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S-ADENOSYL-L-METHIONINE PROTECTS THE HIPPOCAMPAL CA1 NEURONS FROM THE ISCHEMIC NEURONAL DEATH IN RAT

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SUMMARY: We investigated the effect of S-adenosyl-L-methionine (SAMe) on the prevention of the delayed neuronal death in rats subjected to transient and brief forebrain ischemia. As the results, SAMe dose-dependently protected the hippocampal CA1 neurons from degeneration and necrosis, whose effect was suppressed by simultaneous administration of S-adenosyl-L-homocysteine, a potent inhibitor in transmethylation. No protective effect was observed in CDP-choline, phosphatidylcholine and L-methionine. Therefore, it is necessary for the prevention of the delayed neuronal death to enhance cerebral SAMe level and to activate transmethylation using SAMe as a methyl donor in post-ischemic brain.

S-Adenosyl-L-methionine (SAMe) is synthesized enzymatically from ATP and L-methionine in every living cell and mainly takes part as a methyl donor in transmethylation of such biological substances as biogenic amines, phospholipids, nucleic acids and proteins (1). Since the discovery of the stable salt, SAMe sulfate tosylate, SAMe has been the subject of many experimental and clinical investigations. The following effects of SAMe were recently reported in experimental animals: the improvement of energy metabolism and EEG spectrum in postischemic brain (2), the reduction of brain edema (3) and the increase of erythrocyte deformability (4), whereby SAMe attracts investigators' attentions as a drug for the treatment of cerebrovascular diseases. In the study of ischemic brain damage, a recent topic

Abbreviations used: SAMe, S-adenosyl-L-methionine; SAH, S-adenosyl-L-homocysteine; PC, phosphatidylcholine; EEG, electroencephalogram.

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is prevention of "the delayed neuronal death" (5), which is a reversible type of neuronal damage following brief ischemic insult. In this model, SAMe has been demonstrated to be effective in preventing the neuronal death (6). In this paper, we speculate the mechanism underlying the protective effect of SAMe in comparison with the results of such drugs as S-adenosyl-L-homocysteine (SAH), L-methionine, CDP-choline and phosphatidyl-choline (PC), which are related to SAMe in metabolic pathways or in phospholipid synthesis. Our results provided evidences that the effect of SAMe was related to transmethylation and that such effect was produced by the administered SAMe, not by the SAMe resynthesized in brain from L-methionine.

MATERIALS AND METHODS

SAMe sulfate tosylate (FO-1561) and SAH were provided by Fuji Chemical Industry Co., Ltd. Other drugs were of analytical grade quality. SAMe sulfate tosylate was dissolved in 0.18M Na_2HPO_4 and the solution was adjusted to pH 6.0 just before use. The other drugs were dissolved in saline, except for PC which was suspended in saline.

Male Wistar rats (230-270 g) were submitted to forebrain ischemia according to the four-vessel occlusion method (7). To be brief, the rats were anesthetized with pentobarbital (40 mg/kg, ip) and their bilateral vertebral arteries were permanently occluded by electrocauterization. On the following day, the rats, normally behaving, were anesthetized with N_2O/O_2 (70:30) containing 1% halothane and the bilateral common carotid arteries were exposed. Then the anesthesia was discontinued and the animals were subjected to forebrain ischemia for 10 min by temporary clipping of bilateral common carotid arteries. Immediately following 10 min of occlusion, the rats were administered a given amount of drugs intravenously or intra-peritoneally. The doses of the drugs were determined on the basis of equivalent mole to SAMe 60 or 120 mg/kg. As control groups, we provided saline group and vehicle group in which the rats were injected 0.18M phosphate buffer, pH 6.0, containing D-mannitol (144 mg/kg as the dose).

Seven days follwing ischemic insult, under pentobarbital anesthesia, the rats were perfusion-fixed with 400 ml of FAM (37% formaldehyde: acetic acid: methanol= 1:1:8). Then the brains were removed out and stored in FAM. The next day, 4-mm-thick coronal sections were cut, dehydrated through graded series of ethanol, soaked in chloroform and embedded in paraffin. Five-micrometer-thick sections, containing dosal hippocampus, were prepared on a sliding microtome and stained with cresyl violet. The preparations were measured according to the method of Kirino et al.(5) The number of surviving neurons in the pyramidal cell layer within the CA1 sector was counted using Optiphot XF-21 (Nikon) at a magnification of 400x. The linear length of CA1

sector was measured on photograph. The neuronal density of the CA1 subfield was defined as the number of neurons per 1 mm of the linear length of CA1 pyramidal cell layer in 5-μm-thick section. The neuronal densities were expressed as the mean value ±SE of the averages of right and left side of each rat. Statistical analysis was carried out by Mann-Whitney's U-test.

