Journal of Life Sciences Research
ISSN : 2408-9184
Vol. 2, No. 3, 72-75, 2015
http://asianonlinejournals.com/index.php/Lifsc/index

The Use of a Rabbit Model to Evaluate the Influence of Age on Excision Wound Healing

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Abstract

Background: The wound healing involves a highly co-ordinated cascade of cellular and immunological response over a period of time including coagulation, inflammation, granulation tissue formation, epithelialization, collagen synthesis and tissue remodeling. Wounds in aged heal more slowly than those in younger, mainly because of co morbidities that occurs as one ages. Present study is about the influence of age on wound healing. 1x1cm^2 (100mm) wounds were created on the back of the animal. The animals were divided into two groups; one group had animals in the age group of 3-9 months while another group had animals in the age group of 15-21 months. Materials and Methods: 24 clinically healthy rabbits in the age group of 3-21 months were used as experimental animals and divided in two groups viz A and B. All experimental parameters i.e Excision wound model, Measurement of wound area, Protein extraction and estimation, Protein extraction and estimation and DNA extraction and estimation were done by standard methods. Results: The parameters studied were wound contraction, hydroxyproline, glucosamine, protein and DNA. Significant increase ($p<0.005$) in the hydroxyproline, glucosamine, protein and DNA and significant decrease in wound area ($p<0.005$) was observed in the age group of 3-9 months when compared to animals of age group of 15-21 months. Wound contraction together with hydroxyproline, glucosamine, protein and DNA estimations suggest that advance age results in retarded wound healing. Conclusion: The decrease wound contraction and accumulation of hydroxyproline, glucosamine, protein and DNA in group B animals may be associated with the reduction or delay in growth factors because of the advancing age.

Keywords: Age, Wound healing, Excision wound, Hydroxyproline, Glucosamine.

Contents

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1. Introduction

Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the shrinkage of wound area. The wound healing involves a highly co-ordinated cascade of cellular and immunological response over a period of time including coagulation, inflammation, granulation tissue formation, epithelialization, collagen synthesis and tissue remodeling [1-3]

Wounds in aged heal more slowly than those in younger, mainly because of co morbidities that occurs as one ages. Ageing results in loss of scaring, less contraction, decreased tensile strength, decreased epithelialization, delayed cell migration and decreased collagen synthesis [4, 5]. Age associated impaired correlates with increased inflammation, increased matrix proteolysis and delayed re-epithelialization leading to chronic wounds [6, 7].

2. Materials and Methods

2.1. Animals

24 clinically healthy rabbits in the age group of 3-21 months were used as experimental animals. These were divided in two groups viz A and B. Each group had twelve animals. The group A had the animals in the age group of 3-9 months while group B in the age group of 15-21 months. All the animals were housed in the cages had free access to fresh water at a room temperature of 22 ± 2^0C. A balanced feed was used throughout the period of study. The experimental protocols involved in this study was approved by the Institutional Animal Ethics Committee, and conforms to the "Guide lines for the Care and Use of Laboratory Animals" published by the "National Institute of Health". All the animals were acclimatized for a period of 7 days prior to the commencement of the experiment.

2.2. Excision Wound Model

The animals were anesthetized locally with injection of xylocaine (10mg/kg) and ketamine (40mg/kg). The dorsal fur of the animal was shaved just distal to scapula. The shaved area was swabbed with alcohol and an area of $1x1cm^2$ (100mm) was demarcated with a self designed stamp on the midline of the shaved area. The marked skin was excised with the help of a scalpel and scissors to the depth of loose subcutaneous tissue, care was taken to restrict the hemorrhage to bare minimum. Animals after recovery from anesthesia were housed individually in properly disinfected cages. The granulation tissue formed on day 4, 9 and 14 was harvested to estimate some biochemical parameters like hydroxyproline, glucosamine, protein and DNA.

2.3. Measurement of Wound Area

The wound area was measured at predetermined interval of time starting at 2 hour interval after creation of the wound. This interval was considered as 0 day measurement and the delay of 2 hour after creation of wound for measurement was allowed to accommodate wound stretching that occurred due to the struggle of animal during recovery from the anesthesia. The subsequent measurement was taken on day 5, 9 and 14 after creation of the wound.

2.4. Estimation of Hydroxyproline and Glucosamine

About 50mg from each stored tissue sample was subjected to acid hydrolysis by adding 1ml 6N Hcl to it in a tube which was tightly sealed and autoclaved at 50 pound pressure for 3 hours. The hydrolysate so produced was used for estimating hydroxyproline and glucosamine [8-10].

