EFFECT OF NIMODIPINE ON VASOSPASM AFTER EXPERIMENTAL SUBARACHNOID HEMORRHAGE IN RATS

(The evaluation of morphometric and ultrastructural changes on basilar artery)

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SUMMARY

This experimental vasospasm model was made by giving the autologous blood into the cisterna magna. Later Nimodipine (ND) was administered by intracisternal or subcutaneous (s.c.) way in defined times. After that, in the light microscopic examination, the wall thickness (W), lumen (L) and diameter (D) of basilar artery were measured and diameter/wall thickness (D/W), diameter/lumen (D/L) ratios were studied and compared with control group with SAH.

In the first group, intracisternal ND was administered immediately after experimental SAH, ND doses were 10 μg/kg (n: 5), 20 μg/kg (n:4), and 50 μg/kg (n:5). There was no significant difference in the measurements between study group and control (n: 9). In the second group which received s.c. 100 μg/kg t.i.d. for 72 hours, the wall thickness significantly decreased when compared to control group ($p<0.01$) but the other parameters did not change.

The double-hemorrhage group which received same dose (100 μg/kg) of ND showed significant increase in the D/W ratio when compared to matching control group ($p<0.01$). But there was no change in other parameters.

Electron microscopy was made only in rats with SAH taking 100 μg/kg t.i.d. ND for 72 hours. Ultrastructural changes were endothelial changes, subendothelial blebs, corrugation of lamina elastica interna, endothelial denudation and intracytoplasmic vacuoles in both control group with SAH and ND receiving ones. Intracytoplasmic smooth-muscle vacuoles were less prominent in the rats receiving ND.

These findings may indicate that ND shows its effectiveness on experimental vasospasm by decreasing the wall thickness and preventing some morphologic and ultrastructural changes.

Key words: Nimodipine, Experimental Subarachnoid Hemorrhage, Vasospasm, Basilar artery

INTRODUCTION

Vasospasm is a serious complication associated with subarachnoid hemorrhage (SAH) (1). The pathogenesis of cerebral vasospasm following aneurysmal SAH has not yet been fully clarified (2). Animal studies had demostrated the ability of calcium channel blockers (CCBs) to prevent experimental cerebral vasoconstriction induced by various agents such as serotoin phenylephrine, potassium chloride, carbocyclic tromboxane A2, ATP and caffeine (3,4).

As a result of the cerebral vasodilator effect of CCBs, increased regional cerebral blood flow and increased cerebral venous outflow were demonstrated in animals (3). Subsequent studies in dogs showed that CCBs were effective in preventing and reversing SAH- induced acute vasospasm (5).

CCBs, cause vasodilation by preventing the influx of extracellular calcium into vascular smooth muscle cells. Clinical and experimental studies indicate that CCBs are effective in the treatment of cerebral ischemia after SAH (5,7-9).

However the appearance and severity of late angiographic vasospasm did not seem to be affected by CCBs (7,10,11).

Numerous studies have shown morphological changes in human cerebral arteries at autopsy in animal models after experimental SAH (12-14).

Electron microscopy showed marked changes such as endothelial swelling, sub endothelial proliferation, corrugation of the elastic lamina and myonecrosis, both placebo and nimodipine group in vasospasm. The pathological findings of vessels were considered to be slightly less severe in the nimodipine group (15).

In this study the effectiveness of ND was investigated by morphometric and ultrastructural studies of basilar artery in rats.

MATERIALS AND METHOD

In this study we used on average weight of 220 gr. white, male, wistar rats. This experimental vasospasm model was made by giving 0.1 ml autologous blood from venous vessel of tail into the cisterna magna after penthotal anaesthesia. Later ND was administered by intracisternal or subcutaneous way in defined times. After that, in the light microscopic examination, the wall thickness, lumen and diameter of basilar artery were measured and diameter / wall thickness (D/W), diameter / lumen (D/L) rations were studied and compared with control group with SAH (Fig. 1).

