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Effect of heparin on wound healing

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Abstract. Heparin with its ability to dissolve the fibrin clot exerts its major effect in the early stages of wound healing by depriving the fibroblasts of their scaffold. Heparin inhibits cross linking of collagen and accelerates its degradation. There is faulty orientation of the collagen fibrils in the heparinized wound. It may be concluded that heparin interferes with wound healing.

Keywords. Heparin; collagen; glycosaminoglycans; wound healing.

Introduction

Many factors have an impact on the healing process from the initial inflammatory reaction to the final maturation of the fibrous scar. The tensile strength gained by the scar may also be due to collagen interactions with mucopolysaccharides which may stabilize the fibres and also control their size or arrangement. Heparin is usually used in the treatment of myocardial infarction. Follow-up studies have shown that there is greater risk of ventricular aneurysm developing when myocardial infarcts are treated with heparin (Anguli and Anantachari, 1980). It is intriguing to speculate whether it would interfere with normal healing process, inasmuch as heparin not only inhibits coagulation and fibrin formation, but has been implicated in osteoporosis due to its stimulation of collagenolysis. If there is a failure in the formation of strong scar tissue in sufficiently quick time, there is the possibility of endangering the myocardial fibres resulting in a liability for aneurysmal weakening. This on theoretical grounds will be an interference in subsequent progress during convalescence. The present study was undertaken to evaluate the efect of heparin on the healing of wounds made in the skins of rabbits. Biochemical and histopathological investigations were carried out.

Materials and methods

Chemicals

Heparin, L-hydroxyproline, glucosamine hydrochloride, acrylamide, methylene bis-acrylamide, sodium dodecyl sulphate and N,N,N',N'-tetramethylethylenediamme were purchased from Sigma Chemical Co., St. Louis, Missourie, USA.

Animals and treatment

Healthy, male albino-rabbits, about two months old, weighing between 0·5 and 0·6 kg were used for the experiment. They were divided into two groups of 12

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animals each. The experimental group was injected with heparin (200 units/kg body weight) subcutaneously, twice a day for 10 days starting 3 days before the making of wounds. The control group was maintained to study the normal healing process. All animals were fed commercial pellets manufactured by Hindustan Lever Ltd., Bombay. Water was given *ad libitum*.

The posterolateral aspect of the gluteal region of the two hind limbs were chosen as sites for incision. An area of about 3 inches in diameter was shaven and prepared. Identical incisions, about an inch long and skin deep, were made on the sites with minimal bleeding. The wounds were approximated by finger pressure. Plain ether was used to anaesthetise the animals for the operative procedure. Every third day biopsies were made across the centre of the incised wound and for every biopsy a different wound was used. Protein was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as Standard.

Extraction of total collagen

Total collagen was extracted by homogenizing the tissue with 5% (w/v) trichloroacetic acid and heating the homogenate in a water bath at 90°C for 30 min. The supernatent was hydrolyzed with 6 N HCl at 110°C for 24 h, neutralized and hydroxyproline estimated by the method of Neuman and Logan (1950). The collagen content was calculated by multiplying the hydroxyproline content by the factor 7·46 (Neuman and Logan, 1950).

Extraction of soluble collagen

Soluble collagen was extracted by the method of Piez *et al.* (1963). The tissue was stirred with 0·5 N acetic acid for 48 h. The solution was hydrolyzed with 6 N HCl at 110°C for 24 h, neutralized and hydroxyproline estimated as in the case of total collagen. Insoluble collagen content was obtained by subtracting soluble collagen from total collagen content.

Extraction of ground substance

Ground substance was extracted by stirring the tissue with 0·5 N acetic acid containing 1 in 50 parts of pepsin for 24 h. The solution was then hydrolyzed with 2 N HCl at 110°C for 6 h. The hexosamine content was estimated by the method of Elson and Morgan (1933) as adopted by Gunnar Blix (1948).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis

The α- and *β*-chains of collagen were separated by subjecting denatured collagen samples to sodium dodecyl sulphate-polyacrylamide gel electrophoresis using 5% gel as described by Furthmayr and Timpl (1971).

Histopathological studies

The biopsy specimens were fixed in 10% formalin. All sections were stained with hematoxylin-eosin.

Results

Figure 1 shows the protein content of normal and heparinized wounds over a period of 18 days. The protein content of heparinized wounds is significantly lesser (P <0·01) than that of control wounds.

The total collagen content of the control animals reaches a peak in 9 days, gradually decreases upto 15 days and then maintains a constant level. In the heparinized wounds the peak, which is much lower than the control, is reached in 12 days and then maintained at that level (figure 2).

