Combined Preoperative Irradiation and Local Hyperthermia Delays Early Healing of Experimental Colonic Anastomoses

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Objective: To determine if a combination of preoperative irradiation and local hyperthermia of a colonic segment is detrimental to subsequent early anastomotic healing.

Design: A prospective randomized experimental trial.

Setting: An animal research laboratory.

Interventions: Eighty male Wistar rats were randomly divided into 4 groups. In each animal, a segment of the colon was treated successively by (sham) irradiation and (sham) hyperthermia. After 5 days, a colonic resection was performed and an anastomosis was constructed; the distal limb consisted of (sham) irradiated, (sham) hyperthermia-treated bowel. The rats were killed 3 or 7 days after surgery.

Main Outcome Measures: Body weight, serum albumin and protein levels, anastomotic bursting pressure, breaking strength, and hydroxyproline content.

Results: All animals tolerated (sham) treatment well. Weight was diminished, though not notably, in treated animals vs the control group. After combined preoperative irradiation and hyperthermia, the frequency of local anastomotic complications increased: 4 of 20 animals had a covered perforation when they were killed. In this group, the bursting pressure was lower 3 days after the operation (P=.008). The breaking strength was also lower but not notably. The serum albumin level was significantly lower in this group vs the control group (P=.006); the serum protein level was not decreased. After 7 days, no differences existed between the groups. The hydroxyproline content of the anastomotic tissue was notably higher in rats treated with radiation plus hyperthermia vs control rats (in both the 3- and 7-day groups). The anastomotic hydroxyproline concentration did not differ between the groups.

Conclusions: The combination of preoperative irradiation and hyperthermia results in increased local anastomotic complications. Anastomotic strength is at risk in the first days after the anastomotic reconstruction. Preoperative irradiation or hyperthermia alone does not lead to impaired anastomotic healing in the early phase.

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Regarding the surgical treatment of colorectal disease, discussion continues about the role of adjuvant treatment modalities. Not all cancers can be cured by surgery alone. Along with distant metastases, the threat of a microscopic disease or a perioperative tumor spill resulting in local recurrence exists. In addition, some tumors have grown beyond local resectability before surgery can be performed. In the latter patients, preoperative radiotherapy can help to obtain local control and to increase resectability.1 The application of hyperthermia is a promising method for increasing the efficacy of radiation therapy.

The biological effects of combined hyperthermia and radiotherapy are well established, although the mechanisms of interaction seem complex.2 Enhanced treatment effects are to be expected in 2 ways.3 The first is the killing effect of heat itself on the hypoxic tumor cells. The second is the enhancement of the radiobiological effect of irradiation. Consequently, lower radiation doses are needed to obtain the same treatment result. Thus, surrounding tissues that are located in the irradiated volume will receive smaller doses of radiation, lessening the potential side effects. In patients with rectal cancer, surgical treatment usually consists of an abdominoperineal resection or a lower anterior resection. In the latter cases, an anastomosis is constructed; after combined prior adjuvant treatment, the fixed, distal limb has received the full dose of radiation plus heat treatment.

Little is known about early anasto-
MATERIALS AND METHODS

ANIMALS

Eighty young-adult, male, outbred Wistar/Cpb:WU rats (body weight=255 g, SD±12.75 g) were used. They received water and standard laboratory chow (diet AM II, Hope Farms, Woerden, the Netherlands) ad libitum. The rats were randomly divided into 4 groups of 20: a control group and 3 experimental groups, which received irradiation (I), hyperthermic treatment (H), or both (I+H). In rats in the I and the I+H groups, part of the colon was irradiated 5 days prior to the operation. Animals in the control and the H groups received sham irradiation, and those in the control and the I groups received sham hyperthermic treatment. All of the animals underwent colonic resection and anastomotic construction; they were killed 3 or 7 days (n=10 for both days) after surgery.

This study was approved by the Animal Ethics Review Committee of the Faculty of Medicine, University of Nijmegen, Nijmegen, the Netherlands.

IRRADIATION, DOSIMETRY, AND HYPERTERMIA

To receive irradiation or hyperthermic treatment, the animals were anesthetized with pentobarbital sodium intraperitoneally. While receiving anesthesia, the core temperature of the animals was kept at a normal level by the use of a heat lamp.

