The Impact of Topical Recombinant Growth Hormone on Wound Healing

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Abstract

Objective. In an effort to improve the wound healing of the ear, the influence of topical recombinant growth hormone was investigated, using hyaluronic acid as biomaterial, on ear burned wounds in rabbits. Material and Methods: 15 New Zeeland male rabbits have been used. A deep 2nd degree burn was inflicted on each ear. On the control side, only hyaluronic acid gel was applied and on the study side, a combination of hyaluronic acid and chicken recombinant growth hormone. The dressings were changed every other day for two weeks. Glycaemia and IGF-1 levels were monitored. On the 10th week, biopsies were harvested for histological and immunohistochemical examination. Results. No differences in serology before and after topical administration of chicken recombinant growth hormone have been observed. Lower degree of inflammation (p<0.05), increased amount of collagen (p<0.05), vascularization (p<0.05), respectively myofibroblasts activity were observed on the study side. Conclusion. The wound healing was improved significantly by topical administration of chicken recombinant growth hormone.

Keywords: burns, hyaluronic acid, biomaterial, IGF-1, scar

1. Introduction

Wound healing is a highly studied and debated topic in the medical literature. However, pathological scars, especially hypertrophic and keloid, are common and their treatment is still a challenge.

Hyaluronic acid (HA), one of the largest components of the extracellular matrix, has identical structure in different species including rabbit and human (1). It is present in almost all types of tissues like skin, cartilage, aorta, brain. Known for its nonimmunogenicity and good biocompatibility, HA is now used in a wide range of areas like pharmaceutical, medical, cosmetic fields. HA is also known as a good drug delivery agent for ophthalmic, nasal, dermal routes (2).

Recombinant growth hormone (rGH), an anabolic hormone, increases glucose uptake and collagen synthesis (3) Used in patients, both children or adults, with burns over 40% of the total body surface area, it leads to a more rapid healing of both, burned areas and donor sites, and no evidence of increased mortality or scarring were reported (4). There are no data in medical literature about topical administration of rGH on burned wounds.
Herein, in an attempt to speed wound healing and to obtain a minimal scar, we developed a deep 2nd degree burn model on rabbits and chicken rGH (crGH) was applied topically using HA as biomaterial.

2. **Material and Methods**

All procedures were approved by the University of Agricultural Sciences and Veterinary Medicine Ethics Committee.

We used a group of 15 male New Zealand rabbits, weighting 2500-3000 grams each. All the procedures were performed under general anesthesia with intramuscular Ketamine (50mg/kg) and Xylasine (5mg/kg). Blood samples were taken and serum glucose and IGF-1 were determined. On the ventral side of each ear, a deep 2nd degree burn was inflicted using a copper device after a protocol previously established (and not published separately). The device of 85 grams was introduced in boiling water. Its temperature was measured, using an infrared thermometer. When the instrument got to 50 degrees Celsius, it was applied perpendicularly on the skin, no other pressure was applied, but its own weight. The contact time with the skin was 6 seconds. On the left ear of each rabbit, considered the control, hyaluronic acid 1.2% gel (Sigma Aldrich, Germany) was applied, while on the right ear, considered the study side, the wound was covered with a gel consisting of hyaluronic acid and crGH (Sigma Aldrich, Germany). The dose for crGH was 0.50 mg/m². All the rabbits received for the first three day 0.1mg/kg/day enrofloxacin intramuscular, 2mg/kg/day ketoprophen intramuscular and 20mg omeprazole orally. The dressings were changed every other day for two weeks. At the end of the two weeks, same blood tests were done as at the beginning of the study. On the 10th week, biopsies from the burned areas were harvested for histological and immunohistochemical studies.

Haematoxiline–Eosine (HE) and Trichrome-Goldner stains were used for histological study. A semiquantitative assessment of inflammation, collagen deposition and fibroblast cellularity, vascularization was done using a scale from 0 to 3 (depending on intensity: 0=none, 1=mild, 2=medium, 3=severe).

