Prostaglandins in Colonic Anastomotic Healing

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In this study we report the effects of flurbiprofen and prostaglandin E_2 on anastomotic tensile strength, collagen synthesis, and collagenolytic activity which are in a particularly fine balance in colonic healing. Colonic anastomoses were fashioned in 150 Sprague—Dawley rats which were allocated to receive either 20 mcg prostaglandin E_2 in 1 ml saline, 1 ml saline alone (control) intraperitoneally for three days postoperatively, or oral 2.5 mg/kg flurbiprofen daily. Anastomotic bursting pressures, collagen content and collagenolytic activity were measured at three, six, and ten days. It was found that prostaglandin E_2-treated animals had significantly weaker anastomoses at three days (102 ± 6.1 mm Hg; m $\pm$ SEM) compared with the control (126 ± 7.3; $P < 0.02$) or flurbiprofen group (128 ± 4.6; $P < 0.01$) with no differences at six and ten days. Collagen levels were higher in flurbiprofen-treated rats at three days (9.7 ± 0.2 μg hydroxyproline/mg tissue) compared with the control (8.1 ± 0.4 μg/mg; P 0.01) or prostaglandin E_2 group (7.2 ± 0.5 μg/mg; P 0.001). These differences were unchanged at six days but were not statistically different at ten days. Collagenolytic activity showed no differences in the three groups during the study. It is concluded that flurbiprofen enhances colonic healing with improved collagen synthesis without affecting collagenolytic activity. [Key words: Colonic healing; Anastomosis; Prostaglandin; Nonsteroidal anti-inflammatory drugs]

ANASTOMOTIC DEHISCENCE following colonic anastomoses is a serious clinical complication. A recent multicenter study of patients undergoing colonic resection for cancer showed that their hospital stay was doubled and mortality increased threefold when this complication occurred.[1] Schrock and associates,[2] in a large series, found that the presence of local infection was a major factor predisposing to leaks.

In an experimental model of diverticulitis, Yamakawa and others[3] found a fourfold increase in anastomotic leakage in the presence of inflammation.

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In 1970, Hawley *et al.*[4] showed, in experimental colonic anastomoses, that production of the enzyme collagenase is increased in the colon in the early inflammatory stages of the healing process adjacent to an anastomosis. Both polymorphonuclear cells and macrophages which infiltrate the wound at this stage produce collagenase. If a wound heals without infection, then the polymorphs are present for a short time only. It has been shown that macrophages play a continued, more important, role in later control of the healing process.[5] Wahl *et al.*[6] demonstrated that endotoxin-stimulated macrophages in cell culture produced collagenase in the presence of prostaglandin E_2, which could be reversed by the addition of indomethacin. Indomethacin is a nonsteroidal, anti-inflammatory drug which exerts its activity by the inhibition of prostaglandin synthetase. Prostaglandins, particularly those of the E series, are involved in the inflammatory process. Prostaglandin E_1, Prostaglandin E_2, and prostaglandin $F_{1\alpha}$ have been recognized as inflammatory mediators by their ability to increase vascular permeability,[7] and by their leukotactic properties[8]; they have also been isolated from inflammatory exudate in man and animals. The implication that prostaglandins might be involved in the production of collagenase prompted us to examine the effects of prostaglandin E_2 and flurbiprofen, a potent, nonsteroidal, anti-inflammatory drug, on colonic healing.

Materials and Methods

One hundred fifty female Sprague—Dawley rats (weighing 180 to 210 gm) were divided into three groups. Under ether anesthesia, all had a left-sided colonic anastomosis fashioned at a distance of 2 to 3 cm above the pelvic brim,

using a continuous 6/0 silk suture. The first group (n = 45) was allocated to receive prostaglandin E_2 (20 mcg in 1 ml of normal saline) injected intraperitoneally at the time of operation and for three days postoperatively. The crystalline prostaglandin E_2 was dissolved in ethanol to produce a stock solution and aliquots were then made up in carbonate buffer immediately prior to usage. The second group (n = 45) received 1 ml of normal saline alone intraperitoneally, at operation, and for three days postoperatively, and acted as controls. The third group (n = 30), in addition to intraperitoneal saline, were given oral flurbiprofen at a dose of 2.5 mg/kg/day.

On days three, six, and ten, animals were sacrificed and the bursting pressure of the anastomoses was measured *in situ*, using a Braun Melsungen pump in series with a Statham pressure transducer and the results recorded on a Bryan Model 27000 chart recorder (Bryans Ltd., U.K.). This was done by passing a soft rectal catheter, per anum, to a level just below the anastomosis, tying the colon above the anastomosis and around the catheter below and filling the segment with saline at a rate of 5 ml per minute until rupture of the anastomosis or adjacent colon occurred. The anastomoses were then excised together with adjacent colon (0.5 cm on either side). These specimens were then hydrolyzed and hydroxyproline content measured using the Woessner method.[9]

