Indian Journal of Plastic Surgery (1990), 23 (1), *pp.* 9-16

MICRO-ANGIOGRAPHIC STUDIES OF END-TO-END ANASTOMOSIS OF FEMORAL ARTERY IN RATS*

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SUMMARY

Fifteen albino rats each weighing 300 to 400 gms were selected for micro angiographic studies. Microsurgical end-to-end femoral vein anastomosis was carried out and cream-o-bar 50% aqueous suspension was injected with the help of a D.K. Injector. A patency rate of 73% was achieved in the six week follow up study. Results of angiographic changes observed in the vascular lumen following anastomosis have been analysed and are being presented.

Micro-angiography is one of the important methods of recording the patency of micro-vascular anastomosis. The aim of our study was to determine the accuracy of anastomosis, patency and associated vascular changes in the femoral artery of rats and analyse the observation for further clinical research.

Review of Literature

Hill (1929) found that Bismuth oxychloride was most suitable for postmortem arteriography and the sample examined possessed approximately uniform particles about 12u (micron) in diameter.

Barclay and Trueta (1947) have used micro-angiography in biological studies and have shown interesting possibilities in study of renal circulation and arteriovenous shunts.

Contrast media, in various concentrations as used for angiograms, have been shown to cause marked changes in the microcirculation. These changes begin first in the venules and include endothelial damage and the formation of thrombi on the vessel walls (Branemark, Jacobassons and Sorensen, 1969). The changes were reversible to some extent wherever the dye was washed away by perfusion with normal saline solution.

Nieuborgh (1979) assessed the patency of microvascular anastomosis by microangiography. The left femoral artery was divided in 25 rats about 30 weeks old. The arteriograms showed that 4 out of the 23 anastomoses were stenotic and the lumen was reduced to half its normal size. The stenotic anastomoses were found at the following intervals after operation : 11 weeks, 12 weeks and 14 weeks. Histological examination confirmed stenosis partial obstruction which was due to thrombus on the vessel wall.

However, four considerations now govern the choice of the injection mass in the technique of micro-arteriography. (i) The injections medium should be sufficiently radiopaque. (ii) The injection mass should be particulate or colloid. (iii) The particulate mass should be uniform, of a definite size and not prone to aggregate and (iv) The compound should not be irritant to blood vessels and tissues. The degree of opacity depends upon the atomic weight and density of the substance to be used, the higher the atomic weight or density, the greater the degree of radiopacity.

Material and Methods

The study was conducted in the microsurgery research laboratory of the Plastic Surgery and the Department of Radiodiagnosis, Institute of Medical Sciences, Banaras Hindu University. The period of study was from October, 1986 to April 1988. Twenty male albino rats, each weighing 300 to 400 gms were chosen as experimental models.

The microangiography was done by an indigenous apparatus fabricated in our Institute Workshop, the D.K. Injector. It was used to

*This paper was presented at the National Conference of A.P.S.I. at Fisherman's Cove, Madras, 1988.

perfuse normal saline and aqueous suspension of radio-opaque dye into the vascular system of the experimental animals and cadavers. It contains two compartments in upright position. The right compartment accommodates the collapsible polythene bags with a system of compression, with springs and adjustable screws. The left compartment contains the pressure gauze which measures and adjusts the chamber pressure. The outlet of these bags are controlled by two different controllers.

The common outlet is attached by an aneroid manometer which monitors the perfusion pressure. The perfusion pressure can be adjusted as per requirement for perfusion (Fig. 1).

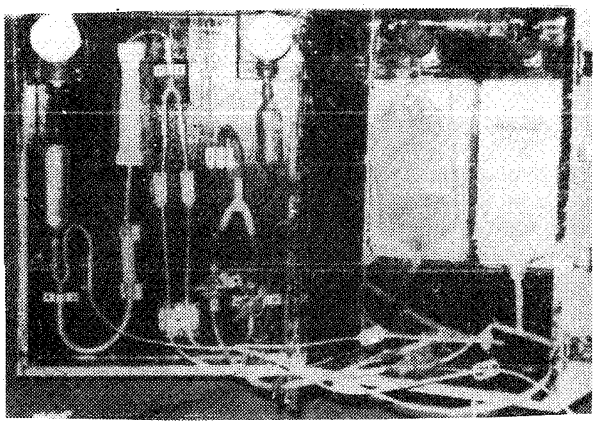

Fig. 1. Perfusion apparatus (D.K. Injector).

