EFFECT OF BENTONITE ON SKIN WOUND HEALING: EXPERIMENTAL STUDY IN THE RAT MODEL

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Abstract- Wound healing in the skin depends upon the availability of appropriate trace metals as enzyme cofactors and structural components in tissue repair. The present study is a part of a series of experimental investigations to examine the influence of Bentonite on skin wound healing. Surgically induced skin wounds in 48 young adult male rats were exposed topically to Bentonite (12 round wound and 12 incisional wound) and control wounds (12 round wound and 12 incisional wound) received de-ionized water only. Skin wounds (round and incisional) treated with Bentonite exhibited no significant difference in margins with erythema and edematous changes. Scab and wound debris was more extensive and persisted for at least 7 days after surgery in control group ($P < 0.05$). Skin wounds exposed to Bentonite exhibited a mild retarded re-epithelialization, the treatment wounds were characterized by a prominent central mass of inflammatory cells, cell debris and wound exudate. The intense infiltrate of lymphocytes, macrophages, monocytes and fibroblasts extended from the wound margin into the region of the panniculus carnosus muscle and hypodermis. Vascular dilatation and dermal oedema were prominent features of these wounds. External utilization of Bentonite for wound healing is safe and feasible, and we finalized that macroscopic healing of wound that treated by Bentonite was superior versus control group.

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INTRODUCTION

Wound healing immediately starts after an injury and proceeds with a complicated but well-organized interaction among various types of tissues and cells. Skin wound healing is composed of the inflammatory, proliferative, and maturation phases.

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In the inflammatory phase, the recruitment of leukocytes such as neutrophils and macrophages into the wound site is a hallmark. In the proliferative phase, the migration and proliferation of keratinocytes, fibroblasts, and endothelial cells result in re-epithelialization and tissue granulation. In the maturation phase, excess collagen in the wound site is degraded by several proteolytic enzymes, leading to the completion of tissue repair (1, 2). It is well known that biological substances such as cytokines, chemokines and growth factors are closely involved in every phase of wound healing process. Trace metals have a major role in tissue repair systems as cofactors in metalloenzyme systems and as structural

components (3). Recent studies in the rat model have demonstrated that as wounds heal, local concentrations of zinc, calcium, copper and magnesium change according to the phase in the wound healing cascade and associated biochemical events (4). Clinical observations in humans and experimental studies have demonstrated that deficiencies in the availability of these metals, imbalance in local concentrations, or defects in metabolism are potential causes of defective or nonhealing wounds (5, 6).

The present study is a part of a series of experiments designed to investigate the action of bentonite on skin wound healing in the rat model.

MATERIALS AND METHODS

Animals

Male young adult rats, SD (250+/-50 g body weight) were used in all experiments. Rats were housed in groups of four in plastic solid-bottomed cages provided with sterile dust-free bedding and temperatures of 22 ± 2 °C, relative humidity of 45–55%, and 12 h:12 h day/night cycles. All experiments on animals were performed in accordance with UK legal requirements.

Chemicals

Type of mineral elements of the bentonite was distinguished in previous studies: commercial term for clays containing montmorillonite type minerals (7), Na 0:33[Al_1: 67 Mg 0:33] Si4 $[OH]_2$ (8), Native hydrated colloidal aluminum silicate clay (9).

The principle constituent is montmorillonite. However, other minerals such as illite, kaolinite and nonargillaceous detrital minerals can be present. Most bentonites appear relatively pure and other mineral contributions rarely exceed 10%.

Cristobalite is often present. Montmorillonite compositions frequently vary either in its lattice structure or in the exchangeable ions present. We analyzed Bentonite by X ray distribution that summarized in Table 1. Bentonite functions as an absorbent, bulking agent, emulsion stabilizer, opacifying agent, suspending agent-nonsurfactant, and viscosity-increasing agent-aqueous in cosmetic formulations (9).

Table 1. Analysis of bentonite by X-ray distribution.