The irradiation procedure was based on techniques developed in a prior experiment (J.B., J.H., T.W., and T.H., unpublished data, 1996). To ascertain that the same tissue area was irradiated in each rat and to mark this area for subsequent surgery, a laparotomy was performed. The colonic segment to be irradiated, which was 1 to 3.2 cm proximal to the rectoperitoneal fold (Figure 1), was marked by a serosal stitch at its proximal border. The irradiated area measured 2.2x0.5 cm. The adjacent bowel and other organs were covered with a lead cone, and the rest of the body was also shielded with lead (thickness, 2.5 mm). Radiation dosimetry was performed by means of thermoluminescent dosimeters and film densitometry in separate animals. Irradiation was performed with a 250-kV x-ray unit that had a 1-mm copper filter (target colon distance, 25 cm). The dose of radiation was 1.29 Gy/min. All of the rats in the I and the I+H groups received a dose of 25 Gy. Animals in the control and the H groups were treated similarly without actually being irradiated.

To deliver hyperthermic treatment, water washing through the distal colon and rectum was used (Figure 1). A small, noninjuring clamp was placed on the colon at the level of the marking stitch to isolate this bowel segment from the proximal colon, and a 1.75-mm plastic tube was gently inserted into the rectum up to the clamp. This tube was used to pump clean tap water through a coil, which was immersed in a temperature-regulated water bath, into the rectal cavity (flow rate, 132 mL/min), while a small, intrarectal temperature probe was used to monitor the intraluminal temperature. A second 1.75-mm tube served as a drain, preventing increased pressure inside the rectal cavity. The infusion of water into the rectum was continued for 30 minutes while the intrarectal temperature was kept at 44°C (this temperature was usually reached within 3 minutes [H and I+H groups]). The intrarectal temperature quickly returned to normal after the heating was discontinued. Subsequently, the clamp was removed from the bowel, and the abdomen was closed with a running catgut suture for the fascia and staples for the skin. Therefore, the animals in the H and the I+H groups received either hyperthermia or a radiation dose that had moderate adhesions at the time of resection. The bowel wall increased in diameter because of edema, and it was more vulnerable in these animals vs the sham-treated animals. These rats also suffered from minimal to moderate diarrhea.

In rats that received both treatments, some gross abnormalities were found. Six rats in the I+H group had small patches of transmural bowel-wall necrosis immediately distal from the marking stitch in the control segment that was removed during surgery. Four rats had anastomotic leakage, which was apparent by a covered perforation when they were killed. Small abscesses around the anastomoses were occasionally seen in all groups: in the control group, 2; in the I group, 3; in the H group, 1; and in the I+H group, 2. Rectal stasis or functional stenosis with a proximal dilated colon was seen in 2 rats, 1 each in the I and the H groups.

BODY WEIGHT

After receiving the first dose of anesthesia, all the rats lost weight. Hyperthermia-treated animals (ie, the H and the
received hyperthermic treatment from 10 minutes after the conclusion of the (sham) irradiation procedure onward. The animals in the control and the I groups were treated similarly with water of a normal body temperature (37°C).

OPERATIVE PROCEDURE

After 5 days, the animals were anesthetized again intraperitoneally with pentobarbital. The median laparotomy wound was opened and a 1.6-cm colonic segment was resected. This segment was identified by the marking stitch left during the initial laparotomy and measured 0.5 cm proximally and 1.1 cm distally (Figure 1). Thus, the proximal limb to be used for the anastomosis consisted of nonirradiated, unheated tissue, while the first 1.1 cm of the distal limb had been irradiated (ie, for the I group), heated (ie, for the H group), or both (ie, for the I+H group). Continuity was restored by an inverted, 1-layer end-to-end anastomosis with 8 interrupted monofilament sutures (Ethilon 8-0, Ethicon, Norderstedt, Germany) using microsurgical techniques. The fascia and the skin were closed with a catgut running suture and staples, respectively.

