Scripta Scientifica Medica, vol. 28 (1993), pp 67-72

HISTOLOGICAL AND ULTRASTRUCTURAL CHANGES IN THERMAL SKIN WOUNDS TREATED BY GENTAMID

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The healing of thermal skin wounds was investigated under experimental conditions on 96 male Wistar rats comparing the effect of Gentamycin ointment with that of the combination of Gentamycin and urea (i.e., with Gentamid, an ointment suggested by Zhelyazkov et al., 1984). The healing process was dynamically followed-up on the 3rd, 7th, 14th, and 21st day after burn. It was established that Gentamid treatment accelerated significantly more the regeneration process than that with 0,1 % of Gentamycin ointment alone. This was manifested by reduced tissue oedema and polymorphonuclear leukocyte infiltration and by stimulated granulation tissue formation as well leading to faster wound healing in Gentamid-treated animals.

Key-words: Thermic injury, Gentamid, Gentamycin, urea, wound healing, ultrastructure, rats

INTRODUCTION

Treatment of thermal skin wounds is an actual problem of modern medicine. That is why the need of research and application of new drugs aiming at accelerating wound healing has been growing continuously. In this aspect a decade ago, Zhelyzakov et al. (5) proposed an ointment, i. e. a combination of 0,1 % of Gentamycin and 0,2 % of Carbamide (urea) designated as "Gentamid". Urea was used in preparations with dehydratant, necrolytic, and detoxifying action which stimulate regenerative processes as well (2, 4, 11, 16).

The purpose of the present study was to investigate the histological and ultrastructural alterations in rat skin induced by thermic trauma during Gentamid treatment.

MATERIAL AND METHODS

The experiments were carried out on 96 male Wistar rats. Of them, 72 were used

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for experimental series but 24 - for control ones. Ironlike electric device of Kochetigov was applied to provoke thermal skin injury of 3/4 cm in size. The animals of the first experimental group were treated with 0.1 % of Gentamycin ointment and these of the second one - with Gentamid ointment. No medicamentous treatment was administered to control animals at all. Ointments were applied in a thin layer of 1-2 mm over the wound surface. Samples from the wounds and surrounding tissues were taken for histological examination on the 3rd, 7th, 14th, and 21st day after injury. They were fixed in 10 % solution of formalin and in Carnua solution. Sections were stained with hematoxylin-eosin, Van Gieson, toluidine-blue with pH 2 and pH 4 for demonstration of acid glucosaminoglycans (GAG), and PAS-reaction of McManus. Reaction of Brachet was used to demonstrate RNA but reaction of Feulgen was applied to detect DNA. Materials were fixed in 5 % glutaraldehyde solution in 1 % osmium tetraoxide, embedded in Durcupan and contrasted with uranyl acetate to be examined by JEM 7A electron microscope.

RESULTS

On the 3rd day after combustion, on the bottom of the wounds numerous necrotic epithelial cells and collagen fibres, fibrin and leukocytes were established in all the animals. Oedema, haemostasis, and haemorrhages were seen in surrounding tissues in animals on Gentamycin treatment and in controls. Blood vascular walls showed fibrinoid necrosis or leukocyte infiltration. Single macrophages, newly-formed capillaries and poorly differentiated fibroblasts were observed, too.

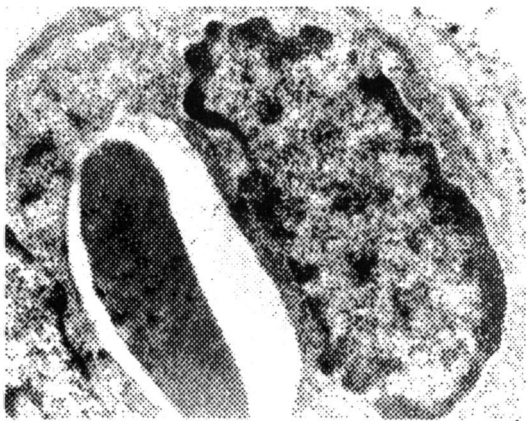

Fig. 1. *Gentamid-treated skin wound on the 3rd day after burn. Many blood vessels with young epithelial cells in granulation tissue. Magn. x 12000*

