Effects of Methylprednisolone on Serum Neuron-Specific Enolase Levels After Global Ischemic Brain Damage in Rats

Ratlarda Global İskemik Beyin Harabiyetinde Metilprednizolonun Serum Neuron-Spesifik Enolase Düzeylerine Etkisi

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Received : 30.11.2000 ⇔ Accepted : 11.1.2001

Abstract: Neuron-specific enolase (NSE) is a mitochondrial enzyme that is found at high levels in neurons. Changes in NSE levels may provide quantitative information about the extent of certain types of neurological injury, including cerebral infarction, subarachnoid hemorrhage, intracerebral hemorrhage, and head trauma. This study investigated serum levels of NSE by enzyme immunoassay after global cerebral ischemia in rats, and evaluated the effects of low-dose methylprednisolone on serum NSE concentration. The results confirmed that serum NSE is a sensitive marker of cerebral ischemia, and revealed a positive correlation between NSE levels and low-dose methylprednisolone treatment.

Key Words: Cerebral ischemia, methylprednisolone, neuron-specific enolase, rat

Özet: Nöron-spesifik enolaz (NSE) nöronlarda yüksek oranda bulunan mitokondrial bir enzim olup, serebral infarkt, subaraknoid kanama, intraserebral kanama ve kafa travmasında nörolojik yaralanmanın derecesi hakkında önemli bilgiler verdiği bilinmektedir. Bu çalışmada, ratlarda global serebral iskemide serum NSE düzeyleri enzim immunoessey metodu ile çalışılmış, düşük doz metilprednizolonun enzim serum konsantrasyonlarına etkisinin araştırılması amaçlanmıştır. Elde edilen sonuçlar serum NSE sensitivitesinin serebral iskemide önemli bir gösterge olduğunu ve düşük doz metilprednizolon tedavisi ile serum enzim düzeyleri arasında anlamlı bir ilişki olduğunu belirgin olarak göstermiştir.

Anahtar Kelimeler: Metilprednizolon, nöron-spesifik enolaz, rat, serebral iskemi

INTRODUCTION

Biological markers for the central nervous system (CNS) can be used to improve diagnostic accuracy and assess the effects of treatment. An early marker of neuronal damage would be valuable for gauging the timing and extent of cerebral injury, and would assist with prognostication. The diagnosis of CNS ischemia is seldom in doubt; thus, the main interest in neurological injury markers relates to therapeutic guidance. A marker may be specific, indicating the severity of a particular aspect of the disease, or it may be general, indicating the patient's potential survival time. In addition to this

information, it would be valuable to be able to quantify the severity of the injury, and to then correlate severity with outcome or responses to different therapies.

Clearly, a reliable biochemical index of neurological injury would be extremely useful. The ideal marker would be uniformly present and specific to neurological tissue. Uniform presence of the marker substance ensures that the amount of tissue damage is reflected independent of the location of injury. The distribution of the marker should be relatively uniform in order to indicate the amount of tissue damaged, but, in order to assist with prognosis, ideally there should be slightly higher concentrations in areas vital to survival. In addition to the importance of distribution, the marker must be specific to the CNS. Another characteristic of the ideal indicator of CNS damage is that it should be released only upon cell death, and not in reversible injury. Although theoretically desirable, this qualification is most difficult to achieve. In order to be readily detectable, a marker enzyme must be soluble; however, to avoid reflecting minor fluctuations in membrane permeability, it must not leak during physiological or pharmacological alterations. In summary, the ideal serum marker of neurological injury should have high specificity for the brain and high sensitivity for cerebral tissue. Also, it should be measurable in the serum immediately after injury, and should be released in a time-dependent manner in accord with damage. An early prognostic indicator is needed in order to select appropriate therapies and quantify their effects.

