LASER - ASSISTED MICROSURGICAL ANASTOMOSIS OF CAROTID ARTERY IN THE RAT USING Nd-YAG LASER

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SUMMARY:

End-to-end anastomosis in the A.Carotis communis of rats (average diameter 0.8-1.0mm) was achieved using Nd-YAG laser. Morphological and histological findings of laser anastomosis and suture anastomosis specimens were compared by the LAMA technique. The rats were examined 1 day, 1 month and 2 months postoperatively. Macroscopic, angiographic, histological and SEM studies showed no development of pseudoaneurismus reported in previous studies. We concluded that laser anastomosis and vessel repair techniques caused less or minimal fibrotic reactions and minimized intraluminal thrombosis which itself means minimal haemodynamic disturbance. In addition, using a laser makes surgical manipulation that might be traumatising unnecessary and may be preferable to classical suture techniques.

KEY WORDS:

Anastomosis, Nd-YAG laser, Microsurgery, Vascular surgery, Rat.

INTRODUCTION:

The technique of microvascular anastomosis which is applied to tumours of the skull base involving ischaemic attack, giant aneurysm, and branches of the internal carotid artery is used with increasing popularity in present day neurosurgical practice. Together with publications which report the long-term results, a great many studies have been published, for and against the techniques of classical suturing used widely in present day clinical practice (2,5,10).

The authors in favour of the technique have reported that suture anastomosis which are well-chosen and fully applied according to their technique, have a success rate of up to 90 percent. However, there are some opinions against, such as traumatization of the vessel by the technique employed, material used for suturing causing the occurrence of reaction tissue, and the long time required for the application of anastomosisi. For this reason, many techniques have been tried with or without sutures to find the ideal solution. Suspicion has been expressed about whether these techniques are completely reliable, or whether they are superior to the suture technique (1,3,6,9).

We performed microvascular anastomosis in rats by using Nd-YAG laser. The purpose of our study was to examine the regions with laser anastomosis and suture materials and compare the reactions.

MATERIAL AND METHOD:

In our study, we used 24 male Wistar albino rats 5 to 6 months of age with average body weight of 180 to 260g. After anaesthetizing the

rats by intraperitoneal administration of sodium pentobarbital, 40mg/kg., they were fixed in the, supine position. After the skin had been opened by a midline neck incision the common carotid arteries of diameter ranging from 0.8 to 1.0mm were with the aid of a surgical microscope and clips were placed proximally and distally. After partially stripping the adventitia of the vessels, they were cut regularly in the area between the clips by microscrissors. The lumen interior in the proximal and distal parts were washed with a solution of heparine-saline 200IU/ml, after clearning the blood clots inside the lumen. Then, 3 traction sutures were placed at equall distances from the vessel wall using a 10:0 monofilament polyamid black suture material of 75mic. in diameter with a rounded needle. Anastomosis was achied by a total of 6-9 shots on the vessel wall, 2 to 3 shots between each suture of 0.6mm spot diameter, 0.2 second duration and 16W power using a Nd-YAG laser micro-manipulator (Medi-Las 2MBB, München). We used a total of 1214 Joule laser energy for each vessel. Later, the vessel was opened to circulation by unfastening both clips, the distal first. After checking whether there was any leakage, the skin was closed with a silk suture of 4:0 (Fig. 1 a-b)

The rats were divided into three groups and after one day for the first group, one month for the second, and two months for the third group, the animals were anaesthetized, their neck regions incised and after checking the site of anastomosis, the incision was extended toward the mediastinum. The thoracic cavity was opened, and an arteriotomy performed by placing clips proximally and distally on the aortic arch. A polyethylene catheter 0.6mm diameter was passed through the arteriotomy opening to reach the anastomosed vessel. Angiography was carried out by giving 1cc of urovision containing 325mg/ml iodine through the catheter. Later, the regions of anastomosis were removed and studied under light and scanning electron microscopes (SEM).