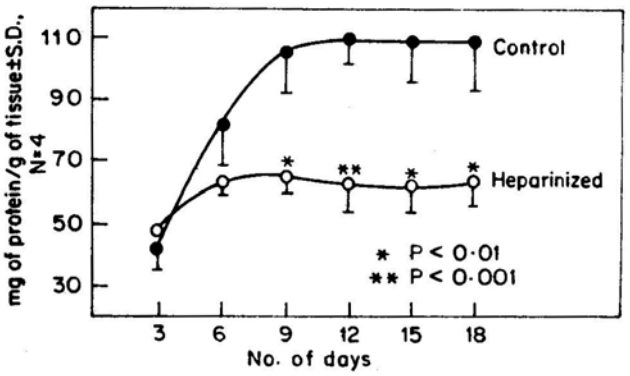


Figure 1. Protein content of normal and heparinized wounds for a period of 18 days.

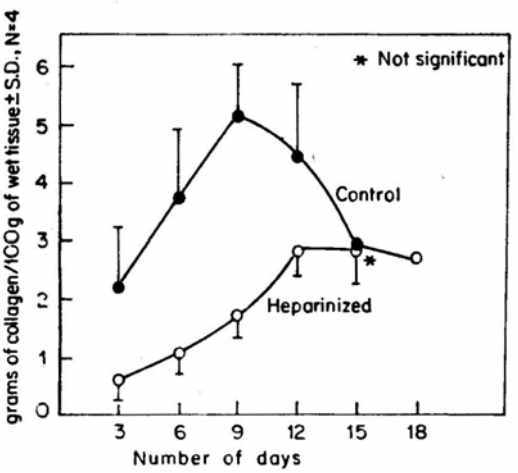


Figure 2. Total collagen content of normal and heparinized wounds.

Table 1 shows per cent ratio of soluble to insoluble collagen in normal and heparinized wounds. The large increase in soluble collagen over the insoluble or more cross linked collagen in heparinized animals can be clearly seen in the data presented.

Table 1. Per cent ratio of soluble to insoluble collagen in normal and heparinized wounds.

	$\frac{\text{Soluble collagen}}{\text{Insoluble collagen}} \times 100$	
Days	Control	Heparinized
3	101·79	556·70
6	57·45	606·67
9	43·47	576·00
12	14·41	142·86
15	13·39	67·06
18	9·27	59·41

The amount of ground substance was judged by measuring the hexosamine contents of the wounds of control and heparinized animals. The hexosamine content of normal wounds follows the expected pattern. As the collagen content increases the hexosamine content decreases. A significant increase in hexosamine content is noted in heparinized animals (figure 3).

Figure 4 shows the sub-unit pattern of collagen of control and heparinized animals. There is a significant decrease in the β component in heparinized animals.

