

The Effect Of Amlodipine On Chronic Vasospasm In Rats

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Abstract : The effect of a new long-acting calcium antagonist, Amlodipine, on experimental vasospasm was tested using a rat femoral artery vasospasm model and morphometry. Twenty-four rats were divided into 3 groups. In group 1, the femoral arteries were removed after cardiac perfusion. In groups 2 and 3, the right and covered with a silastic cuff. In these groups the left femoral arteries were covered with an empty silastic cuff. Animals in group 3 were treated with Amlodipine (10 mg/kg) for 7 consecutive days. After perfusion-fixation the femoral arteries were examined by light and transmission electron microscopy and processed for morphometric analysis.

Vessels from animals in group 2 showed significant luminal narrowing and morphological changes throughout the vessel wall. In animals treated with Amlodipine, the vessel lumen was slightly decreased and light and transmission electron microscopy demonstrated less prominent morphological changes in the vessel wall. These results suggest that Amlodipine can prevent chronic morphological vasospasm in rats.

Key Words : Amlodipine, Calcium Channel Blockers, Cerebral Vasospasm, Morphometry, Subarachnoid Haemorrhage.

INTRODUCTION

In large clinical series, it has been shown that calcium antagonists decrease morbidity and mortality from vasospasm following subarachnoid haemorrhage (1,13,20,23,24,25), but angiographic studies in animals (6,14,19,29,34) and patients (1,9,23,24,25) have failed to demonstrate reversal or prevention of vasospasm. Thus, alternative hypotheses such as the vasodilator effect of calcium antagonists have been put forward to explain the beneficial results of calcium antagonists in clinical trials (18). More recently, some authors found a more beneficial effect of intrathecal calcium antagonists in reversing angiographic vasospasm in postoperative aneurysm patients (3) and animals (11,22,31,34). Therefore in the light of present literature, it can be predicted that new calcium antagonists with more selective vascular or neuronal effects can be developed (18). In this study the effect of a new calcium antagonist Amlodipine [3-ethyl-5 methyl 2-(2 aminoethoxymethyl) 4-(2 chlorophenyl) 1,4 dihydro 6 methyl 3,5-pyridine-dicarboxylate benzene sulfonate] (Norvasc, Pfizer) on

experimental chronic vasospasm was tested. This evaluation was made with a recently developed model of rat femoral artery vasospasm and morphometry (21).

MATERIALS AND METHODS

The details of rat femoral artery vasospasm have been described previously (21). Twenty-four male Sprague-Dawley rats weighing from 200 to 250 g were divided into three groups. Animals were anaesthetized with intramuscular ketamine and xylazine and allowed to breathe spontaneously. With sterile microsurgical technique, 1cm segments of both proximal femoral arteries were exposed in the inguinal region in animals of group 2 (10 animals) and group 3 (10 animals). 0.1ml fresh autologous whole blood was applied directly to the right femoral artery and covered with a silastic cuff. The left femoral artery was covered with an empty silastic cuff and the animals were allowed to recover.

Animals in group 3 received 10 mg/kg/day Amlodipine orally for 7 days. Seven days after the

experiment, all animals were anaesthetized and the vessels were perfusion fixed via intracardiac infusion with 100 ml of 0.03M phosphate buffer (ph 7.4) followed by 200 ml of 4% paraformaldehyde and 1% glutaraldehyde in phosphate buffer. After perfusion fixation 1cm segments of both femoral arteries were removed and stored in 0.03M phosphate buffer. Vessels from 4 unoperated animals in group 1 were also removed after perfusion fixation in a similar fashion. For light microscope examination proximal 5mm segments of the vessels were dehydrated in graded ethanols embedded in ethly methacrylate sectioned and stained with haematoxylin and eosin. For transmission electron microscopy, the distal 5mm segment of the vessel was placed in buffered 1% osmium tetroxide, dehydrated in graded ethanols and examined by JEOL electron microscope, for morphometric analysis, 5 consecutive light microscopic sections were photographed and cross sectional areas of lumen and radial wall thickness were measured. For each parameter statistical analysis of the morphometric study was performed with a Mann-Whitney U test. Significance was assumed for comparisons in which the probability level was less than 0.01.

