The effects of 5-fluorouracil and interferon-\(\alpha\) on early healing of experimental intestinal anastomoses

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Summary  The continuing search for effective adjuvant therapy after resection of intestinal malignancies has prompted a growing interest in both immediate post-operative regional chemotherapy and the combination of 5-fluorouracil (5-FU) and interferon-\(\alpha\) as drugs of choice. We have compared the effects of both compounds, alone and together, on early healing of intestinal anastomoses. Four groups \((n=26\) each) of rats underwent resection and anastomosis of both ileum and colon: a control group and three groups receiving intraperitoneal 5-FU, interferon-\(\alpha\) or both on the day of surgery and the next 2 days. Animals were killed 3 or 7 days \((n=10\) each) after operation in order to measure anastomotic strength and hydroxyproline content. The remaining six animals in each group were used to study anastomotic collagen synthetic capacity at day 3. Three days after operation, ileal anastomotic bursting pressure was lowered by 37% in the 5-FU/interferon-\(\alpha\) group \((P=0.0104)\). At day 7, anastomotic breaking strength was reduced significantly in ileum \((P=0.0221)\) and colon \((P=0.0054)\) of the 5-FU/interferon-\(\alpha\) group and in colon of the interferon-\(\alpha\) group \((P=0.0221)\). Collagen synthetic capacity was strongly suppressed by 5-FU but not by interferon-\(\alpha\). However, no differences in anastomotic hydroxyproline content were observed between groups at both days 3 and 7. Thus, post-operative use of interferon-\(\alpha\), in particular in combination with 5-FU, may be detrimental to anastomotic repair in the intestine.

Keywords: anastomosis; collagen; fluorouracil; interferon-\(\alpha\); intestine

Despite the high resectability rate and general improvement in surgical therapy, nearly half of all patients with colorectal cancer will eventually die from recurrent disease. Candidates for post-operative adjuvant therapy are patients at risk for disease relapse, as judged by clinical evaluation, surgical examination and pathological examination of resection specimens. In general, safe and effective adjuvant therapy would be highly desirable in patients with Dukes’ stage B2 disease relapse, as judged by clinical evaluation, surgical

Materials and methods

Animals

Altogether, 104 male outbred Wistar/Cpb:WU rats, weighing between 200 and 300 g, were used. They were housed with two animals per cage and had free access to water and standard laboratory chow (diet AM II, Hope farms, Woerden, The Netherlands).

For the measurement of anastomotic strength and hydroxyproline content, 80 animals were randomly divided
into four groups of 20 animals each: a control group, a 5-FU group, an interferon group and a group receiving 5-FU plus interferon. Within each group, ten rats were killed at 3 and 7 days after operation. Collagen synthesis was measured in similar groups of animals (n = 6 each in each group). These rats were killed 3 days after operation. The study was approved by the Animal Ethics Review Committee of the Faculty of Medicine, University of Nijmegen.

**Drug administration**

5-FU (Abic, Netanya, Israel) was given intraperitoneally in a dose of 20 mg kg⁻¹ body weight (concentration: 1 mg ml⁻¹ saline). This is the same dose we used before (de Waard et al., 1993, 1995a,b) and represents the highest dose which, in combination with surgery, did not result in a significant mortality. Recombinant rat interferon-α (van der Meide et al., 1986; specific activity 6 × 10⁸ U mg⁻¹ protein) was administered intraperitoneally in a dose of 2 × 10⁶ U kg⁻¹ body weight (concentration: 2 × 10³ U ml⁻¹ saline). The drugs were given once a day, on the day of operation and the next 2 days. The animals in the control groups received intraperitoneal saline daily.

**Operative procedure**

After an intraperitoneal injection of sodium pentobarbital, a midline incision was made and 1 cm of both small and large bowel was resected, at 15 cm proximal to the ileocecal junction, and 3 cm proximal to the rectal peritoneal reflection respectively. Continuity was restored microsurgically by the construction of an inverted one-layer seromuscular end-to-end anastomosis with eight interrupted sutures of 8x0 monofilament material (Ethicon, Sommerville, USA). The abdomen was closed in two layers with a continuous 3x0 silk suture for the fascia and staples for the skin.