RESULTS:

Macroscopic findings:

Using a surgical microscope it was established that in the anastomosis f all 24 rats, all the vessels were working and pulsations were normal. There were no adhesions to the surrounding tissues, but the surfaces of the suture material were covered by granulation tissue With the aid of a penset it was found that the anastomosis was working completely normally

Fig 1 : a) Vessel sutured with the LAMA technique before laser application.

b) Laser anastomised vessel.

Angiographic findings:

When angiography was performed using the method described, it was found that in all the rats there was complete passage through the anastomosed region and even the opposite carotid artery was filled by retrograde flow. In no case was, there any stenotic or aneurysmal development (Fig. 2).

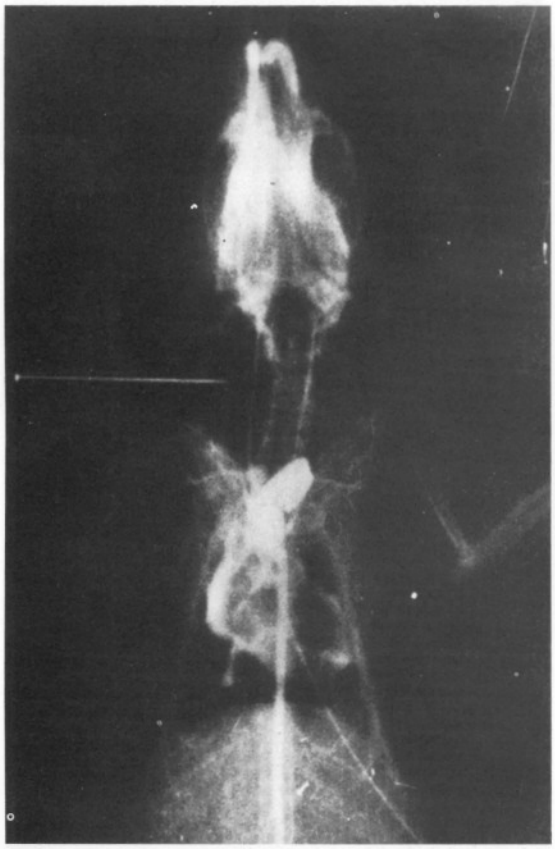


Fig. 2 : Angiography: 2 months after anastomosis.

SEM findings:

The surfaces of anatomosis were studied by scanning electron microscopy with a magnification of 300-500-1000. It was noted that lumen endothelialisation completed in the one month group, and there was thrombus formation composed of fibrin cover, erythrocytes, lymphocytes and thrombocytes on the suture material and its surroundings. This thrombus formation was also seen in the two months group though decreased. There was no aggregation on the region over which laser anastomosis had been applied, and endothelial regeneration was without defect. This thrombus formation and aggregation on the suture material gradually thinned toward the periphery and disappeared in the region of laser anastomosis (Fig. 3 a-b-c).

Fig. 3 : a) Cell accumulation localized around the suture in the 2 months.

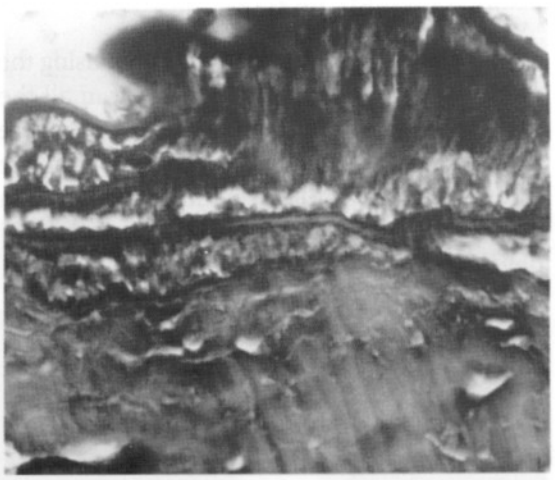
b) Decrease in cell count in the 2 months group (SEM 1000 magnification).

c) Area of laser application in the 2 months group. Endothelial regeneration completed and no pathological cell findings.

