

INFLUENCE OF PYRAMID ENVIRONMENT ON THE WOUND HEALING EFFECTS OF DEXAMETHASONE AND INDOMETHACIN – A COMPARATIVE STUDY

KAMATH SUREKHA

Department of Physiology Melaka Manipal Medical College, Manipal, India, E mail: surekharkamath@yahoo.com

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ABSTRACT

Shapes and angles of The Great Pyramid of Egypt are balanced and renowned for their energy properties. Studies have proved that beneficial effects are found on human health in pyramidal environment. The present study was designed to determine the efficacy of dexamethasone and indomethacin on incision and dead space wound models in rats under the influence of pyramid environment. A wooden pyramid model fabricated locally was used for the study. The parameters chosen were breaking strength of incised wound, breaking strength of granulation tissue, hydroxyproline content and histology. The results showed that the dexamethasone and indomethacin slow down all phases of wound healing and the inhibitory action of dexamethasone on wound healing was stronger than that of indomethacin. Thus pyramid exposure reversed the action of indomethacin more effectively than that of dexamethasone.

Key words: Pyramid environment, dexamethasone, indomethacin, incision wound, excision wound, dead space wound

INTRODUCTION

The Pyramids of Egypt are one of the most mystifying structures. These pyramids have been planned with mathematical and geometrical accuracy so as to create an energy force which is active within them and can do many things that are beneficial to both non living and living creatures including human beings¹. There is evidence to show that the pyramid environment enhances wound breaking strength in incision wound² model. Wound healing is the process of repair that follows injury to the skin and other soft tissues. Inflammation is the reaction of vascularized living tissues after injury. Steroidal and nonsteroidal anti-inflammatory drugs suppress wound healing. Steroids are widely used for the treatment of various diseases, despite their known side effects such as skin atrophy and immune suppression³. There is enough data, which says that steroidal anti-inflammatory agents suppress wound healing^{4, 5}. The healing suppressant effect of NSAIDs has been further confirmed for ibuprofen⁶, enfenamic acid⁷ and tolmetin⁸. Since NSAIDs are being used routinely to suppress postoperative edema and pain, they may interfere with wound healing. With this in view, the present study has been designed to monitor the effects of dexamethasone and indomethacin on different phases of wound healing in rats.

MATERIAL AND METHODS

Dimensions of pyramid- A wooden pyramid model (height of 20", base of 30" and sides measuring 28.55") as explained by Toth and Nielsen⁹ was used. Holes were drilled on all sidewalls for ventilation and a glass window was fixed on one of the sidewalls for observation. The pyramid was positioned such that the walls faced north, south, east and west, while the corners aligned with North West, south west, north east, and south east¹⁰.

Rats- Healthy Wistar albino rats (80) of either sex, weighing 150-250g, bred locally were used. They were individually housed in clean polypropylene cages, maintained on standard conditions (12; 12 hr L: D cycle). The rats were fed with standard chow diet and water ad libitum. They were starved for 12 hr before infliction of wounds.

Experimental procedure- Wounded rats were divided into following 6 groups of 8 animals each for both incision and dead space wound models: Group I [home cage control]; rats were kept outside the pyramid without any drug treatment: Group II [pyramid control]; rats were kept inside the pyramid without any drug treatment: Group III [dexamethasone control]; rats were treated with dexamethasone kept outside the pyramid: Group IV [dexamethasone +pyramid]; rats were treated with dexamethasone kept inside the pyramid: Group V [indomethacin control]; rats were treated with indomethacin kept outside the pyramid: Group VI [indomethacin+pyramid]; rats were treated with indomethacin kept inside the pyramid.

The wounding procedures were carried out using pentobarbitone anesthetized rats in these wound models, at the dose level of 30mg/kg bodyweight. Dexamethasone (4mg/2ml) was injected in the dose of 0.34mg/kg, intramuscularly, on the day of operation ('0' day) and 0.17mg/kg thereafter on alternate days till wounds healed or the 21st day whichever was earlier. Indomethacin was used in the dose of 0.2 mg/kg body weight. It was given orally and daily from the day of wounding. The tablets were powdered and suspended in gum acacia (4%) mucilage to provide the required dose in vehicle volume of 0.5 ml/100 gm body weights.

