Effects of mannitol on lipid peroxidation and Na+-K+/ATPase enzyme activity in experimental head trauma

Mehmet Aköz*, Emrah Karagözoglu**, Cemile Topçu*, Mehmet Gürbilek*, Hülagü Barişkaner***, Mehmet Erkan Üstün****, Alper Yosunkaya*****.

*Department of Biochemistry, Faculty of Meram Medicine, University of Selçuk,Konya,Turkey.
**Konya Numune Hospital Clinical Biochemistry Laboratory Konya,Turkey.
***Department of Pharmacology, Faculty of Selçuklu Medicine, University of Selçuk, Konya,Turkey.
****Department of Neurosurgery, Faculty of Meram Medicine, University of Selçuk,Konya,Turkey.
*****Department of Anesthesiology and Intensive Care, Faculty of Meram Medicine,University of Selçuk,Konya,Turkey.

Özet

Deneysel kafa travmasinda Na^+-K^+/ATPaz aktivitesi ve lipit peroksidasyonu üzerine mannitolün etkileri

Amaç: Bu çalişma tavşanlarda deneysel kafa travmasindan sonra doku Na^+/K^+-ATPaz aktivitesi ve malondialdehyde (MDA) seviyeleri üzerine mannitolün etkilerini belirlemek için dizayn edildi. Metod: Otuz Yeni Zelanda tavşani eşit olarak üç gruba ayrildi: Grup S: Şam grubu (şam operasyonu yapilan), grup C: Tedavi edilmemiş grup, grup M: Mannitol ile tedavi edilen. Grup C ve grup M üzerine ağirlik düşürme metodu ile travma uygulandi. M grubuna, travmadan hemen sonra 1,0 g/ kg i.v. olarak mannitol uygulandi. Kafa travmasi kafatasinin sağ tarafina uygulandi. Travmadan bir saat sonra her iki taraf beyin korteksi cerrahi olarak çikarildi ve beyin dokularinda Na^+/K^+-ATPase enzim aktivitesi and MDA seviyeleri belirlendi. Bulgular: Mannitol verilmesiyle Na^+/K^+-ATPase enzim aktivitesi artarken MDA seviyeleri düşük bulundu. Ancak bu değişimler istatistiksel olarak anlamli değildi ($p>0,05$). Sonuç: Bu modelle oluşturulan travma tavşanlarin beyin dokularinda Na^+/K^+-ATPase enzim aktivitesinde ve lipid peroksidasyonunda bir değişikliğe sebep olmamiştir. Travmadan sonra uygulanan mannitol tedavisi bu parametreler üzerinde etkili olmamiştir.

Anahtar kelimeler: Mannitol, Kafa travmasi, Na^+/K^+-ATPaz, Malondialdehyde.

Abstract

Objective: This study was designed to determine the effects of mannitol on tissue Na^+/K^+-ATPase enzyme activity and malondialdehyde (MDA) levels in rabbit brain after experimental head trauma. Methods: Thirty New Zealand rabbits were divided equally into three groups: Group S (sham-operated group), group C (untreated group) and group M (mannitol treated group). Grup C and group M received head trauma with the weight drop method. Mannitol was administered 1,0 g/ kg i.v. immediately after the head trauma to group M. Head trauma was applied to the right side of the head. One hour after trauma, brain cortices were resected from both sides and the Na^+/K^+-ATPase enzyme activity and MDA levels were determined. Results: Na^+/K^+-ATPase activities were increased and MDA levels were decreased in mannitol treated rabbits but the differences were not statistically significant ($p>0,05$). Conclusion: Head trauma did not cause to lipid peroxidation and any changes in Na^+/K^+-ATPase activity in brain tissue of rabbits in this model. Mannitol treatment after the trauma does not affect these parameters.

Key words: Mannitol, Head trauma, Na^+/K^+-ATPase, Malondialdehyde.

Introduction

While mechanical processes may cause tissue damage by tearing or stretching tissue in brain trauma, a number of secondary factors initiate biochemical or physiological events that may lead to neuronal damage or cell death in hours to days after trauma.

