A STUDY OF THE EFFECT OF HESA-A ON THE WOUND HEALING PROCESS IN RATS

ALIZADEH ALIMOHAMMAD*
PAKNEGAD MALIHE**
AHMAD AMROLLAH*
MOHAMMADZADEH MOHAMMAD***
MOHAGHEGHI MOHAMMADALI*
GHIACI SEDDIGEH****

SUMMARY: Wound healing is the restoration of physical integrity of internal and external structures and involves intricate interactions between the cells and numerous other factors. Appropriate treatment and care are essential for acceleration of the healing process, prevention from infection and chronicity of the wound; in addition different means and approaches have been used to this end. The aim of this study was to evaluate the effect of HESA-A (a drug of marine-plant origin with active biological ingredients patented by Dr. Ahmadi) on the wound healing process.

The effect of HESA-A; on the 35 mm long full thickness wound in the paravertebral area 1,5mm from the midline on the back of rats. Appliying a concentration of 2,5% (mixture of 2.5% drug and 97.5% chow) and 5% and 10% on the healing process. The results were evaluated measuring the length and area of the healed region on different days to conduct tensiometry experiments after complete wound healing.

The percentage of wound healing on days 10, 12, 14, 16 and 18 in the control group changed in the group treated with 2.5% HESA-A from 51.27%, 62.54%, 73.11%, 86.71% and 100% to 59.34%, 75.53%, 91.17% and 100%, respectively; in the group treated with 5% HESA-A to 77.53%, 88.27%, 95.58%, 100% and 100%, respectively; and in the group treated with 10% HESA-A to 67.81%, 92.81%, 100%, 100%, and 100%, respectively.

Stress (maximum tensile force causing skin rupture) changed from 16.54 in the control group to 19.2 Newton ($P<0.001$), 24.23 Newton ($P<0.001$), and 32.12 Newton ($P<0.001$) in the groups treated with 2.5%, 5%, and 10% HESA-A, respectively.

Strain (tissue length under maximum strain) increased from 14.83 mm in the control group to 16.44 mm, 25.25 mm ($P<0.001$) and 35.96 mm ($P<0.001$) in the groups treated with 2.5%, 5%, and 10% HESA-A, respectively.

Our findings suggest that HESA-A may have accelerated the skin wound healing process in rat in a concentration-dependent fashion and increased tissue strength through stimulating collagen formation.

Key Words: Wound healing, HESA-A, Strain, Stress.

INTRODUCTION

Wound healing is the restoration of physical integrity of internal and external structures and involves complex interactions between the cells and various factors (1). Wound healing is a homeostatic mechanism for restoration of physiological balance and is triggered by the interruption of the connection between adjacent cells or cell death. The healing process consists of a sequence of overlapping events including inflammatory responses, regeneration of the epidermis, shrinkage of the wound and finally connective tissue formation and remodeling (2, 3). Appropriate treatment and wound care accelerate the

*From Institute of Cancer Research-Medical Sciences of Tehran University, Tehran, Iran.
**From Biochemistry Department, Medical Sciences of Tehran University, Tehran, Iran.
***From Physiology Department, Medical Sciences of Tarbiat Modarress University, Tehran, Iran.
****From Physiotherapy Department, Medical Sciences of Tarbiat Modarress University, Tehran, Iran.

Table 1: The means percentage of wound healing in the control and drug groups (2.5%, 5% and 10%) with computation changes length relation to 0 day.

Groups / Day	Control	HESA-A 2.5%	HESA-A 5%	HESA-A 10%
2	14.97 ± 3.4	20.45 ± 3.4	27.99 ± 2.6	31.66 ± 2.3
4	22.45 ± 4.5	28.15 ± 3.5	40.15 ± 2.2	44.27 ± 4.5
6	30.1 ± 3.9	38.28 ± 4.4	[a] 53.69 ± 9.3	[a] 52.4 ± 3.3
8	39.44 ± 5.3	[a] 47.46 ± 3.4	[a] 66.49 ± 4.4	[a] 65.31 ± 5.3
10	51.27 ± 6.2	[a] 59.34 ± 5.4	[a] 77.53 ± 6.2	[a] 67.81 ± 6.3
12	62.54 ± 6.4	[a] 75.53 ± 3.3	[a] 88.27 ± 6.2	[a] 92.81 ± 6.4
14	73.11 ± 4.4	[a] 91.17 ± 3.5	[a] 95.58 ± 2.3	100
16	86.71 ± 2.1	100	100	
18	100			

Values are Means ± S.E.M. Sample size is six rats in each group. Tests: Two-way ANOVA, LSD. a $p < 0/001$ Compared to control.

healing process and prevent infection and chronicity of the wound. Thus far, different methods and approaches have been used to achieve shorter healing times. Despite extensive efforts to improve wound healing, the outcomes of existing methods are far from optimal.