RESULTS

Histological changes:

In light microscopy the left femoral arteries in groups 2 and 3 were similar in appearance to normal vessels in group 1 with monolayer endothelium overlying a thin nonconvoluted internal elastic lamina. Concentrically-oriented smooth muscle cells surrounded the intima and no smooth muscle proliferation or necrosis was observed in these vessels (Fig. 1A). Electron microscopy also demonstrated the normal appearance of the vessels at the ultrastructural level (Fig. 2A). The right femoral arteries in group 2 (visospastic arteries) demonstrated all morphological changes described previously (15.21.28). Light microscope examination showed prominent reduction in luminal diameter and marked thickening of vessel wall (Fig. 1B). Endothelial cells were distorted by convolutions of adjacent internal elastic lamina and protruded into the lumen. Transmission electron microscopy substantiated distortion, vacualization and loss of cytoplasmic density in endothelial cells (Fig. 2B). The internal elastic lamina was markedly thicker and convoluted. Vacuolar

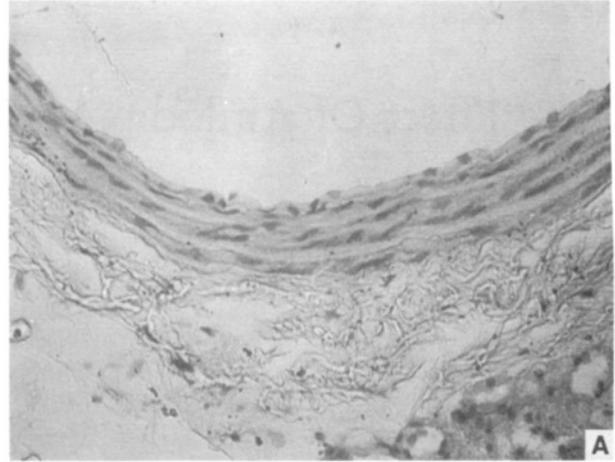


Fig. 1A : Light photomicrograph showing normal rat femoral artery with nonconvoluted internal elastic lamina and concentrically oriented smooth muscle cells. (H&Ex200)

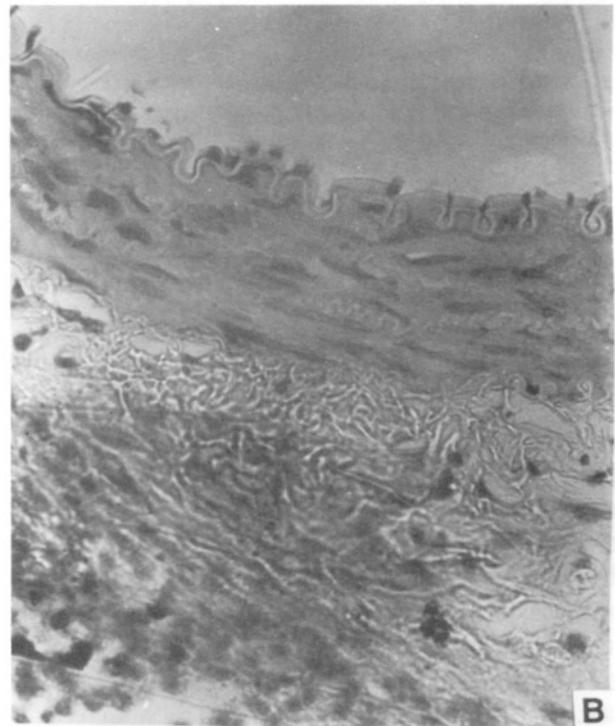


Fig. 1B : Light photomicrograph showing vasospastic rat femoral artery with marked luminal narrowing and increased wall thickness. Convolutions of internal elastic lamina and degenerative changes in endothelial cells and myonecrosis in smooth muscle cells are also noted. (H&EX200)

degeneration of the smooth muscle cells and an increase in extracellular matrix in media were less prominent (Fig. 2C).