**Analytical procedures**

The rats were killed by an intraperitoneal overdose of sodium pentobarbital. After opening the abdominal wound and identifying the anastomoses, the adhesions were cut as far as possible without injuring the intestine. An intestinal segment with the anastomosis in the middle was removed, with the sutures left in place. This segment was attached to an infusion pump filled with methylene blue-stained saline. The pressure was raised with an infusion rate of 4 ml min⁻¹ and recorded graphically. Both the bursting pressure, i.e. the maximum pressure recorded immediately before sudden loss of pressure, and the site of rupture were noted. Thereafter, the segment was placed in a tensiometer, and the breaking strength was recorded. Thus, both the bursting pressure and breaking strength were measured in the same anastomotic segment. The validity of this procedure has been confirmed in a pilot experiment. Anastomotic breaking strength was compared in two groups of rats, either measured directly or after the procedure used for measuring the bursting pressure, and found to be similar in both groups (de Waard et al., 1995b). The anastomotic segment was then cleaned from the surrounding tissue and a 5 mm segment with the suture line in the middle was collected. The samples were frozen immediately and stored in liquid nitrogen until processing. After weighing, the samples were pulverised and lyophilised in the middle was collected. The samples were frozen immediately and stored in liquid nitrogen until processing.

**Relative collagen synthesis (%)**

\[
\text{Relative collagen synthesis} = \frac{\text{CDP}}{(\text{NCP} \times 5.4) + \text{CDP}} \times 100
\]

Incorporation is expressed on the basis of sample wet weight, DNA (Burton, 1956) content or protein (Smith et al., 1985) content.

**Results**

No animals died prematurely during the experimental protocol. Up to 24 h after operation, all rats lost approximately 8% of their body weight. Thereafter, animals regained weight, although clear differences were observed between groups (Figure 1). Weight gain in the 5-FU group was significantly slower than in the control group. Administration of

![Figure 1](image-url)

**Figure 1** Course of body weight. Data represent mean values (n = 10) and, for the control group only, the s.d. (O), control group; (•), interferon-α group; (Δ), 5-FU group; (▲), 5-FU/interferon-α group. Significant differences (P < 0.033, see Materials and methods) between groups are denoted by # (interferon-α vs control group), * (5-FU vs control group) and $ (5-FU vs 5-FU/interferon-α group).

was counted. Subsequently, in order to determine proline incorporation into collagens, excess purified collagenase was added. The radioactivity in the supernatant represents CDP, as a measure of the amount of collagen synthesised. Subtraction of the radioactivity in the CDP fraction from that in total protein yields the incorporation into non-collagenous protein (NCP). The relative collagen synthesis (RCS) was calculated with the formula (Peterkofsky et al., 1981) that takes into account the enrichment of proline in collagen compared with other proteins:

Relative collagen synthesis (%) = \[ \frac{\text{CDP}}{(\text{NCP} \times 5.4) + \text{CDP}} \times 100 \]
interferon-α appeared to increase the rate of weight gain over the first post-operative days: mean body weight was significantly higher in the interferon-α group than in the control group from day 3 onwards, and in the 5-FU/interferon-α group than in the 5-FU group from day 5 onwards.

Anastomotic strength may be assessed both from the bursting pressure, which represents its resistance to intraluminal pressure, and from the breaking strength, which reflects its ability to withstand longitudinal forces.

Figure 2 depicts the outcome of all measurements of anastomotic bursting pressure performed at 3 days after operation. At this time the bursting site was always within the anastomotic area. In altogether six (out of 80) anastomoses the bursting pressure could not be measured because of technical problems. Neither 5-FU nor interferon-α administration led to a significant change in bursting pressure. However, the mean bursting pressure of ileal anastomoses in the 5-FU/interferon-α group (44 ± 7 mmHg) was significantly (P = 0.0104) lower than that in the control group (70 ± 20 mmHg). In addition, it was also significantly reduced with respect to the 5-FU group (P = 0.0037) and the interferon-α group (P = 0.0062). In colon, these differences did not reach statistical significance. At 7 days after operation the bursting site was always outside the suture line and therefore the bursting pressures measured (data not shown) did not reflect actual wound strength.

When measuring the breaking strength (Figure 3) the breaking site was invariably located within the wound area. At 3 days after operation no differences were found between groups, but after 7 days the breaking strength of both ileal and colonic anastomoses was significantly lower in the 5-FU/interferon-α group than in the control group. Also, anastomotic breaking strength in the colon was reduced in the interferon-α group.

