The Efficacy of Gallium-Aluminum-Arsenide Laser on Skin Wound Healing in Guinea Pigs

Mohamed E.A. Mostafa
Anatomy Department, Faculty of Medicine, Cairo University

ABSTRACT

Background: Skin wounds have serious human consequences including pain, lost work days and marked reduction in quality of life. Many studies were conducted to seek new therapeutic methods for resolving or minimizing failures of tissue repair including therapeutic ultrasound, electrical stimulation and low power laser light. The laser technique was used in the treatment of wounded tissue by inducing vascular dilatation, collagen synthesis and by increasing tissue oxygenation. The suitable energy density of laser is still of debate.

Aim of the Work: This study was conducted to evaluate the effect of gallium-aluminum-arsenide laser of different energy densities on the skin healing process.

Material and Methods: This study was carried out on 45 adult guinea pigs. In each animal two full-thickness wounds (A & B) were induced with surface area of 200 mm² each. Wound A was irradiated by GaAlAs laser daily at a wavelength of 905 nm and wound B was used as a sham control. The animals were randomly divided into three equal groups (I, II & III) which were exposed to laser energy densities of 4, 30 & 60 J/cm² respectively. Each group was subdivided into 3 equal subgroups (5 animals each) a, b & c and received laser daily for one, two & three weeks respectively. The wounds’ surface areas (WSA) were measured. The animals were sacrificed one day after last laser application and pieces of unwounded skin as a control and skin fragments at the different wounds edges were collected. They were subjected to Hematoxylin & Eosin and Mallory Trichrome techniques and examined by light microscopy.

Results: There was very highly significant decrease in WSA and enhancement of healing in laser-treated wounds compared to the sham control ones.

Conclusion: Treatment with GaAlAs laser of different intensities was able to induce several modifications that accelerated the wound healing with best results obtained for energy density of 60 J/cm².

Key Words: laser- gallium- skin wounds- healing.

Corresponding Author: Dr. Mohamed Ehab Al-din Mostafa, Anatomy Department, Faculty of Medicine, Cairo University, Egypt, Email: dr_ihab_anatomy70@yahoo.com, Mobile: 0122162752.

INTRODUCTION

The skin is the largest organ of the body and represents a barrier to the environment. It protects the body from water loss and penetration of harmful substances. It can be damaged by mechanical, thermal or ultraviolet rays and different diseases such as psoriasis and atopic dermatitis (Lipozencic & Wolf, 2007).

Skin wounds are ranked second as the most common cause of work absenteeism. They are present in approximately 10% of hospitalized patients and 20% of bedridden patients treated at home, with consequent high financial costs (Blanes et al., 2004). Skin wounds have serious human consequences including pain, lost work days and marked reduction in quality of life. Several putative therapeutic approaches for wounds have been proposed including the use of antiseptics, growth factors, pressurized oxygen and physical therapy modalities (Houghton et al., 2003). Injury to the skin triggers the healing process which involves a cascade of events including inflammatory cell migration, granulation tissue synthesis, collagen and proteoglycan deposition and scar maturation associated with intense remodeling (Reddy, 2004). This may occur due...
to release of cytokines, growth factors, some hormones and several low-molecular weight substances from plasma or activated platelets (Medrado et al., 2003; Meirelles et al., 2008). Morphological and biochemical studies in humans have revealed a sophisticated mechanism for skin wound healing including replacement of the affected subcutaneous tissue with a new matrix and re-epithelization. This eventually leads to total or partial restoration of the injured area. Various aspects of this complex process have attracted the attention of researchers over the years, particularly the factors which may hinder it (Werner & Grose, 2003).

The kinetics of the wound healing process is influenced by endogenous and exogenous factors (Fu et al., 2007). The analysis of this kinetics is important for therapy control and for the development of efficient drugs and cosmetic products which stimulate wound healing (Alborova et al., 2007).

The most important repair failures are those which occur in the initial stages which lead to accentuation of edema, reduced vascular proliferation and decrease of the cell elements such as leukocytes, macrophages and fibroblasts. Such alterations give rise to low collagen synthesis and increase risk of infections (Carvalho et al., 2003).

Most therapeutic interventions were designed to facilitate the wound healing process by protecting wounds to prevent complications (Posten et al., 2005). Currently, studies were conducted to seek new therapeutic methods for resolving or minimizing failures of tissue repair. Whatever the type of wound, many experimental trials were set up to confirm the benefits of therapeutic ultrasound, low power laser light and electrical stimulation (Demir et al., 2004).

Laser has been used in many medical applications (Baxter, 2002). Experimental low intensity laser was used in many clinical trials including painful osteoarthritis of the knee (Gur et al., 2003a), chronic low back pain (Gur et al., 2003b), repair of medial collateral ligament of the knee (Fung et al., 2003) and management of chronic myofascial pain in the neck (Gur et al., 2004). It was used to improve the appearance of photodamaged skin (Jeffrey et al., 2004). Also, laser resulted in significant quantitative improvements in skin topography in patients with mild to moderate atrophic acne scars (Friedman et al., 2004). Moreover, laser was used for healing of burn (Meirelles et al., 2008; Ezzati et al., 2009).