2.5. Protein Extraction and Estimation

The tissue (30 mg) already stored at -80 ^{0} C was pulverized in ice cold lysis buffer containing 100 mM Tris-Hcl, 0.05 mMEDTA with a proportion of 300 µl of lysis buffer/30 mg tissue with the help of chilled pestle and motar and a pinch of glass wool. After pulverization, homogenous mixture was transferred to 1.5 ml micro centrifuge tube and centrifuged for about 10 minutes at 10000 rpm. Supernatant protein lysate was collected and estimated for protein [11-13].

2.6. DNA Extraction and Estimation

DNA was extracted and estimated by phenol-chloroform method [14, 15].

2.7. Statistical Analysis

Analysis was done by Students t test using R software [16]. The level of significance α was taken 5%.

3. Results

Table 1 shows significant decrease in wound area of group A animals from day 4 as compared to group B animals. This trend continued up to day 14 i.e., last day of observation. On day 14 the group A animals had 20.10 ± $0.54mm^2$ wound area unhealed while group B animals had 28.80 ± 0.62 mm^2 wound area unhealed. On day 14 the group A animals exhibited 82.6 % contraction in wound contraction as against 75.2 % those of group B animals. There was a significant increase in the hydroxyproline and glucosamine content of group A animals from day 4 to day 14. The highest accumulation of hydroxyproline and glucosamine was observed on day 14; the group A animals had 13.20 ± 0.95 mg/g and 6.16 ± 0.52 mg/g of hydroxyproline and glucosamine as against 9.16 ± 0.74 mg/g and 4.58 ± 0.43 mg/g of group B animals. There was also a significant increase in protein and DNA content of group A animals on day 4 and 9 when compared to group B animals and a decrease in protein and DNA on day 14 in group A animals as compared to group B animals but the decrease is not significant (Table 2)

Table-1. Effect of age on wound contraction in excision wound model (mm^2)

Time duration	Group A	Group B
Day 0	116.0±0.09 (0.00)	116.0±0.07 (0.00)
Day 4	102.7±0.89* (11.5)	106.9±0.85 (7.8)
Day 9	69.75±0.91* (39.9)	73.4±0.67 (36.7)
Day 14	20.10±0.54* (82.6)	28.80±0.62 (75.2)

Values are expressed as mean ±SEM, n=12 animals in each group; number in parenthesis indicate percentage of wound contraction *p<0.005 when compared to control wounds.

Table-2. Effect of age on biochemical parameters in excision wound model

Biochemical parameters	Post wounding days	Group A	Group B
Hydroxyproline (mg/g)	4^{th} day	6.20±0.86*	3.86±0.14
	9^{th} day	10.16±0.83*	5.86±0.54
	14^{th} day	13.20±0.82*	9.16±0.74
Glucosamine (mg/g)	4^{th} day	3.10±0.43*	2.00±0.14
	9^{th} day	5.08±0.53*	3.43±0.31
	14^{th} day	6.16±0.52*	4.58±0.43
Protein (mg/100mg wet weight)	4^{th} day	2.97±0.11*	1.82±0.11
	9^{th} day	6.82±0.14*	4.91±0.13
	14^{th} day	5.30±0.17	5.18±0.12
DNA (mg/100mg wet weight)	4^{th} day	1.89±0.23*	1.17±0.14
	9^{th} day	5.47±0.18*	4.26±0.13
	14^{th}day	4.92±0.12	4.97±0.08

Values are expressed as mean ±SEM, n=12 animals in each group. Values are significant at *p<0.05 when compared to control wounds.

4. Discussion

The group A animals revealed increased wound contraction, enhanced accumulation of hydroxyproline, glucosamine, protein and DNA at the wound site than group B animals. It may be due to the advancing age in group B animals, increased age results in delayed appearance of epidermal growth factor (EGF), basic epidermal growth factor (bEGF), transforming growth factor-β and platelet derived growth factor A and B [17]. These growth factors stimulate various aspects of wound healing. EGF enhances keratinocytes migration and cellular proliferation there by accelerates wound contraction [18].

bEGF induces DNA and protein synthesis, angiogenesis, ECM synthesis and wound contraction. PDGF is released from a number of cells including platelets, endothelial and monocytes/macrophages [19]. This acts as chemoaltractant for inflammatory cells and fibroblasts there by stimulating fibronectin, glycosaminoglycans and collagen synthesis [20]. So the decrease wound contraction and accumulation of hydroxyproline, glucosamine, protein and DNA in group B animals may be associated with the reduction or delay in growth factors because of the advancing age.

Competing interests: Authors declare that they have no competing interests.

