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Short communication

Beneficial effects of *S*-adenosyl-L-methionine on blood–brain barrier breakdown and neuronal survival after transient cerebral ischemia in gerbils

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Abstract

We have studied the beneficial effects of *S*-adenosyl-L-methionine (SAM) tosylate on blood–brain barrier (BBB) breakdown and neuronal survival after transient cerebral ischemia in gerbils. BBB breakdown experiments were performed in pentobarbital anesthetized gerbils subjected to 10 min of bilateral carotid artery occlusion and 6 h of reperfusion. For BBB breakdown measurements, SAM (120 mg/kg, i.p.) was administered to gerbils just after occlusion and thereafter every hour up to 5 h. Fluorometric measurements quantified the blood–brain permeability tracer, Evans blue (EB). SAM treatment significantly reduced the BBB breakdown as indicated by reduced levels of EB fluorescence. Neuronal count experiments were conducted in gerbils subjected to transient ischemia and 7 days of reperfusion. For neuronal count experiments SAM (15–120 mg/kg) was administered at 6 and 12 h after reperfusion, and twice each day thereafter for 7 days. SAM dose dependently protected the hippocampal CA1 neurons assessed by histopathological methods. SAM has a beneficial effect on the outcome of ischemic injury by reducing the BBB breakdown and neuronal death.

Keywords: Cerebral ischemia; Blood–brain barrier; Edema; Delayed neuronal death; Gerbil; Hippocampus

Transient cerebral ischemia causes an increase in ornithine decarboxylase (ODC) activity (rate limiting step for polyamine production) [20] and putrescine levels associated with a decrease in spermidine, spermine and *S*-adenosylmethionine decarboxylase (SAMDC) activity [18]. Both the post-ischemic increase in putrescine and decrease in spermidine and spermine levels [16] may contribute to neuronal death. Several studies have presented evidence that the polyamines spermidine and spermine modulate signals mediated through the NMDA receptor complex [4,28]. Stimulation of intracellular spermidine and spermine may provide better neuronal protection during and after ischemia [3]. Some studies indicate that the administration of *S*-adenosyl-L-methionine (SAM) can enhance the intracellular levels of spermine and spermidine [1,3], thus reducing cerebral edema [13] and improving neuronal counts [14,22] after transient cerebral ischemia. SAM is known to be involved in three intracellular reactions: transmethylation, glutathione biosynthesis, and polyamine biosynthesis. Transmethylation of biological substances could regulate membrane fluidity [7], calcium transport [8], and excitatory amino acid-related neurotoxicity [25]. The mechanism by which SAM prevents neuronal degeneration after ischemia is not clear, but some groups have argued that the protective effects of SAM are through the transmethylation process [26,29].

Recently it has been proposed that SAM prevented apoptotic cell death in cerebellar granule neurons through the involvement of the polyamine biosynthetic pathway [5]. Harada et al. [5] also showed that the polyamine, spermine, and the spermine analog N^1,N^{12}-bis(ethyl)-spermine (BESPM) protected granule cells. Previously SAM beneficial effects on neuronal death in a transient ischemia model [14,22] and brain edema in gerbils and spontaneously hypertensive rats were studied [13]. But all these studies utilized mannitol as a filler solution which

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might have confounded the results. Secondly, the effect of SAM on physiologic parameters as well as blood–brain barrier (BBB) breakdown in the hippocampus has not been investigated previously.

We now report the protective effects of SAM on ischemia-induced BBB breakdown in the cortex and the hippocampus and neuronal degeneration in the CA1 region of the hippocampus in transient ischemia model of gerbil and confirm the previous observations made by other groups [14,22]. In this study we exclude any contributions made by mannitol.

S-Adenosyl-L-methionine sulfate (SAM) *p*-toluenesulfonate (Research Biochemicals Int., Natick, MA) was dissolved in 0.18 M Na_2HPO_4 and the solution was adjusted to pH 6.0 just before use. For BBB breakdown measurements SAM (120 mg/kg, i.p.) was administered to gerbils just after occlusion and thereafter every hour for 5 h subsequently. For neuronal count experiments SAM (15–120 mg/kg) was administered at 6 and 12 h after reperfusion, and twice each day thereafter for 7 days.