Corresponding Address: Mehmet Aköz,M.D.,
Selçuk Üniversitesi Meram Tip Fakültesi
Biyokimya Anabilim Dali
Konya 42080,Turkey
Phone: +90 332 223 66 55 Fax: +90 332 223 61 81
E-Mail:mehmetakoz2003@yahoo.com

Müracaat tarihi: 26.04.2010
Kabul tarihi: 13.05.2010

Although the exact mechanism of the delayed injury is unclear, a variety of factors such as perturbations of cellular energy metabolism (1,2) ion changes (3), tissue lactic acidosis (2,4) membrane depolarization by release of excitatory amino acids(5), free-radical generation and lipid peroxidation (6) production of prostaglandins and leukotrienes (7) were identified in experimental brain injury models.

Ischemia results in lowered oxygen and glucose delivery to brain tissue. As a result, cells have less ATP production, ie, less available energy. Although the brain may represent only 1% of body weight, it can account for 20% of its energy production. Most of this energy is consumed by ATP-driven ion pumps which keep calcium (Ca^{++}) and sodium (Na^{+}) outside of cells and potassium (K^{+}) inside of cells. In ischemia, the loss of energy means that the ion gradients essential for functional neurons cannot be maintained (8). Trauma to the central nervous system causes cellular energy failure that leads to adenosine triphosphate (ATP) depletion, and an increase in anaerobic glycolysis and glycogenolysis. These processes result in lactate accumulation in tissue (1,2,9). Energy failure causes ion pump failure and an increase in excitatory amino acids (glutamate and aspartate) which results in excessive intracellular calcium (Ca^{++}) overload, which in turn triggers many Ca^{++} dependent lytic enzymes including proteases, lipases and nucleases (5,10,11). These enzymes cause proteolysis, depletion of membrane phospholipids, elevation of oxygen free radicals and lipid peroxidation resulting in membrane disruption, altered permeability and further Ca^{++} influx (3,12). Free radicals have been implicated in the injury that occurs to brain tissue in response to ischemia and reperfusion insults. Free radicals may be produced during ischemia/reperfusion as a result of multiple mechanisms involving normal regulatory systems of intra-extracellular metabolism. For example, metabolism of free fatty acids by the cyclo-oxygenase pathway and adenine nucleotides by xanthine oxidase has been proposed to produce free radical adducts during reperfusion (13). Oxygen radical generation and lipid peroxidative reactions appear to be early biochemical events subsequent to CNS injury (14).

Tissue lipid peroxidation is evaluated by measurement of thiobarbituric acid-reactive substances (15). Malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acids serves as a convenient index for determining the extent of the peroxidation reaction (16,17).

Mannitol is an osmotic agent and a free radical scavenger so it might decrease oedema and tissue damage in stroke (18). Mannitol has yet another benefit in that it scavenges hydroxyl radical (19). Free radical scavengers such as superoxide dismutase, desferrioxamine, thiopentone, vitamin E, vitamin C, glutathione, chlorpromazine, mannitol, and some dextrans have been used in an attempt to reduce the effects of reperfusion injury of several organs including the brain, but their clinical impact is not yet clear (20).

The aim of the present study was to investigate the effects of mannitol on lipid peroxidation and brain tissue Na^{+}/K^{+}-ATPase activities in an experimental head trauma model.

Materials And Methods

The animals in this study were cared for in the Medical School of Selçuk University in accordance with the 'Guide for the care and use of laboratory animals'. These protocols were approved by the Animal Use Committee of Selçuk University. A total of 30 New Zealand white rabbits of both sexes weighing approximately 3 – 3,5 kg were used in the experiment. The rabbits were randomly divided into three groups; sham (n=10), control (n=10), and treatment (n=10). All the rabbits were anesthetized with xylazine HCI 15 mg/kg intramuscularly (i.m.) and ketamine 35 mg/kg i.m. Supplemental doses were given as needed. Spontaneous breathing was maintained throughout the entire experiment. A femoral vein was cannulated for fluid and drug administration, and a femoral artery was cannulated for arterial blood gas sampling and continuous arterial blood pressure monitoring. Temperature was monitored by a rectal thermistor probe. Peripheral oxygen saturation (pulse oximetry), heart rate (HR), systolic, diastolic and mean arterial blood pressures were monitored continuously. Standard head traumas were applied by using the method of Feeney (21). Anesthetized rabbits were fixated in prone position. A 4 cm long median vertical incision was made and the frontoparietal region was exposed at each side. A craniectomy was carried out by use of a high-speed drill and rongeurs to the parietal region on both sides. The craniectomies performed were 2 cm in diameter. In this model, the dura remained intact in order to prevent puncture or tearing of the cerebral cortex.

