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The Therapeutic Effect of Nifedipine as a Ca⁺² Entry Blocker on Experimental Transient Ischemic Retina

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Abstract: We evaluated the effect of nifedipine, an L-type voltage sensitive Ca⁺² channel (VSCC) blocker, on the onset of neuronal damage induced by transient ischemia of guinea pig retinas in vivo. The animals were divided into two groups. In the first group 2 mg/kg Nifedipine was administered intraperitoneally, while only 2 cc/kg NaCl 0.9% solution was given to the second group thirty minutes before the ischemic insult. Seven days after the temporary ligation of bilateral

common carotid arteries for 45 minutes, the animals were dispatched and the eyes were enucleated for histopathologic analysis. It was observed that in the Nifedipine treated group, ganglion cells of the sensorial retina were well preserved.

Key Words: Nifedipine, Ischemia, Retina, VSCC Blocker.

Introduction

Ischemia may cause serious retinal damage with disruption of the normal retinal cellular metabolism and accumulation of cellular debris from disrupted axoplasmic flow (1). Loss of cellular homeostasis and a shortage of available adenosine triphosphate (ATP) causes the release of glutamate and some other influxes of sodium, calcium and chloride (2, 3). One of the most important causes of neuronal damage is known to be influx and mobilization of calcium (4, 5). In the literature some calcium antagonists have been reported to prevent the neuronal damage following ischemia by reducing the entry of Ca⁺² into the cell and by increasing the blood flow into the tissue (6-8).

Even though the prevention of neuronal dysfunction by calcium antagonists has been well documented especially in stroke and cerebral ischemia (9-11), only a few studies have been performed on these drugs in ocular ischemia (12-13).

In this experiment we aimed to detect and demonstrate the neuroprotective effect of Nifedipine, an L-type voltage sensitive Ca⁺² channel blocker, on the ischemic retinas of guinea pigs.

Materials and Methods

The eyes of 12 guinea pigs weighing 250-530 g were

used in our experiment. The animals were divided into two groups each consisting of 6 guinea pigs. In the first group 2 mg/kg nifedipine was administered intraperitoneally while only 2 cc/kg NaCl 0.9% solution was given to the second group in the same way, thirty minutes before the ischemic insult.

Following a deep anesthesia with 40 mg/kg ketamin intramuscularly, the animals were immobilized by fixing their legs and the head and neck were fully extended. After shaving the cervical region, a 2cm horizontal skin incision was made. Using an operation microscope (Zeiss OPMI-1) and microsurgical instruments, the common carotid arteries were dissected from the vagus and the cervical sympathetic nerves. Then bilateral temporal ligation of the common carotid arteries was performed using microvascular clips, simultaneously, as we have described previously (14). The enucleated eyes were fixed in 10% neutral buffered formaldehyde solution, dehydrated in graded alcohols and embedded in paraffin. Sections of the whole eye were stained with hematoxylin and eosin. The histologic sections of the eyes were examined. With high power fields (10x40) ganglion cells of the sensorial retina in the peripapillary region and posterior pole were counted. At least three areas on each slide were examined and then the average number of intact ganglion cells per slide calculated. The statistical

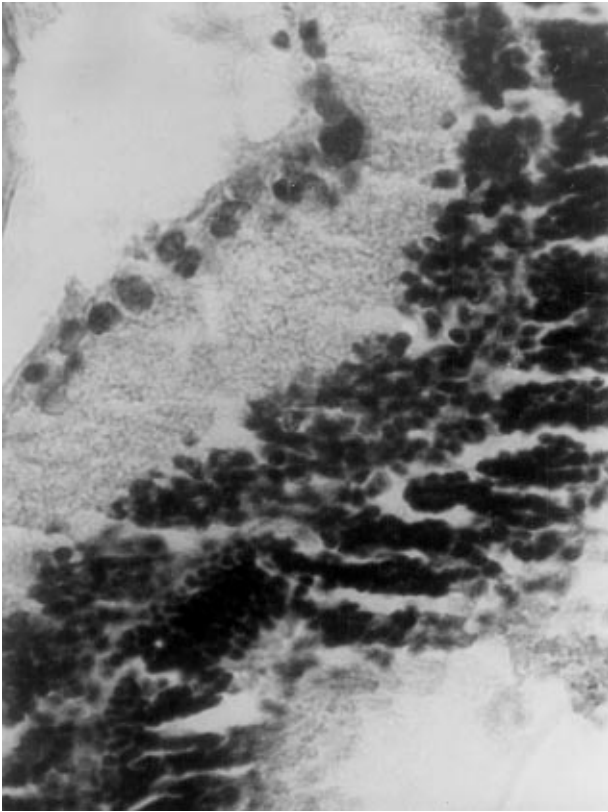


Figure 1. Retinal ganglion cells were well preserved in the Nifedipine treated group (H&E X400)

