplastic agents. Eur J Surg Oncolog 1987; 13: 27-33.

de Roy van Zuidewijn DBW, de van, Hendriks T, Wobbes T, Klompmakers AA, de Boer HHM. The effect of antineoplastic agents on the healing of small intestinal anastomoses in the rat. Cancer 1986; 58: 62-66.

Ruberg RL. Role of nutrition in wound healing. Surgical Clinics of North America 1984; 64: 705-714.

Saclarides TJ, Woodard DO, Bapna M, Economou SG. Fibrin glue improves the healing of irradiated bowel anastomoses. Dis Colon Rectum 1992; 35: 249-252.

Sandberg N. Time relationship between administration of cortisone and wound healing in rats. Acta Chir Scand 1964; 127: 446-455.

Santi DV, McHenry CS, Sommer H. Mechanism of interaction of thymidylate synthetase with 5-fluorodeoxyuridylate. Biochemistry 1974; 13: 471-480.

Schauer RM, Bubrick MP, Feeney DA, Johnston GR, Rolfsmeyer ES, Strom RL, Hitchcock CR. Effects of low-dose preoperative irradiation on low anterior anastomosis in dog. Dis Col & Rect 1982; 25: 401-405.

Smith SRG, Connolly JC, Gilmore OJA. The effect of faecal loading on colonic anastomotic healing. Br. J. Surg. 1983; 70: 49-50.

Speyer JL, Collins JM, Dedrick RL, et al. Phase I and pharmacological studies of 5-fluorouracil administered intraperitoneally. Cancer Res 1980; 40: 567-572.

Speyer JL, Sugarbaker PH, Collins JM, Dedrick RL, Klecker RW, Myers CE. Portal levels and hepatic clearance of 5-fluorouracil after intraperitoneal administration in humans. Cancer Res 1981; 41: 1916-1922.

Staley CJ, Trippel OH, Preston FW. Influence of 5-fluorouracil on wound healing. Surgery 1961; 49: 450-453.

Stegemann H, Stalder K. Determination of hydroxyproline. Clin Chim Acta 1967; 18: 267-273.

Stockholm Rectal Cancer Study Group. Preoperative short-term radiation therapy in operable rectal carcinoma. Cancer 1990; 66: 49-55.

Sugarbaker PH, Cunliffe WJ, Belliveau J, de Bruijn EA, Graves T, Mullins RE, Schlag P. Rational for integrating early postoperative intraperitoneal chemotherapy into the surgical treatment of gastrointestinal cancer. Seminars in Oncology 1989; 16: 83-97.

Swedish Cancer Registry. Cancer incidence in Sweden 1989.

Taylor I, Machin D, Mullee M, Trotter G, Cooke T, West C. A randomized controlled trial of adjuvant portal vein cytotoxic perfusion in colorectal cancer. Br J Surg 1985; 72: 359-363.

Turnbull RB, Kyle K, Watson FR, Spratt J. Cancer of the colon: the influence of the no-touch isolation technique on survival rates. Ann Surg 1967; 166: 420-427.

Turunen MJ, Peltokallio P. Surgical results in 657 patients with colorectal cancer. Dis Colon Rectum 1983; 26: 606-612.

Törnqvist A, Blomquist P, Jiborn H, Zederfeldt B. Anastomotic healing after resection of a left colon stenosis. Effect on collagen metabolism and anastomotic strength. Dis Col & Rect 1990;33:217-221.

Wallace JL, Keenan CM. An orally active inhibitor of leucotriene synthesis accelerates healing in a rat model of colitis. Am J Physiol 1990; 258: 527-534.

Wereldsma JCJ, Bruggink EDM, Meijer WS, Roukema JA, van Putten WLJ. Adjuvant portal liver infusion in colorectal cancer with 5-fluorouracil/heparin versus urokinase versus control. Results of a prospective randomized clinical trial (colorectal adenocarcinoma trial 1). Cancer 1990; 65: 425-432.

Windle R, Bell PRF, Shaw D. Five year results of a randomised trial of adjuvant 5-fluorouracil and levamisole in colorectal cancer. Br J Surg 1987; 74: 569-572.

Wolmark N, Fisher B, Rockette H, et al. Postoperative adjuvant chemotherapy or BCG for colon cancer: results from NSABP protocol C-01. J Natl Cancer Inst 1988; 80: 30-36.

Wolmark N, Rockette H, Wickerham DL, et al. Adjuvant therapy of Dukes' A, B, and C adenocarcinoma of the colon with portal-vein fluorouracil hepatic infusion: preliminary results of National Surgical Adjuvant Breast and Bowel Project protocol C-02. J Clin Oncol 1990; 8: 1466-1475.

Yamada Y, Marshall S, Specian RD, Grisham MB. A comparative analysis of two models of colitis in the rat. Gastroenterology 1992; 102: 1524-1534.

Öhman U. Colorectal carcinoma - trends and results over a 30 year period. Dis Colon Rectum 1982; 25: 431-440.