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The Effect of HESA-A, an Herbal-marine Compound, on Wound Healing Process: An Experimental Study

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Abstract: Appropriate treatment and wound care accelerate the healing process and prevent infection. The aim of present study was to evaluate the effect of HESA-A (a drug of marine-plant origin) on the wound healing process. The effect of HESA-A at concentrations of 2.5% (mixture of 2.5% drug and 97.5% chow), 5 and 10% on the healing process of a 35 mm long full-thickness wound was prepared dorsally on rats, evaluated through measuring the length of the healed region on different days and conducted tensiometery experiments after complete wound healing. The mean percentage of wound healing on days 12, 14, 16 and 18 in control group changed in the group treated with 2.5% HESA-A from 62.5, 71.1, 86.7 and 100-75.5, 91.2, 100 and 100 ($p<0.001$), respectively; in the group treated with 5% HESA-A to 88.5, 98.6, 100 and 100 ($p<0.001$), respectively and in the group treated with 10% HESA-A to 92.8, 100, 100 and 100 ($p<0.001$), respectively. The stress changed from 16.5±0.9 Newton (N) in the control group to 19.2±1.5 N, 24.2±1.3 N ($p<0.001$) and 32.1±0.7 N ($p<0.001$) in the groups treated with 2.5, 5 and 10% HESA-A, respectively. The strain increased from 14.8±0.6 millimeter (mm) in the control group to 16.5±0.8, 25.7±0.4 ($p<0.001$) and 35.7±1.1 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 dose-dependent manner and it seems increased tissue strength through stimulating collagen formation.

Key words: Wound healing, HESA-A, strain, stress, herbal-marine compound, healing process

INTRODUCTION

Wound healing is a homeostatic mechanism for restoration of physiological balance and is triggered by the interruption of the connection between adjacent cells (Brunicardi and Schwartz, 2005). Appropriate treatment and wound care accelerate the healing process and prevent infection (Leaper and Harding, 1998; Choucair and Phillips, 1997). Thus far, different methods and approaches have been used to achieve shorter healing times. Patino *et al.* (1996) used wound area and circumference measurements to evaluate the wound healing process (Patino *et al.*, 1996). 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 (Watson, 1995; Bargeson, 1987).

HESA-A is a drug of herbal-marine origin (patented by Iranian researchers) consisting of an array of various non-organic elements. HESA-A includes mineral constituents (50%), organic constituents (45%) and water (5%). The mineral constituents are a mixture of calcium carbonate, magnesium sulfate, potassium sulfate, sodium sulfate, magnesium phosphate, potassium phosphate and sodium phosphate. Low percent of other elements such as Br, Sr, Ti, Mn, Ni, As, Ag, Cu, Tm, Lu TI, Er, V, Cs, Ba, Te and so forth are found in salt or complex forms in HESA-A mixture (Ahmadi *et al.*, 2001, 2003) given their physiological properties, these components may be able to accelerate the healing process and form collagen

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(Rizoiu *et al*., 1996; Simoes *et al*., 2002; Bang and Dashti, 1995; Gumustekin *et al*., 2004). Our previous study showed hepatoprotective effect of HESA-A against hepatic damage in rabbits (Ahmadi *et al*., 2005) and anti-inflammatory effect (in press). Hence, The present study, was conducted to evaluate the effect of HESA-A on the wound healing process in rat.

MATERIALS AND METHODS

Male Wistar rats, weight 250-300 g (supplied from Razi Vaccine and Serology Research Center) were caged individually in a controlled environment at 23-25°C and 50% humidity with a 12 h light-12 h dark cycle and allowed free access to food and water. The NIH guide for the care and use of laboratory animals was used for animal study. Two groups of rats were studied, control and case groups. The control group (n = 6) was given normal chow after incision to create wound until the end of the study. The case group divided into three subgroups; 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 (daily weigh), the 5% group (n = 6) was given a mixture of 5% drug and 95% normal chow after incision until the end of the study (daily weigh) and the 10% group (n = 6) was given a mixture of 10% drug and 90% normal chow after incision until the end of the study (daily weigh).

Prior to incision, the rats were anesthetized by intraperitoneal injection of ketamine (50 mg kg^{-1}) and Xylazine 2% (5 mg kg^{-1}). 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 prepared 2 mm from midline dorsally. The wounds were left open. After incision, the wound was thoroughly disinfected using cotton and alcohol and injected gentamicin (5 mg kg^{-1} daily, 3 days) as antiseptic (Dana *et al*., 1993).

