Effect of Reactive Skin Decontamination Lotion on Skin Wound Healing in Laboratory Rats

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Reactive skin decontamination lotion (RSDL) is a proposed replacement for the existing skin and equipment decontamination kit. Because RSDL may need to be used to decontaminate wounded personnel, we conducted an assessment of the effect of this agent on wound healing. A skin incision model was chosen because of its long history of use. A 7.0-cm incision was made through the skin, and RSDL was applied directly to the open wound; the wound edges were then approximated with sutures. Seven days later, animals were euthanized and wound samples were taken. Healing was assessed by measuring mechanical strength, collagen content, and histological appearance. RSDL-treated wounds had 23% lower tensile strength (p < 0.05) and 11% lower collagen content (p < 0.05) than did the untreated control wounds. Histological assessments did not differ significantly between groups. The results of this investigation demonstrate that the application of RSDL directly to an open wound impairs wound strength and decreases collagen content in the early phases of wound healing. This may have clinical implications for the treatment and outcomes of chemical casualty combat trauma.

Introduction

Preparation for chemical attack occupies an important place in current military medical doctrine. The readiness of U.S. forces to withstand a chemical attack depends on the ready availability of chemical decontamination kits for personnel and equipment. Reactive skin decontamination lotion (RSDL) is a Food and Drug Administration-approved medical device (K023969) that is the proposed replacement for the current M-291 skin decontamination kit. It is for use by individuals to remove and to neutralize chemical warfare agents from the skin immediately after exposure. RSDL is a yellow liquid with the consistency of transmission fluid, packaged in a single-use soft pack with an foam pad applicator. The Food and Drug Administration has approved RSDL as a novel chemical decontaminating agent for use on intact skin. This agent has shown improved efficacy, compared with the M-291 kit; however, the effect of the current formulation on wound healing is unknown. Previous formulations have had adverse tissue effects on wounded skin in animal models. Because many chemical casualties may sustain soft tissue injuries as well, the effect (if any) of RSDL on wound healing must be investigated before fielding.

The purpose of this study was to determine the impact of RSDL on the healing process in wounded skin after direct application of the agent to a standardized animal wound. The rat skin incision model was chosen because of its long history of use in tissue injury and wound-healing studies. Our primary outcome measure of wound healing was a biomechanical property, namely, wound breaking strength at 7 days. Our hypothesis was that there would be no difference in wound breaking strength at 7 days between RSDL-treated and untreated wounds. Secondary endpoints included measures of wound collagen content and microscopic analysis.

Methods

The entire protocol was conducted under good laboratory practices. RSDL was obtained from the manufacturer. All animal procedures and protocols were approved by the U.S. Army Institute of Surgical Research institutional animal care and use committee. Animals were housed and cared for in accordance with the Guide to the Care and Use of Laboratory Animals, in a vivarium accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. Animals were provided with food and water ad libitum before and after all procedures. All procedures were performed under anesthesia with 1.5 to 2.5% isoflurane in oxygen, administered via a nosecone and adjusted to maintain a surgical plane. Postprocedural pain was controlled with buprenorphine (0.1 mg/kg, administered through intraperitoneal injection). Male Sprague Dawley rats (age, 90-120 days; weight, 250-350 g) were obtained from Harlan (Indianapolis, Indiana).

The dorsum of the anesthetized animals was shaved with an electric clipper, and the area was wiped with 70% isopropanol and draped in aseptic fashion. A template was used to mark the proximal and distal portions of the incision and the locations of sutures (Fig. 1A). The ends of the incision area were grasped with forceps, and the incision was made in the dorsal midline, beginning cranially 4.0 cm from the skull base and extending 7.0 cm (Fig. 1B and C). In treatment group animals, 0.25 mL of RSDL was applied directly into the surgical wound with a sterile pipette. Simple interrupted 4-0 monofilament nylon sutures were placed 0.5 cm from the skin edge, beginning 0.5 cm from the end of the incision, for closure (Fig. 1D). For RSDL-treated animals, closure was performed over the pooled agent (Fig. 1E).

