A Comparison of Microvascular Anastomoses With The Techiques of CO_2 Laser And Classical Suture

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Abstract : In this study end-to-end anastomoses were made in the left carotid arteries of 90 adult rats, in 45 of which the classical suture technique was used and in the remaining 45 the CO2 laser technique. Anastomoses made with CO2 laser and classical suture techniques were compared in the 1st, the 3rd, and the 9th weeks postoperatively in equal groups using the same histopathological parameters. Endothelium destruction, necrosis in media and elastic lamina disorders were seen in both techniques. In the anastomoses with the classical suture more foreign body reaction was found whereas in those with the CO_2 technique there was more inflammation. Anastomoses with the suture technique showed aneurysms at the rate of 4.4%. There were no aneurysms in the anastomoses with the CO_2 laser technique. After this study, the CO_2 laser technique was concluded to be more suitable because it produced less foreign body reactions, and aneurysms, and could be performed in a shorter time.

Key Words: Microvascular anastomosis, CO_2 laser, Suture techniques Aneurysm formation, Inflammation.

INTRODUCTION

In 1958 Bernard Seidenberg made the first experimental studies which shed light on the anastomosis of small arteries (3). In 1960 Jacobson and Suarez published microvascular (MV) anastomosis techniques using a operation microscope (14). In 1967 Yaargil and Donaghy achieved clinical anastomosis of extracranial arteries with intracranial arteries for the first time (6,44,45).

As some problems related with microvascular anastomosis still remain unsolved in spite of numerous modern techniques and facilities, CO_2 and Nd-YAG laser started to be used.

In 1980 Jain made an experimental sutureless MV anastomosis with Nd: YAG laser. In 1984 he applied it clinically and published the results of sutureless extracranial bypass (15,16).

The first original study using the CO_2 laser was in 1980 by Morris and Carter (36). In the following years the results of experimental MV anastomoses with CO_2 laser were published by Serune in 1983 (33), Quigley in 1985 (26), in 1986 by Neblet et al. (21), Oishi (22,23), and Vale et al (42). In 1988 Travers et al. (40,41) published the results of anastomoses in bigger groups; in 1990 Razura et al. (30) published the results of end to side MV anastomoses, and Jacobowitz et al. (13) R-13 the results of MV anastomoses made at different milliwatts and different laser beat times.

MV anastomosis with CO_2 laser is explained in two ways:

1) Collagen melts and then takes part in coagulation (21).

2) The thermal effect welds the tissues by activating tissue fibrinogen (42).

In this study we aimed at minimizing the destruction due to carbonization and thermal effect by decreasing the power used for anastomosis to 40-60

milliwatts and compared the anastomoses with light and electron microscope studies.

MATERIALS AND METHODS

This study was carried out at Glhane Medical Faculty, Department of Neurosurgery, Microsurgery Training and Research Centre between June 1991 and April 1992. A total of 90 albino rats was used, ranging 12 to 14 months in age and 210 to 330 gr. in weight. The ratio of male rats to females was equal. The experimental rats were separated into two groups of 45.

In each group the rats were anaesthetized with 2 mg/100 m (sn) Ketalar (Parke Davis) l.m. 20 minutes preoperatively. 0.01 mg Atropin sulfate was administered intraperitoneally to decrease respiratory tract secretion. After cleaning of neck region and the sterile covering, an artery segment of about 20-25 mm was explored as far as the left common carotid artery bifurcation with the help of an operation microscope (Zeiss, Opmi-6). Temporary clips were placed first in the proximal then in the distal allowing the artery to turn 180. The artery segment between the clips was cut at one time to be anastomosed again. Each cut end was irrigated with 9 saline solution contaning heparin.

In the first group in which the classical suture technique was used, end-to-end anastomoses were made in the left carotid arteries of 45 adult albino rats following minimal adventitiectomy with 8-10 sutures with (Ethicon Bv. 75-3) silk placed at equal intervals 10-0.