In 1996, Patino studied the effect of electromagnetic fields on the healing of rectangular wounds on the back of rats and monitored changes in wound length compared to day 0 (4).

In a different experiment, Patino used wound area and circumference measurements to evaluate the wound healing process.

Another highly valuable measure for evaluating the wound healing process is the tissue tensile strength.

There is evidence of increased collagen synthesis in the hours immediately following injury. Multiple bonds and special arrangement of collagen fibers are responsible for tissue strength, hence tensiometry can be used to assess the tensile strength of the healed wound (5-7).

HESA-A is a drug of plant and marine origin (patented by Iranian researchers) consisting of an array of various non-organic elements. X-ray studies have revealed the presence of certain oxides including CaO (43.787%), P_2O_5 (6.63%), Na_2O (3.689), MgO (2.897%), SO_3 (2.193%), K_2O (1.988%), SiO_2 (1.09%), Fe_2O_3 (0.375%), Al_2O_3 (0.354%) and elements such as Tm, Zn, Cu, Ag, As, Mn, Ti, Sr, Br, Ca, Se, Te, Cd, Cs, Er, Lu and other trace elements at very low quantities (8, 9); given their physiological properties, these components may be able to accelerate the healing process. Hence this study was conducted to evaluate the effect of HESA-A on the wound healing process in laboratory rats (10-18).

MATERIAL AND METHODS

The experiments were conducted on wistar rats weighing 200-300 grams (supplied from Razi Vaccine and Serology Research Center). The animals were caged individually in a controlled environment at 23-25°C and 50% humidity with a 12 h artificial light cycle. Two groups of rats were studied:

a) Control group (n=6): This group was given normal chow after incision to create wound until the end of the study.

b) Case group: This group was divided into three sub-groups:

1. The 2.5% group (n=6) was given a mixture of 2.5% drug (powdered) and 97.5% normal chow after incision until the end of the study.

2. The 5% group (n=6) was given a mixture of 5% drug and 95% normal chow after incision until the end of the study.

3. The 10% group (n=6) was given a mixture of 10% drug and 90% normal chow after incision until the end of the study.

Prior to incision, the rats were anesthetized by intraperitoneal

injection of ketamine (50mg/kg) and Xylazine 2% (5mg/kg). Then the animals were shaved on the back and the skin was disinfected using cotton and alcohol wipes. Using sterile surgical scalpels, full-thickness incisions, 35 mm in length were created in the paravertebral area, 1.5 mm from the posterior midline. After incision, the wound was thoroughly disinfected using povidone iodine and injected gentamicin (5mg/kg, single dose) as antiseptic. The control group was given normal chow and the case group was given the combination of drug and chow until complete healing of the wound.

Evaluation of the healing process

Two methods were used to evaluate the wound healing process.

a) Measurement of wound length and area: Every other day as of the first day of creating the incisions (day 0) until complete wound healing, the animals were anesthetized with ether and the shape of the wound was drawn on a piece of transparent plastic using a special marker. To measure the wound area, using a negatoscope and the software Video Image Analyzer. The wound area was accurately measured and the percentage of healing was calculated according to the following formula on different days.

$$\text{The wound size percentage} = \frac{\text{Difference wound length or area on Xday relation to 0 day}}{\text{Wound length or area on 0 day}} \times 100$$

$$\text{Healing percentage on Xday} = 100 - \text{Wound size percentage on Xday}$$

b) Measurement of tissue tensile strength (tensiometry): To conduct this measurement, the animals were killed by chloroform inhalation after complete healing of the wound. The skin of the back was excised at the deep fascia region and submersed in normal saline to prevent drying.