RESULTS AND DISCUSSION

It is well known that neuronal cells are apt to be damaged by ischemic insult and that the neurons in hippocampal CA1 subfield are especially susceptible (8). When the animals are subjected to transient and brief ischemia as in this study, the CA1 neurons degenerate and die in a few days though showing temporary recovery after recirculation. This phenomenon, called "the delayed neuronal death" (9,10), is a reversible type of neuronal injury, and it can be prevented by effective drugs such as pentobarbital (5).

Our results are shown in Table 1 . In the rats submitted to forebrain ischemia for 10 min, if no effective drugs are given,

Table 1. Effect of drugs on survival of hippocampal CA1 neurons in rats subjected to forebrain ischemia for 10 min

drug	dose (mg/kg)	N	cell density
Sham operated		10	276± 6**
Saline		10	48±12
Vehicle		10	50±14
SAMe	15	11	48± 7
	30	10	78±16
	60	12	104±15*
	120	10	223±20**
SAMe + SAH	60 + 57.7	10	74±12
	120 + 115	10	75±10
CDP-choline	76.1	10	58±11
	152	11	33± 7
Phosphatidylcholine	250 (ip)	11	42±12
L-Methionine	22.4	11	58±14
	44.8	10	46±10

Values are expressed as mean ±SE in number of neurons per mm of linear length of CA1 sector. Significantly different from Saline: *, $p<0.05$; **, $p<0.01$ (by Mann-Whitney's U-test).

most of neuronal cells in CA1 sector degenerated and disappeared and the neuronal densities decreased as shown in saline and vehicle group. When the rats were treated with SAMe, the CA1 neurons survived dose-dependently. Especially with 60 and 120 mg/kg of SAMe treatment, the neuronal densities increased significantly in contrast to those of both control groups. Accordingly, SAMe showed a definite, reproducible and favorable effect in the prevention of the neuronal death. However, this effect of SAMe was suppressed by simultaneous administration of SAH, which is a demethylated metabolite of SAMe and a potent inhibitor in transmethylation. Therefore, it is suggested that transmethylation takes part in the effect of SAMe.

SAMe has been reported to participate as a methyl donor in the biosynthesis of phosphatidylcholine (PC) from phosphatidylethanolamine by transmethylation in cell membranes (11). Since phospholipids are decomposed by the activation of phospholipases induced by ischemia (12), it seems to be important that the cell membranes are repaired by resynthesis of PC via this pathway in the postischemic brain. In fact, Trovarelli et al. (13) demonstrated that SAMe prevented change of choline lipids in ischemic brain. Therefore, it is suggested that SAMe prevents the delayed neuronal death by stabilizing and repairing the neuronal membranes.

There is another main pathway for PC biosynthesis using CDP-choline as a cofactor (14), so we examined the effects of CDP-choline and PC in the same system. However, both CDP-choline and PC did not prevent the neuronal death. Consequently, only PC synthesis using SAMe may play an important role for the preventive effect of neuronal death mentioned above or either PC synthesis may not be related to this effect. If the former would be the case, it is necessary to look into the difference in

molecular kind (kind of esterificated fatty acids) of PC synthesized through these two pathways and the difference in their respective physiological action as well, and if the latter would be the case, it requires that the possibility due to transmethylation of nucleic acid, protein, biogenic amines, etc. other than phospholipids will be examined.

Meanwhile, we also estimated the protective effect of L-methionine, however, it showed no effect. The ineffectiveness of L-methionine is probably owing to the disturbance of SAMe synthesis by ATP depletion in postischemic brain. Though SAMe is not considered to cross the blood-brain barrior so much, Stramentinoli et al. (15) demonstrated that cerebral SAMe concentration increased to twice as much as its control value by the administration at the dose of 100 mg/kg. Moreover, Trovarelli et al. (13) showed that SAMe was used in transmethylation in ischemic brain. Accordingly, under unphysiological conditions such as ischemia, SAMe becomes easy to be incorporated into brain and is used in transmethylation, by which the neuronal death might be prevented.

From our results described above, SAMe is expected to be effective for the clinical treatment in acute stage of cerebrovascular diseases.

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