Effect of transforming growth factor beta and basic fibroblast growth factor on steroid-impaired healing intestinal wounds

A longitudinal intestinal wound model in the pig was used to assess the effect of parenteral steroids (betamethasone 12 mg 50 kg⁻¹ intramuscularly twice daily) on breaking load. Steroid treatment significantly decreased the breaking load of wounds in the ileum and colon in comparison with wounds from saline-treated animals. In a further group of animals receiving steroids, paired longitudinal wounds were constructed. One wound of a pair was treated with a local application of transforming growth factor beta (TGF-β) (5 μg per wound) or basic fibroblast growth factor (5 μg per wound) in a collagen suspension. The other wound was treated with a collagen suspension alone. Local wounds treated with TGF-β were significantly stronger than collagen treated controls at 7 days. The steroid-induced impairment of breaking load in intestinal wounds is partially reversed by a local application of TGF-β in a collagen suspension at the time of surgery.

Peptide growth factors are increasingly implicated in the control of wound healing. Recombinant biotechnology provides amounts of these previously rare peptides which allows their use in in vitro experiments and may permit their clinical use. Epidermal growth factor, tumour necrosis factor, transforming growth factor beta (TGF-β) and platelet-derived growth factor, applied in slow release suspensions or vehicles to healing incisional skin wounds at the time of wounding, accelerate the gain in breaking load of these wounds. Mustoe and colleagues recently demonstrated acceleration of the gain in strength of healing rabbit gastrotomy wounds with topical TGF-β in a collagen suspension. Local applications of peptide can be shown to reverse the deleterious effects of steroid therapy, cytotoxic treatment and local radiotherapy in simple skin incisional wound models. It remains unclear to what extent these various effects are organ- or species-specific.

The failure of intestinal wounds is associated with considerable morbidity and mortality rates and usually occurs in situations of impaired healing. Steroid impairment of cutaneous healing is well documented. Few studies of the effects of steroids on healing intestinal wounds have been published. One recent experimental study failed to demonstrate impairment of healing following steroid treatment. Clinical studies have failed to demonstrate any deleterious effects on intestinal healing. The numbers of treated patients in any particular trial would, however, be small and demonstrating any negative effect would be difficult. The effect of steroid therapy on healing longitudinal enterotomy wounds in pigs was assessed by measurement of breaking load. The effect of local applications of two peptide growth factors, TGF-β and basic fibroblast growth factor (bFGF), each in a collagen suspension, was tested on the steroid-impaired healing of enterotomy wounds.

Materials and methods

Weanling Landrace pigs weighing between 16 and 25 kg were used throughout. Pigs were housed in groups of up to four and maintained on a constant day/night cycle. Animals were weighed 48 h before the operation, at the time of operation and when killed.

An intramuscular betamethasone preparation (Betrolan™; Coopers Pittman/Moore, Crewe, UK) was used. Biological activity of TGF-β, and bFGF was ensured by a simple in vitro assay utilizing Swiss 3T3 fibroblasts between the tenth and 14th passage grown to confluence in 96-well plates. Mitogenicity was assessed by uptake of tritiated thymidine. The vehicle used for delivery of peptide was 200 μl of a dilute collagen suspension (Zyderm II; Collagen Corporation, Palo Alto, California, USA) to provide a viscous preparation allowing easy application to wound edges that may delay release of peptide for a short period at a site of local application.

Demonstration of steroid impairment of healing

One group of seven pigs was treated with betamethasone 12 mg 50 kg⁻¹ intramuscularly twice daily, beginning 48 h before the operation and continuing until killing. Seven other animals received a similar volume of saline intramuscularly. At operation animals had 6 cm antimesenteric enterotomy wounds constructed at standard sites (three in the ileum and two in the colon) and were killed at 7 days. Statistical comparisons were between wounds in steroid- and saline-treated animals.

Effect of growth factors

A further 19 pigs were treated with betamethasone 12 mg 50 kg⁻¹ intramuscularly twice a day beginning 48 h before the operation and continuing until killing. Animals had paired wounds constructed at standard sites, three pairs in the ileum and one in the colon (Figure 1). Each member of a pair was 30 cm from its partner. One wound in a pair was treated with growth factor in a collagen suspension, the other was treated with collagen suspension alone. All control wounds in a particular animal were completed before growth factor-treated wounds to prevent contamination. A standard wounding protocol was followed; the seromuscular layer was divided with cutting diathermy until the mucosa bulged, and growth factor or control solution was applied to the wound to allow even application to the whole surface. The mucosa was then opened with a blade before suture. Animals were treated with only one growth factor. In six animals the effects of saline were compared with collagen suspension. In nine animals the effects of TGF-β (5 μg) in a collagen suspension were compared with collagen and in four animals the effects of bFGF (5 μg) in a collagen suspension were compared with collagen. Statistical comparisons were between paired wounds.

Wounding model

Fluids only were allowed for the 12 h preceding operation. Anaesthesia was induced with halothane and maintained after intubation with a mixture of halothane, nitrous oxide and oxygen. A single dose of penicillin (Triplopen™; Glaxo, Greenford, UK) was given with induction.
Effects of steroids

There were no significant changes in weight between the steroid- and saline-treated groups. It was common to find small intraperitoneal collections of fluid in steroid-treated animals. Steroid treatment significantly decreased the mean(s.e.m.) breaking load of ileal (160(20) g cm \(^{-1}\) versus 320(32) g cm \(^{-1}\)) and colonic (200(12) g cm \(^{-1}\) versus 390(33) g cm \(^{-1}\)) wounds (both \(P < 0.01\), Wilcoxon rank sum test, Table 1 and Figure 2). There were no differences in breaking load between the three different sites within the ileum or the two sites in the colon (Table 1). No histological differences were apparent at 7 days between steroid- and saline-treated animals.