Incision wounds

Two 6 cms long, paravertebral straight incisions were made one cm lateral to the vertebral column on either side through the entire thickness of the skin¹¹. Intermittent sutures were placed 1 cm apart. The sutures were removed on the seventh day. Wound breaking strength was measured by continuous constant water flow technique as described by Lee KH¹² on the 10th day.

Dead- space wounds

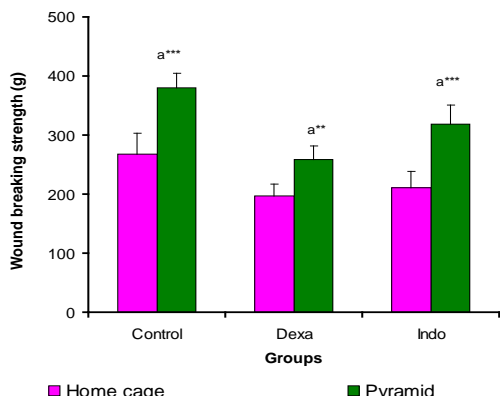
Wounds were prepared by implanting subcutaneously, a 2.5x0.5cm polypropylene tube in the paravertebral lumbar region through a small 0.5cm transverse incision. On the tenth post-wounding day, the granulation tissue formed on the implanted tubes was dissected out carefully. Granulation tissue from one tube was used for the determination of tensile strength¹², after which it was dried in an oven at 60°C for 24 h and the dry weight noted. The acid hydrolysate of the dry tissue was used for the estimation of hydroxyproline content in the tissue¹³. Granulation tissue from the other tube was subjected to histopathological studies.

Statistical analysis- Results are expressed as mean \pm SD. One-way analysis of variance (ANOVA) followed by Bonferroni's post-test was applied. *P* values < 0.05 were considered as significant.

Institutional Ethics Committee (IEC) - Actual experiments started after IEC approved and cleared the research project.

RESULTS AND DISCUSSION

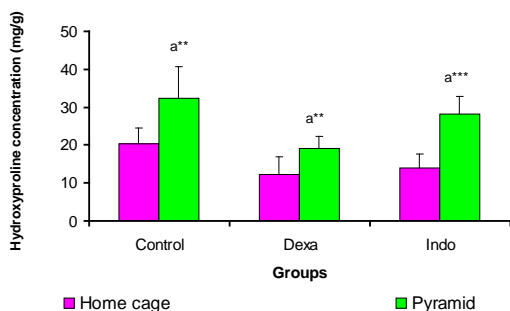
In the incision wound model, a significant increase was observed in the skin tensile strength of the pyramid exposed group. The pyramid exposed control and drug treated animals of the dead space wound model showed a significant increase in dry weight of granulation tissue, breaking strength and the level of hydroxyproline content. The histopathological study revealed improved neovascularisation and increased collagen formation in all pyramid exposed control and drug treated rats. However this effect was better in indomethacin treated pyramid exposed rats as shown by the maximum number of blood vessels and collagen fibres.



a with home cage. *=0.05, **= 0.01, ***=0.001. Dexa= Dexamethasone, Indo= Indomethacin

Graph 1: Effect of environment within the pyramid alone and in presence of dexamethasone and Indomethacin on wound breaking strength in incision wound (*p<0.05, **p<0.01, *p<0.001)**

The results of our study showed that dexamethasone and indomethacin slow down all phases of wound healing. The poor breaking strength observed may be due to their adverse influence on formation of collagen and inter-intra molecular cross linking of collagen. This is confirmed by the reduced hydroxyproline concentration in dexamethasone treated and indomethacin treated control rats. The granulation tissues of these rats revealed poor neovascularisation, reduction in collagen content and moderate number of inflammatory cells in comparison to home cage and drug treated pyramid groups.

