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Preliminary evaluation of [1-^{11}C]octanoate as a PET tracer for studying cerebral ischemia: A PET study in rat and canine models of focal cerebral ischemia

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Octanoate is taken up into the brain and is converted in astrocytes to glutamine through the TCA cycle after β-oxidation. We speculate that [1-^{11}C]octanoate may be used as a tracer for astroglial functions and/or fatty acid metabolism in the brain and may be useful for studying cerebral ischemia. In the present study we investigated brain distribution of [1-^{11}C]octanoate and compared it with cerebral blood flow (CBF) by using rat and canine models of middle cerebral artery (MCA) occlusion and a high resolution PET. In rats brain distribution of [^{15}O]H_2O measured 1–2 h and 5–6 h after insult was compared with that of [1-^{11}C]octanoate measured 3–4 h after insult. Radioactivity ratios of lesioned to normal hemispheres determined with [^{15}O]H_2O were lower than those determined with [1-^{11}C]octanoate. These results were confirmed by a study on a canine model of MCA-occlusion. Twenty-four hours after insult, CBF decreased in the MCA-territory of the occluded hemisphere, whereas normal or higher accumulation of [1-^{11}C]octanoate was observed in the ischemic regions. The uptake of [1-^{11}C]octanoate-derived radioactivity therefore increased relative to CBF in the ischemic regions, indicating that [1-^{11}C]octanoate provides functional information different from CBF. In conclusion, we found that [1-^{11}C]octanoate is a potential radiopharmaceutical for studying the pathophysiology of cerebral ischemia.

Key words: [1-^{11}C]octanoate, positron emission tomography, cerebral ischemia, rat, dog

INTRODUCTION

ISCHEMIC STROKE is one of the most common neuronal disorders and diagnostic imaging of the disease is one of the most important subjects in nuclear medicine, but the clinical use of positron emission tomography (PET) for the assessment of pathophysiological changes in the disease has been limited exclusively to the determination of cerebral blood flow (CBF) and oxygen and glucose metabolism.[1] This limitation is caused by the lack of radiopharmaceuticals suitable for diagnostic imaging of the pathophysiology of ischemic stroke. Increasing interest has recently been focused on interactions between glial cells and neurons. It is now a matter of the greatest importance to elucidate the glial functions in the pathophysiology of cerebral ischemia, and the development of new PET tracers for studying glial functions may be useful in the diagnosis of cerebral ischemia. In this regard, several peripheral-type benzodiazepine receptor (ω_3) antagonists have been positron labeled[2,3] and their utility has been reportedly demonstrated.[4,5]

Octanoate, an 8-carbon monocarboxylic acid, readily enters the brain.[6–8] Acetyl-CoA released from the first

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Table 1 Asymmetry indices of radioactivity in the brain after injection of $[^{15}O]H_2O$ or [1-^{11}C]octanoate in rats with or without focal cerebral ischemia

Animal	Tracer	Time after MCA-occlusion	AI* (Mean ± SD)
Normal rats	$[^{15}O]H_2O$	—	1.02 ± 0.03
	[1-^{11}C]octanoate	—	1.00 ± 0.02
MCA-occluded rats	$[^{15}O]H_2O$	1.3–2.3 h	0.88 ± 0.04[a,b]
	[1-^{11}C]octanoate	3.3–4.3 h	0.95 ± 0.03
	$[^{15}O]H_2O$	4.9–5.9 h	0.93 ± 0.05[a]

*Asymmetry index (right/left hemisphere). a: $p < 0.05$, vs. normal rats ($[^{15}O]H_2O$), b: $p < 0.05$, vs. [1-^{11}C]octanoate (MCA-occluded rats).

acetyl moiety by β-oxidation is rapidly metabolized to glutamine through the tricarboxylic acid (TCA) cycle and a small pool of glutamate.[9] In the brain, astrocytes, but not oligodendrocytes or neurons, are capable of metabolizing octanoate by β-oxidation.[10–12] Glutamine synthetase, an enzyme that catalyzes the conversion of glutamate to glutamine, is localized predominantly in astrocytes.[13] Consequently, [1-^{11}C]octanoate may be applicable as a radiopharmaceutical for studying astroglial functions with PET. We previously evaluated its brain uptake and metabolism in rats,[14] brain distribution in cats,[15] and uptake in astrocytoma cells.[16] These studies indicated its utility as a PET tracer for assessing glial functions and/or fatty acid metabolism.

