RESEARCH ARTICLE

WOUND HEALING PARAMETERS AT DIFFERENT TIME INTERVALS IN EXCISION WOUNDS OF RABBIT

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ABSTRACT

Wound healing is a fundamental response to tissue injury and several integrated cellular and biochemical events are set in motion immediately after injury leading to re-establishment of structural and functional integrity with regains of strength of injured tissue. Present study was under taken to study the accumulation of different biochemical molecules at the wound site at specific time intervals. 36 clinically healthy rabbits in the age group of 3-9 months were used as experimental animals. Wound contraction was recorded on day 2, 4, 6, 8, 10, 12 and 14 after creation of wound. Biochemical parameters hydroxyproline, glucosamine, protein and DNA was estimated at 1 hour, 3 hour, 6 hour, 24 hours, 5 days, 8 days, 11 and 14 post wounding days. There was a decrease in the wound area from day 2 and this trend continued up to the last observation of the study. On day 14 there was 100% contraction of the wound area. There was also an increase in the accumulation of hydroxyproline and glucosamine at the wound site from 1 hour and this trend continued up to last day of observation. The maximum accumulation of hydroxyproline and glucosamine, 17.20 ± 0.82 mg and 7.16 ± 0.52 mg respectively was observed on day 14. There was also an increase in protein and DNA content at the wound site from 1 hour. The peak in the accumulation of protein and DNA, 6.82 ± 0.14mg and 5.47 ± 0.18 mg respectively was observed on day 11 and a decrease on day 14, 5.30 ± 0.17 mg and 4.99 ± 0.31 mg. We observed complete healing on 14th post wounding day indicating active proliferation and remodeling that resulted in the reduction of wound size. There was increase in the hydroxyproline and glucosamine from 1 hour and this trend continued up to 14 days after creation of the wound, the increase in hydroxyproline content is the reflection of increased level of collagen levels, which indicates better maturation and proliferation of collagen while glucosamine is an essential moiety of proteoglycans.

INTRODUCTION

Wound may be defined as the disintegration of cellular and anatomic continuity of a tissue and may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue. Wound healing is a process by which a damaged tissue is restored to its normal state and wound contraction is the shrinkage of the wound area (Singh, et al., 2005). In normal skin, the epidermis and dermis exists on steady-state equilibrium, forming a protective barrier against external environment. Once this protective barrier is broken, the normal process of wound healing is set in motion. Wound healing can be divided in two major phases early phase and cellular phase (Ngyuen, et al., 2009). The early phase begins immediately after injury involves cascading molecular and cellular events leading to homeostasis and formation of an early, makeshift extracellular matrix, thereby providing structural support for cellular attachment and subsequent cellular proliferation. The cellular phase follows the early phase and involves several types of cell working together to mount an inflammatory response, synthesize granulation tissue and restore the epithelial tissue. These steps are orchestrated in controlled manner by a variety of cytokines including growth factors. Some of these growth factors like platelet derived growth factor (PDGF), transforming growth factor-β (TGF-β), fibroblast growth factor (FGF) and epidermal growth factor (EGF) etc. have been reported to stimulate various aspects of wound healing viz keratinocytes migration, cellular proliferation, DNA synthesis, wound contraction 'Tsobo and Rifkin. 1999', ECM synthesis, glycosaminoglycans and collagen synthesis (Pierce, et al., 1989.).

MATERIALS AND METHODS

Animals: 36 clinically healthy rabbits in the age group of 3-9 months were used as experimental animals, out of these 12 were used for contraction studies and 24 for biochemical analysis. All the animals were housed in the cages had free access to fresh water at a room temperature of 22±20C. A balanced feed was used throughout the period of study. The experimental protocols

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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.

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 1x1cm² (100mm²) 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.

Tissue collection

Tissue of appropriate quantity was excised from each wound at time intervals of 1 hour, 3 hour, 6 hour, 24 hours and then at a gap of two days up to 14th post wounding day to estimate some biochemical parameters like hydroxyproline, glucosamine, protein and DNA.

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 2, 4, 6, 8, 10, 12 and 14 after creation of the wound.

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 (Woessner, 1961) and glucosamine (Rondle and Morgan, 1955).

Protein extraction and estimation

The tissue (30 mg) already stored at -800 C was pulverized in ice cold lysis buffer containing 100 mM Tris-Hcl, 0.05 mM EDTA with a proportion of 300 l of lysis buffer/30 mg tissue with the help of chilled pestle and motor 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 (Lowry, 1951).