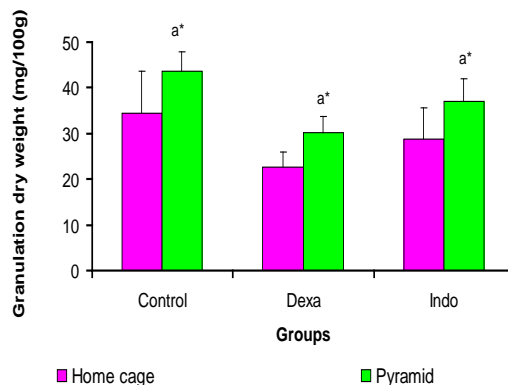


a with home cage. *=0.05, **= 0.01, ***=0.001. Dexa= Dexamethasone, Indo= Indomethacin model

Graph 2: Effect of environment within the pyramid alone and in presence of dexamethasone and Indomethacin on hydroxyproline concentration in dead space wound model

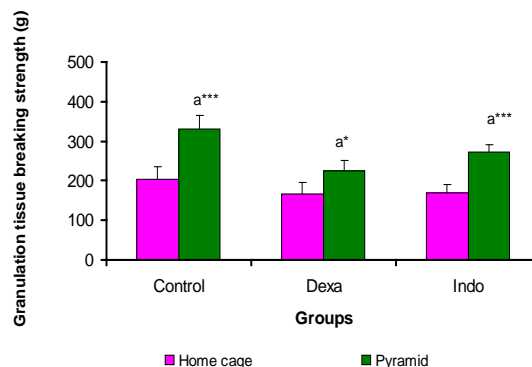
Dexamethasone is known to reduce collagen synthesis^{14, 15, 16}. In our study, there was no statistical difference in dry weight and hydroxyproline content between the pyramid and dexamethasone control groups, which suggests that the pyramid exposure might not have induced increase in collagen synthesis. Hence, pyramid exposure probably increased the wound breaking strength by altering the maturation process where it could have affected the cross linking or could have improved the quality of collagen fibrils. This effect is exhibited in the histology of granulation tissues. The histology of pyramid exposed group showed moderate cell

population with some matrix formation and better neovascularisation and collagen bundles. Hence, it showed better healing as compared to dexamethasone control groups. Thus, it appears that the pyramid was able to antagonize the actions of dexamethasone not by promoting collagen synthesis but by improving the quality of collagen fibrils.



a with home cage. *=0.05, **= 0.01, ***=0.001. Dexa= Dexamethasone, Indo= Indomethacin

Graph 3: Effect of environment within the pyramid alone and in presence of dexamethasone and Indomethacin on granulation tissue dry weight in dead space wound model



a with home cage. *=0.05, **= 0.01, ***=0.001. Dexa= Dexamethasone, Indo= Indomethacin

Graph 4: Effect of dexamethasone and indomethacin on granulation tissue breaking strength in dead space wound model

Pyramid environment effectively antagonized the anti-healing actions of indomethacin on breaking strength of skin and granulation tissues when compared to indomethacin home cage and indomethacin square box groups. This shows that pyramid energy has also reversed the anti-healing effects of indomethacin on the maturation phase of collagenation. Enhanced healing activity of pyramid can be attributed to increased collagen formation and angiogenesis, as is indicated by the increase in the hydroxyproline concentration in the indomethacin pyramid group when compared to home cage /square box exposed groups. Increased capillary proliferation was seen in the histology of indomethacin pyramid group and also the well-arranged collagen bundles showed better healing as compared to home cage group. Indomethacin treated

control and square box rats showed increase in cell population (fibroplasias) in the whole thickness of granulation tissue while collagen bundles were sparse, as compared to indomethacin pyramid group.

The results of our experiments on wound healing and effect of anti-healing drugs within the pyramid showed that the pyramid environment has reduced the anti-healing effects of these drugs and enhanced the wound healing process. Decreased suppression in wound healing following anti-healing drugs by pyramid is due to its enhancing the early inflammatory reaction to wounding, including increasing the number of total leukocyte count, possible effect on modulating collagenase activity, effect on epithelial cell

differentiation and stimulation of immune responsiveness. Thereby, pyramid acts as a wound-healing promoter. It also appears that the energy field developed within the pyramid may work either by inhibiting the release of agents that delay healing or by reducing the negative effects of these agents on healing process.

In conclusion, it can be said that in our study pyramid environment reversed the wound healing suppressant effects of dexamethasone and indomethacin and restored the wound healing ability to the home cage levels as compared to their respective drug control groups. Also, the pyramid environment reversed the actions of indomethacin more effectively than those of dexamethasone.