Blood–brain barrier (BBB) breakdown measurements. For BBB breakdown measurements male Mongolian gerbils (50–80 g) were anesthetized with pentobarbital (60 mg/kg i.p.). The gerbils were subjected to 10 min of bilateral carotid occlusion and reperfused for 6 h [2,10,20]. In this model of the transient ischemia significant ODC activity and edema formation was observed after 6 h reperfusion [20]. A 6 h reperfusion period was therefore chosen for BBB breakdown studies. A 2% solution of Evans blue (EB) was given at a dose of 3 ml/kg (60 mg/kg) i.p. At the end of the reperfusion, gerbils were reanesthetized with pentobarbital (60 mg/kg i.p.)., and transcardially perfused with saline before decapitation. EB fluorescence method was used to evaluate the BBB breakdown [27].

Histopathology. For histopathology, gerbil carotid arteries were occluded [2,10,20] and reperfused for 7 days [10]. Histopathology was performed according to Kindy et al. [9] and Kirino et al. [11]. Gerbils were anesthetized with pentobarbital (60 mg/kg, i.p.) and perfused transcardially with cold phosphate buffered saline, followed by 4% paraformaldehyde, 7 days after transient ischemia and reperfusion [9]. Brains were removed and fixed with paraformaldehyde for 24 h, followed by 20% sucrose for 24 h. The tissue was placed in Tissue-Tek O.C.T. compound (Miles, Inc.), frozen on dry ice and stored at −80°C until sectioning. Tissue blocks were sectioned coronally (10 μm) and stained with Cresyl violet. The number of neurons in the CA1 region of the hippocampus was counted (1 mm in length) as described by Kirino et al. [11]. The body temperature was monitored with a rectal probe and maintained at 37–38°C with heating blanket and lamp during the ischemia and the reperfusion period. Mean arterial blood pressure (MABP) was monitored continuously for all the animals and measurements were recorded just before, during, and 30 min after ischemia.

Statistical analysis. All measurements were expressed as the mean ± S.E.M. Data were analyzed (GraphPad Software, San Diego, CA) using a one-factor ANOVA with the Bonferroni test to compare between the groups. A value of $P < 0.05$ was considered significant.

Physiologic parameters. No significant changes were noted in the body temperature. During ischemia, the mean arterial blood pressure increased in the experimental group (EXP) (compared to pre-ischemia), but the experimental group treated with SAM (EXP + SAM) did not show any significant changes (compared to the experimental group) (Table 1).

Blood–brain barrier breakdown after transient cerebral ischemia: effect of SAM. The amount of EB was quantified by fluorescence, in the cortex and the hippocampus at 6 h of reperfusion after 10 min of ischemia. This is shown in Fig. 1. The amount of EB was low in the control group (cortex, 9.5 ± 0.6 μg EB/g tissue; hippocampus 9.8 ± 0.7). After 6 h of reperfusion, the amount of EB in the experimental (EXP) group was 4.5 times higher (42.9 ± 4.7) in the cortex and 6 times higher (61.6 ± 9.2) in the hippocampus. SAM treatment returned EB levels to near control levels (cortex, 19.8 ± 1.4; hippocampus, 20.7 ± 1.5). These results indicate that SAM treatment attenuated the ischemia-induced BBB breakdown by ~70% in the cortex and ~79% in the hippocampus.

Neuronal death after ischemia: effect of SAM. The effect of SAM on the CA1 region of the hippocampus of gerbils subjected to transient ischemia was assessed (Fig. 2). Gerbils subjected to ischemia showed degeneration of 75% neurons in the CA1 region of the hippocampus. SAM treatment dose dependently showed significant improvement. SAM, at the doses of 60 and 120 mg/kg, improved the neuronal counts by ~43% and ~63% respectively over the EXP group.

We now report the protective effects of SAM on ischemia-induced BBB breakdown in the cortex and hippocampus and confirm previous findings that treatment of gerbils with SAM protects CA1 hippocampal neurons from delayed degeneration after transient cerebral ischemia [14,22].