On the other hand, Ishiwata et al. and Sakiyama et al. recently examined the potential of [1-^{11}C]hexanoate as a radiopharmaceutical for assessing fatty acid metabolism in biodistribution studies in mice[17,18] and cats.[19] They concluded that it would be difficult to evaluate fatty acid metabolism by the total radioactivity uptake or clearance in the cat brain with [1-^{11}C]hexanoate,[19] because peripherally originating $[^{11}C]CO_2/HCO_3^-$ was redistributed into the brain. The utility of medium-chain fatty acids labeled with a positron emitter is therefore still controversial and remains to be elucidated.

The purpose of the present study was to evaluate the utility of [1-^{11}C]octanoate as a PET tracer for studying cerebral ischemia, by comparing its distribution in the brain with CBF in rat and canine models of focal cerebral ischemia.

MATERIALS AND METHODS

[1-^{11}C]Octanoate

[1-^{11}C]Octanoic acid was synthesized by a Grignard reaction of $[^{11}C]CO_2$ and heptylmagnesium bromide[20] in an automated synthesis apparatus (CUPID, Sumitomo Heavy Industries, Tokyo, Japan). The radiochemical purity of [1-^{11}C]octanoic acid obtained was > 97% as determined by HPLC on a reverse-phase column (COSMOSIL 5C18-AR, 150 mm × 4.6 mm i.d.; NACALAI TESQUE, Kyoto, Japan) with CH_3CN/0.12 N HCl (55 : 45, v/v).

PET Scanner

PET studies were performed with a multi-slice positron emission computed tomography apparatus (ECAT EXACT HR/47, Knoxville, TN).[21] The scanner provides 47 tomographic images at 3.1-mm intervals per frame. The transaxial and axial resolutions of the scanner were 3.7 mm and 4.1 mm, respectively, at full width at half maximum (FWHM) for a point source at the center of the field of view.

Animal Preparations

Ten male Sprague-Dawley rats weighing 290 to 344 g and a male beagle dog weighing 11.7 kg were used. All procedures were performed in accordance with an institutional guideline, IBR Guidelines for the Care and Use of Laboratory Animals.

The rats were fasted overnight before the experiment but allowed free access to water. Those selected for inducement of focal cerebral ischemia were anesthetized with 400 mg/kg body weight i.p. chloral hydrate. The ostium of the right middle cerebral artery (MCA) of each rat was occluded intraluminally by a method described in detail previously.[22,23] MCA-occluded and normal rats were prepared by venous and arterial catheterization under chloral hydrate anesthesia for subsequent PET studies.

The dog was fasted overnight before the experiment but allowed free access to water. We used an experimental thromboembolic stroke model reported by De Ley, et al.[24] with slight modifications. Briefly, the animal was anesthetized with pentobarbital (10 mg/kg body weight i.v.) after premedication with xylazine (2 mg/kg body weight i.v.) and implanted with a cronic catheter in the left internal carotid artery. The catheter, filled with heparin solution, was tunneled subcutaneously through the neck toward the mid-dorsal region. The animal was allowed to recover and was observed before embolization to ensure that no neurological deficit had occurred due to the operation. Twenty-four hours after the operation, acute obstruction of the MCA was obtained by injecting a single autologous blood clot into the internal carotid artery through the carotid catheter and its neurological deficits were confirmed.

