Original Paper · Travail original · Originalarbeit

Ophthalmologica

Ophthalmologica 2001;215:55–60

Received: June 8, 1999
Accepted after revision: January 19, 2000

Early Ultrastructural Findings and Superoxide Dismutase Levels in Experimental Ischemic Optic Neuropathy: Effect of Hypertension and Hypotension on Ischemic Changes

Gülhanım Hacıyakupoğlu[a] İsmail İşigüzel[a] Suzan Zorludemir[b]
Handan Zeren[b] Hüseyin Bağdatoğlu[c] Şule Menziletlioğlu[d]
Sebahattin Hacıyakupoğlu[c] Kıymet Aksoy[d] Kenan Dağlıoğlu[e]

Departments of [a]Ophthalmology, [b]Pathology, [c]Neurosurgery and [d]Biochemistry, Medical Faculty, and [e]Medical and Experimental Research Center, Çukurova University, Adana, Turkey

Key Words

Optic nerve · Experimental ischemia · Optic neuropathy · Light microscopy · Transmission electron microscopy · Hypertension · Hypotension · Superoxide dismutase

Abstract

We induced ischemia, hypertension and hypotension in 15 rabbits in order to evaluate the ischemic changes in the optic nerve and the effect of hypertension and hypotension on ischemia. We cauterized the right internal and external carotid arteries of 15 rabbits and applied dopamine hydrochloride and glycerol trinitrate to 5 each of these rabbits. Two rabbits were used as controls. We enucleated both eyes of all animals at the 24th hour. All of the optic nerves underwent biochemical, histopathological and ultrastructural examination. Histopathological and transmission electron-microscopic changes were found to be more prominent in the hypotensive group. We observed decreased superoxide dismutase levels in all groups, but it was more evident in the third group. In comparison to hypertension, hypotension is found to be a more important factor in the development of early degenerative changes.

Copyright © 2001 S. Karger AG, Basel

Ischemic optic neuropathy (ION) is an important cause of visual loss over the age of 50 years. A frequent association with HT, DM, atherosclerosis, collagen and cerebrovascular disorders has been reported [1–4]. ION is thought to develop from the obstruction of the blood flow in the posterior ciliary arteries; however, the pathogenesis is still uncertain [4]. The nonexistence of a barrier in the choriocapillaris, such as the blood-retina barrier, enables the leakage of vasoconstrictor agents to the choroidal extracellular space through the pores which causes vasoconstriction and optic disk ischemia [5]. The acute drop of arterial tension in hypertensive cases increases the existing optic nerve ischemia and causes bilateral infarction of the optic nerve head [6, 7].

We obstructed the internal carotid arteries (ICA) of 15 rabbits in order to evaluate the ischemic changes in the optic nerve and the effect of hypertension and hypotension on these changes.

Materials and Methods

The study group consisted of 17 rabbits weighing 2–3 kg. Four globes of 2 rabbits were used as controls. The following procedures were performed in the remaining 15 rabbits. The arterial tension of all the rabbits was recorded through the right arteria femoralis. The

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2001 S. Karger AG, Basel
0030–3755/01/2151–0055$17.50/0

Accessible online at:
www.karger.com/journals/oph

Gülhanım Hacıyakupoğlu
Çukurova Üniversitesi, Tıp Fakültesi
Balcalı Hastanesi
TR–01330 Adana (Turkey)

Downloaded by:
Cukurova Universitesi
193.255.199.2 - 1/12/2018 12:29:46 PM

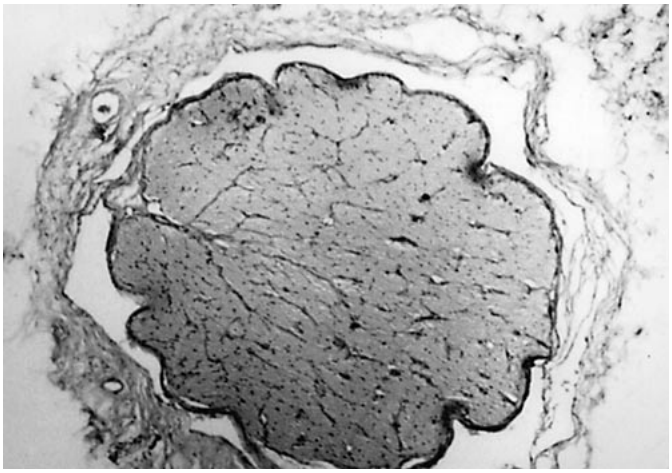


Fig. 1. Control group. Axons and pial septa of the optic nerve surrounded with meningeal sheaths. HE. × 40.



