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Histopathological effects of nimodipine and pentoxifylline on the vessel wall in end-to-end anastomoses in rat carotid arteries

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Abstract
When reperfusion following ischemia occurs, oxygen returns to the ischemic tissue, increasing free oxygen radicals and inducing paradox secondary damage. Before infarction, revascularization may influence the morbidity rate. Successful revascularization is not always achieved due to stenosis incidence, proliferation of smooth muscle cells, and intimal hyperplasia. This study compares the effects of nimodipine that prevents vasospasm and pentoxifylline, which stimulates growth factors and reduces collagen synthesis on intimal hyperplasia. Eighteen randomly selected Sprague-Dawley rats were divided into three groups: Group 1, the control group; Group 2, intraperitoneally administered nimodipine; Group 3, orally administered pentoxifylline. Their right-sided carotid arteries were used for anastomosis and the left-sided ones for the control. After a 7-day treatment, both the right and left carotid arteries were removed. In the biopsy, the lumen's area and diameter, thickness of tunica media thickness, thrombus, edema, intimal hyperplasia, vessel wall injury, and inflammation were analyzed. Pentoxifylline was effective in preventing intimal hyperplasia and tunica intima was similar to that in untreated carotid arteries. However, nimodipine inhibited intimal hyperplasia, but it was not as effective as pentoxifylline. The effects of pentoxifylline after anastomosis should be further assessed in vasoprotective treatment taking into account its efficacy against intimal hyperplasia

Keywords: nimodipine, pentoxifylline, end-to-end anastomoses, carotid arteries, cerebral ischemia

1. Introduction

Cerebral ischemia occurs when the required oxygenation for cerebral tissues cannot be maintained as the arterial blood flow is reduced (1). When reperfusion occurs following ischemia, the oxygen reaches the ischemic tissues, causing an increase in free oxygen radicals and inducing paradox secondary damage (1, 2). Thus, performing revascularization before infarction may affect the morbidity rate.

Owing to the occurrence of stenosis, proliferation of smooth muscle cells, and intimal hyperplasia, revascularization attempts are always not successful. These complications, following vascular reconstructive interventions, result in higher mortality and morbidity rates. Neointimal hyperplasia, resulting from smooth muscle cell migration, proliferation, and extracellular matrix accumulation, plays a crucial role in late narrowing or restenosis (3, 4).

Nimodipine is a dihydropyridine calcium channel blocker, which is mainly effective against central nervous system disorders and particularly useful in preventing and treating arterial vasospasm resulting from subarachnoid hemorrhage (5). Pharmacological agents inhibiting calcium entry into the cell or intracellular calcium-dependent events may delay the proliferation and migration of smooth muscle cells, thus reducing neointimal thickening after arterial injuries (6). Pentoxifylline is a xanthine derivative, phosphodiesterase inhibitor elevating cAMP (cyclic adenosine monophosphate) levels by inhibiting phosphodiesterase. Increased cAMP levels obstruct the growth of vascular smooth muscle cells. Therefore, pentoxifylline stimulates several growth factors in vascular smooth muscle cells and reduces collagen synthesis and the rate of neointimal hyperplasia occurring after vascular injury (7).

In this study, we compared and analyzed the effects of both drugs, nimodipine and pentoxifylline, on intimal hyperplasia. For this purpose, we conducted common carotid artery anastomosis in rats, administered drugs postoperatively, performed and histopathological evaluation.

2. Material and Methods

This experimental study was carried out after obtaining approval from the Dicle University Medical Faculty Animal Experiments Local Ethics Committee, approval number 9 dated 20/01/2016.

The study design consisted of two steps: first, common carotid arterial anastomosis; then, drug administration in rats.

In this study, eighteen randomly selected male and female Sprague-Dawley rats were used. The right carotid artery was used for anastomosis and the left as control. After one week of

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adaptation, the rats were randomly divided into three equal groups: Group 1 rats were the control group; Group 2 were intraperitoneally given a single dose of 10 mg/kg nimodipine (8) daily for 7 days; Group 3 were orally given a single dose of 100 mg/kg pentoxifylline (Trental® 400 mg; Sanofi Aventis, Turkey) (9) daily for 7 days.

The average time to develop pseudointima in the anastomotic line is 7 days (10); therefore, the effects of the anastomosis were evaluated at a limited treatment period of 7 days, when intimal hyperplasia is most prominent and fibrin and thrombus wastes are disposed of (10).

