



Effect Of Topical Application Of Zinc Oxide On Early Phase Of Wound Healing

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ABSTRACT

30 Albino rats [Wister strain] weighing between 160-240 gm., were used for the study. The animals were grouped into three with one group serving as the control and the other two groups treated with 3% and 5% zinc oxide solutions respectively. Paramedial incisions measuring about 2cm were made bilaterally under light ether anesthesia and subsequently sutured with plain catgut. Smear biopsies were taken from the wound edges at different time points and analyzed for connective tissue cell count. Results show a zinc oxide dose dependent fast response to injury with higher cell counts indicating a rapid inflammatory response, indicative of an effective wound healing process that may ultimately lead to a reduced total healing time and probably better quality scar tissue.

Key words: Zinc Oxide. inflammation. wound.

Wound healing comprises an ordered sequence of events that include cell migration and proliferation, synthesis of extracellular matrix, angiogenesis and remodelling (Amento & Beck, 1991; Piercp et al., 1998). The process of wound healing is fundamentally the same in all wounds.

There are, however, marked quantitative variations. These variations are dependent on the amount of issue destruction and to a certain extent, on the presence or absence of sepsis. Undisturbed wound healing is a special kind of inflammation and is subject to fixed time schedule of biochemical and cellular events (Wokalek & Ruh, 1991). These series of events produce a more or less replica of the former structure.

The sequence of events comprising wound healing is influenced by the following factors: age, diet, nutritional status, vascularity, drugs, adequate supply of oxygen and presence or absence of local irritants.

Wound healing process can be divided into 3 phases viz:-

- (a) Lag Phase: This occurs during the first several days and is usually associated with an acute inflammatory response with cellular migration into the wound area. These cells are

mostly connective tissue cells (both migrants and native). During this phase, the first 24-48 hrs. is dominated by neutrophil infiltration.

- (b) Proliferative phase
- (c) Maturation phase

Zinc Oxide, a component of many proprietary topical emollients is said to significantly accelerate reduction of wound area and enhance re-epithelization of partial thickness wounds (Agren et al., 1991). Its mode of delivery is probably critical for achieving beneficial healing effects.

The aim of this study is therefore to compare the lag phase of wound healing in albino rats treated with topical zinc oxide and those not treated to see any difference in the population of connective tissue cells recruited into the wound area.

MATERIALS AND METHOD

Thirty (30) Albino Wister Rats of both sexes weighing between 160 and 240gm were used. The rats were bred at the Animal House of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. The animals were then divided into three groups. They were weighed, sex matched and divided into three groups. Group A served as control while Group B

and C were treated with 3% and 0.5% zinc oxide solution.

Surgical Procedure

Under light anaesthesia the hair on each animals back and flanks was closely shaved on both sides and wounds of about 2cm inflicted bilaterally. The wounds inflicted were full thickness skin wounds. These wounds were then immediately sutured with plain catgut suture to enable healing by first intention.

Under ether anesthesia, smear biopsies were taken from the wound edges at different time points. 6h, 12h, 72h, 96h, 144h and 168h after surgery.

The biopsy from one of the wound sites was subjected to histological analysis, while the biopsy from the second wound was subjected to biochemical estimation of alkaline phosphatase.

The connective tissue sample was thinly spread out to make smears on clean slides. These preparations were then fixed by immersing the slides in 10% formal saline and left for one week and then stained with Leishman's stain.

The slides were analysed by identifying and counting the individual cells at x 400 magnification using an eye piece graticule with squares of 3 x 5mm. Squares. Counting was done along horizontal line of slides for 100 squares per slide.

RESULT

Table 1. Neutrophils

Time post Surgery	Normal Wound Healing State	With 3% Zn0	With 0.5% Zn0
6 hours	60	88	72
18 hours	110	126	115
24 hours	190	209	196
48 hours	136	210	198
72 hours	136	150	136
96 hours	100	110	105
144 hours	44	60	55
168 hours	40	50	43

By 6 hours post surgery, neutrophils were already more in the Zinc oxide treated groups and remained so throughout the experimental period. This effect was found to be concentration dependent. The figures were statistically significant at 18h, 24h and

48h. While the normal state achieved a peak at 24h the Zinc oxide treated groups achieved a peak at 48h and thereafter declined.

TABLE 2. - Fibroblast Cell Population Count At Various Time Points For Normal And Zn0 Influenced Wound Healing.