References

[1] P. Masoko, J. Picard, and J. N. Eloff, "The use of a rat model to evaluate the in vivo toxicity and wound healing activity of selected combretum and terminalia (Combretaceae) species extracts, onderstepoort," *Journal of Veterinary Research*, vol. 77, pp. 2-7, 2010.

[2] S. Bilal, I. Hussain, and S. A. Bhat, "Wound healing parameters at different time intervals in excision wounds of rabbit," *International Journal of Recent Scientific Research*, vol. 5, pp. 590-592, 2014.

[3] A. K. Tsirogianni, N. M. Moutsopoulos, and H. M. Moutsopoulos, "Wound healing: Immunological aspects," *Injury*, vol. 37, pp. 5-12, 2006.

[4] S. Bilal, S. A. Bhat, and A. A. Ahanger, "Healing potential of rheum emodi (Rhubarb) root powder on excision wounds in rabbit," *World Journal of Pharmacy and Pharmaceutical Sciences*, vol. 3, pp. 1317-1323, 2014.

[5] M. J. Hardman and G. S. Ashcroft, "Estrogen, not intrinsic aging, is the major regulator of delayed human wound healing in the elderly," *Genome Biology*, vol. 9, pp. R80.1-80.17, 2008.

[6] G. S. Ashcroft and S. J. Mills, "Androgen receptor mediated inhibition of cutaneous wound healing," *J. Clin. Invest.*, vol. 110, pp. 615-624, 2002.

[7] A. Andreza Miranda, D. O. Dhelfeson Willya, M. Sandra Aparecida, L. Nadia Lages, d. M. Joao Luiz, and V. Flaviana Dornela, "Effect of topical application of different substances on fibroplasia in cutaneous surgical wounds," *ISRN Dermatol*, vol. 10, pp. 1ISBN-10: 15848854ISBN-10: 1584885459 Edition: 1st59 Edition: 1st-5, 2012.

[8] P. Prajapati, *A handbook of medicinal plants, a complete source book*. Jodhpur: Agrobios, 2003.

[9] J. J. Woessner, "The determination of hydroxyproline in tissue and protein sample containing small proportions of this imino acid," *Arch. Biochem. Biophys.*, vol. 93, pp. 440-447, 1961.

[10] C. Rondle and W. Morgan, "The determination of glucosamine and galactosamine," *J. Biochem.*, vol. 61, pp. 586-589, 1955.

[11] S. McLennan, J. Bonner, and S. Milne, "The anti-inflammatory agent propolis improves wound healing in a rodent model of experimental diabetes," *Wound Repair and Regeneration*, vol. 16, pp. 706–713, 2008.

[12] S. Suthar, R. Patel, and V. Kumar, "Wound healing activity of aqueous extract of leucas urticifolia leaves in experimental animals," *IJRPS*, vol. 1, pp. 141-150, 2011.

[13] O. H. Lowry, N. J. Rosebrough, and A. Farr, "Protein measurement with the folin phenol reagent," *J. Biol. Chem.*, vol. 193, pp. 265-275, 1951.

[14] S. Joseph and W. R. David, *DNA extracted by phenol-chloroform-protenase-K method. Molecular cloning*, 3rd ed. NY, USA: Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 2001.

[15] M. Robson, B. Stenberg, and J. Heggers, "Wound healing alterations caused by infection," *Clinics in Plastic Surgery*, vol. 17, pp. 485–492, 1990.
[16] Maria L. Rizzo, *Statistical computing with R*: Chapmen & Hall/CRC, ISBN-10: 1584885459 EISBN-10: 1584885459 Edition: 1stdISBN-10: 1584885459 Edition: 1stiISBN-10: 1584885459 Edition: 1sttion: 1st, November 15, 2008.
[17] E. K. Legrand, J. K. Burke, and D. E. Costa, "Dose responsive effects of PDGF-BB, PDGF-AA, EGF and bEGF on granulation tissue in guinea pig partial thickness excision model," *Growth Factors*, vol. 8, pp. 307-311, 1993.
[18] A. M. Delaney and C. E. Constance, "Post-transcriptional regulation of collagenase and stromelysin gene expression by epidermal growth factor and dexamethasone in cultured human fibroblasts," *J. Cell Biochem.*, vol. 50, pp. 400-410, 1992.
[19] R. Ross, E. W. Raines, and D. F. Bowen-pope, "The biology of PDGF," *Cell*, vol. 46, pp. 155-169, 1986.
[20] S. P. Blatti, D. N. Foster, and G. Ranganathan, "Induction of fibronectin gene transcription and m RNA is a primary response to growth factor stimulation of AKR-2B cells," *PNAS (USA)*, vol. 85, pp. 1119-1123, 1988.