Orijinal Araştırma

The Inhibition of Cerebral Vasospasm by Using Chelerythrine After Experimental Subarachnoidal Haemorrhage in Rats

Ratlarda Deneysel Subaraknoidal Kanama Sonrası Gelişen Serebral Vazospazmın, Seleritrin Kullanılarak Engellenmesi

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Özet

Amaç: Önlenmesi için büyük çapta deneysel ve klinik araştırmalar yapılmış olmasına rağmen, anevrizmal subaraknoidal kanamanın (SAK) yıkıcı bir tıbbi komplikasyonu olan serebral vazospazm, yüksek oranda morbidite ve mortalite ile birliktedir. Deneysel sonuçların çoğu güçlü vazokonstrüktör protein kinaz C'nin (PKC) serebral vazospazmda anahtar bir rol oynadığı görüşünü desteklemektedir. Biz vazospazmı önlemek için, deneysel subaraknoidal kanama modelinde güçlü, selektif ve hücre içine geçebilen protein kinaz C (PKC) inhibitörünü (seleritrin klorid) sistemik olarak kullandık.

Materyal ve Yöntem: Yirmi sekiz sıçan dört gruba ayrıldı: grup 1 kontrol grubu; grup 2, SAK grubu, grup 3, SAK artı plasebo grubu; ve grup 4, SAK artı chelerythrine chloride (5 μmol /kg/gün) grubu ayrıldı. 3. ve 4. gruplara Seleritrin klorid veya eşit hacimde % 0.9 salin sırasıyla, intraperitoneal olarak 5 gün süreyle verildi. Sıçanlar beşinci günde sakrifiye edildi. Kesitler ışık mikroskobu ile incelendi. Pons seviyesinde yapılan kesitlerde, baziler arterin lümen alanı ve duvar kalınlığı ölçümleri mikrometre ile alındı.

Bulgular: 4. gruptaki baziler arter daralması 2 ve 3 gruplarla karşılaştırıldığında, önemli ölçüde ($p<0,001$) daha azdı.

Sonuç: Bu sonuçlar Seleritrin klorid'in geç serebral vazospazmı önlemede sistemik olarak etkili olduğunu ve vazospazm oluşumunda PKC'nın önemli bir rolü olduğunu göstermektedir.

Anahtar Kelimeler: Sıçan, Subaraknoidal Kanama, Vazospazm, PKC İnhibitörü.

Abstract

Background: Cerebral vasospasm, a devastating medical complication of aneurysmal subarachnoid hemorrhage (SAH), is associated with high morbidity and mortality although a great deal of experimental and clinical research conducted to prevent this complication. The most of the experimental results support the view that potent vasoconstrictor proteine kinase C (PKC), plays a key factor in cerebral vasospasm. To inhibit vasospasm, we used a potent, selective, and cell-permeable protein kinase C (PKC) inhibitor (chelerythrine chloride) systemically in an experimental subarachnoidal hemorrhage (SAH) model.

Methods: Twenty eight rats were divided into four groups: group 1; control, group 2; SAH, group 3; SAH plus placebo, and group 4; SAH plus chelerythrine chloride (5 μmol/kg/day). Chelerythrine chloride or an equal volume of 0.9 % saline was intraperitoneally administered for 5 days to groups 3 and 4, respectively. The rats were sacrificed on the fifth day. Sectioned slices were examined by light microscopy. Measurements were made for the cross-sectional areas of the lumen and the vessel wall of basilar artery in the sections of pons by a micrometer.

Findings: The vasoconstriction of the basilar artery significantly attenuated in group 4 compared with the groups 2 and 3 ($p<0.001$).

Conclusions: These results suggest that chelerythrine chloride is systemically effective in preventing delayed cerebral vasospasm and PKC has a significant role in the pathogenesis of vasospasm.

Keywords: Rat, Subarachnoid Haemorrhage, Vasospasm, PKC Inhibitor.

Introduction

Protein kinase C (PKC) plays a pivotal role in development of vasospasm after subarachnoid hemorrhage (SAH), although studies suggested that the development of vasospasm is multifactorial (1-6).

Studies showed that vasospasm after (SAH) is a free radical disease (7-9). The interaction between free radicals, released by oxyhemoglobin, causes the production of many vasoconstrictor agents especially PKC and the inhibition of vasodilator agent Nitric Oxide (NO) (10-15). Although endogenous intracellular enzymatic antioxidants such as superoxide dismutase (SOD) prevent these interactions, these mechanisms are disturbed in SAH as a result of excessive production of free radicals (16, 17).

