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THE IMPORTANCE OF TOPICAL HEPARIN IN MICROVASCULAR ANASTOMOSES: A STUDY IN THE RAT

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It is obvious that surgical technique is the most important single factor in achieving patent microvascular anastomoses. But in clinical microsurgery where one tiny thrombus can cause so much destruction, many investigators have sought an adjuvant to technical expertise. Various anticoagulants, antiplatelet agents and vasodilators have been investigated and assessed (Daniel and Terzis, 1978; O'Brien, 1977).

Low dose subcutaneous heparin, shown to be effective (Kakkar *et al.*, 1971) against postoperative deep vein thrombosis, has not been tested in microsurgical practice. The project was designed *initially* to assess the effect of low dose subcutaneous heparin on patency rates.

CHOICE OF EXPERIMENTAL MODEL

Any benefit conferred by an adjuvant regime should be most apparent in difficult conditions. Therefore, (1) the vessels anastomosed should be small by microsurgical standards and (2) a free flap is preferable to simple division-and-anastomosis in situ, for patency is harder to achieve when flow in "large" vessels is reduced to supply only an island of skin.

An additional advantage of a free flap is that the result can be checked without re-exploration. Accordingly the lower abdominal flap in the rat was chosen, as described by Strauch and Murray (1967).

MATERIALS AND METHODS

Male and female Sprague Dawley rats, weighing approximately 300 G, were anaesthetised by ether inhalation.

A right lower abdominal flap was raised, based on the superficial epigastric vessels. A cuff of the femoral vessels was included by dividing them between ligatures distally and between clamps proximally. The accompanying branch of the femoral nerve was severed to allow complete detachment of the flap.

Replantation was then performed. The vessels were sutured end to end with 11/0 (Davis and Geck) monofilament nylon, 9 interrupted sutures for the artery and 12 for the vein, using the standard "triangulation" technique. A warm pack was then applied to the flap and the clamps released. After cessation of any anastomotic leakage, patency tests were performed and the skin sutured.

Ether for anaesthesia and heparin, as detailed below, were the only drugs

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used in this investigation. To prevent the animals from mutilating their flaps, they were fitted with plastic collars approximately 8 cm in diameter.

Flap survival was decided by its appearance at 10 days, though a judgment made at 4 days was found invariably to be correct.

To check that the technique was developed to an acceptable standard, a group of 10 flaps (Group 1) was replanted, using heparinised saline (10 i.u./ml) to irrigate the vessel ends, but no systemic heparin.

A double blind controlled trial was then launched giving either subcutaneous heparin 100 i.u./kg (0·3 ml of 100 i.u./ml) or an equal volume of normal saline 1 hour pre-operatively and 12 hourly post-operatively for three days. The vessel ends were meticulously irrigated with *normal saline only* as it was felt that heparinised saline applied locally might, in a small animal, have a significant systemic effect relative to the subcutaneous dose. However, after 10 flaps (Group 2) the trial was abandoned because of repeated failure.

Finally, to exclude the possibility that an unintentional factor had crept into the technique, a further 10 flaps (Group 3) were replanted using heparinised saline topically and no subcutaneous heparin, as in Group 1.

Results

The results are detailed in the table. When the vessel ends were irrigated with heparinised saline, flap survival was 75 per cent. With normal saline irrigation, flap survival was nil whether low dose subcutaneous heparin was used or not.

Results

	Flaps Survived	Flaps Necrosed
GROUP 1 (Heparinised saline irrigation; no subcutaneous heparin)	8	2
GROUP 2 (Normal saline irrigation: 5 subcutaneous heparin, 5 controls)	0	10
GROUP 3 (Identical to Group 1)	7	3

In each of the 30 flaps, immediate patency of the arterial anastomosis was achieved and venous return noted. In Groups 1 and 3 flow across the venous anastomosis was moderate or brisk at the time of skin closure whereas at this time in Group 2 venous flow was either sluggish or already obstructed.

Discussion

Clearly, any slight positive effect that the low dose subcutaenous heparin regime had on patency was swamped by the large negative influence of withholding heparinised saline irrigation (HSI) of the vessel ends.