In the first group, intracisternal ND was administered immediately after experimental SAH. ND doses were 10 µgr/kg (n:5), 20 µgr/kg (n:4) and 50 µgr/kg (n:5), second group consists of five rats that received ND sc, 100 µgr/kg, tid for 72 hours. The double-hemorrhage group which received the same dose (100 µgr/kg) of ND was the third one.

Electron microscopy was made only in rats with SAH taking ND 100 µgr/kg tid for 72 hours (n: 4).

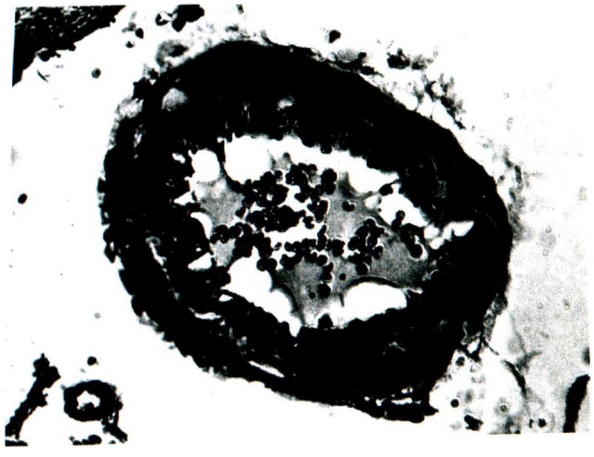

Fig 1. Photomicrograph of transverse section of the basilar artery

Electronmicroscopic study was made at the İstanbul University Medical Faculty, Histology and Embriology Department by using Zeiss EM 9 transmission.

RESULTS

In the first group, there was no significant difference in the measurements between control group and study group ($p>0.05$). In the second group, the wall thickness (W: 0.093 mm) significantly decreased when compared to control group (W: 0.116 mm) ($p<0.01$), but the other parameters have not changed ($p>0.05$). The double hemorrhage group (D/W: 4.852 mm) showed significant increase in the D/W ratio when compared to matching control group (D/W : 3.580 mm) ($p<0.05$) but there was no change in other parameters ($p>0.05$).

Ultrastructural changes were endothelial changes, subendothelial blebs, corrugation of lamina elastica

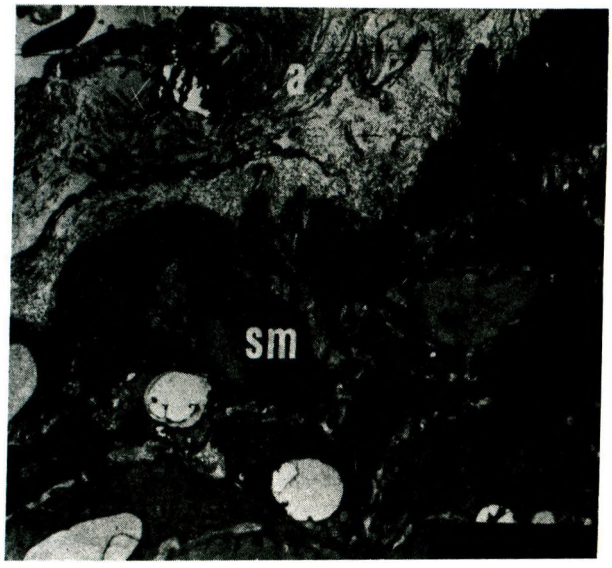

Fig 2. Electronmicrograph showing endothelial denudation, swelling, intracytoplasmic smooth-muscle vacuoles (control) 1850 x 1.2

