Effects of Chronic Corticosteroids and Vitamin A on the Healing of Intestinal Anastomoses

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The ability of vitamin A to reverse the inhibitory effects of chronic corticosteroids on cutaneous and facial wound healing is well established. To investigate this in the unique low-collagen environment of the intestinal anastomosis, 35 rabbits received twice-daily injections of either saline (control), dexamethasone (0.1 mg/kg/day), dexamethasone plus low-dose vitamin A (1,000 IU/kg/day); or dexamethasone plus high-dose vitamin A (10,000 IU/kg/day) for a 2-week period. Animals then underwent creation of single-layer, inverting small and large intestine anastomoses. All injections were continued postoperatively. A fifth group received only dexamethasone preoperatively and dexamethasone plus high-dose vitamin A postoperatively. On postoperative day 7, animals underwent in situ assessment of anastomotic bursting pressure and subsequent histologic examination using a modified Ehrlrich/Hunt scale. Corticosteroids significantly impaired the healing of small and large intestine anastomoses, with decreased bursting pressures and histologic parameters at 1 week. Only high-dose vitamin A significantly reversed this inhibitory effect, whether given preoperatively or only postoperatively.

Clinically evident anastomotic leakage, or dehiscence, occurs in approximately 5% to 10% of patients following colonic anastomosis (reported range: 1% to 23%) and somewhat less often with small intestine anastomosis [1-3]. Retrospective studies have shown that, if leakage occurs, the duration of hospital stay doubles and the postoperative mortality rate triples [1,3]. Twenty percent to 80% of all postoperative deaths can be attributed to anastomotic leakage with its subsequent sequelae, which include peritonitis, abscess formation, and sepsis [2,3,5].

Ehrlrich and Hunt [6] demonstrated over 20 years ago that in rats corticosteroids significantly impair the healing of cutaneous wounds and that vitamin A has the unique ability of partially reversing this effect. Subsequent studies by other investigators have shown similar effects of corticosteroids on wounds of tendons [7], bones [8], and arteries [9], and the formation of granulation tissue [10]. However, healing in these tissues differs greatly from that in the gastrointestinal tract, which tends to develop with less strength and at a slower rate [11]. Although most retrospective series have shown a trend toward higher leakage rates in corticosteroid-treated patients, these differences were usually not statistically significant [2,4]. Few authors have been willing to state that steroids predispose a patient to anastomotic dehiscence [3]. Indeed, there is a relative paucity of objective data concerning the effects of chronic doses of corticosteroids on the healing of bowel anastomoses.

This study was designed to determine, in an animal model, whether the administration of chronic supraphysiologic doses of exogenous corticosteroids impairs the healing of intestinal anastomoses and if, as with cutaneous wounds, the administration of vitamin A in the perioperative period might reverse this effect.

MATERIALS AND METHODS

Thirty-five adult male New Zealand white rabbits (3.0 to 3.7 kg), obtained from a commercial breeder, were randomly assigned to five groups (Table 1). For 2 weeks preoperatively, rabbits received twice-daily intramuscular injections of either saline (control group), dexamethasone sodium phosphate (0.1 mg/kg/day) (Elkins-Sinn, Inc., Cherry Hill, NJ), dexamethasone plus low-dose vitamin A (1,000 IU/kg/day) (Aquasol A, Armour Pharmaceutical Co., Kankakee, IL), or dexamethasone plus high-dose vitamin A (10,000 IU/kg/day). The fifth group received only dexamethasone preoperatively and then dexamethasone plus high-dose vitamin A postoperatively. The dexamethasone dosage would be approximately equivalent to 40 mg of prednisone per day in the average 70 kg adult human. Both vitamin A doses are well below presumed toxic doses in rabbits [12]. All injections were continued postoperatively.

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ileocecal valle peritoneal arcades. Immediate anastomosis of both small and large intestine was performed using inverting, interrupted 6-0 polypropylene suture (Prolene, Ethicon, Inc., Somerville, NJ). This technique was chosen because it has been determined to minimize cellular reaction, luminal stenosis, interference with anastomotic blood flow, and disruption of collagen metabolism [13], all of which might potentially interfere with study results. Rabbits were allowed chow and water ad libitum, beginning on postoperative day 1.

