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Neuroprotective effects of preischemia subcutaneous magnesium sulfate in transient cerebral ischemia[1]

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Abstract

Objective: Neurological injury due to transient cerebral ischemia is a potential complication of cardiovascular surgery. The neuroprotective effect of magnesium, when given subcutaneously before the ischemia, was assessed in a rat model of transient global cerebral ischemia. **Methods**: Thirty-six male Wistar albino rats were included to this randomized, controlled, prospective study. In 24 animals, ischemia was induced with four-vessel occlusion technique with the duration of 15 min. $MgSO_4$ was given 600 mg/kg subcutaneously 48 h before the procedure in group 1 ($n = 12$). Similar volume of saline solution was used in animals of control group (group 2, $n = 12$). The animals in group 3 (sham group, $n = 12$) were anesthetized and subjected to operative dissections without vascular occlusion. Physiological parameters and somatosensory evoked-potentials (SEP) were monitored in animals before ischemia, during ischemia and in the first 30 min of reperfusion. Their neurological outcome had been clinically evaluated and scored up to 4 days postischemia. The intergroup differences were compared. Then the animals were sacrificed and their brains were processed for histopathological examination. **Results**: In group 3, SEP amplitudes did not change during the procedures, and all animals recovered without neurologic deficits. At the end of ischemic period, the average amplitude was reduced to 5 ± 3% of the baseline in all ischemic animals. This was followed by a gradual return to 87 ± 10% and 83 ± 8% of the initial amplitude after 30 min of reperfusion in group 1 and group 2, respectively ($P > 0.05$). The average neurological score was significantly higher in group 1 than in group 2 at 48, 72 and 96 h after the ischemic insult ($P < 0.05$). Histological observations were clearly correlated with the neurological findings. **Conclusion**: The results suggest that subcutaneous $MgSO_4$ reduces cerebral injury and preserves neurologic function when given two days before the transient global ischemia in rats.

Keywords: Magnesium; Cerebral ischemia; Cerebral protection; Somatosensory evoked potentials; Rat

1. Introduction

Permanent neurological injury due to transient cerebral ischemia is a potential complication of cardiac surgery that may occur during deliberate hypotension, with the temporary clamping of cerebral vessels or total circulatory arrest. The reported prevalence of neurologic injury for these operations ranges up to 25% [1,2]. A reliable pharmacological regimen designed to reduce or eliminate ischemic injury when given before the procedure could be of great benefit in cardiovascular surgery.

In the pathogenesis of cerebral ischemic injury, release of excessive amounts of excitatory amino acids (EAA) from terminals of ischemic neurons into the extracellular space

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are thought to be important [3,4]. Two possible mechanisms for the induction of cell death by EAA have been suggested. The first is the induction of Cl^- and Na^+ influx leading to neuronal swelling, and the second is the induction of Ca^{2+} influx into neurons via the *N*-methyl-D-aspartate (NMDA) receptor complex, resulting in delayed damage [3–5].

To date, a variety of pharmacological agents designed to prevent NMDA receptor activation have been investigated in an attempt to reduce the neural damage in those situations. Most of these have met with varying success and some are presently being evaluated in several clinical trials [6,7]. However, the use of these drugs have been limited because of their neurotoxic and behavioral side effects [8].

$MgSO_4$ is a readily available, inexpensive NMDA receptor antagonist with a well-established profile in cardiovascular practice. The cytoprotective effects of magnesium ions have been shown in both in vivo and in vitro experiments [9–12]. They play an important role in neural cellular physiology by competing with calcium ions in the extracellular space, acting as an endogenous calcium channel blocker and gating NMDA receptor-associated ion channels [13]. Additionally, they function as non-competitive antagonists of EAA receptors [10]. Magnesium may also be of value in decreasing the metabolic rate and oxygen demand of the postsynaptic cell because of its blocking effect on neurotransmitter release at synaptic junctions [14]. On the basis of these data, we investigated the effects of magnesium pre-treatment on histopathological changes and neurological recovery in a rat model of transient global cerebral ischemia.