After a 7-day treatment, 1 cm segments of the right and left carotid arteries were removed and dispatched for further pathological examinations. The obtained segments were stained in formol solution, followed by hematoxylin-eosin, periodic acid–Schiff (PAS), and Masson's trichrome stains. The same pathologist conducted the pathological and staining examinations, blindly. Inflammation, vessel wall damage, intimal hyperplasia, lumen's area and diameter, thickness of tunica media, thrombus, and edema were analyzed.

2.1. Surgical technique

The rats were fasted for 4 h prior to the surgery. On the day of the experiment, rats were anesthetized with 80 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride (intraperitoneally) and were fixed in the supine position (11).

After sterilization, a horizontal right-sided neck incision was performed. Fatty tissues were raised toward the cranial as a pedicle flap. The sternocleidomastoid muscle was removed laterally with a retractor and the neurovascular bundle near the paratracheal muscle was reached (Figure 1A). To avoid respiratory distress, it is necessary not to pull the muscles too hard during the operation. A clamp was placed prior to the dissection.

The carotid artery was transected 1 cm proximal to the bifurcation with microscissors and washed with Ringer's lactate solution using a silicone tube (Figure 1B). The adventitia layer was peeled off using microforceps. Sutures were passed through the media layer with 10-0 prolene suture. First, 0- and 180-degree sutures were made (Figure 1C) and then the anterior and posterior surface sutures. Subsequently, suturing was completed with 8 sutures (Figure 1D). Then, the clamp was removed and pressure was applied with a moist tampon for 5 min. When the bleeding stopped completely, the layers were closed anatomically.

2.2. Preparation of pathology specimen

Following the treatment, the procedure was carried out again under anesthesia. To reveal the anastomosis line, 1 cm segments of the anastomosis and common carotid arteries were removed. Moreover, the left common carotid artery sample without anastomosis was removed, including the area 1–1.5 cm proximal to the bifurcation. Then, the removed vascular segments were sent to the pathology laboratory, preserved in formol solution, for analysis.

To evaluate the effects of the surgical procedure, the control group received anastomosis surgery but were not given any medication. For assessing the drug effects, a sample from the left carotid artery of the rat was taken as control.

2.3. Statistical analysis

Bivariate correlation (Pearson's R and Spearman's tests) was utilized for estimating the correlation between the datasets, while Chi-square test was used for categorical data. Mann–Whitney *U* test was employed for analyzing independent data not considered within the normal distribution. Statistical Package for the Social Sciences (SPSS) for Windows (version 20.0) was used for analysis and $p < 0.05$ was considered statistically significant.

3. Results

The lumen's diameter and area, thickness of tunica media, edema, inflammation, damage to the vessel wall, thrombus, and intimal hyperplasia were compared between the groups.

In the control group (Group 1), the lumen diameter and area were significantly decreased when comparing the right and left carotid arteries ($p = 0.023$; $p = 0.012$, resp.). In Group 2, the decrease in the lumen diameter was less than that in Group 1 but insignificant ($p = 0.079$). In Group 3, the decrease in the lumen diameter was less than that in Group 1, but statistically significant ($p = 0.026$). No difference was observed between Groups 2 and 3 ($p = 0.826$) (Table 1).

The lumen area in Groups 2 and 3 was bigger than that in Group 1; the difference was statistically significant ($p = 0.002$, $p = 0.005$); however, there was no difference between Groups 2 and 3 ($p = 0.918$) (Table 1). The increase in media thickness was significant for Group 1 and for the right and left carotid arteries was statistically significant ($p = 0.003$). However, it was less for both Groups 2 and 3. Although the thickness of tunica media was increased in all groups, when compared it was found to be insignificant ($p > 0.05$) (Table 1).

Edema and inflammation in Groups 2 and 3 were less than those in the control group; however, the difference was statistically insignificant ($p = 0.220$; $p = 0.220$, resp.). No difference regarding thrombus was observed in all groups ($p = 1.000$) (Table 2). When the right and left carotid arteries were compared in Groups 1 and 2, vascular wall damage was found to be statistically significant ($p = 0.014$; $p = 0.045$, resp.). Damage to the vessel wall was not observed in Group 3; however, the difference was insignificant when compared with that of Groups 1 and 2 ($p = 0.070$; $p = 0.220$, resp.) (Table 2). The occurrence of intimal hyperplasia was less frequent in Group 3 and comparing the right and left carotid arteries, the difference was not statistically significant ($p = 0.296$). The treatment in Group 2 was more effective than that in Group 1, and the results when comparing Groups 2 and 3 with Group 1 were statistically significant ($p = 0.035$; $p = 0.009$, resp.) (Table 2) (Fig. 2).

