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DENEYSEL SUBARAKNOİD HEMORAJİ MODELİNDE NİMODİPİN VE NİKARDİPİNİN NÖRONAL KORUYUCU ETKİSİNİN KARŞILAŞTIRMASI

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ÖZET

Nimodipinin subaraknoid kanama sonrası vazospazma bağlı gelişen serebral iskemideki terapotik etkisi iyi bilinmektedir. Bu etkinin tam mekanizması halen tartışmalıdır. Bu çalışmanın amacı, nimodipinin oluşturduğu nöronal protektif etkiyi ortaya koymak ve yeni bir kalsiyum kanal blokeri olan nikardipinle karşılaştırmaktır.Dört guinea pig grubuna intrasisternal kan enjeksiyonu yapıldı: 1) sham grubu 2) SAK grubu 3) SAK sonrası nimodipin tedavi grubu 4) SAK sonrası nikardipin tedavi grubu. Serebral iskeminin bir parametresi olarak serebral doku lipid peroksidasyon seviyeleri ölçüldü. Her iki grupta da lipid peroksidasyonunda istatistik anlamı olmayan bir düşüş gözlendi. Bulgular 1-4 dihidropridin kalsiyum kanal blokerlerinin serebral vazospazmda olası bir çift etki mekanizmasıyla etkili olduğunu göstermektedir.

Anahtar Sözcükler: Serebral İskemi, Serebral Vazospazm, Subaraknoid Kanama, Kalsiyum Kanal Blokerleri, Lipid Peroksidasyonu

THE COMPARISON OF THE NEURONAL PROTECTIVE EFFECTS OF NIMODIPINE AND NICARDIPINE IN AN EXPERIMENTAL SUBARACHNOID HEMORRHAGE MODEL

Nimodipine has a well-known therapeutic effect on cerebral ischemia due to vasospasm after subarachnoid hemorrhage (SAH). The exact mechanism of this effect is still controversial. The aim of this study is to determine the possible neuronal protective effect of Nimodipine, and to compare Nimodipine with a newer Calcium-channel blocker, Nicardipine. intracisternal injection was performed in four guinea pig groups: 1) sham operation group 2) SAH group 3) Group with Nimodipine after a short period of SAH 4) Group with Nicardipine treatment after SAH. Cerebral tissue lipid peroxidation levels were measured as a parameter of cerebral ischemia. The results revealed a non-significant decrease in lipid peroxidation in both of the groups. These data represent a possible dual action mechanism for 1-4 dihydropyridine Ca-channel blockers in case of cerebral vasospasm.

Key Words: Subarachnoid hemorrhage, Cerebral Vasospasm, Cerebral İschemia, Lipid Peroxidation, Calcium-channel Blockers

INTRODUCTION

Cerebral vasospasm is a serious complication of aneurİsmal SAH. The mechanism of this entity is not fully understood yet. Nearly all of the theories accuse "something in the blood" as the causative agent; except for the Svendgaard's "neuronal mechanism" concept (1, 2, 3, 4)

Despite the presence of different theories, the results of the cerebral vasospasm does not change; cerebral ischemia occurs and neuronal injury due to hypoxia and lack of ATP follow it. Uncontrollable intraneuronal calcium ions (Ca++) entrance is an important mechanism of neuronal injury (5, 6, 7, 8). The excessive amounts of free intraneuronal Ca++ cause loss of integrity and function of intracellular organelles and neuronal membranes (5, 8). Peroxidation of membrane phospholipids is probably the most important contributor of cellular membrane damage (5, 6, 7, 8, 9, 10, 11). Measurement of lipid peroxidation products is a sensitive and reliable method for the determination of neuronal ischemia (10, 11, 12, 13, 14, 15). Some authors suggest that, lipid peroxidation products also cause cerebral vasospasm (11, 14, 16). Free oxygen (O2) radicals are another causative factors of neuronal injury after aneurismal SAH (8, 10, 11, 17). Free intraneuronal Ca++ was reported to have a trigger effect for free O2 radical production at the same time (8, 17).

A great number of different drugs and methods have been suggested for the prophylaxis and treatment of cerebral vasospasm in the last four decades. 1-4 dihydropyridine Ca-channel blocker Nimodipine has a unique position among these alternatives. This drug inhibits the action of L-type voltage operating Ca-channels (VOC) of both the vascular smooth muscle and neuronal membranes (18, 19). Cerebral vascular smooth muscle cells are high sensitive to this drug (18). A high number of 1-4 dihydropyridine sensitive neuronal membrane receptors were identified in different regions of mammalian brain (20).

Nicardipine is a newer commercially available member of this family. The membrane stabilizing action of Nicardipine is stronger than Nimodipine (21). Alps et al. showed that, the activity of Nicardipine is superior than Nimodipine in a cerebral ischemia model (22).

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Despite the proven clinical efficacy of both drugs; mechanism of action is still controversial. Ca-channel blockers do not relieve the angiographic cerebral vasospasm (4, 23, 24, 25). Recently, many studies suggest that the Ca-channel blockers don't improve the cerebral blood flow significantly; thus the therapeutic action may depend on neuronal protection against ischemia (13, 23, 26, 27, 28, 29, 30, 31).