Fig. 2. Control group. Axons surrounded with healthy myelin sheaths and fibrils of astrocytic cytoplasm. × 24,000.

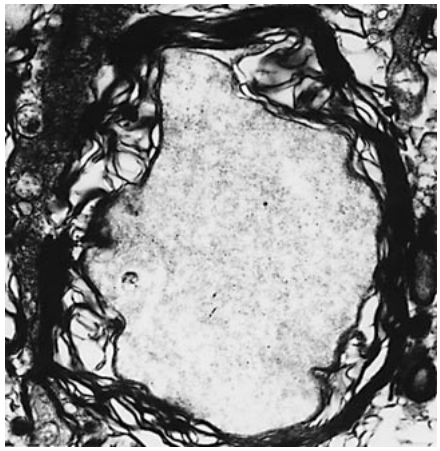


Fig. 3. Ischemic group. Myelin sheath degeneration, axonal edema and degeneration. × 24,000.

animals were sedated with 1 mg diazepam and anesthetized with intramuscular ketamine 50 mg/kg injected through an ear vein. We cauterized the right ICA and external carotid artery (ECA) for 2 min following ligation of the right common carotid artery. The animals were divided into three groups; each group consisted of 5 rabbits. We induced only ischemia in the first group. We applied intravenous dopamine hydrochloride 10 μg/kg/h to the second and 5 μg/kg/h glycerol trinitrate for 24 h to the third group. Both globes of all the animals were removed at the 24th hour before sacrificing the animals with high-dose Nembutal. No intervention was made on the left orbits prior to extirpation of the nerves.

After the enucleation and dissection of the globes, the proximal parts of the optic nerves were transsected into three equal pieces of 2 mm. The first two pieces were fixed in 10% formaldehyde and Karnovsky's solution. The most anterior part was processed for transmission electron microscopy (TEM) and the middle part for light-microscopic examination. The third tissue samples were immersed in isotonic sodium solution (10% NaCl) for the analysis of Cu, Zn superoxide dismutase (SOD) enzyme using biochemical techniques.

Light-microscopic examination was performed on paraffin-embedded tissues and 5-μm-thick hematoxylin-eosin (HE) sections. Periodic acid-Schiff, reticulin, van Gieson, Masson's trichrome and Bielschowsky special stains were also used. After being immersed in Karnovsky's fixative at 4 °C for 1 h, tissue samples for TEM were postfixed in 1% osmium tetroxide, dehydrated by graded series of ethanol and finally embedded in Epon. Semithick (1 μm) sections were stained with toluidine blue special stain and examined under the light microscope. Following the trimming of the tissues, 500-Engström sections supported with 200-mesh copper grids and stained with uranyl acetate and lead citrate were examined using a Zeiss EM 900 transmission electron microscope. The SOD enzyme analysis was performed using the Randox/Ransod kit technique (Product No. ORD04, Lot No. 108RD, Randox Laboratories, UK). Student's t test was used for statistical analysis.

Findings

Prior to intervention, the mean arterial tension of all the animals was 105/65 mm Hg whereas it increased to 135/85 mm Hg in the second and dropped to 85/45 mm Hg in the third group.

Downloaded by:
Cukurova Universitesi
193.255.199.2 - 1/12/2018 12:29:46 PM



Fig. 4. Ischemic and hypertensive group. Degeneration of the myelin sheaths, focal areas of widened and edematous axons, and axonal degeneration and vacuolization. × 24,000.