Major phase
Montmorillonite
Minor phase
1. Illite-Montmorillonite [K-$Al_4(SiAl)_8O_{20}(OH)_4$,XH_2O]
2. Nontronite-15A [$Na_{0.3}Fe_2Si_4O_{10}(OH)_2$,$4H_2O$]
3. Albite [(Na,Ca) $Al(Si_3Al)O_8$
4. Hslloysite-7A [$Al_2Si_2O_5(OH)_4$]

Full-thickness wounds

Rats were anaesthetized by Ketamine 50mg/Kg and Xylazine HCL 5mg/Kg and all subsequent wounding procedures were carried out on a heated table. The dorsal region of the animal was shaved and sterilized using 70% alcohol. Using sterile 1 cm punch biopsies, a full-thickness excisional wound (round wound) and 1 cm longitudinal full thickness wound (incisional wound), (4, 10) were created on each dorsolateral flank equidistant from the midline. Wounds were separated by a margin of at least 15 mm. Each wound region was digitally photographed at the indicated time intervals, and the areas of the wounds were calculated by PhotoShop software, version 7.0. Changes in the wound areas were expressed as the percentage of the initial wound areas. After preparation of Bentonite (2 gr/5cc H2o), the covering was applied in round and incisional wound in case group and the treatment was repeated 10 days until the end of the experiment. Measurements of the inner wound edge were repeated on days 3, 5, 7 and on day 10 to enable calculation of wound closure as a result of re-epithelialization. Bentonite treated and control rats after 10 days and wound sites excised. The tissue was preserved in 10% phosphate buffered formalin for histology and haematoxylon and eosin stained sections. Comparable numbers of control animals received de-ionized water only.

Clinical assessment of wounds healing

During and the end of the treatment wound sites were examined daily for evidence of hemorrhage, ulceration, scab formation, suture loss or other changes. For this, wound outlines were transferred to software capable of determining the area and the extent of wound closure expressed as a percentage of the original wound size: % wound closure = 100×

[(area on day 0- area of open wound)/area on day 0] (11).

Pathological assessment of wound healing

At time end of the study, animals were scarified by cervical dislocation and the wounded skin was excised and fixed overnight in a 4% buffered formalin solution. The tissue was washed in PBS, embedded in fibrowax, and sections (10 µm) were stained using standard procedures with haematoxylon and eosin for morphological assessment. Using planimetry software, captured images of the stained tissue were analyzed. Analysis of stained tissue comprised of cell degeneration, giant cell, epidermis, collage, infiltration and vessel formation. Granula-tion tissue thickness was assessed by dividing the measured granulation tissue area (mm^2) by the length measured (mm) for each treatment. Re-epithelialization was assessed by measuring the length, area, and average thickness of the epidermal 'tongue' for each wound site (12).

Statistical methods

Statistical differences were determined using the Student's t test. All data are presented as the mean ± standard deviation. P value < 0.05 was accepted as statistically significant.

RESULTS

Macroscopic observations

Wound healing in control rats progressed with crusting, scab formation and re-epithelialization around the round and incisional wound for approximately 10 days. Skin wounds (round and incisional) treated with Bentonite exhibited no significant difference in margins with erythema and edematous changes. Scab and wound debris was more extensive and persisted for at least 7 days after surgery in control group ($P < 0.05$) (Table 2, Fig 1).

Microscopic observations

Differences in the histological profile between wounds treated with Bentonite and controls were marginal through the 10 days following incision. After 10 days, wound sites were re-epithelialized but some epidermal hyperplasia was present in most sections. Skin wounds exposed to Bentonite exhibited a mild retarded re-epithelialization, the treatment wounds were characterized by a prominent central mass of inflammatory cells, cell debris and wound exudate (Fig. 2). The intense infiltrate of lymphocytes, macrophages, monocytes and fibroblasts extended from the wound margin into the region of the panniculus carnosus muscle and hypodermis. Vascular dilatation and dermal oedema were prominent features of these wounds.

DISCUSSION

Skin injury immediately causes clot formation and local inflammation characterized by an infiltration of neutrophils and macrophages into the wound sites. These pathological changes are hallmarks of the inflammatory phase of wound healing. The inflammatory response is believed to be instrumental in supplying the growth factors, cytokines, and chemokines that orchestrate the cell movement necessary for wound repair (1, 2).

