The Influence of Early Postoperative Intraperitoneal Chemotherapy on Human Wound Healing

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Cell ingrowth, hydroxyproline accumulation, and mRNA expression of collagen I were measured in two polytetrafluoroethylene grafts implanted subcutaneously at the time of colorectal cancer surgery to evaluate the influence of early postoperative chemotherapy on human wound healing. Eleven patients treated with intraperitoneal 5-fluorouracil and intravenous folinic acid Days 1–6 after operation were compared with 15 patients who underwent surgery alone. At 1 week, chemotherapy-treated patients had accumulated less hydroxyproline (mean 0.35 ± 0.33 μg/cm) compared with untreated patients (mean 0.73 ± 0.37 μg/cm, P < 0.05). By 2 weeks, the hydroxyproline content had increased sixfold in the chemotherapy group (P < 0.01) and threefold in the nonchemotherapy group (P < 0.01) and there was no difference between the groups. Cell and connective tissue ingrowth and total RNA content did not differ between the groups at any point in time, but at 1 week the mRNA expression of collagen I was higher in the chemotherapy group (P < 0.05). These results indicate that collagen accumulation in human subjects is reduced during a short course of postoperative chemotherapy and normalizes after the end of treatment.

INTRODUCTION

Adjuvant intraperitoneal 5-fluorouracil (5-FU) infusion has been associated with an improved survival after curative surgery for colorectal cancer [1]. Intraperitoneal administration has been suggested as an alternative to intraperitoneal infusion to expose also peritoneal and local tumor remnants to the cytotoxic drug [2]. However, several experimental studies have shown that 5-FU reduces the strength of surgical wounds [3–5], most likely due to reduced collagen formation [5,6]. Particularly, intraperitoneal administration appears to impair postoperative collagen synthesis [7]. Although numerous animal studies have shown that 5-FU inhibits wound healing, it is not known if there is an analogous effect in human subjects treated with doses commonly used in adjuvant therapy. Such knowledge would be valuable for the application of results from animal studies to humans and hence for assessing the risks connected with early postoperative chemotherapy.

This study was undertaken to analyze the effects of early postoperative intraperitoneal 5-FU infusion on human wound healing through comparing treated patients with those submitted to surgery alone with respect to cell migration and collagen accumulation in a standardized subcutaneous wound.

PATIENTS AND METHODS

Patients

Surgical morbidity and tolerance to adjuvant intraperitoneal chemotherapy were studied in a randomized multicenter phase I trial involving patients not older than 75 years who were scheduled for a colorectal cancer resection with curative intent between February 1990 and April 1992. Fifty patients were randomized to receive intraperitoneal 5-FU and intravenous folinic acid, and 51 to receive placebo, in a double-blind manner. Folic acid was given with the aim of improving the anti-tumor activity of 5-FU [8]. The active treatment consisted of 500 mg/m²/day 5-FU (Cyanamid Nordiska AB, Stockholm, Sweden) as an intraperitoneal infusion given over a 30-min period and 60 mg/m²/day folinic acid (Leucovorin, Cyanamid Nordiska AB) as an intravenous infusion lasting 5–10 min and commencing 1 hr after the start of the intraperitoneal infusion. The 5-FU was dissolved in 500 ml of 0.9% NaCl and the Leucovorin in 100 ml of 0.9% NaCl. Patients in the placebo arm received only the vehicle according to the same schedule. The treatment started on the day after surgery...
The investigators had no knowledge about the ran-
preoperative hematological values (data not shown). Nei-
disease and in two suspected Dukes',A lesions), giving a
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cluded in the study were four patients eligible preopera-
and continued for 6 consecutive days with a break on
Sundays.

Twenty-two patients treated within the trial at our
department were included in the present study. Also in-
cluded in the study were four patients eligible preopera-
tively for the randomized trial, but in whom exclusion
criteria were found at surgery (in two cases metastatic
disease and two suspected Dukes',A lesions), giving a
total of 11 patients treated with chemotherapy postoper-
avely and 15 undergoing surgery alone (Table 1).

The average (±SD) preoperative weight loss was 2.9 ±
4.3 in the chemotherapy group and 2.5 ± 2.9 in the non-
chemotherapy group. The corresponding values for body
mass index (body weight/length²) and S-albumin were
24.8 ± 5.0 and 23.9 ± 3.7 and 39 ± 4.9 and 41 ± 3.4,
respectively. There was no indication of a difference in
immunocompetence between the groups as reflected by
preoperative hematological values (data not shown). Nei-
ther did variables reflecting the surgical trauma (i.e.,
operating time, preoperative bleeding, and number of
units of blood transfused) differ between the groups
(data not presented). During the conduct of the analyses,
the investigators had no knowledge about the ran-
don allocation of the patients in the trial but knew
which patients were outside the trial. The study was ap-
proved by the regional ethics committee and informed
consent was obtained from all patients.

