Heparin and Acute Inflammation in the Rat

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Abstract

The subcutaneous injection of heparin into the dorsal cervical region of rats 30 min before the intrapedal administration of carrageenin significantly impaired the local inflammatory reaction from 2 h through to 6 h. This observation implies that the release of endogenous heparins from tissue mast cells may play a role in modulating the extent of the response to inflammatory insults.

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Allergic and inflammatory reactions involve increased capillary permeability and oedema. Thus, damaged extravascular areas are selectively bathed by plasma released from the circulation via the secretion of mast cell amines. This displaced plasma contains the protein Hageman factor (clotting factor XII) which on contact with the wettable surfaces of non-endothelial cell tissues is activated to generate the kinin and complement systems as well as the blood clotting cascade [1]. Once generated, these systems contribute to the allergic and inflammatory phenomena by further increasing capillary permeability and initiating movement of inflammatory cells. However, mild injury consisting of only mast cell discharge and capillary leakage does not necessarily lead to kinin and complement activation.

In addition to histamine, mast cells also contain heparins [2] which are released on stimulation of the cell. This family of sulphated glycosaminoglycans prevents blood clotting by activating antithrombin III, a naturally occurring inhibitor of factor XIIa and other serine proteases. Thus, it is possible that heparins released from mast cells may play a modulatory role in determining the severity of the response to tissue damage, by limiting the reaction to a histamine-like triple response. Continuation of the injurious insult may deplete mast cell heparins, at which time the kinin, complement and fibrin systems of the extravasated plasma may be activated via Hageman factor, leading to a continuing and enhanced response.

Heparin has been demonstrated to be of clinical value in external otitis, hay fever and asthma, actions attributed to the attenuation of histamine activity [3, 4]. Additionally, prior intravenous injection of heparin inhibits inflammation in the rat's foot measured 3 h after the local injection of carrageenin, an action attributed to impairment of fibrin formation [5].

Carrageenin-induced rat oedema involves the sequential release or activation of mast cell amines, Hageman factor derived products and eicosanoids [6], and we have used this technique to examine the anti-inflammatory effects of heparin.

Groups of 12 female Wistar rats were pre-treated by a subcutaneous injection into the dorsal cervical region with either sterile 0.9% saline or heparin (6, 60 or 600 U/kg; CP Chemicals, Wrexham, UK) 30 min before the injection of carrageenin (Marine Colloids Ltd., USA; 1 mg in 0.1 ml sterile saline), prepared by the method of Butts and Rehm [7], into the plantar region of

the right hind-foot. The left hind-foot received 0.1 ml saline. The inflammatory response in the right foot was measured by monitoring the paw thickness, to the nearest millimetre, with vernier calipers (Matui, Japan) and comparing it with the contralateral foot. Blood clotting time was determined by the method of Dale and Laidlaw [8].

The biological half-life of heparin is approximately 69 min [9], but the measurement of the whole blood clotting time in groups of 5 animals showed that at 600 U/kg its anticoagulant properties lasted

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0.7 η

Time, h

Fig. 1. Effect of prior (30 min) subcutaneous (dorsal cervical) administration of saline or heparin on carrageenin (1 mg)-induced local oedema in the hind-paw of the rat. The values shown are means from 12 observations $\pm$ standard error bars. Asterisks indicate that the oedema in the heparin-pre-treated animals is significantly different from that in the control rats: * $p < 0.05$, ** $p < 0.01$; one-tailed Student's *t* test for non-paired data. O = Carrageenin and saline pretreatment; □ = carrageenin and 6 U/kg heparin pre-treatment; • = carrageenin and 60 U/kg heparin pretreatment.

over 6 h (control clotting time, 209 ± 15 s; 2 h after heparin, 344 ± 24 s; 6 h after heparin, 240 ± 7 s). At doses of 60 and 600 U/kg, heparin significantly inhibited the inflammatory response of the foot to carrageenin from 2 h through to 6 h. Figure 1 shows that at 5 h 60 U/kg heparin inhibited the response by approximately 30% compared to the control animals at this time; 600 U/kg produced a similar degree of inhibition (not illustrated). A dose of 6 U/kg significantly reduced the inflammatory reaction at 2 h, but inhibition at later times did not reach statistical significance.

Di Rosa et al. [6] have demonstrated that the first 90 min of carrageenin foot oedema is associated with histamine and serotonin release, and that the reaction is then maintained for the next hour by kinins, after which a third phase (2.5–6 h), involving eicosanoids, sustains the response. Cellular migration of poly-morphs into the inflamed foot begins at about 2 h [10]. Alongside this, the time course of inhibition by heparin indicates that its effects are associated with inhibition of vascular-derived mediators, such as kinins, complement and clotting cascade systems, and not to the binding of endogenously released histamine [3].

Since carrageenin itself impairs fibrin formation [11], it is unlikely that heparin owes its anti-inflammatory effect to impairment of fibrin formation [5]. These data suggest that heparin

reduces carrageenin foot oedema by impairment of Hageman factor, and therefore imply that endogenous heparin may play a modulatory role in determining the degree of response to inflammatory insults.

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