It has recently been shown that brain tissue contains a specific form of enolase that is structurally, immunologically, and functionally distinct from the enolase present in other tissues. This isoenzyme, designated neuron-specific enolase (NSE), is located in differentiated neurons (1,2,13,23). In this study, we investigated serum levels of NSE after induction of global cerebral ischemia in rats. We also evaluated the effects of methylprednisolone treatment on the serum concentrations of this enzyme. The broader purpose was to determine whether changes in enzyme levels during the destructive process of cerebral ischemia reflect the therapeutic efficiency of medical interventions.

MATERIALS AND METHODS

Twenty-one adult male Sprague-Dawley rats (250-350 g) were used in the study. Food was withheld for 12-16 hours prior to the experiment, but the animals were allowed free access to water. The rats were randomly assigned to three groups: Group 1 (n=7) controls were sham-operated and treated with physiological saline solution; Group 2 rats (n=7) sustained ischemia-reperfusion and were treated with physiological saline; and Group 3 (n=7) rats sustained ischemia-reperfusion and were treated with methylprednisolone (Mustafa Nevzat Laboratories, Istanbul, Turkey).

Global cerebral ischemia was produced using a four-vessel occlusion method (22). The rats were anesthetized with intraperitoneal ketamine (90 mg/kg) and xylazine (10 mg/kg). Using a posterior cervical approach, an incision was made behind the occipital bone directly over the first two cervical vertebrae. The paraspinal muscles were dissected and retracted from the midline, and both vertebral arteries were electrocauterized and severed.

Twenty-four hours later, the rats were anesthetized with intraperitoneal urethane (1.2 g/kg). Thirty minutes prior to this, we administered 2-ml physiological saline solution (Group 2) and 8-mg/kg methylprednisolone (Group 3) intraperitoneally. Then the animals were placed in supine position and the common carotid arteries were isolated through a ventral midline cervical incision. The exposed common carotid arteries were occluded bilaterally for 30 minutes using miniature aneurysm clips. After this period, recirculation was achieved by releasing the clips, and we visually confirmed spontaneous reperfusion under the microscope. Sixty minutes after recirculation, a small amount of blood was obtained from the tail artery of each rat, and the samples were frozen and kept at -20 ºC until they were analyzed. The rats were all sacrificed with an intraperitoneal injection of 100-mg/kg thiopental. The control animals (Group 1) underwent all the surgical procedures described except for the vessel occlusions.

Serum NSE concentrations were measured by radioimmunassay, as described previously (11,20). All determinations were performed under blind coding. The data were analyzed on a personal computer using commercial software (Sytat for Windows, version 6.0, SPSS), and the differences within each group were statistically tested using analysis of variance (ANOVA). The groups' mean enzyme concentrations were compared using the Mann Whitney U-test. All results were expressed as mean ± standard deviation (SD), and p values <0.05 were considered significant.

RESULTS

The NSE values for each group (µg/L) are shown in Table 1. In the sham-operated saline-treated group (Group 1), the maximum and minimum NSE values were 1.6 µg/L and 1.1 µg/L, respectively. In the ischemia-reperfusion and saline-treated rats (Group 2), these values were considerably higher, at 4.4 µg/L and 2.2 µg/L, respectively. In the ischemia-reperfusion methylprednisolone-treated rats (Group 3), the maximum and minimum concentrations (2.0 µg/L and 1.0 µg/L, respectively were slightly lower than the control values, but the difference was not significant. The respective means for the three groups were 1.31±0.18 µg/L, 3.26±0.89 µg/L, and 1.67±0.35 µg/L (Table 2). The differences among the group medians were highly significant ($p<0.0005$), and this comparison is illustrated in Figure 1.

Histological Evaluation

The rats' brains were removed and then post-fixed in 4% formaldehyde overnight. After 12 hours, we cut 3 mm-thick coronal slices and embedded these in paraffin. The embedded tissues were then cut in 8-µm sections and stained with hematoxylin and eosin, and S-100. All slides were examined under the light microscope (Olympus, BH-2, Olympus, Japan) by one examiner who was blinded to the treatment condition. Standardized sections of cerebral tissue were qualitatively evaluated to assess edema formation. In the ischemia-reperfusion saline-treated (Figure 2) and methylprednisolone-treated (Figure 3) groups, neuronal histopathology was evaluated in the coronal sections at the level of the caudate nucleus and hippocampus.