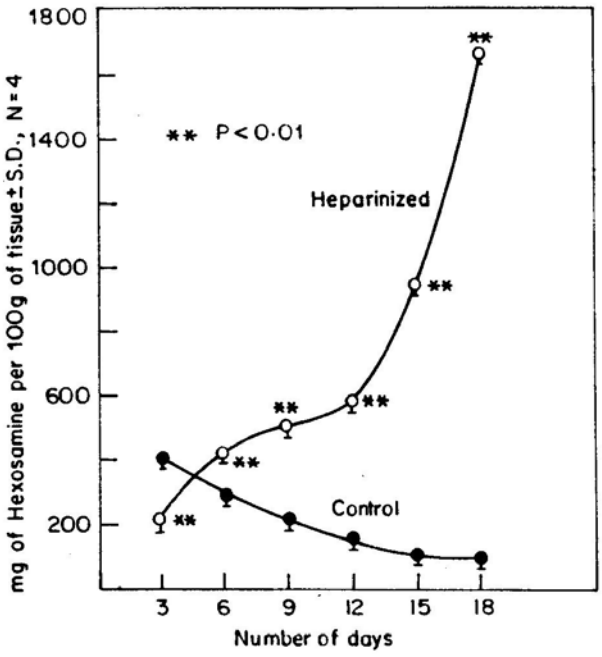
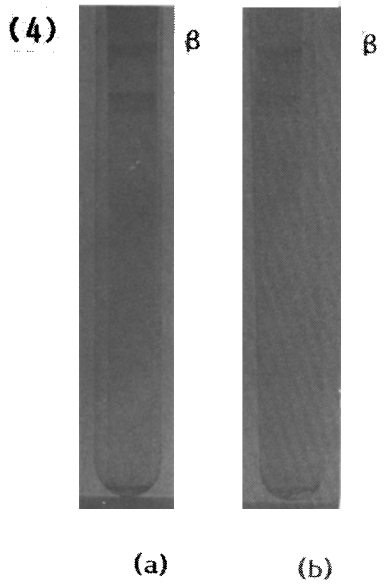
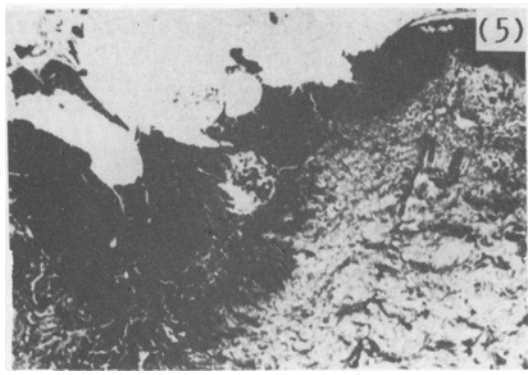
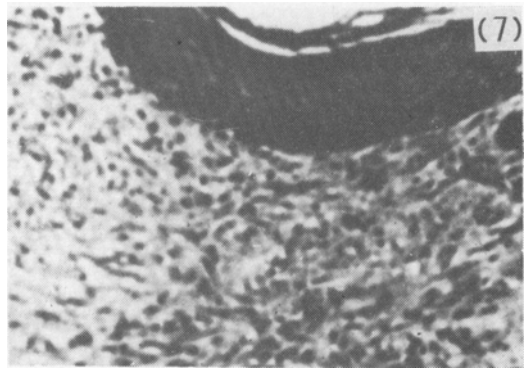
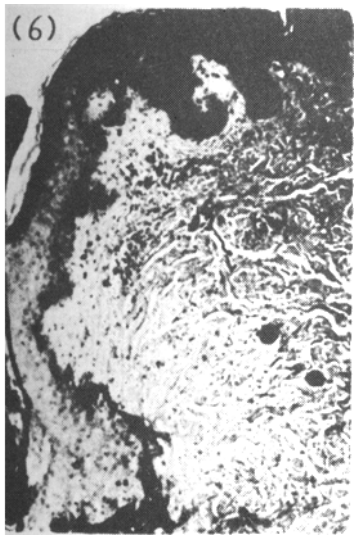


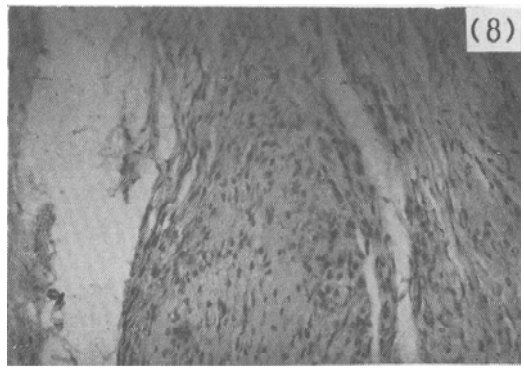
Figure 3. Hexosamine content of normal and heparinized wounds during the process of healing.











Figures 4–8. 4. Sodium dodecyl sulphate-polyacrylamide gel electrophoretic subunit patern of collagen from control (a) and heparinized (b) wounds. **5.** Section of normal wound, 48 h after wounding (H and E × 60). **6.** Section of a heparinized wound 48 h after wounding (H and E × 60). **7.** Section of normal wound at the end of the healing process (H and E × 120). **8.** Section of a heparinized wound at the end of the healing process (H and E × 120).

Histopathological findings

Figure 5 shows normal healing 48 h after wounding. The cavity of the wound is seen to be filled with clot and necrotic debris. Early inflammatory changes are seen in the periphery. Figure 6 shows that in the heparinized wound there is more oedema and oozing of blood than the control animals. The cavity of the wound is filled with clot. A vigorous downgrowth of the epidermis over the edge of the wound can be seen.

Towards the end of the healing process the normal wound shows mild keratinization of the epidermis. The inflammatory cells are sparse (figure 7). It is high power view presenting the young fibroblasts occupying the wound in a manner almost horizontal parallel to the epidermis. A section of the heparinized wound indicates that the fibroblasts are shrunken and the fibrous scar is nodular. The orientation of the collagen fibrils is vertical and perpendicular to the surface (figure 8).