Evaluation of the healing process:

- Length of wounds measured every other day until complete wound healing. The animals were anesthetized by ether and then the shape of the wound was drawn on transparent film by a special marker. The wound area was accurately measured and the percentage of healing was calculated by negatoscope and Video Image Analyzer software according to the following formula on different day: The percentage of wound healing on day

$$X = 100 - \frac{\text{Wound length or area on day X}}{\text{Wound length or area on day 0}} \times 100$$

- Measurement of tissue tensile strength (tensiometery): To conduct this measurement, the animals were killed by chloroform inhalation, after complete healing of the wound. The dorsal skin was excised at the deep fascia and the specimens were put immediately in saline 9% to prevent drying. The strength of tissue tensile was measured by a tensiometer. In this method, a narrow strip of skin, 5 cm in length and 2 cm in width, is attached to tensiometer holders. The healed wound lies at the center. The movement of holders is controlled by computer. The stress (maximum force tensile leading to skin rupture) and strain (tissue length under maximum tension) parameters are calculated by tensiometer and their results analyzed by computer

Statistical methods: Data pertaining to the duration of healing and tissue strength were evaluated using one-way ANOVA and Tukey post test. Significance level was set at 0.05. All results were reported as Mean±SEM.

RESULTS

The mean percentage of healing on days 8, 10, 12, 14, 16 and 18 in the control group increased from 39.4, 51.3, 62.5, 71.1, 86.7 and 100 to 47.5, 59.3, 75.5 ($p<0.001$), 91.2 ($p<0.05$) and 100 in the 2.5 group, to 65.3 ($p<0.001$), 77.5 ($p<0.001$), 88.5 ($p<0.001$), 98.6 ($p<0.001$) and 100 in the 5% group and to 66.5 ($p<0.001$), 81.7 ($p<0.001$), 92.8 ($p<0.001$) 100 in the 10% group (Table 1).

Table 1: The mean percentage of wound healing in the control and drug groups of length changes

	Groups			
Days	Control	HESA-A 2.5%	HESA-A 5%	HESA-A 10%
2	14.4±2.8	20.5±1.5	28.0±1.9[b]	31.8±3.2[c]
4	22.5±3.6	28.2±2.0	40.4±1.3[b]	44.2±4.6[ce]
6	30.9±3.5	38.3±1.9	52.3±3.9[cd]	53.4±3.6[ce]
8	39.4±4.1	47.5±2.8	65.3±3.4[cd]	66.5±4.6[cd]
10	51.2±3.7	59.3±2.5	77.5±6.2[cd]	81.7±5.2[b]
12	62.5±6.4	75.5±3.3[b]	88.5±6.2[c]	92.8±6.4[c]
14	71.1±4.4	91.2±3.5[a]	98.6±2.3[c]	100
16	86.7±2.1	100	100	
18	100			

Wound healing in the drug groups has occurred at a faster pace than in the control group and a significant difference was seen between the 2 groups in the percentage of healing on different days. Acceleration of healing in the drug subgroups seems to be dose-dependent. Values are Means±SEM. Sample size is 6 rats in each group. Tests: One-way ANOVA, Tukey. [a]$p<0.05$ Compared to control. [b]$p<0.01$ Compared to control. [c]$p<0.001$ Compared to control. [d]$p<0.01$ Compared to 2.5% group. [e]$p<0.01$ Compared to 5% group

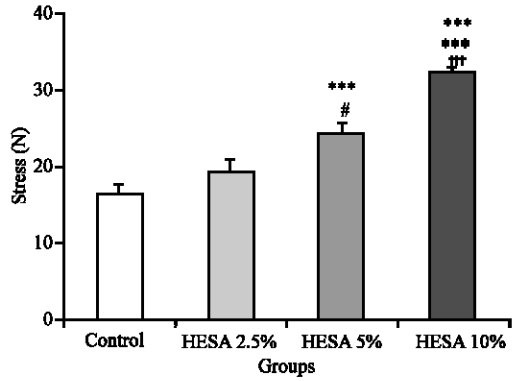

Fig. 1: The stress in control and drug (2.5, 5 and 10%) groups at the end of study. The tensile strength of the skin tissue from rats receiving HESA-A increased compared to the control group and a significant difference is seen between the groups. Acceleration of healing in the drug subgroups seems to be dose-dependent. Tests: One-way ANOVA, Tukey. N: Newton. $^{***}p<0.001$ compared to control group. $^{\#\#\#}p< 0.001$ compared to 2.5% group. $^{\#}p<0.05$ compared to 2.5% group. $^{\dagger\dagger\dagger}p<0.001$ compared to 5% group

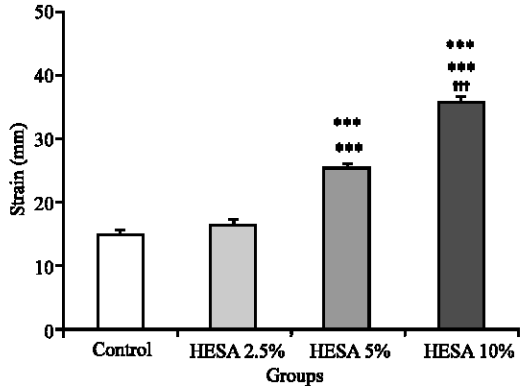

Fig. 2: The strain in control and drug (2.5, 5 and 10%) groups at the end of study, The tensile strength of the skin tissue from rats receiving HESA-A increased compared to the control group and a significant difference is seen between the groups. Acceleration of healing in the drug subgroups seems to be dose-dependent. Tests: One-way ANOVA, Tukey. mm: millimeter. $^{***}p<0.001$ compared to control group. $^{\#\#\#}p<0.001$ compared to 2.5% group. $^{\dagger\dagger\dagger}p< 0.001$ compared to 5% group

The stress changed from 16.5±0.9 Newton (N) in the control group to 19.2±1.5 N, 24.2±1.3 N ($p<0.001$) and 3.12±0.7 N ($p<0.001$) in the groups treated with 2.5, 5 and 10% HESA-A, respectively (Fig. 1).