The aim of this study is, to determine the mechanism of action of Nimodipine and Nicardipine; and to compare the efficacy of these drugs in an experimental SAH model.

MATERIAL AND METHOD

28 adult guinea pigs in both sexes were used in this study. The body weights of the animals varied between 310-830 gms. (mean: 504 gm). All animals had been allowed to free access to food and water during the experimental period. The environmental temperature and humidity were kept constant for all animals.

Experimental Groups: Four experimental groups, each including 7 animals have been designed: Sham operation:The surgical procedure was performed in this group; isotonic saline was given intracisternally instead of blood and was given intraperitoneally instead of test drug according to the experimental protocol. SAH only: Fresh autologous arterial blood was given intracisternally in these animals. After a 60 minute period of SAH; the animals were sacrificed and the brains were removed. SAH + Nimodipine: SAH procedure was performed in these animals. After a 30 minute of waiting period; 2 mg/kg Nimodipine (Nimotop, Bayer AG) was given intraperitoneally. The animals were sacrificed and the brains were removed 30 minutes later. SAH + Nicardipine: The same time regimen with the former group was applied and 2 mg/kg Nicardipine (Loxen, Sandoz) was given intraperitoneally in this group.

SAH Procedure: All animals were anesthetised with intraperitoneal injection of 100 mg/kg Ketamine + 12 mg/kg Xylazine. The atlanto-occipital membranes of animals were cannulated and fresh autologous arterial blood samples were taken by this route. A stainless steel, 26G microneedle with 90° angled tip was inserted into the cisterna magna; a few drop of cerebro-spinal fluid drained and 0,25 ml. fresh autologous arterial blood was given into the cisterna. After the microneedle was removed, the hole in the atlanto-occpital membrane was occluded with a surgi-cell pack and the animal was kept in 45° head-down position for 15 minutes. At the end of the 1 hour SAH period, the animals were sacrificed and the brains were rapidly removed and freezed in the fluid Nitrogene. These samples were stored in a deep freeze in -70 °C until the biochemical analysis.

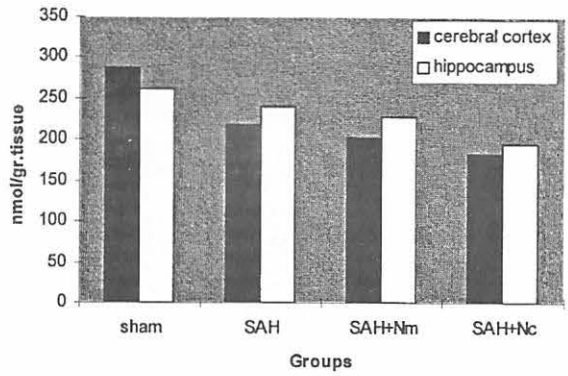
Measurement of Lipid Peroxidation Products: Analysis of the samples have been performed by the Biochemistry Dept. staff, who hasn't any knowledge about the experimental groups. From all removed brains, randomly selected right or left brain hemispheres were divided. A brain tissue sample from cerebral cortex, and another sample from the hippocampal region were obtained from the selected hemisphere. Tissue samples were homogenized with 1.15 % KCL solution to make a 10 % homogenate. One-half milliliter of the homogenate was pipetted into a 10 ml centrifuge tube, and 3 ml 1 % H3PO4 and 1 ml of 0,6 % TBA aqueous solution were added. The mixture was heated for 45 min. in a boiling-water bath. After cooling, 4 ml of n-butanol was added and mixed vigorously. The butanol phase was separated by centrifugation and the absorbances at 532 and 520 nm were measured. The difference was taken as the MDA (Malondealdehyde) value. With this method, the level of MDA, which is an intermediate product of lipid peroxidation, was determined in brain tissue samples; as nanomoles in Per gm. of brain tissue (nmol/ gr. tissue) (32).

Statistical Analysis: Results were analised with the methods of Analysis of Variance (ANOVA), Kruskal-Wallis analysis and Mann-Whitney U test.

RESULTS

Fig. 1 shows the whole results in a graphic design. The measured MDA values of cortical gray matter tissue samples are showed in Table I. Both Nimodipine and Nicardipine caused a decrease in MDA values, but the difference between the SAH and drug groups are not statistically significant ($p > 0.05$). Table II shows the reduced ratios of MDA values in drug groups. There isn't any significant difference between the drug groups either ($p>0.05$). Both drugs caused a decrease of MDA values in hippocampal tissue samples (Table III). When the MDA values of SAH and drug groups are compared, the difference is not statistically significant ($p>0.05$). As seen in the table IV, Nicardipine causes a more evident decrease in MDA values; but the difference between the groups is not significant either ($p>0.05$). When the MDA values of cortical gray matter and hippocampal region samples were compared (Table V), we couldn't determined any significant difference between the SAH and SAH+drug groups (Mann-Whitney U test, $p> 0.05$).