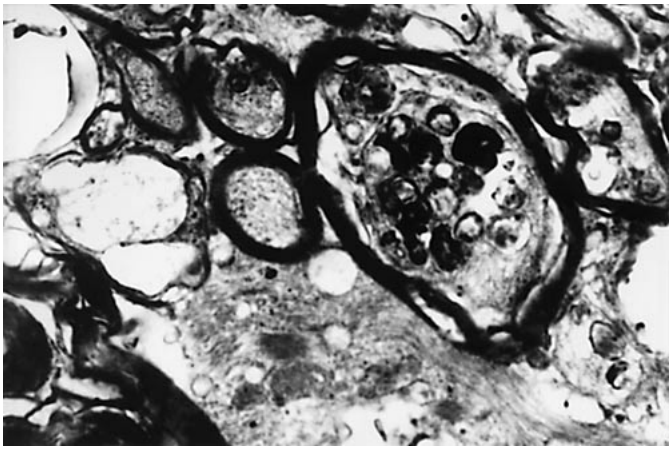


Fig. 5. Ischemic and hypertensive group. Axonal organelle accumulation. × 24,000.



Fig. 6. Ischemic and hypotensive group. Features of serious injury by diffuse myelin sheath damage, axonal degeneration, swelling, vacuolation, organelle accumulation and loss. × 24,000.



Fig. 7. Ischemic and hypotensive group. Axonal degeneration and organelle accumulation. × 40,000.

The control group's light-microscopic examination of HE sections of the optic nerves did not show any pathological findings (fig. 1). Ultrastructural examination showed axons surrounded with a healthy myelin sheath and astrocytic cytoplasm (fig. 2).

In group 1, light-microscopic examination of HE and toluidine blue sections of the ischemic optic nerves revealed interstitial edema and disorganized axons while ultrastructural examination showed a degeneration of the myelin sheaths, focal areas of widened and edematous axons, and axonal degeneration (fig. 3). Vascular structures were intact.

In group 2, interstitial edema was prominent. Ultrastructurally, in addition to the axonal swelling and degeneration of the myelin sheaths in focal areas, vacuolation of the myelin and axons as well as accumulation of the organelles in the axons were noted (fig. 4, 5). Swelling of the endothelium and obliteration of the lumina in vascular structures were common features.

In group 3, light microscopy showed diffuse interstitial edema and thickening of the vessel walls as well as pial septa. TEM showed features of serious injury by diffuse myelin sheath damage, axonal degeneration, swelling, vacuolation, organelle accumulation and loss (fig. 6, 7).

Downloaded by:
Cukurova Universitesi
193.255.199.2 - 1/12/2018 12:29:46 PM

Table 1. Optic nerve mean SOD enzyme levels of all the rabbits and the statistical difference between the eyes with and without ischemia

	Ischemic eyes (n = 5), U/mg	Nonischemic eyes (n = 5), U/mg	t value	p value
Group 1	9.1 ± 2.1	16.9 ± 1.8	5.64	<0.001
Group 2	12.9 ± 2.6	18.8 ± 2.4	3.335	<0.05
Group 3	10.3 ± 2.2	10.2 ± 1.6	0.074	>0.05

Table 2. Statistical difference between the control group and the eyes with and without ischemia

	Ischemic eyes (n = 5)		Nonischemic eyes (n = 5)	
	t value	p value	t value	p value
Control and group 1	6.324	<0.001	2.027	>0.05
Control and group 2	3.086	<0.05	1.588	>0.05
Control and group 3	4.831	<0.001	4.758	<0.01

The optic nerve mean SOD enzyme level of the control group was 14.8 ± 2.1 U/mg.

The comparison of eyes with and without ischemia in groups 1 and 2 revealed low SOD enzyme levels in the ischemic eyes and was found to be statistically significant ($p < 0.01$), whereas in the third group the difference was not found to be statistically significant ($p > 0.05$; table 1).

The comparison of the optic nerve mean SOD levels of the control group and the ischemic eyes of the study group revealed decreased SOD levels. The difference was found to be statistically significant ($p < 0.05$). In comparison with the control group, SOD levels of the eyes without ischemia in groups 1 and 2 were not found to be statistically significant ($p > 0.05$). In group 3, these values were decreased and the difference was statistically significant ($p < 0.01$; table 2).