For the immunohistochemistry study, antibodies anti alpha-smooth muscle actin (α-SMA), anti-collagen type I and anti-collagen type III were used. For α-SMA a semiquantitative assessment was done: 0- no expression, 1-minimal expression, 2-mild expression and 3-intense expression.

The statistical analyses were performed using IBM SPSS 21. Mann-Whitney non-parametric test was used to evaluate the histological and immunohistochemical parameters. Paired t test was used for comparison of glycaemia and IGF-1 means. Statistical significance was accepted at a p-value of less than 0.05 and the confidence interval was set at 95%. The graphics were performed using IBM SPSS and Microsoft Excel 365.

3. **Results**

One rabbit died right after anesthesia administration and was excluded from the study, 28 wounds were evaluated, 14 in the control group (left ears, treated with HA gel) and 14 in the study group (right ears, treated with HA+crGH gel). No infections were present at the wounds site during the study. Clinical hypertrophic scars were present in 3 cases on the study side and in 6 cases on the control side (Figure 1). One rabbit presented bilateral ear hypertrophic scars.
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Seric glucose level varied from 131.2-245.5 mg/dl at the beginning of the study to 133.6-192.1 mg/dl after the two weeks treatment with topical rGH (p=0.40). IGF-1 baseline varied between 206.7-372.7 ng/ml and after the two weeks treatment with rGH, between 160.6-323.8 ng/ml (p=0.90).

*Histology and immunohistochemistry exam*

The inflammation was discrete or even absent in all cases on the right side compared with the left side (p=0.01) (Figure 2). The auricular cartilage on the control side showed necrosis in all cases.

There were significant differences between the two anatomical sides regarding dermal fibroblastic cellularity, collagen deposition (p=0.03) and neovascularization (p=0.01) (Figure 2).
The evaluation of fibrosis and myofibroblastic activation was realized using trichrome-Goldner stain and immunohistochemical exam for α-SMA expression. Dermal fibrosis with the presence of numerous fusiform cells in the papillary dermis along with numerous collagen bundles were present. Immunohistochemistry for α-SMA proved that most of these cells were myofibroblasts. (Figure 3, Figure 4)

Figure 3. Evaluation of fibrosis and myofibroblastic activation; HA treated (left) side. Complete reepitelization, dermal fibrosis (A, B, D, E); Numerous α-SMA positive spindle shaped cells and capillaries in the subepidermal connective tissue (C, F).

Figure 4. Evaluation of fibrosis and myofibroblastic activation; rGH treated (right) side. Complete reepitelization, superficial dermal fibrosis, numerous spindle shaped cells in the dermis (A, B, D, E); Numerous α-SMA positive myofibroblasts and capillaries in the subepidermal connective tissue (C, F).

The number of myofibroblasts and the intensity of the α-SMA expression were higher on the study side, compared to the control side (p=0.06) (Figure 5). Numerous capillaries were present in the reticular dermis, the pericytes of these vessels being positive to the α-SMA antibody. The number of newly formed capillaries was higher on the right side comparative to the left.
The distribution of collagen I and III was also followed. In our study, in both anatomical sides, the intensity of collagen III expression was higher in the superficial papillary dermis, while the collagen I expression was higher in the reticular dermis.

4. Discussions

Wound healing is a complex, interactive process and represents an exactly organized activity of various cells, different cytokines, growth factors, and collagen (5). Several treatment methods, locally or systemically, have been tested.

In systemic administration, the rGH side effects are present. The most frequent side effects are increased blood sugar levels and insulin resistance. IGF-1 levels are sometimes used to test the efficacy of rGH treatment. IGF-1 is increased by rGH administration. In our study, no increased serum glucose and IGF-1 levels were noticed after topical chicken rGH administration.

Regarding the two main parameters evaluated, in order to monitor a possible general metabolization of locally applied rGH, it can be concluded that used topically, rGH has not the same side effects as in systemic administration. The systemic absorption of chicken rGH used was non-existent.