Before the craniectomy, a pre-traumatic electroencephalogram (EEG) was recorded with a sensitivity of approximately 20 μV/mm from both parietal cortices with two channels of an EEG recorder (Nihon Kohden, Inc., Irvine, CA). The low filter was set to 15 Hz and the high filter was set to 120 Hz with the 60 Hz line filter on. The paper speed was 5 mm/s. Time constant was 0,3 second. The disc electrodes were placed over the dura of the parietal cortices. After EEG recording, a constant weight of 10 grams was dropped onto the right craniectomy

area from a height of 80 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 times height, the rabbits were subjected to a trauma that was 800 g/cm^2.

After the trauma, more than a 50% decrease in amplitude or a decrease in alpha frequency to delta band or even disappearance of electrocortical activity when compared with pre-traumatic EEG recordings in the traumatized side was considered as sufficient evidence of head trauma. If there was not sufficient change in EEG recordings, trauma was repeated. In group S (sham operated group) only craniectomy was performed: trauma was not applied. In group C (control group), rabbits were observed untreated for one hour following head trauma. In group M, 1 mg /kg mannitol was administered after ten minutes head trauma with a bolus injection via femoral vein. In group C and group M, one hour after the head trauma, approximately 0,5 grams of traumatized (right side) and non-traumatized (left side) brain tissues was resected from each side. The traumatized sides were labeled as C_1 and M_1 and the non-traumatized sides as C_2 and M_2. The samples were stored below -70°C until the homogenization procedure.

After thawing, brain tissues were homogenised for 3 minutes in the cold phosphate buffer in order to provide a 10 % homogenate. These homogenates were centrifuged at 6000 g for 10 minutes to obtain supernatants. The levels of protein, MDA and the activities of Na^+/K^+-ATPase were determined in the supernatants.

Protein content of homogenates was determined by Lowry method (22). MDA level was measured by Drapper and Hadley's method (16). To assess lipid peroxidation, MDA, an end product of lipid peroxidation was measured by the thiobarbituric acid reaction. Tissue supernatants were deproteinized with trichloracetic acid (TCA). 2500µl of TCA reagent was added to 500µl of supernatants and heated in water bath at 90^{0}C for 15 min. These samples were then centrifuged at 3000 (g) for 10 min. 1000µl of TBA (0,675 %) reagent was added to the supernatant and heated in water bath at 90 ^{0}C for 15 min. Briefly, MDA level was estimated by measuring the characteristic absorbance at 532 nm after reaction with thiobarbituric acid. The concentration of MDA was calculated using extinction coefficient of $1{,}56\times10^5\times M^{-1}$.

Na^+/K^+-ATPase activity was determined by the method of Kitao and Hattori (23). The total ATPase activity was assayed by incubating ghost at 37 ^{0}C in a medium containing 5 mM MgCl, 140 mM NaCl and 14 mM KCL in 40 mM Tris-HCl, pH 7,7. The reaction was started by the addition of 3 mM Na_2ATP and stopped 20 min later by the addition of 14 % trichloracetic acid. Inorganic phosphate (Pi), hydrolyzed from the reaction, was measured. The results were expressed as mol of Pi per mg of proteins per 10 min. All values were expressed as means ± SD. One-way ANOVA test were used for the evaluation of Na^+/K^+-ATPase activities and MDA levels using SPSS program. Two way ANOVA for repeated measures and unpaired t test and paired t test were used for evaluating hemodynamic, blood gas analysis and rectal temperature results between groups. A p-value less than 0,05 was considered statistically significant.

Results

The mean Na^+/K^+-ATPase activities and MDA levels are demonstrated at the Table 1.

Table 1. The Na^+/K^+-ATPase activities and MDA levels of all groups (mean ± SD).

Study Groups	Na^+/K^+-ATPase (mol Pi/ mg protein/10 min)	MDA (nmol/mg protein)	P values
Group S	0.43±0.16	1.31±0.26	P >0.05
Group C1	0.43±0.08	1.47±0.39	P >0.05
Group C2	0.43±0.10	1.27±0.34	P >0.05
Group M1	0.55±0.16	1.26±0.13	P >0.05
Group M2	1.32±1.47	1.01±0.18	P >0.05

Group S: Sham operated group, Group C1: Traumatized side of non-treated group (right side), Group C2: Nontraumatized side of non-treated group (left side), Group M1: Traumatized side of manitol-treated group (right side), Group M2: Nontraumatized side of manitol-treated group (left side)