Tissue tensile strength was then measured using a tensiometer. In this method, a narrow strip of skin, 5 cm in length and 4 cm in width, is attached to tensiometer holders. The healed wound lies at the center and at right angle to the length of the skin. The movement of holders is controlled by computer. The tension exerted on the skin is increased and is stopped as soon as the skin ruptures. The following parameters are calculated by tensiometer and the results are displayed by computer:

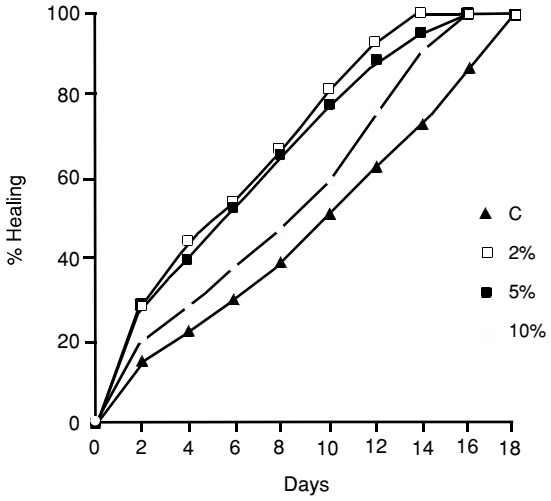
Stress: Maximum tensile force causing skin rupture.

Strain: Tissue length under maximum tension.

Statistical methods

Data pertaining to the duration of healing and tissue strength were evaluated using one-way ANOVA and t-test. Comparison of data pertaining to duration and percentage of healing was performed using two-way ANOVA and LSD test. Significance level was set at 0.05. All results were reported as Mean ± SEM.

Figure 1: Percentage of changes in wound area during the healing process in the control and drug groups (2.5%, 5% and 10%). Sample size is 6 rats in each group. Tests: Two-way ANOVA, LSD; Wound healing in the drug group has occurred at a faster pace than in the control group and a significant difference is seen between the two groups in the percentage of healing on different days. Acceleration of healing in the drug subgroups seems to be dose-dependent.



RESULTS

Percentage of healing on days 10, 12, 14, 16 and 18 in the control group increased from 51.27%, 62.54%, 73.11%, 86.71%, and 100% to 59.34%, 75.53%, 91.17% and 100% in the 2.5% group, to 77.53%, 88.27%, 95.58%, 100% in the 5% group, and to 67.81%, 92.81%, 100% in the 10% group (Figure 1, Table 1).

Stress (maximum tensile force causing skin rupture) in the control group increased from 16.54 Newton to 19.2, 24.23 ($P<0.001$), and 32.12 Newton ($P<0.001$) in the 2.5%, 5%, and 10% groups, respectively (Figure 2).

Strain (skin length under maximum tension) increased from 14.83 mm in the control group to 16.44 mm, 25.25 mm ($P<0.001$), and 35.96 mm ($P<0.001$) in the 2.5%, 5%, and 10% groups, respectively (Figure 3).

Figure 2: Stress (Maximum tensile force causing skin rupture) in the control and drug groups (2.5%, 5% and 10%). Sample size is six rats in each group. Tests: Two-way ANOVA, LSD. Wound healing in the drug group has occurred at a faster pace than in the control group and a significant difference is seen between the two groups in the percentage of healing on different days. Acceleration of healing in the drug subgroups seems to be dose-dependent.

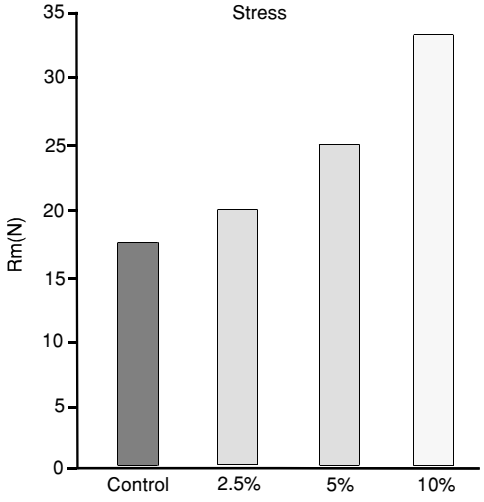
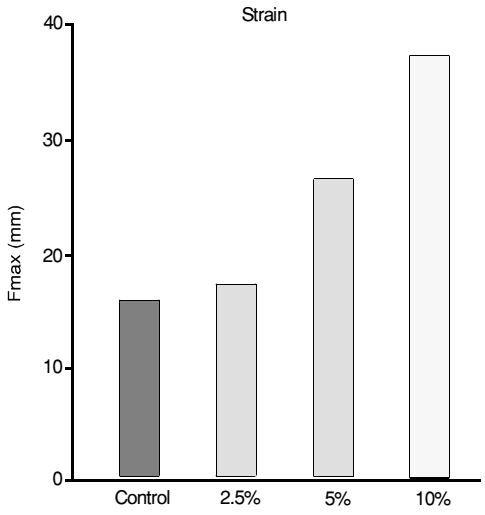