Table 1: The serum NSE values for all rats in each study group as measured by radioimmunassay (µg/L). (Group 1: sham-operated and saline-treated; Group 2: ischemia-reperfusion and saline-treated; Group 3: ischemia-reperfusion and methylprednisolone-treated)

n	Group 1	Group 2	Group 3
1	1.2	4.4	1.0
2	1.1	3.2	1.9
3	1.5	2.2	1.6
4	1.6	2.5	1.5
5	1.3	3.8	2.0
6	1.2	2.5	1.9
7	1.3	4.2	1.8

Table 2: The mean ± SD for the NSE concentrations in each of the three study groups. The differences among the means were all highly significant (Mann Whitney U-test, $p<0.0005$).

Group		mean ± SD
1	Sham operation, saline treated	1.31±0.18
2	Ischemia-reperfusion, saline treated	3.26±0.89
3	Ischemia-reperfusion, methylprednisolone treated	1.67±0.35

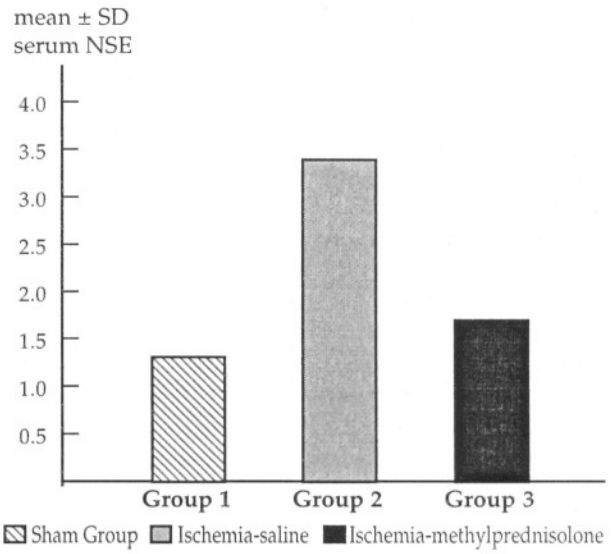

Figure 1: The mean serum NSE values for each study group presented in bar graph format. The median enzyme concentration in the methylprednisolone-treated rats (Group 3) was significantly lower than that in the saline-treated animals (Group 2).

DISCUSSION

Enolase is a potentially useful glycolytic and mitochondrial enzyme that is involved in energy-yielding metabolism. It is a dimeric cytoplasmic enzyme composed of three immunologically distinct subunits (a, b, and g) that give rise to five isoenzymes (aa, bb, gg, ab, and ag) (17). These forms have differential cellular distributions, and of particular interest are the isoenzyme patterns in the nervous system. Isoenzyme gg is confined to the neurons, and is called neuron-specific enolase, whereas aa-enolase has been found in astrocytes, ependymal cells, endothelial cells, and Schwann cells (24).

NSE is released into both the cerebrospinal fluid and serum after CNS damage. This enzyme is a very

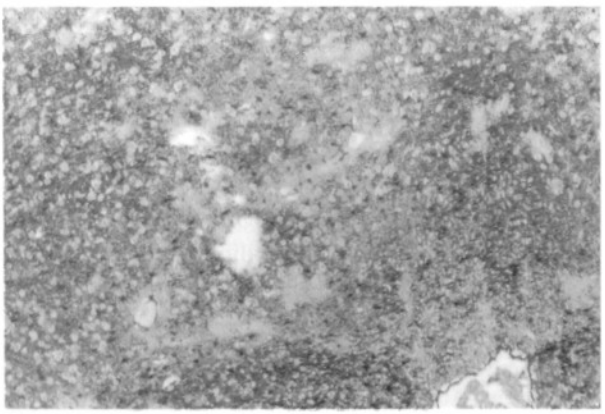

Figure 2: An S-100-stained brain section from the ischemia-reperfusion saline-treated group shows high-density staining. (x100)

