Preventive Effect of Ticlopidine on Postoperative Thrombus Formation after Experimental Microsurgical Carotid Endarterectomy in Rats

Sıçanlarda Deneysel Mikrocerrahi Karotid Endarterektomi Sonrası Oluşan Trombusa Tiklopidinin Önleyici Etkisi

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Abstract: The purpose of this study was to investigate the preventive effect of Ticlopidine on thrombus formation after surgical arterial injury. The model of microsurgical carotid endarterectomy was performed on the common carotid arteries in rats. Twenty rats were equally divided into two groups: a control group that received no drugs and a Ticlopidine-treated group (100mg/kg orally 3 hours before surgery). The vessels were prepared for scanning electron microscopy and were evaluated based on the platelet aggregates at the endarterectomized site. The Mann-Whitney U test identified a statistically significant difference between the control group and the drug-treated group ($U=82.50$, $p< 0.05$). This study proves that Ticlopidine has a preventive effect on platelet aggregation in endarterectomized carotid arteries and that it can be used to reduce postoperative thrombus formation after carotid endarterectomy.

Key Words: carotid endarterectomy, microneurosurgery, postoperative thrombosis, scanning electron microscopy, thrombus formation, ticlopidine

Özet: Bu çalışmanın amacı arteriyel cerrahi travma sonrasındaki trombus oluşumu üzerine Ticlopidin'in önleyici etkisini araştırmaktır. Mikrocerrahi ile yapılan karotid endarterektomi ratlar üzerinde, ana karotid arterlerde gerçekleştirildi. 20 adet rat kontrol grubu ve cerrahiden 3 saat önce 100mg/kg ilaç verilen grup olarak ikiye ayrıldı. Damarlar scanning electron mikroskopi için hazırlanarak endarterektomi alanındaki trombosit agregasyonuna göre puan verildi. Mann-Whitney U testi kullanılarak yapılan istatistiksel incelemede kontrol grubu ile Ticlopidin verilen grup arasında belirgin fark olduğu görüldü ($U=82.50$, $p< 0.05$). Bu çalışma Ticlopidin'in endarterektomi yapılan arterlerdeki trombosit agregasyonunu önleyici etkisinin olduğunu ve postoperatif trombus oluşumunu azalttığını göstermiştir.

Anahtar Kelimeler: Karotid endarterektomi, mikronöroşirürji, postoperatif tromboz, scanning electron mikroskopi, ticlopidin, trombus oluşumu

INTRODUCTION

Carotid endarterectomy is a well recognized operation that is widely performed in the treatment of transient ischemic attacks (TIA). Platelet deposition and thrombus formation at the endarterectomy site play important roles in perioperative and postoperative cerebral ischemia (5,10,11,14,15). Several antithrombotic drugs are being used preoperatively and postoperatively to prevent this

complication. Heparin and aspirin are the agents more commonly used.

Ticlopidine is known to reduce platelet aggregation. It has been found to be a potent and long-lasting inhibitor of platelet aggregation induced by various agents including adenosine diphosphate (ADP), platelet-activating factor (PAF), thrombin, collagen and arachidonic acid (1,2,17).

In this study, we investigated the preventive effect of Ticlopidine on thrombus formation after microsurgical carotid endarterectomy in rats. We examined the platelet aggregation at the endarterectomy site using scanning electron microscopy (SEM).

MATERIALS and METHODS

Animal preparation:

Twenty albino rats weighing 200 to 300 g were used in this study. Animals were anesthetized by an intramuscular injection of a mixture of ketamine (60mg/kg) and xylazine (10mg/kg). The rats were equally divided into two groups: a control group that received no drugs and a Ticlopidine-treated group (100 mg/kg orally, 3 hours before the surgery).

Surgical Technique:

The rats were placed in a supine position and fastened to a dissecting stand. One common carotid artery was exposed using microsurgical techniques and the operating microscope. The artery was occluded with microvascular clips proximally and distally, and was then incised longitudinally for approximately 5mm. Under magnification and with the operating microscope, we used the tip of a 26-gauge needle to make two transverse incisions in the intima that were separated by 3 mm. Using microforceps, we then removed the intervening intima and part of the media. The arteriotomy was closed with 10-0 monofilament polyamide suture and the artery was unclipped.