The laser technique is one of the methods used to hasten the recovery of tissue functionality in the treatment of wounded tissues. The efficacy of laser procedures in the healing process is due to induction of vessel dilatation, collagen synthesis and by increase of tissue oxygenation (Ihsan, 2005). All phases of the healing process are positively affected by laser treatment, for which wavelength and energy density are crucial factors for successful treatment (Enwemeka et al., 2004).

The frequency of the laser light, as well as the type of tissue being irradiated, determines the depth to which light penetrates (Webb & Dyson, 2003). Laser therapy with a wavelength between 600 and 1300 nm optimizes the depth of penetration in human tissue at 1 to 4 mm and is therefore most frequently used in the clinical settings. It is capable of influencing the collagen percentage in skin wounds by increasing the mean quantity of collagen fibers (Camillo De Carvalho et al., 2006).

Laser light with shorter wavelength such as red light, produced by the helium-neon laser, promotes collagen formation and restores the baseline cellularity increasing the skin healing process; however, it penetrates human skin very superficially (Medrado et al., 2003; Pugliese et al., 2003; Maiya et al., 2005; Gonçalves et al., 2007). Laser light with longer wavelength, such as the infrared produced by the gallium-arsenide (GaAs) or gallium-aluminum-arsenide (GaAlAs) laser, penetrates deeper (Ng et al., 2004).

Demir et al. (2004) reported that laser treatment has an antibacterial effect by inhibiting proliferation of bacteria in cultures and stimulating the phagocytic activity of leukocytes in vitro. Also, Reddy (2004) found that GaAs laser was effective in healing of wounds in diabetic and nondiabetic rats. Moreover, Posten et al. (2005) reported that GaAlAs laser (904 nm) promotes dilatation of the irradiated arterioles, followed by an increase in arteriolar blood flow. Furthermore, Gonçalves et al. (2010) described efficient collagen deposition after GaAs and GaAlAs laser promoting skin wound healing.
On the other hand, Gul et al. (2008) tested helium-neon laser of 1 and 3 J/cm² on healing of experimental wound in rabbits. They found no difference of healing rate between control and low dose group. However, they found a significant improvement after use of high dose laser. Moreover, Rodrigo et al. (2009) examined the repair process of experimental wounds on the back of rats after aluminium gallium indium phosphide & GaAlAs laser therapy of wave length 685 and 830 nm and energy density of 10 J/cm² and 20 J/cm², respectively. They found that the healing was more advanced in the wound located farthest from the point of laser application.

The identification of adequate healing-promoting techniques is highly beneficial for patient care and immediate cost reduction. This is to establish the modality which promotes faster, safer and infection-free circumstances thus reducing the healing and recovery time and improving the quality of life of the patients at a lower cost. It has been settled that the laser is useful in wound healing but the suitable energy density of laser is still of debate. The investigations of the healing effect of different laser modalities and determining their action on collagen fibers throughout the healing process are of fundamental importance. Therefore, the aim of the current study was to determine the effect of gallium-arsenide and gallium-aluminium-arsenide of different energy densities on the skin healing processes.

MATERIALS AND METHODS

Laser device
Portable Lasermed 2100, (MEDICAL ITALIA, Physiotherapy & Rehabilitation Division, Italy).

Experimental animals
This study was carried out on 45 adult male guinea pigs, weighing 390-640 g. Their age ranged from eight to eleven months. They were obtained from the Animal House, Faculty of Medicine, Cairo University. The animals were housed in stainless steel cages under normal hygienic conditions and allowed water and food (laboratory chow) ad libitum throughout the study.

The animals were anesthetized with 50 mg/kg ketamine hydrochloride injected intramuscularly along with 5 mg/kg diazepam (Ezzati et al., 2009). The hair on both dorsolateral regions was shaved and antisepcticised with povidon-iodine (betadine) according to Mehmandoust et al. (2007) and Talebi et al. (2008). Following the sterilization, two equal circles of 12mm diameter were marked on the shaved area. Using a sterilized surgical scalpel, two full-thickness round excisional wounds (A & B) were performed (Fig.1). The wounds were of 16mm diameter (200 mm² surface area) after excision due to skin retraction (Mehmandoust et al., 2008; Gonçalves et al., 2010). The depth of the surgical incision was controlled by removing the epithelial tissue until the dorsal muscular fascia was exposed (Camillo De Carvalho et al., 2006). The wounds were cleaned with 0.9% saline and betadine solution once a day in the morning (Demir et al., 2004). In each animal, wound A was submitted to laser application and wound B was used as sham control. All therapies were initiated 6 h after surgery and repeated daily for the 21 days of the experiment. The animals were randomly divided into three groups:

- **Group I:** 15 animals, their wounds A were submitted to GaAlAs laser radiation at a wave length of 905 nm and energy density of 4 J/cm² (0.8 J/mm² per point). This group was subdivided into three equal subgroups (five animals each):
  - **Subgroup I-a:** animals were submitted to laser radiation for 7 days then sacrificed and skin from their wounds were excised and examined.
  - **Subgroup I-b:** animals, were submitted to laser radiation for 14 days then sacrificed and skin from their wounds were excised and examined.
  - **Subgroup I-c:** animals, were submitted to laser radiation for 21 days then sacrificed and skin from their wounds were excised and examined (Camillo De Carvalho et al., 2006; Mehmandoust et al., 2007; Talebi et al., 2008; Ebrahimian et al., 2009; Mortazavi et al., 2009).