Seven days later, all rabbits underwent relaparotomy under general anesthesia for determination of anastomotic bursting pressure in situ without interruption of normal mesenteric blood supply or adhesions to the anastomosis. Using a modification of the technique described by Jiborn et al [14], intestinal segments were ligated with heavy silk 1 cm distal to each anastomosis. Polyethylene pressure tubing was then introduced 1 cm proximal to each anastomosis and held in place with a heavy suture. Air was then insufflated at a constant rate of 5.0 mL/min. By filling the open abdominal cavity with warmed saline, bursting was defined as the point at which air bubbles were seen streaming from the anastomosis (or adjacent bowel) into the saline solution. This correlated with an abrupt fall in the measured intraluminal pressure, continuously monitored on a Hewlett-Packard recorder (model 7833A).

Adhesions were assessed, and each intestinal segment was excised. Animals were then killed, polypropylene sutures were carefully removed, and anastomotic cross-sections were fixed in 10% formalin. After being stained with hematoxylin and eosin, anastomoses were graded histologically in a blinded fashion, using a modified 0 to 4 Ehrlich and Hunt numerical scale (Table II) [15]. Evaluated parameters were inflammatory cell infiltrate (white blood count), fibroblast and blood vessel ingrowth, and collagen deposition. Data are expressed as mean ± the standard error of the mean (SEM). Statistical comparisons were made using analysis of variance and Fisher’s PLSD tests with p <0.05 considered significant.

RESULTS

Thirty-one rabbits survived the entire study period. Three animals died of premedication overdosage early in the study period, and one rabbit died on the first postoperative day of undetermined cause. There were no spontaneous anastomotic dehiscences, intra-abdominal abscesses, or other infections. Mean weights of each study group decreased by 7% to 13%, but no group had a significantly different mean weight at any time point. There were no appreciable differences between groups in the incidence of adhesions at the anastomotic sites (Table III).

On the day of surgery, following a 12-hour fast, rabbits were premedicated with intravenous ketamine hydrochloride (2 mg/kg) and acepromazine maleate (0.8 mg/kg) and then received xylazine sodium 100 mg/kg intramuscularly (Ketol, Eli Lilly and Co., Indianapolis, IN). Rabbits were anesthetized with halothane in oxygen (1.0% to 4.0%) by mask. Using sterile technique, each rabbit underwent midline laparotomy. One-centimeter segments of distal ileum (10 cm proximal to the ileocecal junction) and sigmoid colon (5 cm proximal to the peritoneal reflection) were excised between prominent vascular arcades. Immediate anastomosis of both small and large intestine was performed using inverting, interrupted 6-0 polypropylene suture (Prolene, Ethicon, Inc., Somerville, NJ). This technique was chosen because it has been determined to minimize cellular reaction, luminal stenosis, interference with anastomotic blood flow, and disruption of collagen metabolism [13], all of which might potentially interfere with study results. Rabbits were allowed chow and water ad libitum, beginning on postoperative day 1.

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of controls (Table IV). Low-dose vitamin A did not significantly increase bursting pressures, although high-dose vitamin A, given to steroid-treated rabbits, significantly increased bursting pressures, to within approximately 30% of normal levels, when administered either preoperatively or only postoperatively (p < 0.05).

This salutary effect of vitamin A was even more notable in large intestine anastomoses (Table IV). As for the small intestine anastomosis, corticosteroids reduced bursting pressures in the colonic anastomoses by approximately 50%. Although low-dose vitamin A appeared to increase large intestine bursting pressures above levels found with the corticosteroids, this increase was not statistically significant. High-dose vitamin A, however, increased bursting pressures to within 12% of control values, and over 55% above those in rabbits receiving dexamethasone alone (p < 0.05).

Four small intestine and five large intestine anastomoses were damaged during suture removal and thus deemed unsuitable for histologic evaluation. A typical control rabbit small intestine anastomosis in cross section is shown in low power in Figure 1. A large area of scar tissue is present on the serosal surface, with evidence of neovascularization and fibroblast proliferation. The two large holes are artifacts from polypropylene sutures. In contrast, the small intestine anastomotic cross-section shown in Figure 2, from a dexamethasone-treated rabbit, demonstrates a relative paucity of scar tissue. Although there are some new blood vessels present and a small number of fibroblasts, there are few inflammatory cells and minimal collagen.

