SERUM NEURON SPECIFIC ENOLASE LEVELS IN EXPERIMENTAL GLOBAL CEREBRAL ISCHEMIA*

Meral FADILOĞLU*, Necati GÖKMEN**, Hilal (ERSÖZ) KOÇDOR*, Yüksel ERKİN**, Semih KÜÇÜKGÜÇLÜ**, Atalay ARKAN**, M.Ali KOÇDOR***

Dokuz Eylül University, Faculty of Medicine, Department of Biochemistry*
Dokuz Eylül University, Faculty of Medicine, Department of Anesthesia**
Dokuz Eylül University, Faculty of Medicine, Department of Surgery***

SUMMARY

The purpose of the present experimental study in rabbits, was to investigate the correlation between global cerebral ischemia and serum Neuron Specific Enolase (NSE) levels and the effects of magnesium sulfate infusion (MgSO4) on serum NSE levels.

We used twelve male New Zealand rabbits divided randomly into two groups. Hypotensive cerebral ischemia model was performed by clipping common carotid arteries for one hour under propofol anesthesia. One hour later, clips were opened. The six rabbits (first group) were perfused only with sodium chloride 0.9% and, the other six (second group) were perfused with magnesium sulfate solution 2.5 mmol/kg/h for one hour. Blood samples were obtained for measuring serum NSE and Mg levels before cerebral ischemia, after ischemia and after reperfusion periods. Serum NSE levels were measured using enzyme immunoassay (Cobas Core NSE EIA ®Roche Kit). Serum magnesium levels were determined using spectrophotometric Menarini Cod Kit. Statistical analysis were performed using Mann Whitney U test. We obtained statistically significant increases in serum neuron specific enolase concentrations after global cerebral ischemia ($p<0,05$). Magnesium sulfate infusion has increased serum magnesium levels($p<0,05$) but not changed serum NSE levels. In conclusion, in addition to the cerebrospinal fluid, serum neuron specific enolase estimation may have a predictive value in the evaluation of the cerebral infarction and a prognostic parameter during post ischemic course. MgSO4 infussion doesn't have any effect on serum NSE levels.

This study has been performed in the research laboratories of Dokuz Eylül University Medical Faculty.

Key words: Global Cerebral Ischemia, Serum Neuron Specific Enolase, Magnesium, Experimental Model.

ÖZET

Tavşanlar üzerinde gerçekleştirilen bu deneysel çalışma Fakültemiz araştırma laboratuvarlarında gerçekleştirilmiştir. Çalışmanın amacı, oluşturulan global serebral iskemi ile serum nöron spesifik enoaz (NSE) değerleri arasindaki ilişkiyi ve magnezyum sülfat infüzyonunun serum NSE düzeyine olan etkisini incelemek olmuştur.

12 erkek Yeni Zelanda tavşanı rastgele iki gruba ayrılarak çalışıldı. Propofol aneztezisi altında hayvanlara bir saat süren arteria karotis kommünis klipi uygulanarak serebral akım durduruldu. Klipler açıldıktan sonra birinci gruptaki toplam 6 tavşana %0.9 sodyum klorür infüzyonu; ikinci gruptaki 6 tavşana ise bir saat süreli magnezyum sülfat (2,5 mmol/kg/h) infüzyonu yapıldı. Serebral iskemi oluşturulmadan önce, oluşturulduktan bir saat sonra ve bir saatlik reperfüzyon döneminden sonra kan örnekleri alındı. Serum NSE değerleri enzim immunoassay (Cobas Core NSE EIA Roche kit) kullanılarak, serum magnezyum değerleri ise spektrofotometrik yöntemle (Menarini Cod kit) ölçüldü. Veriler Mann Whitney U testi kullanılarak değerlendirildi. Sonuçta serebral iskeminin birinci saati sonunda NSE değerlerinde anlamli artış olduğu ($p<0,05$), magnezyum sülfat infüzyonunun ise serum NSE değerlerini etkilemediği saptandı.

Bu verilerle serebral iskemide oluşacak serebral enfarktusun değerlendirmesinde beyin omurilik sıvısının yaninda, serum NSE ölçümünün de, serebral iskeminin tanınmasında ve iskemi sonrası dönemde, yol gösterici bir parametre olabileceği, MgSO4 infüzyonunun serum NSE düzeylerini etkilemediği kanatina varıldı.