After closure of the incision, animals were allowed to recover from anesthesia and were monitored until they exhibited normal behavior. They were then returned to their cages; they were weighed daily and assessed twice-daily for 7 days. Anesthetized animals were euthanized with an intracardiac injection of sodium pentobarbitol (150 mg/kg) on postoperative day 7. After euthanasia, sutures were carefully cut and removed. The entire wound area was sharply excised in full thickness, with a 2-cm margin around the entire wound. All underlying connective tissue, fascia, and adipose tissue were removed. The specimen was sectioned transversely into six 0.8-cm-wide pieces by using a standard block with imbedded blades. The distribution of tissue...
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Effects of RSDL on Wound Healing

samples from head to tail is shown in (Fig. 2). Duplicate pieces were (1) analyzed for tensile strength, (2) snap-frozen and used for an assay of tissue collagen (a marker of healing), and (3) fixed in formalin and sectioned for histological analysis.

The tensile strength of the wound was tested by using a materials testing system (model LRX Plus, Lloyd Instruments, Fareham, Hampshire, United Kingdom) with a 50-N load cell (Fig. 3). Both ends of the specimen were clamped in the device, with the direction of tensioning perpendicular to the wound. A preload of 0.5 N was placed on the specimen (Fig. 4A), and a calibrated image of the specimen was captured by using a digital camera (Coolpix 995, Nikon, Tokyo, Japan) mounted on the test platform. This image was later used to determine the width of the sample, which was used to normalize wound breaking strength. The specimen was then stretched to its breakpoint at a rate of 200 mm/s (Fig. 4B).

Collagen content was measured with a modified version of the technique described by Reddy and Enwemeka.5 Samples were weighed, homogenized in 2 N NaOH, and stored at −80°C until processing. Concentrated HCl was added to the samples (run in triplicate), controls, and standards, and samples were autoclaved for hydrolysis. Chloramine-T reagent was added, followed by Ehrlich’s reagent, resulting in colorimetric changes proportional to the hydroxyproline content in the samples. Absorbance at 545 nm was measured, and hydroxyproline content was calculated by using a standard curve.

Tissue sections for histological analysis were stained with hematoxylin and eosin. All samples were graded by a board-certified veterinary pathologist, who was blinded to the sample group. The following parameters were determined for each slide: (1) gap width (reported as mean ± SD; all other values are

**Fig. 1.** Photographs of surgical procedures. (A) Template marking. (B) Raising of incision line. (C) Incision. (D) Application of test agent. (E) Suture closure.

**Fig. 2.** Schematic diagram of tissue samples taken from each animal. The wound and 2 cm of surrounding tissue were sectioned into six 1-cm-wide strips. Segments 3 and 4 were submitted for assessment of collagen concentration, segments 2 and 5 for biomechanical assessment, and segments 1 and 6 for histological assessment.

**Fig. 3.** Lloyd Instruments materials testing system (model LRX Plus; Lloyd Instruments, Fareham, Hampshire, United Kingdom). The specimen (narrow white arrow) is grasped and tensioned by the vertical movement of the 50-N load cell (wide white arrow). The digital camera used for imaging of the sample should be noted. Calibrated images were used to determine the width of the sample.
reported as medians with ranges), (2) collagen density (1 = sparse; 2 = same as surrounding dermis; 3 = more than surrounding dermis), (3) collagen bundle orientation (0 = cannot determine; 1 = perpendicular to skin surface; 2 = parallel to skin surface), (4) collagen polarization (0 = cannot determine; 1 = parallel arrays; 2 = basketweave pattern), (5) cellularity (quantity of endothelial and connective tissue cells) in the incision (1 = sparse; 2 = same as surrounding dermis; 3 = more than surrounding dermis), (6) epithelialization (1 = partial; 2 = complete), and (7) inflammation (0 = undetectable; 1 = minimal; 2 = mild).

A power analysis was performed before the experiment, assuming that the difference between the mean breaking strength values for the control and treatment groups was =10%, with a common within-group SD of 10%. The number of animals required was 19 per group to achieve 80% power to show that the mean breaking strength for the treatment group was the same as (neither lower nor higher than) that for the control group, with α set at 0.05. Therefore, 38 animals were randomly assigned to either the treatment group or the control group. Data from duplicate samples for each outcome measure were averaged. Continuous data were compared by using Student’s one-tailed t test and are reported as group mean ± SD. Ordinal data were analyzed by using the Mann-Whitney rank sum test and are reported as median and range. Statistical significance was attributed to p < 0.05.