A potent vasoconstrictor PKC may also play a role as a key factor linking several other signaling pathways such as calmodulin, MLCK, intracellular Ca 2+ , protein tyrosine

kinase (PTK), or MAPK. (3, 18-23). Consequently, the balance between vasoconstricting and vasodilating influences to the arterial endothelium alters in favor of vasoconstricting and a delayed but prolonged vasospasm of major arteries begins.

Numerous experimental subarachnoid hemorrhage studies have been performed to resolve cerebral vasospasms caused by subarachnoidal hemorrhage (24-30). Although the PKC inhibitors had been used by local application to prevent or reverse cerebral vasospasm in many experimental subarachnoidal hemorrhage studies, these studies has yielded inconsistent and discrepant data because probably many of them are nonselective kinase inhibitor and they could not administered systemically (21-23, 31).

These studies also are unable to show if these drugs attain sufficiently high concentrations in the cerebrospinal fluid (CSF) in the systemic trials. According to us, the lack of systemic trials of PKC inhibitors is an important deficiency. For that reasons, to prevent vasospasm, we used a cell-permeable and selective protein kinase C inhibitor (chelerythrine chloride) systemically in a experimental rat model of SAH.

Materials and Methods

Experiments were performed on 15 week-old 28 male Wistar rats ranging in weight from 225 to 250 g (a mean weight of 235 g) obtained from Inonu University Animal Research Laboratory. Rats were divided into four groups: group 1; control (no SAH), group 2; SAH, group 3; SAH plus intraperitoneal saline (0.9 % NaCl), and group 4; SAH plus intraperitoneal Chelerythrine.

Experimental model of SAH and study protocol: All rats were pretreated with an antibiotic, enrofloxacin (Baytril, Bayer, Germany), (2.27 mg/kg, subcutaneously) one day before surgery. The rats in groups 2, 3, and 4 were anesthetized with intraperitoneal ketamine HCl (60 mg/kg) and xylazine HCl (6 mg/kg) and placed on a heated surgical table at 37°C during surgical procedures. Anesthesia was continued by repeated injections of ketamine as needed. A 0.3 ml of blood sample was drawn from tail vein into a heparinized syringe. Under sterile conditions and a surgical microscope, occipital bone was explore by a midline incision from middle of the calvarium to the lower cervical spine. After the atlanto-occipital membrane was dissected, a 27-gauge needle was inserted into the cisterna magna. 0.3 ml of heparinized blood sample was injected into cisterna magna over a 10-minute period. After the needle was withdrawn, dural opening plugged with an absorbable sponge and the wound was sutured. The rats were injected 5 mL of saline (warmed at 37 °C) subcutaneously to prevent dehydration before recovery from anesthesia. During observation, the rats were allowed access to food and water ad libitum. 1 ml of placebo (saline) and the chelerythrine (5 μmol/day) was administered by intraperitoneal injection twice daily for 5 days to groups 3 and 4, six hours later SAH.

48 hours later the initial intracisternal blood injection, the rats in groups 2, 3, and 4 were reanesthetized and 0.3 ml of blood from tail vein reinjected into the cisterna magna. Intraperitoneal chelerythrine or saline injections (warmed at 37 °C) were continued up to fifth day after SAH. Control rats were sacrificed as described below for determination of the baseline basilar artery diameter.

Sample collection and sacrificing of rats: The animals reanesthetized as described above on fifth day after first application of blood or saline. The ascending aorta was cannulated retrogradely through a thoracotomy. The craniocervical circulation was perfused with 200 ml of heparinized iso-osmotic phosphate buffer saline (0.1 M, pH 7.4) at a physiological mean arterial pressure (80-90 mm Hg) via a peristaltic pump (May/ PRS9508/ 991129-1). The perfusion was followed by 200 ml of 0.1 M phosphate buffer saline containing 4% paraformaldehyde at a physiological mean arterial pressure as above. The control group's rats were sacrificed without any surgical procedure for SAH and was perfused as above.

The sample was taken by cutting brain stem at above and belove the pons. The sample has subdivided into two segments at the level of middle pons. The preparations of samples in the levels of upper and lower pons were embedded in liquid paraffin and sectioned at 6 μm thickness, mounted on glass slides, and stained with hematoxylin and eosin. Sectioned-slices were examined by light microscopy and photographed. Planimetric measurements were performed in light microscopy by a micrometer (Olympus BX 50**,** Japan) for the cross-sectional areas of the lumen and the vessel wall (intima plus media).