Bu çalışma Dokuz Eylül Üniversitesi Tıp Fakültesi araştırma laboratuvarında gerçekleştirilmiştir.

Anahtar sözcükler: Global serebral iskemi, serum nöron spesifik enoaz, magnezyum, deneysel model.

Neuron specific enolase (NSE), a dimeric glycolytic enzyme, originate predominantly from cytoplasm of neurons and neuroendocrine cells. It is structurally and immunologically distinct from non-neuronal enolase. NSE is stabl in biological fluids and is a major neuronal protein which constitutes a significant

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percentage (about 1,5%) of total soluble brain proteins (1). In neuronal injury, changes in membrane integrity may cause leakage of proteins such as NSE from cytosol into the extra cellular space. Indeed, increased NSE in cerebrospinal fluid (CSF) is a good predictor of brain damage in experimental cerebral ischemia . Increased NSE levels in the CSF have also been reported following a variety of nervous system diseases such as cardiac arrest, head injury, ischemic stroke, hemorrhagic stroke, multiple sclerosis and brain neoplasm, West syndrome, Coronary artery syndrome, vascular dementia (2-13). In man, the level of CSF - NSE has been positively correlated with the degree of cerebral tissue damage, neurological outcome and mortality (14-17).

While multiple reports have shown the presence of elevation in the CSF NSE concentrations following nervous tissue damage, there are very few studies concerning blood NSE changes related with experimental global cerebral ischemic damage and the effects of magnesium sulfate (MgSO4) on it. The purpose of the present experimental study in rabbits was to investigate the correlation between global cerebral ischemia and serum NSE levels, and the effects of MgSO4 infusion on serum NSE levels.

MATERIALS AND METHODS

We used 12 male New Zealand rabbits weighted 1900-2400g, randomly grouped in two. Before the surgical procedure they were infused with NaCl O,9 %, 2ml/ kg/ hour. Tracheotomy and mechanical ventilation were performed under profobol anesthesia. Both Common Carotid Arteries were clipped with 721 FE Yasargil's microvascular clip and haemoragic hypotension was performed by drawing blood on CPDA sol. (Citrate, Phosphate, Dextrose, Adenosine) until the blood pressure decreased 25-30% of total. After an ischemic period for one hour, the clips were opened. Then immediately six of the rabbits (first group) were perfused with their own blood taken into CPDA sol. and NaCl 0,9 %. The other six (second group) were also perfused with their own blood plus MgS04 infusion, 2.5mmol/kg/h for one hour. Blood samples were collected before ischemia, after ischemia and after reperfusion periods. Serum samples were stored at -70°C until analysis. Since red blood cells contained considerable amount of NSE, serum samples with any haemolysis had not been studied (18). Serum NSE levels were determined using enzymoimmunoassay (EIA) kits (Cobas core NSE EIA Roche kit) and the EIA incubator, EIA washer, EIA photometer. This solid phase EIA, based on the sandwich technique, was described in detail by Jacobi and oneReiber (19). The only difference was the reduction of the incubation time to 15 minutes using the EIA incubator and tetramethylbenzidine as the substrate solution with a final reading at 450 nm.

Serum magnesium levels were determined using spectrophotometric Menarini (R) Cod Kit. Statistical analysis were performed by using Data Sigma Stat version 1.02 Statistical Analysis System 1992 and Mann Whitney U test.

This study has been performed in the multidisciplinary research laboratories of Dokuz Eylül University Medical Faculty.

RESULTS

Serum NSE and Mg levels obtained are shown in Table I, Figure 1 and Table II, Figure 2 respectively. The following results were obtained :

1. For the first group serum NSE concentrations obtained were (36,33±6,39) before ischemia, (87,66 ± 18,9) after one hour ischemia and (76,33±13,5) after reperfusion with NaCl. For the second group serum-NSE concentrations were (47,16±13,34) before ischemia, (96,00±20,63) after one hour ischemia, (79,83±16,91) after reperfusion withMgSO4 sol. Serum-NSE concentrations were found significantly increased after one hour ischemia and after reperfusion either with NaCl or with MgSO4 ($p<0,05$) Table I, Figure 1.

