“to assess second-degree burn wound treatment with Water Jel“
University of Miami
School of Medicine
Department of Dermatology & Cutaneous Surgery
Department of Pathology

“Evaluations of the effects of a new Water Jel system on specific bacterial and yeast strains in laboratory conditions”
The Center for Antibiotics of the Hygienic Institute, Ostrava
Medical School Hospital, Ostrava
Burns Unit of the Medical School Hospital, Ostrava

“Primary Skin Irritation Test“
To assess second-degree burn wound treatment with Water-Jel®

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DATE:

October 12, 1990
INTRODUCTION

The beneficial effect of local cooling on burn wounds is not completely understood. Hydro gel dressings have been shown to have an immediate cooling effect on wound surfaces. Water-Jel® burn dressing is an emergency burn-care product designed to cool and protect while easing the pain of burns. Previously, we have examined the use of Water-Jel® in reducing the temperature of burn wounds when applied at different time intervals. The result from this preliminary study demonstrated that the temperature declined sharply when Water-Jel® was applied. Tissue sections from this preliminary experiment appeared to show cellular differences on day 4 post burning when Water-Jel® dressing was applied. A thinner band of coagulative necrosis was observed in both the epidermis and dermis when compared to either the air exposed or gauze treated burn wounds. This finding suggests that tissue cooling seen when Water-Jel® was applied may deter the amount of eventual dermal necrosis. In order to substantiate the reduction in temperature and examine any possible indication of hypothermia, we performed the following experiments.

MATERIALS AND METHODS

Experimental Animals

Five young specific pathogen free (SPF) pigs weighing 20-25 kg were conditioned for two weeks prior to experimentation. Four animals were used for these studies and one additional animal served as a reserve conditioned animal. The animals received water and a basalt diet without antibiotics (Purina Control Factor) ad libitum and were housed individually in our animal facilities (meeting American Association for Accreditation of Laboratory Animal Care [AAALAC] compliance) with controlled temperature (19°C-21°C) and light and dark (12h/12h LD).

Burn Wounding and Treatment

The experimental animals were clipped with standard animal clippers. The skin on the back and both sides of the animals were prepared for wounding by washing with a non-antibiotic soap (Neutrogena®). Antiseptics were not used because of their potential influence on the wound healing process.

Burn wounds were made according to the methodology of our established burn model. On the day of burning (Day 0), the pigs were anaesthetized with ketamine (I.M.) and inhalation of a halothane, oxygen and nitrous oxide combination. Four specially designed cylindrical brass rods weighing 358 g each were heated in a boiling water bath to 100°C. A rod was removed from the water and wiped dry before it was applied to the skin surface to prevent water droplets from creating a steam burn on the skin. The brass rod was held at a vertical position on the skin, with all pressure supplied by gravity, for six seconds, to make a burn wound 8.5 mm diameter x 0.8 mm deep. Immediately after burning, the roof of the burn blister was removed with a sterile spatula. The burn wounds were made approximately 2 cm from each other.

ASSESSMENTS

Temperature

Two animals received thirty burns, ten burn wounds were assigned to one of the following treatment groups: 1) air exposed, 2) gauze, or 3) Water-Jel® dressing. Water-Jel® dressing was applied at three different time intervals (t=0, 15 and 60 seconds) after burning. Gauze treated burns received treatment immediately after burning. The temperature of the burn wounds was recorded every five seconds post burning for a five minute period.

Hypothermia

Two animals received one hundred burn wounds and then were treated with Water-Jel® dressing. The temperature of one of the burns was recorded for one hour by using a hypodermic temperature probe that was

placed underneath the skin at a consistent depth at a $10^\circ$ angle (Figure 1). This procedure places the hypodermic probe in the papillary dermis at a depth of approximately 0.3 mm. The burn was made directly over the implanted temperature probe and the lowest skin temperature during this time period was measured. One hundred burn wounds represent a 25 % to 35 % total body burn and these animals were monitored for hypothermia. Rectal temperatures were recorded before burning, post burning and at 15 minute intervals to detect hypothermia. Animals also were observed for any physical signs of hypothermia.