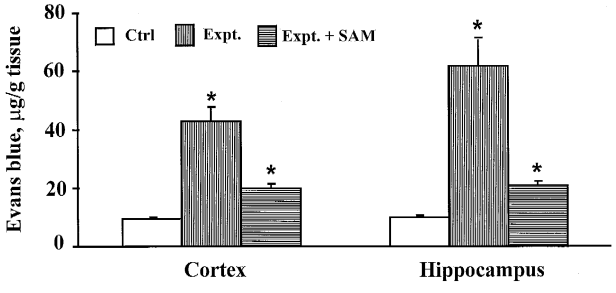

Fig. 1. Regional changes of BBB breakdown and effect of SAM on post-ischemic changes in BBB breakdown in transient ischemia produced in gerbils subjected to 10 min of bilateral carotid artery occlusion followed by 6 h reperfusion. Values are mean ± S.E.M. ($n = 6$). * $P <$ 0.05 compared with control animals.

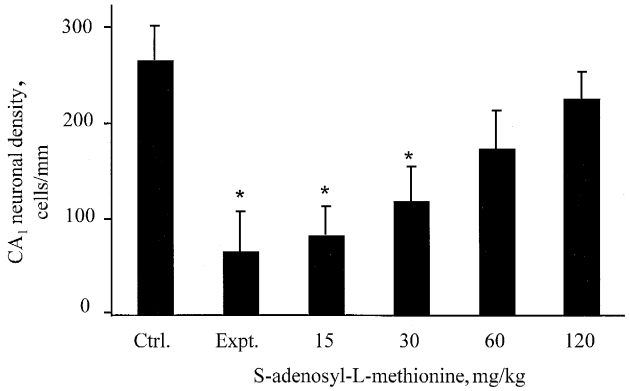

Fig. 2. Neuronal counts in the CA1 region of the hippocampus and effect of SAM on neuronal survival after transient ischemia followed 7 days of reperfusion in gerbils. Values are mean ± S.E.M. ($n = 6$). * $P < 0.05$ compared with control animals.

An early increase in putrescine biosynthesis is a characteristic of brain regions sensitive to neuronal degeneration [10,16,18]. These findings have suggested that the increase in putrescine content might have played a role in ischemia-induced delayed neuronal death [16–19]. Gilad and Gilad [3] demonstrated that polyamine treatment, including putrescine, can protect forebrain neurons from degeneration, and α-difluoromethylornithine (DFMO), an inhibitor of ODC could not protect CA1 hippocampal neurons after ischemia. A recent study utilizing transgenic mice overexpressing the human ODC gene also suggested that ODC activation and subsequent accumulation of endogenous putrescine are not neurotoxic [30]. These results indicated that the increased ODC activity and accumulation of endogenous putrescine are indicative of an ischemic insult and that these changes reflect an adaptive response rather than acting as causative factors of neuronal damage [30]. Another alternative rationale speculates that the increased ODC activity and putrescine levels in the post-ischemic brain may be an indication of a plastic response [30]. On the contrary, studies from our laboratory and others [10,20] showed that DFMO treatment reduced the edema formation [20] and protected the CA1 hippocampal neurons [10] and reduced the BBB breakdown [23], and infarct volume [15] in transient and focal ischemia models. DFMO treatment in combination with putrescine elevated the putrescine levels in CA1 region and increased the CA1 neuronal death [10]. DFMO treatment alone attenuated the BBB breakdown, and putrescine in combination with DFMO nullified the DFMO protective effect in focal cerebral ischemia [23] and cryo injury models [12]. Evidence suggests that any activation of polyamine synthesis (a sharp increase in putrescine levels) in brain may trigger BBB breakdown [12]. Paschen [18] has shown an independent relationship between ODC and putrescine levels suggesting that besides ODC activation, inhibition of SAMDC may contribute to the increase in putrescine content. Since SAMDC is necessary for the synthesis of spermidine and spermine from putrescine, a low SAMDC activity is considered to influence putrescine levels [6].