ANALYTICAL PROCEDURES

The condition of the animals was monitored, and their weight was measured daily. Three or 7 days after the operation, the rats were killed by cardiac puncture. A blood sample was obtained to measure albumin and protein levels to monitor the general nutritional condition of the animals. The abdomen was inspected for adhesions, rectal stenosis, abscesses, or other abnormalities. The anastomoses were resected en bloc. Healing was assessed by measuring the anastomotic strength and the hydroxyproline content. To determine the anastomotic strength, the anastomotic segment was washed in a saline solution and connected to an infusion pump on one side while the other side was clamped. A manometer was connected by a side line. The bursting pressure was measured by raising the intraluminal pressure by infusing a methylene blue–saline solution at a rate of 2 ml/min. The procedure was performed in water for better visualization of the bursting site. The bursting pressure was defined as the maximal intraluminal pressure the segment resisted, which was expressed in millimeters of mercury. The bursting site was noted. The breaking strength of the segment, as a measure of the resistance to longitudinal forces, was measured immediately after determination of the bursting pressure. The segment was placed in a tensiometer that provided a constantly increasing distraction. The peak force (in gravity) necessary to induce the total disruption of the segment was perceived as the breaking strength. The breaking site was noted. Then, adhesions and fat tissue were removed from the segment, and a 0.5-cm sample containing both sides of the suture line was collected (Figure 1), stored in liquid nitrogen, and assayed for hydroxyproline content.

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Anastomotic samples were weighed, lyophilized, pulverized, and stored at −30°C. The hydroxyproline content, as a measure of the collagen content, was measured by high-performance liquid chromatography after hydrolysis with 6N hydrochloric acid and derivatization with dabsylchloride.

Differences between the control group and the 3 experimental groups were tested for significance (P=.03) using a 2-tailed Mann-Whitney U test. To correct for the fact that multiple comparisons were made, pairwise comparisons were performed using a level of significance of α'=α/k, where k is the total number of pairwise comparisons. Thus, differences between groups were considered significant (α=.05 at P<α', where α'=0.03).

I+H groups) seemed to lose more weight than those in the other groups (Figure 2). However, recovery seemed to be similar in all groups from postoperative day 2 onward. Colonic surgery induced a transient loss of body weight, but the size of this effect and the subsequent course of recovery were similar in all groups. No notable differences in body weight were found between groups on the day that the rats were killed.

SERUM ALBUMIN AND PROTEIN

The levels of serum albumin and protein are given in the Table. Three days after the operation, the serum albumin level was slightly but significantly (P=.006) lower in the I+H group. This difference was not found after 7 days. The only significant (P=.01) reduction in serum protein levels was found in the I group after 7 days.

ANASTOMOTIC STRENGTH

Individual measurements of bursting pressure and bursting site area are depicted in Figure 3. In all groups, the bursting strength increased between days 3 and 7 after the operation. The average bursting pressure in the I+H group was significantly (P=.008) reduced vs the control group 3 days after the operation. In addition, the bursting site was more often within the suture line in the treatment groups vs the control group, particularly in the I+H group.

Seven days after the operation, anastomoses usually ruptured outside the anastomotic area, indicating that anastomotic healing had progressed beyond the values of normal bowel-wall resistance. The average bursting pressures in the experimental groups did not differ notably from that in the control group.

The mean values for the anastomotic breaking strength are depicted in Figure 4. The breaking strength, like the bursting pressure, increased from day 3 to day 7.

Three days after the surgery, the values were slightly lower in the I+H group vs the control group. The breaking site was inside the anastomotic area in all groups except for 1 rat in the I group. No notable differences in breaking strength were observed between experimental and control groups 7 days after operation. The breaking site was within the suture line in 6 of 10 cases in the control group and in 2 of 9, 3 of 9, and 3 of 10 cases in the H, the I, and the I+H groups, respectively.
ANASTOMOTIC HYDROXYPROLINE CONTENT

The hydroxyproline content of the anastomotic segments increased between days 3 and 7 after operation (Figure 5). On day 3, the anastomotic hydroxyproline content was significantly higher in the H (P=.02) and the I+H (P=.003) groups vs the control group. On day 7, only the hydroxyproline content of the I+H group was significantly (P=.02) elevated.

The average±SD hydroxyproline concentrations in the anastomotic segments from the control group were 10.1±1.0 µg/mg of dry weight on day 3 and 13.4±1.4 µg/mg of dry weight on day 7. The values found in the experimental groups were not notably different.