2. Materials and methods

2.1. Animal preparation

After approval of the study by the local Institutional Committee, experiments were performed on 36 male Wistar albino rats weighing 235 ± 27 g (mean ± SD). During the surgical procedures, anesthesia was induced and then maintained with intraperitoneal ketamine 25 mg/kg fractionally as needed. No animal received haemodynamic or ventilatory support. The animals placed in a nose cone to breath oxygen at a rate of 0.5 l/min. Rectal temperature was monitored and maintained close to 38°C under a warming light. After the surgical procedures, the rats were returned to their individual cages. Rat diet and water were freely available.

2.2. Experimental groups and surgical procedures

Rats were randomly allocated into three groups each consisting of 12 rats. They were prepared for transient global cerebral ischemia by using a four-vessel occlusion model. On day 1, both vertebral arteries were electrocauterized at the level of first cervical vertebra within alar foramina. Then, another small longitudinal scalp incision was made and two stainless steel screws placed on the skull over the right parietal region in order to connect active and reference electrodes and to record somatosensory evoked potentials (SEP). The incisions were then closed and the rats were allowed to awaken. In group 1, $MgSO_4$ 600 mg/kg was given subcutaneously. A similar volume of saline solution was given in control groups correspondingly.

Two days later, rats were reanesthetized. After the surgical preparation using aseptic techniques, a femoral artery was exposed and catheterized. The catheter was connected to a blood pressure/heart rate transducer and monitor (Hewlett-Packard 1495C). Determinations of blood glucose, blood gases and pH were also accomplished with this line (Stat Profile 5 autoanalyser). With a small midline ventral neck incision, both carotid arteries were exposed and nylon threads placed around them. In group 1 and group 2, ischemia was induced by pulling the nylon threads for 15 min. Restoration of blood flow was verified visually. The incision was closed after establishing the reperfusion. In group 3 of animals (sham operated) neck incision was left open for 15 min corresponding to cerebral ischemia period but carotid arteries were not occluded. This group of animals were used for eliciting the effects of anesthesia and operation on results.

2.3. SEP recording

In order to assess the recovery of cerebral electrical function, SEP were monitored before the occlusion of the carotid arteries, during ischemia and first 30 min of reperfusion. Nihon Kohden Neuropack II plus (MEB 5000) was used for recordings. The active electrode was connected to the screw located 2 mm lateral to the midline and over the coronal suture. The reference electrode was connected to the other screw located at the midline over the frontal sinus (maxillary bone). A needle ground electrode was placed on the left upper extremity. The left sciatic nerve was stimulated by surface electrodes. The stimulus rate was 3/s with a duration of 0.1 ms and the stimulus intensity was 3–5 mA. Two-hundred and fifty-six responses were averaged. Bandpass filter was set at 20 Hz to 1 kHz. SEP responses were evaluated for recovery of the percentages of their baseline control values obtained before carotid occlusion.

2.4. Postoperative care and assessment

Femoral arterial line was removed at the 30th min of reperfusion. When the animals awakened from anesthesia, they were returned to their cages. Four animals (2 rats originally assigned to the group 1 and others to group 2) died in the early postoperative period (within the first 12 h) and they were excluded from the study. Others survived without any pathological finding except neurologic deficit. All animals were weighed daily and had food and water intake monitored.

Neurological outcome was evaluated on each day using a scoring system described by Capdeville et al. [15]. Briefly, items rated were cranial nerve reflexes and sensory motor functions. The total neurologic score for a normal rat was 24 (the sum of the scores of following items: corneal reflex, 2; pinna reflex, 2; grasping reflex, 2; placing reaction, 6; righting reflexes, 4; equilibrium tests, 4; flexion reflex, 2; spontaneous motility, 2). The neurological examinations of the animals were performed by an independent neurologist who was blinded to the study.