Data analysis: Data were expressed as mean±SEM. Statistical differences between the control and SAH, SAH plus intraperitoneal saline, and SAH plus intraperitoneal chelerythrine were compared by independent samples t-test. For all comparisons, $p<0.05$ was considered statistically significant.

Results

Histopathologic appearances of groups 1, 2, 3, and 4 were shown on figures 1, 2, 3, and 4. Qualitative histological observations these groups revealed significant reduction in luminal diameter and marked thickening of the vessel wall and endothelial cells and substantial corrugation of the internal elastic lamina of the basilar artery. Microscopic examination of group 4 (Figure 4) were similar in appearance to normal vessels in the control group (Figure 1) that presented with a monolayer endothelium overlying a thin no convoluted internal elastic lamina. Concentrically-oriented smooth muscle cells surrounded the intima (Figure 4). Corrugation of the internal elastic lamina was less prominent in group 4.

In groups 1, 2, 3 and 4; the diameters of basilar artery lumens were found to be 267±1.5, 79±10.3, 77 ±10.2, and 254±1.5 (μm, respectively. There was marked narrowing in the lumens of basilar arteries in groups 2 and 3 compared to control group ($p <0.001$) (figure 5). The mean thicknesses of basilar artery walls in groups 1, 2, 3 and 4 were 22±1.1, 50.3±1.1, 52±1.4, and 37.8±1.3 μm, respectively. Compared to control group, the thicknesses of basilar artery walls were found increased in groups 2 and 3. While the changes of internal diameter and wall thickness of group 4 were not statistically significant compared to control group, but they were statistically significant compared to groups 2 and 3 ($p < 0.001$) (Figure 5).

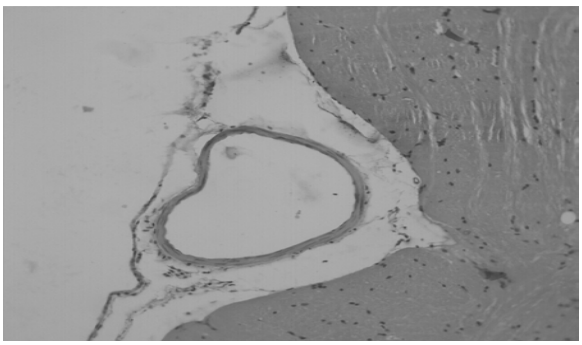

Figure 1. The light microscopic appearance of a cross-sectional area of the basilar artery in Control group (group 1), (H&E x 66).

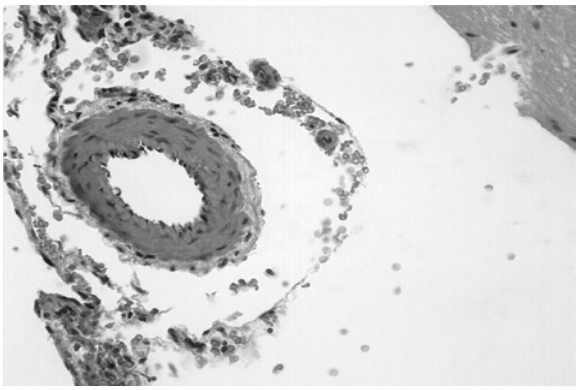

Figure 2. The light microscopic appearance of a cross-sectional area of the basilar artery in SAH group (group 2), (H&E x 66). The reduction in lumen diameter and increase in wall thickness are evident.

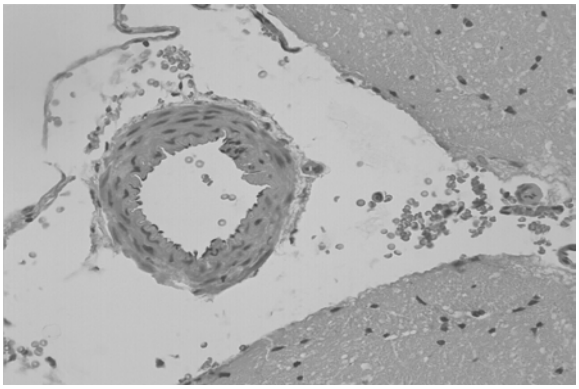

Figure 3. The light microscopic appearance of a cross-sectional area of the basilar artery in SAH plus placebo group (group 3), (H&E x 66). It is seen a significant degree of reduction in lumen diameter and increase in wall thickness.