Perfusion Technique

The experimental rats were anaesthetised by giving Inj. Nembutal, intaperitonealy and sacrified later. The anaesthetised rat was placed on the rat board and legs were fixed with sutures. The thoracotomy was done and the heart was delivered out from the window. The apex was caught by artery forceps and the root of the ascending aorta was secured in two ligatures. An incision was made in the left ventricle and a polythene cannula (22 G) was introduced into the left ventricle, root of aorta up to the arch of aorta. Then the cannula was snugly ligated. The cannula was attached to the injector and the saline flow circuit was put on. The vascular system was washed with the normal saline at a pressure of 5 to 10 mm of mercury for 10 minutes, till the clear solution had started flowing through the incision of the left ventricle.

Now the saline circuit was closed and the radio-opaque dye (Cream-o-bar 50% aqueous suspension) circuit was opened and the dye was allowed to flow in the vascular system till it returned as a clear white suspension through the ventricular opening. The dye was perfused at a pressure of 5 to 10 mm of mercury monitored by the aneroid manometer fitted in the D.K. injector. Then the dye circuit was closed and cannula was taken out of the ascending aorta and the wound was ligated (Fig. 2). After half an hour the radiographs were taken and general survey of the vascular perfusion was assessed. Then the rat was preserved in 10% formal saline for 24 hours. Next day the skin and viscera of the abdomen were removed. The upper part of the chest was removed. The remaining part of the body was x-rayed.

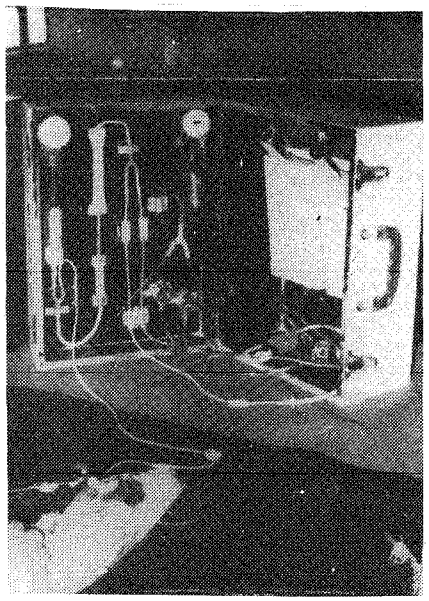

Fig. 2. Perfusion in situ.

Observations

Twenty albino rats weighing approximately 300 to 400 gms were seleted for the study out of which two rats were used to establish the experimental procedure, the third one died during anaesthesia, and two more died due to post-

operative complications. Rest fifteen rats were used for experinmental study. The rat's right femoral artery was subjected to microvascular anastomosis using an operating microscope. The left femoral artery of the same animal served as the control. The external diameter of femoral artery ranged from 0.5 mm to 1.0 mm. The angiographic study of the right femoral artery (Experimental) were compared with those of left femoral artery (Control) at two days, four days, six days, nine days and then at four, five and six weeks intervals. End-to-end anastomosis of femoral artery was done by Acland (1980) technique on the right side in all the rats.

Angiographic Observations

— Vessel at the anastomosis site.
— Main arterial trunk in thigh and leg.
— Medium and small arteries of thigh and leg.
— Capillaries around thigh and leg.

The micro-arteriography was shown to be an adequate method of testing the patency of the anastomosis in experimental microvascular surgery (Nieuborg, 1979).

The patency of a microvascular anastomosis is under the influence of many important factors. Some of them are not under the control of the surgeon, for example, the size of the vesel and pressure of blood column inside them. But a majority of the factors can be influenced by the efforts of a surgeon (Hayhurst, 1976).

The perfusion of isotonic saline followed by injection of radio-opaque dye should be done at a appropriate temperature, pressure, viscosity and radiography should be performed under suitable radiographic factors (Rai, 1970). To achieve this purpose a new apparatus (D.K. Injector) was deviced in our laboratory.

Nieuborgh (1979) and Hoynck et al. (1982, 1983) have used urografin as contrast medium for microangiography. We have used cream-o-bar 50% aqueous suspension in our experiments because it remains in the vascular channels for longer period of time and does not diffuse to cells. So, after 24 hours of preservation in formal saline and removal of skin and viscera, the vascular channel could be seen clearly, with cream-o-bar perfusion.

