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—Effect of S-Adenosyl-L-Methionine on Postischemic Brain Injury
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Introduction

In use of positron-emitters produced by cyclotron, the path for multi-tracer autoradiography(ARG) opened rapidly. The multi-tracer ARG is possible to acquire several kinds of images in the same brain slices by applying the differences on the half-life times of the nuclei. In our laboratory, the double-tracer ARG is mainly performed using ^{14}C -Iodoantipyrine as the tracer of cerebral blood flow and ^{18}F -Fluorodeoxyglucose as the tracer of glucose utilization. By using this technique, ^{31}P -NMR spectrometry and biochemical assay of energy metabolites, we estimated the effect of S-Adenosyl-L-methionine(SAM) on the energy metabolism and the microcirculation of postischemic brain. And we examined the distribution of ^{11}C -SAM in gerbil brain by ARG.

Methods and Materials

Chemically stable salt of S-adenosyl-L-methionine (FO-1561) were dissolved with 0.18M disodium phosphate(Solvent) and neutralized before use.

INTRODUCTION OF CEREBRAL ISCHEMIA

Male wistar rats (250-300g) were anesthetized with pentobarbital (40mg/kg i.p), then their vertebral arteries were electrically cauterized (Pulsinelli and Brierley, 1979). The animals were allowed to recover for 24 hours until they were used for the experiments described bellow.

After anesthesia with diethyl ether, the rats were given pancronium bromide (0.5mg/kg i.p), tracheotomized, and mechanically ventilated by Rodent respirator (Harvard). The right femoral artery was cannulated for monitoring blood pressure and periodic sampling of arterial blood gases and pH, and the right femoral vein was also cannulated for the administration of drug and radioisotopes. Besides, needle electrodes were settled on the carvarium to record electroencephalograms(EEGs). PaO_2 , PaCO_2 and pH were maintained within $100\pm 10\text{mmHg}$, $38\pm 4\text{mmHg}$ and 7.35 ± 0.05 respectively by adjusting the tidal volume at the respiratory rate of 56/min. Rectal temperature was maintained at 37°C by heating mats.

The animals were heparinized (1000units/kg) and subjected to a whole brain ischemia by temporary clipping of both common carotid arteries for 60min. We used only animals that the EEGs became isoelectric within one minute after four-vessel occlusion and that the mean blood pressure was not lower than 100mmHg. Afterwards, both carotid clips were removed and SAM or its solvent was administered intravenously at the dosage of 0, 30 or 100mg/kg; the volume of injection was 0.2ml. And then 60 min of recirculation was performed. This experimental system is abbreviated to 60-60 SAM(or Solvent).

DOUBLE-TRACER AUTORADIOGRAPHY

In this experimental system, two radioisotopes were given intravenously: ^{18}F -FDG (2mCi) at 30 min and ^{14}C -IAP (10 μ Ci) in just one minute from 59min after recirculation. As soon as the administration of ^{14}C -IAP was completed, the rat was decapitated. Then the brain was removed, frozen in powdered dryice and coronally at 30 μ m in thickness in a cryostat(-20°C). Less than 20 min after the decapitation, the slices were contacted to a X-ray film for 8 hours to acquire the images of glucose uptake. At an interval of 48 hours for being decayed out ^{18}F (half-life time: 109.8 min), the next X-ray film was contacted for 14 days to acquire the images of cerebral blood flow(CBF).

ASSAY OF ENERGY METABOLITES

After 60 min of recirculation, the brain was frozen in situ by trans-calvarial application of liquid nitrogen while the animal was ventilated (Pontén et al., 1973). The frozen cortical tissue was homogenized with perchloric acid, and the homogenate was centrifuged. The clear supernatant was used for assays after neutralization. Phosphocreatine(PCr), glucose, glucose-6-phosphate(G-6-P), lactate and pyruvate were assayed by the enzymatic fluorometry (Lowry and Passonneau, 1972). Adenine nucleotides(ATP, ADP and AMP) were assayed by the anion-exchange HPLC according to the method of Onodera and Kogure (in preparation). Statistical differences between means were determined by Student's t-test.