DNA extraction and estimation

DNA was extracted and estimated by phenol-chloroform method with some modifications (Sambrook and Russell, 2001).  
- Homogenize the tissue in liquid nitrogen with the help of motor pestle.
- Add ice cold 600 l cell lysis buffer (10mM Tris-HCl, pH=8 i.e. 0.3028gm/250ml, 1mM EDTA i.e., 0.09306gm/250ml, 0.1% SDS i.e., 0.25gm/250ml) and mix thoroughly by vortexing.
- Add Proteinase K (20mg/ml) @ 2.5 l, incubate at 650C for 1 hour followed by 15 min. at 950C (or incubate at 500c overnight).
- Add equal vol. of Saturated Phenol (pH 8.0), mix thoroughly by gentle inversions for 10 min.
- Centrifuge at 14,000 rpm for 5 min. at 40C.
- Collect the supernatant and air dry DNA.
- Add 150μl of potassium acetate (60ml of potassium acetate & 28.5ml DH2O) and mix thoroughly.
- Add equal volume of Isoamyl alcohol or Isopropyl alcohol.
- Centrifuge at 14,000 rpm for 5 min. at 40C.
- Remove carefully the supernatant and air dry DNA pellet.
- Add 100 l of 1X TAE buffer and dissolve the pellet at 650C for one hour (or keep overnight at room temperature).
- The DNA samples are alliqouted and stored at -200C for future use.

Concentration of DNA was detected by Spectrophotometer (Hitachi-U-1800)

Statistical analysis: Analysis was done by Students t test using R software (Maria, 2008).

RESULTS

Table 1 indicates the trend in wound area from day 2 and this trend continued up to day 14 after creation of the wound. There was a decrease in the wound area from day 2 and this trend continued up to the last observation of the study. On day 2 the animals had wound area of 108 ± 0.46 mm² as against 116 ± 0.09 mm². On day 14 the wound area was 0.00. On day 14 there was 100% contraction of the wound area. There was also an increase in the accumulation of hydroxyproline and glucosamine at the wound site from 1 hour and this trend continued up to last day of observation.

<table>
<thead>
<tr>
<th>Time duration</th>
<th>Wound area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>116 ± 0.09</td>
</tr>
<tr>
<td>Day 2</td>
<td>108± ±0.09</td>
</tr>
<tr>
<td>Day 4</td>
<td>98.7 ± 0.89</td>
</tr>
<tr>
<td>Day 6</td>
<td>92.2 ± 0.72</td>
</tr>
<tr>
<td>Day 8</td>
<td>70.5 ± 0.67</td>
</tr>
<tr>
<td>Day 10</td>
<td>48.1 ± 0.91</td>
</tr>
<tr>
<td>Day 12</td>
<td>22 ± ±0.43</td>
</tr>
<tr>
<td>Day 14</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SEM, n=12 animals in each group.
The maximum accumulation of hydroxyproline and glucosamine, 17.20 ± 0.82 mg and 7.16 ± 0.52 mg respectively as depicted in table-2 was observed on day 14. There was also an increase in protein and DNA content at the wound site from 1 hour. The peak in the accumulation of protein and DNA, 6.82 ± 0.14mg and 5.47 ± 0.18 mg respectively was observed on day 11 and a decrease on day 14, 5.30 ± 0.17 mg and 4.99 ± 0.31 mg as shown in table 2.

DISCUSSION

Wound contraction is the movement of wound edges towards each other in a centripetal fashion (Tejero, 2001). Measurement of wound contraction is an important tool to ascertain the progress of wound healing. Wound repair starts with the migration and proliferation of keratinocytes forming a new epithelial layer (Falanga, 2005). The excision wound may undergo 80-90% reduction in mammals other than humans in 10-12 days (Rudolph, 1979). WE observed complete healing on 14th post wounding day indicating active proliferation and remodeling that resulted in the reduction of wound size. There was increase in the hydroxyproline and glucosamine from 1 hour and this trend continued up to 14 days after creation of the wound, the increase in hydroxyproline content is the reflection of increased level of collagen levels, which indicates better maturation and collagen remodeling. These two along fibronectin are the major constituents of extra cellular matrix (Deodhar and Rana, 1997). There was also an increase in the protein and DNA content from 1 hour to 11th day after creation of the wound. Protein and DNA during shortens the inflammatory phase; promote collagen and proteoglycan synthesis, wound remodeling and tensile strength of the wound (Chernof, R. 2004).

CONCLUSION

The maximum incorporation of protein and DNA occurred on the 11th day after creation of the wound. It is not known why the peak in incorporation occurred at this point; it is possible that the need for new proteins and DNA is high and the wound size has not decreased enough to increase the level of inhibition that may occur with contact inhibition.

References

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