Figure 3: Strain (tissue length under maximum tension) in the control and drug groups (2.5%, 5% and10%). Tests: Two-way ANOVA, LSD. Wound healing in the drug group has occurred at a faster pace than in the control group and a significant difference is seen between the two groups in the percentage of healing on different days. Acceleration of healing in the drug subgroups seems to be dose-dependent.



DISCUSSION

Figure 1 and Table 1 show the effect of HESA-A at different concentrations on wound healing in rats treated with the drug compared to control rats. The figure shows the percentage of healing based on changes in wound area on different days compared to the first day (day 0) of the experiment.

Statistical analyses, including ANOVA and LSD show a significant difference in the percentage of healing on days 12, 14, 16, and 18 of the study.

Spectrophotometric studies have demonstrated the role of trace elements in healing of burn wounds (18). The role of elements such as strontium in the movement and immigration of non-muscle cells in the tissue healing process has also been shown (20). Obtaining counts of fibroblasts, capillaries, and polymorphonuclear leukocytes, as well as measurement of immunoglobulin G levels on the surface of wound using the immune-centrifuge technique has shown the notable effect of selenium on the wound healing process (21).

A number of studies conducted by Grzesiak showed that elements such as magnesium cause migration of keratinocytes and mediators of the healing process such as E-cadherin and integrin to the wounded area (22, 23).

In the present study, HESA-A apparently led to a notable decrease in length and area of the wound, as demonstrated by the significant difference in the percentage of healing on different days of the study as compared to the control group.

In view of the results (Table 1, Figure 1), it can be concluded that HESA-A significantly accelerated the wound healing process. The percentage of healing increased with increased drug concentration, hence it can also be concluded that the effect of HESA-A on healing is dose-dependent. Measurement of tissue strength after complete healing of the wound was among the parameters assessed by the present study. As shown in Figures 3 and 4, the tensile strength of the skin tissue from rats receiving HESA-A increased compared to the control group. It has been demonstrated that the tensile strength of the skin is related

to the number of collagen fibers and how they are connected. HESA-A probably increases collagen synthesis by increasing fibroblasts, as well as increasing DNA and protein production. It may also have positive effects on the maturation, deposition, and correct orientation of collagen fibers. Experiments conducted by Grzesiak and colleagues (22) in 1995 showed that the amount of magnesium and calcium increases in wound exudates. Analysis of wound exudates showed that elements such as magnesium promote tissue adhesion, migration of macrophages, keratinocytes, fibroblasts and production of type I collagen, all of which contribute to the wound healing process (24-27).

Neovascularization is an important factor in wound healing. Povies and colleagues studied the metabolism of minerals in the healing arterial walls of rats by measuring the accumulation of some radioisotopes. Full thickness incisions were made in the aorta of rats injected with radioisotopes. A significant accumulation of radioisotopes such as zinc, selenium and chromium was seen in the healing area compared to the control group (28). The role of vanadium, another trace element in the healing process has also been studied (29). Separate studies have investigated the role of trace elements in healing (30, 31).

It can be concluded from the obtained evidence that HESA-A has probably had a dose-dependent accelerating effect on the healing process.

Study limitations

The main limitation of this study was obtaining a fixed chow/drug percentage ratio. To achieve this, the appropriate chow/drug ratios were supplied to the Razi Vaccine and Serology Research Center to produce plates easily consumable by rats.

REFERENCES

1. Schwartz's Principles of Surgery.7th Ed, 1999.

2. Leaper PJ, Harding KG: Wounds, Biology and management Sanders, 1998.

3. Choucair MM, Phillips TG: What is new in clinical research in wound healing. Clin Dermatol, 15:45-54, 1997.

4. Patino O, Grana D, Beenaim F: Pulse electromagnetic fields in experimental cutaneous wound healing in rats. J Burn Rehabil, 17:528-531, 1996.

