

A Study of Anti-inflammatory effect of Heparin and Enoxaparin on Experimentally Induced acute Inflammation in Albino Rats.

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Abstract:

Objective: To evaluate and compare anti-inflammatory effect of Heparin and Enoxaparin with the standard drug Diclofenac sodium.

Methodology: Rat paw edema induced by carrageenan was the experimental model. Albino rats weighting 130 to 180 grams of either sex were taken and divided into 4 groups of 6 rats each. Heparin 800 units/kbw was given to test group I, Enoxaparin 200 mcg/kbw was given to test Group II, Diclofenac sodium 25mg/kbw was given to standard group and 0.5ml normal saline was given to control group. Rats were injected intraperitoneally (ip) with respective drugs 30minutes prior to administration of carrageenan. 0.1ml of 1% carrageenan was injected into the sub-plantar space of rat right hind paw. Rat paw volumes were measured at 0, 1, 2, 3 hours with the help of mercury plethysmometer.

Results: Anti-inflammatory effect is expressed as percentage of inhibition of rat paw edema. Percentage of inhibition of rat paw edema at 3hours was 66% with Enoxaparin and 51% with Heparin and 78% for Diclofenac sodium.

Conclusion: Enoxaparin and heparin have significant anti-inflammatory activity and enoxaparin shows more anti-inflammatory effect than heparin but less than Diclofenac sodium.

Keywords: Anti-inflammatory effect, Carrageenan, Enoxaparin, Heparin, Rat paw edema.

I. Introduction

Inflammation is basically a protective response against tissue damage either due to infection or local injury. Without inflammation infection would go unchecked, wound would never heal and become chronic. When inflammatory response is far greater than protective response, it would cause damage to the tissues, such inflammation can be controlled mainly either by non steroidal anti-inflammatory drugs or steroids. In spite of availability of large number of anti-inflammatory drugs there is continuous search for newer drugs.

Cost and time incurred on development of a new drug becomes waste if it is withdrawn from the market, as seen in the case of Rofecoxib a cox-II inhibitor approved by U.S. FDA in 1999 and withdrawn from market in 2004. Number of drugs being approved per year by regulatory authorities has declined over the years. These difficulties introduced a concept of searching for new therapeutic uses for an established drug.

Heparin has been used as an anticoagulant since 1937. It has wide range of biological effects including anti-inflammatory effects. Enoxaparin is a low molecular weight heparin, possess anti-inflammatory action. Heparin and Enoxaparin were found to reduce the leukocyte recruitment into the site of injury or inflammation by down regulating TNF α induced leukocyte rolling, adhesion and migration. Heparin inhibits adhesion of leukocyte to endothelial cells without altering expression of adhesion molecules.[1]

In this study carrageenan induced rat paw edema was used as an experimental model for acute inflammation. The parameter observed was percentage of inhibition of rat paw edema. Statistical analysis revealed that inhibition of rat paw edema was seen with Heparin and Enoxaparin. Percentage of inhibition of rat paw edema with enoxaparin was more than heparin.

II. Methodology

This study was conducted in the Dept. of Pharmacology, Osmania Medical College, Hyderabad. A pilot study was done to decide anti-inflammatory dose of heparin and enoxaparin. This dose is much lower than the anticoagulant dose. Experimental protocol was presented to Institutional Animal Ethics Committee and approval was taken. Chemicals used are Carrageenan to induce inflammation, Diclofenac sodium is the standard drug. Heparin and enoxaparin are the test drugs.

Carrageenan induced hind paw edema in rats is the experimental model used in this study to evaluate anti-inflammatory effect of heparin and enoxaparin.[2] Albino rats weighting 130 to 180 grams of either sex were taken for the experimental study. They were allowed free access to food and water ad libitum. They were

divided in to four groups of six rats each. Four groups were test group-I, test group-II, standard group and control group.

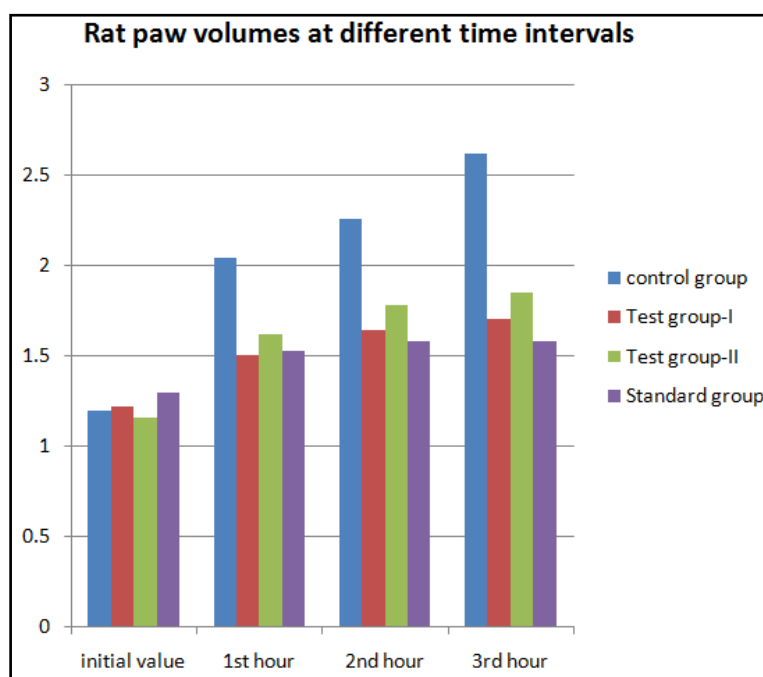
Animals were identified and an anatomical marking made at the level of the malleolus of the right hind paw, in order to a fix a constant level, up to which the animal paw must be dipped in the mercury plethysmometer , to measure rat paw edema. Diclofenac sodium 25 mg/kbw, Heparin 800 units/kbw, Enoxaparin 200 mcg/kbw and 0.5ml of normal saline was given intraperitoneally to Standard group, test group-I, test group-II and control group respectively. After 30 min, 0.1ml of 1% freshly prepared solution of carrageenan was injected in to subplantar space of rat hind paw to induce inflammation. Rat paw volumes were measured just before (0 hour) and at end of 1, 2, 3 hours after carrageenan injection. The difference in the paw volume was taken as edema. Values were tabulated.[3]

