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WOUND HEALING AND VITAMIN A ACID

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Abstract. A study was undertaken to observe the gross and ultrastructural results when vitamin A acid was applied to wounds. Wounds were made on mice and vitamin A acid in lanolin and mineral oil, or vitamin A acid in acetone, or lanolin and mineral oil only, or acetone only, were applied to the wounds. It was observed that vitamin A acid in lanolin and mineral oil topically applied to wounds, stimulated wound healing. Further investigation seems warranted, promising that vitamin A acid may prove to be an important drug in the control of healing.

Vitamin A is known to affect epithelial cells. Numerous studies have shown that an excess of vitamin A will inhibit keratinization (4–6, 9, 11, 12). Other studies have shown that when vitamin A is applied topically to the skin tumor, keratoacanthoma, the tumor will exude a thick viscous mucus. Cessation of the vitamin applications results in a reversion in the tumor to the usual dry keratotic state (15, 16).

Knowing that vitamin A acid can dramatically affect epithelium and possibly even the dermis, a study was undertaken to observe the gross and microscopic results when vitamin A acid was applied topically to wounds. The results presented report the effect of vitamin A acid on the rate of wound healing.

MATERIAL AND METHODS

One hundred and ninety-five Swiss male mice, 3 months old, had their backs shaved with an electric shaver. Wounds were made on the back of the mice with a standard 2 mm dermatologic punch biopsy. Two punch biopsies were made, 2 cm apart, on each animal. The wounds were all exactly 2 mm in diameter, full skin thickness, with no blood visible on any wound. The animals were divided into 13 groups of 15 mice per group. Each group of 15 mice was divided into three cages (5 mice per cage). The animals were treated with either vitamin A acid in lanolin and mineral oil, lanolin and mineral oil only, vitamin A acid in acetone, or absolute acetone alone. The drugs were applied direct to one of the two open wounds on an animal. The other, untreated wound on the animal served as a control. The materials and methods are summarized in Table 1.

Biopsies were performed on treated and untreated wounds. The tissues were incubated in 5% glutaraldehyde in phosphate buffer for 1.5 hrs followed by fixation in 2% osmium tetroxide buffered to pH 7.4. The tissues were dehydrated in graded strengths of ethanol and embedded in Epon 812. One micron thick and ultrathin sections were cut on a Reichert ultramicrotome. The ultrathin sections were stained with uranyl acetate followed by lead citrate and examined in an RCA-EMU 2E electron microscope.

RESULTS

Gross results. Observations of wounds that received only one dose of drug or placebo and examined daily showed that the wounds treated with vitamin A acid in lanolin and mineral oil, with vitamin A acid in acetone, or left untreated, appeared to have similar results with a thicker scab at the periphery of the wound than at the center of the wound. Wounds treated with lanolin and mineral oil, or acetone, did not produce a thick scab anywhere. By the end of the second week after a single drug application, no wound, treated or untreated, had completely re-epithelialized.

Gross examination of wounds that received two doses of drugs or placebo and were examined daily for 2 weeks showed that those wounds receiving vitamin A acid in lanolin and mineral oil formed a thick crust over the wound after 1 week, and by 2 weeks the wounds were almost but not quite completely re-epithelialized. Wounds treated with vitamin A acid in acetone also had a crust but it was unlike the thicker

Table I. *Summary of materials and methods used in wound healing*

Time of biopsy after last drug application: 3 mice 1 day, 3 mice 3 days, 3 mice 5 days, 3 mice 7 days, 3 mice 12 days

Experiment no.	No. of animals	No. of wounds treated	Drugs applied	No. of doses
1	15 (30 wounds)	15 treated 15 untreated (control)	3% vitamin A acid in lanolin and mineral oil	One dose (0.1 g) delivered via wooden stick applicator
2	15 (30 wounds)	15 treated 15 untreated	Lanolin and mineral oil only in equal parts	One dose (0.1 g) delivered via wooden stick applicator
3	15 (30 wounds)	15 treated 15 untreated	3% vitamin A acid in acetone	One dose (0.1 ml) delivered via pipette
4	15 (30 wounds)	15 treated 15 untreated	Absolute acetone only	One dose (0.1 ml) delivered via pipette
5	15 (30 wounds)	30 untreated	No drug applied	
6	15 (30 wounds)	15 treated 15 untreated	3% vitamin A acid in lanolin and mineral oil	Two doses (0.1 g/dose) delivered 1 day apart via wooden stick applicator
7	15 (30 wounds)	15 treated 15 untreated	Lanolin and mineral oil only in equal parts	Two doses (0.1 g/dose) delivered 1 day apart via wooden stick applicator
8	15 (30 wounds)	15 treated 15 untreated	3% vitamin A acid in acetone	Two doses (0.1 ml/dose) delivered 1 day apart via pipette
9	15 (30 wounds)	15 treated 15 untreated	Absolute acetone only	Two doses (0.1 ml/dose) delivered 1 day apart via pipette
10	15 (30 wounds)	15 treated 15 untreated	3% vitamin A acid in lanolin and mineral oil	Three doses (0.1 g/dose) delivered 24 hrs apart via wooden stick applicator
11	15 (30 wounds)	15 treated 15 untreated	Lanolin and mineral oil only in equal parts	Three doses (0.1 g/dose) delivered 24 hrs apart via wooden stick applicator
12	15 (30 wounds)	15 treated 15 untreated	3% vitamin A acid in acetone	Three doses (0.1 ml/dose) delivered 24 hrs apart via pipette
13	15 (30 wounds)	15 treated 15 untreated	Absolute acetone only	Three doses (0.1 ml/dose) delivered 24 hrs apart via pipette

crust of the vitamin A acid in lanolin and mineral oil treated wounds. Untreated wounds did not heal so rapidly as the vitamin A acid treated wounds but re-epithelialized more quickly than wounds treated with acetone only or lanolin and mineral oil only.