PET Studies in Rats

Each anesthetized rat was placed in the PET scanner in the prone position. The anesthetized state was maintained with 0.5% to 2.0% halothane delivered in an air-oxygen gas mixture through a face mask. $[^{15}O]H_2O$ (185 to 370 MBq/kg body wt., n = 3) or [1-^{11}C]octanoate (87 to 94 MBq/kg body wt., n = 4) was administered to normal rats via the venous catheter. Dynamic PET scanning (six 5-sec, three 10-sec, and three 20-sec frames for $[^{15}O]H_2O$

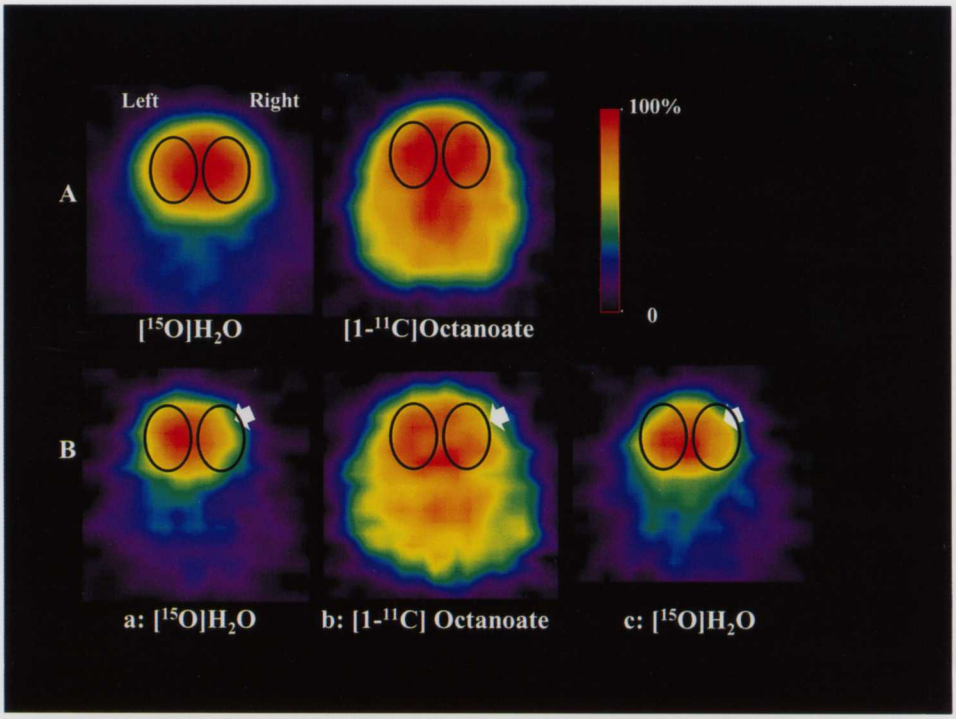

Fig. 1 Representative images of radioactivity and regions of interest (ROIs) for a coronal slice (hippocampus level) of the brain after the injection of $[^{15}O]H_2O$ (0–2 min) or [^{11}C]octanoate (5–15 min) in rats with or without focal cerebral ischemia. A: Normal rats, B: MCA-occluded rats 1–2 h (a), 3–4 h (b), and 5–6 h (c) after MCA-occlusion. Arrows show reduced radioactivity in the lesioned (right) hemisphere.