In the second group, three fixative sutures were put in the left carotid artery of 45 rats at 120 and at equal intervals without adventitiectomy. Then end-to-end microvascular anastomosis was completed between the two fixative sutures with a CO_2 laser (Cooper LS-860 Sonics) by a total of 6 laser beats with a spotsize of 0.2 mm and 45-50 milliwatts.

Of the experimental rats in each group, 15 after the first week, 15 after the third week, and 15 after the ninth week were re-explored and the anastomosis regions were evaluated morphologically. After the rats were sacrificed their anastomosed carotid artery segments were extracted for histopathological studies.

In both groups anastomoses were checked with the milking test to see whether they functioned properly after the first hour on completion of the anastomoses, namely the early postoperative period, because the most common complication after microvascular anastomoses is white (platelet) thrombosis seen within the first 20 minutes which is capable of developing on a large scale (19). In our study this period was 1 hour in order to gain sufficient and effective time for lests evaluation.

Each rat was put in a separate cage following the early postoperative period after completion of the anastomoses and the dates and procedures involved were recorded.

Procedures in the Post Operative Period

Fifteen rats in each group were re-explored in the first, third and ninth weeks, and thus the anastomosis line was determined. It was observed that all 90 functioned properly, but aneurysm developed in 2 and these were photographed. Of the re-explored rats, ten chosen at random underwent angiographic examination. Control images were loaded into Digitron-2 and Digital Subtraction Angiography instrument(Siemens-Germany). A contrast agent of 0.55 cc non-ionic (340 mg. Omnipaque, Nycomed-Norway) was spelking administered manually through a catheter. Images were recorded into discettes at 2 f/second.

The anastomosed artery segments extracted from all the rats in the first, third and ninth weeks were kept in 10% tamponed formalin solution for one day for examination under a light microscope. After that they were put in alcohol, ksilol and paraffin in the follow up instrument for a period and blocked in paraffin. Cross sections with a thickness of 3-5 microns taken in the anastomose line were cut with a microtome (Rotary microtome, Detroit), put on slides, stained by the haemotoxilen-eosin method and examined under a light microscope. In the histopathological evaluation five parameters were taken as the basis for scoring. Different values between the groups were statistically compared using the "Z" test. The criteria for histopathological evaluation are shown in (Table I.)

Three artery segments taken from each group in the 9th week were fixed in 5% spelling gluteraldehide solution. They were processed by gluteraldehide fixation and alcohol series and blocked with "Epon

812". Semi thin cross sections were taken from the blocks, the suitable ones were selected and thin cross sections were obtained with "Supernova ultra microtome" (Reichert-Jung-Austria). These were stained with uranil spelling acetate and lead citrate and examined under a "Carl Zeiss EM 9 A Transmission Electronmicroscope."

Table I : Histopathological Evaluation Criteria

A. Foreign Body Granulation
- 0 - Reaction to foreign body was not seen
- 1 - Slihgt reaction to foreign body
- 2 - Medium reaction to foreign body
- 3 - Severe reaction to foreign body

B. Endothelial Changes
- 0 - The endothelium was completely protected and intact
- 1 - Deficiencies in the endothelium were present in some parts.
- 2 - Deficiencies in the endothelium were present a large area.
- 3 - Obvious endothelial proliferation in the surrounding area.

C. Density of Inflammation
- 0 - Inflammation was not seen.
- 1 - Slight inflammation was seen.
- 2 - Medium inflammation was seen
- 3 - Severe inflammation was seen.

D. Necrosis in Media
- 0 - No necrosis was seen in the media layer
- 1 - Necrosis as thick as 1/3 of the media layer.
- 2 - Necrosis as thick as 2/3 of the media layer.
- 3 - Necrosis as thick as the diameter of the media layer.

E. Elastic Lamina disorders
- 0 - Elastic laminae were protected and regular
- 1 - Elastic laminae were partly apparent
- 2 - Elastic laminae were hardly apparent.
- 3 - Elastic laminae had disappeared

Results

In this study the average anastomosis durations obtained from the microvascular anastomoses with both techniques were compared. The anastomosis periods were 163 minutes in anastomoses with suture technique, and 9+2 minutes with the CO_2 laser technique.