^{31}P -NMR SPECTROMETRY

The brain was frozen in situ in the same way. Then it was powdered and put into a NMR tube(10mmi.d). ^{31}P -NMR spectrograms were obtained on Bruker CXP-300 FT-NMR spectrometer at 121.5MHz, collecting 6000 transients at -5°C.

AUTORADIOGRAPHY STUDY OF ^{11}C -SAM IN GERBIL BRAIN

^{11}C -SAM was synthesized enzymatically from ^{11}C -methionine and ATP, and it was purified by SP-Sephadex C-25 cation exchange gel column (radiochemical purity: 98.0%).

After anesthesia with pentobarbital (40mg/kg i.p) and heparinization (1000units/kg), male mongolian gerbil (80g) was subjected to right hemispheric ischemia for 30 min by temporary clipping of right common carotid artery. Then carotid clip was removed, ^{11}C -SAM(5mCi) was given intravenously and 30 min of recirculation was performed. Subsequent procedures were the same as those of the double-tracer ARG.

Results

Fig. 1 shows the ARGs of ^{18}F -FDG and ^{14}C -IAP. Without treatment of SAM, glucose uptake and CBF were not recognized at all. In contrast, fair improvements were seen in both ARG images by the drug treatment: the glucose uptake almost recovered, the regional distribution of ^{14}C -IAP recovered heterogeneously as compared with that in a normal brain. The improvement of glucose uptake was confirmed in the assayed values of glucose and G-6-P (Table 2).

The assayed values of adenine nucleotides and other energy metabolites are shown in Table 1 and 2. Most of metabolites tend to recover significantly by the drug treatment. Moreover, ATP, EC, lactate and La/Py in the high-dose treatment are different significantly from those in the low-dose treatment. And EC almost shows the normal value in the high-dose treatment, though the total adenine nucleotides indicated only a little increase.

Representatives of NMR spectrograms are illustrated in Fig. 2. The spectrograms are obviously different in the case that the drug treatment has done or not. Without the drug treatment, the spectrogram shows no PCr, no ATP and very large inorganic phosphate (Pi) signal, and the Pi chemical shift, which is greatly influenced by pH, differs evidently from that in the SAM-treated animal. This indicates the acidosis and it is confirmed by the lactate values in Table 2, because acidosis is mainly induced by the accumulation of lactate. Besides, the drug treated groups have similar signals with the normal group, showing the recovery of PCr and ATP.

Fig. 3 shows the ARG of ^{11}C -SAM in gerbil brain. The uptake of ^{11}C -SAM was very little in 30 min after the administration.

Discussion

A whole brain ischemia is possible to be made with small individual differences by the occlusion of four major arteries, if the isoelectric changes of EEGs are monitored. According to our pilot experiment using ^{31}P -NMR spectrometry in this stroke model (Izumiya, 1983; Ohtomo, in press), the energy metabolism was able to recover by postischemic recirculation, if the ischemic period was less than 30 min. However, when the brain was submitted to 60 min of ischemic insult, no recovery was recognized during 60 min of recirculation. But the schedule of 60-60 was performed, the cerebral energy metabolism was possible to be recovered by the administration of drugs, for example, glycerol and mannitol (hyperosmotic agents). Therefore, the model of 60-60 is suitable for the estimation of drug effects to the ischemic brain injury, so we used this model in this study.