A summary of the histologic data from the small intestine anastomoses is shown in Figure 3. Corticosteroids caused reductions in all assessed parameters, including inflammatory cell infiltration, blood vessel and fibroblast ingrowth, and collagen deposition, when compared with controls (p < 0.05). Although low-dose vitamin A increased fibroblast ingrowth above dexamethasone levels, only high-dose vitamin A, administered either preoperatively or only postoperatively, returned all assessed parameters to near-control levels.

Similar findings were observed in large intestinal anastomoses and are shown in Figure 4. Although collagen levels did not appear to differ significantly between the
groups, a marked improvement in the other wound-inhibitory effects caused by corticosteroids was apparent when high-dose vitamin A was administered.

There was no evidence of hypervitaminosis A in any rabbits from the three groups of animals that received vitamin A. Liver biopsies obtained at the time of death failed to show any morphologic changes or evidence of hypervitaminosis A [16].

COMMENTS

Wound healing remains an incompletely understood series of events, with initial tissue disruption and hemorrhage, the release of various chemoattractants and vaso-dilatory factors, inflammatory cell infiltration, and capillary ingrowth. Fibroblasts then proliferate and synthesize collagen that, in time, is remodeled to form a mature scar.

This process has been studied extensively in various animal models with respect to the healing of intestine anastomoses [11,13,17,18]. Although similar to the frequently studied skin and fascial wounds, collagen synthesis and scar formation in the intestine has a different time course and magnitude. This may be, in part, a function of the relative paucity of collagen in excised intestinal wounds (5% by dry weight) when compared with what is found in the skin or fascia (50% by dry weight), or the marked difference in collagen turnover [19]. In addition, healing has been shown to be quite different in small versus large intestinal anastomoses [17,18,20]. As shown in the present study, normal bursting pressures in large intestinal anastomoses were only one half to two thirds as high as those in the small intestine. This is probably a result of the relative paucity of collagen in the colonic
Figure 3. Small intestine (SI) anastomosis histologic grading (mean ± SEM). * = p < 0.05 versus control. ANOVA and Fisher’s PLSD tests. See Table III for definitions of abbreviations.

Figure 4. Large intestine (LI) anastomosis histologic grading (mean ± SEM). * = p < 0.05 versus control. ANOVA and Fisher’s PLSD tests. See Table III for definitions of abbreviations.
at only one time point, the observed reductions in healing may merely be delays in the normal healing process. Indeed, the rat studies mentioned above [26–28] and studies of skin and fascial healing have indicated that, after several weeks, wounds in both steroid-treated animals and in controls are capable of achieving equal strength. However, if intestinal anastomotic healing is delayed, as in steroid-treated patients, when the usual postoperative ileus resolves and luminal bacterial concentrations increase, contractions begin, intraluminal pressure increases, and intestinal contents may be forced through weakened areas in the anastomosis causing peritoneal contamination.

Corticosteroids appear to disrupt anastomotic healing primarily by interfering with the normal inflammatory phase [15], perhaps by stabilizing membranes of the intracellular lysosomes of the white blood cells [5]. If one assumes that the steps of the wound healing cascade must occur in sequence, then simply interfering with the inflammatory phase could delay subsequent phases and therefore explain the histologic findings in this study of diminished leukocyte infiltration, fibroblast and blood vessel ingrowth, and collagen deposition in the corticosteroid-treated animals. However, corticosteroids are also believed to inhibit fibroblast proliferation [29], antagonize the effects of circulating angiogenesis factors and thus limit capillary budding [30], and inhibit collagenase activity [31], and thus the histologic observations may have varied explanations.

Data from the present study that demonstrate that high-dose vitamin A partially reverses the inhibitory effects of steroids on wound healing are in agreement with earlier studies using other tissues [6,10,15,32]. Vitamin A has been shown to increase colonic anastomotic bursting strength in control (nonsteroid-treated) rat colon anastomoses and those subjected to preoperative irradiation [22,33]. However, the vitamin A doses given in these studies were expressed as IU vitamin A per g chow and are thus difficult to compare with the present data.