All the anasotomoses with suture and CO_2 laser techniques were checked by observation and the milking test in the first hour, namely the early postoperative period. No early thrombosis occurred.

One of the undesired complications of microvascular anastomosis is aneurysm formation. No aneurysms were seen in the anastomoses with the CO_2 laser technique (Fig. 1-A) and only two in those with the suture technique (Fig. 1-B). The incidence of aneurysms in the anastomoses with the suture technique was 4.4%.

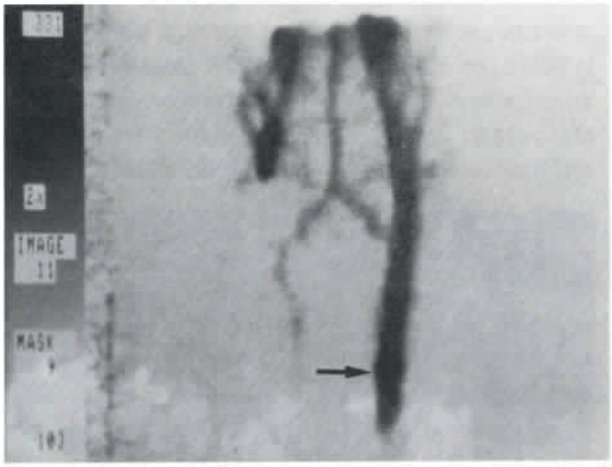

Fig. 1 :A - Angiography demonstrating an anastomosis (arrow head) with CO_2 laser technique.

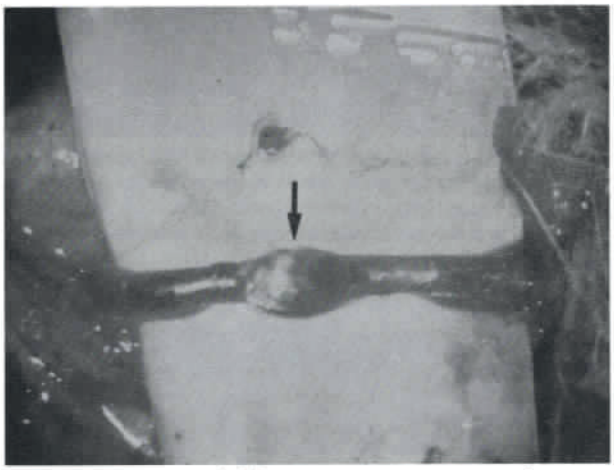

Fig. 1 : B - Aneurysmal dilatation in anastomosis with suture technique (arrow head).

Angiography on five rats from each group in the 9th week postoperatively showed that the non-ionic contrast substance had a very easy passage through the anastomose line and this was photographed. The contrast substance had an easy passage without obstruction in both techniques.

Histopathological Findings

1. Foreign body granulation

In the first week foreign body granulation was found to be less in the anastomoses with the CO_2 technique due to the number of sutures. It was found statistically significant with the suture technique.

In the third week foreign body granulation in the anastomoses with the CO2 laser technique was much less compared with the suture technique (Fig. 2-A).

It was statistically significant ($p>0.05$). The anastomoses with the CO_2 laser technique had only three sutures. However 8-10 sutures were used in anastomoses with the suture technique (Fig. 2-B). For this reason foreign body granulation was more in anastomoses with the suture technique.

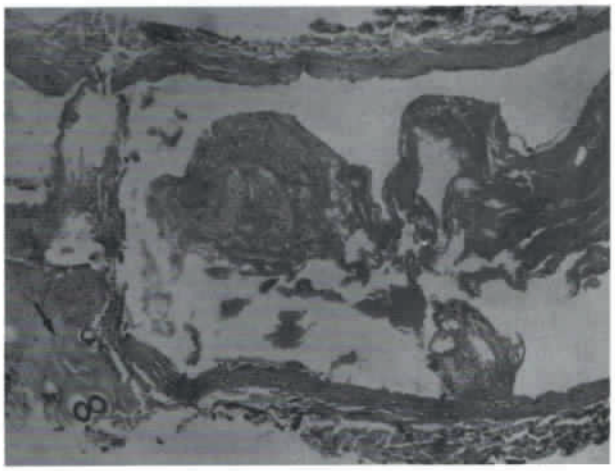

Fig. 2 : A - A cross section of the anastomosis with suture technique: in post-operative 3th week endothelium has died out completely, reendothelization is insufficient, necrosis around silk suture, foreign body reaction present.

