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Administration of $MgSO_4$ failed to improve the neurological recovery after complete global brain ischemia in dogs

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Abstract: The cerebral protective effects of $MgSO_4$ after complete global brain ischemia were evaluated with EEG, evoked potentials (EP) and the neurological recovery score (NRS) in the dog. Complete global brain ischemia for 15 min was achieved by occluding the ascending aorta and the caval veins. The $MgSO_4$ group (N = 7) were injected with a 10% $MgSO_4$ solution and the control group (N = 7) were administered a normal saline intravenously from the beginning of the resuscitation to 48 h after ischemia. The EEG grades (1 = normal, 5 = flat) in the control group and the $MgSO_4$ group were 3.9 ± 0.1 (mean ± SEM) and 3.7 ± 0.3, and the EEG-EP scores (6 = normal, 0 = serious deterioration) were 2.6 ± 0.4 and 2.7 ± 0.4 4h after ischemia, respectively. The 7-day survival rates for ischemia were equal in both groups (5/7:71%). The NRSs (0 = death, 100 = normal) in the control group and the $MgSO_4$ group were 50 ± 3 (n = 7) and 43 ± 9 (n = 7) on the 3rd day after ischemia, and were 56 ± 5 (n = 5) and 42 ± 12 (n = 5) on the 7th day. The differences between the two groups were not significant. We conclude that $MgSO_4$ administered after ischemia has no beneficial effects on the recovery of EEG, EP and the NRS after 15 min of complete global brain ischemia in the dog.

Introduction

The excessive increase of intracellular Ca^{2+} has been thought to aggravate the damage of the nerve cells after brain ischemia [1]. Drugs which inhibit Ca^{2+} influx into nerve cells have been expected to protect the neural cells from ischemic damage. However, the Ca^{2+} antagonists have only a slightly beneficial effect [2], and MK-801, which is a glutamate receptor antagonist that blocks Ca^{2+} influx mediated by N-methyl-D-aspartate (NMDA), has no effect on the protection of neural cells after complete brain ischemia [3].

Since magnesium ion is a non-specific Ca^{2+} antagonist [4,5], the neural protection with Mg^{2+} has been examined in several animal models of brain damage, such as brain injury [6] and forebrain ischemia [7]. For complete global brain ischemia, Okawa reported that $MgSO_4$ caused marked improvement of the neurological recovery in the canine model [8].

For confirming further the neural amelioration by Mg^{2+} after global brain ischemia, we investigated the efficacy of $MgSO_4$ on the recovery of EEG, evoked potentials and neurological functions after 15 min of complete global brain ischemia in dogs.

Materials and Methods

The present experiment was approved by the Animal Care Committee of Tohoku University School of Medicine.

Fourteen male mongrel dogs, weighing 9.0–12.5 kg, were used. Standard methods for the study of complete global brain ischemia were described elsewhere [9,10]. Briefly, the anesthesia was induced with 25 mg·kg^{-1} of thiopental sodium, and was maintained with 1% halothane in oxygen during the surgical procedure after the tracheal intubation. The dog was ventilated with an animal ventilator (R-60, Aika, Japan) to maintain $Paco_2$ between 35 and 40 mmHg under muscle paralysis with pancuronium bromide. A femoral artery and a forepaw vein were catheterized for blood pressure monitoring and administration of fluid and drugs, respectively. Lactated Ringer's solution was administered at the rate of

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10 ml·kg^{-1}·h^{-1} during the study. Following aseptic thoracotomy of the left side, tapes were placed around the bases of the ascending aorta, and the superior and inferior caval veins. Complete global brain ischemia for 15 min was achieved by occluding these great vessels by tightening the tapes. Recirculation was established by releasing the occlusion, accompanied by an iv injection of 5–10 ml of 7% $NaHCO_3$ and 1–3 mg of ethylephrine. The concentration of halothane was decreased to and maintained at 0.3% from 30 min before ischemic insult. The upper esophageal temperature before ischemia was maintained between 37.0 and 38.0°C. Four hours after ischemia, the chest wall was closed and the dog was allowed to recover from anesthesia by discontinuance of halothane and by reversal of muscle paralysis with iv neostigmine and atropine. After adequate spontaneous breathing in room air was confirmed by the arterial blood gas analysis, the dog was extubated and returned to the animal cage. A mixture of Lactated Ringer's solution and 5% glucose solution (1:1) was infused intravenously (500 ml·day^{-1}), and amikacin (50 mg·day^{-1}) was injected intramusculary for 7 days after ischemia. The body was covered with blankets, and the operated wounds and the decubitus were sterilized by povidone iodine once a day.