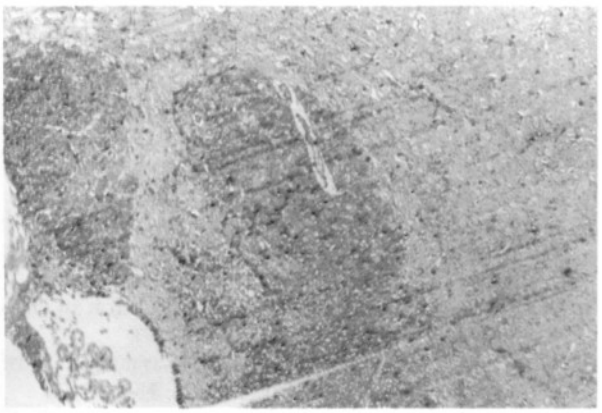

Figure 3: An S-100-stained brain section from the ischemia-reperfusion methylprednisolone-treated group shows low-density staining. (x40)

sensitive marker for many types of neurological injury, including cerebral ischemia, subarachnoid hemorrhage, intracerebral hemorrhage, and head injury. It is well established that neurons are the CNS cells that are most sensitive to ischemia, and studies of patients with neurological lesions have demonstrated a relationship between the degree of cell damage in the CNS and the concentration of NSE (1,6,17,26). Thus, it appears that NSE may be a marker of CNS damage. Research has also shown elevated NSE levels in patients with mild cerebral ischemia, including those who have suffered transient ischemic attacks and minor strokes that have not been visualized radiologically (16,21).

Cerebral ischemia followed by reperfusion results in a host of biochemical events that aggravate the initial damage and cause threshold-dependent injury. The flow rates in the areas of primary necrosis are known to be below the rate required to maintain energy metabolism (12). One important molecular cascade involves an intracellular accumulation of calcium followed by activation of cell membrane phospholipases. These enzymes degrade membrane phospholipids and release arachidonic acid. Arachidonate is a precursor of vasoconstrictor substances, the thromboxanes and prostaglandins. The synthesis of these substances produces free radicals, which are unwanted byproducts. These molecules not only damage CNS enzymes, but also cause further injury to cell membranes through lipid peroxidation. In conjunction with secondary ischemia, lactate is formed and the resultant acidosis exacerbates CNS damage. A variety of cellular functions are disturbed as a result of these conditions. These processes include transmitter mechanisms, energy metabolism, enzymatic mechanisms, and Na+/K+ ionic gradient maintenance. Phospholipid breakdown induces membrane instability as well as injury to the blood-brain barrier and lysosomes. This breakdown leads to capillary leakage and lysozyme release, increasing the damage already associated with secondary ischemia (12,19).

A major goal of this research was to assess whether methylprednisolone is capable of preventing the consequences of secondary ischemia. We determined the serum concentrations of NSE associated with cerebral ischemia, and then assessed whether the drug attenuated the expected rise in NSE. Methylprednisolone is known to help stabilize cell membranes and prevent edema, inhibit the activation of phospholipases, and scavenge free radicals. Studies of several indicators of ischemia-induced damage to cerebral tissue have demonstrated that this agent provides therapeutic benefits (5,8,918,27,29).

To our knowledge, the present study is the first in the literature to have evaluated the effects of methylprednisolone based on serum NSE levels after cerebral ischemia. One earlier investigation found that minimum doses of glucocorticoids did not affect peroxidative metabolism (9); however, another study showed that low and megadoses of glucocorticoids inhibited transcription of the glucose transporter gene, and directly inhibited glucose transport by moving glucose transporter molecules from the plasma membrane to intracellular sites (28). The finding of no effect is consistent with reports that have documented a lack of glucocorticoid-mediated

improvement in several types of neurological insult (4,5,7,25). However, it disputes other reports of positive glucocorticoid effects after ischemia and CNS injury (9,15,29).