2.5. Histopathology

After the 4-day recovery period, rats were reanaesthetized and killed with intracardiac perfusion of 10% neutral formol. After decapitation, the heads were stored in the same fixative overnight at 4°C. Afterwards, the brains were removed and fixed in 10% buffered formalin solution for 7 days. Right and left hippocampal regions were obtained from coronal sections in frontal planes. Formaline fixed, paraffin embedded sections (4 μm thickness) were stained with haematoxylin eosin and cresyl violet. The CA1 and CA3 subfields of the hippocampus were examined in both sides of the brain. Using an ocular micrometer (Olympus ocular eye ×10), all pyramidal neurons in four different microscopic fields of both CA1 and CA3 regions were counted. Ischemic damage were based on the percentage of damaged neurons. Shrunken eosinophilic cytoplasm and pyknotic nucleus were accepted as criteria for determination of ischemic neuronal damage. This procedure was conducted in a blinded fashion.

2.6. Statistical analysis

Control variables such as blood pressures, blood gases and pH were compared among groups with one way analysis of variance. Non-parametric analysis with Wilcoxon-Mann–Whitney *U*-test using the Bonferroni correction were performed on the data of the neurological status score. Comparison of the weight loss and recovery of SEP amplitude values among the groups were made with *t*-test and Bonferroni corrections. All data are expressed as the mean ± SD. A *P* value less than 0.05 was considered significant.

3. Results

3.1. Physiological parameters

The vertebral electrocauterization induced a decrease in body weight. The weight loss during the first 2 days did not differ among the groups (group 1, 92 ± 2% of initial body weight; group 2, 91 ± 3%; group 3, 92 ± 1%). At the end of the 4-day recovery period, the animals in groups 1, 2, and 3 had 91 ± 2, 89 ± 3 and 92 ± 2% of their initial weights, respectively. These differences among the groups were not significant.

No differences in arterial blood gases, pH, blood glucose, arterial pressures or body temperatures were noted among the control groups and the group treated with magnesium before the occlusion of carotid arteries. These physiological parameters were in normal limits and stayed quite similar during the procedure in all groups.

3.2. SEP recordings

SEP recordings consistently showed a small negative peak (N1) with an average latency of 7.2 ± 0.6 ms and a large positive peak (P1) with an average latency of 10.6 d± 1.1 ms. These were followed by a large negative peak with some individual variations. The peaks were stable during the procedure in group 3 (sham operated group). In ischemic groups, All peaks progressively declined with an average of 4 ± 1.6 min after the occlusion of carotid arteries. At the end of ischemic period, N1-P1 amplitude decreased to 0–12% of preischemic baseline level in all animals. The average amplitude was reduced to 4 ± 3 and 5 ± 4% of the baseline in groups 1 and 2, respectively ($P > 0.05$). This was followed by a gradual return to 87 ± 10% of the initial N1-P1 amplitude after 30 min of reperfusion in group 1. This recovery was 83 ± 8% in group 2 animals ($P > 0.05$). The typical changes in SEP amplitudes are shown in Fig. 1.

3.3. Neurological status

Sham operated animals showed full neurological recovery after the procedure. The evaluations of the neurologic scores in groups 1 and 2 are presented in Table 1. All ischemic animals exhibited neurologic deficiencies especially with loss of spontaneous motility and righting reflexes

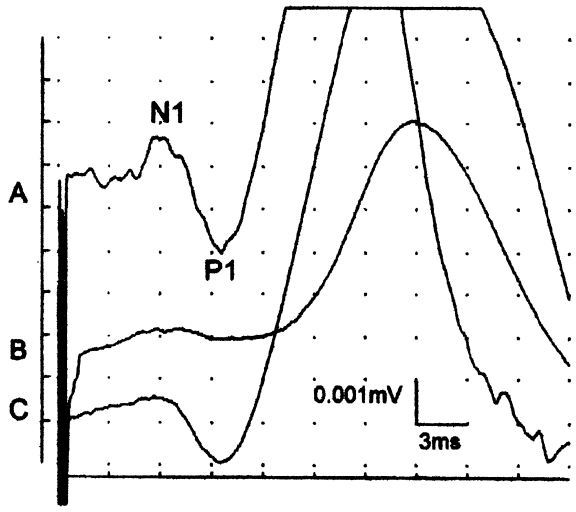

Fig. 1. The typical changes in SEP recordings during the procedure. (A) Preischemia. (B) At the end of ischemia. (C) At 30th minute of reperfusion.