The central problem of this study is whether SAM is effective on postischemic brain injury. Fortunately, we could obtain the results that it was effective. However, we can't explain clearly how SAM affects the ischemic brain, because there are very few reports about the problem. But we supposed two possibilities. One possibility is the improvement of cerebral microcirculation by the enhancement of erythrocyte deformability. Hirata and Axelrod

(1978a,b) have reported as follows. Phospholipid distribution is asymmetrical in bilayers of erythrocyte membranes, phosphatidylcholine(PC) being mainly located on exterior side of membrane, whereas phosphatidylethanolamine(PE) principally on the interior surface. When the enzymatic methylation of PE to PC takes place in which SAM takes part as a methyl doner, the phospholipids transfer rapidly from the inside to outside of the membrane. This phospholipid rearrangement causes the increase of the membrane fluidity. Therefore, it is considered that the erythrocyte deformability might be enhanced by this event. In fact, Artale et al.(1982) have reported in the hemorheological study that SAM could affects the erythrocyte flexibility in the condition that the deformability was reduced by the exercise-induced hyperlactacidemia metabolically. They supposed that SAM might enhance the erythrocyte deformability by decreasing the hyperlactacidemia and by the increase of the membrane microfluidity due to the phospholipid methylation. Besides, another possibility is the facilitation of the metabolic reactions related to SAM. Since SAM is synthesized from ATP and L-methionine in living cells, it is expected that the endogenous SAM level would be reduced by ATP depletion owing to ischemia. Therefore, this metabolic failure should be improved by the treatment of SAM. And it has been reported that polyamines, important metabolite of SAM, might enhance glycolysis, protein synthesis, and so on. For these reasons, the cerebral energy metabolism might be improved by the treatment of SAM. But the important problem is a poor permeability of SAM to the blood-brain barrier(BBB), which agrees with our result in ARG study of ¹¹C-SAM(tracer dose). However, Strametinoli et al.(1978) have shown that though SAM given in relatively small doses crossed the BBB only slowly and sparingly, SAM accumulates in brain tissue to exceed control values by as much as 100% after the administration of relatively large doses of the substance. And Placidi et al.(1978) have supposed in the ARG study using ¹⁴C-SAM that SAM might reach the brain via the cerebrospinal fluid, because the radioactivity accumulated in the choroid plexuses and hippocampus. Moreover, the drug might pass through the BBB in postischemic brain owing to the destruction of the BBB by the ischemic insult. Since our results show the dose-dependent effect in ATP, EC, lactate and La/Py, SAM and/or its metabolites might affect directly the energy metabolism of the postischemic brain. Anyway, we hope to resolve how SAM could affect.

To summarize our interpretation of the results, we can explain that SAM could improve the microcirculation in the post-ischemic brain and that it could enhance the energy state of the ischemic brain.

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	ATP	ADP	AMP	Σ Ad nucl.	EC
Normal (n = 5)	2.83 ± 0.22	0.239 ± 0.008	0.0190 ± 0.0011	3.09 ± 0.23	0.955 ± 0.002
60-60 Solvent (n = 5)	0.111 ± 0.026	0.168 ± 0.038	1.04 ± 0.20	1.32 ± 0.24	0.148 ± 0.017
60-60 SAM(30mg/kg) (n = 6)	0.929* ± 0.441	0.216 ± 0.079	0.331* ± 0.314	1.59 ± 0.15	0.683* ± 0.222
60-60 SAM(100mg/kg) (n = 5)	1.95*▲▲ ± 0.48	0.216 ± 0.031	0.0762* ± 0.0979	2.25*▲ ± 0.46	0.913*▲▲ ± 0.056

Table 1. Cerebral concentrations of adenine nucleotides and energy charge. Values are means±SD in μmol/g wet weight except for EC.
 Σ Ad nucl. = ATP + ADP + AMP
 EC = (ATP + 0.5ADP) / (ATP + ADP + AMP) by Atkinson (1968)
 * Different from 60-60 Solvent (Control), with p 0.01.
 ▲,▲▲Different From 60-60 SAM(30mg/kg) : ▲, p<0.05 ;▲▲, p<0.01.