### Technique

Polytetrafluoroethylene (PTFE) grafts, 1 mm in diam-
eter and with a 90-μm pore size, were purchased from
Gore, Swedish AB (Mölndal, Sweden). The weight/
length ratio was close to constant (0.46 ± 0.03 mg/mm).

The grafts were cut into 7-cm lengths and sterilized in an
autoclave. During anaesthesia for colorectal cancer sur-
gery, the extensor part of the right upper arm was
cleaned with chlorhexidine and wiped dry. A small stab
wound was made with a surgical blade approximately
halfway between the elbow and shoulder. A 2 × 50-mm
crissa biopsy needle (Cameco AB, Täby, Sweden) was
inserted subcutaneously, with the tip emerging at a dis-
tance large enough to allow the graft to be placed in
straight position. The trochar was removed and a guide
wire with the graft threaded on it was inserted through
the needle, and while the graft was held with a forceps,
first the guide wire and then the needle were withdrawn.
Two 7-cm grafts were placed parallel to each other ap-
proximately 3-cm apart. One centimeter of the graft
protruded outside the skin and was fixed with a suture.
The grafts were covered with a sterile dressing. This
method was originally described by Goodson and Hunt
[9]. One graft was extracted after 1 week and the other
after 2 weeks. After extraction, the grafts were divided in
a standardized fashion; the innermost 8 mm was taken
for histology, the next 20 mm for RNA extraction, and
the next 30 mm for determination of hydroxyproline; the
last part, the 1 cm outside the skin and a further 2 mm,
was discarded. The pieces were immediately measured to
the nearest millimeter, weighed, and thereafter frozen in
liquid N₂ and stored at ~70°C until analysed.

### Hydroxyproline Assay

The hydroxyproline content was measured as de-
scribed by Stegemann and Stalder [10]. Briefly, the graft
was cut into 2-mm pieces and hydrolyzed in 1 ml HCl 6
mole/liter at 110°C for 21 hr. The hydrolysate was eva-
porized to dryness and dissolved in 500 μl deionized
water. Buffer was added to a volume of 2 ml. The sample
was then incubated with 1 ml chloramine T at room tem-
perature for 20 min. One milliliter of aldehyde/perchlo-
ric acid solution was then added, and the admixture was
shaken thoroughly and immersed in a 60°C water bath
for 15 min. After cooling, the absorbance was read in a
spectrophotometer at 550 nm. To assess the variation in
the hydroxyproline content along the graft, determina-
tions based on two different parts of the 30-mm graft
segment were performed in 35/52 measurements. There
was a close correlation between the two determinations
(𝑟 = 0.93, 𝑃 < 0.0001) (Fig. 1). The total amount of hy-
droxyproline per total length of graft (i.e., the weighted
mean) was used in the presentation of results. The
compositions of the various solutions are detailed else-
where [10].

### RNA Analyses

Total RNA was extracted by the acid–guanidinium–
phenol–chloroform method [11] and quantitated by spec-
trophotometry at 260 nm, and the results were expressed
as μg/cm. Serial dilutions of formaldehyde-denatured RNA were dot blotted onto nylon membranes (Amersham, UK) with the aid of a vacuum chamber device (Bio-Rad, Richmond, CA), fixed on a bed of 0.05 mole/liter NaOH, and washed briefly in standard saline phosphate EDTA (0.30 mole/liter NaCl, 20 mmole/liter, NaH₂PO₄, 2 mmole/liter EDTA, pH 7.4). The membranes were then subjected to hybridization with a 372-bp cDNA probe corresponding to a conserved region of the collagen α1(I) chain (a gift from Dr. E. Vuorio, University of Turku, Finland). The probe was labeled with ³²P by the random priming method (Amersham, UK). Autoradiographs were evaluated by laser densitometry. As an internal reference for the mRNA measurements, two different methods were used. Initially, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) method was employed in which the membranes were reprobed with a 700-bp cDNA probe corresponding to the GAPDH enzyme of the glycolysis, labeled exactly in the same way. This probe served as a positive control and as a reference for comparison of the different RNA samples in the densitometry assay, as it is considered to be a constitutively expressed gene product. In the later part of the study (n = 14), the oligo(dT) method was used in which mRNA was semiquantitated as described by Harley [12]. Briefly, RNA was dot blotted onto a nitrocellulose filter and subsequently hybridized with a ³²P end-labeled oligo(dT)₁₈ probe which detects total mRNA by binding to its poly(A) tail. The oligo(dT)₁₈ was removed by stringent washing and the filter was finally exposed to the collagen pro α1(I) cDNA probe. After laser densitometry of autoradiographs from the hybridized dot blot filters, a pro α1(I)/oligo(dT) index was created for each sample and these figures were compared for relative values of pro α1(I) mRNA levels. Absolute levels of mRNA expression of collagen I were calculated only in the 14 patients evaluated with the oligo(dT) method, but relative changes were calculated for all patients.