The other supportive evidence is the finding that treatment with SAM can protect neurons from ischemia-induced degeneration [14,22]. Besides being a general methyl donor [7], SAM is also source of a propylamine group for spermidine and spermine following its decarboxylation by SAMDC [18]. After ischemia, SAMDC activity decreases, which may be a cause for the observed lack of increase in spermidine and spermine. An increased supply of the substrate SAM after treatments with exogenous SAM may therefore lead to increased production of spermidine and spermine in vivo. This suggests that administering exogenous polyamines (or their biological precursors) should lead to increased blood levels of these metabolites and make them available to the neurons. Some studies have provided evidence for functional recovery in damaged nerves after polyamine administration [3,24].

Spermine influences the calcium buffering capacity of mitochondria by considerably reducing the set point at which the calcium ions are taken up [21]. Thus a decrease in spermine levels after cerebral ischemia [16] may disturb the intracellular calcium homeostasis by increasing the set point for the uptake of calcium ions by mitochondria. The activation of polyamine metabolism after transient ischemia reduces the polyamine levels in the severely injured areas and depletes the polyamines into the blood through the injured neurons [19]. This may be partly responsible for the disturbances in BBB observed in severely injured areas after ischemia and reperfusion. Several studies provided evidence that the polyamines spermine and spermidine modulate signals mediated through the NMDA receptor complex [4,28] by interacting at the polyamine binding site and induce pathological effects.

Table 1
Physiological variables before, during and 30 min after bilateral carotid artery occlusion of gerbils

Treatment	Temperature			MABP [b]		
	Before ischemia	During ischemia	30 min after ischemia	Before ischemia	During ischemia	30 min after ischemia
Control	37.1 ± 0.1	37.1 ± 0.1	37.3 ± 0.3	58 ± 2	67 ± 6	59 ± 7
EXP	37.2 ± 0.3	37.4 ± 0.3	37.6 ± 0.2	64 ± 5	107 ± 5 [a]	66 ± 7
EXP + SAM	37.1 ± 0.1	37.1 ± 0.3	37.4 ± 0.4	81 ± 6	128 ± 11 [a]	91 ± 9

Data are presented as mean ± S.E.M. ($n = 8$); [a] $P < 0.05$ compared to pre-ischemic group; [b] MABP: mean arterial blood pressure; arterial blood gases were maintained within normal limits (p_aO_2: 75–100 mmHg; p_aCO_2: 25–40 mmHg).

Sato et al. [22] reported the protective effects of SAM on CA1 hippocampal neurons and attributed these protective effects of SAM to activation of the transmethylation process. Matsui et al. [14] also reported the prevention of ischemic hippocampal CA1 neuronal death by SAM, but did not fully support the SAM protective effects through a transmethylation mechanism. However, in all these studies, mannitol was used as a filler solution which might have some confounding effects. Harada et al. [5] showed the protective effects of *S*-adenosyl-L-homocysteine (SAH), SAM, spermine and a spermine analog N^1,N^{12}-bis(ethyl)-spermine (BESPM) in cerebellar granule neurons and suggested that either glutathione and/or polyamine biosynthetic pathways but not transmethylation process are responsible for the protective effects.

Kozuka et al. [13] attributed that the SAM ameliorating effect on brain edema was partly due to improvement of the microcirculation in the ischemic brain including the cortex and hippocampus. Our present study shows that significant BBB breakdown occurs in the hippocampus region and loss of pyramidal neurons in the CA1 region of the hippocampus. Hippocampal CA1 neurons are susceptible to delayed neuronal death to ischemia and reperfusion [11] and, in the present study SAM attenuated the delayed neuronal degeneration.

It is conceivable that part of the protective effect of spermine during nerve degeneration processes is due to the activation of calcium uptake which holds intracellular calcium to levels that promote growth without producing toxic effects [1]. The present study also suggests that exogenous treatment with SAM leads to increased production of spermidine and spermine in vivo which may be playing a role in attenuating the BBB breakdown and hippocampal CA1 neuronal death. This report shows the protective effects of SAM on ischemia-induced BBB breakdown in the cortex and the hippocampus and neuronal degeneration in the CA1 region of the hippocampus in the gerbil model of transient ischemia. This confirms the previous observations made by other groups [14,22]. These observations suggest the need for further studies into the protective effect of SAM after an ischemic event.

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