Preparation of Animal for Perfusion

The rat was anaesthetised and an incision was made in the 5th intercostal on the left side. The heart was brought out of the chest cavity and a cannula was inserted into the left ventricle. It was advanced upto the ascending aorta where it was snugly tied by means of ligatures. The free end of cannula was attached to the D.K. Injector and the process of perfusion was carried out.

We had chosen direct perfusion through the left ventricle and therefore expected contrast media to return to the right ventricle. It was an advantage to deliver the heart and perfuse the rat. There was no spillage of the contrast media during the procedure which otherwise would have spilled out from the collateral vessels of the abdominal wall during subsequent laparotomy. Secondly, the whole animal could be perfused with uniform pressure. The only problem in this procedure was some spillage of the contrast media from the chest wall wound which was minimal and as a safe guard the rat was washed thoroughly before X-rays were taken.

Radiography

The radiographic problem in microangiography mainly depends upon the type of X-ray and quality of film employed. The routine diagnostic X-ray plant was used in our studies and we have observed that this unit also gives excellent results in microangiography. The photographic film of fine gain emulsion was used to achieve a clear image of vessels.

Microangiographic Picture

(1) Two animals were sacrificed at the end of two days and angiographic studies were carried out (Fig. 3). It revealed :

(i) The common femoral artery had decreased in calibre at the site of anastomosis in the thigh.

(ii) The femoral artery distal to the anastomosis had decreased in calibre and the two main branches showed decreased calibre.

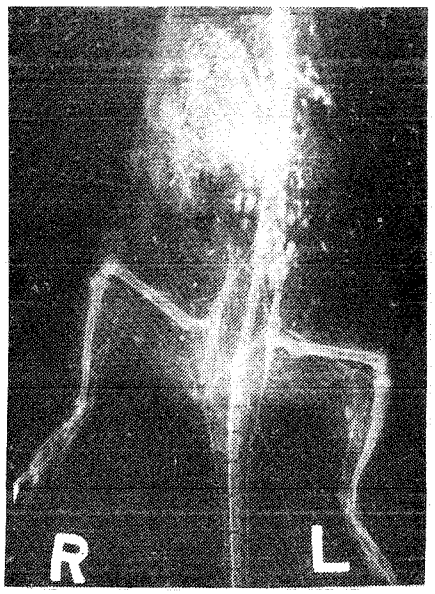

Fig. 3. Micro-angiographic picture at 2 days after anastomosis.

Fig. 4. Micro-angiographic picture at 4 days after anastomosis.

Fig. 5. Micro-angiographic picture at 6 days after anastomosis.

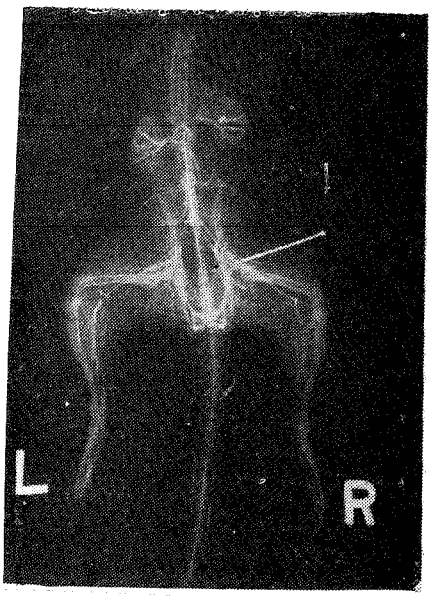

Fig. 6. Micro-angiographic picture at 9 days after anastomosis.

(iii) The medium and small sized arteries of thigh and leg showed comparatively less number of branches in the experimental limb.

(iv) The capillary net work around thigh and leg was decreased.

(2) Two animals were sacrificed at the end of four days and angiographic studies were carried out (Fig. 4). The findings were as follows :

(i) The common femoral artery has achieved the normal calibre at the site of anastomosis in the thigh.

(ii) The femoral artery distal to anastomosis and main branches showing normal calibre.

(iii) The medium and small sized arteries of the thigh and leg appeared normal.

(iv) The capillary net work around thigh and leg were dilated.

(3) Two animals were sacrificed at the end of six days and angiographic studies were carried out (Fig. 5). The findings were as follows :

(i) The common femoral artery had decreased in calibre at the site of anastomosis. There was dilatation of artery proximal of the anastomosis.

(ii) The femoral artery distal of the anastomosis and its main branches had decreased in calibre in thigh and leg. In one rat the femoral artery was not seen distal to anastomosis showing complete stenosis.

(iii) The medium and small sized arteries of the thigh and leg showed moderate dilatation.