### Histological Examinations

After fixation in 10% formaldehyde overnight, the grafts were dehydrated in ethanol and embedded in the plastic medium JB4 (Bio-Rad, Richmond, CA), and 2- to 3-μm thick sections were cut longitudinally. A hematoxylin-eosin-stained coded section was inspected for cell and connective tissue ingrowth (Figs. 2 and 3). A quantification of the degree of invasion of tissue into the pores was performed after identification of the fibroblast-containing organized granulation tissue and for each pore described as the location of the edge of this compartment (arrowhead in Fig. 3). This was estimated to be 0, 25, 50, 75, or 100% of the total length of the pore. The mean of 40 randomly selected transversally sectioned pores was given for each patient and each time.

### Statistical Methods

Results are presented as means ± standard deviation. Statistical comparisons between the groups were made with the Mann-Whitney U test and temporal changes within the groups with the Wilcoxon signed rank test. All presented P values are two-tailed.

### RESULTS

#### Postoperative Course

Anastomotic dehiscence was diagnosed in one patient in each group, one of whom required a second laparotomy. Furthermore, two patients treated with 5-FU experienced surgical complications; one had severe localized abdominal pain and rebound tenderness, which resolved spontaneously, a condition judged as chemical peritonitis [13], and the other had a perineal infection after an abdominoperineal resection. A leukopenia was observed in one patient in the chemotherapy group (white blood cell count 1.1 × 10⁹/liter on the third day of treatment). In all other patients the postoperative course was uneventful. Among the 11 patients treated with chemotherapy, 8 received the intended six treatments, whereas it was terminated on Days 2, 3, and 4 in the remaining three patients.

#### Graft Weights and Hydroxyproline

No signs of infection appeared around the grafts and removal was always easy. At 1 week, the graft weight was 1.45 ± 0.22 mg/mm in the chemotherapy group and 1.52
± 0.28 mg/mm in the nonchemotherapy group (P > 0.10). By two weeks the corresponding figures were 1.65 ± 0.34 and 1.69 ± 0.23 mg/mm, respectively (P > 0.10). The difference over time was significant in both groups (P < 0.05). By 1 week, the hydroxyproline content in the chemotherapy group was lower than that in the nonchemotherapy group (P < 0.05). This was also true when hydroxyproline accumulation was expressed as concentration (P < 0.05, Table 2). Compared with 1 week, a marked increment in hydroxyproline accumulation had occurred at 2 weeks in both groups (P < 0.01), when it was approximately equal in the two groups (Table 2).

RNA Analyses

The total RNA content at 1 week was 5.64 ± 2.02 μg/cm in the chemotherapy group and 6.17 ± 4.26 μg/cm in the nonchemotherapy group (P > 0.10). At 2 weeks the corresponding values were 7.53 ± 3.13 and 10.7 ± 5.24 μg/cm, respectively (P > 0.10). The difference in RNA content between 1 and 2 weeks was significant in the nonchemotherapy group (P < 0.01), but not in the chemotherapy group. By 1 week, the mRNA expression of collagen I was 0.70 ± 0.40 in the chemotherapy group (n = 7) and 0.28 ± 0.19 in the nonchemotherapy group (n = 7) (P < 0.03). By 2 weeks the corresponding figures were 0.88 ± 0.64 and 0.38 ± 0.27, respectively (P = 0.10). The relative change in mRNA expression of collagen I was 223 ± 213% in the chemotherapy group and 191 ± 165% in the nonchemotherapy group (P > 0.10).