Fig. 2 : B - In the third week in the anastomoses with CO_2 laser the endothelium has mostly. Mild foreign body reaction around the silk suture and inflammation density is present.

2. Endothelial changes

In the first week the anastomoses with both techniques (CO_2 laser and suture) were examined for endothelial changes. It was seen that endothelial layer was protected better in the anastomoses with the CO_2 laser technique. However the difference between the two was not statistically significant.

In the third week the reendothelialisation was almost completed in the MV anastomoses with the CO_2 laser technique (Fig. 3-A). It was less in those using the suture technique (Fig. 3-B). The difference between the two was statistically sinificant ($p>0.01$).

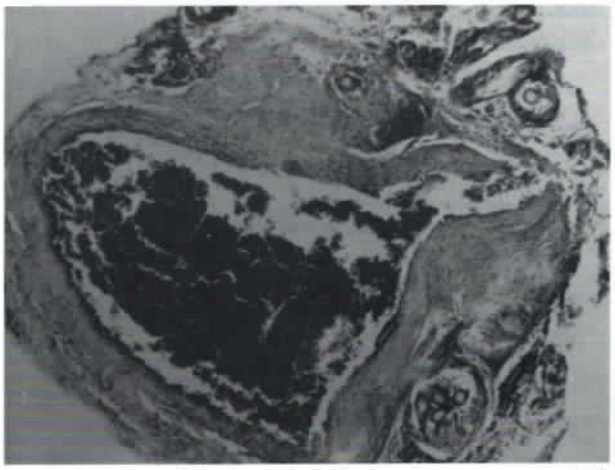

Fig. 3 : A - Accomplished reendothelization in anastomoses with CO_2 laser technique, the layers of the lamina elastica interna and media are normal, foreign body granulation tissue around silk sutures is present.

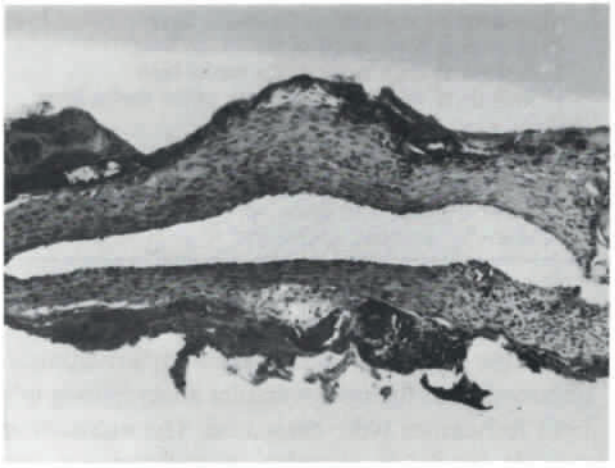

Fig. 3 : B - Reendothelization has been accomplished in anastomoses with suture technique, foreign body granulation tissue around silk sutures is present. Lamina elastica interna and media disorders, in suture, regions. (arrow head).

The endothelium is damaged in anastomoses made with each technique. However damage is less in the CO_2 laser technique and proliferation is completed in a shorter time. In spite of this, reendothelialisation was found to be complete after the third<ninth week in both techniques. This was not statistically significant (Fig. 5 - 6).

3. Density of inflammation

In the frst week there was dense inflammation

in the anastomoses with CO_2 laser technique (Fig. 2-B) but less in those with the suture technique. The difference was significant statistically ($p>0.01$).

In the third week dense inflammation was observed in anastomoses with CO_2 laser technique and compared with the suture technique, the difference was significant ($p>0.05$).

In the ninth week the density of inflammation was almost equal in both techniques and the difference was not significant.