Morphometric analysis :

Figure 3 shows the measured cross sectional

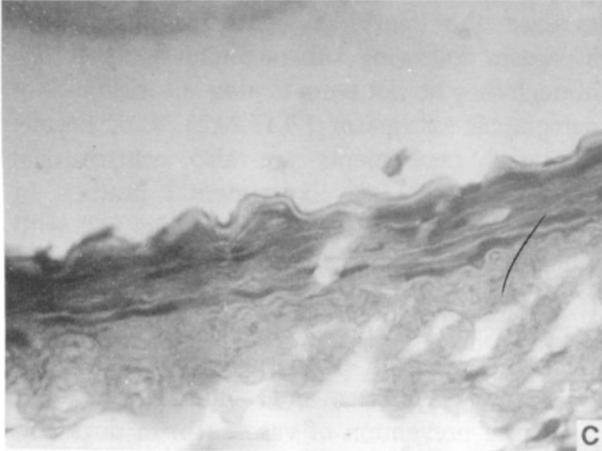


Fig. 1C : Light photomicrograph demonstrating right rat femoral artery from an animal treated with Amlodipine. Minimal luminal narrowing associated with slightly increased wall thickness is seen. Convolution of internal elastic lamina is less prominent than that of vasospastic rat femoral artery. (H&EX200)

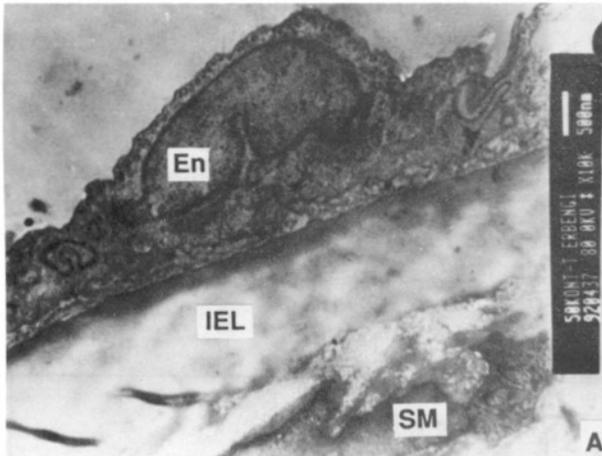


Fig. 2A : Transmission electron micrograph of a normal rat femoral artery. (En) Endothel, (IEL) Internal elastic lamina, (SM) Smooth muscle cell. Scale bar: 0.5 m.

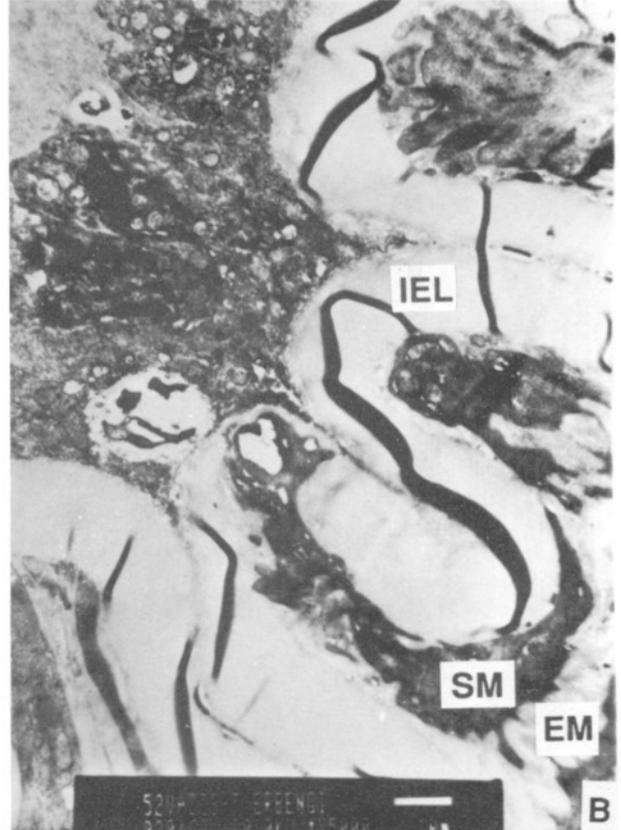


Fig. 2B : Transmission electron micrograph of vasospastic rat femoral artery. The artery shows distortion and degeneration of endothelial cells (En), convolution of internal elastic lamina (IEL), vacuolar degeneration of smooth muscle cells (SM) and increased extracellular matrix (EM). Scale bar: 1 m.

areas of lumen and radial wall thickness in normal, vasospastic and Amlodipine-treated femoral arteries. There was significant reduction in the luminal area and a significant increase in radial wall thickness in vasospastic vessels when compared with normal vessels. In contrast, animals treated with Amlodipine exhibited only a small insignificant reduction in luminal area and an insignificant increase in wall thickness compared with normal vessels. The left femoral arteries in groups 2 and 3 were not significantly different from normal unoperated vessels for any morphometric parameter.