The remaining 30 rats were subdivided into three groups of ten. All rats had a left colonic anastomosis performed as above and received prostaglandin E_2 intraperitoneally (n = 10), intraperitoneal normal saline (n = 10) or oral flurbiprofen at the same dose as previously described. On the third postoperative day, all animals were sacrificed and the colon was excised as before. The specimens were homogenized in 1 ml of 100 millimolar tris buffer containing 15 millimolar calcium chloride at *p*H 7.6. The homogenate was assayed for collagenolytic activity using the acetylated C^{14} method for Cawston and Barrett.[10]

Results

The anastomotic bursting pressures are shown in Figure 1. At three days, the prostaglandin E_2-treated group was significantly weaker (102 ± 7.3 mm Hg; m ± SEM) when compared with either saline controls (126 ± 7.3 mm Hg; $P < 0.02$; Student's unpaired *t*-test) or the flurbiprofen-treated group (128 ± 4.6 mm Hg; $P < 0.01$). At six and ten days, there were no significant differences among the three groups. At ten days, bowel rupture occurred at sites away from the anastomoses.

Hydroxyproline levels (Fig. 2) were significantly lower in the prostaglandin E_2-treated animals at three days (7.2 ± 0.47 μg/mg dry wt; m ± SEM) compared with the flurbiprofen-treated group (9.7 ± 0.23 μg/mg; $P < 0.001$; Student's unpaired *t*-test) but not with the saline controls (8.1 ± 0.37 μg/mg). The flurbiprofen-treated group contained significantly more hydroxyproline than the saline control ($P < 0.01$). At six days, both the prostaglandin E_2-treated (9.1 ± 0.38 μg/mg) and control animals (9.5 ± 0.37 μg/mg) had significantly lower hydroxyproline levels than the flurbiprofen group (11.1 ± 0.4 μg/mg; $P < 0.01$ and $P < 0.05$, respectively). At ten days, the mean anastomotic hydroxyproline content of the three groups was not statistically different.

The results of collagenolytic activity in the three groups are shown in Figure 3. There were no statistically significant differences using nonparametric tests.

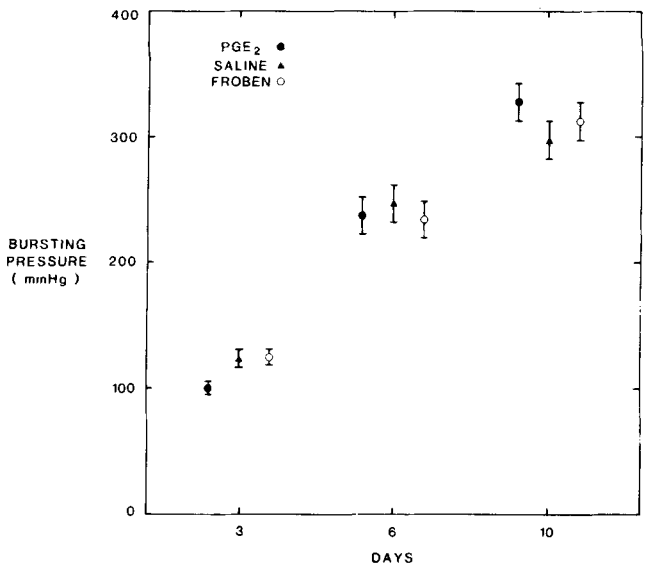

FIG. 1. Anastomotic bursting pressure of colonic anastomoses (Mean ± SEM).

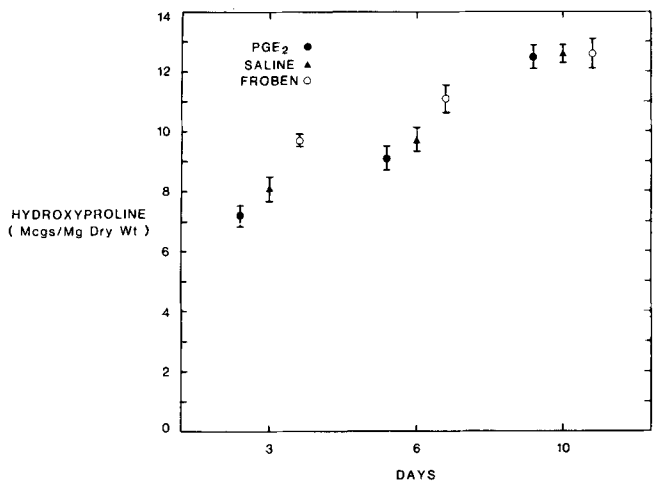

FIG. 2. Hydroxyproline content of anastomoses (Mean ± SEM).

Discussion

The normal healing process in the colon represents a particularly fine balance between synthesis and lysis of collagen.[4] Raisz and Koolemans-Beynen[11] showed that prostaglandin E_2 inhibited the incorporation of labeled proline into collagenase-digestible protein in fetal rat calvaria in tissue culture. A similar inhibition of hydroxyproline accumulation has been demonstrated when prostaglandin E_2 was administered early during granuloma formation.[12] This suggested that excess prostaglandin may interfere with collagen synthesis. Wahl *et al.*[6] showed an increased production of collagenase in endotoxin-stimulated macrophages which is enhanced by the addition of prostaglandin E_2, but is reversed by indomethacin. Thus, there are two possible ways in which prostaglandin can adversely affect the collagenous equilibrium of the healing process. The importance of collagenase in healing colonic anastomoses has been emphasized by Hawley *et al.*[4] He found enhanced activity throughout the gastrointestinal tract following surgery, even at sites distant from the colon, and considered it to be a major factor in the etiology of anastomotic dehiscence.