Administering megadoses of methylprednisolone is known to provide neuroprotective advantages in patients with spinal cord injuries (3,14). Beneficial effects of megadoses of this drug have also been documented after CNS trauma in several animal models (9,10). It has been hypothesized that this protective effect reflects the ability of extremely high doses of methylprednisolone (30 mg/kg) to inhibit lipid peroxidation induced by oxygen free radicals, as has been demonstrated in rats (8,14,15,18). In the present study, we found that even low-dose methylprednisolone (8 mg/kg) had significant effects on serum NSE levels in rats. The mean NSE concentrations in Groups 1 through 3 were 1.31±0.18 µg/L, 3.26±0.89 µg/L, and 1.67±0.35 µg/L, respectively, and the differences among the means were all highly significant ($p<0.0005$). These results confirm that serum NSE is a sensitive marker of cerebral ischemia, and indicate that low-dose methylprednisolone does attenuate NSE levels after CNS injury.

In conclusion, these results suggest that investigation of NSE levels during the cerebral ischemic process may be useful for assessing the therapeutic efficacy of medical interventions. Our observations are of potential clinical relevance to various aspects of neurosurgical practice. Pretreatment with glucocorticoids for expected ischemia during temporary occlusion, or for expected intraparenchymal trauma during intracranial or spinal surgery, may prevent some of the deleterious effects of neuronal ischemia. NSE is a reliable biochemical index of neurological injury. Our findings suggest that it may be of significant clinical value as a predictor of outcome, and as a means of evaluating patient management and therapeutic interventions. Future studies of this isoenzyme may show that it is a very important marker for the pathophysiological process in nervous tissue damage.

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REFERENCES

1. Bakay RAE, Ward AA: Enzymatic changes in serum and cerebrospinal fluid in neurological injury. J Neurosurg 58: 27-37, 1983
2. Bakay RAE, Sweeney KM, Wood JH: Pathophysiology of cerebrospinal fluid in head injury: Part 2. Neurosurgery 18: 376-382, 1986
3. Bracken MB, Shepard MJ, Collins WF Jr, Holford TR, Young W, Baskin DS, Eisenberg HM, Flamm E, Leo-Summers L, Maroon J: A randomized, controlled trial of methylprednisolone or naloxone in the treatment of acute spinal cord injury. Results of the Second National Acute Spinal Cord Injury Study. N Engl J Med 322: 1405-1411, 1990
4. De Reuck J, Vandekerckhove T, Bosma G, De Meulemeester K, Van Landegem W, De Waele J, Tack E, De Konick J: Steroid treatment in acute ischemic stroke. Eur Neurol 28: 70-72, 1988
5. De Reuck J, De Bleecker J, Reyntjens K: Steroid treatment in primary intracerebral haemorrhage. Acta Neurol Belg 89: 7-11, 1989
6. Ergün R, Bostanci U, Akdemir G, Be?konakli E, Kaptano?lu E, Gürsoy F, Ta?kin Y: Prognostic value of serum neuron-specific enolase levels after head injury. Neurol Res 20: 418-420, 1998
7. Francis TJ, Dutka AJ: Methylprednisolone in the treatment of acute spinal cord decompression sickness. Undersea Biomedical Res 16: 165-174, 1989
8. Hall ED: Inhibition of lipid peroxidation in CNS Trauma. J Neurotrauma 8: 831-840, 1991
9. Hall ED: The neuroprotective pharmacology of methylprednisolone. J Neurosurg 76: 13-22, 1992
10. Hall ED, Braughler JM, Mc Call JM: Antioxidant effects in the treatment of spinal cord injury. J Neurotrauma 9: 165-172, 1992
11. Hardemark HG, Persson L, Bolander HG: Neuron-specific enolase is a marker of infarction development and size in a focal ischemia model. Stroke 19: 1140-1144, 1988
12. Hata R, Maeda K, Hermann D, Mies G, Hossmann KA: Evolution of brain infarction after transient focal cerebral ischemia in mice. J Cereb Blood Flow Metab 20: 937-946, 2000
13. Hay E, Royds JA, Davies-Jones GAB, Lewtas NA, Timperley WR, Taylor CB: Cerebrospinal fluid enolase in stroke. J Neurol Neurosurg Psychi 47: 724-729, 1984
14. Hilton G, Frei J: High-dose methylprednisolone in the treatment of spinal cord injuries. Heart Lung 20: 675-680, 1991
15. Kalayci O, Cataltepe S, Cataltepe O: The effect of bolus methylprednisolone in prevention of edema in hypoxic ischemic brain injury: An experimental study in 7-day-old rat pups. Brain Res 569:112-116, 1992
16. Kawasaki H, Wakayama Y, Okayasu H, Takahashi H, Shibuya S: Levels of serum and cerebrospinal fluid enolase in patients with cerebral vascular disease and other neurological diseases. Jpn J Stroke 10: 313-318, 1988