COMMENT

This study shows that high-dose preoperative irradiation of the colon with a dose of 25 Gy, combined with local hyperthermia (44°C, 30 minutes), results in increased local anastomotic complications. The high incidence of covered perforations in the I+H group suggests that local re-

action is such that late anastomotic problems, like stenosis, can be expected as well. In addition, the data demonstrate that in this model neither local hyperthermia nor irradiation alone has an adverse effect on subsequent anastomotic healing. The fact that hyperthermia increases the biological effects of irradiation2'6 may explain the finding that a combination of 2 tolerated treatment modalities results in serious complications.

Hyperthermia seems to improve the tumor-killing effects of radiotherapy in 2 ways. First, hyperthermia directly affects the cells. Hypoxic, poorly nourished cells in an environment with a relatively low pH and poor perfusion are susceptible to hyperthermic damage. These cells are often found in tumors, and they are usually relatively radioresistant. The killing of tumor cells by heat is established within 24 hours after application and is caused by damage to membrane lipoproteins and by denaturation of thermolabile cellular enzymes.3,6 Second, hyperthermia has a radiosensitizing effect. This effect can only be expected if hyperthermia precedes irradiation,3 and it seems to be caused by inhibition of sublethal damage repair and blockage of cell proliferation.6,11 The combined effect of radiation therapy and hyperthermia as an adjuvant treatment for surgery seems to be promising; although only reports of incidents or small series are avail-

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**Figure 1.** A schematic of the experimental procedure. A, Irradiation of the colonic segment. B, The hyperthermia procedure. A clamp is placed at the proximal border of the irradiated segment. The inflow tube, the outflow tube, and the temperature probe are placed in the rectum. C, Resection of the colonic segment and construction of anastomosis with a distal, irradiated limb. D, The sample used for hydroxyproline analysis.

**Figure 2.** Weight changes in the 7-day groups. The average values of the control group, the H group, the I group, and the I+H group, are shown. The SD of the C group is shown.

<table>
<thead>
<tr>
<th>Serum Albumin and Protein*</th>
<th>Serum Albumin, g/L</th>
<th>Serum Protein, g/L</th>
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</thead>
<tbody>
<tr>
<td>Group</td>
<td>3 d</td>
<td>7 d</td>
</tr>
<tr>
<td>C</td>
<td>22.6±0.9</td>
<td>22.8±1.0</td>
</tr>
<tr>
<td>H</td>
<td>22.8±1.0</td>
<td>22.2±0.7</td>
</tr>
<tr>
<td>I</td>
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<td>22.0±1.0</td>
</tr>
<tr>
<td>I+H</td>
<td>21.1±0.9†</td>
<td>23.5±1.4</td>
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</tbody>
</table>

*Data are given as the mean±SD (n=9 or n=10). C indicates control animals; H, animals that received hyperthermic treatment; I, animals that received irradiation; and I+H, animals that received irradiation and hyperthermic treatment. †P<.05.

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able, the outlook for patients with locally irresectable colorectal cancer seems to be favorable.12-21 However, numerous questions need to be answered if hyperthermia is considered for widespread clinical use.

Discussion continues about the optimal timing and sequence for the combination of radiotherapy and hyperthermia. The sequence where hyperthermia precedes irradiation has been advocated as the most effective, primarily because of a radiosensitizing effect.5 However, radiotherapy followed by hyperthermia has also been proposed to be more effective; using this sequence, the increased effect is mainly owing to the direct heat killing of radioresistant cells.3,17,19,22-25 Because it has also been noted that human colon cancer cells show evidence of radioresistance,10 the latter sequence might be more appropriate for patients with colorectal cancer.

Because radiotherapy in patients with colorectal cancer is often given preoperatively, we have chosen to investigate the potential detrimental effects of preoperative irradiation followed by hyperthermia on anastomotic healing in the colon. Hyperthermia was applied immediately after radiotherapy because this method has been reported to yield a maximal effect on tumor cells and less of an effect on normal cells.24 A temperature of 44°C for 30 minutes seems to yield the maximal effects, and it is still safe.7 A single application was chosen to prevent the influence of factors like fractionated treatment, thermotolerance (ie, the phenomenon that a second dose of hyperthermia is less effective), and step-down heating (ie, increased effectiveness if maximal hyperthermia is immediately followed by a period of mild hyperthermia).3,5,6 A 30-minute treatment is biologically possible in animal experiments and is derived from other experimental work.7,26

Three days after the operation, the anastomotic bursting pressure was clearly and notably reduced in the I+H group, while the breaking strength was only marginally affected. Early anastomotic strength is mainly determined by the capacity of the submucosal collagen network to retain the sutures. A localized loosening of this structure, by the enhanced activity of collagenolytic enzymes, may lead to easier local rupture at inflation (and lowered bursting pressure) while the breaking strength remains largely unaffected. Whether local degradation of anastomotic collagen occurs within the I+H group still needs to be established. The existence of such a process cannot be derived from the current measurement of the anastomotic collagen (as hydroxyproline) content, which is too insensitive to demonstrate the localized loss of collagen.