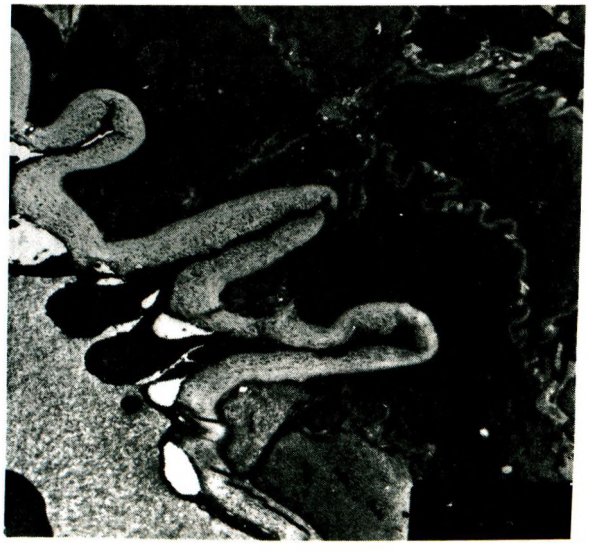

Fig 3. Electronmicrograph showing corrigation of lamina elastica interna, subendothelial blebs and vacuoles (Nimodipine group) 1850x1.2

interna, endothelial denudation and intracytoplasmic vacuoles in both control groups (n:4) with SAH and receiving ND ones intracytoplasmic smooth muscle vacuoles were less prominent in the rats receiving ND (Figure 2-3).

DISCUSSION

Vasospasm is generally observed by angiogram which has unsatisfactory resolution for arteries having an inner diameter of < 200 μm (1).

The incidence of angiographic vasospasm has been reported to be as high as 50-70% with 20-30% of these patients developing clinical ischemic symptoms "Symptomatic Vasospasm" and approximately 15% of patients dying or becoming permanently disabled (16).

The blood injections give rise to angiographically visible constriction of the major brain vessels, associated with a decrease in CBF. Also the oxygen consumption of the brain is reduced to a corresponding degree. Infusion of nimodipine increased CBF by 25% above baseline values after the cisternal blood injection (17).

Nosko et al have shown that ND causes varying degrees of vasodilation when applied to the basilar artery of dog, monkey and man (18).

In our study, in the first group there was no significant difference in the measurements between study group and control. In the second group the wall thickness significantly decreased, but the other parameters have not changed. The double- hemorrhage group showed significant increase in the D/W ratio, but there was no change in other parameters.

Decreasing of the wall thickness and increasing of D/W ratios by vasodilatory affect of ND have been considered.

CCBs may have a greater effect on intracerebral penetrating arterioles than on angiographically visible larger arteries (19). The incidence and severity of late angiographic vasospasm was not obviously reduced with ND treatment. There is no absolute correlation between delayed angiographic spasm and symptoms of late cerebral ischemia.

These data provide a possible explanation for the apparent disparity between clinical efficacy and angiographically determined vessel diameter when patients with cerebral vasospasm are treated with CCBs (10).

The luminal narrowing seen angiographically in SAH patients is due not merely to contraction of smooth muscle cells but also to various histological changes such as cellulofibrous thickening of the intima, subendothelial proliferation and organization of luminal trombus (12,20).

Hughes and other investigators found that the magnitude of structural and morphological changes in the arterial wall was related to lapse of time after SAH. Early changes were swelling of the endothelium with areas of displacement from basement membrane, migration of smooth muscle cells to the subendothelium, fragmentation and corrugation of the internal elastica, swelling and necrosis of smooth muscle cells and an inflammatory reaction of the adventitia containing lymphocytes, plasma cells and macrophages (13).

Electron microscopy showed vacuolization and fibrosis of the media and some areas of myonecrosis, the elastic lamina was more dense than normal and the endothelial cells looked rounded (21). Other studies showed necrosis, vacuolization and widening of the interstitial space of the media. They also described vacuoles and dense bodies in endothelial cells, detached endothelium and thickening of the intima (22,23).

Espinosa et al, showed marked changes such as endothelial swelling, subendothelial proliferation, corrugation of the elastic lamina and myonecrosis. The pathological findings of vessels in spasm were considered to be slightly less severe in the ND group (15).

In our study, ultrastructural changes were endothelial changes, subendothelial blebs, corrugation of lamina elastica interna, endothelial denudation and intracytoplasmic vacuoles in both control groups with SAH and ND receiving ones. Intracytoplasmic smooth muscle vacuoles were less prominent in the rats receiving ND.

Our findings are similar to those described in the literature. These findings may indicate that ND shows its effectiveness on experimental vasospasm by decreasing the wall thickness and preventing some morphologic and ultrastructural changes.

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