Yonsei Medical Journal
Vol. 44, No. 6, pp. 955 - 960, 2003

Original Article

Preventive Effects of Intracisternal Alphatochopherol on Cerebral Vasospasm in Experimental Subarachnoid Hamorrhage

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Vasospasm is an important cause of morbidity and/or mortality with a subarachnoid haemorrhage (SAH). The roles of lipid peroxidation in a vasospasm caused by a SAH remain to be investigated. The effect of an intracisternal administration of alphatochopherol on a cerebral vasospasm was investigated in an experimental model. The authors assessed whether the administration of alphatochopherol reduced the vasospasm.

By means of an intracisternal blood injection model, a SAH was induced in 30 rats, which were randomly divided into three groups, as follows: group I (G1), without a SAH and drug, group II (G2), a SAH alone, group III (G3), a SAH and alphatochopherol. Following the withdrawal of cerebrospinal fluid (CSF), a fresh unheparinized arterial blood was injected into the cisterna magna to induce a SAH. In G3, 20 U (0.4ml) alphatochopherol was intracisternally injected forty-five hours after induction of the SAH. All rats were sacrificed 72 hours after the induction. The basilar artery, with surrounding tissue, was removed from the cranium. The cross-sectional diameter of the lumen and vessel wall of the rat basilar artery was assessed from a planimetric analysis, and changes compared with G1 and G2.

The reduction in the luminal cross-sectional diameter of the vessels exposed to subarachnoid blood was found to be 29.01 % (p=0.001). The group treated with alphatochopherol had a 9% reduction (p=0.004).

The role of lipid peroxidation on a vasospasm caused by SAH is well known to be critical. Data from the present study indicated that antioxidant therapy, with topical alphatochopherol, may be promising on a vasospasm caused by a SAH.

Key Words: Subarachnoid haemorrhage, cerebral vasospasm, Vitamin E, rat

INTRODUCTION

Cerebral ischemia, secondary to cerebral arterial luminal narrowing (vasospasm), complicates more than two-thirds of subarachnoid haemorrhages (SAH), and causes significant morbidity and mortality.[1-4] Despite extensive clinical and experimental investigations for more than 30 years, the pathogenesis of cerebral vasospasms has not been fully explained.[5-9]

The presence of blood, and blood breakdown products, in cerebrospinal fluid (CSF) has been related to the etiology of a vasospasm.[7,8,10,11] A vasospasm caused by a SAH is not only due to a primarily vascular spasm, but also to a thickened hyperplastic arterial wall.[7,8,10,12]

It is unknown whether these changes reflect the damage incurred by prolonged intense vasoconstriction, or the direct effect of vasotoxins released by a decaying subarachnoid thrombus. However, the evidence revealed from these processes implicates an inflammatory and /or immunoreactive cause. It has been reported that there was a correlation between the CSF lipid hydroperoxide content and the occurrence of vasospasm in a

Received March 13, 2002
Accepted June 26, 2003

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SAH.[13-17] Lipid peroxidation has been hypothesized as a potential cause of a cerebral vasospasm following a subarachnoid haemorrhage (SAH). Vitamin E can prevent the vasospasm because of its role in specific anti-oxidant mechanisms.[1,14]

An intracisternal administration of lipid hydroperoxide produces an immediate mild chronic contraction, which become stronger after 72 hours.[13,14] Topical alphatochopherol, functioning as a free-radical scavenger, may inhibit lipid peroxidation by combining with lipid peroxide radicals to form alphatochopherol quinine.

The aim of this study was to investigate the therapeutic effects of an intracisternal administration of alphatochopherol on a cerebral vasospasm in a SAH experimental model.

MATERIALS AND METHODS

Thirty male Sprague Dawley rats, each weighing between 280-350 gm, were used in this study. All procedures were performed in accordance with the Animal Care Guidelines of Dicle University.

The rats were randomly divided into three groups, as follows: Group I (G1), the control group, without a SAH and drug, Group II (G2), the SAH group, a SAH alone, without treatment), Group III (G3), the treatment group, with a SAH and alphatochopherol as a therapeutic agent.

The rats were anaesthetized with Ketamine hydrochloride (Ketalar, Parke-Davis) (5 mg/kg, intramuscularly) and Xylazine (Rompun, Bayer) (10 mg/kg, intramuscularly). Atropinemetylnitrate (0.18 mg/kg) was administered intraperitoneally as a premedication to prevent obstruction of the airway due to mucus formation. The animals were allowed to breathe spontaneously. They were laid in a supine position on a standard rat operation board, and fixed by the tail, legs and teeth. The rectal temperature was maintained at 37℃ with a heating pad. The femoral artery was cannulated for arterial blood sampling.

