THE ROLE OF SINGLE DOSE HEPARIN IN PREVENTION OF THROMBUS FORMATION IN A NEW EXPERIMENTAL THROMBOSIS MODEL

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SUMMARY: Vascular thrombosis continues to remain a major problem in microvascular surgery. Various antithrombotic agents have been used in experimental models in order to study their influence on the incidence of thrombosis. A new microvascular thrombosis model which utilizes a crushing artery forceps on the rat femoral vessels was used to evaluate the effect of a single dose of heparin administered subcutaneously just prior to release of the crushing clamp. Patency across the traumatized segments was determined after 24 hours, by the "milking" test and by dividing the vessels downstream to the site of trauma.

In the arterial model there was an increase in patency from 83% to 96% and that in the vein model, from 35% to 89%. The results suggest that single dose heparin significantly increases patency rate in veins, in the immediate and early postoperative period. The arterial thrombosis model was however insufficiently thrombogenic and needs further evaluation.

INTRODUCTION

The major obstacle to successful anastomosis of small blood vessels is thrombosis. Despite advances in microsurgery small vessel thrombosis persists as a major cause of patient morbidity in 7 per cent of free tissue transfers. The thrombosis rates are higher in replantation surgery. Majority of thrombosis in microvascular surgery occur in the immediate and early postoperative period. Single dose heparin administered at the time of clamp release has been shown to reduce thrombosis in a microarterial thrombosis model. The "arterial inversion graft", the "crush-intimal abrasion and stasis" and the "arteriotomy/venotomy-intimectomy" are recently discussed models, used to evaluate the efficacy of antithrombotic agents. These models involve multiple influences, promoting thrombosis. We have developed a single, economical and easily reproducible model of microvascular injury having a single thrombogenic stimulus in the form of vessel wall trauma, inflicted by a crushing clamp. Using this model, this study evaluates the role of single dose heparin administered at the time of clamp release in both arteries and veins.

MATERIALS AND METHODS

The study population comprised of 48 adult male Wistar rats, each weighing between 250 and 300 grams. The rats were anaesthetized with sodium pentothal 30 mg/kg body weight, intraperitoneally. Under aseptic precautions, groin incisions were made and the femoral vessels exposed on both sides. Dissection was done with loupe magnification (6X). The right femoral artery and the left femoral vein were identified and their branches ligated and cut for a segment of 2 cms. A small crushing artery forceps (Speedways Surgical) was placed to occlude these vessels for thirty minutes. The vessels were placed in the third groove from the tip of the artery forceps, which was pressed up to the third ratchet (Fig. 1).
The same pair of identical artery forceps were used for the artery and vein in all the rats by the same operator. No clamps were applied to the right femoral vein and the left femoral artery. These vessels served as controls for the contralateral clamped vessels. The rats were divided into two groups, Group A (n=23) and Group B (n=25), using random numbers generated by a computer. The Group B rats were administered heparin 1000 IU/kg body weight, subcutaneously (Gland Pharma Pvt Ltd) immediately prior to release of the crushing clamp. All vessels were patent at the time of wound closure.

Postoperatively the rats were kept in separate chambers inside a room with temperature ranging between 25 and 30°C. Water and rat feed were available ad libitum.

After 24 hours the rats were re-explored. Presence of any haematoma was noted. The patency of the femoral vessels across the traumatised segment was assessed by the "milking" patency test and confirmed by incising the vessel downstream to the traumatised segments. These segments were then excised, washed in a jet of saline and preserved in 10% formalin. The rats were then sacrificed with an overdose of sodium pentothal. Several haematoxylin and eosin stained sections of the arterial and venous segments from a representative sample of 8 nonheparinised and 9 heparinised rats, were examined.

The chi square test was used for comparing the difference in patency rates caused by heparin in the vein model (Table 1). Fisher’s exact test was used for making a similar comparison in the arterial model, since a column value of less than 5 is encountered in Table 2.