17. Mabe H, Suzuki S, Mase M, Umemura A, Nagai H: Serum neuron-specific enolase levels after subarachnoid haemorrhage. Surg Neurol 36: 170-174, 1991
18. Marzatico F, Gaetini P, Buratti E, Messini AL, Ferlenga P, Rodriguez y Baena R: Effects of high-dose methylprednisolone on Na+-K+-ATPase and lipid peroxidation after experimental subarachnoid haemorrhage. Acta Neurol Scand 82: 263-270, 1990
19. Palmer GC, Palmer SJ, Christie-Pope BC, Callahan AS, Taylor MD, Eddy LJ: Classification of ischemic-induced damage to Na+-K+-ATPase in gerbil forebrain. Modification by therapeutic agents. Neuropharmacol 24: 509-516, 1985
20. Paus E, Nustad K: Immunoradiometric assay for a and g-enolase (neuron-specific enolase), with use of monoclonal antibodies and magnetizable polymer particles. Clin Chem 35: 2034-2038, 1989
21. Persson L, Hardemark HG, Gustafsson J, Rundström G, Mendel-Hartvig I, Esscher T, Pahlman S: S-100 protein and neuron-specific enolase in cerebrospinal fluid and serum: Markers of cell damage in human central nervous system. Stroke 18: 911-918, 1987
22. Pulsinelli WA, Brierley JB: A new model of bilateral hemispheric ischemia in the unanesthetized rat. Stroke 10: 267-271, 1979
23. Royds JA, Timperley WR, Taylor CB: Levels of enolase and other enzymes in the cerebrospinal fluid as indices of pathological change. J Neurol Neurosurg Psychi 44: 1129-1135, 1981
24. Royds JA, Davies-Jones GAB, Lewtas NA, Timperley WR, Taylor CB: Enolase isoenzymes in the cerebrospinal fluid of patients with disease of the nervous system. J Neurol Neurosurg Psychi 46: 1031-1036, 1983
25. Shapira Y, Artru AA, Yadid G, Shohami E: Methylprednisolone does not decrease eicosanoid concentrations or edema in brain tissue or improve neurologic outcome after head trauma. Anesth Analg 75: 238-244, 1992
26. Skogseid IM, Nordby HK, Urdal P, Paus E, Lilleaas F: Increased serum creatine kinase BB and neuron-specific enolase following head injury indicates brain damage. Acta Neurochir (Wien) 115: 106-111, 1992
27. Uhler TA, Frim DM, Pakzaban P, Isacson O: The effects of megadose methylprednisolone and U-78517F on toxicity mediated by glutamate receptors in the rat neostriatum. Neurosurgery 34: 122-128, 1994
28. Virgin CE Jr, Taryn PH, Packan DR, Tombaugh GC, Yang SH, Horner HC, Sapolsky RM: Glucocorticoids inhibit glucose transport and glutamate uptake in hippocampal astrocytes: Implications for glucocorticoid neurotoxicity. J Neurochem 57: 1422-1428, 1991
29. Zarem HA, Hayden B, Saderberg R, Ringham LM, Gabriel K: Effect of corticosteroid administration in ischemia: ischemic injury. Plast Reconstr Surg 82: 865-871, 1988