5. Watson T: Bioelectric correlates of musculoskeletal injury and repair. Bioelectromag, 17:447-504, 1995.

6. Bargeson RE: The collagen of skin. Dermatol, 17:61-75, 1987.

7. Luther L, Kloth M: Wound healing alternative management, 1990.

8. Ahmadi A, Hajhashemi V, Balali M: Toxicological Studies on an Anticancer Drug (HESA-A) with Marine Orign. Med J Islam Acad Sci, 14:145-149, 2001.

9. Ahmadi A, Sadeghi-Aliabadi H: Cytotoxicity and Antitumor Properties of A Marine Compound, HESA-A, on Cancer cells. Daru, 11N: 3, 2003.

10. Rizoiu IM, Eversole LR, Kimmel AI: Effects of an erbium, chromium: yttrium, scandium, gallium, garent on mucocutanous soft tissues. Pathol Oral Radiol Oct, 82:386-395, 1996.

11. Simoes RM, Teixeira D, Maldonado EP: Effects of 1047-nm neodymium laser radiation on skin wound healing. J Clin Laser Med Surg, 20:37-40, 2002.

12. Tanzi EL, Alater TS: Side effects and complication of variable-pulsed erbium: yttrium-aluminum-garnet skin resurfacing. Plast Reconstr Surg, 11:1524-1529, 2003.

13. Agren MS, Stromberg HE: Selenium, zinc, iron and copper levels in serum of patients with arterial and venous leg ulcers. Acta Derm Venereol, 66:237-240, 1986.

14. Bang RL, Dashti H: Keloid and hypertrophic scars: trace element alteration. Nut, 11:527-531, 1995.

15. Urban J, Antonowicz J: Vanadium, threat and hope. Med Pe, 52:125-133, 2001.

16. Gumustekin K, Seven B, et al: Effects of nicotin and selenium on wound healing in rats. Int J Neurosci, 114:1433-1442, 2001.

17. Moore L, Pastan I: A calcium requirement for movement of cultured cell. J Cell Physiol, 101:101-108, 1979.

18. Selmanpakoglu AN, Cetin C: Trace element (Al, Se, Zn, Cu) levels in serum, urine and tissues of burn patients. Burns, 20:99-103, 1994.

19. Byl NN, Mckenzie AL, et.al: Low-dose ultrasound effects on wound healing: A controlled study with yucatain pigs. Arch Phys Med Rehabil, 73:656-664, 1992.

20. Moore L, Pastan I: A calcium requirement for movement of cultured cells. J Cell Physiol, 101:101-108, 1979.

21. Gumustekin K, Seven B, Karabulut N, et al: Effects of sleep deprivation, nicotine and selenium on wound healing in rats. Int J Neurosci, 114:1433-1442, 2004.

22. Grzesiak JJ, Pierschbacher MD: Shifts in the concentration of magnesium and calcium in early porcine and rat wound fluids activate the cell migratory response. Clin Invest, 95:227-233, 1995.

23. Kuwahara M, Hatoko M, Tada H, Tanaka A: E-cadherin expression in wound healing of mouse skin. J Cutaneo Pathol, 28:191-199, 2001.

24. Bang RL, Dashti M: Keloid and hypertrophic scars: trace element alteration. Nut, 11:527-531, 1995.

25. Wayne K: Physiology and healing dynamics of chronic cutaneus wounds. Am J Surg, 176:26-38, 1998.

26. Jensen L: Collagen, scaffold for repair or execution. Cardiovascular Res, 33:535-539, 1997.

27. Witt M, Thornton F: Enhancement of fibroblast collagen synthesis by nitric oxide. NO, 4:572-582, 2000.

28. Pories WJ, Atawneh A, Peer RM, Childers RC, Worland RL, Zaresky SA, Strain WH: Mineral metabolism of the healing arterial wall. Arch Surg, 114:254-257, 1979.

29. Urban J, Antonowicz-Juchniewicz J, Andrzejak R: Vanadium, threat and hope. Med Pro, 52:125-133, 2001.

30. Agren MS, Stromberg HE, Rindby A, Hallmans G: Selenium, zinc, iron and copper levels in serum of patients with arterial and venous leg ulcers. Acta Derm Venereol, 66:237-240, 1986.

31. Stanisstreet M: Calcium and wound healing in Xenopus early embryos. J Embryol Exp Morphol, 67:195-205, 1982.

Correspondence:
Alizadeh Alimohammad,
Institute of Cancer Research
Medical Sciences of
Tehran University, Tehran, IRAN.
e-mail: ama_alizadeh@yahoo.com