Time post Surgery	Normal Wound Healing State	With 3% Zn0	With 0.5% Zn0
6 hours	0	0	0
18 hours	2	19*	16*
24 hours	6	25*	21*
48 hours	18	25*	22*
72 hours	11	10*	15
96 hours	7	10*	18
144 hours	10	18*	16
168 hours	10	18*	15

* Show that those points are statistically significant P.0. 05 level.

In both the Zinc oxide treated and normal states fibroblast begins to appear at 18 hrs with more cells being recruited in the Zinc oxide group.

TABLE 3 Macrophage cell population count at various time point for normal and Zn0 influenced wound healing.

Time post Surgery	Normal Wound Healing	With 3% Zn0	With 0.5% Zn0
6 hours	1	2	1
18 hours	1	2	1
24 hours	1	3*	1*
48 hours	0	2*	1*
72 hours	0	0	0
96 hours	0	0	0
144 hours	0	3*	2*
168 hours	2	3*	3

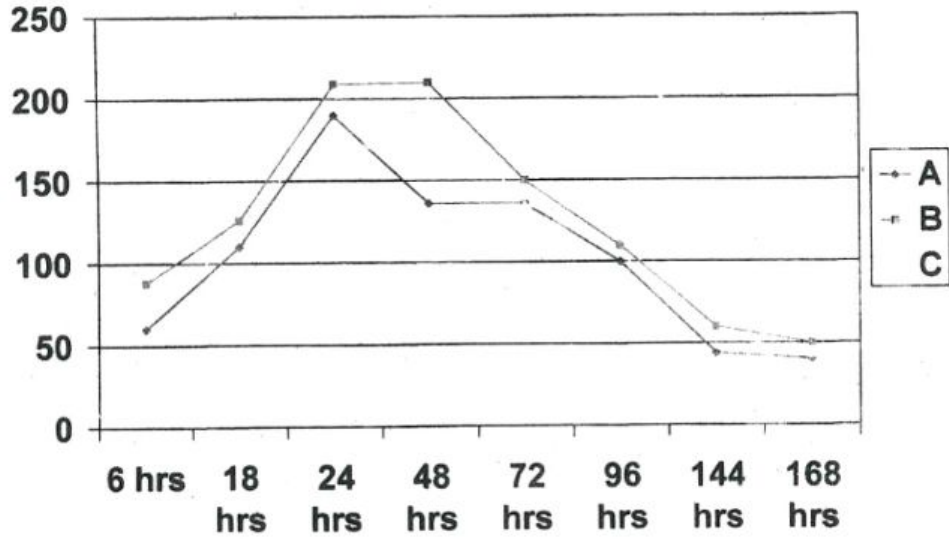
* Statistically significant $P \leq 0.05$.

From the table significantly more cells are recruited by the 3% Zn0 group over the normal as compared to the 0.5% Zn0 group.

DISCUSSION

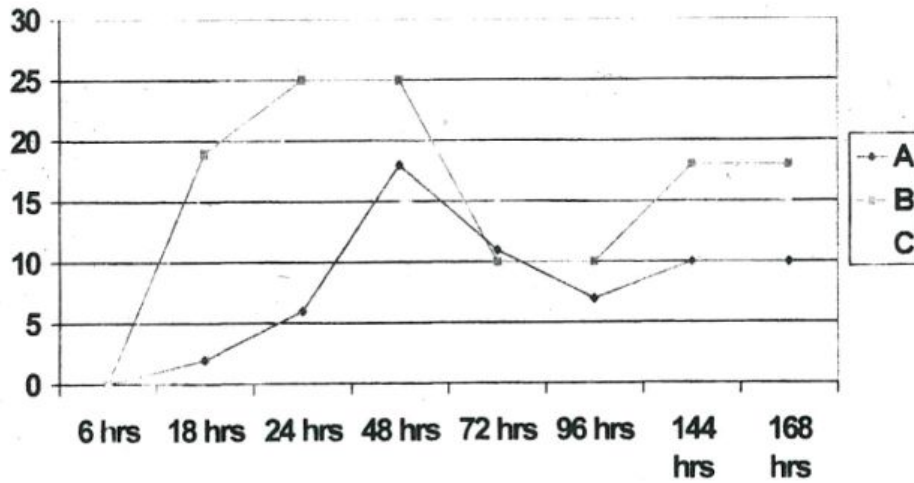
The effect of wound healing on the sate of connective tissue cells both morphologically and numerically has been investigated (Agren et al., 1991; Tongroup et al., 1980). These investigators paid attention to neutrophils and fibroblasts. The literature is however scanty about the other connective tissue cells. This is more so

