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Effects of nimodipine on tissue lactate and malondialdehyde levels in experimental head trauma♣

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ÖZET

Bu çalışmada, deneysel kafa travmasından 1 saat sonra nimodipinin doku laktat (LA) ve malondialdehid (MDA) seviyeleri üzerine etkilerini araştırdık. Yeni Zelanda tipi tavşanlara kafa travması oluşturmak için ağırlık düşürme metodu uygulandı. Tavşanlar 3 gruba ayrıldı. Grup 1 sham grubu idi. Grup 2'ye yalnızca travma yapıldı. Üçüncü grupdaki tavşanlara travmadan hemen sonra juguler ven yoluyla 20 mgr/kg dozunda nimodipin verildi. İkinci ve üçüncü grupta travma yapılmayan taraftan alınan örnekler "a", travma yapılan taraftan alınan örnekler "b" olarak adlandırıldı. Ortalama doku LA ve MDA seviyeleri sırasıyla grup 1'de 15.33 ± 1.89 mikromol/gram yaş doku (mmol/gww) ve 66.21± 8.69 nanomol/gram yaş doku (nmol/gww); grup 2a'da 18.24 ± 1.84 mmol/gww ve 89.64 ± 9.65 nmol/gww; grup 2b'de 25.50 ± 1.78 mmol/gww ve 142.43 ± 8.69 nmol/gww; grup 3a'da 18.67 ± 1.81mmol/gww ve 91.09 ± 11.86 nmol/gww; grup 3b'de 25.25 ± 1.95 mmol/gww ve 137.60 ± 9.59 nmol/gww olarak bulundu. Grup 2 ve grup 3 arasındaki farklılıklar istatistiksel olarak anlamlı değildi. Bu sonuçlar, deneysel kafa travmasının erken döneminde verilen nimodipinin doku LA ve MDA seviyeleri üzerine etkili olmadığını gösterdi.

Anahtar Kelimeler: *Kafa travması, lipid peroksidasyon, laktat, malondialdehid, nimodipin*

SUMMARY

In this study, we investigated the effects of nimodipine on tissue lactate (LA) and malondialdehyde (MDA) levels in rabbit brain one hour after experimental head trauma. The weight drop method was used to produce head trauma in 25 New-Zeland rabbits. The rabbits were divided into three groups. Group 1 was the sham operated group. Group 2 (control group) received head trauma only and in group 3 nimodipine was administered 20 µgr./kg (i.v) immediately after the head trauma via the jugular vein. In group 2 and 3 samples from the non contused side was named as "a" and contused side as "b". The tissue mean LA and MDA levels were 15.33±1.89 micromoles per gram wet weight (µmol/gww) and 66.21± 8.69 nanomoles per gram wet weight (nmol/gww) in group 1, 18.24±1.84 µmol/gww and 89.64 ± 9.65 nmol/gww in group 2a, 25.50±1.78 µmol/gww and 142.43±8.69 nmol/gww in group 2b, 18.67±1.81 µmol/gww and 91.09±11.86 nmol/gww in group 3a, and 25.25±1.95 µmol/gww and 137.60±9.59 nmol/gww in group 3b respectively. The results were found similar between the group 2 and 3. These results demonstrated that nimodipine has no effect on tissue LA and MDA levels in the early period of experimental head trauma.

Key Words: *Head trauma, lactate, lipid peroxidation, malondialdehyde, nimodipine*

Postischemic or traumatic brain injury causes an energy failure that leads to adenosine triphosphate (ATP) deplation and an increase in anaerobic glycolysis and calcium overload (1-4). This process

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results in tissue lactate (LA) level elevations, generation of oxygen free radicals (OFR) and lipid peroxidation (LP) which can be measured by tissue malondihaldehyde (MDA) levels (5-7). All these mechanisms are held responsible for the irreversible cellular damage. Nimodipine is a calcium-channel blocker that crosses the blood-brain barrier. By blocking the calcium channels in the cerebral neurons and blood vessels it is supposed to reduce intracellular calcium and to protect the brain from harmfull calcium overload in ischemic or traumatic brain injuries (8-10).

In a head trauma model we aimed to investigate the effects of nimodipine on cerebral LA and MDA levels.