Vessel preparation:

Three hours after recirculation, a fixative consisting of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.4) was perfused through a left ventricular cannula for 30 minutes. The common carotid arteries were excised under the microscope, and were placed in 2.5% glutaraldehyde (pH 7.4) for 24 hours at 4°C. They were then prepared with a cacodylate-buffered fixative. The arteries were postfixed in 1% osmium tetroxide, dehydrated in a graded series of acetone and critical-point dried with CO_2. The specimens were placed on aluminum stubs, coated with gold-palladium and examined with an JEOL scanning electron microscope (SEM-ASID-10).

Evaluation of scanning electron microscopy findings:

Two observers blindly evaluated the specimens according to the scale described by Spallone et al. (21) in 1985:

Score 1, Almost normal appearance of the endarterectomized surface, absent or scanty platelet aggregates.

Score 2, moderately abnormal appearance, obvious platelet aggregates or isolated small thrombi.

Score 3, definitely abnormal appearance, large or massive thrombi.

Statistical evaluation:

The scores were evaluated for statistical significance using the Mann-Whitney U test.

RESULTS

Vessel patency:

Arteries examined 3 hours after recirculation (before perfusion with the fixative), were patent and pulsatile.

Scanning electron microscopy studies:

The scores for the control and drug-treated groups are listed in Table I.

Large, visible platelet aggregates and fibrin strands covered the endarterectomized surface (Figure 1) in the control group. Scanning electron microscopy revealed massive thrombi in 6 of these 10 animals. The mean score for the control group was 2.5.

In the Ticlopidine-treated group, platelet aggregates were scanty or obvious in 9 of the 10 animals (Figure 2). Massive thrombi were seen in only one animal. The mean score for this group was 1.5.

Table I. Scores for the scanning electron microscopy findings.

Animal No.	Group I Control group	Group 2 ticlopidine-treated group
1	3	2
2	3	2
3	2	1
4	3	3
5	2	1
6	2	1
7	3	1
8	1	2
9	3	1
10	3	1

(U=82.50, $p< 0.05$)

Statistical analysis:

Using the Mann-Whitney U test, we found a statistically significant difference between the control and Ticlopidine-treated groups (U=82.50, $p< 0.05$).

DISCUSSION

The first reports of carotid artery reconstruction were published in early 1950s. In his overview, Callow (7) notes that DeBakey (9) is credited with performing the first endarterectomy for carotid disease in 1953. Today, carotid endarterectomy is a widely performed operation in the treatment of occlusive cerebrovascular disease. The primary indication for the procedure is the presence of a radiologically proven lesion in the carotid artery and this lesion should explain the patient's cerebrovascular symptoms. Though carotid endarterectomy is usually performed in patients who have had transient ischemic attacks, it may also be effective in asymptomatic patients. Although it is a common and safe procedure, perioperative and postoperative cerebral infarction due to platelet deposition at the endarterectomized site remains an important potential complication.

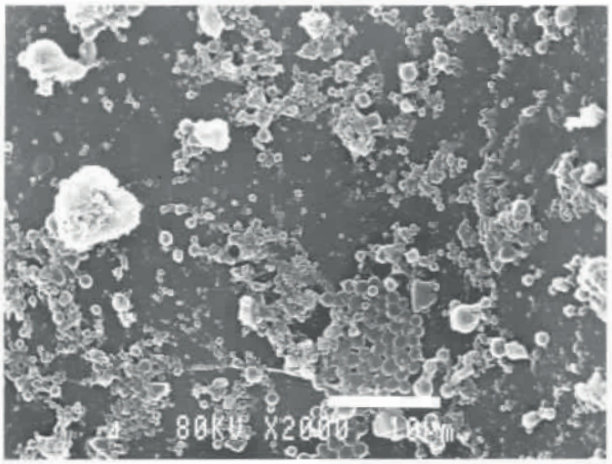

Figure2: Scanning electron micrograph of a Ticlopidine-treated vessel showing insifnificant platelet aggregates and groups of red blood cells.

Several experimental models have been used to evaluate thrombus formation and assess the effectiveness of antithrombotic drugs. These models include arteriovenous shunting, sodium laureate injection in femoral artery, de-endothelialization of the carotid artery by air injection, metallic coil insertion, electrical stimulation and microsurgical carotid endarterectomy (3,4,6,19,20,21,22).