Immediately after recirculation, the dogs were randomly divided into two groups, 7 dogs for the $MgSO_4$ group and 7 dogs for the control group. The $MgSO_4$ group was administered 10% $MgSO_4$ in 10% glucose (Magnesol, Toriiyakuhin, Japan) at the infusion rate of 3.2 ml·kg^{-1}·h^{-1} for the first 15 min after ischemia, at 0.4 ml·kg^{-1}·h^{-1} for the next 3 h, and then at 0.1 ml·kg^{-1}·h^{-1} until 48 h. This infusion rate of $MgSO_4$ solution followed the study of Okawa [8]. The concentration of plasma Mg^{2+} in the $MgSO_4$ group was measured by an automatic clinical analyzer (aca sx, Du Pont, USA) before and after ischemia. The control group received normal saline at the same rate as the infusion of $MgSO_4$ solution.

The evoked potentials of auditory brainstem response (ABR), middle latency response (MLR) and somatosensory evoked potential (SEP) were measured (7S12, Nihondenki-Sannei, Japan) using needle electrodes inserted into the head skin [9]. The EEG grade (1 = normal alpha-beta wave and 5 = flat) was evaluated according to the system of Hockaday et al. [10,11]. The appearance of the 5th wave in ABR, Pa wave in MLR, N2 and N3 waves in SEP and the EEG grade were expressed as the EEG—Evoked potential score (EEG-EP score; 6 = normal and 0 = serious deterioration of electric function) described in our previous studies [9, 10]. The neurological recovery after ischemia was assessed with the neurological recovery score (NRS; 0 = death and 100 = normal), which was same as the score used in the previous studies [9,10].

Blood pressure, heart rate, upper esophageal temperature, hematocrit, blood glucose concentration, arterial blood gases, plasma Mg^{2+} concentration, EEG and evoked potentials were measured before and until 4 h after ischemia. The NRS of each day was counted once a day for 7 days after ischemia.

The differences of survival rate and appearance rates of evoked potentials were compared using Fisher's Exact Probability Test. The NRS, EEG grade and EEG-EP score were evaluated utilizing the Mann-Whitney U-test. Physiological variables were examined using the unpaired Student's *t*-test. The concentrations of plasma Mg^{2+} were compared using analysis of variance (ANOVA) followed by the paired Student's *t*-test. A P value of <0.05 was considered significant. The data were presented as mean ± SEM.

Results

The difference in the body weight between the control group (10.5 ± 0.5 kg) and the $MgSO_4$ group (9.8 ± 0.3) was not significant. Before ischemia, there were no significant differences in the physiological variables between the two groups (Table 1). After ischemia, the heart rate of the control group was significantly higher than that of the $MgSO_4$ group 30 min after ischemia. The mean blood pressures 1 h and 2 h after ischemia in the control group were significantly lower than those of the $MgSO_4$ group (Table 1). The plasma Mg^{2+} of the two cases in the control group was 1.2 (Mean, mEq·l^{-1}) before ischemia, 1.5 at 10 min after ischemia, 1.3 at 30 min, 1.3 at 1 h, 1.0 at 2 h and 1.1 at 4 h. The administration of $MgSO_4$ after ischemia significantly increased the concentration of plasma Mg^{2+} (Table 2).

The 7-day survival rates after ischemia were equal in both groups (5/7: 71%). The changes in the NRS of the two groups are illustrated in Fig. 1. The NRSs on the 3rd day after ischemia were 50 ± 3 in the control group (n = 7) and 43 ± 9 in the $MgSO_4$ group (n = 7). The NRSs on the 7th day were 56 ± 3 in the control group (n = 5) and 42 ± 12 in the $MgSO_4$ group (n = 5). The best NRSs observed during 7 days were 53 ± 4 in the control group (n = 7) and 45 ± 9 in the $MgSO_4$ group (n = 7) (Fig. 1). There were no significant differences in the NRSs between the two groups.

