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ORIGINAL ARTICLE

EFFECTS OF LOCAL GROWTH HORMONE THERAPY ON IGF-1 AND TGF-β DURING FACIAL SKIN WOUND HEALING IN RABBITS

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Summary

Growth hormone (GH) the most abundant hormone secreted by the anterior pituitary gland could have a role with other growth factors in wound healing because they can help in the physiological wound healing process.
Aims: To investigate the effects of GH on facial skin wound healing in rabbits and to evaluate its effect on "insulin-like growth factor (IGF-1)" and "transforming growth factor- β (TGF-β)" in serum.
Material and Method: Thirty healthy male rabbits included in this study were classified into two groups according to the day of euthanization 7 and 14 days of study, each group was subdivided into three groups; negative control group, positive control group, and treatment group, full-thickness circle 1 cm wounds were excised in the skin of the forehead for each rabbit without any medication.3-(treated group) full-thickness circle 1 cm wounds will excise in the skin of the forehead for each rabbit, 0.1ml [contain 1.2mg /3.6 IU] of growth hormone injected subcutaneously around the incision, the injection process is every other day.
Result: showed a highly significant difference among all study groups in serum TGF-β (ng/L) and IGF (ng/ml) during the first and second weeks. the serum TGF-β at the end of the first and second weeks showed a significant elevation in the treatment group when compared to the other study groups. There is no significant difference between the two control groups. The serum IGF at the end of the first and second weeks showed a significant difference in IGF levels among all study groups.
Conclusions: Topical GH has a role in skin wound healing since it can increase the serum level of TGF-β. GH also causes a decrease in serum IGF. Topical GH may have a positive impact on skin wound healing.

Key words: Growth hormone; Wound healing; TGF-β; IGF

Introduction

Growth hormone is secreted by the anterior pituitary gland most abundantly. Its secretion is regulated by hypothalamic neurons that release neuropeptides of both stimulatory and inhibitory effects into the hypophyseal portal system to control the GH synthesis and release by somatotropic cells. In this concern, GH-releasing hormone

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(GHRH)-expressing neurons stimulate GH release, whereas somatostatin (SST) expresses neurons that inhibit GH secretion (1). The most important action of GH is to promote tissue growth, which includes regulating cell divisions, regeneration, and proliferation of different tissues (2).

The tissue repair process (wound healing) is regulated by the communication of growth factors with specific receptors; these interactions promote cell migration, activate epithelialization, and angiogenesis, and enhance matrix formation and also the remodelling of the injured area. TGF-β is one of the growth factor families being explored for wound healing (3). Growth factors are important in healing because they can help in the physiological process of wound healing (4). The success and enhancement of the wound healing process depend on growth factors, chemokines, and cytokines which participate in a multifaceted integration of signals that organize cellular processes. These factors are active polypeptides that regulate the growth, differentiation, and metabolism of a specific cell (5).

IGF-1 can encourage the activity of wound cells, so it plays a vital role in tissue repair. It is released locally by wound cells, and IGF-1 derived from the liver is also available at a high concentration in the blood circulation, but their influences on the wound healing process are unknown (6).

TGF-β is a group of growth factors that play a role in a variety of cellular processes. The isoforms of TGF-β "TGF-β1, -β2, -β3" are released as inert, dormant precursors that must be activated before binding to the TGF-β receptors. In wound healing, all three isoforms are present (7).

This study was carried out to evaluate the effect of topical growth hormone on facial skin wound healing and to study serum changes of both IGF-1 and TGF-β during the wound healing period.

Material and Methods

Experimental animals: In this case-control experimental study, thirty healthy black and white male rabbits (age 11-13 months old; the weight of 1.25-1.5 Kg) were purchased from the local market. Animals were permanently kept indoors at a temperature of 20±°C with a photoperiod cycle of light and dark under the standards required of animal housing with applicable guidelines for the care and use of animals. The animals were fed a consistent diet, had access to water and were subjected to daily clinical examination daily by a veterinarian until they were euthanized.

Animals grouping: The animals were distributed randomly into two groups according to euthanizing day:

Group A: included 15 rabbits euthanized on day 7 after surgical procedure.