2. For the first group serum-Mg values were (4,52± 0,66) before ischemia, (5,40 ±0,53) after one hour ischemia, (5,46± 0,82) after reperfusion. For the second group serum-Mg values were(3,44± 0,21) before ischemia, (5,08± 0,63) after one hour ischemia, (8,10±0,44) after reperfusion. Serum-Mg values were increased significantly after reperfusion with MgSO4 in the second group ($p<0,05$) Table II, Figure 2.

Table I. Serum-NSE concantrations (ng/ml) of two groups were significantly increased after ischemia and reperfusion ($P<0.05$) compared to preischemia

	Group1	Group 2
Before ischemia	36.33 ± 6.39	47.16 ±13.34
After ischemia	87.66 ± 18.91*	96.00 ± 20.63*
After reperfusion	76.33 ±13.51*	79.83 ±16.91*

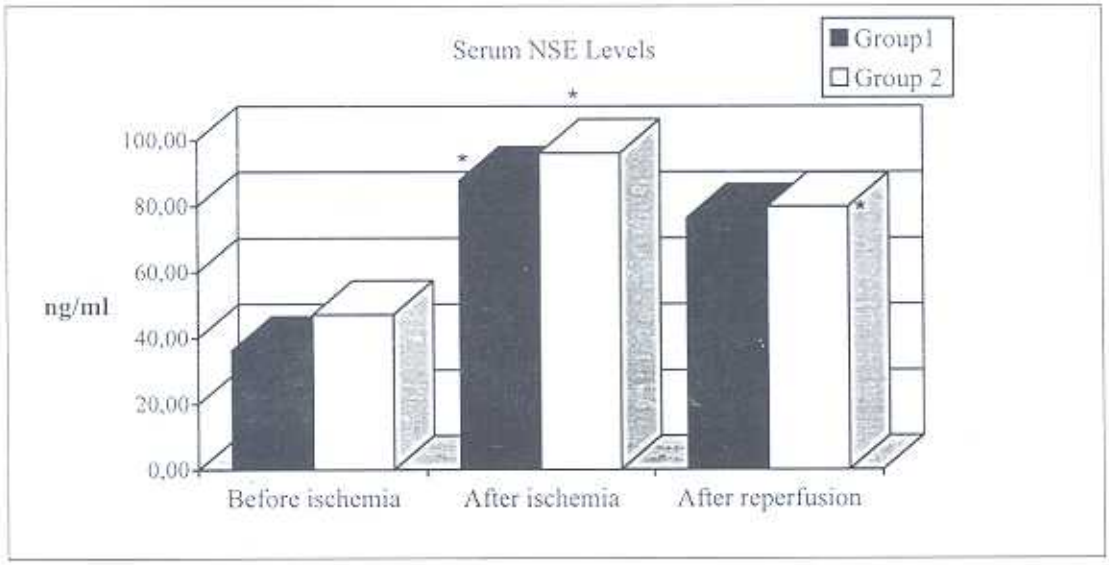

Figure 1. Serum NSE alterations after ischemia, and after reperfusion compared to pre-ischemia were significant ($p<0.05$)

Table II. Serum Mg mean levels (mg/dl) increased significantly after MgSO4 reperfusion in group 2 ($p<0.05$) compared to preischemia

	Group 1	Group 2
Before ischemia	4.52 ± 0.66	3.44 ± 0.21
After ischemia	5.40 ± 0.53	5.08 ± 0.63*
After reperfusion	5.46 ± 0.82	8.10 ± 0.44*