Na^+/K^+-ATPase was not affected by trauma. But administrating mannitol after trauma caused to increase the Na^+/K^+-ATPase activity in traumatized side and decreased nontraumatized side of the brain. Bu these changes are not statistically significant (p>0,05). MDA, as a marker of lipid peroxidation increased by trauma and decreased by mannitol treatment, insignificantly (p>0,05). When compared to both sides of the brain, Na^+/K^+-ATPase activity did not differ by trauma. But treating mannitol after trauma caused different effect on the enzyme. While the activity in traumatized side increasing, nontraumatized side decreased. But, these changes were not significant (p>0,05). MDA levels in traumatized side higher than nontraumatized side in C and M groups, insignificantly (p>0,05).

Discussion

In this research head trauma did not change Na^+/K^+-ATPase activity. But the treatment with mannitol increased in traumatized and decreased in nontraumatized side of the brain. Lipid peroxidation increased only in traumatized side of the brain and mannitol administration reduced this increase. However, the changes of Na^+/K^+-ATPase activity and the level of MDA were not significant. Oxidative stress may contribute to many pathophysiologic changes that occur after traumatic brain injury (24). Oxygen radical generation and lipid peroxidation appear to be early biochemical events subsequent to CNS trauma (25).

Intracranial perfusion regulation is usually maintained by means of vascular constriction / dilation in a normal brain. In an injured brain, this ability may be lost. $PaCO_2$, pH, and PaO_2 influence the autoregulation response of the cerebro-vasculature. Increased $PaCO_2$ and hypoxemia, frequent findings in traumatized patient, will cause vasodilatation and increase intracranial pressure (ICP). Ischemia triggers the Cushing reflex, ultimate guarantee in cerebral blood flow (CBF) maintenance, by increasing the systemic arterial pressure. ICP will finally increase too. Brain edema is the source of the increased ICP. The traumatic disruption of the blood brain barrier explains its vasogenic extracellular component (26). Brain edema is a frequent complication of cerebral ischemia; however, its mechanism of formation is not well understood. Sodium is known to accumulate in brain during the early stages of partial ischemia (27).

Patients with severe head injury frequently have evidence of elevated intracranial pressure (ICP) and ischemic neuronal damage at autopsy. Mannitol has been used clinically to reduce ICP with varying success and it is possible that is more effective in some types of head injury than in others (28). The effectiveness of mannitol for the treatment of cerebral edema after stroke has long been debated and the diffusion of mannitol through a disrupted blood-brain barrier has been the focus of many contradictory studies (29). Mannitol is a hyperosmolar molecule that dehydrates the cerebral interstitium, lowering ICP. Mannitol also decreases blood viscosity promoting cerebral perfusion; it also has free radical scavenger ability which limits the deleterious molecular cascade induced by ischemia. After fluid resuscitation, the risk of a rebound effect on the ICP is minimized (26). Mannitol has replaced other diuretics as the agent of first choice for control of raised intracranial pressure (ICP) after brain injury. Mannitol should be given as a bolus intravenous infusion, over 10 to 30 mins, in doses ranging from 0,25 to 1 g/kg body weight (26,30).

Mannitol is sometimes dramatically effective in reversing acute brain swelling, but its effectiveness in the on-going management of severe head injury remains open to question. There is evidence that in prolonged dosage mannitol may pass from the blood into the brain where it might cause reverse osmotic shifts that increase intracranial pressure (31). Mannitol is effective in reducing ICP, and was recommended its use in the menagement of traumatic intracranial hypertension (32).

Compromised blood flow rapidly impairs ATP synthesis and thus the ability of excitable cells to maintain polarized resting membrane potentials. The resulting membrane depolarization favors an influx of sodium and Ca^{++} ions through voltage sensitive channels and promotes the release of neurotransmitters from neuronal pre-synaptic endings (33,34). The most important one of these neurotransmitters is glutamate . This released glutamate acts postsynaptically by activating N-methyl-D-aspartate (NMDA) and non-NMDA types of glutamate receptors which, in turn results in a large increase in intracellular Ca^{++} and leads to calcium-mediated cell death (35) Ca^{++} overload inhibits pyruvate dehydrogenase (PDH), suppression of PDH activity inhibits the decarboxylation of pyruvate to Acetyl-CoA, and consequently causes anaerobic glycolysis, glycogenolysis, and lactate accumulation. This accumulation leads to the retardation of the extrusion of Ca^{++}, which results with further lactate accumulation (36,37).