After completion of the preparing process, the rats were placed in the prone position, with their heads were slightly flexed downwards. Following the micro surgical dissection of the atlantooxipital membrane, the cistern magna was punctured and 0.1 ml of CSF withdrawn. The operation table was tilted 300 Trandelenburg.

In G1, no process was performed. In G2, fresh non-heparinized arterial blood (0.3 ml) was withdrawn from the femoral artery, then autolog fresh non-heparinized arterial blood and CSF were mixed, 0.4 ml of this mixture was injected into the cisterna magna for approximately 3 minutes and 20 IU (0.4 ml) of alphatochopherol (Ephynal amp. 100 IU, Roche, Istanbul/Turkey) was then injected through the same puncture hole exactly 45 minutes after the blood injection.

In G3, fresh non-heparinized arterial blood (0.3 ml) was withdrawn from the femoral artery, then autolog fresh non-heparinized arterial blood and CSF were mixed and 0.4ml of this mixture was injected into the cisterna magna for approximately 3 minutes. A 2 × 2 mm muscle graft of was placed over the puncture site immediately after withdrawal of the needle.

All the rats were sacrificed 72 hours later by an intramuscularly injection of high dose Ketamine-hydrochlorur. The brains of rats were removed immediately, and specimens placed in 10% formaldehyde solution for five days. After fixation of the tissues, the samples were dehydrated by a graded ethyl alcohol series, and then cleared in xylene. The tissues were transferred to an incubator (30℃), processed using two different kinds of paraffin preparation (56℃) for 30 minutes and cut into 4-micron sections with a steel knife rotator microtome. An albumined slide was floated and allowed to dry 12 hours at 40℃.

Routine staining was performed using Harris' haematoxylin counter and stained with alcoholic eosin. The samples were examined, and photographed using an Olympus microscope. The planimetric measurements were made via an occulometer at × 41 magnification using light microscopy. The luminal cross-sectional diameter and wall thickness of the rat basilar arteries were measured.

The planimetric measurements of the cross-sectional diameter of the basilar arteries were made, as with the luminal diameter. The calibre of the basilar artery was measured on the specimens at three locations; near the vertebrobasilar junction, at the midpoint and close to the basilar tip. The wall thickness was measured separately in the sulcus basilaris, ventrally and dorsally, and

their median value calculated. Two different pathologists, blinded to the conditions, performed the measurements independently, with the average value being used in our study.

In the present study, the type of arterial wall changes, and the luminal diameter of the artery in specimens stained by H&E were histopathologically investigated. The Cross-sectional diameters of the lumen and vessel walls of the rat basilar arteries were assessed in the planimetric measurements. The inter- and intra-observer reliabilities were calculated. Morphometric analyses of the vessels measurements were evaluated with the Mann-Whitney U test (Table 1). Inter group comparisons were made between G1 and G3, and G2 and G3. P values less than 0.05 were considered statistically significant.

RESULTS

In G1, light microscopy of the rat basilar arteries was consistent with normal rat cerebral arteries. The endothelium had a continuous monolayer overlying a thin convoluted internal elastic lamina. There were five to six layers of concentrically oriented smooth muscle cells surrounding the intima. The layers had no pathology (Fig. 1).

In G2, 72 hours after the SAH and treatment with alphatochopherol, the rat basilar arteries showed certain morphological changes. The histopathological study with H&E staining mainly revealed the desquamation of the endothelial cells and vacuolar degeneration of the vascular wall. There was also evidence of arterial narrowing and vascular wall thickening in both G2 and G3 (Fig. 2 and 3).

In G3, the arterial wall in some areas had thickened and in others had narrowed. Endothelial cell desquamations and convolution in the internal elastic lamina were observed. The smooth muscle cells showed vacuolar degeneration and hypertrophy (Fig. 2 and 3). The adventitia showed inflammatory reactions with the mononuclear cells. The histopathological findings in the SAH control group were similar to those of the SAH group.

The intra- and inter-observer reliabilities for the luminal diameter and vascular wall thickness were 91.7 and 81.5%, and 87.5 and 78.2% respectively.

On an inter-groups comparison of the luminal diameters, between G2 and G3, a statistically significant difference was found (p=0.0001). The thickening of the arterial walls in G3 was 27.2%,

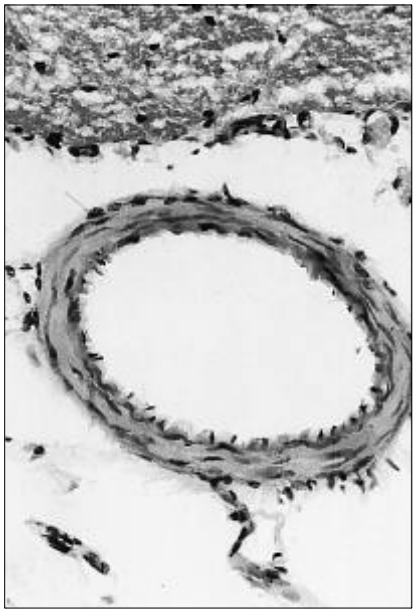

Fig. 1. In normal cerebral arteries, a continuous monolayer of endothelium formed, overlying the thin convoluted smooth muscle cells surrounded the intima (H&E × 82).