Discussion

The protein content of the control wounds follows the expected pattern. In view of the fact that the animals were fed the normal diet which was not deficient in proteins, the fibroblastic stage has set in at the expected period. There is evidence that fibroblasts reach the area and begin to proliferate within 24 h of injury (Grinnell *et al.,* 1980). In the heparinized animals on the other hand the protein values are low because during the lag phase it would be only fibrin and epidermal cells which would be holding the wound. The fibroblasts use the fibers of the fibrin clot as a scaffold to move into and within the damaged area (Grinnell *et al.,* 1980). Heparin would dissolve the fibrin and thus deprive the fibroblasts of their scaffold. While, fibrin is a good scaffold for fibroblast migration (Ross and Benditt, 1961) large amounts of fibrin would certainly inhibit the movement of fibroblasts and epithelial cells. On the other hand, heparin would prevent fibrin formation but it would help mobilization and migration of epithelial cells. It would help remove debris and necrotic tissue. The relative value of heparin as a help or hindrance in wound healing would thus seem to be rather delicately balanced and nothing unequivocal can be said.

It is known that collagen accumulation is the sum of synthesis and destruction and both occur simultaneously during the wound healing process (Minor, 1980). By the fifth day the fibroblasts lay down large quantities of collagen (Ross and Benditt, 1961; Kurkinen *et al.,* 1980). The low collagen content in heparinized wounds is due to decreased collagen synthesis. It is obvious that heparin influences the turnover rate of collagen.

Even though soluble collagen represents mainly newly synthesized collagen (Jackson and Bentley, 1960), the degradation of insoluble collagen may also contribute to the amount of soluble collagen (Prockop and Kivirikko, 1968). The collagenases involved in the degradation come from epithelial cells (Grillo and Gross, 1967), from fibroblasts encountering the new epithelium (Perez-Taymo, 1978; Welgus *et al.,* 1980) and from macrophages containing lysosomes (Perez-Taymo, 1978). It is known that heparin activates the enzyme collagenase (Sakamoto *et al.,* 1974) thus resulting in conversion of insoluble to soluble collagen. The sequential synthesis of collagen can be represented as precursor→soluble collagen ⇄ insoluble collagen. The results suggest that heparin interferes to a small extent only, in the first phase of

collagen synthesis, but interferes to a large extent in the conversion of soluble to insoluble collagen.

The gain in strength of scar tissue comes from the rearrangement of the collagen in the wound and perhaps from increased crosslinking of the collagen (Minor, 1980). The low insoluble collagen content in the heparinized wound suggests that heparin interferes with the cross linking of collagen besides activating the enzyme collagenase.

Since *ß*-component is a dimer of α-chains, a decrease in *ß*-component indicates a decrease in intra-molecular cross linkages (Golub *et al.,* 1978). Sodium dodecyl sulphate-polyacrylamide gel electrophoretic pattern confirms that cross linking is impaired in heparinized animals.

For the first few days after an injury fibroblasts synthesize and secrete ground substance materials including proteoglycans, glycosaminoglycans and fibronectin (Peacock and van Winkle, 1976). There is no evidence that mucopolysaccharides or other elements of the ground substance make any contribution to tensile strength. There is overwhelming evidence that almost all the tensile strength of the wound is due to the collagen content (Oxlund and Andreassen, 1980). Collagen content of the wound increases rapidly following the lag period and as the collagen content increases the hexosamine content of the tissue declines (Alitalo *et al.,* 1980). Mucopolysaccharides are involved in the extracellular formation of collagen fibrils and in collagen maturation (Highberger *et al.,* 1951; Jackson *et al.,* 1960; Watts *et al.,* 1964; Jackson, 1970). Since heparin itself is a component of the ground substance the large increase in the hexosamine content of heparinized wounds is inevitable. There is evidence that heparin stimulates proteoglycan synthesis by arterial smooth muscle cells (Wight, 1985). It remains to be determined whether heparin stimulates proteoglycan synthesis by fibroblasts or not. Nevertheless a large amount of ground substance would hardly leave any space for collagen fibrils to be laid, which is the usual way that a wound starts healing.

Histopathologically it can be seen that in the heparinized wounds there is a vigorous downgrowth of the epidermis due to the abundant haemorrhagic exudates which pulls down the epidermis. There is evidence that if the orientation of the collagen fibrils is improper, tensile strength remains at a low ebb (Stearns, 1940). The vertical orientation of the collagen fibrils in the heparinized wounds as against the horizontal orientation in normal wounds would decrease the tensile strength of the integument.

In the light of the above discussion, it is possible to speculate that heparin interferes with wound healing at every stage. It inhibits cross linking of collagen and accelerates degradation thus interfering with the formation of normal, mature and strong collagen which explains why the aneurysms result in fatal ruptures.

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