Discussion

ION is one of the most common causes of permanent loss of vision in the presenescent and elderly population [1, 8, 9]. Disturbance in optic nerve circulation and drop in perfusion pressure due to short posterior ciliary artery occlusion are suggested to be the important factors in the pathogenesis [10].Occlusion or stenosis of the ophthalmic artery, ICA and common carotid artery, systemic arterial hypertension, embolus and hematological diseases lower the blood flow of the short posterior ciliary arteries resulting in a drop in optic nerve perfusion pressure [11–14].

Studies of blood flow with radioactively labeled microspheres demonstrated that the autoregulation mechanism of the optic nerve is limited in comparison to the retina and optic nerve neural tissue is more sensitive to anoxia than other tissues supplied by the choroidal circulation [15].

Waybright et al. [16] stated that in cases with ION and ipsilateral ICA occlusion, despite the retrograde circulation provided by the ECA, the development of optic nerve head hypoperfusion and ION cannot be prevented. In cases with acute ICA occlusion, the optic nerve head blood supply will not be provided by the collateral circulation of the cerebral hemispheres. We considered to occlude the ipsilateral ICA and ECA in order to achieve an ischemia [17–19].

Lessel and Miller [20] did not observe pathological changes in optic nerves of normotensive monkeys following circulatory arrest of 12–24 min and proposed that the optic nerve is resistant to circulatory arrest just like brain tissue. Histopathological examination of the rabbits with ischemia revealed interstitial edema, diminished staining concentration, myelin sheath degeneration in focal areas, enlarged, edematous axons and axonal degeneration. These ischemic changes imply that 24 h are an adequate period for the beginning of degenerative changes in the optic nerve.

It is known that segmental infarcts and disturbances of vascular structures in the optic nerve head due to hypotensive shock result in ION [12, 13, 21–24]. Lessell and Miller [20] observed mild edema in the optic nerve head, hypercellularity at the temporal retrobulbar portion of the

Downloaded by:
Cukurova Universitesi
193.255.199.2 - 1/12/2018 12:29:46 PM

optic nerve and loss of myelin sheaths in 2 of 7 rhesus monkeys who underwent hypotension and circulatory arrest for 16–20 min.

Antihypertensive agents have been used for the relief of arterial spasm due to anoxia but were found to be ineffective [7, 25–30]. We observed widespread axonal loss and degeneration as well as intensive organelle accumulation at the 24th hour in rabbits who underwent hypotensive and ischemic procedures. The detection of ultrastructural changes in the control eyes of the hypotensive group demonstrated the importance of hypotension in the development of hypoperfusion and anoxia resulting in ischemic changes.

We induced hypervolemia and hypertension in order to increase perfusion pressure. Kishi et al. [6] caused renovascular hypertension in 12 rhesus monkeys and observed endothelial edema and pericystic degeneration in the optic nerves at the 21th month. The histopathological examination of the optic nerves with ischemia and hypertension at the 24th hour revealed intramyelinic and axonal vacuolization and organelle accumulation besides ischemic histopathological changes. We can suggest that, in cases with ischemia, hypertension accelerates the development of specific ultrastructural changes.

Hayreh et al. [31] induced experimental hypertension and observed optic nerve edema on the 7th–637th day. Light-microscopic and ultrastructural findings of these subjects demonstrated the ischemic nature of hypertensive optic neuropathy, but the mechanism of the optic nerve ischemia remained unclear [31].

The optic nerve is known to be ischemic in malignant hypertension and a sudden drop in arterial pressure accelerates ischemia and results in bilateral optic nerve head infarction. In cases with malignant hypertension, arterial tension must be reduced slowly in order to prevent permanent visual loss [13].