FIG. 1: NEUTROPHIL CELL COUNT AT VARIOUS TIME POINTS FOR NORMAL AND ZINC OXIDE INFLUENCED WOUND HEALING



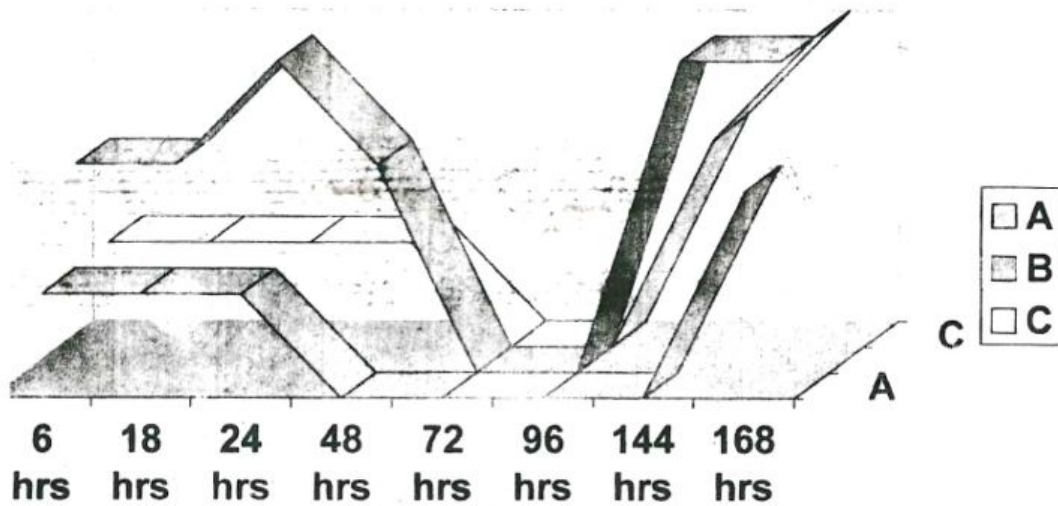
A=Normal wound healing B=3%Zinc oxide influenced C=0.5% Zinc oxide influenced.

FIG. 2: FIBROBLAST CELL POPULATION COUNT AT VARIOUS TIME POINTS FOR NORMAL AND ZINC OXIDE INFLUENCED WOUND HEALING



A=Normal wound healing B=3%Zinc oxide influenced C=0.5% Zinc oxide

FIG. 3: MACROPHAGE CELL COUNT AT VARIOUS TIME POINTS FOR NORMAL AND ZINC OXIDE INFLUENCED WOUND HEALING



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when these cells are considered in relation to the effect of drugs that alter or affect wound healing. Since the initial process of wound healing is predominantly cellular, the states of these cells become important.

Neutrophils are usually the first group of cells to be recruited into a wound site. The normal group exhibited an earlier peak though quantitatively less occurring at 24h while for both groups treated with ZnO, peak time was at 48h with a higher value. For the 30% group, the difference was significant at 6h 48h and 72h. ($P < 0.05$). While the 0.5% was only significant at 48h. The higher numerical response in terms of neutrophils may be contributory to the better quality of wound healing associated with zinc oxide application.

The synthesis of collagen by fibroblast perhaps represents a very important aspect of wound healing (Tengrup et al., 1998). Fibroblast form the most important cellular element after the inflammatory phase of wound healing until probably healing is complete (Wokalek & Ruh, 1991). ZnO treated rats show evidence of more fibroblast at wound sites 48h and 72h and also at 144h and 168h.

Agren et al. (1991) working with parabiotic rats have shown that fibroblasts involved in wound healing are of local origin.

In wound healing macrophages play a very essential process in remodeling laid down fibres, thus functioning as a regulator of the replicate and synthetic activities of fibroblasts. In both control and treated groups, after being present for 24h the macrophages disappear and then reappear later at 144h.

The initial appearance may be in relation with wound debridement, while the more sustained late appearance is probably accounted for by the remodeling need of laid down collagen. Despite the similar picture presented, more macrophages are seen with the treated groups having significantly higher numbers at 24h, 48h and again at 144h and 168h ($P < 0.05$).

From the ongoing, it shows that topical application of ZnO on skin wounds in rats results in more profound cellular reaction and most probably better quality healing.

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