MATERIAL AND METHOD

A total of 25 New-Zeland rabbits of both sexes weighing approximately 2-2,5 kg. were used in the experiment. The rabbits were divided into three main groups. The first group consisted of 5, while the others consisted of 10 rabbits. All the rabbits were anesthetized with xylazin HCL 5 mg/kg and ketamine 35 mg/kg intramuscularly (i.m.). Supplemental doses were given im. as needed. Spontaneous breathing was maintained throughout the entire experiment. The electrocardiogram, periferic oxygen saturation, heart rate, systolic-diastolic and mean arterial blood pressures were monitored continously.

Standard head traumas were applied by using the Feeney method which is modified from the spinal cord contusion method of Allen (11). Anesthetized rabbits were fixated at prone position. The scalp was shaved and swabbed with polyvinil iodide. A 4 cm long median vertical incision was made and the frontoparietal regions were exposed on each side. Craniectomies were performed with a high speed drill and rangeurs in the parietal region on both sides. The craniectomies were 2 cm in diameter. The dura remained intact in order to prevent puncture or tearing of the cerebral cortex.

After the craniectomy a pretraumatic electroencephalogram (EEG) was recorded at a sensitivity of approximately 20 mV/cm from both parietal cortices. Disc electrodes were placed over the dura of the parietal cortices. After the EEG recording, a weight of 20 grams was dropped on to the right craniectomy area from a height of 40 cm through a stainless steel guide tube kept at a vertical angle. The guide tube was perforated at 1 cm intervals to prevent air compression. Since the power of trauma is equal to weight multiplied by height, the rabbits were subjected to a trauma which was 800 gr/cm. The supression of cerebral electrical activity in EEG after the trauma (a decrease in amplitude and/or frequency) was considered as a sufficient head trauma. If there was no difference in EEG recordings the trauma was repeated.

In group 1 (sham - operated group) only craniectomy was performed, trauma was not applied. This group consisted of 5 animals. In group 2 (control group) rabbits were observed untreated for one hour following head trauma. In group 3 (nimodipine treated group) nimodipine was administered 20 μg/kg/min. iv. continously for 30 minutes via the jugular vein, after the trauma.

One hour after the trauma approximately 0,5 grams of contused (right) and non-contused (left) brain tissue samples were resected from both sides in group 2 and 3 in order to see the effect of trauma on the non-- contused side. In group 1 tissue samples were taken only from the right side. The samples were stored below -70°C until the homogenization procedure. After the experiment was completed, the animals were sacrificed. LA and MDA levels were assesed as described below.

Biochemical studies

Tissue lipid peroxidation was evaluated by measurement of thiobarbituric acid-reactive substances (12). MDA, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the peroxi-dation reaction. MDA has been identified as the product of LP that reacts with thiobarbituric acid to give a red species absorbing at 535 nm. The assay procedure for lipid peroxide in brain tissue was set up as follows. Samples, weighing less than 0.2 ml of 10% (weight/volume) tissue homogenate was added to 0.2 ml of 8.1% sodium dodecyl sulfate and a 1:5 aqueous solution of thiobarbituric acid. The mixture was diluted to 4.0 ml with distilled water and heated in an oil bath at 95°C for 60 minutes using a glass ball as a condenser. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of a mixture of N-

butanol and pyridine (15:1, volume/volume) were added, and the mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, the organic layer was taken, and its absorbance at 535 nm. was measured. Tetramethoxy propane was used as an external standard, and the level of lipid peroxides was expressed as nanomoles of MDA per gram wet weight (nmol/gww).

The concentrations of LA in cerebral cortices were determined using spectrophotometric enzymatic methods (13). The brain was homogenized at 0°C in 3 M frozen perchloric acid and the LA concentration in the cortical homogenate was determined using a LA dehydrogenase kit (Sigma). The level of LA was expressed as micromoles per gram wet weight (μmol/gww).

Statistical analysis

One way ANOVA and tuckey's HSD tests were used for the evaluation of the results. P<0.05 was considered as significant.

RESULTS

The non-contused sides were labeled as 2a and 3a and the contused sides as 2b and 3 b. The tissue mean LA and MDA levels are listed in table 1. The LA and MDA contents were higher in group 2 and 3 than group 1 (P< 0.05). The difference between the contused and non-contused sides were significant in group 2 and 3 (P< 0.05). The differences between group 2b and 3b were not satistically significant.