- **Group II:** 15 animals, their wounds A were submitted to GaAlAs laser at a wave length of 905 nm and energy density of 30 J/cm² (6 J/mm² per point). This group was divided into three equal subgroups: II-a, II-b & II-c and were submitted to laser radiation and examined like group I.

- **Group III:** 15 animals, their wounds A were submitted to GaAlAs laser at a wave length of 905 nm and energy density of 60
J/cm² (12 J/mm² per point). This group was divided into three equal subgroups: III-a, III-b & III-c and were submitted to laser radiation and examined like group I.

The wounds (A & B) were observed daily and tracing method was performed one week, two weeks and three weeks to measure the unhealed wound surface area (WSA) and percentage of total wound healing.

**Histological Examination**

Biopsies were taken weekly from the wound edges to evaluate the healing process (Gul et al., 2008). The mean and standard deviation were calculated for WSA and post-hoc test was done to determine the level of significance between the different subgroups.

Sacrifice was done one day after the last laser application for each group by inhalation of chloroform (Mehmandoust et al., 2007). Pieces of unwounded skin (control) and skin pieces at the different wound edges were collected. They were dehydrated and embedded in paraffin. Sections of 5 μm-thickness were cut, subjected to Hematoxylin & Eosin and Mallory trichrome techniques and examined by light microscopy (Bancroft & Gamble, 2002).

Data were statistically described in terms of mean ± standard deviation (± SD), frequencies (number of cases) and percentages when appropriate. Comparison of quantitative variables between the study groups was done using Kruskal Wallis analysis of variance (ANOVA) test with Conover Inman test for independent samples as posthoc multiple 2-group comparisons. A probability values (p values) < 0.05, < 0.01 and < 0.001 were considered statistically significant, highly significant and very highly significant, respectively. All statistical calculations were done using computer programs Microsoft Excel 2003 (Microsoft Corporation, NY, USA) and Stats Direct statistical software version 2.7.2 for MS Windows, Stats Direct Ltd., Cheshire, UK (Mould, 1989).

**RESULTS**

**The wound surface area**

After one week, the wound surface area (WSA) of wound B in all examined animal groups was of mean value 158.8 ± 16.15 mm². However, the mean WSA of wound A in subgroup I-a was 123.2 ± 6.27 mm². The mean WSA of wound A in subgroup II-a was 107.4 ± 7.33 mm². The mean WSA of wound A in subgroup III-a was 100.4 ± 3.97 mm².

Statistical analysis revealed very highly significant decrease in the WSA of wound A in subgroups I-a, II-a & III-a (P< 0.001) compared to wound B in the same subgroups. There was also a very highly significant decrease (P< 0.001) of wound A in subgroup II-a compared with subgroup I-a and in subgroup III-a versus subgroup I-a while there was a highly significant decrease (P= 0.003) in WSA of subgroup III-a compared with subgroups II-a (Table 1; Graph 1).

After two weeks, the mean WSA value of wound B in all groups was 84 ± 4.74 mm². The mean WSA of wound A in subgroup I-b was 47.2 ± 6.22 mm², subgroup II-b was 30.6 ± 5.32 mm² and subgroup III-b was 12.8 ± 0.84 mm². The surface area of wound A showed a very highly significant decrease (P< 0.001) compared to wound B in all subgroups. Comparing surface area of wound A in different subgroups, there was a very highly significant decrease (P< 0.001) of subgroup II-b versus I-b, of subgroups III-b versus I-b and of subgroup III-b versus subgroup II-b (Table 1; Graph 1).

After three weeks, there was complete closure of wound A in all animals in subgroups II-c and III-c. The mean WSA of wound B was 7.2 ± 0.84 mm². The WSA mean in wound A in subgroup I-c was 1.4 ± 0.55 mm². There was a very highly significant decrease (P< 0.001) in surface area of wound A in subgroup I-c compared with wound B in the same subgroup (Table 1; Graph 1).

The mean percentage of WSA closure after one week in wound B in all subgroups was 19.94 ± 3.16 while, that of wound A in subgroup I-a was 38.05 ± 1.70, in subgroup II-a was 46.31± 2.23 and in subgroup III-a was 49.80+ 1.51. There was a very highly significant increase (P< 0.0001) in percentage of WSA closure of wound A of subgroups I-a, II-a & III-a compared to wound B. There was also a very highly significant increase (P< 0.0001) of subgroup II-a versus I-a and in subgroup III-a compared to I-a. While, subgroup III-a showed a highly significant increase (P= 0.001) compared to subgroup II-a (Table 2).
After two weeks, the mean percentage of WSA closure in wound B was 58.03 ± 1.76 and that of wound A was 76.13 ± 2.22, 84.70 ± 2.22 and 93.61 ± 0.32 for subgroups I-b, II-b and III-b, respectively. Statistical results revealed a very highly significant increase (P< 0.0001) of percentage of WSA closure of wound A of all subgroups compared to that of B. There was also a very highly significant increase (P= 0.0009) of subgroup II-b versus I-b, in subgroup III-b compared to I-b and subgroup III-b compared to subgroup II-b (Table 2).