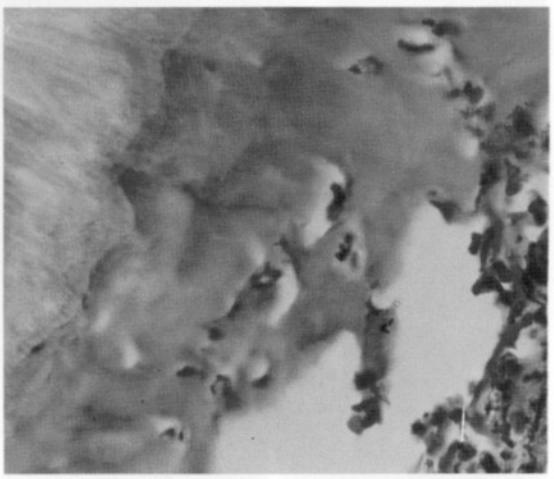
Light microscopy findings:

Areas of anastomosis were stained by hematoxylineosin and Elastica Vangiesen and studied under a light micrscope to see the fibres of lamina elastica more clearly, and by phosphotungustic acid hematoxylin to determine the changes in the cells of smooth muscles (Fig. 4 a-b-c-d).

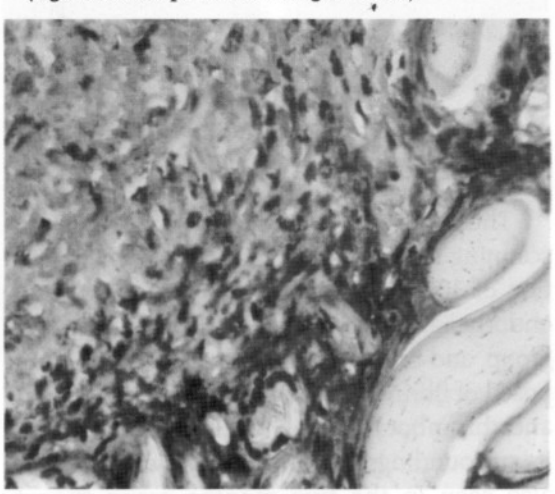
Fig. 4 : a) Disruption of lamina elastica interna fibers, laser necrosis in smooth muscle cells and place of laser spot in the 1 day group (Light microscope, EvG 500 magnification).



b) Muscle necrosis in the 1 day group (Light microscope, PTAH 500 magnification).



c) Cell infiltration and foreign body giant cells with foreign body granulation tissue formation in the 2 months group (Light microscope, HE 500 magnification).



d) Regeneration in necrotic areas of smooth muscle tissue in the 2 months group (Light microscope, HE 500 magnification).

Group 1: Smooth muscle cells in the media layer of the vessel which had undergone laser anastomosis were lacking in nuclei and had a necrotic apperance. This amorphous appearance due to the thermal effect of the laser beam was also observed in the subepithelial and lamina elastica interna layers. It was found that fibres of lamina elastica interna had lost their integration and a few elastic connective tissue fibres appeared as fragmented thin lines. It was noted that on anastomotic surfaces there were fissures related to endothelial damage and which formed elements of blood had entered and filled, moreover, these preparations had an appearance which may be described as adventitial coagulum.

Group 2: There was cellular infiltration of mononuclear cells mostly lymphocytes and macrophages around the suture. It was noted that mononuclear cells had formed foreign body giant cells around the suture. The amorphous appearance in smooth muscle cells and the fragmented condition of the lamina elastica interna fibres was maintained.

Group 3: Foreign body granulation tissue and giant cells were manifest in the regions where the sutures were placed, but hand, there was no such appearance in the laser region. The necrotic appearance of the smooth muscle cells was no longer widespread and could only be observed locally. Fragmentation in the fibres of the reticular connective tissue of the lamina elastica interna continued.