RESULTS

The mean temperatures of this study were combined with the data from the previous preliminary study. A curve was generated from the data and is presented in Figure 2. Water-Jel® dressing applied at the different time intervals sharply reduced the temperature of the burn wounds. Applying the dressing immediately after burning prevented the temperature from reaching it’s peak. When the time of assessment was extended to one hour the temperature levelled off (Figure 3). The rectal temperature was recorded for this time period and no significant decline in temperature was observed from the normal temperature of 39.7 °C (Figure 3). No other signs of hypothermia were observed during any of the experiments.
CONCLUSIONS

Water-Jel® dressing treatment reduced the temperature at the burn wound site without significantly reducing the core body temperature. This data suggest that the animals were not at risk for hypothermia following Water-Jel® treatment. We believe that the ability of this dressing to reduce the burn wound temperature may reduce the progression of injury as indicated by the preliminary histological results. The presence of a thinner band of epidermal and dermal necrosis four days post burning in Water-Jel® treated burns, would be an important justification for the use of the burn blanket as a first-aid therapy. However, more tissue specimens need to be examined to confirm this preliminary finding before a substantiated claim can be established. It is possible that Water-Jel® treatment might also stimulate epithelization of second-degree burn wounds.
Evaluations of the effects of a new Water-Jel system on specific bacterial and yeast strains in laboratory conditions

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In three previous studies the Water-Jel (WJ) system was found to protect burn wounds from microbial contamination, to have excellent analgesic and cooling effects when used as a first-aid dressing and to be bactericidal to 15 microorganisms including yeasts tested from the Ostrava Burn Unit. Now a new WJ system has been introduced without povidone iodine. An extensive bacteriological laboratory evaluation of the new WJ system showed quite clearly its excellent antimicrobial and antymycotic properties for the 13 of the 15 strains of microorganisms tested, the only exception being Clostridium difficile and partially Streptococcus faecalis. In a preliminary study, the new WJ system was used for 24-48 h in 74 burned patients with superficial partial and deep partial skin thickness burns. In 89 per cent of them there were no signs of infection on their burn wound after 48 h. The new WJ system was well tolerated and no allergic reactions appeared.

Introduction

The original Water-Jel (WJ) system of burn wound dressing was tested during 1986-89 in collaboration with the Burn Unit of the Medical School Hospital in Ostrava when its clinical and laboratory effects were evaluated1-3. The main clinical features of the WJ system when used after burn trauma included: (a) excellent analgesic effects; (b) cooling of burn surfaces; (c) protection from bacterial contamination; (d) easily modelled, and (e) an aid in removing any adherent clothing after burning. In nine burned patients (eight males) treated with the WJ system, triiodothyronine, thyroxine, cortisol, aldosterone and testosterone levels were measured, as well as the blood picture, urinalysis, urea, creatinine, transaminases, ions and blood glucose. Their levels were similar to those found in other burned patients with similar severity injuries who were not treated with the WJ system, i.e. no specific changes due to the use of this system were found2.

In a preliminary study Boswick (JA Boswick, unpublished results) found in experimentally burned rats that the colloidal Water Jel prevented the transfer of heat from a heat source into living tissue as well as lowering the temperature of tissue that had been heated to abnormal levels. This was not achieved when water at room temperature was used. The use of the WJ system after burn trauma in man had been suggested by other authors4. Remarkable results with the WJ dressings were observed in patients with heavily contaminated, lacerated wounds and with microorganisms that were multi resistant to antibiotics3. In the latter study a micro biological semi quantitative method using templates ensured the identical evaluation of the number of microorganisms on a given surface directly in burned patients, on flat injured tissues in other patients, as well as under laboratory conditions. The numerical evaluation was performed after 24 and 48 h.

Compared with original WJ system, the new WJ does not include povidone iodine but does include:

- Octoxyanol 9 N.F. – which increases the wetting, penetrating and spreading properties for WJ.
- Glycerine (USP) – as a solubilizer.
- Kelgum – as a thickener and stabilizer.
- Germaben II – as a clear liquid preservative.
- Oil of Melaleuca alternifolia – also known as Tea Tree Oil, a natural oil with bactericidal properties.
- DI Water – de-ionized water as a base.

The manufacturers carried out extensive laboratory studies of WJ on rabbits and rats, an irritation index on rabbit skin, an ocular irritation test, acute dermal and acute oral toxicity studies, intravenous and intraperitoneal injection assays in mice, etc., details of which can be obtained from the manufacturers. There were no pathological necropsy finding in the animals tested. All the tests showed a very good tolerability and safety of the WJ system.