DISCUSSION

The pathophysiology of chronic vasospasm after subarachnoid haemorrhage is still unclear (17,21). One of the major current theories is that vasoactive agents in subarachnoid blood permeate into the vessel wall and promote prolonged contraction of cerebral arterial smooth muscle (17,34). Regardless of the pathway of stimulation, calcium ion is essential for activation of the myosin-actin complex that produces muscle contraction (26). Therefore calcium antagonists that block the entry of calcium into the cell can prevent this prolonged smooth muscle contraction. In recent years, more than fifteen kinds of calcium antagonists have been tested to prevent cerebral vasospasm (32). Dihydropyridine calcium antagonists (Nimodipine, Nicardipine, and Nifedipine) have also been used in the treatment of vasospasm (1,9,13,20,23,24,25).

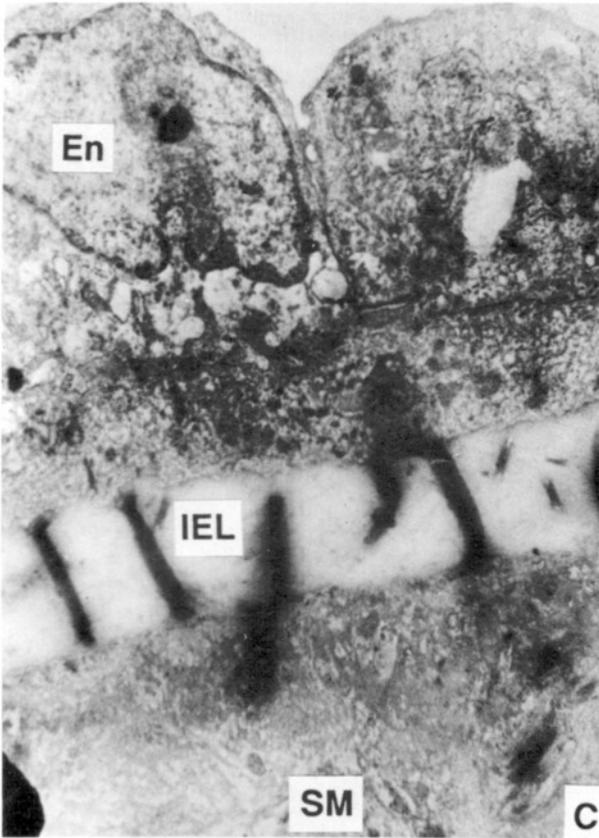


Fig. 2C: Transmission electron micrograph of right femoral artery from an animal treated with Amlodipine. Slight degeneration of endothelial cells (En) with vacuol formation and decreased cytoplasmic electron density is seen. (IEL) Internal elastic lamina. (SM) Smooth muscle cell. Scale bar:1 m.

In large clinical trials, calcium antagonists decrease the morbidity and mortality from vasospasm following subarachnoid haemorrhage although they do not seem to alter the incidence of angiographic vasospasm (1,9,13,20,23,24,25). Results of animal experiments are also controversial (2,6,10,11,14,19,29,34). Experimental studies in monkeys (6,7,19), dogs (34) and rabbits (22) with Nimodipine, Nifedipine or Nicardipine administered orally or intravenously proved that calcium antagonists could not prevent angiographic vasospasm of basal arteries. In two other reports, oral administration of Diltiazem and Nifedipine were effective in the prevention of vasospasm in dogs and monkeys (2,10). However more recently, some authors found a more beneficial effect of intrathecal calcium antagonists in reversing angiographic vasospasm in animals and humans (3,11,22,31,34). By intrathecal administration of calcium antagonists a higher cerebrospinal fluid or better access to the smooth muscle cells of the media can be obtained (22). However, repeated intrathecal infusion of calcium antagonists may present technical difficulties, significant side effects and complications (22). Oral or intravenous Nimodipine and Nicardipine treatment also needs repeated daily doses because of the short half life of these agents. Higher dosages of Nimodipine and Nicardipine can produce significant peripheral cardiovascular effects, reducing mean arterial pressure (9,34).