Hayhurst and O'Brien (1975) performed rabbit femoral vein anastomoses without the use of anticoagulants, topical or systemic, and reported an early patency of 80 per cent. This achievement supported the omission of HSI in

the design of the present trial and at first sight is at variance with the results reported here. However, for the reasons outlined earlier, free flap anastomoses are more at risk than those of in situ division-and-anastomosis experiments and this may resolve the apparent conflict. In fact the same unit (O'Brien and Shanmugan, 1973) had earlier anastomosed the same (rabbit femoral) vessels in the free flap context, reporting 100 per cent survival "without supposed aids such as perfusion, anticoagulants or antibiotics", but later stated that "the open ends of the vessel were irrigated with heparinised saline".

The use of HSI in clinical microsurgery is commonplace. Perhaps this explains the failure to mention the irrigating fluid in a number of experimental studies and, as above, the tendency to overlook it as an antithrombotic aid.

Is the importance of HSI then underestimated? Certainly, the present results suggest it has a major role. The speculation is unavoidable that heparin forms a protective coat on the exposed endothelium and on other tiny thrombotic sites in the anastomotic zone not covered by endothelium.

More than 25 years ago, Samuels and Webster (1952) demonstrated that thrombosis in large veins in dogs was initiated by platelet adherence to intercellular cement lines and that this was abolished by pre-treatment with full dose systemic heparin. Their toluidine blue staining technique suggested that heparin may become attached to these same intercellular cement lines.

A search of the recent thrombosis research literature uncovers weighty supporting evidence of the affinity of endothelium for heparin.

Glimelius *et al.* (1978) using tritium-labelled heparin, have shown that heparin binds in a time dependent, reversible way to cultured human endothelial cells. They found that the amount of cell-bound heparin increased over 2 to 4 hours whereafter further binding was small. After 5 hours incubation in a heparin free medium about half of the cell associated heparin had been released.

Hiebert and Jaques (1976) injected rats with heparin and, using a quantitative biochemical technique, showed that isolated endothelium contained concentrations of heparin 30 to 7,500 times greater than had been present in the circulating blood.

In experiments in vitro Awbrey *et al.* (1975) showed binding of radioactive-labelled thrombin to human endothelial cells and, separately, to platelets, while Essien *et al.* (1978) have demonstrated that pre-treatment with heparin inhibited adherence of platelets to damaged or undamaged rabbit aortic endothelium, provided that the endothelium had been in contact with thrombin. If thrombin-endothelium contact was prevented, the number of platelets adhering was low and not influenced by heparin.

Conclusion and Recommendations

Although the results of the original experimental design to protect the patency of microvascular anastomoses with low dose subcutaneous heparin were negative, they serve to highlight the importance of heparinised saline irrigation of the vessel ends.

While the irrigation protects mechanically by evacuation of blood and blood products, it appears that the heparin protects pharmacologically by binding to the exposed endothelium. The effect of this adsorbed heparin is, possibly, to prevent formation of a thrombin coat which may be the prelude to platelet adhesion and thrombus formation. The possibility that small

thrombogenic areas not covered by endothelium, inevitable after even the finest of microvascular anastomoses, also become protectively heparin-coated remains speculative.

The results of this trial taken with the evidence summarised above lead to a number of recommendations:

1. As contact with thrombin may pave the way for platelet adherence:
   (a) the heparin should be applied immediately the "virgin" endothelium comes in contact with the extra-luminal environment (possibly, of course, only in elective surgery);
   (b) during the period of endothelial exposure to this environment, blood from the operating field should be fastidiously prevented from entering the open vessels.

2. Saturation of the heparin binding process may take more than 2 hours. With the aim of maximising the amount of adsorbed heparin:
   (a) HSI should be applied frequently and, particularly where clamps will not be removed immediately, just before inserting the final suture in the anastomosis;
   (b) the microsurgeon should consider increasing the heparin concentration of his irrigating fluid (e.g. from 10 i.u./ml to 50 i.u./ml).

SUMMARY

The importance of heparinised saline irrigation of the vessel ends in microvascular anastomoses may be under-estimated. Recent thrombosis research is reviewed which suggests that heparin is selectively adsorbed onto endothelium, thereby preventing thrombus formation. This evidence is invoked to explain the results of a free flap experiment in the rat and to make some practical recommendations.

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