The mean percentage of WSA closure after three week in wound B was 96.40 ± 0.36 and in wound A in I-c was 99.30 ± 0.21. However, there was complete closure (100%) in wound A in subgroups II-c and III-c. Statistically, there was a very highly significant increase in percentage of WSA closure in wound A in subgroups I-c, II-c and III-c compared to that of wound B (P= 0.0054, 0.0049 and 0.0049, respectively). Also, there was a significant increase (P= 0.0274) of subgroups III-c and II-c compared to I-c while there was no significant difference (P > 0.9999) between the subgroups II-c and III-c (Table 2).

The intact skin showed normal histological structure of the epidermis and dermis. The epidermis (epithelium) is formed of many layers of epithelial cells covered by a thin horny layer. It contains many short hair follicles. The dermis is formed of papillary layer which interdigitates with epidermis and reticular layer which contains many long hair follicles, sebaceous glands (Figs. 2, 3) and normal amount of clear organized collagenous fibers (Fig. 3).

After one week, wound B showed a large unepithelialized depressed area (absence of epidermis) with an area of unhealed dermis. There was complete absence of the hair follicles (Figs. 4, 5).There was a minimal amount of well formed collagenous fibers in the healing dermis (Fig. 5). The wound A in subgroup I-a showed a large unepithelialized depressed area (absence of epidermis). However, epithelialization started to appear at the periphery of the wound with short hair follicles in the healthy area only (Fig.6). There was a reasonable amount of well arranged collagenous fibers (Fig.7). The unepithelialized area in subgroup II-a became reduced compared to subgroup I-a. The epidermis started to cover the wound area with appearance of few long hair follicles and sebaceous glands in the dermis(Fig.8). There was a reasonable amount of well arranged collagenous fibers (Fig.9). Subgroup III-a showed a smaller area of absence of epidermis compared to that of subgroup II-a. There were many long hair follicles and sebaceous glands in the dermis (Fig.10). There was a moderate amount of well arranged collagenous fibers (Fig.11).

After two weeks, wound B showed large unhealed area with absence of epidermis (Figs.12, 13). The collagenous fibers became moderate in amount (Fig.13). The wound A area in subgroup Ib was still showing a small depressed non-completely epithelialized area. However, there were many long hair follicles and sebaceous glands denoting a nearly healed dermis (Figs.14, 15). There was a moderate amount of collagenous fibers (Fig.15). In subgroup II-b there was nearly complete healing of the wound area apart from a small unepithelialized area. There were many long hair follicles and sebaceous glands. The papillary layer of the dermis was well developed in the healed area (Figs.16, 17). The collagenous fibers in the dermis were abundant and well organized (Fig.17).

Subgroup III-b showed nearly complete healing and epithelialization of the wound area apart from a very small unepithelialized area. There were many long hair follicles and sebaceous glands. The papillary layer of the dermis was well developed (Figs. 18, 19). The collagenous fibers in the dermis were abundant and well organized (Fig.19).

After three weeks, wound B showed an unepithelialized area. The dermis showed long hair follicles and sebaceous glands (Figs. 20, 21). The dermis showed moderate amount of well organized collagenous fibers (Fig. 21).

The wound A in subgroup I-c showed nearly complete healing of the skin. However, there was a small area in the center of the wound which showed a small layer of epithelium (not completely healed epidermis). The dermis was well formed with many long hair follicles and sebaceous glands (Figs. 22, 23). The collagenous fibers in the dermis were abundant and well organized (Fig.23). Subgroups II-c & III-c showed complete healing of the skin which retained its normal structure (Figs. 24, 25).
**THE EFFICACY OF GALLIUM-ALUMINUM-ARSENIDE LASER ON SKIN WOUND HEALING IN GUINEA PIGS**

**Table 1:** The wound surface area in mm² in different subgroups.

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<tr>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
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<tr>
<td>Wound</td>
<td>A Ia</td>
<td>A Ib</td>
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<td></td>
<td>M=107.4±7.33 &amp;&lt;.001*** &amp;&lt;.001*** &amp;&lt;.001***</td>
<td>M=100.4±3.97 &amp;&lt;.001*** &amp;&lt;.001*** &amp;&lt;.001***</td>
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<tr>
<td></td>
<td>B M=158.8±16.15</td>
<td>A Ib</td>
</tr>
<tr>
<td></td>
<td>M=123.2±6.27</td>
<td>A Ib</td>
</tr>
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*: Significant (P<0.05). **: Highly significant (P<0.01). ***: Very highly significant (P<0.001).

