Effect of Flunarizine on Arterial Thrombosis after Endarterectomy in Rats

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Abstract

Unilateral carotid endarterectomy was performed microsurgically in 60 rats. The effects of peri-operative administration of aspirin and flunarizine, a Ca^{2+} entry blocker, on thrombus formation were examined 24 hours after endarterectomy. An untreated group of endarterectomized rats served as controls. Platelet adhesion, blood cells, and fibrin cords were observed at the cut edge of the intima as well as in the endarterectomized area in all three groups. However, these findings were significantly more extensive in the control group than in the drug-treated groups. Flunarizine was as effective as aspirin in inhibiting platelet adhesion and fibrin cord formation. Both drugs significantly increased the patency rate after carotid endarterectomy.

Key words: carotid endarterectomy, flunarizine, aspirin

Introduction

Carotid endarterectomy is now generally accepted as a useful procedure for the prevention of major stroke in patients with ischemic symptoms whose angiograms demonstrate significant atheromatous plaque at the ipsilateral common carotid artery bifurcation. However, major postoperative complications occur in 1.1–21.1% of patients;⁷⁾ the complication rate varies greatly depending on the institutes reported in the literature. Two of the most dangerous complications of carotid endarterectomy are acute postoperative occlusion and recurrent stenosis. In the hope of finding ways to prevent these complications, much experimentation has been conducted in rabbits,¹⁰⁾ dogs,^{5,6)} cats,¹⁴⁾ and, more recently, rats.¹⁶⁾

Loss of endothelium or exposure of the collagen of the media after carotid endarterectomy leads to rapid activation of platelets and may subsequently cause thrombus formation.^{10,12)} Aspirin's antithrombotic effect involves suppression of thromboxane A_2 production through inhibition of platelet cyclo-oxygenase.¹⁵⁾ Ca^{2+} entry blockers, such as flunarizine, protect the endothelial lining against the des-

quamating effect of citrate and inhibit stimulation of platelet aggregation by epinephrine and arachidonic acid.^{11,13)}

We compared the effects of flunarizine and aspirin on thrombus formation following microsurgical carotid endarterectomy in rats.

Materials and Methods

Sixty male Wistar rats weighing 200–250 gm were randomized into a control group ($n = 20$), a flunarizine-treated group ($n = 20$), and an aspirin-treated group ($n = 20$). Each group received three intraperitoneal injections 1 hour before, immediately after, and 12 hours after endarterectomy. The controls were given saline, and the experimental rats received 1.5 mg/kg flunarizine or 3 mg/kg aspirin.

Carotid endarterectomy was performed under 40 mg/kg sodium pentobarbital, injected intraperitoneally. An anterior skin incision was made from shoulder to shoulder. The right common carotid artery was exposed and temporarily occluded with two polyethylene vascular occluders. A longitudinal incision of approximately 8 mm in length was made in the artery under an operating microscope. The intimal surface was cut transversely in two locations 4 mm apart with the tip of a 27-gauge needle,

Fig. 1 Diagrammatic illustration of the endarterectomy procedure. A longitudinal incision 8 mm in length was made in the anterior wall of the common carotid artery. The endarterectomized segment was 4 mm in length.

after which the intervening intima and a portion of the media were excised circumferentially with the tips of microforceps (Fig. 1). The vessel was then closed with 10-0 monofilament nylon suture and the vascular occluders were removed.

All animals were sacrificed 24 hours after surgery. The cardiovascular system was perfused with 30 ml of saline followed by 30 ml of 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate. The perfusion pressure was maintained at 100 cmH₂O. The common carotid artery was excised and divided into two pieces, one of which was prepared for scanning electron microscopy (SEM) and one for transmission electron microscopy (TEM). The SEM specimens were stored in cacodylate-buffered 2.5% glutaraldehyde for 2 hours and postfixed in 1% osmium tetroxide in the same buffer for 1 hour at 4°C. They were dehydrated in graded alcohols and amyl acetate, critical point dried with CO₂, coated with gold in an evaporator (EIKO 1B-3), and examined with a Hitachi S-450 SEM. The TEM specimens were fixed and dehydrated in the same manner, then embedded in Epon 812. Semi-thin sections were cut with an LKB ultramicrotome and stained with toluidine blue. Ultra-thin sections were cut from selected areas and stained with uranyl acetate and lead citrate and examined with a Hitachi H-600 TEM. The data concerning patency *versus* occlusion were analyzed by the Chi-square test between the control group and the drug-treated groups.

Results

Table 1 shows the patency rates after carotid endarterectomy, which were 60% in the control group, 100% in the flunarizine-treated group, and 95% in

Table 1 Patency rates in control and drug-treated endarterectomized rats

Control	Flunarizine-treated	Aspirin-treated
12/20 (60%)	20/20 (100%)*	19/20 (95%)**

* $p < 0.01$, ** $p < 0.05$ *versus* control (Chi-square test).

the aspirin-treated group. Both the flunarizine- and aspirin-treated groups exhibited significantly greater patency than did the controls.