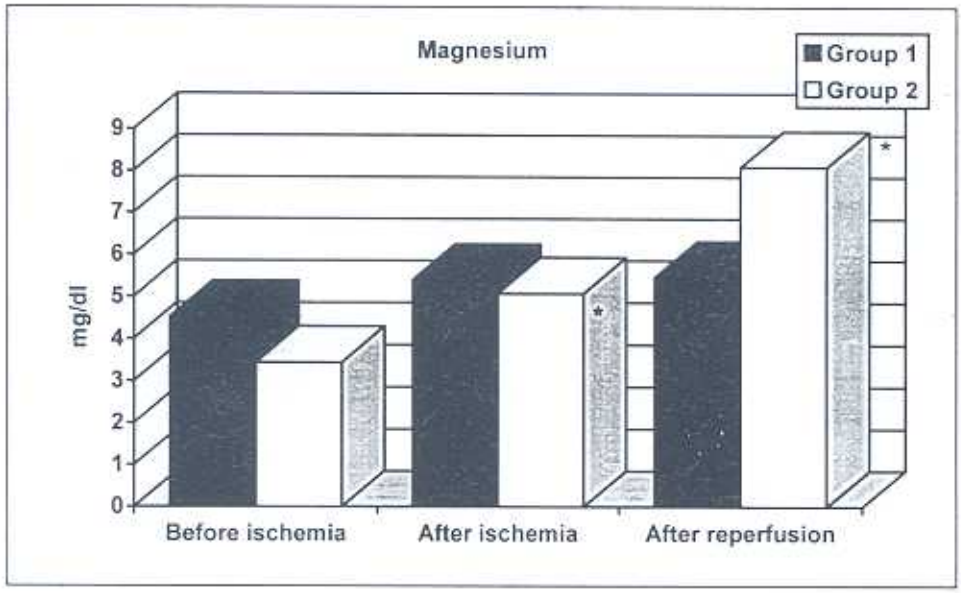

Figure 2. Serum Mg. Alterations after ischemia and after reperfusion compared to pre-ischemia were

DISCUSSION

Although many modern imaging techniques such as CT, MRI and positron emission tomography yield important information concerning the status of the brain ischemic insult, they cannot distinguish irreversibly damaged tissue from reversible changes (2-4,19). The analysis of nervous tissue specific, cell-damaged related proteins, such as NSE, may contribute to the actual structural brain damage (9,13,20). Indeed, in experimental and clinical studies a correlation between NSE concentrations in the CSF and brain has been shown by various investigators (1,5,10-12,16,19-24). But temporal profile of NSE in the CSF and brain during ischemic process remains incompletely understood.

Baron et al. in their experimental study have suggested that NSE was leaking from damaged ischemic neuron into the systemic circulation and that it could be detected in the peripheral circulation (19). Given the difficulties with CSF sampling, measurement of plasma NSE may be valuable (2-4,19) and gives implications for the treatment and prognostic parameter during post-ischemic course (25,28). In our experimental model we produced complete global cerebral infarction and studied blood NSE levels at the end of the first hour of infarction and after reperfusion (Table 1, Fig.1). Some experimental studies have shown that neuronal damage in ischemic process is dynamic, beginning at 2 h after infarction and continuing to develop up to several days (2-4,19). Under certain pathological conditions, such as ischemia, protein may pass the blood brain barrier (BBB). In cerebral ischemia induced by occlusion of common carotid arteries, it was found that the permeability of BBB changes occur immediately after ischemic insult. In the initial stage of ischemia, micropinocytosis take place in the BBB and later it appears to be passive leakage through necrotic vessel walls. In the present study we found significantly increased serum NSE levels($p<0,05$) after one hour global cerebral ischemia (Table I, Figure 1) and reperfusion. It appears that, in our case, one hour common carotids ligation resulted with complete cerebral infarction and leakage of NSE to the circulation through damaged nervous and vascular tissues. However, NSE has also been demonstrated in the platelets although 30 folds less than that

present in the brain tissue and also influenced from haemolysis (15,29). Althought we did not use haemolysed in this study, it is possible that platelet alterations following cerebral complete infarction might also contributed to the serum NSE increases in our cases.

Slight decreases in serum NSE levels measured after reperfusion in the present study could be due to the possible degradations of NSE by macrophage and/or proteinase locally and hemodilution produced by auto-transfusion plus MgSO4 infusion.

Experimental studies have shown that inorganic magnesium ions may prevent anoxic damage (17,24). Post ischemic injury in CA1 neuron of the rats is prevented by magnesium (17). But in the present study we did not find any difference in serum NSE levels in animals which were infused with MgSO4 after ischemia. It appears that MgSO4 did not have any effect on serum NSE levels. Figure 2, Table II.

In conclusion, it appears that in complete global experimental cerebral infarction serum NSE level increases as early as one hour after infarction. Serum NSE estimation may have some predictive value in the developing ischemic injuries and prognostic parameter during post-ischemic course. Mg SO4 infusion doesn't have any effect on serum NSE levels.

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