Group B: included 15 rabbits euthanized on day14 after the surgical procedure.

Each group is subdivided into 3 groups (5 rabbits/subgroup)

Group I (negative control group): the rabbits are not undergoing surgical procedures or treatment.

Group II (positive control group): the rabbits in this group undergo the surgical procedure without any medication.

Group III (treatment group): the rabbits in this group undergo a surgical procedure, and each rabbit, was treated with 0.1ml [contain 1.2mg/3.6 IU] of growth hormone as a subcutaneous injection at the surgical area, the injection process was carried out every other day till the euthanizing day. The drug used in this study is listed in Table 1.

Table 1. Drug-used in the present study.

Drug used	Purpose of use	Trade Name	Origin	Description
Growth hormone	Tested drug	Genotropin	Pfizer, USA	prefilled pen contain 36 IU (12 mg)/ml
Xylazine	Sedation and Muscle relaxant	Xylazine	Interchemie, Holland	2% solution (20 mg/ml)
Ketamine	General anaesthesia	Ketamine	Dutch Farm, Holland	1% solution (100 mg/ml)

Surgical procedure: On the first day of the study, each rabbit in both group II (positive control) and group III (treatment) was anaesthetized by giving an IM dose of a mixture of xylazine hydrochloride and ketamine hydrochloride at 5, 50 mg/Kg respectively. The forehead of the animal was shaved, washed with water, and disinfected with povidone-iodine solutions to be ready for surgery.

A circle of 1 cm in diameter was measured using a ruler on the skin of each animal's forehead, and full-thickness circular excision was carefully done. After that, the animals in group III (treatment) were treated topically with 0.1ml [contain 1.2mg/3.6 IU] of growth hormone (8) as a subcutaneous injection in the surgical area, the injection process was carried out every other day till the euthanizing day (7 days' group A and 14 days' group B).

Blood samples collection: Fresh blood was collected from each rabbit at the time of euthanizing, for analysis of biochemical parameters, and the serum was separated by centrifuge and stored at (-20°C) till analysis by using Rabbits Transforming Growth Factor β, TGF-β ELISA kit (Bioassay Technology Laboratory, Cat.No. E0133Rb) and IGF-1 CLIA kit (MAGLUMI®).

Statistical Analysis

The variance between five experimental groups was statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan test. p values ≤ 0.01were considered significant (9).

Results

Clinical Wound healing: Macroscopic view of wound-healing progression in experimental groups shown in Figure (1). The observation is a clinical estimation of the wound only.