Recent studies have demonstrated that concentrations of essential trace metals change in skin wounds to reflect their requirements in metalloenzyme complexes in sequential events in the wound healing cascade (4). Since bivalent trace metals compete for binding sites on carrier proteins and in metabolic events, the balance of trace metals in the wound site is critical for the stage in healing. Imbalances in the relative concentrations of calcium:zinc, zinc:copper, etc., or the presence of a xenobiotic ion like cadmium, are potential causes of impaired wound healing (6, 13, 14).

Table 2. Clinical observation of wound healing (ring and long) that expose to Bentonite compared to control group after 7 days.

Round wound	**Wound healing**	**Inflammation**
Bentonite group	10 (83.3%)	4 (33.3%)
Control group	5 (41.6%)	4 (33.3%)
Incisional wound	**Wound healing**	**Inflammation**
Bentonite group	11 (91.6%)	5 (41.6%)
Control group	4 (33.3%)	6 (50%)

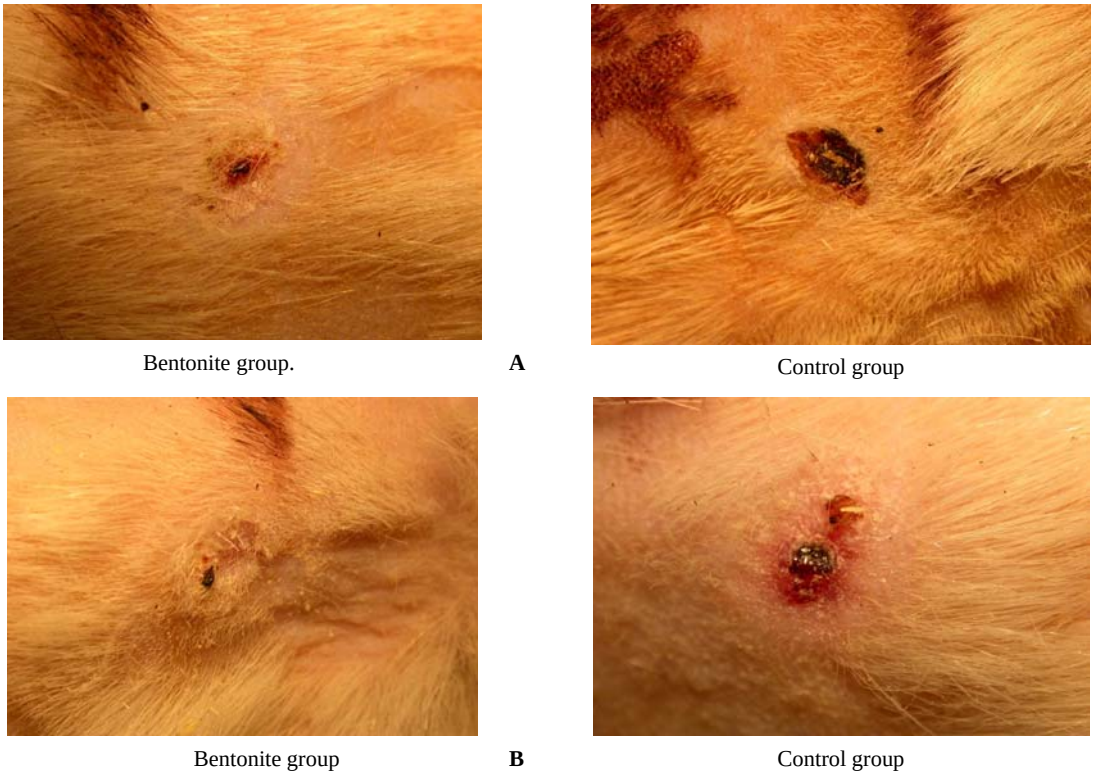

Fig 1. Macroscopic observations of Skin round (A) and incisional (B) wounds exposed to Bentonite compared to control group after 7 days.