Table 1. Comparison of lumen diameter, lumen area, and tunica media thickness between groups

Group / Parameter	Control group (n = 6)		*p*	Nimodipin group (n = 6)		*p*	Pentoxifylline group (n = 6)		*p*	*p* *
	Right	Left		Right	Left		Right	Left		
Lumen diameter (µm)	383.6	501.2	*p=0.023*	484.8	547.9	p=0.119	518.6	568.4	p=0.396	cxn=0.079 *cxp=0.026* nxp=0.826
Lumen area ($µm^2$)	122433	200422	*p=0.012*	233516	253612	p=0.203	222961	254509	p=0.468	*cxn=0.002* *cxp=0.005* nxp=0.918
Tunica media thickness ($µm^2$)	65.03	52.54	*p=0.003*	63.05	56.60	p=0.143	57.20	50.20	p=0.204	cxn=0.831 cxp=0.196 nxp=0.449

* *p* values for the right-side analysis; cxn = control group versus nimodipine group, cxp = control group versus pentoxifylline group, nxp = nimodipine group versus pentoxifylline group.

Table 2. Comparison of edema, vessel wall injury, intimal hyperplasia, and inflammation between groups

Group / Parameter		Control group (n = 6)		*p*	Nimodipin group (n = 6)		*p*	Pentoxifylline group (n = 6)		*p*	*p* *
		Right	Left		Right	Left		Right	Left		
Edema (+)		3	0	*p=0.045*	1	0	p=0.296	1	0	p=0.296	cxn=0.220 cxp=0.220 nxp=1.000
Vessel wall injury (+)		4	0	*p=0.014*	3	0	*p=0.045*	1	0	p=0.296	cxn=0.550 cxp=0.070 nxp=0.220
Intimal hyperplasia	+	2	0	*p=0.002*	4	0	*p=0.014*	1	0	p=0.296	*cxn=0.035* *cxp=0.009* nxp=0.078
	++	4	0		0	0		0	0		
Inflammation		2	0	p=0.121	0	0	p=1.000	0	0	p=1.000	cxn=0.120 cxp=0.120 nxp=1.000

* *p* values for the right-side analysis; cxn = control group versus nimodipine group, cxp = control group versus pentoxifylline group, nxp = nimodipine group versus pentoxifylline group

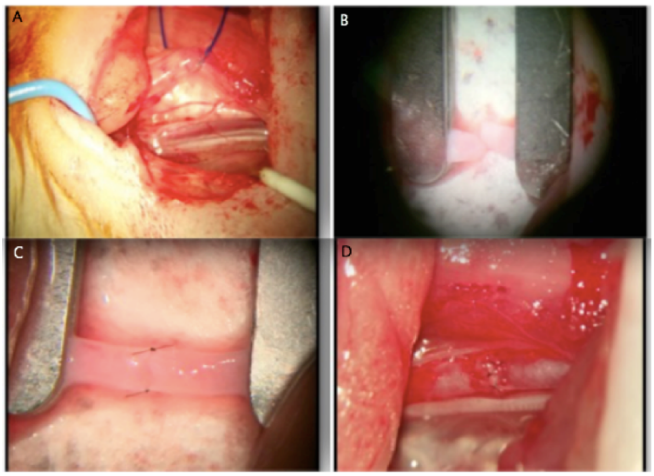

Fig. 1. (A) The carotid and vagus nerve after the sternocleidomastoid muscle is removed medially (black arrow); (D) removal of fixation sutures, completion of the anastomosis, and control of vessel patency