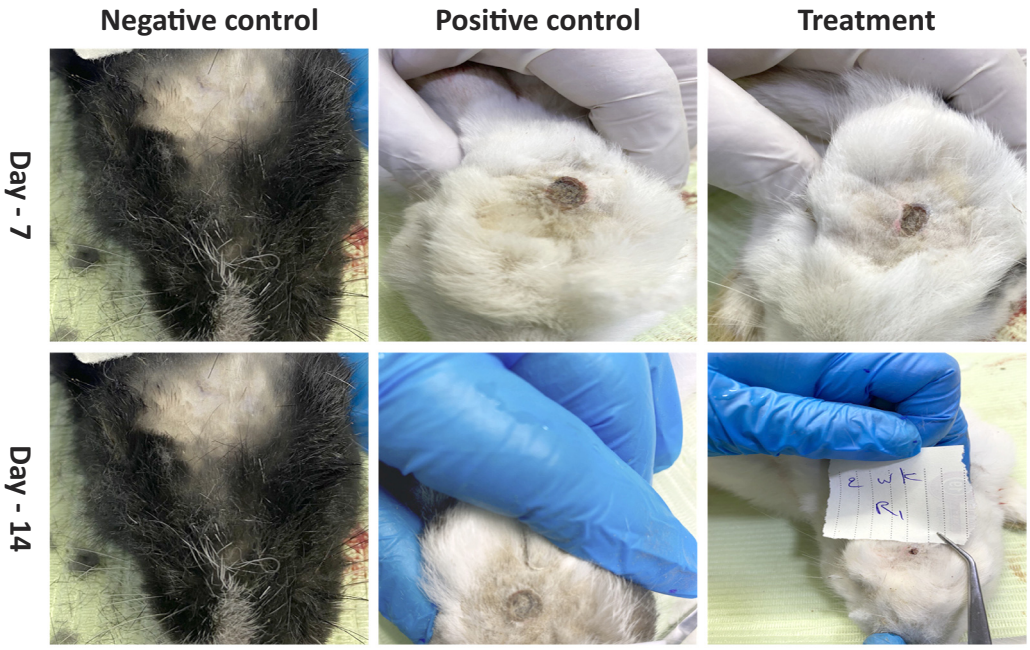

Figure 1. Gross appearance of wound-healing progression in experimental groups.

Comparisons of TGF-β and IGF among all study groups.

The first week: A highly significant difference among all study groups in serum TGF-β and IGF as shown in Table (2).

Table 2. Comparison of serum TGF-β and IGF among study groups at the end of the first week.

Test	S.O.V.	Sum of Squares	d.f.	Mean Square	F	Sig.
TGF (ng\L)	Between Groups	60201.845	2	30100.923	15.719	0.001**
	Within Groups	22978.803	12	1914.900		
	Total	83180.648	14			
IGF-1 (ng\ml)	Between Groups	3530.497	2	1765.249	585.813	0.001**
	Within Groups	36.160	12	3.013		
	Total	3566.657	14			

** Highly Significant at P ≤ 0.01
d.f (degree of freedom) number of independent observations or measurements that can be made in order to calculate some statistics
S.O.V(source of variance) is a measurement of the spread between numbers in a data set.
Sum of Squares used in to determine the dispersion of data points from their mean value.

When comparing the treatment group to the other groups. The serum TGF-β at the end of the first week showed a significant increase in TGF-β levels. There is no significant difference between the two other control groups. Figure (2).

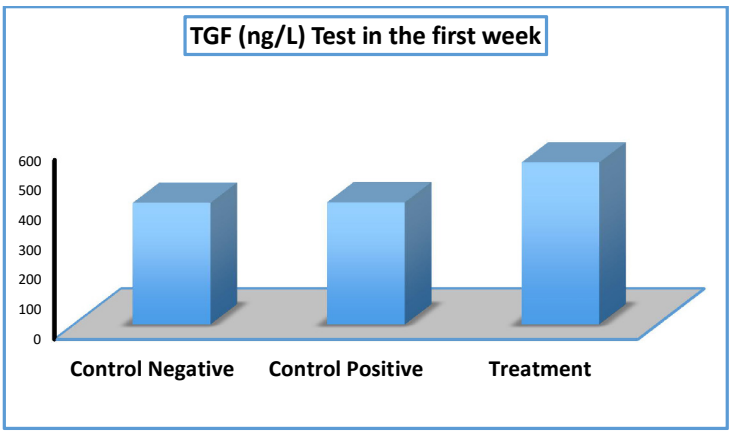

Figure 2. The TGF-β concentration among study groups at the end of the first week.

A significant difference in IGF levels among all study groups was shown in the serum IGF levels at the end of the first-week Figure (3).

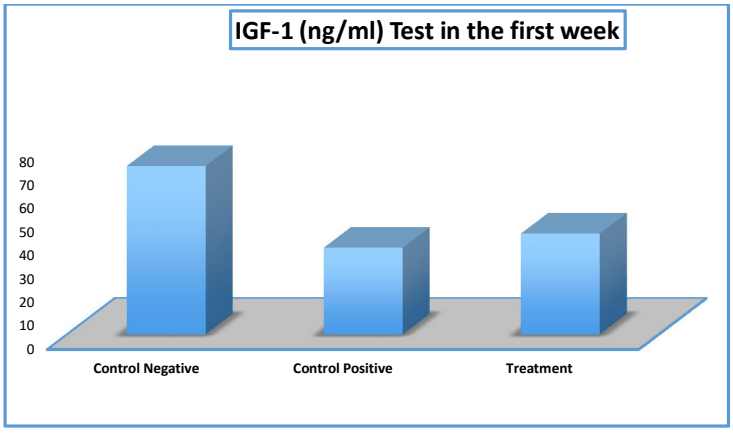

Figure 3. The serum IGF levels among study groups at the end of the first week.