Gross examination of wounds that received three doses of drugs or placebo and examined daily for 2 weeks showed that those wounds receiving vitamin A acid in lanolin and mineral oil came close to re-epithelialization after 1 week and were completely re-epithelialized after 2 weeks. When wounds treated with vitamin A acid in acetone are compared with wounds treated with lanolin and mineral oil only, the results are found to be very similar. However, only about half to three-fourths of the wounds had re-epithelialized after 2 weeks. Untreated wounds did not heal so rapidly as triple-dose vitamin A acid treated wounds nor as fast as lanolin and mineral oil treated wounds, but did heal faster than wounds treated with acetone alone.

Table II. *Summary of results of tissue treated with vitamin A acid or placebo*

Drug applied	No. of doses	Time of examination 1 day after last drug application	1 week after last drug application
Vitamin A acid in lanolin and mineral oil	1	Granulation tissue present. Many polys. Dermis has spongy appearance	Migrating epidermis over wound which is filling in
Vitamin A acid in lanolin and mineral oil	3	Thick granulation tissue present. Migrating epidermis present	New epidermis, 6–7 cell layers has formed over the wound
Vitamin A acid in acetone	1	Granulation tissue. Many polys	Thin epidermis migrating over wound. Many inflammatory cells present
Vitamin A acid in acetone	3	Migrating epidermis present. Granulation tissue present	Epidermis, 2 cell layers in thickness has almost covered wound. Some polys are present in dermis. Dermis has slightly spongy appearance.
Lanolin and mineral oil	1	Granulation tissue present. Dermis has spongy appearance	Migrating epidermis over wound. Dermis has spongy appearance
Lanolin and mineral oil	3	Wound has much granulation tissue	Much granulation tissue. Migrating epidermis over wound
Acetone	1	Inflammatory cells present. Dermis has very spongy appearance	Inflammatory cells present. A few epidermal cells are migrating over periphery of wound
Acetone	3	Polys are present. Dermis has spongy appearance	Some migrating epidermal cells with lipid present in cytoplasm. Dermis still has a spongy appearance
Wound left untreated		Inflammatory cells present 1 day after wound was made	Inflammatory cells present 1 week after wound was made. Migrating epidermal cells present

Microscopic results. Ultrastructural examination of tissue treated with vitamin A acid or a placebo are summarized in Table II.

DISCUSSION

Gross observations as well as ultrastructural studies have been carried out previously in experimental animals. Tarin & Croft (19) have described wound healing in mouse skin and have noted that the epithelium cuts through the connective tissue to separate out an area which forms the scab. They also observed that 16 hrs after wounding, the surrounding dermis had many polymorphonuclear leucocytes (3). In time, the healing process involved dermal and epidermal cells coming into contact. Ross & Odland (17) reported epithelial pseudopodia coming into contact with dermal cells. In another study (20) it was noted that epithelial basal cells divide as well as migrate toward the center of the wound. Those cells which are left behind by the migrating cells divide, differentiate, and keratinize. Krawczyk has stated that epidermal cells appear to move by rolling or sliding over one another with fine fibers oriented in the cortical cytoplasm playing a role in the movement of the epidermal cells (10). Many investigators have also noted that epidermal cells originating from the periphery of the wound extend over the wound (1–3, 7, 14).

It is known that vitamin A has a controlling action on the basal cells of epithelium (21). Sherman noted that vitamin A, whether applied topically or administered orally in physiologic amounts, causes an increase in epithelial mitotic index (18). In a recent study, vitamin A promoted wound healing by increasing acid mucopolysaccharide synthesis in the granulation tissue of skin wounds in rats (13). Further studies

have shown that topical vitamin A can stimulate cortisone-retarded healing of open wounds in animals as well as in man (8).

In the present study, vitamin A acid in lanolin and mineral oil applied topically to wounds stimulated wound healing. Although all the wounds, whether treated or untreated, were not covered by an occlusive dressing, it is nevertheless interesting to note that when vitamin A was in lanolin and mineral oil, the results were more positive than when vitamin A was in an acetone vehicle. Lanolin and mineral oil as a vehicle agent probably retained vitamin A acid over the wound longer than did acetone.

In an unpublished communication, vitamin A acid was applied to a few wounds in humans whereby the wounds demonstrated a faster healing rate than untreated wounds. Further investigation seems warranted, but it would seem that vitamin A acid may prove to be an important drug in the control of healing.

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