	PCr	Glucose	G-6-P	Pyruvate	Lactate	La/Py
Normal (n = 5)	4.42 ± 0.28	3.69 ± 0.25	0.169 ± 0.026	0.133 ± 0.024	1.96 ± 0.41	11.8 ± 1.3
60-60 Solvent (n = 5)	0.408 ± 0.127	0.144 ± 0.032	0.0316 ± 0.0201	0.0651 ± 0.0403	35.5 ± 3.4	509 ± 98
60-60 SAM(30mg/kg) (n = 6)	2.15** ± 0.99	3.46** ± 1.18	0.241** ± 0.077	0.150** ± 0.018	18.6** ± 8.2	127** ± 53
60-60 SAM(100mg/kg) (n = 5)	2.98** ± 0.84	3.17** ± 0.62	0.224** ± 0.084	0.146* ± 0.052	6.35**▲ ± 2.79	45.6**▲ ± 14.4

Table 2. Cerebral concentrations of energy metabolites and Lactate:Pyruvate ratio (La/Py). Values are means±SD in μmol/g wet weight except for La/Py.
 *,**Different from 60-60 Solvent (Control) : *, p<0.05 ;**, p<0.01.
 ▲ Different From 60-60 SAM(30mg/kg), with p<0.01.

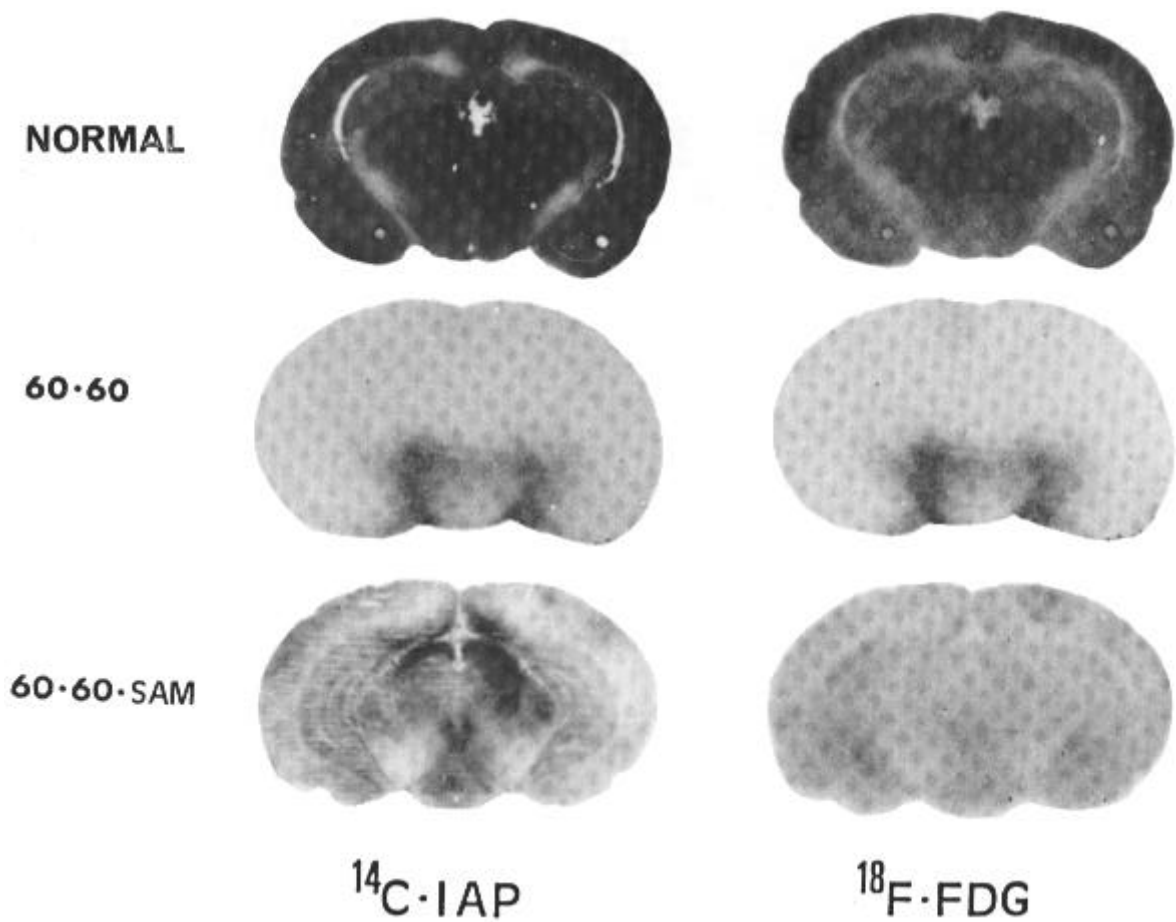


Fig. 1. The autoradiograms of $^{14}\text{C}\cdot\text{IAP}$ and $^{18}\text{F}\cdot\text{FDG}$ by double tracer autoradiography.

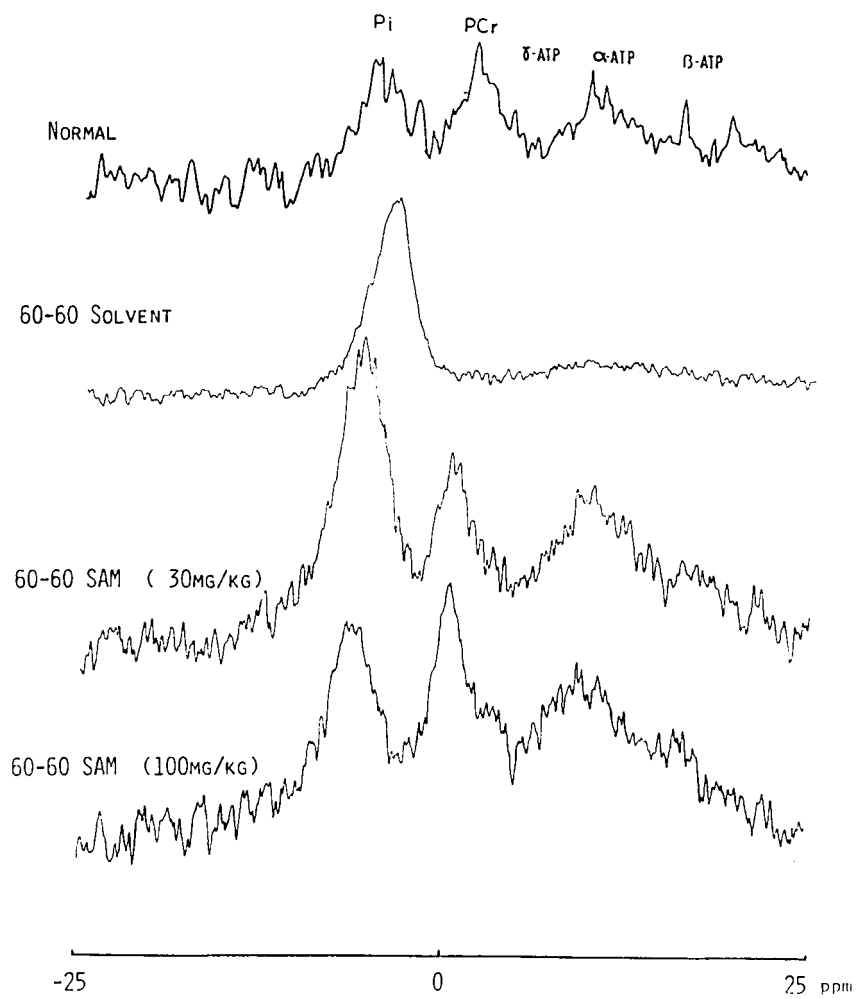


Fig. 2. Representatives of ^{31}P -NMR spectrograms.

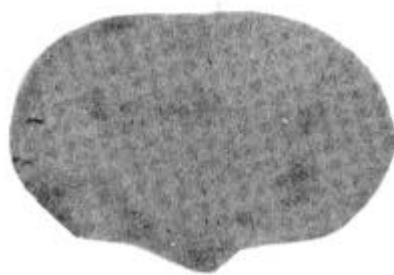


Fig. 3. The autoradiogram of ^{11}C -SAM in gerbil brain.