The strain increased from 14.8±0.6 millimeter (mm) in the control group to 16.5±0.8, 25.7±0.4 ($p<0.001$) and 35.7±1.1 mm ($p<0.001$) in the groups treated with 2.5, 5 and 10% HESA-A, respectively (Fig. 2).

DISCUSSION

Table 1 show the effect of HESA-A at different concentrations on wound healing in rats treated with the drug compared to control rats and also shows the percentage of healing based on changes in wound length on different days compared to the first day (0 day) of the experiment. Statistical analysis, including ANOVA and Tukey show a significant difference in the percentage of healing on days 4, 6, 8, 10, 12, 14 and 16 of the study. In the present study, HESA-A apparently led to a notable decrease in length of the wound, as demonstrated by the significant difference in the mean percentage of healing on different days of the study as compared to the control group. In view of the results (Table 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.

A number of studies conducted by Grzesiak and Pierschbacher (1995) 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 (Kuwahara *et al*., 2001). 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 (Oore and Pastan, 1979). 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 (Gumustekin *et al*., 2004). Also spectrophotometric studies have demonstrated the role of trace elements in healing of burn wounds (Selmanpakoglu and Cetin, 1994).

Measurement of tissue strength after complete healing of the wound was among the parameters assessed by the present study (Fig. 1 and 2). 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 (Watson, 1995; Bargeson, 1987). Experiments conducted by Grzesiak and

Pierschbacher (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 (Bang and Dashti, 1995; Wayne, 1998; Jensen, 1997; Witt and Thornton, 2000). As shown in Fig. 1 and 2, 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 may has positive effects on the maturation, deposition and correct orientation of collagen fibers. 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 selenium and chromium was seen in the healing area compared to the control group (Pories *et al*., 1979). The role of vanadium, another trace element in the healing process has also been studied (Urban and Antonowicz, 2001). Separate studies have investigated the role of trace elements in healing (Agren and Stromborg, 1986; Stanisstreet, 1982).

CONCLUSION

It can be concluded, from the obtained evidence that HESA-A may have accelerated the skin wound healing process in the rat in a dose-dependent manner and seems increased tissue strength through stimulating collagen formation.

Study limitation: 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

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

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

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

Ahmadi, A., G. Naderi and S. Asgary, 2005. Evaluation of hepatoprotective potential of HESA-A (a marine compound) pretreatment against thioacetamide-induced hepatic damage in rabbits. Drugs Exp. Clin. Res., 31: 1-6.

Bang, R.L. and H. Dashti, 1995. Keloid and hypertrophic scars: Trace element alteration. Nut., 11: 527-531.

Bargeson, R.E., 1987. The collagen of skin. Dermatol, 17: 61-75.

Brunicardi, F.C. and S.I. Schwartz, 2005. Schwartz's Principles of Surgery. 8th Edn. McGraw-Hill, New York.

Choucair, M.M. and T.G. Phillips, 1997. What is new in clinical research in wound healing? Clin. Dermatol., 15: 45-54.

Dana, G., K. John, A. Dale and M. Petra, 1993. Handbook of veterinary drugs (J B lippincott company).

Grzesiak, J.J. and M.D. Pierschbacher, 1995. 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.

Gumustekin, K., B. Seven, N. Karabulut, O. Aktas, N. Gürsan, S. Aslan, M. Keles, E. Varoglu and S. Dane, 2004. Effects of nicotin and selenium on wound healing in rats. Int. J. Neurosci., 114: 1433-1442.

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

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

Leaper, P.J. and K.G. Harding, 1998. Wounds, Biology and management (Oxford University Press, UK).

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

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

Pories, W.J., A. Atawneh, R.M. Peer, R.C. Childers, R.L. Worland and S.A. Zaresky *et al*., 1979. Mineral metabolism of the healing arterial wall. Arch. Surg., 114: 254-257.

Rizoiu, I.M., L.R. Eversole and A.I. Kimmel, 1996. Effects of an erbium, chromium: Yttrium, scandium, gallium, garent on mucocutanous soft tissues. Pathol. Oral. Radiol., 82: 386-395.

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

Simoes, R.M., D. Teixeira and E.P. Maldonado, 2002. Effects of 1047 nm neodymium laser radiation on skin wound healing. J. Clin. Laser Med. Surg., 20: 37-40.

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

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

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

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

Witt, M. and F. Thornton, 2000. Enhancement of fibroblast collagen synthesis by nitric oxide. Nitric Oxide, 4: 572-582.