The Effect Of Defibrotide On Reendothelialisation In Microarterial Anastomosis

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Abstract : Endothelial injury during microaterial anastomosis typically results in mural thrombus formation. However, endothelial cells produce various substances which suppress thrombus formation and maintain normal vascular wall architecture by pereventing muscular proliferation. Therefore, in order to reduce the risk of thrombus formation following microarterial anastomoses, drugs which stimulate endothelial cells and active reendothelialization might be beneficial.

Using a rat model, we studied the effect of defibrtide on reendothelialization following microarterial anastomosis and concluded that defibrotide is an effective agent which supports reendothelialization and decreases the incidence of posanastomotic thrombi.

Key Words : Anastomosis, Surgical, Endothelium, Microscopy, Electron, Scanning

INTRODUCTION

The functions of endothelial cells have been widely investigated during recent years (3,6,7,11,12,14). It is now known that these cells are not just "cobble stones" covering the inner surface of the vascular wall, but also produce PG12, endothelial tPA, and heparin-like substances. These chemical agents both suppress thrombus formation and maintain the normal architecture of tha vascular wall by suppressing mascular proliferation of the tunica media(13). The most important function of the endothelium in maintaining lumenal patency is inhibition of platelet adhesion which naturally reduces platelet aggregation and thrombus formation.

On the other hand, endothelial injury during microarterial anastomosis typically results in mural thrombus formation (9,11). Surgical damage to the arterial wall, especially the endothelium, may be reduced by using appropriate microsurgical techniques. And in this manner, thrombus formation can be prevented to a certain extent and the patency of

the vascular lumen protected. Therefore, in addition to preoperative antiaggregant and antithrombotic agents, drugs which stimulate endothelial cells and activate reendothelialization may be recommended to reduce the risk of thrombus formation and vascular occlusion following microarterial anastomosis. The process of reendothelialization following microanastomostic procedures has not been studied in depth (1,6,11,12). We previously reported the antithrombotic effect of defibrotide following microarterial anastomosis in a rat model (8). Now in this study, we have studied the effect to defibrotide on the reendothelialization process.

MATERIALS AND METHODS

Surgical Technique :

Twelve albino Swiss-Webster rats, with a mean weight of 250g were anaesthetized with intraperitoneal pentobarbital 30 mg/kg. Rats were then fixed to an operating flatform in the supine position, and their necks and upper chests were shaved and

disinfected with Hibiscrub (Imperial Chemical Industries PLC, Cheshire, England). Surgery was performed on all animals using the identical surgical technique for each animal with a Kapps surgical microscope (Kapps Son I Karl Kapps GmbH and Co. Wetzlar, Germany). Following a 4 cm transverse neck incision and the necessary dissection, the thyroid gland was retracted upwards and the sternocleidomastoid muscle laterally. Both common carorid arteries were identified. The sympathetic chain and vagal nerve lying adjacent to the carotid artery were dissected free. After preparation of the carotid artery, the adventia was stripped as described previously (16). The carotid artery was clipped with 3 mm wide straight Mayfield clips distally and proximally, and cut with a straight microscissor.

The lumen wsa irrigated with saline and a microvascular anastomosis was performed using 10.0 Ethicon sterile monofilament polyamide suture (W 2850) with eight interrupted sutures (Ethicon Ltd., Edinburg, Scotland). The approximate time for anastomosis was twenty minutes and a splint was not employed during the procedure.

Experimental and Control Groups :

Both common carotid arteries of six control rats were anastomosed. The rats were sacrificed ten days later. The carotid arteries with the anastomotic area were removed and cut in horizontally for inner surface examination under the scanning electon microscope (SEM) (JEOL) Electron Microscope, Tokyo, Japan) with low (x600) and high (x3000) power magnification. The sutures adjacent the edge were not taken under consideration because of possible damage during preparation. As a whole from all anastomoses the middle four sutures, a total of 48, were examined in the control group.

Defibrotide group: Both common carotid arteries of six rats were anastomosed with the identical procedure as the control group and defibrotide (Crinos Sp A, Biological Research Laboratories, Como, Italy) of 10 mg/kg/day was administered, via an external jugular vein catheter fon ten days. Rats were sacrificed on the tenth day following anastomosis and again 4 sutures from each anastomosis, a total of 48, were examined under the SEM, in two different magnifications.

Statistical analysis was performed using the Chi-square test with continuity correction factor.