Table 1

The neurologic scores of ischemic animals in group 1 ($n = 10$) and group 2 ($n = 10$) at postoperative 24th, 48th, 72nd and 96th hour

	24th hour	28th hour	72nd hour	96th hour
Corneal reflex				
Group 1	1.9 ± 0.3	1.9 ± 0.3	1.9 ± 0.3	1.9 ± 0.3
Group 2	1.6 ± 0.5	1.7 ± 0.5	1.7 ± 0.5	1.7 ± 0.5
Pinna reflex				
Group 1	1.7 ± 0.5	1.8 ± 0.4	1.8 ± 0.4	1.8 ± 0.4
Group 2	1.5 ± 0.5	1.7 ± 0.5	1.7 ± 0.5	1.7 ± 0.5
Grasping reflex				
Group 1	1.3 ± 0.5	1.5 ± 0.5	1.8 ± 0.4	1.8 ± 0.4
Group 2	1.2 ± 0.4	1.6 ± 0.5	1.6 ± 0.5	1.6 ± 0.5
Placing reaction				
Group 1	4.6 ± 1.1	4.8 ± 0.9	5.5 ± 0.7*	5.5 ± 0.7*
Group 2	4.1 ± 1.0	4.2 ± 1.1	4.3 ± 1.2	4.3 ± 1.2
Righting reflexes				
Group 1	2.2 ± 0.6	2.9 ± 0.7	3.4 ± 1.0	3.4 ± 1.1
Group 2	2.0 ± 0.5	2.5 ± 1.0	2.7 ± 0.8	2.8 ± 0.8
Equilibrium test				
Group 1	3.1 ± 0.7	3.2 ± 0.6	3.5 ± 0.7	3.5 ± 0.7
Group 2	2.9 ± 0.9	2.8 ± 0.9	3.1 ± 0.9	3.1 ± 0.9
Flexion reflex				
Group 1	1.6 ± 0.5	1.6 ± 0.5	1.8 ± 0.4	1.8 ± 0.4
Group 2	1.4 ± 0.5	1.4 ± 0.5	1.6 ± 0.5	1.6 ± 0.5
Spontaneous motility				
Group 1	0.8 ± 0.6	1.4 ± 0.5	1.7 ± 0.5	1.7 ± 0.5
Group 2	0.7 ± 0.7	1.2 ± 0.8	1.5 ± 0.7	1.5 ± 0.7
Total average scores				
Group 1	17.2 ± 2.2	19.1 ± 1.9	21.4 ± 2.1*	21.4 ± 2.1*
Group 2	15.4 ± 2.1	17.1 ± 3.6	18.2 ± 3.8	18.3 ± 3.8

*Significantly different from group 2 ($P < 0.05$).

after the procedure. This was followed by a gradual recovery in most animals, but full neurologic recovery was not seen in any of these animals. Neurological deficits persisted in two animals in groups 1 and 4 animals in group 2 during the 4-day recovery period. One animal in group 2 exhibited additional deterioration in neurologic score within 24 to 48 h. The average neurologic score was significantly better in magnesium pretreated animals than in group 2 postoperatively.

3.4. Histopathology

Pyramidal neurons in the CA1 and CA3 subfields of the hippocampus were completely normal in appearance in sham operated animals (Fig. 2). Surviving neurons was markedly decreased specially in CA1 subfield in ischemic animals (Figs. 3 and 4). In comparison with magnesium pretreated animals, control group showed significantly more damage in both CA1 and CA3 subfields. The percentage of damaged neurons in CA1 was 36 ± 9% in group 1, whereas group 2 had 47 ± 12% ($P < 0.05$). Similar but less severe histopathological changes were seen in CA3 fields. This field had 14 ± 10 and 23 ± 7% damaged neurons in groups 1 and 2, respectively ($P < 0.05$). Pyramidal neurons observed in CA3 were essentially unaffected in some animals in group 1. There was no additional remarkable focal ischemic or hemorrhagic cerebral lesions in the examined hippocampal regions in both groups 1 and 2.