The hydroxyproline content in 5 mm segments containing the anastomosis was quantitated as a measure of wound collagen levels (Figure 4). No differences between the control group and the experimental groups were observed. The hydroxyproline content increased similarly from 3 to 7 days after operation independent of medication. Likewise, no differences were found for hydroxyproline concentrations. Mean hydroxyproline concentrations were 7.0 ± 1.3 and 9.6 ± 1.8 µg mg⁻¹ dry weight in 3- and 7-day-old ileal anastomoses respectively; corresponding values in colonic

![Figure 2](image2.png)

**Figure 2** Anastomotic bursting pressure after 3 days. Points represent measurements in individual animals with bars indicating mean values. *Significantly (P < 0.033, see Materials and methods) different from control group.

![Figure 3](image3.png)

**Figure 3** Anastomotic breaking strength. Bars represent mean values (n = 9 or 10) ± s.d. 1, control group; 2, 5-FU group; 3, interferon-α group; 4, 5-FU/interferon-α group. *Significantly (P < 0.033, see Materials and methods) different from control group.

![Figure 4](image4.png)

**Figure 4** Anastomotic hydroxyproline content. Bars represent mean values (n = 10) ± s.d. 1, control group; 2, 5-FU group; 3, interferon-α group; 4, 5-FU/interferon-α group.
Discussion

The continuing search for effective adjuvant therapy after resection of colorectal carcinoma has resulted in a growing interest in the efficacy of both immediate post-operative regional chemotherapy (see Pahlman, 1995) and the combination of 5-FU with interferon-α as the cytostatic drugs of choice (see Grem et al., 1995). The present results indicate that caution should be exerted in the use of interferon-α as an adjunct to 5-FU therapy in the early post-operative period since such treatment might constitute a threat to undisturbed anastomotic healing. The combination of interferon-α and 5-FU, administered intraperitoneally on the day of operation and the first two post-operative days, reduces the development of anastomotic strength during the first week after its construction. Administration of 5-FU alone has no significant deleterious effect on wound strength, but interferon-α in itself significantly lowers strength below control values in 7-day-old colonic anastomoses.

The wound healing process is characterised by massive cell migration and proliferation. Cytostatic drugs are by nature anti-proliferative and may therefore be expected to interfere with wound healing. Indeed, 5-FU administered daily from the day of operation onwards until sacrifice after 7 days severely impairs anastomotic healing in the rat intestine (Graf et al., 1992; de Waard et al., 1995b). In earlier experiments, we tried to mitigate this negative effect by concomitant administration of either interleukin 2 or granulocyte-macrophage colony-stimulating factor, but 5-FU impaired repair was not essentially altered by either cytokine. On the other hand, retinol significantly promoted 5-FU suppressed anastomotic healing (de Waard et al., 1995a). Also, we reported before that the negative effect is limited if 5-FU is given only three times (de Waard et al., 1993, 1995a). Since we expected any additional effect of interferon-α to be observed more easily under the latter conditions, we limited drug administration to the first 3 days.

Table I  Increase in anastomotic collagen synthetic capacity 3 days after operation

<table>
<thead>
<tr>
<th></th>
<th>Control segment</th>
<th>Anastomosis</th>
<th>Ratio</th>
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<tbody>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>D.p.m. µg⁻¹ DNA</td>
<td>41 ± 6</td>
<td>194 ± 30</td>
<td>4.9 ± 1.2</td>
</tr>
<tr>
<td>D.p.m. mg⁻¹ wet weight</td>
<td>85 ± 24</td>
<td>595 ± 72</td>
<td>7.6 ± 2.2</td>
</tr>
<tr>
<td>D.p.m. mg⁻¹ protein</td>
<td>2578 ± 654</td>
<td>14786 ± 1953</td>
<td>6.0 ± 1.4</td>
</tr>
<tr>
<td>RCS (%)</td>
<td>0.47 ± 0.08</td>
<td>1.02 ± 0.18</td>
<td>2.2 ± 0.5</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D.p.m. µg⁻¹ DNA</td>
<td>86 ± 18</td>
<td>221 ± 62</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>D.p.m. mg⁻¹ wet weight</td>
<td>305 ± 45</td>
<td>773 ± 150</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td>D.p.m. mg⁻¹ protein</td>
<td>7317 ± 1019</td>
<td>17454 ± 3214</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>RCS (%)</td>
<td>1.12 ± 0.12</td>
<td>1.72 ± 0.32</td>
<td>1.5 ± 0.2</td>
</tr>
</tbody>
</table>