DISCUSSION

The mechanisms involved in neuronal cell damage that occurs after head trauma and cerebral ischemia have been related to calcium(Ca++) overload (2,4,10,14-17). Three mechanisms have been proposed for this damage. 1- Following cell injury the cellular energy stores are decreased. This leads to ion pump failure. An increase of Ca++ entry via voltage sensitive Ca++ channels and a decrease of Ca++ extrusion is observed (10,16,17). 2- The increase in excitatory amino acids; glutamate and aspartate leads to the activation of N-Methyl-D-Aspartate (NMDA) receptors. This activation causes influx of Ca++ via agonist operated Ca++ channels, as well as release of Ca++ from intracellular stores (16-20). 3- Ca++ overload inhibits pyruvate dehydrogenase (PDH). Suppression of PDH activity inhibits the decarboxylation of pyruvate to acetyl CoA, as a result causes anaerobic glycolysis, glycogenolysis and LA accumulation. This accumulation leads to the retardation of the extursion of Ca++ (21). Intracellular increase of Ca++ may overload the mitochondria, blocking oxidative phosphorylation and ATP production (1-4,14,17,22,23).

LA accumulation causes an increase in perhydroxyl radicals which contribute to peroxydation (22,23). The degree of tissue LA accumulation is a prime feature associated with the progressively worsening cellular effects and in development of irreversible ischemic cell injury (5,6,12,24). Normally, the membrane phospholipids are recycled, through a series of energy-requiring processes; however, during LA energy stores are depleted and the action of phosplolipase on the cell membrane leads to the depletion of membrane phospholipids, resulting in altered permeability and further Ca++ influx (5,6,12,24). The intracellular Ca++ overload triggers

Tablo 1. *Tissue mean lactate (micromoles per gram wet weight) and malondialdehyde (nanomoles per gram wet weight) levels and standart deviation.*

Mean ± SD	Group 1	Group 2a	Group 2b	Group 3a	Group 3b
LA	15.33±1.89	18.24±1.84[a]	25.50±1.78[a,b]	18.67±1.81[a,d]	25,25±1.95[a,c,e]
MDA	66.21±8.69	89.64±9.65[a]	142.43±8.69[a,b]	91.09±11.86[a,d]	137.60±9.59[a,c,e]

[a] Compared with group 1 P<0.05
[b] Compared with group 2a P<0.05
[c] Compared with group 3a P<0.05
[d] Compared with group 2a P>0.05
[e] Compared with group 2b P>0.05

Ca++ dependent lytic enzymes that cause proteolysis, accumulation of free fatty acids (FFA) and oxygen free radicals (OFR) (14,25-28). Accumulation of FFA may be involved in irreversible cell damage (14,17,29-31). The OFR causes lipid peroxidation which results in membrane disruption (22,23,26-28).

There are numerous studies on nimodipine's action in cerebral ischemia and subarachnoid hemoragia but studies on head trauma is lacking. Nimodipine promotes cerebral blood flow and increases tolerance to ischemia (8-10,14,32,33). Some studies claim that nimodipine has a beneficial effect on neuron protection (10,16,19,34), several other studies have shown that nimodipine has no beneficial effect on neurons (35-38).

It was previously reported that MDA and LA levels in injured tissue reaches its maximum at the first hour after trauma (39,40). Therefore, we obtained the brain tissues 1 hour after the trauma. Brain tissue samples were also taken from the non-contused side, in order to see the indirect effects of the trauma.

The ineffectivenes of nimodipine might be due to its failure to prevent excessive elevation of intracellular Ca++. Nimodipine can prevent Ca++ influx within physiological limits, while it blocks voltage sensitive channels, but as mentioned above different mechanisms are involved in cellular damage. It has been stated that receptor activated Ca++ influx is much more effective in increasing intracellular Ca++ than voltage activated Ca++ increase(10,17,20)

The inefficiency in preventing Ca++ accumulation intracellularly,leads to LA accumulation and trigger of the Ca++ dependent lytic enzymes.These enzymes cause the accumulation of OFRs which results with lipid peroxidation and elevation of MDA.We have found a strong correlation between tissue LA and MDA levels in each group. Also our dose and method of administration may have played a role in the ineffectiveness of nimodipine. We studied LA and MDA in the acute phase, in the chronic phase the results may have been different.

In conclusion; nimodipine has been found to be ineffective in the acute phase of the head trauma in preventing cellular damage, but furtlıer studies are needed to support our results.

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