Table 1. Measurements of the Mean Cross Sectional Diameter and Wall Thickness of Rat Basilar Arteries (n=10 each group)

Groups	Luminal Diameter		Wall thickness	
	Mean ± SD	p	Mean ± SD	p
Control (G1)	0.312 ± 0.027	r	0.062 ± 0.09	R
SAH (G2)	0.228 ± 0.018	0.01	0.051 ± 0.005	0.47
SAH / Alphatochopherol (G3)	0.346 ± 0.019	0.0001	0.032 ± 0.007	0.004

A significant statistical difference was found between G3 and G1, and G3 and G2 according to both the cross sectional diameter and wall thickness.

Fig. 2. In the SAH group, the arterial wall had thickened, accompanied by narrowing of the luminal diameter (H&E × 82).

Fig. 3. In the treatment group, the wall thickness and diameter of the rat basilar arteries were more dilated than in the SAH group (H&E × 82).

which was higher than G2 ($p<0.001$). When G3 was compared with G2, there was a 44.4% reduction in the arterial wall thickness ($p<0.001$). In G3, the reduction in the luminal cross-sectional diameter in the vessels exposed to the subarachnoid blood was 29.01% ($p<0.001$).

DISCUSSION

Despite intensive study, the etiology of a cerebral vasospasm in a SAH has not been totally resolved. The development of a vasospasm is a complex process, where various components of the blood interact.[1,2,5] The actual mechanism of a vasospasm is unknown. Myogenic, metabolic and neurogenic theories have all been proposed to explain a vasospasm after a SAH.[5,15,17]

There is some evidence suggesting this phenomenon may occur partly due to progressive micro vascular lipid peroxidation. The participation of lipid peroxidation in the occurrence of a vasospasm has been supported by many published reports, and the intercellular mechanism underlying the sustained contraction of arterial smooth muscle due to lipid peroxidation has partly been elucidated.[2,6,13-17]

Enzymatic and non-enzymatic peroxidations participate in the development of a cerebral vasospasm after a SAH.[13,16-19] The inhibition of the release of cyclooxygenase, or an endothelium derived relaxing factor, abolishes the pressure- or stretch-induced constriction in canine cerebral arteries.[14,17]

Lipid hydroperoxide and malondialdehyde cause toxic cell damage in both vascular smooth muscle and endothelial cells. Endothelial cells have been quoted as being more susceptible to lipid hydroperoxide than smooth muscle cells.[13,14,16]

In this study, intracisternal alphatochopherol was used to evaluate its possible effects on neural and vascular structures.[20] At the end of the study, it was histologically determined that there were only small changes in the neural and vascular structures.

Previous observations of some investigator have shown the maximal degree of spasm to occur in rats on the 3rd post SAH day, therefore, an interval of 72 hours post SAH was chosen for the sacrifice of our rats. In this model, a blood clot can still clearly be identified in the cisternal subarachnoid spaces.[2,7,20]

The effect of chronic two-fold dietary vitamin E

supplementation, on subarachnoid haemorrhage-induced brain hypo perfusion, has been investigated.[19] The study reported that chronic dietary supplementation with vitamin E can completely antagonize acute cerebral hypo perfusion due to a vasospasm following an experimentally induced SAH, as reflected by the preservation of the cerebral blood flow. Alphatochopherol acts as a natural fat-soluble antioxidant, and appears to be the first line of defense against peroxidation of cellular and subcellular membrane phospholipids.[6]

The administration of topical alphatochopherol into the subarachnoid space may be more advantageous in relieving systemic adverse effects, and obtaining the desired concentration, in a SAH setting. In this study, to this end, vitamin E was intracisternally injected. In G3, the vessel wall thicknesses and cross-sectional luminal diameters were higher than either G2 or G1. In G2, the morphological changes more evident than in G3 (p=0.001, p=0.004).

In conclusion, the role of lipid peroxidation on a vasospasm caused by SAH is well known to be critical. The topical administration of alphatochopherol was found to be considerably effective against developing a cerebral vasospasm following a SAH in rats. However, further work is necessary to confirm these preliminary findings and to clarify the mechanisms of action.

ACKNOWLEDGMENT

The authors would you like to thank Miss Elif Kemaloğlu for her contribution to this manuscript.

