Platelet-Derived Growth Factor and Wound Contraction in the Rat

Bradley P. Karr, B.A.,* Paul J. Bubak, M.D.,* K. H. Sprugel, Ph.D.,† Edward G. Pavlin, M.D.,‡ and Loren H. Engrav, M.D.*

*Division of Plastic Surgery, Harborview Medical Center, University of Washington, Seattle, Washington; †ZymoGenetics, Inc., Seattle, Washington; and ‡Department of Anesthesiology, Harborview Medical Center, University of Washington, Seattle, Washington 98104

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This experiment was undertaken for three purposes: (1) to determine a dose–response curve of acute steroid inhibition of wound contraction in the rat; (2) to confirm the results of our preliminary study that platelet-derived growth factor (PDGF) enhanced wound contraction in acutely steroid impaired rats; and (3) to examine the histology of the PDGF-treated wounds.

To determine the dose–response of acute steroid inhibition of wound contraction, the rats were suppressed with daily doses of methylprednisolone and wound contraction measurements revealed no improvement in the amount or rate of wound contraction. Histologically, the wounds were all very similar in the patterns of cellularity, granulation tissue maturity, collagen content, and epithelial migration. We have clarified the dose response of acute steroid inhibition of wound contraction in rats, data previously unavailable, and have concluded that PDGF in reasonable doses does not improve wound contraction in steroid-impaired rats nor does it alter the histology of the wounds.

INTRODUCTION

Growth factors derived from platelets have been shown to be important mediators in wound healing processes. Platelet-derived growth factor (PDGF), in particular, exhibits potent chemotactic activity for monocytes, fibroblasts, and smooth muscle cells and accelerates the healing and increases the breaking strength of linear wounds in rats [1–16].

Monocytes and fibroblasts are necessary for normal wound contraction to occur and the number of these cells in wounds and wound contraction is diminished by steroids [17–22]. Since PDGF is chemotactic for monocytes and fibroblasts and since the absence of monocytes and fibroblasts impairs wound contraction, PDGF may augment wound contraction in acutely steroid-impaired animals. In fact, we have previously reported in a preliminary study that PDGF did augment wound contraction in the acutely steroid-impaired rat but the observed effect was minimal [23]. On the contrary, Pierce et al. [24] have reported that, in steroid-treated animals, PDGF will not reverse the effect of steroids on wound breaking strength, does not attract monocytes into the wound, and does not increase synthesis of type I procollagen. Increased numbers of fibroblasts were observed, however. Pierce et al. [25] have also reported that PDGF depresses the number of myofibroblasts in excisional wounds. Neither of these articles actually studied wound contraction. In fact, we could only find two articles in which the effect of PDGF on wound contraction was evaluated and the authors reported no effect in normal animals [26] and the diabetic mouse [10]. So the effect of PDGF on wound contraction remains unclear. Furthermore, we could not find in the literature a dose–response curve of the effect of steroids on wound contraction, which is essential to studies such as these since oversuppression can mask the effects of the growth factors.

We undertook the following experiments to (1) establish the dose–response curve of acute steroid inhibition of wound contraction in rats; (2) confirm the results of our preliminary study of the effect of PDGF on wound contraction in acutely steroid-impaired rats; and (3) to examine the histology of the PDGF-treated wounds.

MATERIALS AND METHODS

To clarify the dose–response of acute steroid inhibition of wound contraction in the rat, 34 male, albino rats, averaging 300 g, were divided into eight groups and maintained on rat chow and water according to University of Washington animal care guidelines. The
animals were given daily methylprednisolone doses of 0.0, 0.4, 0.8, 1.2, 1.6, 2.0, 3.0, and 4.0 mg. The primary investigator was blinded to the steroid dose. Daily intramuscular injections of steroids were given for 5 days prior to wounding and for the remainder of the experiment. On Day 5, according to the previously described model, a circular, 4-cm diameter wound was made on the dorsum of each rat [27]. All wounds were dressed with Adaptic, 4 × 4 gauze and Tubigrip. The rats were anesthetized daily with halothane for the next 12 days to change dressings, measure wounds, inject steroids, and obtain photos. Three acetate tracings of each wound were made daily. The areas of the daily tracings were determined by computer graphics. The means were calculated, expressed as a fraction of the original mean wound area, and plotted versus time. The areas under the curves were compared using Student’s t test, with P < 0.05 considered significant, as described in Part II [23].