The EEG of grade 1 and all waves of evoked potential were present before ischemia. EEG and evoked potential waves disappeared during complete brain ischemia. One hour after ischemia, the first and third waves of ABR and the N1 wave of SEP reappeared in all dogs. The appearance rates of ABR 5th wave, MLR Pa wave, SEP N2 and N3 waves for 4 h after ischemia were not significantly different between the two groups

Table 1. Physiological variables before and after ischemia

			After Ischemia			
		Before	30 min	1 h	2 h	4 h
mean BP	Cont.	115 ± 4	119 ± 7	99 ± 6	95 ± 6	92 ± 7
(mmHg)	$MgSO_4$	128 ± 6	120 ± 9	117 ± 4*	123 ± 9*	105 ± 6
HR	Cont.	167 ± 12	216 ± 5	182 ± 10	163 ± 8	160 ± 10
(min^{-1})	$MgSO_4$	137 ± 8	181 ± 13*	169 ± 12	151 ± 10	154 ± 8
Temperature	Cont.	37.6 ± 0.1	35.7 ± 0.2	35.9 ± 0.2	36.4 ± 0.3	36.8 ± 0.4
(°C)	$MgSO_4$	37.5 ± 0.1	36.1 ± 0.1	36.4 ± 0.2	36.9 ± 0.2	37.5 ± 0.1
Hematocrit	Cont.	32 ± 2	41 ± 2	40 ± 2	39 ± 2	37 ± 3
(%)	$MgSO_4$	35 ± 3	43 ± 2	45 ± 2	44 ± 3	43 ± 3
Glucose	Cont.	133 ± 10	297 ± 47	260 ± 36	183 ± 18	159 ± 14
($mg \cdot dl^{-1}$)	$MgSO_4$	129 ± 8	326 ± 28	307 ± 26	240 ± 21	183 ± 14
Pao_2	Cont.	510 ± 18	487 ± 46	522 ± 14	500 ± 17	524 ± 16
(mmHg)	$MgSO_4$	488 ± 22	459 ± 37	471 ± 26	438 ± 25	513 ± 12
$Paco_2$	Cont.	38.0 ± 0.8	39.7 ± 3.4	36.1 ± 2.4	36.1 ± 2.0	37.8 ± 1.7
(mmHg)	$MgSO_4$	39.7 ± 0.4	40.4 ± 1.2	38.7 ± 1.4	38.4 ± 1.3	38.8 ± 1.0
BE	Cont.	−3.0 ± 0.6	−8.6 ± 0.8	−6.3 ± 1.1	−5.1 ± 1.3	−4.9 ± 1.0
($mEq \cdot l^{-1}$)	$MgSO_4$	−2.1 ± 0.9	−7.9 ± 0.4	−5.8 ± 0.3	−3.6 ± 0.5	−3.4 ± 0.7

* $P < 0.05$ between the two groups. Each value represents mean±SEM.
Cont., the control group; $MgSO_4$, the $MgSO_4$ group; BP, blood pressure; Pao_2, arterial O_2 pressure; $Paco_2$, arterial CO_2 pressure; BE, base excess.

Table 2. Changes in the plasma Mg^{2+} concentrations of the $MgSO_4$ group before and after $MgSO_4$ administration

		After Ischemia					
	Before	10 min	30 min	1 h	2 h	4 h	1 week
Mg^{2+}	1.3	5.3*	4.7*	4.3*	3.7*	2.7*	1.7
($mEq \cdot l^{-1}$)	±0.1	±0.3	±0.4	±0.4	±0.1	±0.1	±0.2
(*n*)	(7)	(7)	(7)	(7)	(7)	(7)	(5)

* $P < 0.001$ vs the pre-ischemic value. Each value represents mean ± SEM. Numbers in parentheses represent the number of the dogs.