Bentonite is widely used in many, many industries. However, what make it unique for consideration in natural medicine are its very unique properties. When properly hydrated, it creates and sustains its own subtle electromagnetic field (negatively charged particles). It has extremely powerful Absorptive properties, i.e. it attracts and holds to its surface many toxic substances. U.S. Army studies show that bentonite may be a successful treatment for exposure to chemical warfare. One Army emergency livestock protocol calls for immediate administration of bentonite internally to counter effects of radiation poisoning in livestock (food sources). The immunotoxicity of Bentonite through environmental and dietary exposure is recognized in humans and experimental animals (15-17). Beck and Bignon dosed peritoneal macrophages with two samples of Bentonite and the

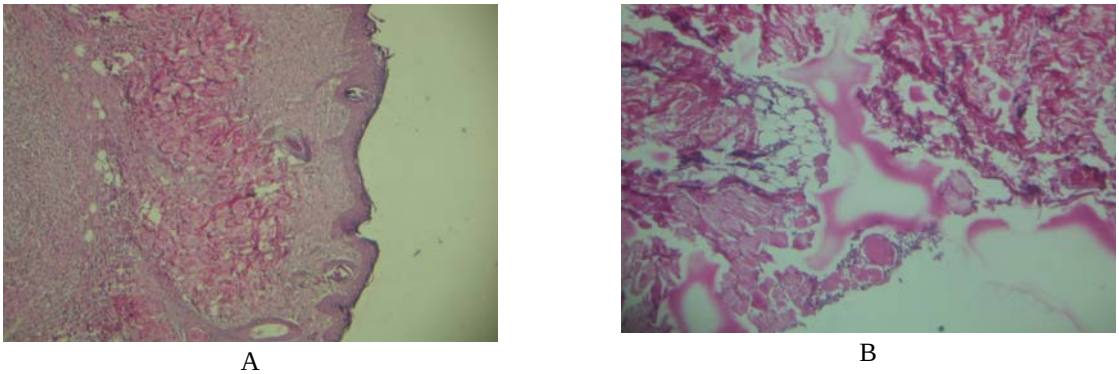

Fig 2. Microscopic observations of skin wounds exposed to Bentonite exhibited a mild retarded re-epithelialization, the treatment wounds were characterized by a prominent central mass of inflammatory cells, cell debris and wound exudates (A) and control group (B).

triphenyltetrazolium chloride (TTC) reduction, LDH activity, and methylene blue absorption were used to assess cytotoxicity (18). Bentonite inhibited TTC reduction similar to the fibrogenic dusts such as quartz. However, the extracellular LDH activity was not increased and methylene blue absorption was very high. Hatch examined the cytotoxicity of Bentonite to rabbit alveolar macrophages (19).

The viability percentage of the macrophages and the ATP content of the cells as index of cytotoxicity were determined. Bentonite caused a large reduction in both the viability and ATP levels.

TTC reduction, LDH activity, and methylene blue absorption were measured as indexes of cytotoxicity in a study by Adamis (20). TTC reduction was much greater and proved Bentonite to be cytotoxic. Extracellular LDH was almost half for Bentonite compared to control values. Methylene blue absorption was significantly higher for Bentonite. Murphy, Roberts, and Horrocks investigated the cytotoxicity of Bentonite to human umbilical vein endothelial (HUVE) cells, undifferentiated N1E-115 neuroblastoma cells, and ROC-1 oligodendroglial cells (21). Indices of cytotoxicity used in this study were morphological examination, LDH activity, and fatty acid release. In a separate study by Murphy, the cytotoxicity of Bentonite was examined in two cell lines: primary murine spinal cord neurons and differentiated N1E-115 neuroblastoma cells and no cytotoxicity were recorded as a result of Bentonite treatment (22).

When used immediately in severe trauma situations (externally), clay packs significantly reduce tissue damage associated with swelling. The protective mechanism of Bentonite is likely to be a combination of allergen absorption and skin barrier function improvement. This may result in significantly reduced percutaneous allergen penetration (23).

In this observation we studied the effect of Bentonite on skin of rat on ring and long wound healing. Wound size both in ring and long group was rapidly improved in Bentonite treated wound compared to control group, but inflammation response was not significantly different between two groups. Moreover, in pathology examination we did not show significant differences in wound healing between two groups and we concluded that Bentonite was not very efficient for improving of wound. Further work by more cases needs to be done on these.

Conflict of interests

We have no conflict of interests.