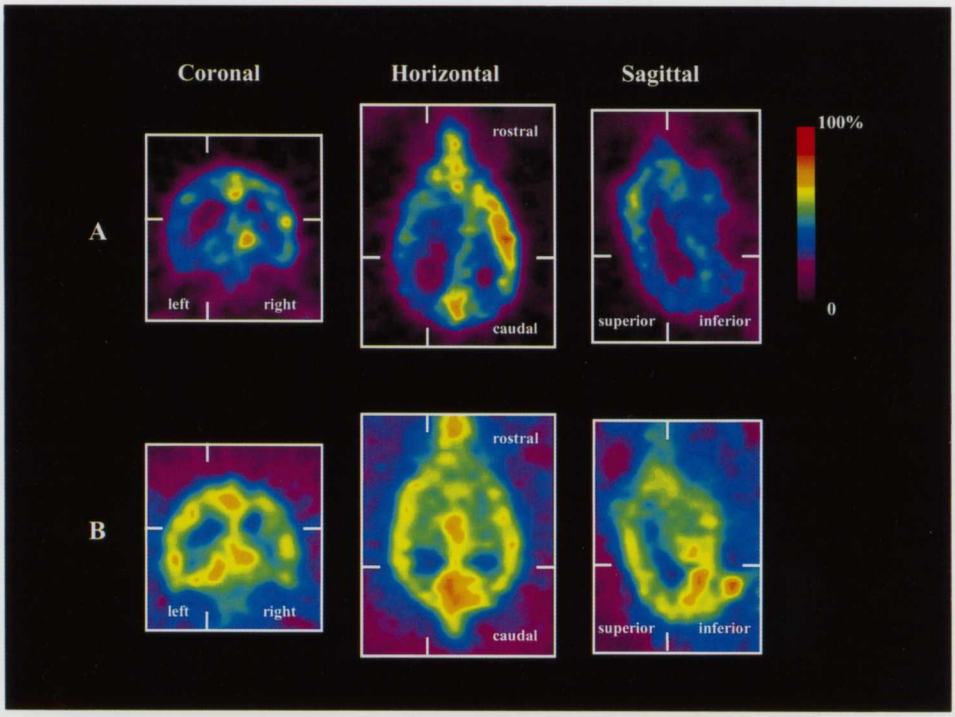

Fig. 2 Three dimensional images of CBF (A) and brain distribution of [^{11}C]octanoate-derived radioactivity (B, 5–15 min after the injection) in a dog 24 h after MCA obstruction.

and four 15-sec, four 1-min, nine 5-min, and one 10-min frames for [1-^{11}C]octanoate) was started at the time of tracer injection. PET measurements were performed three times on each MCA-occluded rat (n = 3). [^{15}O]H_2O (185 MBq/kg body wt.), [1-^{11}C]octanoate (85 to 96 MBq/kg body wt.), and [^{15}O]H_2O (185 MBq/kg body wt.) were administered 1 to 2 (1.8 ± 0.5), 3 to 4 (3.7 ± 0.5), and 5 to 6 (5.2 ± 0.6) hours after MCA-occlusion, respectively.

PET Studies in Dog

Twenty-four hours after clot injection, the dog was re-anesthetized by halothane inhalation and then artificially ventilated. The anesthetized state was maintained with 0.5% to 2.0% halothane delivered in a 70% N_2O/30% O_2 gas mixture during the experiment. Catheters were introduced into the right femoral artery and vein for subsequent experiments. The anesthetized dog was mounted on a custom-made stereotaxic instrument in the supine position, and the head was restrained by tooth- and ear-bars. The brain position was standardized with the aid of laser beams. [^{15}O]H_2O (63 MBq/kg body wt.) was administered via the venous catheter. The right arterial catheter was placed in an automatic blood sampling system detector (Shimadzu, Kyoto, Japan) for continuous measurement of radioactivity in blood. Dynamic PET scanning (six 5-sec, three 10-sec and three 20-sec frames) was started at the time of [^{15}O]H_2O injection. Immediately after the radioactive decay of [^{15}O]H_2O, [1-^{11}C]octanoate (83 MBq/kg body wt.) was administered via the venous catheter. Dynamic PET scanning (four 15-sec, four 1-min, nine 5-min and one 10-min frames) was started at the time of [1-^{11}C]octanoate injection. Immediately after completion of the PET-scanning, the brain was perfused with ice cold saline and the dog was sacrificed by exsanguination under pentobarbital anesthesia. The brain was quickly removed, sectioned coronally at 5-mm intervals, and incubated in a 1% solution of TTC (2,3,5-triphenyltetrazolium chloride) at 37°C for vital staining. Areas not stained red with TTC were considered lesioned.

Data Analysis

PET images were reconstructed according to a standard filtered back-projection procedure with a Ramp (rat) or Hanning (dog) filter.