RESULTS

The low power magnification SEM appearances of the control group (Fig. 1) and the defibrotide group (Fig. 2) were interpreted as similar. On high power

Fig. 1 : Low power magnification of control group ten days after anastomosis. The vessel lumen appears normal (x600)
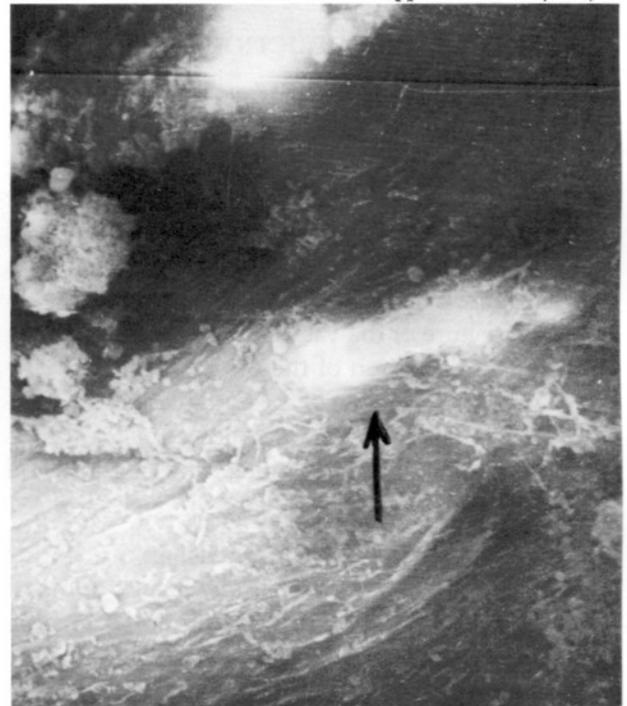

Fig. 2 : In the defibrotide group the same magnification showed no significant difference (x600).

Fig. 3 : Ten days after anastomosis and resumption of blood flow, 36 out of 48 sutures demonstrated patchy areas without endothelium (x3000).

Fig. 4 : In the defibrotide group all sutures and the anastomotic lines were completely covered with endothelial cells on high magnification (x3000).

magnification of the control group (Fig. 3). SEM examination revealed 36 of the 48 (75%) sutures to be unendothelialized. Although there was endothelial proliferation covering the sutures, patchy clear areas were present on 75 per cent. On the contrary all sutures of the defibrotide group were completely covered with endothelium, without any part of suture being visible on high magnification (Fig. 4). This difference was statistically significant ($p > 0.0001$).

DISCUSSION

Following microarterial anastomosis, both damaged endothelium and exposed subendothelial tissue stimulate platelet adhesion and aggregation which results in mural thrombus formation. Rosenbaum et al. (11) and Pamir et al. (9) previously demonstrated that maximum thrombus formation occurs 15 to 20 minutes following arterial anastomosis. Obviously, the extent of the thrombus is directly correlated with the area of endothelium damaged and the amount of aggregated platelets over this endothelium. Therefore, to increase the patency rate of microarterial anastomosis, use of antiaggregant and

antithrombotic agents has been proposed and the importance of the appropriate surgical technique has been emphasized (4,8,9,11). In recent years, various endothelial functions have been widely studied incuding inhibition of platelet aggregation and thrombus formation that have a supportive effect on the endothelium. These functions also induce rapid reendothelialization of the anastomotic line which is important in increasing the patency rate of microarterial anastomosis.

Only a few studies concerning reendothelialization after arterial anastomosis have been published (1,3,5,6,11,12). It is now generally accepted that the reendothelialization process beging three days following anastomosis and lasts about two weeks (3,12). Rosenbaum and Sundt (11) claim that reendothelialization is completed on approximately the ninth day, while O'Brien (6) believes that completion of this process may take as long as four weeks. Tomasello et al. (12) have shown that reendothelialization continues until the fourteenth day following anastomosis. Although it is difficult to state definitively the duration of the reendothelization

process, it is known that the tissue of anastomotic lines is covered by endothelium before the sutures, and this fact was confirmed our observation.

The reendothelialization process has been studied in detaily by Moseley et al. (50) in rabbit aorta, Bannister and Chapman (1), Tomasello et al. (12), and Dernbach et al. (3) in experimental end-to-end anastomosis models. Their experiments demonstrated that the anastomotic line and sutures were covered by new endothelial cells. The surface of these new spindleshaped cells, called proendothelial cells, contain numerous cytoplasmic protrusions and microvilli. Due to their ability to move actively and their high metabolic rate, proendothelial cells are able to perform a fast reparation function.

Defibrotide is a recently-developed polydeoxyribonucleotide of mammalian origin with profibrinolytic and antithrombotic action (15). It also has a modulating and stimulating effect on endothelial cells, which has been demonstrated in various animal models as well as in humans. In experimental models, defibrotide has been proved to decrease MDA and TxA2 formation while increasing cAMP and PGI2 (2,10,14,15). In vascular surgery, it decreases thrombus formation on the suture line (14). The effect of defibrotide on endothelial cell function has previously been studied (10,14). In the present study, we demonstrated a statistically significant difference between the reendothelialization rates of sutures between control and defibrotide groups on high magnification examination. The interpretation of low power magnification did not demonstrate the same fact. Therefore we strongly recommend examination of the sutures in high power magrification to obtain better visual information. The reendothelialization process was completed in ten days in the defibrotide group but was not completed in the control grop. These findings confirm the supportive role defibrotide in reendothelialization.

We concluded that i.v. defibrotide, administered for ten days increased reendothelialization and decreased the extent of thrombus formation following microarterial anastomosis of the carotid artery of rats. Therefore, post-operative defibrotide may be beneficial in speeding reendothelialization following vascular anastomosis in humans.

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