REFERENCES

1. Asono T, Takakura K, Sano K, Kıkuchi H, Nagal H, Tamura A, et al. Effect of a hydroxyradical scavanger on delayed ischemic neurological deficits following aneurismal subarachnoid haemorrhage: Results of multi center, placebo controlled double blind trial. J Neurosurg 1996;84:792-803.
2. Polidori MC, Frei B, Rodof G, Ogilvy CS, Koroshetz WJ, Beal MF. Increased levels of plasma cholesteryl ester hydroperoxides in patients in patients with subarachnoid hemorrhage. Free Radic Biol Med 1997;23:62-76.
3. Saito I, Asono T, Sano K, Takakura K, Abe H, Yoshimoto T, et al. Neuroprotective effect of antioxidant, ebselen, in patients with delayed neurological deficits after aneurismal subarachnoid hemorrhage. Neurosurgery 1998;42:269-76.
4. Sonmez OF, Unal B, Inalöz S, Yılmaz M, Aydın A, Kaplan S. Therapeutic effects of intracarotid infusion of spremine/Nitric oxide complex on cerebral vasospasm. Acta Neurochir (Wien) 2002;144:921-8.
5. Espinosa F, Weir B, Shnitka T. Electron microscopy simian cerebral arteries after subarachnoid haemorhage and after the injection of horseradish peroxidase. Neurosurgery 1986;19:935-45.
6. Travis MA, Hall ED. The effects of chronic two-fold dietary vitamin E supplementation on subarachnoid hemorrhage induced brain hypoperfusion. Brain Res 1987;418:366-70.
7. Aydin IH, Onder A. The effect of very early cisternal irrigation on basilar artery spasm after SAH in rat model. Acta Neurochir (Wien) 1991;113:69-73.
8. Atalay B, Bolay H, Dalkara T, Soylemezoglu F, Oge K, Ozcan OE. Transcorneal stimulation of trigeminal nerve afferents to increase cerebral blood flow in rats with cerebral vasospasm: a non-invasive method to activate the trigeminovascular reflex. J Neurosurg 2002;97:1179-83.
9. Handa Y, Kubota T, Kaneko M. Tsuchida A, Kobayashi H, Kawano H, et al. Expression of intercellular adhesion molecule 1(ICAM) on cerebral artery following subarachnoid haemorrhage in rats. Acta Neurochir (Wien) 1995;132:92-7.
10. Mayberg MR, Okada T, Bark HD. The significance of morphological changes in cerebral arteries after subarachnoid haemorrhage. J Neurosurg 1990;72:626-33.
11. Bergh WM, Zuur K, Kamerling NA, Asseldonk JTH, Rinkel JEG, Tulleken CAF, et al. Role of magnesium in the reduction of ischemic depolarization and lesion volume after experimental subarachnoid hemorrhage. J Neurosurg 2002;97:416-22.
12. Ohta T, Satoh G, Kuroiva T. The permeability change of major cerebral arteries in experimental vasospasm. Neurosurgery 1982;30:331-5.
13. Kajita Y, Takayasu M, Dietrich HH, Dacey RG. Possible role of nitric oxide in autoregulatory response in rat intracerebral arterioles. Neurosurgery 1998;42:834-41.
14. Kajita Y, Suzıki Y, Oyama H, Tanazava T, Takayasu M, Shıbuya M, et al. Combined effect of L-arginine and superoxide dismutase on spastic basilar artery after subarachnoid hemorrhage in dogs. J Neurosurg 1994; 80:476-83.
15. Nakagomi T, Kassel NF, Hongo K, Sasaki T. Pharmachological reversibility of experimental cerebral vasospasm. Neurosurgery 1990;27:582-6.
16. Kanamura K, Weir KAB, Simpson I, Witbeck T, Grace M. Effect of 21-aminosteroid U-74006F on lipid peroxidation in subarachnoid clot. J Neurosurg 1992;74:454-9.
17. Caner H, Oruckaptan H, Bolay H, Kilinc K, Senaati S, Benli K, et al. The role of lipid peroxidation in the genesis of vasospasm secondary to subarachnoid hemorrhage. Kobe J Med Sci 1991;37:13-20.

18. Shcaller C, Rohde V, Meyer B, Hassler W. Amount of subarachnoid blood and vasospasm: Current aspect. Acta Neurochir (Wien) 1995;136:67-71.
19. Yamamato Y, Smith RR, Bernake HD. Accelerated nonmuscle contraction after subarachnoid hemorrhage: Culture cerebral arteries in vasospasm. Neurosurgery 1992;30:337-45.
20. Tekkok ICH, Tekkok S, Ozcan OE, Erbengi T, Erbengi A. Preventive effect of intracisternal heparin for proliferative angiopathy after experimental subarachnoid hemorrhage in rats. Acta Neurochir (Wien) 1994;127: 112-7.