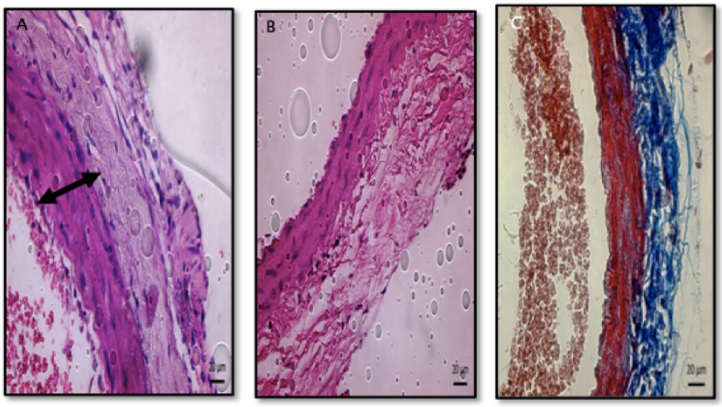

Fig. 2. (A) In the control group, tapering of the tunica intima, tunica media moderate edema, and thickening (black arrow) intimal hyperplasia are observed (hematoxylin & eosin dye, 40×). (B) In the nimodipine group, intimal hyperplasia is observed (hematoxylin & eosin dye, 40×). (C) In the pentoxifylline group, intimal hyperplasia is observed (Masson's dye, 40×)

4. Discussion

Several previous researchers reported the effects of nimodipine and pentoxifylline; however, studies investigating the effects of these drugs on anastomosis are limited.

Pharmacological agents inhibiting the calcium entry into the cells or intracellular calcium-dependent events may delay the proliferation and migration of smooth muscle cells following arterial injuries. Based on this fact, using calcium channel blockers to inhibit neointimal thickening in arterial injuries is recommended (6, 12).

Kadioglu et al. reported that nimodipine diminished endothelial dysfunction, accelerated and increased proliferation, and increased hyperplasia in the smooth muscle layer; however, this effect was reduced with prolonged treatment duration (13).

Hammerman et al. performed a study on rat intestine, demonstrating that pentoxifylline acts as an antioxidant by inhibiting the xanthine oxidase enzyme, thereby playing a role in preventing ischemia-reperfusion injury (14).

Busk et al. found that after balloon angioplasty in rabbit iliac arteries, subcutaneously administered pentoxifylline decreased the neoadventural hyperplasia at the end of 28 days and reduced cytokine and collagen accumulation in the vessel wall in the study group compared to those in the control group. Neointimal hyperplasia in the study group was less than that in the control group (15).

Chen et al. demonstrated that pentoxifylline inhibits platelet-induced growth factor after vessel damage and decreases collagen synthesis stimulated by TGF-β (transforming growth factor beta) in vascular smooth muscle cells; consequently, the vessel diameter in the pentoxifylline group was greater than that in the control group, following vessel damage (7).

Takahashi et al. revealed the proliferation of smooth muscle cells and intimal migration were induced by two growth factors in the media layer, the basic fibroblast growth factor and platelet-derived growth factor. The basic fibroblast growth factor is secreted from damaged smooth muscle and endothelial cells and regulates smooth muscle cell proliferation, whereas the platelet-derived growth factor is released by platelets and vascular cells and provides profiling and migration of smooth muscle cells (16).

In Ustunsoy et al.'s study, pentoxifylline decreased the blood viscosity by enhancing erythrocyte flexibility and preventing platelet aggregation. Accordingly, they stated that pentoxifylline led to increased and improved capillary blood flow and tissue oxygenation (107).

Taking into account the findings of these previous studies, we compared the inhibitory effects of nimodipine and pentoxifylline on intimal hyperplasia and restenosis. In this study, the vasoprotective effects were assessed by comparing the lumen diameter, lumen area, tunica media thickness, edema, vessel wall damage, thrombus, intimal hyperplasia, and inflammation parameters.

After anastomosis, the luminal diameter and area decreased in all groups compared to that those in other carotid arteries; however, Group 3 had the least degree of reduction. Although the reduction by nimodipine was not as effective as that by pentoxifylline in Group 2, it had a positive impact on the luminal diameter and area.

Upon evaluation, in terms of intimal hyperplasia, pentoxifylline effectively inhibited intimal hyperplasia, and tunica intima was similar to that in untreated carotid arteries. Moreover, nimodipine was successful in preventing intimal hyperplasia, but to not the same extent of pentoxifylline. The effects of pentoxifylline after anastomosis should be further evaluated in vasoprotective treatment protocols considering its effectiveness against intimal hyperplasia.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: A.A., P.A.O., Design: A.A., P.A.O., Data Collection or Processing: A.A., P.A.O., Analysis or Interpretation: A.A., P.A.O., Literature Search: A.A., P.A.O., Writing: A.A., P.A.O.

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