Percentage of inhibition of rat paw edema= $(1-(Vt/Vc)) * 100$. Vt is difference in paw volume in test group, Vc is difference in paw volume in control group.[4]

III. Results

Rat paw volumes are tabulated and compared by using ANOVA. P-value was (<0.05) significant.

| Groups | Initial Paw Volumes (ml) | After induction of inflammation with 0.1 ml of 1% carrageenan | | |
|----------------|--------------------------|---|------------------------------------|------------------------------------|
| | | At the end of 1 hour | At the end of 2 nd hour | At the end of 3 rd hour |
| Control Group | 1.20±0.11 | 1.62±0.15 | 2.26±0.21 | 2.62±0.16 |
| Test Group I | 1.22±0.13 | 1.50±0.07 | 1.64±0.14 | 1.70±0.09 |
| Test Group II | 1.16±0.17 | 1.62±0.11 | 1.78±0.19 | 1.85±0.16 |
| Standard Group | 1.30±0.09 | 1.53±0.15 | 1.58±0.21 | 1.58±0.14 |



Percentages of inhibition of rat paw edema:

| Groups | Initial paw volume | Paw volume at the end of 3 rd hour | Difference in paw volume (edema) | Percentage of inhibition paw edema: $(1-(Vt/Vc))* 100$ |
|----------------|--------------------|---|----------------------------------|--|
| Control Group | 1.20 | 2.62 | 1.42 | ---- |
| Test Group I | 1.22 | 1.70 | 0.48 | 66% |
| Test Group II | 1.16 | 1.85 | 0.69 | 51% |
| Standard Group | 1.30 | 1.58 | 0.31 | 78% |

IV. Discussion

In the recent past several animal experimental studies were conducted on anti-inflammatory effect of heparin on chronic inflammatory models, very few on acute inflammatory models. In this study, experimental model was carrageenan induced rat paw edema, an acute inflammatory model.

Inflammation induced by carrageenan is biphasic. Initial phase of inflammation is mediated by histamine, bradykinin and serotonin. Later phase of inflammation is mainly by prostaglandins.[4]

Histamine released from the mast cells at the site of injury contributes to an inflammatory response. Heparin binds with histamine and reduces the inflammation.[5] Heparin down regulates TNF α induced

leukocyte rolling, adhesion and migration in to the inflamed tissue.[6] Heparin binds to P and L selectins and block them there by prevent the leukocyte migration. 6-O-sulfated group of glucosamine is essential for interaction with P and L selectins. Heparin and enoxaparin posses this group.[6][7] Inhibition of angiogenesis is an additional property of heparin, helps to halt cancer progression.[8]

Analysis of paw volumes revealed that heparin and enoxaparin have anti-inflammatory effect. Anti-inflammatory dose is much less than anti-coagulant dose. Low dose heparin and low dose enoxaparin show anti-inflammatory action. Enoxaparin showed better anti-inflammatory action when compared to heparin, this may be because of better bioavailability and less avidity to cells and binding proteins. But anti-inflammatory effect of heparin and enoxaparin is less than the standard drug diclofenac sodium.

This study revealed that low dose heparin and enoxaparin have anti-inflammatory action but the limiting factor to use heparin as anti-inflammatory drug is heparin induced thrombocytopenia (HIT) . Heparin binds with platelet factor 4. This complex stimulates the immune system to produce IgG antibodies against it. IgG antibody, heparin and platelet factor 4 binds to Fc gamma IIa receptors on the surface of platelets and activate it. This leads to platelet aggregation and release of more platelet factor 4 and thrombin generation and thrombus formation. These antibodies cross react with low molecular heparin. 6-O-sulfated group of glucosamine is essential for both anticoagulant and anti-inflammatory action. 2-O, 3-O desulfation of heparin generates a potent anti-inflammatory agent with no anticoagulant activity.[6][7]. More research is required to prepare a tailor made molecule from heparin or enoxaparin, which is devoid of the side effect thrombocytopenia.

V. Conclusion

Heparin and Enoxaparin at lower doses than required for anticoagulation possess anti-inflammatory action. Enoxaparin shows better anti-inflammatory action than heparin and less than the Diclofenac sodium.

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