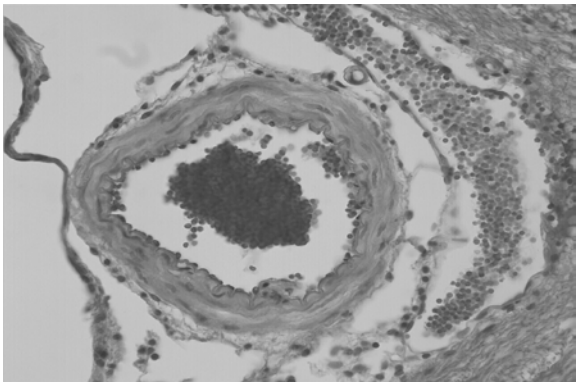

Figure 4. Representing photograph of light microscopic appearance of a cross-sectional area of the basilar artery in SAH plus chelerythrine chloride group (group 4), (H&E x 66). There is minimal increase in wall thickness.

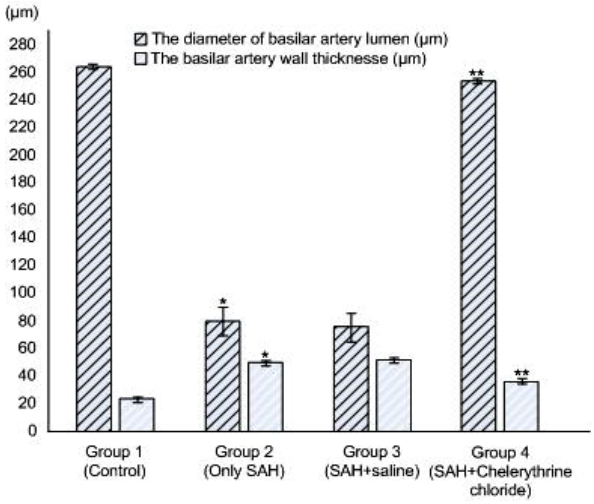

Figure 5. The comparison of internal diameters and wall thicknesses of the basilar arteries of groups 1-4.

* Statistically significant compared to Group 1 ($p<0.001$)
** Statistically significant compared to Group 3 ($p<0.001$)

Discussion

PKC is a serine/threonine kinase and it consists of different isoforms that implicated in numerous processes of signal transduction (32). Although PKC play numerous and important roles in the central nervous system the most important role of it is the regulation of cerebral vascular tone. The role of PKC in the regulation of cerebral vascular is as a vascular contractor in contrast to NO that play a role through vascular relaxation (33-35). Although the PKC inhibitors such as H7 and staurosporine had been used to prevent or reverse cerebral vasospasm in many experimental subarachnoidal hemorrhage studies, these drugs had been used by local application but nonsystemically and they are nonselective kinase inhibitors (22, 23, 31).

Chelerythrine is a potent selective inhibitor of protein kinase C. It inhibits protein kinase C with modulating the activities of some cyclic nucleotide phosphodiesterase isozymes and thus altering the vasoconstrictor potency of this compound (36, 37). In a canine double hemorrhage model, vasospasm after SAH inhibited by local application of Chelerythrine (21). But this study is unable to show if this drug attain sufficiently high and effective concentrations in the cerebrospinal fluid (CSF) in the systemic trials.

In the present study, systemically administration of 5 µmol/day of chelerythrine demonstrated an attenuating effect on the development of vasospasm following experimental SAH in rat.

In our study, experimental SAH elicited vasospasm in all animals of group 2 (SAH only) and group 3 (SAH plus saline). The basilar artery lumen narrowing was higher 337% in group 2 (SAH only) and 346% in 3 (SAH plus saline) than in group 1 (control) ($p< 0.001$) and higher 321% and 329% respectively than in group 4 (SAH plus Chelerythrine) ($p< 0.001$). In groups 2 and 3, in addition, the thickening in basilar artery wall was found to be higher 228% and 236% respectively than control group ($p< 0.001$) and 171% than group 4 (SAH plus chelerythrine) ($p < 0.001$). In animals of group 4 that treated with chelerythrine, narrowing in arterial lumen and thickening in arterial wall were markedly attenuated compared to groups

2 and 3. While the changes of internal diameter and wall thickness of SAH plus chelerythrine group were not statistically significant compared to group 1 (control), they were statistically significant compared to group 2 (SAH only) and group 3 (SAH plus saline), (figur 5) ($p< 0.001$).

The present study is the first report on the systemically effects of chelerythrine (PKC antagonist) on cerebral vasospasm after experimental subarachnoidal hemorrhage in rats. In this study, it was shown that administration of chelerythrine markedly attenuates the basillary artery vasoconstriction, attains sufficiently high concentrations in the cerebrospinal fluid (CSF) and is effective in the systemic trials. Further studies are needed to clarify the issue.

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