Table 2
PATENCY AT 24 HOURS, IN THE ARTERIAL THROMBOSIS MODEL

<table>
<thead>
<tr>
<th>Group</th>
<th>No of rats</th>
<th>Patency Test site</th>
<th>Control site</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>23</td>
<td>19 (83%)</td>
<td>22 (96%)</td>
</tr>
<tr>
<td>(Without heparin)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>25</td>
<td>24 (96%)</td>
<td>25 (100%)</td>
</tr>
<tr>
<td>(With heparin)</td>
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RESULTS
Patency at 24 hours
Observations made on re-exploration of the rats 24 hours after the operation are listed in Tables 1 and 2. The femoral vein was found patent in 35% of rats in Group A against 80% in heparin treated rats (Group B). The chi square test found this difference statistically significant (p<0.01). The power of the test is 80%. In the vein model, the patency increased by 2.3 times in Group B. There was 83% patency rate, across the traumatised segment in the arteries. This increased to 96% on adding heparin (Fig.2). The difference was not statistically significant (Fisher’s exact test, p=0.18).
On re-exploration a small haematoma was found around the traumatised segment of the vein in two rats and both the artery and vein in one rat. These rats belonged to the heparin group. Occluding thrombus was found in two of the above veins.

Histology
The H&E stained transverse sections of the vessels from a sample of 9 heparin treated (Group B) and
8 untreated rats (Group A) were examined. Fifty transverse sections were prepared from each excised segment of 1 cm. Vessels that had no flow across them had sections which showed a completely occluding thrombus (Fig. 3). Most of the other vessels revealed a partially occluding thrombus (Fig. 4). An acute inflammatory reaction characterised by neutrophils in the perivascular tissue was seen at the site of clamping.

DISCUSSION

Virchow described thrombogenesis in terms of stasis, hypercoagulability and endothelial damage. All the three may affect microvascular anastomosis. In order to understand thrombogenesis, a number of models with a variety of vascular insults have been studied, but it remains a difficult problem to find an appropriate model for studying each thrombogenic factor in isolation. Different calibre vessels differ in their morphology, composition and physiology and therefore influence to a varying extent, the adherence of the thrombus to the vessel wall, its recanalisation and rethrombosis.

An accurate study of thrombogenesis in microvascular surgery, requires examining the effect of each thrombogenic factor on vessel patency. Our model of vascular injury created in the rat femoral vessel fulfils the criteria of an ideal model as proposed by Kersh et al.1,8.

Recent models that have been developed include the "arterial inversion graft" (AIG), the "crush-intimal abrasion and stasis" and the "arteriotomy/venotomy-intimectomy" models3,4. The AIG model utilises rat femoral artery segments which are excised, gently turned inside out and resutured into their native positions. Although this model mimics clinical repair, it is very difficult to imagine this happening in clinical practice. It relies on technical proficiency and other influences in the form of adventitia exposed to the blood, the suture material at the anastomosis site and trauma to the vessel wall. The model does not allow evaluation of the role of each one separately.

The models designed by Davidson et al and Salemark et al, too rely on microvascular technical proficiency and multiple thrombogenic influences such as the above3,4. These make the models difficult to reproduce and evaluation of each thrombogenic influence impossible.

Our study used a microvascular thrombosis model that is close to ideal1,8. The thrombogenic influence was the trauma inflicted on the rat femoral vessel by an artery forceps applied to it for a period of 30 minutes.

The vein model was sufficiently thrombogenic and can be utilised for evaluating antithrombotic agents, thrombolytic drugs or for developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thrombus. In the arterial model, although developing methods for early diagnosis of the evolving thromb
models, for use in microvascular surgery. Heparin has been tested in thrombosis models in microvascular surgery and found useful. Heparin dose(s) used in previous rodent models have been approximately 1000 IU/kg body wt. (Heparin sodium 1 mg = 130 I.U. of Heparin - Pharmacopoeia of India). Effects of a single dose heparin do not significantly differ from those of a constant infusion. In fact the side effects of heparin administration can be reduced by using a single dose. Further it seems that intra-arterial administration allows for lower doses and greater efficacy in maintaining vessel patency.

It may be concluded from our study, that single dose heparin used immediately prior to exposure of the damaged intima to blood flow, significantly reduces the incidence of occluding thrombus formation in veins. It appears that the arterial injury model was not sufficiently thrombogenic for the evaluation of antithrombotic properties of heparin.

Acknowledgement

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