In rats, summed radioactivity images taken 0 to 2 min after [^{15}O]H_2O injection were used as an index of CBF. Based on our previous results for the pharmacokinetics of [1-^{11}C]octanoate,[14,15] PET-images obtained 5 to 15 min after [1-^{11}C]octanoate injection were used for the evaluation of [1-^{11}C]octanoate distribution in the brain. A semielliptical region of interest (ROI, 145 mm^3) was placed on the left hemisphere of a coronal radioactivity image at the level of the hippocampus. The mirror image of the ROI was placed on the corresponding right hemisphere. Asymmetry indices (AIs), defined as the ratios of radioactivity for ROIs in the right hemisphere to those for the contralateral ROIs, were used for the data analysis. A paired t-test was used to assess the significance of differences between the AIs obtained with [^{15}O]H_2O and those obtained with [1-^{11}C]octanoate. An unpaired t-test was used for comparison of AIs for normal and ischemic brains. A two-tailed probability value less than 0.05 was considered significant.

In the dog experiment, CBF images were calculated on the basis of diffusible autoradiographic method,[25] and summed radioactivity images obtained 5 to 15 min after [1-^{11}C]octanoate injection were used for the evaluation of [1-^{11}C]octanoate distribution in the brain.

RESULTS

Studies in Rats

Figure 1 shows representative images of radioactivity for a coronal slice (hippocampus level) of the normal and MCA-occluded rat brain after the IV injection of [^{15}O]H_2O or [1-^{11}C]octanoate. As shown in Fig. 1A, the [^{15}O]H_2O- and [1-^{11}C]octanoate-images in the normal rats showed no obvious asymmetry. In the [^{15}O]H_2O-image of the MCA-occluded rats (Fig. 1B), radioactivity was lower in the lesioned (right) than in the normal (left) hemisphere. After the injection of [1-^{11}C]octanoate, a slight radioactivity reduction was observed in the lesioned (right) hemisphere of the MCA-occluded rats (Fig. 1B).

The AIs are summarized in Table 1. In the normal rats, the AIs were 1.02 ± 0.03 for [^{15}O]H_2O and 1.00 ± 0.02 for [1-^{11}C]octanoate. The AIs for [^{15}O]H_2O in the MCA-occluded rats were 0.88 ± 0.04 and 0.93 ± 0.05 at 1 to 2 and 5 to 6 hours after the injection of [^{15}O]H_2O, respectively. These AIs were significantly lower than those in the normal rats ($p < 0.05$). The AI for [1-^{11}C]octanoate in the MCA-occluded rats (0.95 ± 0.03, 3 to 4 h after MCA-occlusion) was slightly lower than in the normal rats. In the MCA-occluded rats, the AI for [1-^{11}C]octanoate (0.95 ± 0.03 at 3–4 h after MCA-occlusion) was significantly higher ($p < 0.05$) than that for [^{15}O]H_2O 1 to 2 h after MCA-occlusion and slightly, but not significantly, higher than that for [^{15}O]H_2O 5 to 6 h after MCA-occlusion.

Studies in the Dog

Three-dimensional CBF and [1-^{11}C]octanoate-images are shown in Figure 2. CBF decreased notably in the MCA-territory of the lesioned (left) hemisphere. On the other hand, normal or higher uptake of [1-^{11}C]octanoate-derived radioactivity was detected in the regions where CBF reduction was observed. No TTC-unstained lesions were detected in the coronal slices, except for a few small lesions (< 3 mm in diameter) in the putamen, hippocampus and white matter at the nucleus caudatus level.