Ischemia in the optic nerve and surrounding vascular tissue results in intracellular and extracellular increases in unsaturated fatty acids which are precursors of eicosanoids and leukotrienes. These substances accelerate the activity of lipo-oxygenase, xanthine oxidase and cyclooxygenase owing to an increase in arachidonic acid metabolism and synthesis of prostoglandins G_2, H_2, D_2, I_2, F_{2a} and thromboxane A_2. Most of these unstable endoperoxides are vasoconstrictive and have toxic effects on aerobic cells. They depress the metabolism which results in proliferative vasculopathy and a vicious circle with a reduction in blood flow. SOD is a scavenger for superoxide radicals and its level gives information about the cellular degeneration and antioxidant substance accumulation [26, 28, 29, 32–37].

We observed a fall in SOD enzyme levels in groups 1, 2 and 3 which may be due to the increased consumption of SOD enzyme in order to remove the increased oxygen radicals, or SOD enzyme may not be synthesized due to the degeneration of the tissue [33, 36, 38, 39].

The comparison of ischemic and nonischemic groups revealed low SOD levels in the ischemic eyes of groups 1 and 2. This may be due to hypotension resulting from hypoperfusion, anoxia and increase in oxygen radicals. Our findings suggest that in comparison to hypertension, hypotension is more effective in the development of early biochemical and ultrastructural degenerative changes.

References

1 Bogen DR, Glaser JS: Ischemic optic neuropathy: The clinical profile and natural history. Brain 1975;98:689–708.
2 Eagling EM, Sanders MD, Muller SJ: Ischaemic papillopathy: Clinical and fluorescein angiographic review of forty cases. Br J Ophthalmol 1974;58:990–1008.
3 Guyer DR, Miller NR, Auer CL, Fine SL: The risk of cerebrovascular disease in patients with anterior ischemic optic neuropathy. Arch Ophthalmol 1985;103:1136–1142.
4 Hayreh SS: Anterior ischemic optic neuropathy: Terminology and pathogenesis. Br J Ophthalmol 1974;58:955–963.
5 François J, Neetens A: Functional importance of the anterior optic nerve supply. Ophthalmologica 1974;168:122–127.
6 Kishi S, Tso MOM, Hayreh SS: Fundus lesion in malignant hypertension. II. A pathologic study of experimental hypertensive optic neuropathy. Arch Ophthalmol 1985;103:1198–1206.
7 Taylor D, Ramsay J, Day S, Dillon M: Infarction of the optic nerve head in children with accelerated hypertension. Br J Ophthalmol 1981;65:153–160.
8 Guyer DR, Miller NR, Auer CL, Fine SL: The risk of cerebrovascular disease in patients with anterior ischemic optic neuropathy. Arch Ophthalmol 1985;103:1136–1142.
9 Johnson LN, Arnold AL: Incidence of nonarteritic anterior ischemic optic neuropathy. J Neuroophthalmol 1994;14:38–44.
10 Hayreh SS; Chopdar A: Occlusion of posterior ciliary artery. V. Protective influence of simultaneous vortex vein occlusion. Arch Ophthalmol 1982;100:1481–1491.
11 Brown GC: Anterior ischemic optic neuropathy occurring in association with carotid artery obstruction. J Clin Neuroophthalmol 1986;6:39–42.
12 Connolly SE, Gordon KB, Horton JC: Salvage of vision after hypotension-induced ischemic optic neuropathy. Am J Ophthalmol 1994;117:235–242.
13 Hayreh SS, Zimmerman MB, Podhajsky PP, Alward WLW: Nocturnal arterial hypotension and its role in optic nerve head and ocular ischemic disorders. Am J Ophthalmol 1994;117:603–624.