Figure 1: Average values of MDA after SAH in Cerebral Cortex and Hippocampus



Average values of MDA after SAH in Cerebral Cortex and Basal Region

Table I: MDA Values of Tissue Samples of Cerebral Cortex (SD:Standard deviation, p> 0.05)

Subject no	Sham	SAH*	SAH+Nm*	SAH+Nc*
1	390	155	213	167
2	275	145	124	171
3	195	273	184	205
4	157	259	160	180
5	441	292	276	200
6	355	191	216	138
7	202	212	240	221
Mean+SD	287.8±109.2	218.1±58	201.8±50.6	183.1±25.3

Table II: Decrease of MDA in Groups* (p> 0.05)

Group	Nimodipine	Nicardipine
Mean (%)	7.79	16.05

*: Tissue samples in cerebral cortex

Table III: MDA Values of Tissue Samples in Hippocampus (SD: Standard deviation, p> 0.05)

Subject no	Sham	SAH*	SAH+Nm*	SAH+Nc*
1	382	213	179	188
2	389	235	297	170
3	164	346	181	174
4	168	297	256	217
5	337	275	191	205
6	171	148	196	218
7	228	160	291	186
Mean+SD	262.7±103.3	239.1±72.2	227.2±52.6	194±19.5

Table IV: Decrease in MDA Values* (p> 0.05)

Group	Nimodipine	Nicardipine
Mean (%)	5.02	18.82

*: Tissue samples in hippocampus

DISCUSSION

Before the discussion of results, the theoretical base and a practical deficiency of the study must be evaluated. The high MDA values of the sham operation group need an explanation. According to Marzatico et al., any injection into the cisterna magna, causes a transient increase of lipid peroxidation in the whole brain (33). Marzatico also emphasized in another study that, an accumulation of the lipid peroxidation products in tissue homogenates occurs during the waiting period of biochemical analysis in aerobic conditions (13). This problem can be solved by using the high performance liquid chromatography for the measurement of lipid peroxidation products (16). Unfortunately we did not have this equipment.

This study has a difference from the majority of other "experimental SAH" studies; because of the used phase of SAH. "Chronic vasospasm" model has been widely accepted in these kinds of experiments but we used the "acute" phase of SAH in this study. There are two main sources of lipid peroxidation in the "milieu" of brain after SAH: 1) the ischemically injured neurons; 2) the subarachnoid clot, the vessel walls and the white blood cells and platelets in the subarachnoid space (10, 11, 12, 13, 14, 15, 16). Sano, Sakaki and Kanamura suggested in different studies that, lipid peroxidation products in the CSF and cerebral vessel walls begin to increase 5 days after SAH and reach the peak level at the eight day of SAH (14). As Wilkins and Delgado already had proved, there is a biphasic pattern of cerebral vasospasm in different species, including primates (4, 34). The first phase of vasospasm occurs in the first 30 minutes of SAH (34). During this period, the size of cerebral vesels and the global cerebral blood flow show a 40 % decrease (34, 35). Thus we assumed that, in the very early period of SAH, the majority of lipid peroxidation products in the brain tissue derived from the ischemically injured neurons. If the neurons could be protected against ischemia in this period of SAH, the lipid peroxidation products would reduce in the brain tissue. As seen in the results of this study, both Nimodipine and Nicardipine decreased the level

of MDA in all studied areas of animal's brains. The results were not statistically significant expect for the comparison of sham operation group versus. Nicardipine group ($p<0.05$). But this comparison hasn't any meaning related to the purpose of this study, so it is excluded from the discussion.

Ca-channel blocker drugs could lower the MDA values independent from the source, in our model. According to our theoretical base, except the contribution of experimental procedure, neuronal ischemia caused the MDA accumulation in the brain at the acute phase of SAH. Another evidence of this ischemic event is the measurement of higher (but unsignificant) MDA level in hippocampal tissue samples. As known already, hippocampus is the most sensitive region of mammalian brain to hypoxia.

These results showed a neuronal protective effect of Ca-channel blockers in the "acute" phase of SAH. According to our findings, this effect is not the "main street" of mechanism of action. Perhaps the determination of more sensitive parameters of the integrity of cellular and subcellular membranes, such as Na-K ATPase, may be more useful in this model (13, 33).

The second purpose of this study was to compare the effectiveness of Nimodipine and Nicardipine after SAH. We couldn't find any significant difference between the "neuronal protective" effects of these drugs. However, we think that, as seen in Table II and IV, the consistency of our results emphasized a possible superiority of Nicardipine than Nimodipine in different brain areas. Alps and Whiting suggested in different studies that, since Nicardipine is a very lipophilic compound, it reaches high concentrations in lipid bilayers (21, 22). This allows a strong membrane stabilizing activity. Our results seems to have been supported by this concept.

As a conclusion, it can be said that; Ca-channel blockers at least have a "minor" neuronal protective effect. Probably Nicardipine is a better choice in the prophylaxis and treatment of SAH; but this topic needs more investigation.

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