In an effort to confirm our preliminary, 100 male Sprague–Dawley rats, averaging 300 g each, were divided into five groups of 20 rats per group. Each rat was treated as described above and received 2.0 mg methylprednisolone daily or the equivalent dose of hydrocortisone (10 mg) beginning 5 days prior to wounding. In addition, recombinant, human PDGF (β-chain homodimer (rPDGF-BB), provided by ZymoGenetics, Inc., Seattle, WA) [28] in doses of 0, 3, 5, 10, and 15 μg was applied daily for 12 days, beginning the day of wounding. The upper dose was chosen to be five times our previous doses, at the upper level of clinical relevance, and similar to the doses of Greenhalgh [10]. The vehicle consisted of phosphate-buffered saline and 5% polyethylene glycol 8000 (Fisher Scientific, 711 Forbes Avenue, Pittsburgh, PA 15219), which is known to have no effect on the activity of PDGF. The investigator was blinded to the dose and order of application and both were randomized. PDGF concentrations were established by amino acid analysis and measurement of mitogenic activity.

To qualitatively evaluate the histology of the wounds, 4-mm punch biopsies were obtained from the center of the wound of 2 rats per group on Days 4 and 8. On Day 12 the entire wound was excised and submitted for H&E and trichrome histology. The histologists were blinded to the concentrations of rPDGF-BB and the steroid dose. Each specimen was qualitatively scored on wound thickness, vascularity, cellularity, collagen content, epithelial migration, and granulation tissue maturity as previously described [10].

RESULTS

In the steroid, dose–response experiment, all rats survived. Figure 1 shows that significant impairment of wound contraction occurred with daily methylprednisolone doses of 2.0 mg and that higher doses did not further impair contraction. Figure 2A shows the fraction of the original wound area remaining over time for subjects that received 0.4, 0.8, and 1.2 mg steroid compared to controls. Only a minimal effect is evident, and these findings are not statistically significant. In contrast, Fig. 2B compares doses of 2.0, 3.0, and 4.0 mg to controls and reveals a statistically significant impairment of wound contraction during the first 4 days after wounding. The subsequent rate of contraction parallels the control group but the total amount of contraction in the impaired subjects remains less than controls at the end of the experiment.

In the experiment designed to confirm our preliminary finding that PDGF improved wound contraction in steroid-impaired rats, data from 80 animals were available for analysis. Twenty rats died intraoperatively, presumably from anesthetic overdose. The surviving rats appeared healthy, maintained their weights, and took food and water well. The surviving rats were given 0, 3, 5, 10, and 15 μg/ml rPDGF-BB and numbered 17, 14, 18, 16, and 15 rats per group, respectively. The mean wound fraction open was plotted versus time, beginning on the day of wounding. The curves are shown in Fig. 3. The curves for the different doses of PDGF are essentially identical. The slopes of the curves show that wound closure occurred at the same rate in each group. When the curves were integrated and the mean area under the curves compared for each of the PDGF groups, no difference was found in the amount of wound contraction in the various treatment groups.

The H&E and trichrome histology of the final wounds at Day 12 was similar for all groups. For each dose of rPDGF-BB, the wounds all appeared very similar in the patterns of cellularity, granulation tissue maturity, and collagen content. Only a modest amount of epithelial migration was evident.

DISCUSSION

Although many studies have used acute steroid-induced impairment of wound contraction as a basis for
analysis of the effects of various growth factors on restoration of wound healing, the dose–response curve for the rat model of wound contraction has not been established. It is important to establish optimal suppression levels to better understand the effect of therapies in wound contraction. Oversuppression of wound healing can render the animal unable to respond to injury [24]. From this study, the threshold and optimum dose of methylprednisolone suppression of wound contraction in rats appears to be 2 mg/wound or 6.7 mg/kg given daily as described. This may, in fact, be the most important contribution of this work since these data were previously unavailable.

The results in our earlier study that suggested rPDGF-BB accelerated wound contraction in a steroid-impaired rat were not reproduced in this study. Rather than a dose–response curve, we found no improvement in the amount or rate of contraction in the wounds using various amounts of rPDGF-BB ranging from 0 to even 15 μg. As mentioned earlier, we attempted to compare our results to others in the literature. But we could find no other studies of the effect of PDGF on wound contraction in steroid-impaired animals. The literature does confirm, however, that PDGF has little effect on reversing the effect of steroids on wound breaking strength [24]. There are two possible explanations for our failure to confirm our earlier report. It is possible that rPDGF-BB does have an effect on wound contraction in steroid-impaired rats but that the effect is minimal and therefore difficult to reproduce with small experiments. A more likely explanation, however, is that PDGF in reasonable doses does not have an effect on wound contraction in acutely steroid-impaired animals and that our first observation was by chance alone.