The inflammatory alterations and oedema in the surrounding zone were less expressed among Gentamycin-treated rats. Foci of granulation tissue with poorly disoriented young blood vessels having young endothelial cells were formed (Fig. 1). Fibroblasts rich in glycogen and cytoplasmic RNA with a well-developed rough endoplasmic reticulum (RER) were more abundant than those in the other animals groups. Numerous collagen fibres were located around them. There were activated macrophages containing lysosomes and lipid vacuoles in their cytoplasm.

On the 7th day, a well-manifested polymorphonuclear leukocyte infiltration in the superficial and deep layers and in the adipose tissue along with vascular wall lesions were established in control animals. Macrophages in the newly-formed poor granulation tissue had only a few of developed organelles and almost no contacts with fibroblasts. However, some

Fig. 2. Gentamid-treated skin wound on the 7th day after burn. A macrophage with well-developed RER, numerous ribosomes and vacuoles containing engulfed collagen fibres. Magn. x 12000

organelles displayed certain trends towards differentiation.

Similar alteration with less expressed inflammatory reaction were established in Gentamycin-treated rats.

Gentamid-treated wounds demonstrated outlined proliferative processes. In the granulation tissue there were many fibroblasts arranged parallelly to the surface with increased cytoplasmic contents of glycogen and RNA as well as with a large nucleus and abundant organelles. In the extracellular matrix there were more glycoproteins and sulfated GAG in comparison with Gentamycin-treated wounds as well as thin collagen fibres. In deep granulation tissue layers there were macrophages with a plenty of ribosomes, mitochondria and vacuoles containing engulfed collagen fibres (Fig. 2). Oedema and leukocyte infiltrations were considerably diminished. At the wound margins the newly-formed epithelium was of varying width; most epithelial cell nuclei

Fig. 3. Gentamycin-treated skin wound on the 14th day after burn. Tissue oedema and destructed collagen fibres. Magn. x 35000

showed mitotic figures and enhanced DNA content.

During the later periods (on the 14th and 21st day after combustion, even at the end of experiment) skin wounds of control animals were characterized by many leukocytes forming at several places abscesses and by persisting oedema. The granulation tissue was loose. Activated fibroblasts and collagen fibres were rare.

The inflammatory reaction reduced in Gentamycin-treated animals during the same period. However, healing was delayed. Tissue oedema, disruption of collagen fibres (Fig. 3), and vasculitis could be detected. There existed more macrophages and fibroblasts in most of which there was a delayed differentiation. Cells of newly-formed epithelium demonstrated some vacuoles in the cytoplasm. Leukocytes were observed among some epithelial cells.

A manifested tendency towards an acceleration of the regenerative processes was established in Gentamid-treated rats. Tissue oedema was less expressed or even disappearing. Many fibroblasts were

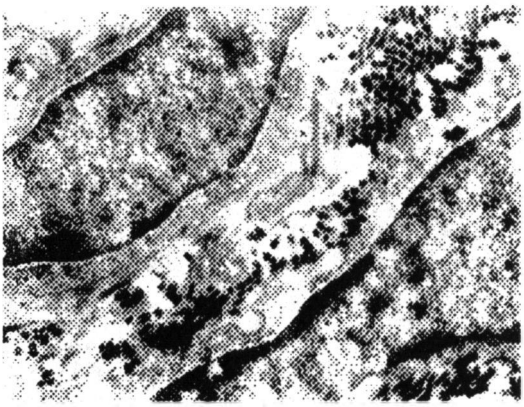

Fig. 4. Gentamid-treated skin wound on the 21st day after burn. Numerous fusiform fibrocytes with poorly developed RER and collagen fibres scattered among them. Magn. x 15000

present in the granulation tissue on the 14th day most of which contacted directly with macrophages. Sulfated GAG predominated in the extracellular matrix and diminished on the 21st day. During this period, granulation tissue maturated and epithelization was completed in many animals. There were numerous fibroblasts with activated collagen-forming function, fibrocytes with spindle-shaped fibres and slightly expressed RER (Fig. 4). Connective-tissue fibres appeared uniformly wide and were located parallelly to the surface.