Histological examination of the vessels disclosed adhesion of platelets, erythrocytes, and leukocytes to the endarterectomized sites. Compared to no treatment, flunarizine and aspirin administration both reduced the number of platelets attached to the luminal surface. Figure 2 is a SEM specimen of the distal end of the endarterectomized segment in a flunarizine-treated rat. Adhesion of platelets and erythrocytes is particularly evident at the cut edge of the intima. Figures 3 and 4 show sutured sites in animals of the flunarizine- and aspirin-treated groups, respectively. Adherent platelets, erythrocytes, and fibrin cords were observed on the nylon suture as well as in the endarterectomized area. In the control group, thrombosed vessels were filled with fibrin cords and blood cells (Fig. 5). Figure 6, TEM specimens from flunarizine-treated rats, show a thrombus at the endarterectomized site. Endothelial cells near the site displayed indentations on the basement membrane side and contained many cytoplasmic vesicles. Leukocytes were found in the endarterectomized region and were also observed in the junctions between and beneath endothelia. These changes were seen in all three groups.

Discussion

Following endarterectomy, platelets adhered to the operated site, including its periphery, and to the exposed suture material. Maximum adhesion of fibrin, platelets, and erythrocytes was observed at the periphery, which was rolled. The degree of endothelial damage resulting from endarterectomy varies according to the proximity of endothelium to the operated site and the surgical technique.⁵ In our study, the endothelial cells had indentations of different sizes on the basement membrane side and many cytoplasmic vesicles. Leukocytes migrated between and beneath endothelial layers. The leukocytes may cause desquamation of the endothelial cells.

- Fig. 2 SEM specimen of the distal end of the endarterectomized segment in a flunarizine-treated rat. Adhesion of platelets and erythrocytes is seen at the cut edge of the intima and endarterectomized area. Bar = 50 μ m.
- Fig. 3 SEM specimen of the sutured site in a flunarizine-treated rat. A fibrin-platelet layer is observed on the nylon suture (*arrow*) as well as in the endarterectomized area. Bar = 50 μ m.
- Fig. 4 SEM specimen of the sutured site in an aspirin-treated rat. Endothelial damage due to the suture (*arrow*) is seen in the non-endarterectomized area. Bar = 100 μ m.
- Fig. 5 SEM specimen from a control rat, showing fibrin cords and blood cells in a thrombosed vessel. Bar = 15 μ m.

- Fig. 6 TEM specimens from flunarizine-treated rats. *left*: A thrombus is seen in the endarterectomized site. *center*: A leukocyte is present in the junction between two endothelia near the endarterectomized site. *right*: Endothelial cells are indented on the basement membrane side and contain many cytoplasmic vesicles. A leukocyte is seen beneath an endothelial layer. Bar = 5 μ m.

Platelets are known to play an important role in the initiation and propagation of arterial thrombus formation.¹⁸⁾ Perioperative administration of aspirin or other antiplatelet drugs has been shown to significantly reduce thrombus formation after vascular surgery.^{1-3,8)} Ercius *et al.*⁶⁾ found that 10 mg/kg aspirin, given to dogs 18 hours before carotid endarterectomy, significantly reduced thrombus formation at the endarterectomy site. However, there have been no reports concerning the effect of flunarizine on thrombus formation.

The production of thromboxane A₂ (TXA₂) by platelets and the synthesis of prostacyclin (PGI₂) by the vessel wall are both important in the regulation of hemostasis and thrombosis.^{9,19)} TXA₂ is a potent platelet aggregator and vasoconstrictor, whereas PGI₂ inhibits platelet aggregation and promotes vasodilatation. Aspirin blocks the production of these two important prostaglandins by inhibiting cyclo-oxygenase (an enzyme common to the synthesis of both).¹⁵⁾ Cyclo-oxygenase within platelets appears to be more sensitive to this inhibition than that within the vessel wall.^{2,9)} Aspirin, although effective, causes gastrointestinal bleeding when given in high doses.¹⁷⁾

Flunarizine, a selective Ca²⁺ entry blocker, was shown to suppress contractions of vascular smooth muscle and protect against loss of functional deformability of erythrocytes.⁴⁾ Oral pretreatment of animals with flunarizine resulted in significant protection of the endothelial lining against the desquamating effect of citrate.¹¹⁾ Our results showed that flunarizine was as effective as aspirin in decreasing the rate of thrombosis after carotid endarterectomy. Sasaki *et al.*¹⁶⁾ reported that all endarterectomized vessels in both control and those rats treated with heparin or TXA₂ synthetase inhibitor were patent 7 days after operation, without apparent mural thrombus formation. However, we observed thrombi in our drug-treated groups as well as in the control group. We used rats weighing 200–250 gm, whereas Sasaki and coworkers used 400- to 450-gm rats. This may have affected the results, since the smaller the animal, the more difficult the surgical procedure itself is. Also, Sasaki *et al.* washed the endarterectomy lumen with heparinized saline, which we did not do. These may account for the differences in the arterial patency rate between our control group and their controls after endarterectomy.

In conclusion, endothelial and medial damage adjacent to the endarterectomized site is one of the major causes of endarterectomy failure. Our results indicate that perioperative flunarizine or aspirin treatment can reduce the incidence of this complication.

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