**Table 2:** The mean percentage of wound surface area closure in different subgroups.

<table>
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<th>Week 1</th>
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<tr>
<td>Wound</td>
<td>A Ia</td>
<td>A Ib</td>
</tr>
<tr>
<td></td>
<td>M=48.31±2.23 &amp;&lt;.001***</td>
<td>M=49.80±1.51 &amp;&lt;.001***</td>
</tr>
<tr>
<td></td>
<td>B M=19.94±1.36</td>
<td>A Ib</td>
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<tr>
<td></td>
<td>M=19.94±1.36</td>
<td>A Ib</td>
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*: Significant (P<0.05). **: Highly significant (P<0.01). ***: Very highly significant (P<0.001).

**Graph 1:** A histogram showing the relations of WSA in wound B and wound A in different subgroups.
Fig. 1: A photograph of the dorsolateral part of a guinea pig showing two surgical wounds (A & B) of diameter about 16 mm.

Fig. 2: A photomicrograph of a section of intact skin of guinea pig showing the normal histological structure of the epidermis and dermis. The epidermis (E) is formed of many layers of epithelial cells covered by a thin horny layer (*). It contains many short hair follicles (F1). The dermis is formed of papillary layer (P) which interdigitates with epidermis and reticular layer (R) which contains many long hair follicles (F2) and sebaceous glands (S). Hx. & E.; X 100

Fig. 3: A photomicrograph of a section of intact skin of guinea pig showing the normal histological structure of the epidermis and dermis. The epidermis (E) is formed of many layers of epithelial cells covered by a thin horny layer (*). It contains many short hair follicles (F1). The dermis is formed of papillary layer (P) which interdigitates with epidermis and reticular layer (R) which contains many long hair follicles (F2), sebaceous glands (S) and normal amount of clear organized collagenous fibers (arrow). Mallory Trichrome; X 100

Fig. 4: A photomicrograph of a section in skin of the wound B after one week showing a large unepithelialized depressed area. The dermis (D) shows an unhealed area (arrow head). There is complete absence of the hair follicles. Hx. & E.; X 100

Fig. 5: A photomicrograph of a section in skin of the wound B after one week showing a large area of complete absence of epidermis. The dermis (D) shows an unhealed area (arrow head) and minimal amount of well formed collagenous fibers (arrow). There is complete absence of the hair follicles. Mallory Trichrome; X 100

Fig. 6: A photomicrograph of a section in skin of the wound A in subgroup Ia showing an area of absence of epidermis (arrow head) on the dermis (D). The epidermis (E) with short hair follicles (F1) starts to cover the edge of the wound area. Long hair follicles (F2) can be seen in the deep part of the repairing area only while there are no hair follicles observed in the superficial layer of dermis. Hx. & E.; X 100
THE EFFICACY OF GALLIUM-ALUMINUM-ARSENIDE LASER ON SKIN WOUND HEALING IN GUINEA PIGS

Fig. 7: A photomicrograph of a section in skin of the wound A in subgroup Ia showing a large area of absent epidermis (arrow head). The epidermis (E) starts to cover the wound periphery. There is a reasonable amount of well arranged collagenous fibers (arrow) in the dermis (D). Blood clot (C) can be seen. Mallory Trichrome; X 100

Fig. 8: A photomicrograph of a section in skin of the wound A in subgroup IIa showing an area of absence of epidermis (arrow head). The epidermis (E) starts to cover the wound area on both sides. The dermis (D) starts to develop with appearance of few long hair follicles (F2) and sebaceous glands (S). Hx. & E.; X 100

Fig. 9: A photomicrograph of a section in skin of the wound A in subgroup IIa showing an area of absence of epidermis (arrow head). The epidermis (E) starts to cover the wound area on both sides. The dermis (D) starts to develop with appearance of few long hair follicles (F2), sebaceous glands (S) and a reasonable amount of well arranged collagenous fibers (arrow). Mallory Trichrome; X 100

Fig. 10: A photomicrograph of a section in skin of the wound A in subgroup IIIa showing a wound area (arrow head) with absence of epidermis (E). The dermis (D) is well developed with many long hair follicles (F2) and sebaceous glands (S). Hx. & E.; X 100

Fig. 11: A photomicrograph of a section in skin of the wound A in subgroup IIIa showing a wound area (arrow head) with absence of epidermis (E). The dermis (D) is well developed with many long hair follicles (F2) and sebaceous glands (S). There is a moderate amount of collagenous fibers (arrow). Mallory Trichrome; X 100

Fig. 12: A photomicrograph of a section in skin of the wound B after two weeks of surgery. There is a large area of complete absence of epidermis (arrow head) with blood clot (C). The dermis (D) can be observed. Hx. & E.; X 100
Fig. 13: A photomicrograph of a section in skin of the wound B after two weeks of surgery. There is large unhealed area (arrow head). There is a moderate amount of collagenous fibers (arrow) which start to be well organized in the dermis (D). Mallory Trichrome; X 100

Fig. 14: A photomicrograph of a section in skin of the wound A in subgroup Ib showing a superficial unhealed area (arrow head) with absence of epidermis (E). The dermis (D) is moderately developed with many long hair follicles (F2) and sebaceous glands (S). Hx. & E.; X 100