DISCUSSION

The development of high-resolution PET scanners[21,26]

has made it feasible to apply PET to studies in rats (Rat-PET). Ingvar et al.[27] reported Rat-PET as a rapid screening method for the evaluation of kinetic properties of PET-tracers. By using high-resolution PET scanners, it was also shown that detection of reduced cerebral metabolic rates of glucose (CMRglc) in rats with focal cerebral ischemia is possible,[28] although extracranial radioactivity prevents quantitative evaluation of CMRglc.[29,30]

We applied the Rat-PET technique to the evaluation of a new tracer, [1-^{11}C]octanoate, in rats with focal cerebral ischemia. The brain distribution of [^{15}O]H_2O measured 1 to 2 hours and 5 to 6 hours after MCA-occlusion was compared with that of [1-^{11}C]octanoate-derived radioactivity measured 3 to 4 hours after the MCA-occlusion. The AIs determined with [^{15}O]H_2O were lower than those with [1-^{11}C]octanoate, indicating that in the ischemic region the uptake of [1-^{11}C]octanoate increased relative to CBF (Table 1). These results suggest that [1-^{11}C]octanoate is a potential imaging agent for studying the pathophysiology of cerebral ischemia. The present experiment had some limitations, due to CBF changes during the three PET measurements and the coarse spatial resolution of the PET scanner as compared with the target size. The AI for [1-^{11}C]octanoate (3–4 h after MCA-occlusion) was significantly higher than that for [^{15}O]H_2O obtained 1 to 2 h after MCA-occlusion, but not significantly higher than that for [^{15}O]H_2O obtained 5 to 6 h after MCA-occlusion. We therefore performed a subsequent experiment with a canine model of cerebral ischemia, in order to confirm the results in rats. Twenty-four hours after the clot injection, CBF was decreased in the MCA-territory of the lesioned (left) hemisphere, whereas normal or higher accumulation of [1-^{11}C]octanoate was observed in the ischemic regions (Fig. 2). No significant infarct was detected by TTC-staining, indicating that the increased uptake of [1-^{11}C]octanoate-derived radioactivity relative to CBF was observed in ischemic, but vital regions. These studies demonstrate that [1-^{11}C]octanoate provides functional information different from CBF and may be useful for imaging and evaluating ischemic damage.

As mentioned above, Sakiyama et al.[19] indicated that radioactivity in the brain does not reflect metabolism of [1-^{11}C]hexanoate, because [^{11}C]CO_2/HCO_3^-, a metabolite of the compound, was redistributed into the brain. The same thing may occur with [1-^{11}C]octanoate, but this is unlikely, because no significant amount of [1-^{14}C]octanoate-derived volatile metabolites was detected in the brain of rats, and the major metabolic products of octanoate in the brain are glutamate and glutamine.[9,14] Based on our previous studies of the metabolic aspects of the compound,[14] it may be possible to image astroglial functions by using [1-^{11}C]octanoate, but the reason for the increased uptake of [1-^{11}C]octanoate in the ischemic region, and the functions which are actually imaged by [1-^{11}C]octanoate, remain to be elucidated. Ischemic response, such as astroglial activation, tissue acidosis, and changes in glutamate turnover, may contribute to distribution of [1-^{11}C]octanoate in the brain.

PET, as the major functional imaging technique, has provided non-invasive access to several parameters of interest in neuroscience. Although many positron labeled radiopharmaceuticals have been developed, few of these have been used clinically, mainly because of the difficulties in radiolabeling and in developing an automated radiosynthesis apparatus. [1-^{11}C]Octanoate is able to be synthesized reproducibly in high yields, by a Grignard reaction of [^{11}C]CO_2 with heptylmagnesium bromide. Commercially available automated synthesis apparatuses can be applied without any modifications to the production of [1-^{11}C]octanoate. This accessibility will be advantageous for extending the clinical application of [1-^{11}C]octanoate.[31] Conversely, its clinical application may be restricted by the intricacy of its metabolism, which renders analysis of the pharmacokinetics difficult. To circumvent this problem, we synthesized three octanoic acid analogs labeled with carbon-11[32] and preliminarily evaluated their pharmacokinetics in small animals,[33] but these compounds did not have a high degree of penetratability into the brain.

In conclusion, the present results in rat and canine models of cerebral ischemia clearly showed that [1-^{11}C]octanoate provides functional information different from CBF. Although experiments confirming the present preliminary results are necessary, [1-^{11}C]octanoate may be useful for clinically imaging and evaluating ischemic damage.

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