Calcium entry into the pre-synaptic membrane of a neuron is a key factor in neurotransmitter release. In the brain glutamate is the predominant excitatory neurotransmitter. In a normal brain, glutamate release at a synapse results in normal signaling. In ischemic brain tissue, glutamate release can result in increased calcium entry into the postsynaptic cell, creating a positive feedback loop of calcium penetration and glutamate-release known as an excitoxicity. Intracellular calcium activates phospholipase, which degrades membrane phospholipids. This not only damages the membrane, but also releases the toxic free fatty acid arachidonic acid, which forms substantial amounts of oxygen free radicals. Calcium

also activates enzymes which degrade protein and DNA, although this effect is less pronounced than the lipase activation (8).

Mannitol is the first-choice therapy for increased intracranial pressure, and barbiturates are still considered as a rescue therapy in case of refractory intracranial hypertension (38). There is currently not enough evidence to decide whether the routine use of mannitol in acute stroke would result in any beneficial or harmful effect. The routine use of mannitol in all patients with acute stroke is not supported by any evidence from randomized controlled clinical trials. Further trials are needed to confirm or refute the routine use of mannitol in acute stroke (39).

In this research, we could not find any significant changes in MDA level and Na^+/K^+-ATPase activity. Small changes could be significant if the number of animal were higher. We cannot build any conclusion on insignificant results. According to these results, we suggested that in this trauma model with this number of cases, head trauma is not cause to lipid peroxidation and any changes in Na^+/K^+-ATPase activity in brain tissue of rabbits. Mannitol treatment after the trauma does not affect these parameters.

References

1. Clifton GL, Robertson CS, Grossman RG, Hodge S, Foltz R, Garza C. The metabolic response to severe head injury. J Neurosurg. 1984 Apr;60(4):687-96.
2. Rabow L, DeSalles AF, Becker DP, Yang M, Kontos HA, Ward JD, et al. CSF brain creatine kinase levels and lactic acidosis in severe head injury. J Neurosurg. 1986 Nov;65(5):625-9.
3. Shapira Y, Yadid G, Cotev S, Shohami E. Accumulation of calcium in the brain following head trauma. Neurol Res. 1989 Sep;11(3):169-72.
4. Bullock R,Lyeth BG, Muizelaar JP. Current status of neuroprotection trials for traumatic brain injury: lessons from animal models and clinical studies. Neurosurgery.1999;45:207-217.
5. Bullock R, Zauner A, Woodward JJ, Myseros J, Choi SC, Ward JD, Marmarou A, Young HF. Factors affecting excitatory amino acid release following severe human head injury. J Neurosurg. 1998 Oct;89(4):507-18.
6. Ikeda Y, Long DM. The molecular basis of oxygen free radicals. Neurosurgery. 1990;27:1-11.
7. Kiwak KJ, Moskowitz MA, Levine L. Leukotriene production in gerbil brain after ischemic insult, subarachnoid hemorrhage, and concussive injury. J Neurosurg. 1985 Jun;62(6):865-9.
8. White BC, Sullivan JM, DeGracia DJ, O'Neil BJ, Neumar RW, Grossman LI, Rafols JA, Krause GS. Brain ischemia and reperfusion: molecular mechanisms of neuronal injury. J Neurol Sci. 2000 Oct 1;179(S 1-2):1-33.
9. De Salles AF, Muizelaar JP, Young HF. Hyperglicemia cerebrospinal fluid lactic acidosis and cerebral blood flow in in severely head injured patients. Neurosurgery. 1987;21:45-50.
10. Peters T. Calcium in physiological and pathological cell function. Eur Neurol. 1986;25:27-44.
11. Trigle DJ. Calcium antagonists:history and perspective. Stroke. 1990;21:49-58.
12. Gutteridge JM, Halliwell B. The measurement and mechanism of lipid peroxidation in biological systems. Trends Biochem Sci.1990;15:129-135.
13. Kirsch JR, Helfaer MA, Lange DG, Traystman RJ. Evidence for free radical mechanisms of brain injury resulting from ischemia/reperfusion-induced events. J Neurotrauma. 1992;23(10):1479-85.
14. Hall ED. Free radicals and CNS injury. Crit Care Clin.1989;5(4):793-805.
15. Callaway JK, Beart PM, Jarrott B. A reliable procedure for comparison of antioxidants in rat brain homogenates. J Pharmacol Toxicol Methods.1998;39:155-162.
16. Drapper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods in enzymology. 1990;186;421-430.
17. Halliwell B Chiroce S. Lipid peroxidation it's mechanism measurement and signifiance. Am J Clin. 1993;57:715-25.
18. Bereczki D, Mihálka L, Szatmári S, Fekete K, Di Cesar D, Fülesdi B, Csiba L, Fekete I. Mannitol use in acute stroke: case fatality at 30 days and 1 year. Stroke. 2003 Jul;34(7):1730-5.
19. Desesso JM, Scialli AR, Goeringer GC. D-mannitol, a specific hydroxyl free radical scavenger, reduces the developmental toxicity of hydroxyurea in rabbits. Teratology. 1994 Apr;49(4):248-59.
20. Christopher SG. The treatment of brain injury. United States Patents. 1996;(5):527- 533.
21. Feeney DM, Boyeson MG, Linn RT, Murray HM, Dail WG. Responses to cortical injury: I. Methodology and local effects of contusions in the rat. Brain Res. 1981 Apr 27;211(1):67-77.
22. Lowry OH, Rosebrough NJ, Farr WL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951; 193: 265-272.
23. Kitao T, Hattori K. Inhibition of erythrocyte ATPase activity by aclacynomycin and reverse effect of ascorbate on ATPase activity. Experientia. 1983;39:1362-64.
24. Tyurin VA, Tyurina YY, Borisenko GG, Sokolova TV, Ritov VB, Quinn PJ, et al. Oxidative stress following traumatic brain injury in rats: quantitation of biomarkers and detection of free radical intermediates. J Neurochem. 2000 Nov;75(5):2178-89.