Fig. 15: A photomicrograph of a section in skin of the wound A in subgroup Ib showing a superficial unhealed area (arrow head) with absence of epidermis (E). The dermis (D) is moderately developed with many long hair follicles (F2) and sebaceous glands (S). Moderate amount of collagenous fibers (arrow) can be seen. Mallory Trichrome; X 100

Fig. 16: A photomicrograph of a section in skin of the wound A in subgroup IIb showing a small superficial unhealed area with absence (arrow head) of epidermis (E). The papillary layer (P) of the dermis (D) is well developed. Many long hair follicles (F2) and sebaceous glands (S) are observed. Hx. & E.; X 100

Fig. 17: A photomicrograph of a section in skin of the wound A in subgroup IIb showing a small superficial unhealed area with absence (arrow head) of epidermis (E). The papillary layer (P) of the dermis is well developed. Many long hair follicles (F2), sebaceous glands (S) and abundant well organized collagenous fibers (arrow) are observed. Mallory Trichrome; X 100

Fig. 18: A photomicrograph of a section in skin of the wound A in subgroup IIIb showing a very small superficial unhealed area (arrow head) in the epidermis (E). The papillary layer (P) of the dermis (D) is well developed with many long hair follicles (F2) and sebaceous glands (S). Hx. & E.; X 100
**Fig. 19:** A photomicrograph of a section in skin of the wound A in subgroup IIIb showing a very small superficial unhealed area (arrow head) in the epidermis (E). The papillary layer (P) of the dermis is well developed with many long hair follicles (F2), sebaceous glands (S) and abundant well organized collagenous fibers (arrow). Mallory Trichrome; X 100

**Fig. 20:** A photomicrograph of a section in skin of the wound B after three weeks showing an unhealed area (arrow head). Sebaceous glands (S) and long hair follicles (F2) start to appear in the dermis (D). A part of healthy skin with epidermis (E) can be seen. Hx. & E.; X 100

**Fig. 21:** A photomicrograph of a section in skin of the wound B after three weeks showing an unhealed area (arrow head). Sebaceous glands (S) and long hair follicles (F2) start to appear in the dermis (D). A part of healthy skin with epidermis (E) can be seen. There is a moderate amount of well organized collagenous fibers (arrow). Mallory Trichrome; X 100

**Fig. 22:** A photomicrograph of a section in skin of the wound A in subgroup Ic showing a depressed area (curved arrow) over which the epidermis (E) is not completely developed. Long hair follicles (F2) and sebaceous glands (S) can be seen in the well formed dermis (D). Mallory Trichrome; X 100

**Fig. 23:** A photomicrograph of a section in skin of the wound A in subgroup Ic showing depressed area (curved arrow) over which the epidermis (E) is not completely developed. Long hair follicles (F2), sebaceous glands (S) and abundant well organized collagenous fibers (arrow) can be seen in the well formed dermis (D). Mallory Trichrome; X 100

**Fig. 24:** A photomicrograph of a section in skin of the wound A in subgroup Ic showing complete healing with the normal histological structure of the epidermis and dermis. The epidermis (E) is formed of many layers of epithelial cells covered by a thin horny layer (*). It contains many short hair follicles (F1). The dermis is formed of papillary layer (P) which interdigitates with epidermis and reticular layer (R) which contains many long hair follicles (F2) and sebaceous glands (S). Hx. & E.; X 100
Skin wounds have serious human consequences including pain, lost work days and marked reduction in quality of life. Several putative therapeutic approaches have been proposed, including the use of antiseptics, growth factors, pressurized oxygen and physical therapy modalities (Houghton et al., 2003). Guinea pig has several advantages as an experimental model for studying cutaneous biology, it is of suitable size, easy to handle and shows structural similarity to the human skin (Sueki et al., 2000).

In the present study, a full-thickness circular excisional wound was performed. This was similar to wound previously done by Ebrahimian et al. (2009) and Gonçalves et al. (2010). On the other hand, Mortazavi et al. (2009) and Matic et al. (2009) performed a rectangular wound and Demir et al. (2004), Gonçalves et al. (2007), Mehmandoust et al. (2007) and Talebi et al. (2008) performed a linear full-thickness incision. However, the circular wound resembles chronic wounds in humans.

A full thickness excisional wound model was utilized in the present study as it resulted in an open wound that requires considerable fibroplasia to fill the defect. This was in agreement with (Devine, 1998) who also reported that the excisional wound resembles the chronic and open wounds encountered in clinical situations. Moreover, in this study the circular incision was performed as healing process will be concentric which facilitates easy and accurate measurement of the wound surface area during assessment of wound healing. It also simulates most of chronic ulcers encountered in clinical practice.

In this study the wounds were kept in moist environment and regularly were cleaned with antiseptic solution. This was in correspondence to the environment achieved by Demir et al. (2004). Moreover, Armstrong and Price (2004) and Richters et al. (2004) confirmed that the moist environment is initially beneficial for the outgrowth of keratinocytes. Dehydrated wounds increase the resistance to wound closure by dead tissue, which must be debrided before normal granulation tissue can develop. On the other hand, Nalty and Sabbahi (2001) reported that antiseptics are cytotoxic agents retard the healing process of wounds. However, Drosou et al. (2003) mentioned that cytotoxicity, found in vitro, was not confirmed and that in the majority of clinical trials, antiseptics appear to be safe and were not found to negatively influence wound healing.