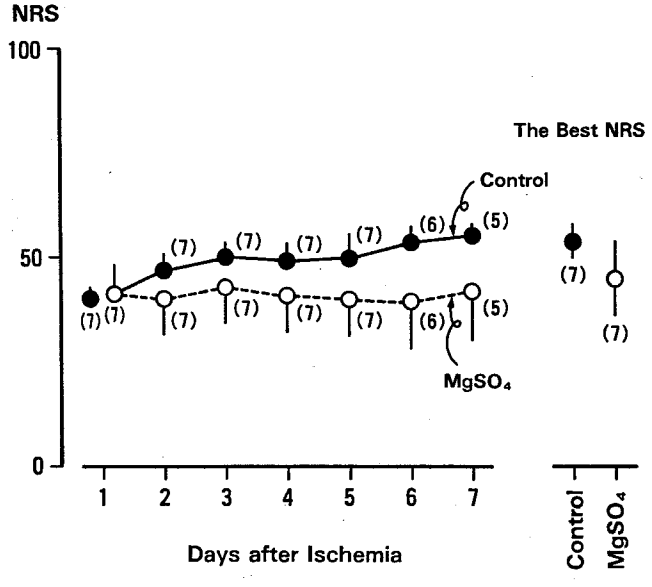

Fig. 1. Changes in the neurological recovery score (*NRS*) (*left side*) and the best NRS (*right side*) of the control group and the $MgSO_4$ group for 7 days after ischemia. The numbers in parentheses are the number of dogs surviving at that time. Each point represents mean ± SEM. Control, the control group, $MgSO_4$, the $MgSO_4$ group. There were no significant differences between the two groups

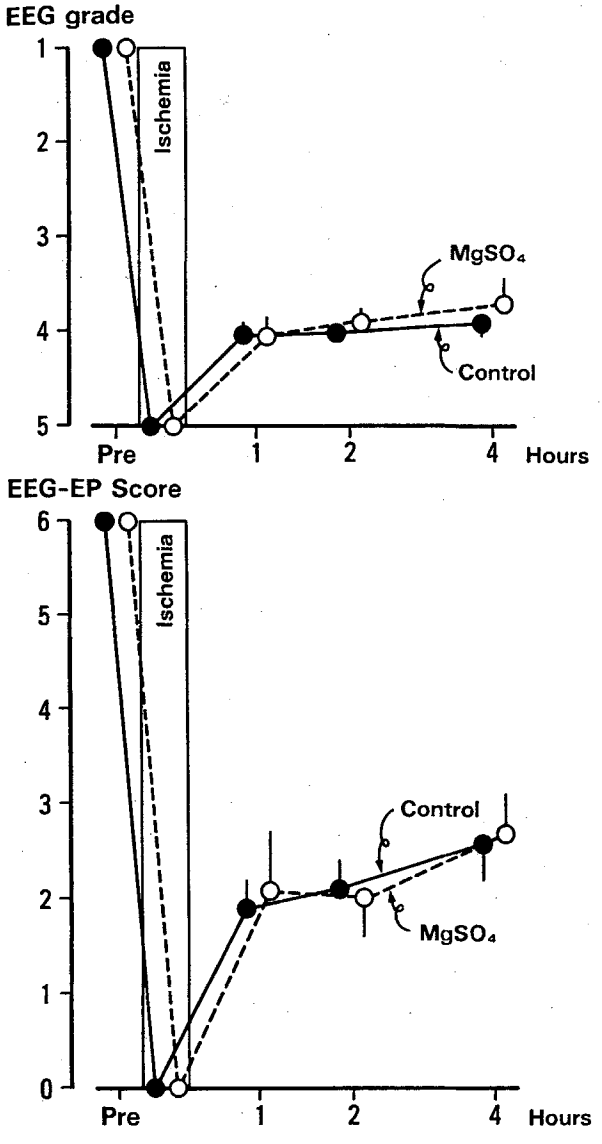

Fig. 2. Changes of the EEG grade (*upper*) and the EEG-evoked potential (*EEG-EP*) score (*lower*) in the two groups before and after ischemia. Each point represents mean ± SEM. Control, the control group, $MgSO_4$; the $MgSO_4$ group, Ischemia: 15 min of complete brain ischemia. There were no significant differences between the two groups

(Table 3). The EEG grades and the EEG-EP scores 4 h after ischemia are illustrated in Fig. 2. There were no significant differences in the grade or the score between the two groups.