Results

The RSDL-treated wounds had significantly less tensile strength than did control wounds. RSDL application resulted in a wound tensile strength of 6.0 ± 1.3 N/cm², compared with 7.8 ± 1.9 N/cm² for the control wounds (p = 0.0023). RSDL treatment also resulted in significantly lower collagen content compared with control wounds. The RSDL group had 67.0 ± 6.7 g of collagen per 100 g of wet tissue, whereas the control group had 75.3 ± 10.5 g per 100 g (p = 0.0164). Despite the biomechanical and biochemical evidence of impaired wound-healing, histopathological analysis failed to reveal any significant differences between RSDL-treated wounds and control wounds (Table I).

Discussion

The results of this study demonstrated a small but statistically significant reduction in the tensile strength of RSDL-treated, suture-closed wounds, compared with that of untreated closed wounds. This finding was corroborated by the significantly lower concentration of collagen within the wounds of treated animals. Results of quantitative histological analysis of gap width were not significantly different between the groups; semiquantitative histological assessments were also not significantly different between groups. These are important observations because they suggest that, despite the statistically significant differences in tensile strength and collagen content, the clinical significance of these differences is undetermined in this model.

Previous formulations of RSDL caused significant damage to intact skin and significantly hindered wound healing. By comparison, we found the current formulation to have only a small impact on wound healing and no observable impact on intact skin, based on gross visual assessment. The latter point is based on the fact that, during application, a volume of RSDL leaked from the wound upon closure. We made no attempt to remove this excess, and it remained on the skin until the end of

| TABLE I |
| RESULTS OF HISTOLOGICAL GRADING |

<table>
<thead>
<tr>
<th>Group</th>
<th>Gap Width [µm]</th>
<th>Collagen Density (0–3)</th>
<th>Collagen Orientation</th>
<th>Collagen Polarization</th>
<th>Cellularity</th>
<th>Epithelialization (0–2)</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSDL</td>
<td>424 ± 160</td>
<td>1 (0, 1)</td>
<td>0 (0–2)</td>
<td>0 (0–2)</td>
<td>3 (0–3)</td>
<td>2 (1, 2)</td>
<td>1 (0–2)</td>
</tr>
<tr>
<td>Control</td>
<td>528 ± 177</td>
<td>1 (0, 1)</td>
<td>0 (0–2)</td>
<td>0 (0–2)</td>
<td>3 (0–3)</td>
<td>2 (1, 2)</td>
<td>1 (0–2)</td>
</tr>
</tbody>
</table>

No differences were noted between RSDL-treated and control wounds. Gap width is presented as mean ± SD. Collagen density is presented as categories and other values as medians, with ranges in parentheses. See text for histopathological grading criteria.

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the experiment. Based on our nonsystematic observations, there was no damage to the intact skin in contact with the agent. Our model is limited by the lack of available data with which to determine the biological significance and direct tissue effects of wound exposure to RSDL, although secondary evidence is present in the form of histological equivalence between the RSDL-treated and control groups. The current model involves a situation in which RSDL was applied in excess, saturating the wound. Furthermore, the agent was sealed within the wound by suturing and tissue debridement, which is the standard of care for all battlefield wounds. Additionally, primary closure of most war wounds is contraindicated because of high rates of infection. RSDL application had the effect of reducing both the tensile strength of the healing wound and the collagen content. These effects may be magnified in the scenario of decontaminated contaminated tissue in many combat wounds. The current study examined a single time point early in the healing process. It cannot be determined from these data whether RSDL reduces the extent of healing at later time points. Characterization of chronic healing and scarring would require examination at later stages of wound healing.

Conclusions

Application of RSDL directly into an open surgical wound, followed by primary closure, resulted in decreased wound tensile strength and collagen content at 7 days. There were no differences noted in the histological appearance of RSDL-treated wounds. In this animal model, RSDL application had a negative impact on wound healing; this may have clinical implications for the treatment and outcomes of chemical casualty combat trauma.

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