In the present study, measurement of wound surface area (WSA) by tracing using metric graph paper technique has been employed. This method was reported by Kloth and Feedar (1988) as a preferable method because it is low in cost, easy to use and non-invasive. Majesk (1992) added that tracing method has been considered reliable for inter-tester and intra-tester measurements of WSA. On the other hand, Mortazavi et al. (2009) and Matic et al. (2009) calculated the surface area by multiplying the largest two dimensions of the wound to serve as an accurate indicator of WSA. However, it can be used only if the wound is rectangular.

The results of this study revealed that there were very highly significant differences in WSA one week and two weeks post-wound induction between the experimental (wound A) and the control (wound B) wounds that could prove the efficacy of laser in decreasing WSA. By the end of the third week there was significant difference between the groups that received 4 J/cm² and the control group. However, in group II and group III that received 30 J/cm² and 60 J/cm², respectively there was 100% closure of WSA before the end of the third week. This is in agreement with
Demir et al. (2004) who studied the effect of gallium-arsenide laser therapy with a wavelength of 904 nm, an energy density of 1 J/cm² for four and ten days. They found that laser treatment had beneficial effects during the inflammatory, proliferation and maturation phases of the wound. It can be used successfully in decubitus ulcers and chronic wounds, in combination with conventional therapies such as daily care and debridement of wound. Also, Enwemeka et al. (2004) and Woodruff et al. (2004) found that the use of wave length 632.8 and 780 nm laser therapy positively influences several tissue repair parameters, including reduced wound area and healing time, while the best energy density results range between 19 and 24 J/cm². Moreover, Camillo De Carvalho et al. (2006) found significant improvement in wound healing in experimental diabetic and non-diabetic rats after 3rd, 7th and 14th days of low-power (632.8 nm) daily helium-neon (HeNe) laser (4 J/cm²). Furthermore, Gul et al. (2008) observed a significant increase in wound healing rate in rabbits after daily HeNe laser of 3 J/cm² for 7, 14, 21 and 28 days.

On the other hand, Walker et al. (2000) found no beneficial effect of GaAlAs laser on rate of wound healing. However they used energy density of 0.5, 1.5 and 4 J/cm² three times weekly for three weeks. Moreover, Woodruff et al. (2004) reported that densities below 8.25 J/cm² and above 130 J/cm² do not produce good wound closure results. Furthermore, Rodrigo et al. (2009) found that the healing was more advanced in the wound located furthest from the point of laser application in rats. They used aluminium gallium indium phosphide & gallium-aluminum-arsenide laser therapy of wave length 685 and 830 nm and energy density of 10 J/cm² and 20 J/cm², respectively. However they applied laser day after day.

In the present study, histological examination provided results that were consistent with those obtained with external examination of WSA. After one week, wound B showed a large unepithelialized depressed area of the dermis with complete absence of hair follicles and a minimal amount of well formed collagenous fibers. The wound A in subgroup I-a showed a large unepithelialized depressed area. This area was gradually reduced (but still large) in subgroups II-a and III-a, respectively. However, epithelialization started to appear at the periphery of the wound in subgroup I-a which gradually increased in subgroups II-a and III-a, respectively. The hair follicles and sebaceous glands appeared in the healed areas of different subgroups. The collagenous fibers were minimal in subgroup I-a and increased gradually in subgroups II-a and III-a. After two weeks, wound B showed large unhealed area with absence of epidermis. The collagenous fibers became moderate in amount. The previous changes in wound A became more obvious with the best results in subgroup III-b compared to other subgroups (I-b & II-b). After three weeks, the unepithelialized area in wound B was still present and the dermis showed hair follicles and sebaceous glands as well as moderate amount of well-organized collagenous fibers. However, there was nearly complete healing in wound A in subgroup I-c while the skin of subgroups II-c and III-c retained its normal structure completely.

In the present study, it was found that the laser enhanced epithelialization (healing of epidermis or wound closure) and collagen formation and arrangement (healing of dermis). These parameters were assayed by Ferguson et al. (2005) as a measurement for wound healing. They added that the recovery of the dermis ultimately determines the strength of the wound preventing the reopening of the epidermis. Thus, ideal treatment will enhance healing events both at the epidermis and dermis. Fung et al. (2003) also reported that GaAlAs laser (63.2 J/cm²) promoted the proliferation of collagen fibers in the medial collateral ligaments of rats' knee. They found that the animals received laser radiation 3 to 6 weeks presented wider well-organized fibers than those in the control group. Moreover, Ng et al. (2004) observed that multiple laser applications to the lesion improved collagen morphology and healing. In addition, Pugliese et al. (2003) and Camillo De Carvalho et al. (2006) found that low-power laser (632.8 nm & 4 J/cm²) gradually increased the mean quantity of collagen fibers in surgically-induced skin wounds 3, 7 and 14 days after operation both for diabetic and non-diabetic rats.