The second week: The serum TGF-β and IGF showed a highly significant difference as shown in Table 3.

Table 3. Comparison of serum TGF-β and IGF among study groups at the end of the second week.

Test	S.O.V.	Sum of Squares	d.f.	Mean Square	F	Sig.
TGF	Between Groups	73801.659	2	36900.830	31.500	0.001**
	Within Groups	14057.624	12	1171.469		
	Total	87859.283	14			
IGF-1	Between Groups	1552.529	2	776.265	82.561	0.001**
	Within Groups	112.828	12	9.402		
	Total	1665.357	14			

** Highly Significant at P ≤ 0.01

The serum TGF-β at the end of the second week showed a significant increase in TGF-β level in the treatment group when compared to the other groups. There is no significant difference between the two other control groups (Figure 4).

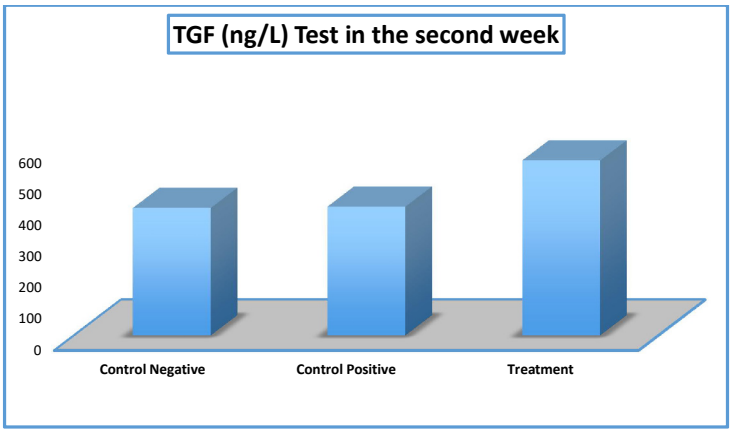

Figure 4. The TGF-β concentration among study groups at the end of the second week.

The serum IGF at the end of the second week of the study showed a significant difference in IGF levels between all study groups (Figure 5).

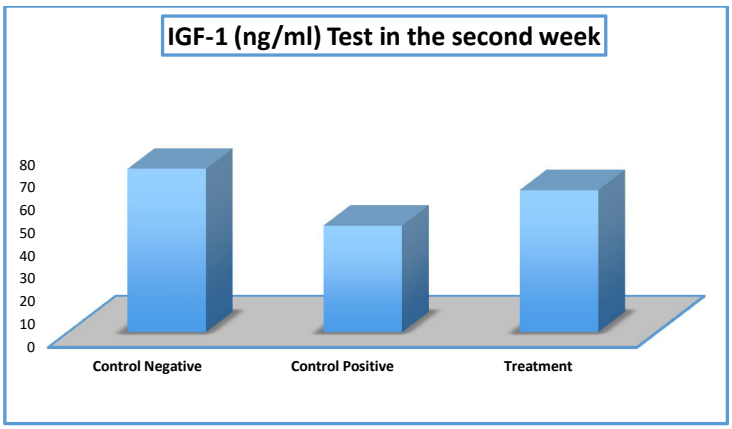

Figure 5. The IGF measurements among study groups at the end of the second week.

Discussion

Skin injuries can challenge healthcare professionals. The healing process starts at the time of injury and includes many complex overlapping stages, extending from hemostasis to tissue regeneration (10). The prognosis of wound healing depends on many factors such as growth factors, cytokines, and chemokines that elaborate on a complex of signals which organize cellular processes. These different biological active polypeptides influence the growth, differentiation, and metabolism of cells. They can affect cell activity and behaviour by binding to particular cell surface receptors or by autocrine, paracrine, juxtacrine, and endocrine mechanisms (11).