In the medical literature are mentioned various doses of rGH, used for therapeutic purposes or for research purposes. Baumgarten et al discussed on account of these doses and noticed that rGH was administered in doses ranging from 2µg / day to 8 mg / kg twice daily in experimental studies. In this study, we used a dose of 0,50mg / m² chicken rGH (6). Applying the formula Dose (mg / kg) x K_m = Dose (mg / m²), where the K_m coefficient is 12 for rabbit (7), chicken rGH dose used in this study is of 21µg / kg/day chicken rGH, administered topically. So, we used a rGH dose within the wide range mentioned in the literature.

The GH effects are produced, either by the direct action of GH, or indirectly through IGF (8). The direct effect of GH on the wound healing process is mentioned in the literature (9). GH stimulates keratinocyte migration, fibroblast proliferation, angiogenesis, and accelerates granulation tissue formation (9,10). GH also influences the wound healing process, through IGF-1. Locally produced IGF-1 is important in this case (9, 11). IGF-1 stimulates the keratinocytes proliferation and collagen and other components of extracellular matrix synthesis by dermal
fibroblasts (12, 13). In our study, higher fibroblast and collagen deposition and vascularization was observed on the side where crGH was applied, compared to the contralateral side.

Reepitelization is essential in skin wound healing, because the epidermis forms a protective and impermeable layer and protects the underlying tissues. In case of a burned wound, the epidermis isolates quickly the lesion, while the collagen synthesis continues in the dermis to strengthen the wound. Our study showed complete reepitelization in all cases both study or control side.

It is known the fact that a prolonged inflammation is responsible for increased scar formation (14). The study side, where crGH was applied, presented a lower degree of inflammation compared to the contralateral side. The number of scars with hypertrophic aspect was lower on the side where crGH was applied.

In our study the most of the activated α-SMA positive myofibroblasts were orientated parallel with the surface of the wound. According to Tomasek, this orientation of the myofibroblasts has a mechanical role, their contraction will promote wound healing by closing the wound. Contractile activity of the myofibroblasts is taking place during wound healing and not after scar formation. Contractile myofibroblast will disappear from the granulation tissue before scar formation (15).

In our study the main stress factor which leads to perturbation of the local homeostasis and myofibroblastic activation was represented by the thermal injury. The applied treatment increased myofibroblastic activation, collagen synthesis and deposition and angiogenesis. The increased number of activated myofibroblasts and their orientation – parallel to the wound surface – had a positive effect over the healing process by quick retraction of the wound and by increasing collagen deposition in the dermis.

The resistance and strength of the healing wound is based on collagen. Proliferation of fibroblasts, myofibroblastic activation and synthesis of collagen predominate in the proliferative phase of the wound healing process. Both type I and type III collagens are found in the granulation tissue during healing process. Previous immunohistochemical studies suggested a sequential appearance of type I and III collagens during wound healing (16). In the early stages of the wound healing process and granulation tissue the proportion of collagen type III is higher than type I, while in the later stages the proportion is inverse. The presence of collagen in the wound bed influences reepitelization, and it's also controls normal wound healing or pathological outcomes such as keloid and hypertrophic scars. In our present study crGH administration increased collagen accumulation, this has stimulated the repair process.

Up to this point, we may conclude that positive effects on wound healing process were observed on the side where topical crGH was applied. It is difficult to anticipate what would happen latter. The study was established for ten weeks, but a longer period of observation might help to better understand the effects of topical used rGH and also to see if hypertrophic scar prophylaxis is done for sure.

5. Conclusions

In this study, crGH applied on deep 2nd degree burns wounds in rabbits ears, had beneficial effects on the wound healing process. The applied treatment increases the number of activated myofibroblasts and collagen deposition in the dermis, increases dermal angiogenesis and decreases the inflammation process.

When applied topically, the rGH does not increase glucose and IGF-1 levels.
6. Acknowledgments

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References