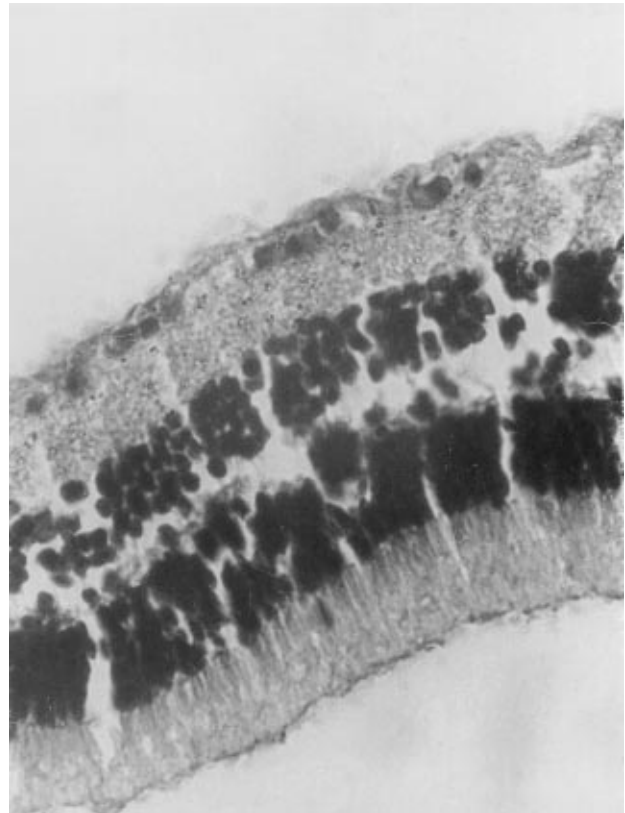


Figure 2. Number of intact ganglion cells were lower in the control group (H&E X400)

analysis was carried out using the unpaired student's t test.

Results

In group I, to which Nifedipine was applied during the preischemic period, the number of ganglion cells and the structure of inner retina were more preserved (Figure 1), while in group II, to which only saline solution was applied during the preischemic period, the number of intact ganglion cells was lower (Figure 2, 3). A statistically significant difference was found between the control group and the Nifedipine treated group as shown in table 1 ($t=2.805$, $p=0.0103$ $p<0.05$).

Discussion

In this study Nifedipine, an L type voltage sensitive calcium channel blocker, was found to be effective in experimental transient ischemic retina for 45 minutes. Since the important involvement of extracellular calcium in the neurodegenerative process induced by excitatory

amino acids in ischemia was observed, a number of attempts were made to evaluate the possible neuroprotective effect of calcium channel blockers. Neuronal cells, especially intact neurons which surround the ischemic area secrete glutamate during ischemia. Glutamate is an important excitatory amino acid (EAA) in many central nervous system synapses and with the other neurotransmitters it causes calcium and sodium influx and finally cell death during ischemia (15). This neurotoxicity occurs via rapid (activation of NMDA receptors) and slow (activation of non NMDA-AMPA/quisqualate and kainate receptors) pathways (16). During the ischemia, the activation of the voltage sensitive calcium channels by glutamate by non NMDA receptors are primarily responsible for calcium influx and neuronal cell death (17). In retinal cells, Ca²⁺ influx is induced through AMPA/KA receptor (non NMDA) stimulation (18). Using the patch-clamp technique, the presence of non NMDA Na/Ca channels in retinal neurons have also been demonstrated (19). To evaluate the possible neuroprotective effect of calcium channel blockers, some important studies have been made. In the primary culture



Figure 3. Pyknosis and thin sensorial retina was observed in the control group (H&E X400)

of rat cerebellar granule cells, Pizzi has found that Nifedipine used at 100nm concentration significantly counteracted the neuronal death induced by 15 min. application of 50um glutamate (20). Crosson also has reported that Nifedipine had a potential therapeutic effect on ischemic retinal dysfunction (21). In all these experiments, nifedipine was found to be effective in inhibiting Ca^{+2} influx into the cell. Contrary to these report, Koh and Cotman have reported that in their in vitro experiment a decrease in intraneuronal calcium levels may trigger synthesis of proteins mediating cell death (22). Some other in vitro experiments have

Table 1. Average number of ganglion cells

Number	Nifedipine Group	Control Group
1	52.33	27.66
2	67.00	68.33
3	70.33	29.00
4	46.00	68.00
5	71.33	29.66
6	70.66	27.66
7	52.00	26.00
8	69.00	11.33
9	68.33	11.66
10	44.00	52.66
11	52.33	67.66
12	68.00	68.33
Mean± SEM	60.94±3.06	40.66±6.55

Unpaired student-t test results:

t=2.805

p=0.0103 (p<0.05)

revealed only a slight effect of calcium channel blockers on neuronal cell loss. However these studies were in vitro and in vitro experiments have revealed only a slight effect of these compounds. On the other hand, it is well known that ischemia has an effect on reducing tissue ATP levels; especially Ca^{+2} reduces the capacity of the mitochondria to synthesize ATP. It is most likely that Nifedipine, raises ATP levels by preventing Ca^{+2} influx into the cell as an additional effect, the same as flupirtine (23).

In conclusion, our in vivo study indicates that Nifedipine, as a Dihydropyridine Ca^{+2} antagonist, reduces ischemic retinal ganglion cell death and it may be recommended as a potent neuroprotective agent in retinal ischemia.

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