Our experimental laboratory study determined the bactericidal properties of WJ using a quantitative dilution method. The method permitted a reliable follow-up of the effects of WJ in vitro. This is important with the new WJ, because its formulation does not include povidone iodine, with its bactericidal properties when compared with the original WJ tested earlier1-3.
Materials and Methods

Under aseptic conditions, 1ml of the new WJ was distributed into tubes prior to the addition of a suspension of bacteria or yeasts (approximately $10^8-9$ CFU/ml). The decline in the number of colony forming units was evaluated after specific time intervals (30, 60, 120, 180, 240 min). The final evaluation of the bacterial/fungal growth took place after 24 h incubation in the suspension of the WJ. The inoculation on solid media was carried out with a standard bacteriological loop with a 10 µl volume; after an appropriate cultivation, the number of micro organisms on a 30 cm² surface was determined. For staphylococci and the gram-negative bacilli Mueller-Hinton agar was used; for the streptococci the agar was enriched with 5 per cent sheep’s blood. The anaerobic bacteria were cultivated on VL agar (IMUNA CSFR) with 7 per cent horse blood under anaerobic conditions. Yeasts were cultivated on Sabouraud agar. The cultivation of bacteria was carried out at 37°C for 24 h, the cultivation of yeasts at room temperature (22°C) for 48 h.

The evaluation of WJ activity was performed using bacterial and yeasts strains isolated in the Burn Unit and in the Traumatological Unit of the Department of Surgery. The following strains were tested: Staph. aureus (10), Strep. pyogenes A. (2), Strep. agalactiae (2), Strep. faecalis (3), Escherichia coli (5), Klebsiella pneumoniae (2), Enterobacter cloacae (2), Serratia marcescens (2), Proteus vulgaris (2), Pseud. aeruginosa (4), Acinetobacter calcoaceticus (2), Clostridium perfringens (3), Clostridium difficile (3), Candida albicans (2) and Candida tropicalis (2). The number of strains are in parentheses.

Results

Laboratory Results
The laboratory results following the use of new WJ are shown in Table I. Gram-positive cocci (the staphylococci and the betahemolytic streptococci) were inhibited within 120 min of exposure to new WJ. The new WJ system had a less marked effect on Strep. faecalis strains, where colony growth was inhibited only after 24 h exposure to new WJ. All the seven species of Gram-negative bacilli, which had a marked resistance to various antimicrobial drugs, were inactive within 60 min exposure to WJ. In these species the effect of WJ was the most marked.

Different results were obtained when evaluating the activity of WJ on spore-forming anaerobic bacteria. The C. perfringens strains were reliably destroyed by WJ within 120 min, whereas C. difficile showed a lower sensitivity. During 240 min there was only a moderate decline in the numbers of bacteria and even after 24 h exposure there was a colony growth in numbers of between $6 \times 10^1-1 \times 10^2$.

A very good effect of WJ was confirmed on yeasts strains which were inactivated in less than 240 min. The new WJ system showed very good laboratory antimicrobial and antimycotic effects. It may be assumed that the same favourable results would be achieved in massively colonized devitalized tissues. In comparison with the original WJ system which included povidone iodine, the new WJ showed less activity against strains of Strep. faecalis and C. difficile which are both important hospital micro organisms.

Clinical Studies
The new WJ System was used in 74 burned patients, with superficial or deep partial skin thickness burns of various extents. The WJ was applied for the first 24-48 h after the burn. No allergic reaction were noticed. In 66 patients, there were no sign of infection on the wound deteriorated and blisters developed in some of them; in three patients the superficial skin thickness burn developed into deep partial thickness burns. In two of the patients a secondary infection was present (unpublished data). Some of the above patients were miners with burns whose emergency services used the new WJ system as the first aid dressing.
Discussion

The results of the above laboratory evaluation of the new WJ system without povidone iodine on selected strains of bacteria and yeasts and used at a density of $10^{8-9}$ CFU/ml, showed, unambiguously, very good antimicrobial and antifungal effects of the new WJ. The above density exceeds, by several times, the numbers of bacteria in massively colonized devitalized tissues.

WJ can significantly decrease the colonization of wound and burned surfaces within the first few hours after its application. It therefore represents an effective method to prevent the development of local infections which could, in critical clinical conditions, give rise to a generalized infection i.e. sepsis.1-3.

The prophylactic application of WJ does not require the simultaneous administration of antibiotics because of its outstanding antimicrobial effects. The new WJ is well tolerated by patients, is non-toxic, biodegradable and water soluble.

