Effect of Topical Mitomycin on Skin Wound Contraction

Gregory K. Sewall, MD; Kevin M. Robertson, MD; Nadine P. Connor, PhD; Dennis M. Heisey, PhD; Gregory K. Hartig, MD

Objective: To evaluate the effect of different dosing regimens of mitomycin on skin wound contraction.

Methods: Full-thickness skin wounds were created in 5 groups of hairless mice, which represented different dosing regimens or a sterile water control: A, control; B, mitomycin (0.5 mg/mL) applied immediately after creation of the lesion (day 1); C, mitomycin (1.0 mg/mL) applied on day 1; D, mitomycin (0.5 mg/mL) applied on days 1 and 3; and E, mitomycin (1.0 mg/mL) applied on days 1 and 3. Wound surface area was measured immediately after drug application (day 1), and thereafter every 3 to 5 days until day 29 by means of computer-assisted image analysis.

Results: All dosing regimens of mitomycin application resulted in an initially exponential rate of wound contraction that was significantly slower than in the sterile water control group, with a significantly larger wound surface area on day 29. Wound area in the control group contracted approximately 9 times more rapidly than in the treatment groups. No difference was observed among the different dosing regimens.

Conclusion: Application of mitomycin, at the lowest dose and frequency of application used in this study, resulted in improved outcomes with regard to contraction of full-thickness skin wounds.

Arch Facial Plast Surg. 2003;5:59-62

Wound Healing involves several complex and overlapping stages, including inflammation, granulation, epithelialization, and wound remodeling. The last stage consists of finely orchestrated interactions between the cellular and extracellular components that typically result in strengthening and contraction of the newly healed wound. Fibroblast migration and myofibroblast intracellular contraction have been shown to be directly responsible for wound contracture, an event that is advantageous and vital to wound closure. However, shrinkage of a healed wound can lead to scar contractions with extensive functional and cosmetic morbidity. Therefore, there is continued interest in the development of interventions that modulate the wound healing process to obtain an improved treatment outcome.

Mitomycin, an antimetabolite isolated from the broth of Streptomyces caespius, has been shown to reduce fibroblast-dependent granulation and scar tissue formation. In vitro, itomycin inhibits fibroblast proliferation in cell cultures, presumably through the inhibition of DNA, RNA, and protein synthesis. These properties have contributed to its interest as a potential agent for reducing scar tissue formation.

Numerous studies have examined the potential benefits of topically applied chemicals, including corticosteroids, antimicrobials, and colchicine, on reducing wound contraction. Mitomycin has been associated with reduction in scar tissue formation in tissues of the trachea, eye, sinonasal passages, and middle ear. Specifically, reductions in airway stenosis and scar formation have been documented after myringotomy, sinonasal, and ophthalmologic surgeries. Recently, this substance has been shown to effectively inhibit subglottic and laryngotracheal stenosis in dogs. Although these studies have examined squamous mucosal tissue, rather than skin wounds, their success is encouraging with regard to extending the application of mitomycin to the prevention of skin wound contraction. However, because of the known bone marrow, renal, and pulmonary toxic effects due to centrally administered mitomycin, attention...
tion to the benefits and potential adverse effects of different mitomycin dosing regimens must be maintained and examined.

The purpose of this study was to determine whether the antifibroblastic properties of mitomycin could be exploited to modulate wound contraction in a hairless mouse model of full-thickness skin wounds. In addition, variable dosing regimens were used to assess dose-response and duration effects.

METHODS

This study was performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act (7 USC et seq, October 2000; available at: http://grants1.nih.gov/grants/olaw/references/phspol.htm); the animal use protocol was approved by the Institutional Animal Care and Use Committees of the University of Wisconsin, Madison.

WOUND CREATION AND DRUG APPLICATION

Forty male hairless mice weighing 20 to 30 g (HRS/HR HR strain; Simonsen Laboratories, Inc, Gilroy, Calif) had general anesthesia induced and maintained by inhalation of isoflurane. The mice were randomly assigned to 1 of 5 study groups, which included different concentrations and frequencies of application of mitomycin (Bristol-Myers Squibb, Princeton, NJ) or a control solution (sterile water) that represented the solvent for mitomycin.

As shown in Figure 1, a full-thickness excisional square wound (1 cm²) was created 0.5 cm from the midline on each side of the dorsum of each mouse. Either mitomycin or the sterile water control was then applied topically to one side, while the other side was left untreated. The concentration and frequency of application varied according to study group assignment. The 5 study groups were as follows: A, sterile water; B, mitomycin (0.5 mg/mL) applied on days 1 and 3; C, mitomycin (1.0 mg/mL) applied on day 1; D, mitomycin (0.5 mg/mL) applied on days 1 and 3; and E, mitomycin (1.0 mg/mL) applied on days 1 and 3. The starting mitomycin concentration of 0.5 mg/mL was chosen to approximate dose levels used clinically at our institution and doses reported for other applications. Repeated application was established on day 3 because of the expectation of rapid, dynamic wound healing at this time point.

To obtain the target concentrations, mitomycin was solubilized in sterile water and then maintained at 4°C between applications. After wound creation, animals were housed in separate cages and maintained on standard rodent chow and water ad libitum.

WOUND MEASUREMENT

Initial measurements of wound surface area were made immediately after drug application (day 1) and thereafter every 3 to 5 days until the final measurement on day 29. Day 29 was chosen as the final day of wound measurement because of the expectation of complete reepithelization of the wound by that time.

To allow digital measurement, the border of each wound was manually traced onto transparent acetate sheets with 1 × 1-cm² calibration grid. Care was taken to trace only the advancing full-thickness margin rather than any inflammatory granulation tissue or the advancing epithelium. At the end of the observation period, the acetate sheets containing the wound tracing were digitally scanned for the purpose of wound area quantification.