The exact mechanism by which vitamin A antagonizes the effects of corticosteroids in the wound healing cascade is unclear. However, it is believed that vitamin A may increase the percentage of macrophages and monocytes in the inflammatory phase of healing [34] and also counteract the effects of corticosteroids on the lysosome [6]. In addition, vitamin A has been shown to increase collagen production when added to cultured fibroblasts [35]. Although previous studies have suggested that vitamin A may increase the formation of peritoneal adhesions (and thus assist in revascularization of ischemic intestine) [36], such an effect was not seen in this study.

In conclusion, corticosteroids significantly impaired the healing of small and large intestinal anastomoses in rabbits, with an approximately 50% decrease in anastomotic bursting pressure and diminished histologic evidence of healing at 7 days. Only high-dose vitamin A appreciably reversed this inhibitory effect, when administered either preoperatively and/or postoperatively. It would appear that patients receiving chronic corticosteroid therapy may benefit from receiving perioperative vitamin A when undergoing intestinal surgery.

REFERENCES

CORTICOSTEROIDS AND VITAMIN A ON INTESTINAL ANASTOMoses

DISCUSSION

Boyd E. Terry (Columbia, MO): I've long practiced the dictums of Dr. Hunt to give high doses of vitamin A to block the effects of steroids on wound healing. Do you have any evidence of a critical serum level of vitamin A to produce the effect in your model?

Jason H. Bodzin (Detroit, MI): The therapeutic levels or therapeutic doses of vitamin A advocated for humans would more closely approximate that of your lower dose vitamin A therapy, which were not found to be therapeutic. What would you suggest that we do to confront that issue?

Arthur H. Aufsers, Jr. (New York, NY): You have shown a decrease in fibroblast activity in the cortisone-treated group, but you have shown an increase in collagen formation over your control group. How do you account for that, since collagen and fibroblast activity are linked?

Jon Thompson (Omaha, NE): We have had an interest in studying a different type of wound healing in rabbits, full-thickness intestinal defects. When we administered steroids chronically, as you did, we found it had a deleterious effect on mucosal function and resulted in weight loss compared with our control animals. Did you observe any weight loss in your animals, and, if so, could this possible malnutrition have an influence on the wound healing parameters that you measured? Are you aware of a differential effect of steroids on the different parameters? I think there is some evidence that vitamin A is more effective at reversing the effects of steroids on mucosa, e.g., epithelialization rather than in the deeper tissues.

Gerald M. Fried (Montreal, Quebec, Canada): You have not reported any data regarding the leak rate in the two groups of animals. If you are going to suggest that the vitamin A is important clinically, we should know whether it, in fact, diminished the incidence of anastomotic leaks. Do you have any information regarding this?

Dr. Terry, we did not measure serum vitamin A levels in this study because levels have not been shown to correlate with systemic effects.

Dr. Bodzin, I would hesitate to encourage the routine use of high-dose vitamin A in human patients receiving corticosteroids. However, our study, as well as previously reported studies in other animal models, has shown that the amount of vitamin A necessary to enhance wound healing is much higher than routine recommended dosages for normal animal function.

Dr. Aufsers, there were no statistically significant differences in collagen content (graded histologically) in the large intestinal anastomoses. I cannot explain this finding. Perhaps other stains would have better demonstrated the collagen.

Dr. Thompson, all animals were weighed twice weekly, and, during the course of the study, rabbits lost between 7% and 13% of their total body weight. However, there were no significant differences in weight loss between the different groups.

Dr. Fried, there were no anastomotic leaks, no intra-abdominal abscesses, and no cases of peritonitis in the five groups. The problem with using leakage as an end point is that one would have to operate on hundreds of rabbits to show a statistically significant difference between one therapy and another, since the overall leakage rate is only 5% to 10%.

Dr. Thorson, your study and a number of others have shown a return to normal healing after 14 to 21 days in steroid-treated animals. However, in the unique environment of the intestine, it is crucial that the healing be at least partially complete within 7 to 10 days when the normal postoperative ileus resolves.