4. Necrosis in the media

Necrosis in the media due to acute trauma is a common finding in CO_2 laser and suture techniques but disappears by the 9th week.

5. Elastic Lamina Disorders

In the first week elastic lamina disorders were seen more with the suture than the CO_2 laser anastomoses. (Fig. 3-A). However the difference was not statistically significant.

In the third week the elastic lamina disappeared in the wall in which aneurysm developed in one case of anastomosis with the suture technique. But when the other cases were compared no significant difference was seen.

In the third week aneurysm was seen in one case of anastomosis with the suture technique (Fig. 4-A,B). Elastic lamina was found to be more protected and regular in the CO2 laser anastomosis (Fig. 3-B). When compared with the suture technique the difference was statistically significant ($p>0.05$).

Elastic lamina dsorders which occur because of fibrosis and inflammation were observed in varying degrees in both techniques (Fig. 4-C).

Transmission electron microscope (TEM) results: Three artery segments with end-to-end anastomosis from each group were studied under TEM in the 9th week postoperatively. It was seen that reendothelialisation was completed in the microvascular anastomoses with CO_2 laser but there were endothelial cell cytoplasms extending toward the lumen and vacuolization in the endothelial cells due to ischaemia (Fig. 5B). In the subintimal layers fibroblastic proliferation and fibrotic changes were observed. The elastic lamina was regular and intact (Fig. 5A).

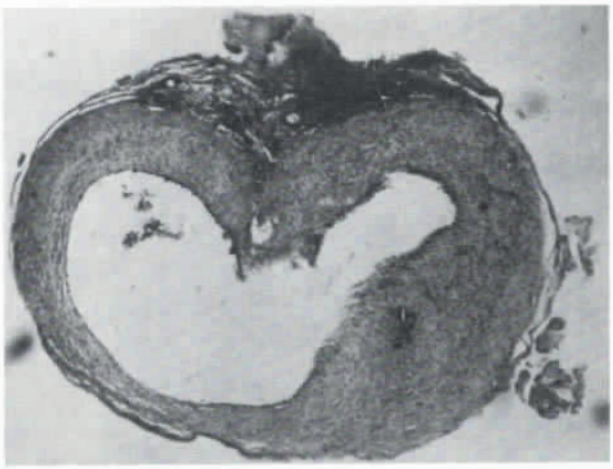

Fig. 4 : Microvascular anastomoses with suture technique: A - In the third week, aneurysmal dilatation and the absence of lamina elastica interna in this region, thickening in the media layer (HEx40).

Fig. 4 : B - In the ninth week, aneurysmal dilatation and the absence of lamina elastica interna in this region, thickening in the media layer (HEx40).

Fig. 4 : C - Lamina elastica interna has died out at the beginning of the aneurysm (HEx200).

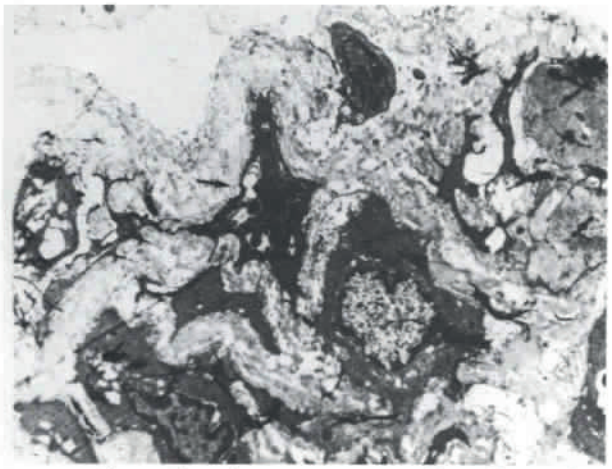

Fig. 5 : A - The ninth week in anastomoses with CO_2 laser technique. reendothelization findings and subintimal fibroblastic proliferation (arrow head) is observed (TEMx56.000).