4. Discussion

The data presented in the study demonstrate that significant cerebral neuroprotection, measured as a reduction in neuronal damage has been achieved with preischemic, subcutaneous administration of $MgSO_4$ in a rat model of transient global ischemia. Animals that were subjected to 15 min of global cerebral ischemia after receiving 600 mg/kg $MgSO_4$ exhibited better neurological outcomes. This confirms the previous reports demonstrating magnesium to be a potent neuroprotective agent.

One possible mechanism for the beneficial effect of magnesium is its positive role in general cellular metabolism and function. Magnesium is essential for normal cell functions such as membrane integrity, cellular respiration, transcription by messenger RNA, protein synthesis, glucose and energy metabolism, maintenance of normal Na^+ and K^+ gradients, and regulation of Ca^{2+} transport and accumulation [11,13,16]. A second neuroprotective mechanism probably includes inhibition of EAA release, and/or blockade of the NMDA-glutamate receptor. It is shown that by inhibiting EAA with magnesium or specific glutamate antagonists, anoxic neuronal death could be prevented in neuronal cell culture [14]. The importance of inhibition of excitatory synaptic transmission for preventing hypoxic neuronal injury was further confirmed in hippocampal slice preparations. In this model, high concentrations of magnesium reduced the vulnerability of neurons by blocking the anoxia-induced depolarization and by enhancing postischemic recovery of high-energy phosphates [12]. Another mechanism by which magnesium may decrease the size of brain infarct is cerebral vasodilatation. Extracellular magnesium affects vascular smooth-muscle contraction by its action on membrane permeability to calcium ions and blocks calcium channels [17]. It has been shown that magnesium improves blood flow to ischemic cortex and attenuates infarct size in focal cerebral ischemia in rats [9].

In most previous studies, the protective effect of magnesium was evaluated in traumatic brain injury or focal ischemia models [9–11]. Thus, they are not as applicable as the present study to the clinical setting of cardiac surgery and total circulatory arrest leading to global cerebral ischemia. In those studies, magnesium was administered just before or after the ischemic insult. In this study, $MgSO_4$ was administered subcutaneously 2 days before the procedure to avoid its acute side effects such as hypotension and hyperglisemia. Intravenous administration of magnesium to humans decreases total peripheral resistance by 20 to 30% [18]. It

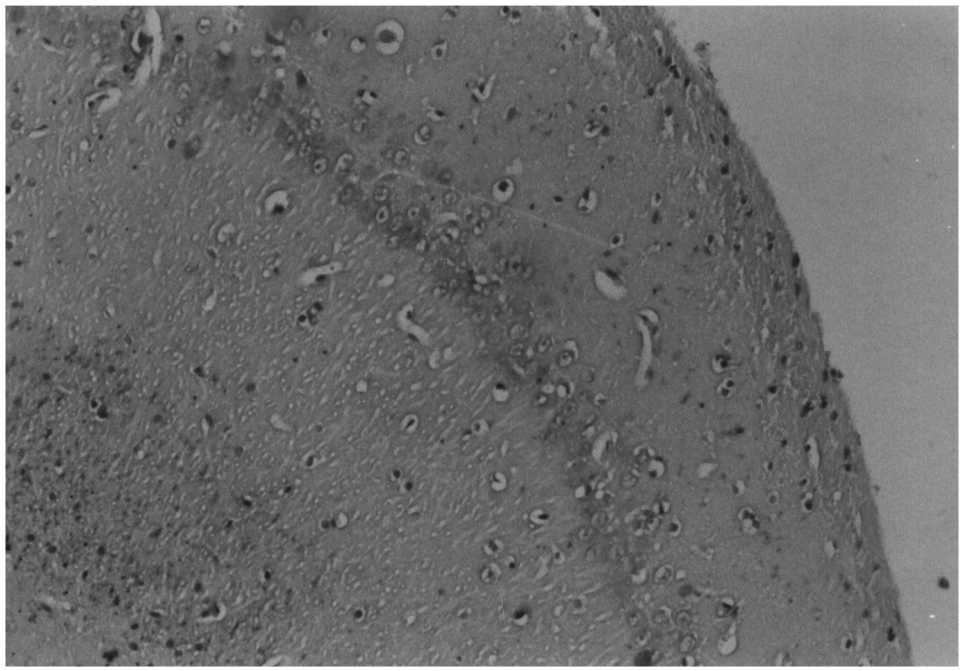

Fig. 2. Normal morphological appearance of unaltered structure of CA1 subfield of hippocampus (group 3, last neurologic score: 24; ×40 hemotoxylin and eosin).