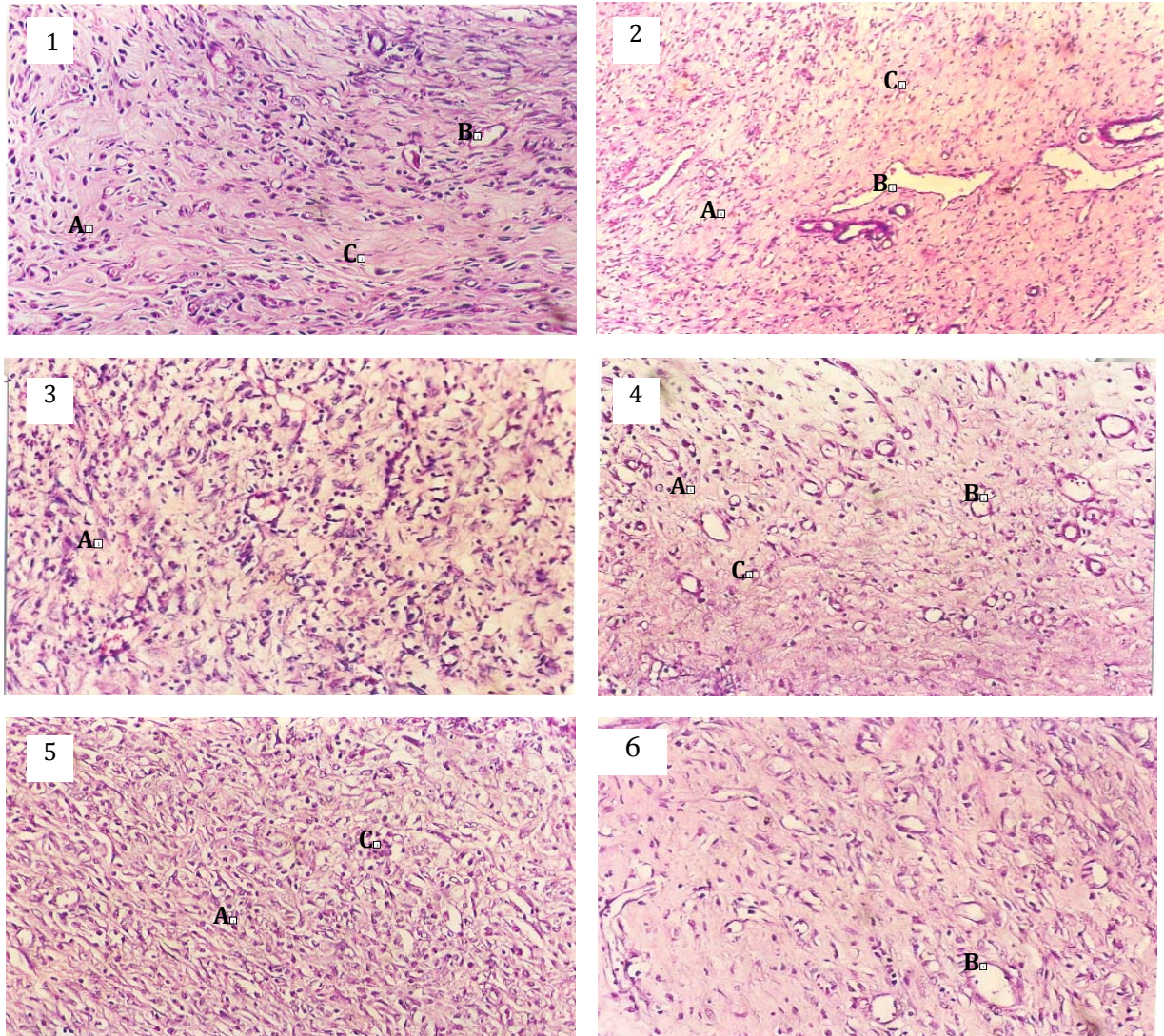


Fig 1: Photomicrographs of 10-day-old granulation tissues (Hematoxylin and Eosin 200x) in control, dexamethasone and indomthacin treated rats. A- Fibroblasts, B- Blood vessels, C- Collagen bundles. Note the decreased inflammatory cells in 2, 4 and 6 as compared to 1, 3 and 5.

1. Home cage - Shows normal progress of healing
2. Pyramid exposed-Healing better than home cage
3. Dexamethasone home cage-Comparatively poor healing
4. Dexamethasone pyramid-Healing comparable to home cage
5. Indomethacin home cage- Comparatively poor healing
6. Indomethacin pyramid - Healing better than home cage

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