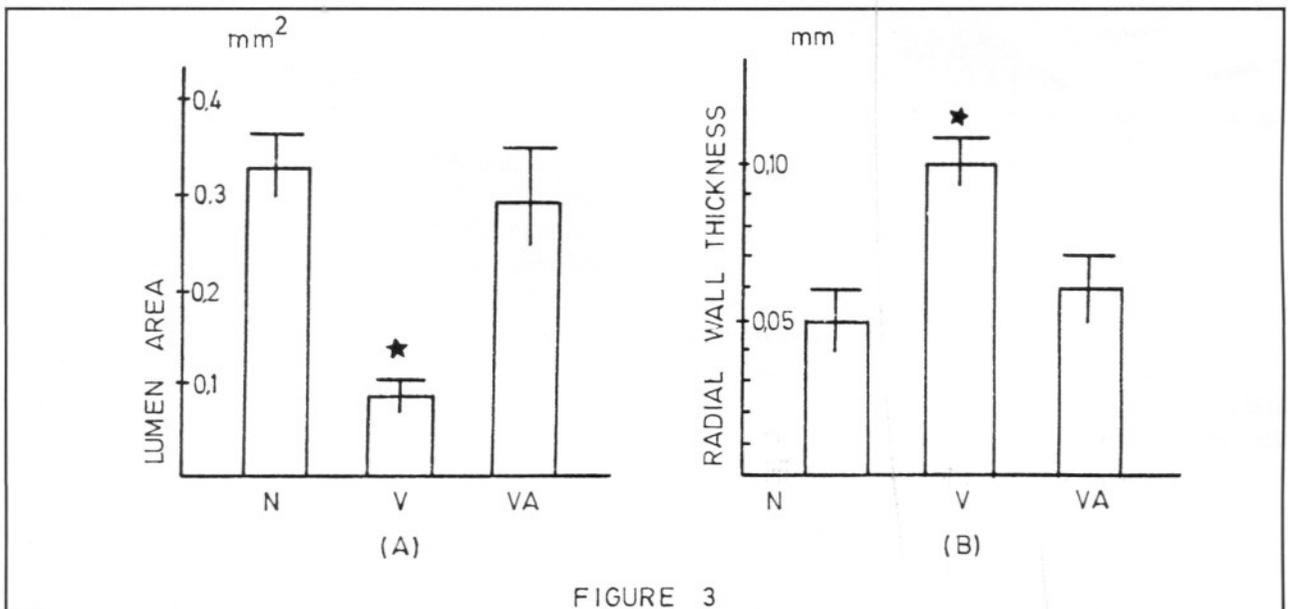


FIGURE 3

Fig. 3: Measured cross sectional area of lumen (A) and radial wall thickness (B), of rat femoral arteries. (N) Normal artery. (V) Vasospastic artery. (VA) Vasospastic amlodipine treated artery. *P<0.01

In our study a new long-acting dihydropyridine calcium antagonist Amlodipine was found to be effective in preventing chronic morphological vasospasm in rats. This drug has been used in the treatment of coronary artery disease and hypertension and it is in the same group as Nimodipine and Nifedipine (12). The major advantage is its long-action (27,30,33). Amlodipine is a potent vasodilator and has a direct relaxant effect on vascular smooth muscle (4,16). In vitro Amlodipine inhibits contractions of rat vascular smooth muscle twice as strongly as Nifedipine (4). It is highly protein-bound and the peak plasma level is reached within 6 hours without side effects due to acute vasodilatation (5,8,12). The half-life of the drug is as long as 45 hours (8,12,33).

We preferred the rat femoral artery vasospasm model in this study for two reasons. First, this model is reliable and inexpensive with a time course similar to clinical vasospasm in humans (21). Second, the absolute bioavailability of Amlodipine is 100% in rats (26). In our study results were encouraging and show that calcium antagonists can be effective in preventing of morphological vasospasm. However, further studies (particularly with primate models) are needed.

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