The observation that the hydroxyproline content of the anastomotic segment, which contains normal bowel wall next to the actual wound area, is actually notably increased in the I+H group indicates the occurrence of a strong fibrotic reaction; if this reaction persists, late complications may also be expected in this group.

Preoperative protein malnutrition may affect anastomotic strength.27 Three days after the operation, the average serum albumin and serum protein levels were 7% and 3% lower, respectively, in the I+H group vs the control group. However, it seems unlikely that the colonic anastomotic strength is affected by this mild hypoalbuminemia.

No studies about the effects of hyperthermia, alone or in combination with radiotherapy, on anastomotic healing have been conducted. The influence of hyperthermia on small-bowel anastomoses was described in 1 study. Hyperthermia was applied intraperitoneally to study the prevention of serosal metastases in patients who un-

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Figure 3. The anastomotic bursting pressure. Each point represents a measurement in 1 animal. The groups are described in the key for Figure 2. Open circles indicate the bursting site within the suture line; closed circles, the bursting site outside the suture line; and bold horizontal lines, the average values for each group.

Figure 4. Anastomotic breaking strength. Bars represent average values±SD (n=9 or n=10). The groups are described in the key for Figure 2.

Figure 5. Hydroxyproline content of the anastomotic segments. Bars represent average values±SD (n=9 or n=10). The groups are described in the key for Figure 2. The asterisk indicates a significant (P<.03, 2-tailed Mann-Whitney U test) difference vs the control group.
underwent gastric cancer surgery. Survival decreased when temperatures of 45°C or higher were used. Local hyperthermia for 30 minutes at 44°C showed no adverse effect on anastomotic healing in the ileum, although an increased adhesion formation might occur.29,30 Our data confirm this result for anastomoses in the colon.

Experiments about anastomatic healing after preoperative radiotherapy have been described in various articles. This adjunct treatment was long considered to be detrimental for anastomotic healing.25-32 However, a few of us (J.B., J.H., T.W., and T.H., unpublished data, 1996) and Weber et al33 have shown that preoperative irradiation without negative effects on anastomotic healing is feasible, depending on factors like total dose, irradiation technique, number of fractions, and irradiated volume.33 We confirmed this belief: irradiated, normothermic rats (ie, the I group) had anastomotic healing similar to the control group.

The results from our treatment protocol raise additional questions. Is the combination of radiotherapy and hyperthermia safe if a smaller radiation dose is used or if the temperature or duration of the hyperthermic treatment is reduced? Are there any late side effects? More experimental work is needed to answer these and other questions before application of this combined modality can be considered for clinical use. We believe that this model is suitable to conduct such investigations.

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REFERENCES


Statement of Clinical Relevance

In the treatment of colorectal cancer, surgical results show that adjuvant therapy is necessary. Adjunct radiation therapy is generally accepted as a method for providing an improved outcome when used in the preoperative setting. The search continues for new treatment modalities to prevent local recurrence and distant metastasis. Few clinical trials in which hyperthermia was used as an addition to irradiation have been published. The first results are promising. A lower anterior resection is performed in most cases; the anastomotic reconstruction is performed with a proximal, untreated limb and a distal limb that has been irradiated and treated with heat. Anastomotic healing under these circumstances is at risk of dehiscence.

We investigated the possible side effects of this adjuvant treatment on anastomotic repair in an animal experiment. Although anastomotic strength was not decreased, there seemed to be increased local anastomatic complications, posing a risk for early anastomotic repair in the first days after the operation.

The results show that further studies about the use of the combination of preoperative irradiation and hyperthermia are needed to provide more insight about the mechanisms of anastomatic wound healing under this condition.