DISCUSSION:

Bypass surgery which is performed in patients with obstructive vessel lesions or in those to whom forced trapping has been applied for various reasons, has become increasingly popular with the aid of microsurgery and its indications have become increasingly widespread. It is reported in the literature that suture anastomosis has a 90 per cent success rate in cases which are well-indicated and correctly manipulated with the proper technique. On the other hand, there have been many adverse reports that anastomosis, working well initially, became stenosed by the development of intimal hyperplasia and gradually completely closed. It has been suggested that suture material on the vessel wall causes a foreign body reaction, even if the least reactive material has been used and that inflammatory reaction which develops after this stage has been followed by fibrotic tissue development and finally led to lumen stenosis. Moreover, the resulting reactionary tissue was directly proportional to the amount of suture used. It has been suggested that leucocytes gathered around the suture weaken collagen bonds, due to the proteolytic enzymes secreted by them, which are already weakened because of traumatisation, and that this is another factor which causes fibrosis resulting in obstruction of the lumen. Another disadvantage of suture anastomosis is that, because of the suture material used the anastomotic region can not adapt itself to the development of the vessel in young patients who are still growing, and it later becomes stenotic, even if functionally. It has been reported that local ischaemic complications may appear in the regions supplied by the clipped cortical branch of the middle cerebral artery, because of the long time taken for the suturing procedure (3,4,6,9).

If we consider the indications for this kind of surgery, we realize why it has such vital value for the patient, due to its additional volume of about 30% and understand better the need for maintenance of this new collateral formed.

The amount of energy sent to the tissues by a laser beam depends on the wavelength of the beam, the characteristics of the exposed tissue, the path of conduction of laser energy, the intensity of the laser beam, the size of the spot and the duration of exposure. When laser energy is applied to the vessel, it is absorbed by tissue chromophores such as haemoglobin, other globulins, melanin and cytochromes and this causes thermal denaturation. The temperature reaching 70-95C, forms new connections in the collagen helix molecules. Thus, if two vessel surfaces rich in collagen are in contact with each other, fusion occurs. But these connections are not very strong, they weaken with time and are vulnerable to strecth. Therefore it is possible for the blood to cause

pseudoaneurysms by passing into the subadventitial region through the separated layers, in anastomosis performed without sutures. For this reason, the LAMA technique has been developed by approximating the anastomotic surfaces of the vessel in order to increase postanastomotic resistance to stretch (3,4,7,9).

In examinations of the anastomosis performed under the light microscope, we determined that reaction developed around the suture but not in the region of the laser beam. Although due to the thermal effect in the laser exposed regions, necrotic smooth muscle cells regenerate with time, we established that fragmentation in the region of the lamina elastica interna layer showed no signs of improvement. In our SEM study, we observed that intimal regeneration was completed in four weeks, and there were cell and fibrin accumulations around the suture material. Aneurysm formation which is reported in e literature to be observed in 7-30% of cases and which attracts much criticism of the technique of laser anastomosis did not occur in any of our cases. Contrary to authors who have reported that loss of elasticity occurring as a result of destruction of muscle and elastic elements in the region of anastomosis due to transmural destruction by the laser beam, causing aneurysm because of failure of regular regeneration, some authors have reported that aneurysms are formed mostly in the region adjacent to the suture, and that large fissures which are formed transmurally by the insertion of sutures have been responsible for the occurrence of aneurysms. In our opinion, the effect of energy diffusing to the vessel wall as a result of uncontrolled application of laser energy, has a major role in the formation of aneurysms. For this reason we consider that careful surgical manipulation and correct adjustment of laser parameters will decrease the formation of aneurysms (6,8).

When laser anastomosis is compared to suture anastomosis, it is reported that the duration of application has been shortened by 30%. In our study, laser anastomosis lasted approximately 11 minutes. It is likely that this shortening of time is effective in the prevention of local ischaemic complications (3).

Consequently, laser anastomosis will have an important place in vascular neurosurgery, especially, bypass surgery.

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