Human GH acts as an anabolic agent in different cell types by stimulating growth and mitosis, they act both directly and indirectly through the "insulin-like growth factor (IGF)-I" (12, 13). Results of this study showed that topical GH appears to enhance tissue regeneration which is per the previous study at which exogenous GH can help in tissue regeneration and healing in patients and animals with severe burns, leading to better results after topical treatment with drug vehicles in skin investigations (14). GH can increase collagen deposition and influences epithelial proliferation and migration and also affects fibroblast secretion and deposition of the extracellular matrix after injury (15).

Results of the current study showed a significant increase in TGF-β level in the treatment group when compared to the other groups which can be due to GH stimulation for the release of TGF-β, similar to its effect on epidermal growth factor, vascular endothelial growth factor, and fibroblast growth factor. Moreover, IGF-I and II mRNAs are modulated during the wound healing process. Human wound fluids contain the highest amount of IGF-I within 24 h after injury, and they return to baseline once healing is complete (16).

In the present study, the concentration of IGF-1 fluctuated within the study period. On day seven, there was a decrease in concentration, while at the end of the study period it increases to almost the normal level which agrees with the fact that IGF-I concentrations fluctuated during GH treatment. A significant decrease in IGF level after skin injury is recorded, which is per previous studies stating that IGF-I is known to fall with acute illness (17, 18). Our results also agree with Rorison and co-workers found that plasma levels of TGF-β1 rapidly increase to significantly higher levels and then rapidly decline in patients with good post-burn healing in the first two weeks post-injury (19).

During the study period, the level of TGF-β1 rises; the rise is most noticeable in the second week. TGF-β1 is released in high amounts from platelets shortly after injury (20). This initial surge of active TGF-β1 from platelets acts as a chemoattractant for neutrophils, macrophages, and fibroblasts, and these cell types further increase TGF-β1 levels in various cell types. In addition to active forms, latent TGF-β is also formed and sequestered inside the wound matrix, permitting proteolytic enzymes to release it over time. This mixture of cellular sources and temporary storage ensures that TGF-β is available at all times during the wound healing process (21).

Every cells in human body secretes a plethora of cytokines and growth factors, some of these factors involved in various biological functions; including proliferation, differentiation, cell adhesion, and immunomodulation (22-25). Main factors involved in wound healing are: Epidermal growth factor (EGF), Transforming growth factors a and B (TGF-b), Fibroblast growth factor (FGF), Insulin-like growth factor-I (IGF-I), Platelet-derived growth factor (PDGF) (26-29). The importance of IGF and TGF in wound healing related to the following reasons (30-36):

1. These are master growth hormones in regulation of wound healing.
2. IGF works in combination of PDGF so measurement of one is same as measuring both of them. Additionally, PDGF is important only in the initial stages of wound for platelet plug formation.
3. TGF-b stimulates or inhibits the growth of many cell types depending upon the presence of other growth factors, therefore its measurement should be in the top list.
4. FGF and EGF are interrelated with TGF-b so measurement of TGF is much more important that FGF and EGF. Moreover, FGF exists in different splice variant which make it difficult to find specific kits for measurement. Similarly, The epidermal growth factor (EGF) family of mitogens comprises several members, including EGF, heparin-binding EGF (HB-EGF), amphiregulin, epiregulin, betacellulin, neuregulins.

Conclusions

The treatment interventions of GH for facial skin wound healing have made great advances from repairing processes to regenerative ones, but the challenge of the ideal method remains. GH has a significant effect on wound healing and has not been investigated enough. Further study in skin wound healing mechanisms and novel methodologies to enhance it will lead to more clinically effective products in treating deep dermal injury and attenuating scar formation. This study indicates that topical growth hormone may have a powerful effect on skin wound healing enhancement supplementary studies are needed to confirm our results.

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Conflict of Interest

The authors declare that no conflict of interest exists for this research.

Adherence to Ethical Standards

The current study was carried out in an animal house and scientific laboratories after approval by the Research Ethics Committee and Scientific Committee/Department of Dental Basic Science/College of the Dentistry/University of Mosul. The approval letter Code is UoM.Dent/A.L.16/22.

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