Discussion

The excessive increase of intracellular Ca^{2+} through the voltage-sensitive Ca^{2+} channel and the NMDA receptor operated Ca^{2+} channel may be induced by brain ischemia and may injure nerve cells [1]. Moreover, the

Table 3. The appearance rates (%) in evoked potential

Wave	Group	Before	After Ischemia 1 h	2 h	4 h
ABR-5	Cont.	100%	71	85	71
	$MgSO_4$	100%	85	85	85
MLR-Pa	Cont.	100%	0	0	14
	$MgSO_4$	100%	14	0	14
SEP-N2	Cont.	100%	85	85	85
	$MgSO_4$	100%	85	71	100
SEP-N3	Cont.	100%	29	42	71
	$MgSO_4$	100%	29	42	57

Cont., the control group; $MgSO_4$, the $MgSO_4$ group; ABR-5, the 5th positive wave of ABR; MLR-Pa, the second large positive wave of MLR; SEP-N2, the second negative wave of SEP; SEP-N3, the third negative large wave of SEP.

post-ischemic cerebral hypoperfusion has been reported to occur after global brain ischemia [2]. These phenomena have been thought to aggravate the neural damage after global brain ischemia. Mg^{2+} blocks the NMDA-induced neural depolarization [4], reduces the current of Ca^{2+} channels [5], and increases the peripheral blood flow by the vasodilatation [12]. Therefore, Mg^{2+} has been expected to ameliorate neurological damage after brain ischemia.

However, the present study demonstrated that the post-ischemic administration of $MgSO_4$ did not accelerate the recovery of EEG, EP or neurological functions after 15 min of complete brain ischemia in dogs. The speculated reasons for this ineffectiveness of $MgSO_4$ are the following. Mg^{2+} is truly ineffective in the treatment of post-ischemic brain damage or the extracellular concentration of Mg^{2+} of brain was not high enough to act effectively. The physiological concentration of Mg^{2+} (below $2\,mEq \cdot l^{-1}$) selectively depresses the NMDA-induced depolarization [4], while the non-NMDA receptor mediated depolarization is depressed weakly even by the higher concentration of Mg^{2+} (more than $10\,mEq \cdot l^{-1}$) [4]. Thus, Mg^{2+} might not block completely the Ca^{2+} influx into ischemic nerve cells. Meanwhile, the Mg^{2+} concentration of cerebrospinal fluid increases very slowly and is kept in a lower level than the serum concentration of Mg^{2+} after intravenous continuous administration of Mg^{2+} [13]. Therefore, it is possible that the intravenous administration of Mg^{2+} may not increase the extracellular Mg^{2+} of the brain sufficiently.

In a canine complete global brain ischemic model, Okawa reported that the post-ischemic administration of $MgSO_4$ dramatically improved the neurological recovery after 18 min of brain ischemia [8]. This result is contradictory to ours. Although the method of $MgSO_4$ administration and the concentration of plasma Mg^{2+} in his study were similar to those of our study, the duration of ischemia was 15 min in our study and 18 min in his

study. The significant increase of Mg^{2+} in the cerebrospinal fluid (CSF), compared with the value before $MgSO_4$ administration, was observed after $MgSO_4$ administration in the ischemic group in his study. He suggested that 18 min of ischemia injured the blood brain barrier (BBB) enough for Mg^{2+} to permeate into brain tissue, and to protect the brain. However, there were no differences in the absolute concentrations of CSF Mg^{2+} after $MgSO_4$ administration between the ischemic group and the non-ischemic control group in his study. Therefore, it is difficult to speculate that only 3 min difference in ischemic duration produces the striking difference in permeability of Mg^{2+} through BBB, resulting in the discrepancy between the two studies.

On a forebrain ischemic model, the post-ischemic infusion of $MgCl_2$ reduced the cortical infarct size in rats [7]. On the other hand, the pre-ischemic administration of $MgCl_2$ showed no protective effects for 10 min of reversible forebrain ischemia in the rat [14]. For complete global brain ischemia, the post-ischemic infusion of $MgSO_4$ had no cerebral protective effects for 12 min of cardiac arrest in the dog [15]. On human cardiac arrest, the administration of verapamil and $MgSO_4$ improved the neurological recovery [16]. Therefore, the effectiveness of Mg^{2+} for brain ischemia was reported variously in the types of brain ischemia, the duration of ischemia, the administered dose of Mg^{2+} and the used drug at the same time. Confirming the efficacy of Mg^{2+} given after ischemia, the neurological outcome study should be performed under the several doses of Mg^{2+} and the several duration of complete global brain ischemia.

We conclude that the post-ischemic intravenous administration of $MgSO_4$ has no beneficial effects on the recovery of EEG, EP, and neurological function after 15 min of complete global brain ischemia in the dog.

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