is also known that hypermagnesemia may cause a reduction in plasma insulin levels, resulting in hyperglicemia [10]. Hyperglycemia is known to aggravate the cerebral injury that follows transient ischemia [19], and it might limit the effectiveness of magnesium treatment for cerebral ischemia [8]. Feldman et al. [11] has shown that 600 mg/kg subcutaneous $MgSO_4$ did not lead significant changes in serum osmolality and provided significant increase of Mg^{2+} concentration in brain tissue 48 h after the administration [11]. In the present study, we observed no significant difference in blood pressure and blood glucose level among all comparable groups. We think this model is relevant to the clinical situation that may be applied when one wishes to increase tolerance of brain to ischemia before elective surgery.

The rat four-vessel occlusion technique is a reliable

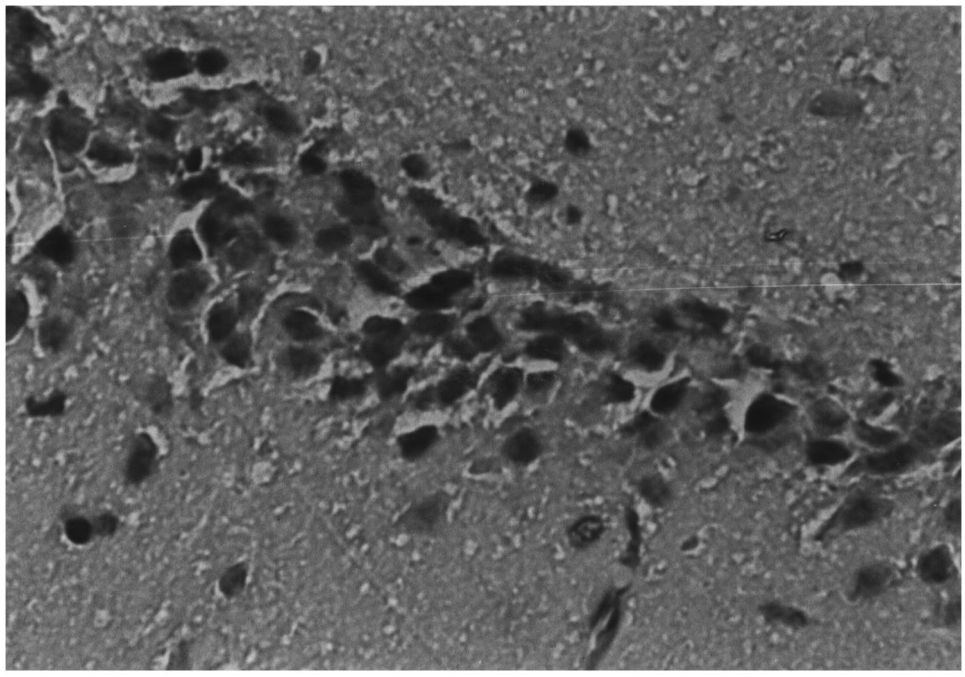

Fig. 3. Severe ischemic damage in CA1 subfield of hippocampus. There are ischemic changes characterized by eosinophilic cytoplasm and pyknotic nuclei in most pyramidal neurons (group 2, last neurologic score: 15, percentage of damaged neurons in CA1: 62%, ×400 hemotoxylin and eosin).