References


<table>
<thead>
<tr>
<th>Species</th>
<th>Strains tested (no.)</th>
<th>Range of numbers of micro organisms after contact with Water-Jel</th>
<th>Growth after 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
<td>60 min</td>
<td>120 min</td>
</tr>
<tr>
<td>Staph. Aureus</td>
<td>10</td>
<td>$1 \times 10^7$-3 $\times 10^8$</td>
<td>$3 \times 10^4$-2 $\times 10^7$</td>
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<tr>
<td>Strep. Pyogenes A.</td>
<td>2</td>
<td>$4 \times 10^2$-1 $\times 10^8$</td>
<td>$2 \times 10^5$-3 $\times 10^5$</td>
</tr>
<tr>
<td>Strep. Agalactiae</td>
<td>2</td>
<td>$1 \times 10^7$-3 $\times 10^7$</td>
<td>$2 \times 10^4$-3 $\times 10^4$</td>
</tr>
<tr>
<td>Strep. Faecalis</td>
<td>3</td>
<td>$5 \times 10^7$-3 $\times 10^8$</td>
<td>$2 \times 10^7$-1 $\times 10^8$</td>
</tr>
<tr>
<td>E. coli</td>
<td>5</td>
<td>0-5 $\times 10^1$</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>2</td>
<td>$3 \times 10^1$-5 $\times 10^2$</td>
<td>0</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
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<td>$2 \times 10^2$-3 $\times 10^2$</td>
<td>0</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>2</td>
<td>$4 \times 10^2$-6 $\times 10^2$</td>
<td>0</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>2</td>
<td>$2 \times 10^2$-3 $\times 10^2$</td>
<td>0</td>
</tr>
<tr>
<td>Pseud. Aeruginosa</td>
<td>4</td>
<td>$5 \times 10^1$-2 $\times 10^3$</td>
<td>0</td>
</tr>
<tr>
<td>Acinetobacter calcoaceticus</td>
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<td>$2 \times 10^2$-1 $\times 10^3$</td>
<td>0</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>3</td>
<td>$5 \times 10^2$-4 $\times 10^5$</td>
<td>$1 \times 10^3$-1 $\times 10^3$</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>3</td>
<td>$7 \times 10^3$-5 $\times 10^6$</td>
<td>$6 \times 10^4$-4 $\times 10^6$</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>2</td>
<td>$3 \times 10^6$-5 $\times 10^6$</td>
<td>$3 \times 10^4$-4 $\times 10^4$</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>2</td>
<td>$3 \times 10^6$-6 $\times 10^6$</td>
<td>$4 \times 10^4$-6 $\times 10^4$</td>
</tr>
</tbody>
</table>
Primary Skin Irritation Test of Water Jel Burn Dressing
INTRODUCTION

The test article identified below was evaluated for primary skin irritation in accordance with the guidelines of the Consumer Product Safety Commission, Title 16, Chapter II, Part 1500. The purpose of this test was to determine the dermal irritation potential of the test article to intact and abraded skin of the rabbit. The study was initiated on July 4, 1988 and terminated on July 7, 1988.

MATERIALS

The following material was provided for this study and prepared as indicated below:

Test Article: Water Jel Burn Dressing

Storage Conditions: Ambient room temperature and humidity

Test Article Preparation: The test article was a cloth patch saturated with a white liquid. The white liquid was drawn from the container and dosed as received; the patch material was not used.

EXPERIMENTAL PROCEDURE

Animals:

Six healthy rabbits of the New Zealand White variety were obtained from USDA licensed suppliers and acclimated to the laboratory. Rabbits, identified by ear tag or tattoo, were individually housed in suspended cages and received Agway® ProLab™ rabbit feed on a daily basis; tap water was available ad libitum.

Animal husbandry was conducted in accordance with the „Guide for the Care and use of Laboratory Animals,” NIH Publication No. 85-23.

Methods:

The backs of the animals were clipped free of fur with an electric clipper at least 4 hours before application of the sample. Just prior to test article application, each rabbit received four parallel epidermal abrasions with a sterile needle at one test site while the skin at the opposite site remained intact.

A 0.5 ml sample of the test article was then applied to each site (two sites per rabbit) by introduction under a double gauze layer to an area of skin approximately 1” x 1” (2.54 x 2.54 cm) square. The patches were backed with plastic, covered with a non reactive tape and the entire test site wrapped with a binder. Animals were returned to their cages.

After a 24 hour exposure, the binders and test article were removed. The test sites were wiped with tap water to remove any remaining test article residue.