Explants from control segments, collected at operation, and anastomotic tissue, collected 3 days after operation, were incubated for 3 h with 4.5 µCi of [³H]proline. Collagen synthesis is expressed as radioactivity in collagen-digestible protein and as percentage relative collagen synthesis (RCS). Data represent average values (+ s.d.) from six animals.
So far, little is clear about the effects of interferon-a on wound healing. Interferon-γ, delivered intraperitoneally through an osmotic pump (Granthan et al., 1989) or injected subcutaneously (Miles et al., 1994), has been found to delay skin repair in mice. In the latter study, wound disruption strength was reduced significantly at a dose of 7 x 10^4 U kg^{-1} body weight and higher. Two experiments, with opposite results, have been reported on the effects of interferon-α/β on cutaneous healing in rodents. Intramuscular injection of interferon-α/β or intraperitoneal administration of polyinosinic-polycytidylic acid, a potent inducer of interferon, seemingly enhances repair (Bhartiya et al., 1992), while local subcutaneous injection of interferon-γ/β in mice in which the wound actually inhibits repair (Stout et al., 1993). In these studies, evaluation of repair was solely on the basis of macroscopic or histological parameters; functional parameters, like wound strength, were not reported.

The present study is the first effort to investigate the effect of purified interferon-α on wound strength. Rat recombinant interferon-α was given in a daily dose of 2 x 10^4 U kg^{-1} body weight. This dose is more than sufficient to protect rats against a lethal pseudorabies virus (PH van der Meide, personal communication), but is substantially lower than the doses of interferon employed in the studies mentioned above. Still, daily doses of this relatively low dose of interferon-α, administered on the first three post-operative days, significantly reduce anastomotic breaking strength in the colon 7 days after operation. The results of treatment with the combination of 5-FU and interferon-α are probably of more immediate interest in terms of potential treatment of patients with colorectal cancer. It seems clear that addition of interferon-α to a regimen of 5-FU, which in itself does not affect anastomotic strength, may lead to a significant and substantial reduction in anastomotic strength during the first week of healing. In this period, where clinically most leakages occur, the strength of the Anastomosed segment is relatively low as compared with the strength of the uninjured intestine: any further reduction constitutes a threat to anastomotic integrity and increases the chances for anastomotic dehiscence. Thus, interferon-α is known to inhibit collagen synthesis (Granthan et al., 1990). This suppressive effect is well established by in vitro experiments with fibroblasts (Jimenez et al., 1984; Duncan and Berman, 1985), which are the primary producers of extracellular matrix in the healing wound. Indeed, histological examination of interferon-α-treated wounds indicates reduced accumulation of collagen (Granthan et al., 1989; Miles et al., 1994). However, our data show that treatment with interferon-α alone does not lead to lowering of either ex vivo collagen synthetic capacity in anastomotic explants or hydroxyproline accumulation in anastomotic segments. Such an effect is indeed observed after 5-FU treatment, but again addition of interferon-α does not lead to further reduction. This lack of effect of interferon-α may be explained by the relatively low doses we used. Alternatively, it could be that interferon-α has less effect on matrix production than the other interferons. Experiments with isolated cells show this to be true for fibroblast collagen synthesis, both on the protein (Jimenez et al., 1984; Duncan and Berman, 1985) and the mRNA level (Duncan et al., 1995).

Anastomotic strength will also be affected by degradation of the existing matrix anchoring the sutures. The methodology used to measure the hydroxyproline content in anastomotic segments, which necessarily contain uninjured tissue next to the actual wound area, does not allow the detection of very localised loss of collagen. It may be that interferon-α increases collagenase expression (Duncan and Berman, 1989; Hujanen et al., 1994), although macrophage metalloproteinase production appears to be inhibited by interferon-γ (Wahl and Corcoran, 1993). It remains to be determined how exactly interferon-α interferes with healing by inhibition of proliferation, either directly or by biochemical modulation of 5-FU metabolism. Interferons are growth inhibitors for a variety of normal and transformed cell lines (see Mallat et al., 1995). The impairment of cutaneous healing by interferon-α/β is thought to be caused primarily by inhibition of proliferation of all cell types involved in wound repair (Stout et al., 1993). More specifically, interferon-α is known to inhibit endothelial cell proliferation and thereby angiogenesis (Polkman, 1995), processes inherent to successful repair.

Whatever the mechanism(s) responsible, our data clearly indicate that administration of interferon-α in the perioperative period may be detrimental to anastomotic repair and that its use in immediate post-operative adjuvant therapy, as a means to enhance 5-FU activity, should be approached with caution.

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References