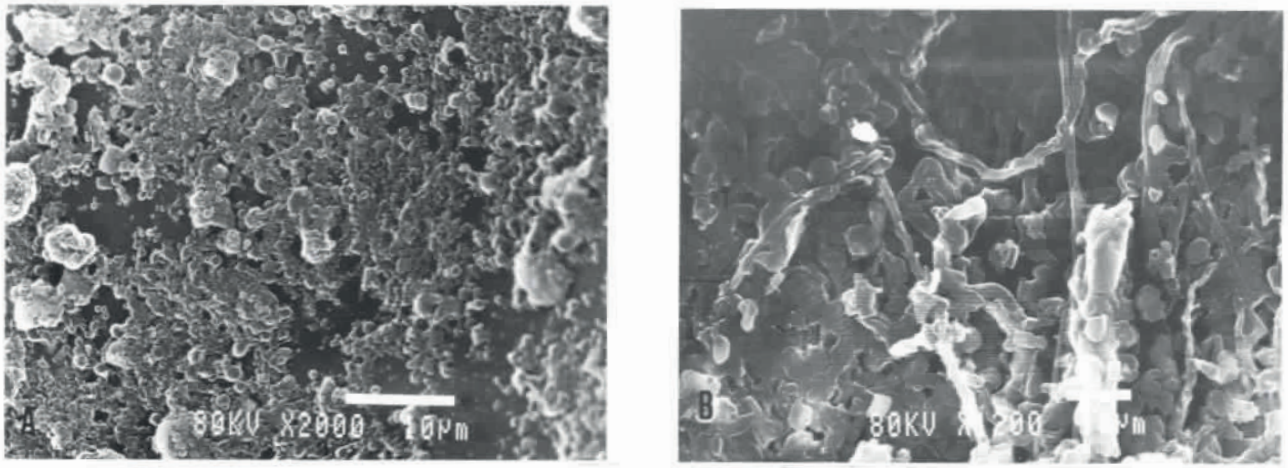

Figure1: Scanning electron micrograph of a control vessel. The endarterectomized surface is covered by obvious platelet aggregates (A) and entrapped red blood cells are visible among the fibrin strands (B).

Experimental microsurgical carotid endarterectomy was described in the early 1980s as an experimental model for the study of thrombus formation. Antithrombotic drugs used to reduce thrombus formation include aspirin, heparin, dipyridamole, prostacyclin(PGI_2), warfarin, OKY-1581, Ticlopidine and hirulog (12,13,19,22).

Ticlopidine has proven to be a potent and long-lasting inhibitor of platelet aggregation induced by various agents such as ADP, thrombin, collagen, adrenaline, arachidonic acid and PAF. Unlike other antiplatelet agents, Ticlopidine inhibits both the first and second phases of platelet aggregation (17,19). The drug's antiaggregant potential has been demonstrated in studies on animals and man (1,2,8,18,19). Approximately 80 to 90% of an oral dose of Ticlopidine is absorbed and peak plasma concentrations occur at 1 to 3 hours after a single oral dose in both the rat and man (19). Ticlopidine has been used at 25 to 300 mg/kg, either as a single dose or for 2 to 3 days, in a large number of animal models of thrombosis (2,8,18,19).

In this study, we investigated the antithrombotic effect of Ticlopidine on an experimental microsurgical carotid endarterectomy model in rats. An oral Ticlopidine dose of 100mg/kg orally was used in this study because this appears to be the most effective dosage in animal models (2,6,16,19). The vessels were studied by scanning electron microscopy, and were evaluated based on the platelet aggregates at the endarterectomized site. We performed our assessment 3 hours after the completion of endarterectomy and recirculation since it is known that thrombus formation peaks 3 hours after microsurgical carotid endarterectomy in the rat (21). Statistical analysis of the scores for the arteries showed a statistically significant difference between the control and Ticlopidine groups.

In conclusion, this study proves that Ticlopidine is effective at reducing thrombus formation after microsurgical carotid endarterectomy in rats. Administration of a single dose has a preventive effect on platelet aggregation after surgical arterial injury. Because it reaches maximal activity at 3 to 5 days, administration of the doses a few days preoperatively may be more effective against thrombus formation after carotid endarterectomy.

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