Histology

There was no difference in the average percentage of ingrowth between the groups at either measurement time (P > 0.10) with values of 5.5 ± 7.8 and 5.8 ± 8.8% at 1 week and 17.1 ± 12.6 and 19.8 ± 13.1% at 2 weeks in the chemotherapy and nonchemotherapy groups, respectively. The increase between 1 and 2 weeks was statistically significant in both groups (chemotherapy group P < 0.05; nonchemotherapy group P < 0.01).

**DISCUSSION**

A large number of studies have explored the effects of chemotherapy on wound healing in experimental ani-
mals, but this is, to the best of our knowledge, the first study of this issue in human subjects. A standardized subcutaneous wound was employed since it is unethical to biopsy a newly constructed anastomosis in humans. The subcutaneous wound and the anastomosis were both exposed to 5-FU in the systemic circulation, but the latter site might also have been exposed to local 5-FU penetration [14]. As far as the anastomosis is concerned, this model is therefore likely to reflect a "minimum" level of influence. In this study, hydroxyproline was determined as a marker of collagen [15]. In contrast to tissue biopsies, hydroxyproline in the PTFE graft represents only "de novo" collagen and is therefore potentially better suited as a marker of collagen synthesis. Histology and total RNA content might be viewed as indicative of the amount of cells in the graft. Finally, mRNA expression of collagen I shows the cellular signal for collagen formation. The variables in this study thus reflect basic events in wound healing, and a defect in any of them might theoretically lead to serious complications.

The present numerical values of hydroxyproline content were in close proximity to those observed in a previous study, where the hydroxyproline accumulation after 1 week was 0.45 µg/cm in control subjects and 0.25 µg/cm in "debilitated" patients [9]. Trauma [16], a brief preoperative illness [17], chronic uremia [18], and poor

### Table 2

Hydroxyproline Content (µg/cm) and Concentration (µg/mg) at 1 and 2 Weeks

<table>
<thead>
<tr>
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<th>Chemotherapy (n = 11)</th>
<th>Nonchemotherapy (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyproline content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td>0.35 ± 0.33</td>
<td>0.73 ± 0.37*</td>
</tr>
<tr>
<td>2 weeks</td>
<td>2.25 ± 1.12**</td>
<td>2.38 ± 1.24**</td>
</tr>
<tr>
<td>Hydroxyproline concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td>0.025 ± 0.020</td>
<td>0.051 ± 0.022*</td>
</tr>
<tr>
<td>2 weeks</td>
<td>0.142 ± 0.065**</td>
<td>0.161 ± 0.074**</td>
</tr>
<tr>
<td>∆ Content</td>
<td>1.90 ± 1.01</td>
<td>1.65 ± 1.23</td>
</tr>
</tbody>
</table>
| ∆ Concentration     | 0.118 ± 0.064         | 0.101 ± 0.083            

Note. The increase in hydroxyproline content (delta content, µg/cm) and concentration (delta concentration, µg/mg) is also shown.
* Difference between groups, P = 0.02 (Mann–Whitney U test).
** Difference between 1 and 2 weeks, P < 0.01 (Wilcoxon signed rank test).
oxygenation [19] have been associated with depressed collagen accumulation in humans, whereas protein deficiency negatively influenced collagen deposition in experimental animals [20].

Hydroxyproline accumulation in the chemotherapy group was reduced to 50% of the control level after 1 week. Because of the larger proportion of women in the chemotherapy group, separate analyses were made according to gender. It was found that the men and the women in the chemotherapy group showed a similar reduction in the hydroxyproline level compared with the corresponding sex in the nonchemotherapy group (data not shown), suggesting that the sex imbalance could not explain the difference. Separate analyses were also performed with exclusion of patients with surgical complications, with essentially similar results (data not shown). The preoperative registration of other parameters that might affect wound healing (tumor stage, nutritional status, hematological values, extent of surgical trauma) did not reveal any major differences between the groups. The present findings thus indicate a depressed collagen accumulation during the chemotherapy course. Another observation in this study was the substantial increase in hydroxyproline amount during the second week which took place irrespective of chemotherapeutic treatment, showing that the negative effect during chemotherapy ceased after the end of the course. A similar rise have previously been observed in experimental animals [9, 16] and hydroxyproline levels were also elevated from Day 5 through Day 7 in human subjects [9]. The present study demonstrates that if the time of grafting is however not known, and there has been no great increase in surgical complications reported after adjuvant intraperitoneal 5-FU infusion [21–25]. Nevertheless, the present results call for considerable caution when treating patients with cytotoxic drugs shortly after gastrointestinal surgery.

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