We have examined the effect of prostaglandin E_2 on colonic healing. The results suggest that inhibition of prostaglandin synthesis by administration of the nonsteroidal, anti-inflammatory agent flurbiprofen may improve healing in the colon. Excess prostaglandin E_2 in the vicinity of the anastomosis did not affect the accumulation of hydroxyproline compared with controls, although the bursting strength was lower at three days. There is no significant difference, however, between the prostaglandin E_2 and saline-treated groups. This does not necessarily mean that excess prostaglandin in the region of the wound is not detrimental. It may be that injected prostaglandin E_2 did not reach the site of early healing or, alternatively, that the half-life of prostaglandin E_2 was too short to allow it to exert its effect.

The effect of nonsteroidal, anti-inflammatory agents in healing is controversial. Several workers have shown poor healing in skin wounds in experimental animals, but there have been no previous reports of these drugs in colonic healing, although the use of related compounds such as sulphasalazine in inflammatory bowel disease is well known.[13] Endotoxemia and endotoxic shock enhance arachidonic acid metabolism, which results in increased production of prostaglandins.[14] In the clinical situation, this may be of importance in relation to operations on inflamed bowel, as in diverticulitis. Our results show that flurbiprofen influences colonic healing favorably and appears to increase collagen production without having any effect on collagenolytic activity.

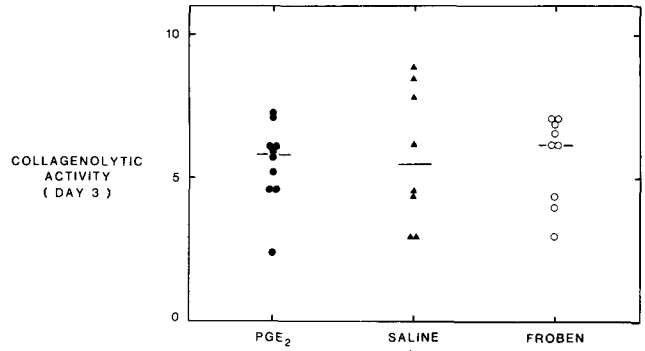

FIG. 3. *Collagenolytic activity of colonic anastomoses.*

Acknowledgments

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References

1. Fielding LP, Stewart Brown S, Blesovsky L. Anastomotic integrity after operation for large bowel cancer: a multicentre study. Br Med J 1980;281:911-8.
2. Schrock TR, Deveney CW, Dunphy JE. Factors contributing to leakage of colonic anastomoses. Ann Surg 1973;177:513-8.
3. Yamakawa T, Snyder Patin C, Sobel S, Morgenstern L. Healing of colonic anastomoses following resection for experimental "diverticulitis." Arch Surg 1971;103:17-20.
4. Hawley PR, Faulk UP, Hunt TK, Dunphy JE. Collagenase activity in the gastro-intestinal tract. Br J Surg 1970;57:896-900.
5. Ross R. Inflammation, cell proliferation, and connective tissue formation in wound repair. In: Hunt TK, ed. Wound healing and wound infection: theory and surgical practice. New York: Appleton Century Crofts, 1980:1-11.
6. Wahl LM, Wahl SM, Mergenhagen SE, Martin GR. Collagenase production by endotoxin-activated macrophages. Proc Natl Acad Sci USA 1974;71:3598-3601.
7. Williams TJ, Morley J. Prostaglandins as potentiators of increased vascular permeability in inflammation. Nature 1973;246:215-7.
8. Higgs GA, McCall E, Youlten LJ. A chemotactic role for prostaglandins released from polymorphonuclear leukocytes during phagocytosis. Br J Pharmacol 1975;53:539-46.
9. Woessner JF. In: Determination of hydroxyproline in tissue and protein samples containing small proportions of the imino acid. Arch Biochem Biophys 1961;93:440-7.
10. Cawston TE, Barrett AJ. A rapid reproducible assay for collagenase using [1-^{14}C] acetylated collagen. Anal Biochem 1979;99:340-5.
11. Raisz LG, Koolemans-Beynen AR. Inhibition of bone collagen synthesis by Prostaglandin E_2 in organ culture. Prostaglandins 1974;8:377-85.
12. Farnham MJ, Shoshan S, Schoenmaker W, Benta IL. Collagen metabolism and phenotype after Prostaglandin E_2 treatment of granuloma: direct and macrophage modulated effects. Prostaglandins 1982;23:85-98.
13. Lee KW, Tong TG. Mechanism of action of salicylates: VIII. Effect of topical application of retinoic acid on wound healing retardation action of a few anti-inflammatory agents. J Pharm Sci 1970;59:1036-8.
14. Cook JA, Wise WC, Halushka PV. Elevated thromboxane levels in the rat during endotoxic shock. J Clin Invest 1980;65:227-30.