DISCUSSION

Our results demonstrate that Gentamid ointment benefits and significantly accelerates the regeneration process of thermal skin wound healing. First, the leukocyte infiltration, which is one of the causes for delayed regeneration (3) is inhibited during the period 7 days after closing. The functional activity of macrophages increases which is related to tissue oedema (9). Contacts between macrophages and fibroblasts are more common and sulfated GAG content is elevated. All these factors enhance collagen synthesis, stimulate vascularization and contribute to faster formation and maturation of granulation tissue (7, 12-15).

The favourable effect of the gentamid ointment can be explained by the following way: during the inflammation, acidosis develops in the traumatic tissue (8) and the acid reaction enhances Gentamysin resorption from the skin through the interstitial fluid in the blood (6). Urea being neutral or slightly alkaline in solutions, probably, inhibits this penetration and thus the antibiotic is maintained on the surface for a longer time and in a higher concentration at the application site (6). This seems to be the most possible mechanism of the inhibition of the inflammatory reaction (1) and the most interesting moment in the present study as well. In vitro investigations of microbial flora isolated from patients with skin burns (of I - III-A degree) clearly indicate a time-dependent reduction in number of pathogenic bacteria (10, 17).

CONCLUSION

It should be emphasized that Gentamid application in the course of treatment of thermal skin wounds in rats possesses essential advantages when compared with that with Gentamycin ointment still used in medical practice. These advantages briefly discussed above make Gentamid ointment a perspective drug and allow its recommendation for a clinical and pharmacological study.

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Histologische und ultrastrukturelle Veränderungen bei der Heilung von thermischen Hautwunden mit Gentamid

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Zusammenfassung: Es wurde die Heilung von thermischen Hautwunden unter experimentellen Bedingungen an 96 weißen männlichen Ratten Wistar untersucht, wobei der Effekt der Gentamycin-Salbe mit diesem der Kombination von Gentamycin mit Urea (des so-genannten Gentamid, das im Jahre 1984 von Zheljazkov und Mitarbeitern vorgeschlagen worden war) verglichen wurde. Der Heilungsprozeß wurde dynamisch am 3., 7., 14. und 21. Tag nach der Verbrennung verfolgt. Festgestellt wurde, daß die Behandlung mit Gentamid im Vergleich zu dieser mit lediglich 1 %-iger Gentamycin-Salbe den Prozeß der Regeneration wesentlich mehr beschleunigte. Das wurde durch die Reduktion der Gewebeschwellung und der Granulozyteninfiltration, wie auch durch die stimulierte Bildung von Granulationsgewebe gekennzeichnet. Als Ergebnis wurde

eine schnellere Wundheilung bei den Tieren, die mit Gentamid behandelt wurden, erzeugt.

Changements histologiques et ultrastructurales lors de la guérison des plaies thermiques de la peau traitées avec "Gentamid"

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Résumé: Sur 96 rats blancs mâles Wistar, dans des conditions d'expériment chronique, la guérison des plaies thermiques de la peau a été étudiée par la méthode de Kotchetigov de traitement journalier avec une pommade - combinaison de Gentamicine 0,1 % et Urée (le nom générique Carbamide) sous le nom "Gentamid", proposée par Zhelyazkov et al., 1984, et préparée dans l'Institut des antibiotiques à Razgrad. Il a été établi que la guérison est essentielement accélérée lors du traitement des animaux avec "Gentamid" en comparaison du traitement avec Gentamicine 0,1 %, la pommade appliquée dans la pratique. "Gentamid" diminue l'oedème des tissues et l'infiltration leucocytaire. Il stimule la formation de la granulation et accélère le processus de guérison.