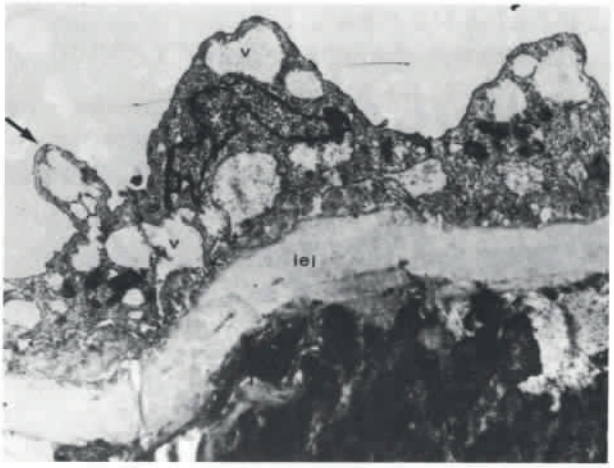

Fig. 5 : B - Endothelial vaculizations (v) and projection (arrow head) to the vessel lumen, vacuolizations (v); protected lamina elastica interna (l.e.i.) in the anastomoses with CO_2 technique in the ninth week (TEMx22.000).

In microvascular anastomosis with the suture technique: reendothelialisation was completed and here also vacuolization in the endothelial cells and endothelial cell cytoplasm extending toward the lumen was observed as in the microvascular anastomoses with the CO_2 laser technique (Fig. 6).

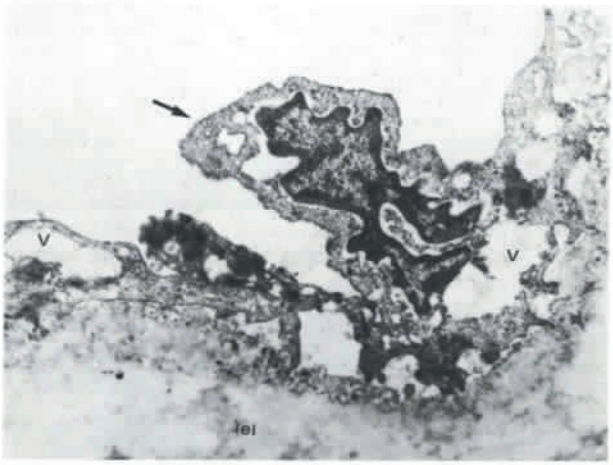

Fig. 6 : Endothelial cell cytoplasm projection (arrow head) to the vessel lumen, vacuolizations (v); protected lamina elastica interna (l.e.i.) in the anastomoses with suture technique in the ninth week (TEMx22.000).

DISCUSSION

The aim of microvascular anastomosis in clinical and experimental applications is to maintain a blood flow and prevent early thrombus formation and pathological factors that can cause a narrowing in the wall of a vessel and late aneurysm formation.

During the initial few days the risk of thrombotic occlusion is said to be the result of a the formation of physiological haemostatic plug made up of platelet aggregates in the anastomotic zone (39). The fibrinolysis of this haemostatic plug which is a step at the natural healing period, is maintained by the transformation of plasmine from plasminogen with the effect of plasminogen activator at the end of the first week. Plasmine is a proteolitic enzyme which causes lysis in the fibrine. Plasminogen activator is released from it's primary origin of endothelial cells locally if there is sufficient in the blood circulation.

Fibrinolotic activator is present in the vasavasorum at the intima layer of vessels as a plasminogen activator. Hobby demonstrated in an experimental canine model that substance produced in the endothelial cells called fibrinoltic activator which prevents thrombus formation. He also stated that this procedure was halted at the traumatized vessels with the exception of fibrine, and an interval of one day was necessary for reproducing fibrinolitic activator (12).

Jing Guo and Ya Du Chao examined end-to-end microvascular anastomosis with both techniques and demonstrated coagulation necrosis at the adventitia and median layer of vessels and also endothelial cell loss at the CO_2 laser anastomosis site. Also transmural necrosis was distinctly seen in most of the anastomotic area because of the thermal effect of the CO_2 laser. In another experiment an increase

was observed in fibroblastic cells and activity around this necrotic zone(17).