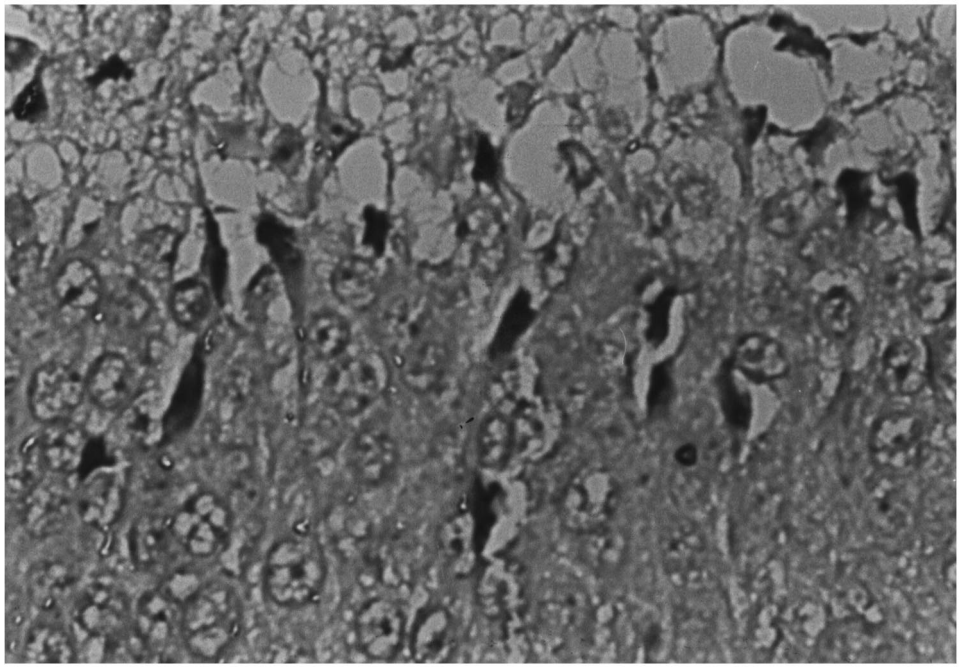

Fig. 4. Ischemic damage in some pyramidal neurons in CA3 subfield of hippocampus characterized by pyknosis and hypercromasia (group 1, last neurologic score: 20, percentage of damaged neurons in CA3: 14%, ×400 hemotoxylin and eosin).

model for observation of the protective effects of investigated agents on ischemia and reperfusion injury. However, blood flow may remains in some animals because of individual variations in the residual collateral circulation or probable surgical insufficiency while cauterizing the vertebral arteries. Monitoring of SEP or electroencephalographic activity has been used in some previous studies for ensuring duration and effectiveness of the global ischemia and exclusion of the animals that did not display a significant changes [20]. Previous studies have shown that the SEP reliably and directly reflect the cerebral electrical function [20,21]. Thus, we preferred SEP monitoring because of it is safe, easy and it does not prolong the operative procedure. In the present study, SEP amplitudes decreased to under 10% of their preischemic values in most animals and there were no differences in this decrement between groups 1 and 2. Monitoring of SEP in this model has permitted observation of similar collateral blood flow properties in studied groups of animals. In the present study, although the difference in recovery of SEP amplitudes did not attain statistical significance, we observed significantly better neurologic outcomes ($P < 0.05$) in $MgSO_4$ treated group. This data demonstrates that the SEP recovery during early reperfusion can not be used as a reliable predictor of neurological outcome. The similar lack of correlation of SEP recovery and postoperative neurologic deficits has been reported in some articles [22].

We used a scoring system described by Capdeville to quantitate the neurological outcome. Although there are some articles reporting lack of correlation of this neurologic score with histological damage [23], our histological observations were clearly correlated with the neurological findings. The pattern of neuronal vulnerability, with the greatest cell lost in the CA1 was similar to that seen in other previous experimental studies and confirms the particular sensitivity of CA1 to ischemia [23,24]. It has been assumed that it is due to a failure of recovery processes following excitatory damage to this particular neural circuitry [25].

In conclusion, the results suggest that subcutaneous $MgSO_4$ treatment 2 days before the ischemic insult reduces ischemic and reperfusion damage in transient cerebral global ischemia and provide better neurologic outcome. However, an animal study such as this may not be fully reproducible in humans. Further studies are needed to define biochemical aspects of the neuroprotective mechanism and to determine the correct dose necessary for maximal benefit.

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