25. Hall ED, Braughler JM. Free radicals in CNS injury. Res Publ Assoc Res Nerv Ment Dis.1993;71:81-105.
26. Cauzinille L. Cranio-Cerebral Trauma: What and When to Decide. 26th WSAVA Congress Proceedings. August 8-11, 2001 Vancouver, British Columbia, Canada.
27. Betz AL, Ennis SR, Schielke GP, Hoff JT. Blood-to-brain sodium transport in ischemic brain edema. Adv Neurol. 1990; 52: 73-80.
28. Mendelow AD, Teasdale GM, Russell T, Flood J, Patterson J, Murray GD. Effect of mannitol on cerebral blood flow and cerebral perfusion pressure in human head injury. J Neurosurg. 1985 Jul;63(1):43-8.
29. Maioriello AV, Chaljub G, Nauta HJ, Lacroix M. Chemical shift imaging of mannitol in acute cerebral ischemia. Case report. J Neurosurg. 2002 Sep;97(3):687-91.
30. Bullock R. Mannitol and other diuretics in severe neurotrauma. New Horiz 1995;3(3):448-52.
31. Schierhout G, Roberts I. Mannitol for acute traumatic brain injury. Cochrane Database Syst Rev 2000;(2):CD001049.
32. Brain Trauma Foundation. The use of mannitol severe head injury. J Neurotrauma 1996;13(11):705-9.
33. Stover JF, Morganti-Kosmann MC, Lenzlinger PM, Stocker R, Kempski OS, Kossmann T. Glutamate and taurine are increased in ventricular cerebrospinal fluid of severely brain-injured patients. J Neurotrauma. 1999;16:135-142.
34. Muir KW. Less KR. Clinical experience with excitatory amino acid antagonist drugs. Stroke.1995;26:503-513.
35. Koura SS, Doppenberg EM, Marmarou A, Choi S, Young HF, Bullock R. Relationship between excitatory amino acid release and outcome after severe human head injury. Acta Neurochir Suppl. 1998;71:244-6.
36. Ustün ME, Gürbilek M, Ak A, Vatansev H, Duman A. Effects of magnesium sulfate on tissue lactate and malondialdehyde levels in experimental head trauma. Intensive Care Med. 2001;27:264-68.
37. Katayama Y, Fukuchi T, Mc Kee A, Terashi A. Effect of hyperglisemia on pyruvate dehyrogenase activity and energy metabolites during ischemia and reperfusion in geril brain. Brain Res. 1998;788:302-304.
38. Damas, Hans P. Management of severely head injured patients during the first 24 hours. Which spesific therapeutics? Ann Fr Anesth Reanim. 2000;19(4):326-32.
39. Bereczki D, Liu M, do Prado GF, Fekete I. Mannitol for acute stroke. Cochrane Database Syst Rev 2001;(1):CD001153.