Acland, Gregorius and Servant reported that sutures played a major role in thrombus formation. They demonstrated by light and electron microscope studies the filling of the needle holes on the sutures with thrombosites even in the initial minutes as soon as microclamps were opened following completion of the anastomosis (1,9,34). The thrombogenic effects of sutures and needle holes led the researchers to develop techniques with less sutures or sutureless. But prevention of thrombus formation and complete opening of the vessel could not be attained even with various anastomosis techniques used as alternatives to the suture technique.

Tissue necrosis and narrowing of the vessel wall was observed in anastomoses with suture or sutureless techniques using synthetic adhesive materials such as methyl -2- cyanoacrylate and Buty -2- cyanoacrylate. Grubbs reported his histopathological findings with end-to-end anastomosis of femoral arteries of rats using the suture technique. CO_2 laser technique and cryoprecipitated fibrinogen 2 (crystallized thrombine 35 mg /cc, cace 2/20 mg/cc, aprothonine 2000 kl U/C). He showed that almost the same amount of damage at the medial layer was seen with both, CO_2 laser and fibrin glue techniques (10) and various authors tried to initiate fibrinolithic activity as soon as possible and lessen trauma during the anastomosis procedure. To date, the main concern has been to stop needle trauma and thus minimize the risk of thromboembolism. But alternative techniques such as CO_2, Nd : YAG laser, Argon and KTP/532 lasers and materials such as cryoprecipitated fibrinogen also cause trauma at the vessel wall and retard fibrinolithic activity at least as much as in the suture technique. (4,7,10,12,23,25).

Vale et al. made 43 end to end anastomoses in rats with suture and CO_2 laser techniques and made observations at the first and 180 th days postoperatively with scanning and transmission electron microscopy. They observed endothelial damage in both techniques, but showed that reepithelisation occurred more quickly with the CO_2 laser technique (42).

Pribil and Powers however, performed anastomosis on the carotid arteries of rats with two sutures at 180 angles and argon laser and studied them on the first day, first week and first month postoperatively with a light microscope. They found a dense inflammation and severe endothelial damage in all the anastomoses as well as progressive fragmentation and segmental stenosis at the vessel wall (24,25). Since the spectral emission curve of the argon laser coincides with the spectral absorption curve of haemoglobin, the argon laser beam is absorbed by haemoglobin in vascularised tissue. The penetration of argon laser is high in non-pigmented tissue since vascularization is less (19,24,36).

Fasano et al. investigated the effects of CO_2 laser, Nd. YAG laser and Argon laser on the vessel wall and observed that CO_2 laser caused less damage compared with the others (7).

Jain, Neblett and Serune et al. states identical histopathological changes occurred in anastomosis of vessels about 1mm diameter with Nd : YAG laser and suture techniques (15,20,33).

In our study we observed that reendothelisation was completed after the third week in microvascular anastomosis with CO_2 laser and suture techniques, but it occurred more slowly in the suture technique. During investigations in a third week with a light microscope this difference was found to be statistically significant.

The pressure exerted by the microclamps during microvascular anastomosis also causes tissue hypoxia. Trauma on the vessel wall affects the outcome of anastomosis. Thurston examined the affects of microclamps on the vessel wall without anastomosis with an electron microscope and observed dilatation of the vessel wall, and alterations in the laminar blood flow due to longitudinal raw disorder as a result of necrosis (in medial layer) and endothelial exfoliation in later periods (37).

On the other hand, Stomatopoulus insisted on the relation between trauma and the nature of the vessel wall and duration and amount of pressure (35). According to Acland, factors such as the decrease of vessel wall nutrition from extrinsic blood circulation, pressure applied by microclamps, and hypoxic and toxic agents cause necrosis at the medial layer (2). The only way to prevent this and keep tissue necrosis to a minimum is to open the microclamps as soon as possible and choose an appropriate anastomosis method. Various authors investigated invented new methods for decreasing the anastomosis application time.