(iv) The capillary net work around thigh and leg was decreased.

(4) Two animals were sacrificed at the end of nineth day and angiographic studies were carried out (Fig. 6). The findings were as follows :

(i) The common femoral artery had decreased in calibre at the site of anastomosis. Persistence of proximal dilatation was observed.

(ii) The femoral artery and its main branches distal to anastomosis were decreased in calibre in thigh and leg.

(iii) The medium and small arteries of thigh and leg showed moderate dilatation and increase in numbers.

(iv) The capillary net work around thigh and leg showed as increase in number.

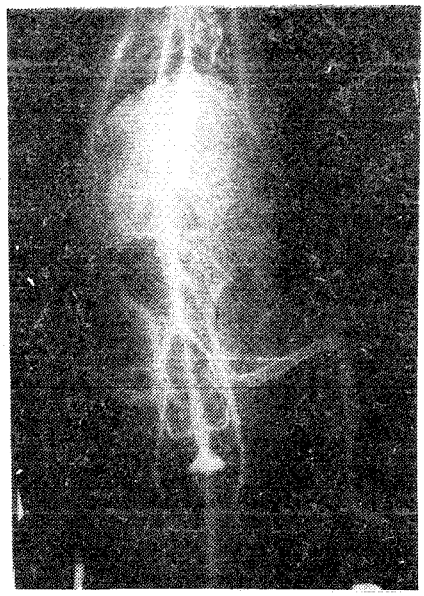

Fig. 7. Micro-angiographic picture at 4 weeks after anastomosis.

(5) Two animals were sacrificed at the end of four weeks and angiographic studies were carried out (Fig. 7). The findings were as follows :

(i) The common femoral artery had decreased in calibre at the site of anastomosis. The dilatation proximal to the anastomosis had decreased.

(ii) The femoral artery and its main branches distal to anastomosis were decreased in calibre in thigh and leg.

(iii) The medium and small arteries of thigh and leg were decreased in number and not dilated.

(6) Two animals were sacrificed at the end of five weeks and angiographic studies were carried out (Fig. 8). The findings were as follows :

(i) The common femoral artery had attained the normal calibre and proximal dilatation had decreased.

(ii) The femoral artery and its main branches distal to anastomosis had attained the normal calibre in thigh and leg.

(iii) The medium and small size arteries of thigh and leg showed mild dilatation.

(iv) The capillary net work around thigh and leg were normal.

(7) Three animals were sacrificed at the end of six weeks and angiographic studies were carried out (Fig. 9). The findings were as follows.

(i) The common femoral artery had attained the normal calibre, proximal to the anastomosis site.

(ii) The femoral artery and its main branches distal to anastomosis achieved normal calibre in thigh and leg.

(iii) The medium and small arteries of thigh and leg showed increase in number and were mildly dilated.

(iv) The capillary net work around thigh and leg were mildly dilated and seen upto the distal part.

Fifteen out of twenty rats were used for experimental study. The different angiographic pictures were found as above. The observations were made by comparing the experimental and the control limbs of the same animal. The important findings pertaining to the angiographic picture have been described.

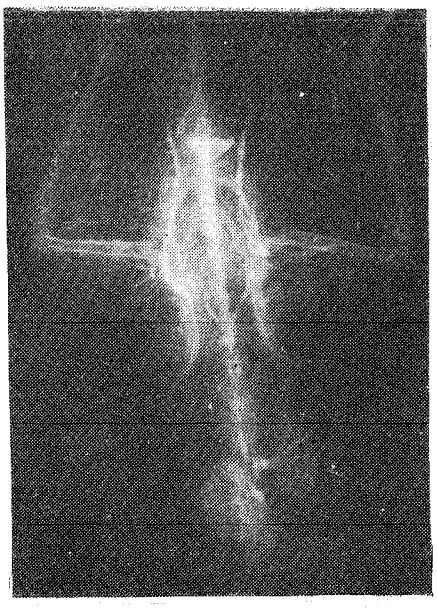

Fig. 8. Micro-angiographic picture at 5 weeks after anastomosis.

In eleven out of fifteen rats the anastomosis was patent. This patency rate has been achieved with the available facilities. It was said by O'Brien (1977) that the microvascular surgeon should be able to achieve patency rates of over ninety percent on experimental repairs of one mm. vessels prior to undertaking clinical anastomosis.