REFERENCES

1. Martin P. Wound healing--aiming for perfect skin regeneration. Science. 1997 Apr 4; 276(5309):75-81.
2. Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med. 1999 Sep 2; 341(10):738-746.
3. Lansdown AB. Physiological and toxicological changes in the skin resulting from the action and interaction of metal ions. Crit Rev Toxicol. 1995; 25(5):397-462.
4. Lansdown AB, Sampson B, Rowe A. Sequential changes in trace metal, metallothionein and calmodulin concentrations in healing skin wounds. J Anat. 1999 Oct; 195 (Pt 3):375-386.
5. Moynahan EJ. Letter: Acrodermatitis enteropathica: a lethal inherited human zinc-deficiency disorder. Lancet. 1974 Aug 17; 2(7877):399-400.
6. Heng MK, Song MK, Heng MC. Reciprocity between tissue calmodulin and cAMP levels: modulation by excess zinc. Br J Dermatol. 1993 Sep; 129(3):280-285.
7. Gamble JF. Silicate pneumoconiosis. In: Merchant JA, editor. Occupational respiratory diseases. Cincinnati: US Department of Health and Human Services, Public Health Service, CDC; 1986. DHHS publication no. (NIOSH). p. 86-102.
8. Rheox Inc. The benefits of hectorite clay and safety data sheet on Bentone MA 1999 (purified hectorite). Unpublished data submitted by Rheox Inc. 3 pages.2
9. Wenninger JA., Canterbery RC, McEwen Jr. GN, eds. International Cosmetic Ingredient Dictionary and Handbook. 8th ed. Baltimore, Maryland: Cosmetic Toiletry & Fragrance Assn; 2000.
10. Lansdown AG. Influence of zinc oxide on the closure of open wounds: an experimental study. Int J Cosmet Sci. 1993; 46: 83-85.
11. Bevan D, Gherardi E, Fan TP, Edwards D, Warn R. Diverse and potent activities of HGF/SF in skin wound repair. J Pathol. 2004 Jul; 203(3):831-838.

12. Low QE, Drugea IA, Duffner LA, Quinn DG, Cook DN, Rollins BJ, Kovacs EJ, DiPietro LA. Wound healing in MIP-1alpha(-/-) and MCP-1(-/-) mice. Am J Pathol. 2001 Aug; 159(2):457-463.
13. Klevay LJ. The relationship of zinc to copper of diets in the United States. Nutr Rep Int.1975; 11: 237.
14. Vicanova J, Boelsma E, Mommaas AM, Kempenaar JA, Forslind B, Pallon J, Egelrud T, Koerten HK, Ponec M. Normalization of epidermal calcium distribution profile in reconstructed human epidermis is related to improvement of terminal differentiation and stratum corneum barrier formation. J Invest Dermatol. 1998 Jul; 111(1):97-106.
15. Descotes J. Immunotoxicology of cadmium. IARC Sci Publ. 1992;(118):385-390.
16. Stelzer KJ, Pazdernik TL. Cadmium-induced immunotoxicity. Int J Immunopharmacol. 1983; 5(6):541-548.
17. Bernier J, Brousseau P, Krzystyniak K, Tryphonas H, Fournier M. Immunotoxicity of heavy metals in relation to Great Lakes. Environ Health Perspect. 1995 Dec;103 Suppl 9:23-34.
18. Beck EG, Bignon J, editors. In vitro effects of mineral dusts. NATO ASI Series, Vol. G3. Berlin: Springer-Verlag; 1985.
19. Hatch GE, Boykin E, Graham JA, Lewtas J, Pott F, Loud K, Mumford JL. Inhalable particles and pulmonary host defense: in vivo and in vitro effects of ambient air and combustion particles. Environ Res. 1985 Feb; 36(1):67-80.
20. Adamis Z, Timar M, Kofler L, Tatrai E, Ungvary G. Biological effects of the respirable dusts from ore mines. Environ Res. 1986 Oct; 41(1):319-326.
21. Murphy EJ, Roberts E, Horrocks LA. Aluminum silicate toxicity in cell cultures. Neuroscience. 1993 Jul; 55(2):597-605.
22. Murphy EJ, Roberts E, Anderson DK, Horrocks LA. Cytotoxicity of aluminum silicates in primary neuronal cultures. Neuroscience. 1993 Nov; 57(2):483-490.
23. Fowler JF Jr. A skin moisturizing cream containing Quaternium-18-Bentonite effectively improves chronic hand dermatitis. J Cutan Med Surg. 2001 May-Jun; 5(3):201-205.