Another factor which causes necrosis at the medial layer is decreased blood supply to the vessel wall. The vasavasorums which supply the vessel wall are in the outer layer of the vessel which is called the adventitia. Adventitial tissue which enters the vessel lumen during anastomosis is an important medium for thrombus formation. Adventitiectomy for prevention of thrombus formation also cuts the blood supply by way of the vasavasorums and causes necrosis in the medial layer. Most authors prefer and advise as little adventitiectomy as possible in order to prevent necrosis of the vessel wall and decrease the number of narrow places at the anastomosis site (1,2,29,38,45).

We applied adventitiectomy in our study on suture anastomoses but not with the CO_2 laser technique, because the thermal effect of the laser beam causes collagen fusion at the adventitial and medial layer. If adventitiectomy is done, the effect of thermal heat increases, the collagen of triple helix molecules degenerates and random fusion occurs causing defects in medial layer (17).

Necrosis at the medial layer is frequently seen with microvascular anastomosis with CO_2 and classic suture techniques. The cause of necrosis is sudden trauma, because, the thermal effect of the CO_2 laser reaches the medial layer even if the duration of microclamps lessened (4,5,7,8,10,17,18,23,27,28,29, 33,40,42).

One of the undesired complications of microvascular anastomosis is aneurysm formation in the postoperative period. We observed two aneurysms with the suture technique but none with the CO_2 laser technique. Trauma, overtension and an insufficient number of sutures are factors listed for aetiopathogenesis of aneurysm formation (30,33,39).

There is a close relationship between aneurysm formation and the anastomosis technique. Eisenhardt explained the pathogenesis as follows : 1) Weakness of the vessel wall, 2) Bulging of the suture material to the lumen, 3) Mechanical factors such as extension and infection (38,39). Sekhar and Heros stated that congenital defects and acquired degenerative factors and the other congenital medial layer defects are responsible for aneurysm formation (32).

Yamazoe et al. examined aneurysms with scanning electron microscope. The first difference observed was the loss of folds originating from the internal elastic laminae (43).

Hazama et al. created experimental aneurysms in rats and found intimal thickening, hypertrophy at the internal elastic lamina and dense dye absorbtion at the entrance of the sac (11). In end-to-end anastomosis with the suture technique the rate of aneurysms was 50%. Krag observed a rate of 24% (20).

Quigley et al. reported 18.6% aneurysms in the early and 29.8% in the late periods in their study of end-to-end anastomosis with CO_2 laser in the femoral arteries of rats but none with the suture technique. They did observe aneurysm especially on the ventral side with CO_2 laser technique. The number of sutures and the distance between the sutures are important in the CO_2 laser technique. Also the thermal effect of the CO_2 laser could cause disturbances in the elastic lamina (29).

Razura et al. found 8 % aneurysms with the suture technique and 44 % with the CO_2 laser technique (30); Vale et al. 7% with at the suture technique and 20% with at the CO_2 laser technique (42); Sartorius et al. had no in their end-to-site microvascular anastomosis with the CO_2 laser technique (31).

Guo ve Chao performed 32 anastomoses with CO_2 laser without suture, 24 with CO_2 laser with 3 sutures, and 24 end-to-end anastomoses with classical suture technique and observed only 16 % aneurysms in anastomoses with CO_2 laser without sutures (17). In the study of Flemming et al. the incidence of aneurysm in microvascular anastomoses with CO_2 was to be found 30.7 % and 15.5% in the suture technique (8).

CONCLUSION

Our observation at the end of this study can be listed as follows:

– Endothelial damage, necrosis at the medial layer and disturbance of the elastic lamina were seen in microvascular anastomoses with both techniques.

– Foreign body reaction was seen more with the classical suture than the CO_2 laser technique.

– More inflammation occurred with the CO_2 laser technique than the classical suture technique.

To lessen aneurysm incidence with both techniques care must be taken to cause minimal damage

to the vessel wall and to apply sutures or lasers at appropriate distances and insufficient numbers. We conclude that the CO_2 laser technique is more suitable than other techniques because when applied with sutures at appropiate intervals, if causes less foreign body reaction and aneurysms and can be performed in a shorter time.

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