With the use of Adobe Photoshop (version 5.5; Adobe Systems Inc, San Jose, Calif), computer-assisted image analysis was performed to calculate the number of pixels encompassing each wound tracing. We then converted the number of pixels to square centimeters.

STATISTICAL ANALYSIS

Semilog plots indicated that, up until 15 days, the decrease in wound area was log-linear, that is, exponential. Beyond 15 days, the decrease was less rapid than exponential. The instantaneous rate of wound area decrease was estimated for each wound from the regression slope of the semilog plot (base e), using the data up to and including day 15. This instantaneous rate of decrease in wound area was referred to as the coefficient of wound contraction (K) in a previous report, such that K × 100 × time provided a very good approximation of the percentage decrease in wound area over some short time interval. The instantaneous rates for each treatment group were analyzed by means of analysis of covariance to assess differences among treatment groups and control group. The rate from the nontreated side for each animal was used as a covariate to adjust for potential interanimal differences in healing. On day 29, analysis of covariance was used to compare the final wound areas. All analyses were performed with SAS statistical software (SAS Institute Inc, Cary, NC). The critical value for statistical significance was set at α = .05.

RESULTS

No significant differences were found (P = .15) in wound area change after application of the sterile water vs the nontreated side, which indicates that sterile water application served as an adequate control for mitomycin application.

Across all 5 groups, the measured wound area increased by 15% to 25% on day 1 after creation of the wound, presumably because of the elasticity of surrounding skin. As shown in Figure 2, a log-linear exponential rate of wound contraction was observed for animals in each treatment group on study days 1 to 15, indicat-
Wound contraction involves a complex set of extracellular and cellular interactions that result in the closure of an open wound. Although there are several theories as to the exact mechanism, it is believed that locomotion of proliferating fibroblasts into the newly open wound contributes to the early stages of wound closure. Likewise, after this early stage of dynamic wound contraction, myofibroblasts predominate within the wound and augment early wound closure, as well as aid in remodeling of the recently contracted wound. Clearly, wound contraction is a fibroblast-driven event that is acutely sensitive to fluctuations in the fibroblast-dependent cellular and extracellular matrix. Although vital to wound closure, wound contraction and shrinkage of the healed scar can lead to scar contractures, which may cause significant functional and cosmetic morbidity.

A cosmetically acceptable end point with the concomitant prevention of scar contractures is the ultimate goal in clinical situations. However, treatment of scar contractures is a demanding task that requires ongoing, meticulous care by the patient and adjunctive therapists. The results of this study suggest that a single application of mitomycin to a newly created, full-thickness epidermal wound significantly reduced both the rate and amount of wound contraction. Wound contraction in all groups occurred at a log-linear rate during days 1 through 15, which indicates that the percentage change in wound area was constant during this period. These results parallel several wound contraction studies in the literature, which reported an active, rapid phase of wound contraction for days 1 to 15 with a log-linear rate of wound contraction on days 16 through 29.

The results of this study parallel those of studies involving the application of mitomycin in the airway to reduce scarring. In each of these studies, mitomycin was effective at its lowest dose, and only a single application was needed to provide a significant effect. Presumably, a single, low-dose application of mitomycin during the early phase of wound healing (days 1-15) blocks the profibrotic and chemotactic markers present in the acute wound milieu. Although there is no clinical evi-
dence of toxicity in topically applied mitomycin to date, the potential exists, and examination of the benefits of lower doses and less frequent application than those used in this study is warranted.

Potential limitations of this study are the failure to examine long-term effects on the wounds after day 29 and the lack of histologic assessment of the wound bed at different milestones in the wound healing process. Specifically, it is not known from the results of this study whether wound contracture continues after 29 days, or if mitomycin application only delays, but does not prevent, the process of wound contracture. Underlying histologic change in the fibroblast and collagen components of the wounds as a function of mitomycin application are also unknown. Accordingly, future studies should examine the course of wound healing with mitomycin treatment for longer periods and should incorporate histologic examination of tissue as a measurement variable.

The results of this study are encouraging for the clinical application of mitomycin in reducing skin wound contractures, and also for further studies investigating expanded uses in reducing or preventing epidermal scar formation. Mitomycin has potential utility in clinical scenarios where skin wounds have a prediction for developing scar contractures: (1) wounds involving a large amount of full-thickness tissue loss; (2) wounds complicated by infection; (3) skin wounds involving mobile surfaces (joints); (4) wounds involving the pediatric population; and (5) wounds involving the neck, axillae, hands, and face, where contracture is more common.

This study examined the effects of reapplication of mitomycin on day 3. After study day 15, the rate of wound contraction became non–log-linear. Future studies may assess reapplication of mitomycin in the second week after initial application, perhaps day 14. In addition, further work is needed in defining the minimal effective dose and frequency of application, as well as the potential benefit of mitomycin in reducing the frequently deforming scar contractures induced by thermal injuries and preventing the recurrence of hypertrophic scar (keloid) formation. Examinations into the cellular mechanism of the beneficial effect demonstrated in this study, with regard to chemotactic factors and fibroblast activity, are needed. Mitomycin is known, however, to be a carcinogenic agent when centrally administered. Therefore, before topical application of mitomycin is used clinically in patients for modulating wound contraction and scar tissue formation, the safety of using mitomycin for skin lesions must be defined.

Accepted for publication September 11, 2001.

Corresponding author: Kevin M. Robertson, MD, K4/722 Clinical Science Center, 600 Highland Ave, Madison, WI 53792-7375 (e-mail: robertso@surgery.wisc.edu).

REFERENCES