Two days old Anastomosis

The common femoral artery had decreased in calibre at the site of anastomosis in the thigh. It may be due to persistence of vascular spasm due to vascular injury. This finding is close to the observations by Spat, Gaynor, 1970, Zucker, 1972, Acland, 1972. The femoral artery and branches distal to anastomosis site showed the patency. The chances of occlusion decreases rapidly after 72 hours (Ketchum et al., 1974). Lesser number of branches of medium and small sized arteries were visualised either due to spasm or less flow of the contrast media.

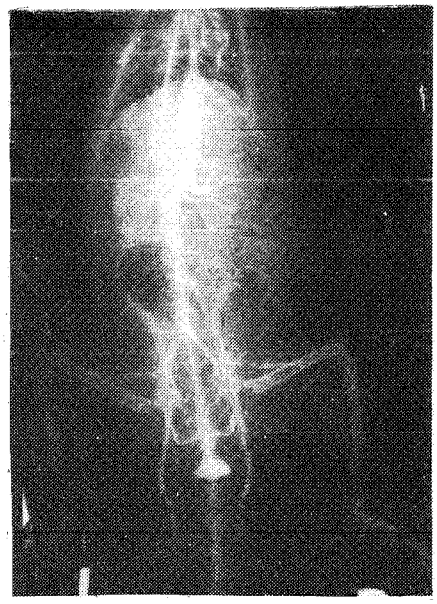

Fig. 9. Micro-angiographic picture at 6 weeks after anastomosis.

Four days old Anastomosis

The common femoral artery had achieved almost normal calibre at the anastomosis site due to relief of postoperative spasm. The femoral artery and branches distal of the anastomosis showed almost normal calibre.

The medium and small arteries of thigh and leg showed better visualisation and were dilated due to reactionary vascularity. It has been shown that fifty percent of thrombosis may develop during actual surgical procedure (Flana, Kakkar and Clarke, 1968). This may be due to increase in platelet responsiveness, factor VIII activity, fibri-

nogen and a decrease in plasminogen and spontaneous fibrinolytic activity (O'Brien, 1974).

Six day old Anastomosis

The common femoral artery showed decrease in calibre at the site of anastomosis. There was proximal dilatation showing signs of stenosis due to formation of partial thrombus. There was complete stenosis in one rat showing absence of distal branches beyond the site of the anastomosis. This was the critical time for occlusion in arteries. The long term effect of the injury produced during microvascular anastomosis like dissection of adventitia, clamping of the vessel, toughing of the intima etc are some of the factors responsible for occlusion.

Nine days old Anastomosis

The common femoral artery showing proximal dilatation decreased in calibre. The branches also showed decreased calibre in thigh and leg. The medium and small arteries of thigh and leg showed moderate dilatation and increase in number due to compensatory mechanism. There was more proliferation of capillaries around thigh and leg due to reaction.

Four weeks old Anastomosis

The common femoral artery and branches had attained the normal calibre. The medium and small sized arteries were decreased in number and dilatation was less. The vascular reaction due to partial stenosis had disappeared. The chances of occlusion in the artery are always more during the first two weeks (O'Brien, 1977).

Five weeks old Anastomosis

The femoral artery and its branches showed normal calibre. The dilatation proximal to anastomosis was not seen. Still there was some dilatation of medium and small sized arteries of thigh and leg. The late occlusion may occur due to growth of thrombus at the anastomosis site. The chances were less in comparison to veins due to the platelet thrombi which were formed but may be swept away by the rapidly flowing arterial blood (Spaet and Gaynor, 1970).

Six weeks old Anastomosis

The femoral artery and its branches were normal. The medium and small sized arteries were mildly dilated in thigh and leg. The patency rates in our series showed better results. In this study it was also demonstrated that arterial re-endothelialization occurs as early as first week of repair. However it seems that a certain degree of stenosis was due to technical imperfection or formation of a mural thrombus.

In our experiments the quantitative information on the severity of stenosis at the anastomosis site could not be obtained. The qualitative reduction in the cross section at the anastomosis site resulted in increased reactions in the surrounding vessels. The exact critical stenotic level could not be determined. The use of Doppler-flow measurement can help in further knowing the degree of stenosis.

Results

A patency rate of 73% was achieved in the present study. The vascular spasm was seen to last for 2-3 days following which the vessels achieved normal size. Thrombotic changes were observed as early as the sixth day and were associated with proximal dilatation of the vessels. Stenotic changes took about 4 weeks time to develop. During the fifth and sixth week the vessels showed normal configuration in cases where the anastomosis was properly accomplished.

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