At 24 and 72 hours after test article application, the test sites were examined for dermal reactions in accordance with the FHSA – recommended Draize scoring criteria (Appendix 1). The Primary Irritation Index (P.I.I.) of the test article was calculated following test completion. As defined in CFR16, Chapter II, Part 1500, a material producing a P.I.I. score of greater than or equal to 5.00 would be considered positive; the material would be considered a primary irritant to the skin.
RESULTS

The Primary Irritation Index of the test article was calculated to be 0.00; No irritation was observed on the skin of the rabbits. Individual results of dermal scoring appear in Table I.

CONCLUSIONS

Under the conditions of this test, the test article would not be considered a primary skin irritant; the Primary Irritation Index was less than 5.00.

RECORD STORAGE

All raw data pertaining to this study are to be stored in the designated archive files at North American Science Associates, Inc. (NamSA®), 2261 Tracy Road, Nortwood; Ohio – 43619.

Remaining test article was discarded following test completion.

TABLE I

DERMAL OBSERVATIONS

Material: Water Jel Burn Dressing

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Reaction</th>
<th>24 Hours</th>
<th>72 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intact</td>
<td>Abladed</td>
</tr>
<tr>
<td>37389</td>
<td>Erythema</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>37362</td>
<td>Erythema</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>37360</td>
<td>Erythema</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>37373</td>
<td>Erythema</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>37379</td>
<td>Erythema</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>37388</td>
<td>Erythema</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Primary Irritation Index: \(0 / 24 = 0.00\)
**APPENDIX 1**

**DRAIZE\(^1\) EVALUATION OF DERMAL REACTIONS**

### SKIN REACTIONS

<table>
<thead>
<tr>
<th>Erythema and Eschar Formation (Most severely affected area graded):</th>
<th>SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No erythema</td>
<td>0</td>
</tr>
<tr>
<td>Very slight erythema (barely perceptible)</td>
<td>1</td>
</tr>
<tr>
<td>Well-defined erythema</td>
<td>2</td>
</tr>
<tr>
<td>Moderate to severe erythema</td>
<td>3</td>
</tr>
<tr>
<td>Severe erythema (beet redness) to slight eschar formation (injuries in depth)</td>
<td>4</td>
</tr>
</tbody>
</table>

**NOTE:** Test sites assigned a “4” score for erythema require further description as to the extent of tissue injury.

<table>
<thead>
<tr>
<th>Edema Formation (Most severely affected area graded):</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No edema</td>
<td>0</td>
</tr>
<tr>
<td>Very slight edema (barely perceptible)</td>
<td>1</td>
</tr>
<tr>
<td>Slight edema (edges of area well defined by definite raising)</td>
<td>2</td>
</tr>
<tr>
<td>Moderate edema (raising approximately 1 millimeter)</td>
<td>3</td>
</tr>
<tr>
<td>Severe edema (raised more than 1 mm and extending beyond the area of exposure)</td>
<td>4</td>
</tr>
</tbody>
</table>

**Results:**

The scores for erythema and edema are totalled for intact and abraded skin for all rabbits at 24 and 72 hours. The primary irritation index (P.I.I.) is calculated, based on the sum of the scored reactions divided by 24 (two scoring intervals multiplied by two test parameters multiplied by six rabbits).

**EVALUATION OF PRIMARY IRRITATION INDEX**

<table>
<thead>
<tr>
<th>INDEX</th>
<th>EVALUATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>No irritation</td>
</tr>
<tr>
<td>0.04 – 0.99</td>
<td>Irritation barely perceptible</td>
</tr>
<tr>
<td>1.00 – 1.99</td>
<td>Slight irritation</td>
</tr>
<tr>
<td>2.00 – 2.99</td>
<td>Mild irritation</td>
</tr>
<tr>
<td>3.00 – 5.99</td>
<td>Moderate irritation</td>
</tr>
<tr>
<td>6.00 – 8.00</td>
<td>Severe irritation</td>
</tr>
</tbody>
</table>

**SUMMARY**

The test article, Water Jel Burn Dressing, was evaluated for primary skin irritation in accordance with the guidelines of the Consumer Product Safety Commission. A 0.5 ml dose of the test article was applied to the intact and abraded skin of six rabbits and left in place for 24 hours. Test sites were graded for erythema and edema at 24 and 72 hours after sample application. The study was initiated on July 4, 1988 and terminated on July 7, 1988.

Under the conditions of this test, the test article would not be considered a primary irritant to the skin.

The Primary Irritation Index was calculated to be 0.00.

\(^1\)Draize, J.H. 1959. Dermal Toxicity. Pages 46-59 in Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics. The Association of Food and Drug Officials of the United States, Bureau of Food and Drugs, Austin, TX.