The mechanism by which laser enhances the wound healing was investigated by many authors. Medrado et al. (2008) reported that GaAlAs of energy density 4 J/cm² application was responsible for edema regression, diminution in the number of inflammatory cells and evident increase in number of actin-positive cells in rats, 3 to 60 days, after surgically-induced skin wounds. The same results were observed by Yasukawa et al. (2007)
who examined the operative wound healing on 1st, 3rd and 5th day after helium-neon laser application. Júnior et al. (2009) also reported that low level laser therapy (3.8 J/cm²) modulated the healing of wounds by inducing an increase in mitotic activity, fibroblast number, synthesis of collagen and neovascularization. They added that many inflammatory cells, fibroblasts and apoptotic epithelial cells were seen in the tissue samples 10 days after laser therapy compared to control animals. They concluded that low level laser therapy may be an important inducer of apoptosis during the process of tissue repair. Moreover, Santos et al. (2010) found that laser of wave length 660, 790 nm and energy density 20 J/cm² resulted in reduced inflammation and an increase in both fibroblast proliferation and collagen deposition. On the other hand, Fujimaki et al. (2003) suggested that low-intensity laser affects the early events in the dynamics of wound healing by inducing attenuation of reactive oxygen species production by neutrophils in inflammatory models. This was based on the study of Rojkind et al. (2002) who described oxidative stress-induced apoptosis by neutrophils in acute inflammation. However, they postulated that oxidative stress and the mechanisms by which reactive oxygen species modulate wound healing are still of debate.

It is concluded that GaAlAs laser of different energy densities was able to induce several modifications that accelerated the wound healing with best results obtained for energy density of 60 J/cm². So, it is recommended to add laser to routine management of surgical skin wounds. Further studies should be carried out on acute or chronic (infected or ischemic) wounds in humans. More researches are needed to establish the best energy density of laser stimulation protocol that can be generalized for human wounds.

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REFERENCES


فعالية ليزر الجاليوم ألمونيوم أرزنيد في التنبم الجروح الجلدية للخلازير الغينية
محمد إيهاب الدين مصطفى
قسم التشريح - كلية الطب - جامعة القاهرة

ملخص البحث

للجروح الجلدية عواقب وخيمة على الإنسان تشمل الألم و فقدان في أيام العمل و انخفاض واضح في جودة الحياة. وقد أجريت عدة دراسات للوصول إلى طرق علاجية جديدة لمنع أو تقليل فشل التنام الأنسجة. وقد اشتملت هذه الطرق على الموجات فوق الصوتية العلاجية و التنبئي الكهربائي و الليزر مدفوع الضوء. وكانت تقنية الليزر تستخدم في علاج الجروح لتحسين اتساع الأوعية الدموية و تكوين الكولاجين وزيادة الأكسجين بالأنسجة. وقد كانت هذه طاقة الليزر الملائمة دائما مثيرة للجدل. ولذلك كان الهدف من الدراسة الحالية تقييم تأثير الجاليوم ألمونيوم أرزنيد بطاقات مختلفة على عملية التنام الجروح.

و قد أجريت هذه الدراسة على خمسة وأربعين حنزا غينيا بالغة. تم إعداد الجرحين (A و B) بشملان السمك الكلي للجدل مساحة كل منها 200 سم مربعاً. وقد تم تعرض الجرح A لأشعة الجاليوم ألمونيوم أرزنيد الليزر يومياً بموجة طولها 900 نانومتر و استخدام الجرح B كضب يطبيقي. تم تقسيم الحيوانات عشوائياً إلى ثلاث مجموعات متساوية (الأولى و الثانية و الثالثة) تم تعرضها لأشعة الجاليوم ألمونيوم أرزنيد ليزر يومياً بطاقات مختلفة 2 و 3 و 4 جول / سم مربع على الترتيب. وقد تم تقسيم جرواح كل مجموعة إلى ثلاث مجموعات فرعية متساوية (خمسة حيويات لكل مجموعة). A، B، C تعرضت للليزر يوميا لمدة أسبوع و أسبوعين و ثلاثة أسابيع على الترتيب. وقد تم قياس ساحة سطح الجروح ثم تم التضليل بالحيويات بعد يوم من آخر جرعة ليزر و أخذ عينات من الجلد الخبي مغضوب (كضب) وأجزاء من الأطراف المختلفة للجروح.

و قد تم تعيين تلك العينات لتصنف برامج بالرنين كهربائي والإيوس و المللوري و ترايكون و تقصيدها بالمحجر الصوني.

و قد أظهرت النتائج أن هناك نقص ذو دلالة إحصائية عالية جداً في ساحة سطح الجروح. كما كان هناك تحسين في التنام الجروح المعالجة بالليزر بالمقارنة مع الجروح الضبابية. وقد تم استباط ان العلاج بالجاليوم ألمونيوم أرزنيد ليزر (باستخدام طاقات مختلفة) كان مؤثراً في احداث تحويرات متحدة بالأنسجة أسرع من في التنام الجروح وقد كانت أفضل النتائج لكثافة الطاقة 60 جول / سم مربع.

MOHAMED MOSTAFA