Effect of growth factors

There were no significant differences in breaking load between collagen-treated wounds and saline controls (Table 2). Wounds treated with TGF-β had increased mean(s.e.m.) breaking loads (ileal 285(32) g cm \(^{-1}\) versus 180(11) g cm \(^{-1}\), colonic 365(59) g cm \(^{-1}\) versus 260(40) g cm \(^{-1}\)) in comparison with collagen-treated controls (Figure 3). This was significant at the 5 per cent level for ileal wounds but just failed to reach significance with colonic wounds. No major differences were noted between wounds treated with TGF-β and collagen-treated controls when histological sections were examined.

Table 1 Breaking strength of intestinal wounds in steroid- and saline-treated animals at specific sites in the ileum (A, B and C) and colon (D and E)

<table>
<thead>
<tr>
<th>Site</th>
<th>Saline (g cm (^{-1}))</th>
<th>Steroid (g cm (^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ileum</td>
<td>Site A (n = 6) 310(63)</td>
<td>150(29)</td>
</tr>
<tr>
<td></td>
<td>Site B (n = 6) 290(58)</td>
<td>170(37)</td>
</tr>
<tr>
<td></td>
<td>Site C (n = 6) 360(37)</td>
<td>170(35)</td>
</tr>
<tr>
<td></td>
<td>Mean (n = 18) 320(32)</td>
<td>160(20)*</td>
</tr>
<tr>
<td>Colon</td>
<td>Site D (n = 6) 370(49)</td>
<td>210(28)</td>
</tr>
<tr>
<td></td>
<td>Site E (n = 6) 415(40)</td>
<td>185(14)</td>
</tr>
<tr>
<td></td>
<td>Mean (n = 12) 390(33)</td>
<td>200(12)*</td>
</tr>
</tbody>
</table>

Values are mean(s.e.m.) expressed in g cm \(^{-1}\); \(n\), Number of wounds

\(* P < 0.01\) (Wilcoxon rank sum test)

Figure 2 Breaking strength of enterotomy wounds in steroid- (■) and saline-treated (□) animals. Values are mean(s.e.m.). There were 18 ileal and 12 colonic wounds in each group. \(* P < 0.01\) (Wilcoxon rank sum test)
Wound healing in the intestine has traditionally been estimated by the measurement of anastomotic bursting strength. A segment of bowel with an anastomosis at its centre is distended with fluid or gas and the pressure at which bursting occurs is taken as a measure of intestinal strength. Unfortunately, after the first few days of healing, disruption characteristically occurs away from the anastomosis; this is not a reflection of supranormal anastomotic wound strength but rather the decreased distensibility of the anastomotic area. The law of Laplace states that the tension in the wall of a distending tube is directly related to its diameter and the tension in the bowel wall distal and proximal to an anastomosis may greatly exceed that at the anastomotic line. For this reason we and a number of others have preferred to measure the breaking load of longitudinal enterotomy wounds or of segments of intestine containing an anastomosis. Collagen is responsible for the strength of a wound. A parallel association has been demonstrated between collagen concentration and wound strength in healing rat gastrotomy incisions. It seems reasonable to speculate, however, that the absolute amount of collagen within a wound and its quality and organization must be important factors.

Mastboom and colleagues recently failed to demonstrate any deleterious effect of steroid therapy (methylprednisolone 10 mg kg⁻¹ day⁻¹) in rats on the healing of intestinal anastomosis as assessed by bursting pressure. At 7 days, however, all the segments of bowel tested burst away from the anastomosis. We have demonstrated that steroid treatment (betamethasone 24 mg 50 kg⁻¹ day⁻¹) significantly decreases the breaking load of both ileal and colonic enterotomy wounds at 7 days.

Steroids might act at a number of points in a wound healing cascade to depress collagen synthesis. Steroid treatment causes profound monocytopenia. Monocytes are now recognized as having a pivotal role within a wound healing cascade. Once present at a site of healing they release peptide growth factors that recruit further cells and stimulate collagen and matrix production. In vitro steroids can be shown to have a direct effect on fibroblasts, decreasing messenger RNA transcript numbers for both procollagen and matrix protein. Clinically a direct depression of both collagen and matrix production might be responsible for the deleterious effects of steroid therapy on fibroplasia.

Basic FGF is ubiquitous and found throughout the body. It has chemotactic and mitogenic effects predominantly on cells of mesenchymal origin (including fibroblasts). In vitro it is also chemotactic and mitogenic for endothelial cells and in simple in vitro assays bFGF is a potent angiogenic factor. These two attributes of bFGF — its mitogenic effect on fibroblasts and its angiogenic properties in vitro — suggest it is a critical factor involved in the formation of granulation tissue.

Injection of bFGF into simple wound chambers increases the cellularity of granulation tissue. The infiltration of recombinant bFGF into healing rat incisional skin wounds on the third day after wounding increases the rate of gain of strength of treated wounds. The inhibition of healing in diabetes shows some similarities to that seen with steroid therapy; bFGF increases the cellularity of treated sponges in diabetic rats. Applications of bFGF in a collagen suspension to ileal enterotomy decreased the strength of treated incisions in comparison with collagen-treated controls at 7 days. Treated wounds appeared to be more cellular than control wounds. We have since confirmed that simple applications of bFGF to