Downloaded by:
Cukurova Universitesi
193.255.199.2 - 1/12/2018 12:29:46 PM

14 Rivkin MJ, Hedges TR, Logigian EL: Carotid dissection presenting as posterior ischemic optic neuropathy. Neurology 1990;40:1469–1470.
15 Alm A, Bill A: Ocular and optic nerve blood flow at normal and increased intraocular pressures in monkeys *(Macaca irus)*: A study with radioactively labeled microspheres including flow determinations in brain and some other tissue. Exp Eye Res 1973;15:15–30.
16 Waybright EA, Selhorst JB, Combs J: Anterior ischemic optic neuropathy with internal carotid artery occlusion. Am J Ophthalmol 1982;93: 42–47.
17 Kearns TP: Ophthalmology and the carotid artery. Am J Ophthalmol 1979;88:714–722.
18 Pitts FW: Variations of collateral circulation in internal carotid occlusions. Neurology 1978; 28:467–471.
19 Sebag J, Feke GT, Delori FC, Weiter JJ: Anterior optic nerve blood flow in experimental optic atrophy. Invest Ophthalmol Vis Sci 1985; 26:1415–1422.
20 Lessel S, Miller JR: Optic nerve and retina after experimental circulatory arrest. Invest Ophthalmol Vis Sci 1975;14:146–148.
21 Drance MS, Morgan RW, Sweeney VP: Shock-induced neuropathy. N Engl Med 1973;288: 392–395.
22 Michaelson C, Behrens M, Jeffrey O: Bilateral anterior ischemic optic neuropathy associated with optic disc drusen and systemic hypotension. Br J Ophthalmol 1989;73:762–764.
23 Rootman J, Butler D: Ischemic optic neuropathy – A combined mechanism. Br J Ophthalmol 1980;64:826–831.
24 Taylor D, Ramsay J, Day S, Dillon M: Infarction of the optic nerve head in children with accelerated hypertension. Br J Ophthalmol 1981;65:153–160.
25 Espinosu F, Weir B, Noseworthy T: Nonoperative treatment of subarachnoid hemorrhage; in Youmans JR (ed): Neurosurgical Surgery, ed 3. Philadelphia, Saunders Co, 1990, pp 1661–1688.
26 Marshall J: Cerebral Blood Flow in the Management of Cerebrovascular Disease. London, Blackwell, 1976, pp 125–126.
27 Millikan CH: Cerebral circulation. Baltimore, Williams & Wilkins, 1968, pp 427–428.
28 Muramatsu I, Fujiwara M, Miura A, Sakakibara Y: Possible involvement of adenine nucleotides in sympathetic neuro-effector mechanism of dog basilar artery. J Pharmacol Exp Ther 1981;216:401–409.
29 Sodeman AW: Pathologic Physiology, ed 6. Philadelphia, Saunders Co, 1979, p 3.
30 Yaşargil MG: Anesthesia for microsurgical procedures in neurosurgery. Microneurosurgery 1984;1:272–278.
31 Hayreh SS; Servais GE, Virdi PS: Fundus lesions in malignant hypertension: Hypertensive optic neuropathy. Ophthalmology 1986;93:75–87.
32 Cooper AJL, Pulsinelli WA, Duffy TE: Glutathione and ascorbate during ischemia and postischemic reperfusion in rat brain. J Neurochem 1980;35:1242–1245.
33 Kinuta Y, Kikuchi H, Ishihamua M, Kimura M, Itohava Y: Lipid peroxidation in focal cerebral ischemia. J Neurosurg 1989;71:421–429.
34 Kontos HA, Vei EP, Poulishock JT, Dietrich WD, Magiera CJ, Ellis EF: Cerebral arteriolar damage by arachidonic acid and prostaglandin G_2. Science 1989;209:1242–1245.
35 Levasseur JE; Patterson JL, Ghatah NR, Kontos H, Choi JC: Combined effect of respirator-induced ventilation and superoxide dismutase in experimental brain injury. J Neurosurg 1989;71:573–575.
36 Rosenkranz B, Fischer C, Frolich JC: Prostacyclin metabolites in human plasma. Clin Pharmacol Ther 1981;29:420–424.
37 Stein JH, Hinder AR, Dosthvizen D: Gastric mucosal injury caused by hemorrhagic shock and reperfusion: Protective role of the antioxidant glutathione. Surgery 1990;108:467–473.
38 McCord JM, Fridovich I: Superoxide dismutase. J Biol Chem 1969;22:6049–6055.
39 Sechettini A, Lippman R, Walsh EK: Attenuation of decompressive hypoperfusion and edema by superoxide dismutase. J Neurosurg 1989;71:578–587.

Downloaded by:
Cukurova Universitesi
193.255.199.2 - 1/12/2018 12:29:46 PM