III. 1. Synthesis of $^{11}$C-Labeled Cocaine and Its Biodistribution in Mice


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Introduction

Cocaine is a naturally occurring local anesthetic and a stimulate of central nervous system. Its chronic administration can cause various medical complication$^1$. Especially, the abuse of this drug caused a cerebropsychosis and the reverse tolerance phenomenon$^2$. It was reported that the cocaine inhibited selectivity the reuptake of released dopamine in striatum of rat$^{3,4}$. These reports suggested that the variation of dopamine in striatum after administration with cocaine developed into important role of cerebropsychosis occurrence. Only one research group$^5$, recently, has synthesized $^{11}$C-cocaine and then its regional distribution and kinetics in human brain has been measured with PET. However, it has been reported that a few investigations about the synthesis of $^{11}$C-cocaine and its distributions in the organ and the brain in mice. In the previous report, we have described the brain distribution of $^{11}$C-methamphetamine in mice$^6$. In this report, we present the result of distribution of $^{11}$C-cocaine in the organ and brain in mice in order to compare with brain distribution of $^{11}$C-methamphetamine and of preliminary experiment of clinical application of $^{11}$C-cocaine for PET by using synthesized $^{11}$C-cocaine.

Materials and Methods

Chemicals

Cocaine hydrochloride was kindly supplied by Eishin Co. Ltd. (Sendai, Japan). Norcocaine$^7$ was prepared in our laboratory. Other chemicals used were of reagent grade.

Animals

Male ddY mice used in all experiments were maintained on a 12-h light-dark cycle with free access to food and water. The mice were approximately 6-7 weeks old and weighed 28-30 g each.
Synthesis of $^{11}$C-cocaine from norcocaine

(1) Preparation of $^{11}$CH$_3$I

$^{11}$CH$_3$I was synthesized from $^{11}$CO$_2$ using an automated synthesis system$^8$. $^{11}$CO$_2$ was produced from the proton bombardment of N$_2$ gas by the $^{14}$N(p,$\alpha$)$^{11}$C nuclear reaction using the Tohoku University Cyclotron (CGR-MeV model 680).

(2) Methylolation of norcocaine (scheme 1)

$^{11}$C-cocaine was prepared by the reaction of $^{11}$CH$_3$I with norcocaine by modifying the method of Denutte$^9$ et al. Briefly, the $^{11}$CH$_3$I was trapped in 2.0 ml of THF solution of norcocaine (7 mg, free base form) at -78°C (cooled by dry ice-acetone) for 5 min. The reaction mixture was stirred at 50°C for 5 min and then the reaction solvent was evaporated to dryness under reduced pressure.

(3) Purification

The labeled products were purified by HPLC, under conditions described in Fig.1. After HPLC separation, the $^{11}$C-cocaine fraction supplemented with 0.01N ethanolic HCl (0.05 ml) was evaporated to dryness under reduced pressure. The residue was dissolved in saline (5-7 ml) for injection. The total synthesis time was within 45 minutes.

(4) Identification of $^{11}$C-cocaine

The HPLC method used for isolation of the compound provided information concerning its chemical purity and the identity of the collected radioactive peak. The retention time of a well-separated radioactive peak was equal to that of an authentic sample of cocaine. Other tests were carried out for identification of $^{11}$C-cocaine. Its UV and mass spectra and its chromatographic behavior (HPLC and TLC) was identical with authentic sample (data not shown).

Animal experiments

(1) Distribution of $^{11}$C-cocaine in organs

A saline solution of $^{11}$C-cocaine hydrochloride (50 $\mu$Ci/0.2 ml) was injected intravenously into ddY mice (28-30 g, body weight). The mice were decapitated at 1, 5, 15, 30 and 60 min after injection. The organs were excised and blotted to remove adhering blood. Radioactivity of those organs were counted by an automated NaI counter. And the organs excised were weighed. The uptake was expressed as the differential absorption ratio (DAR).

The DAR is defined as follows:

$$\text{DAR} = \frac{\text{observed tissue activity}}{\text{(tissue weight) / (injected activity) / (body weight)}}$$

If a compound were uniformly distributed throughout the body and not excreted, the DAR of each organ would be 1.0.

(2) Distribution of $^{11}$C-cocaine in brain

ddY mice (28 - 30 g) were decapitated at various intervals after the injection of $^{11}$C-cocaine (50 $\mu$Ci/0.2 ml). The brain was removed and dissected into eight sections (striatum,
front-panetal cortex, posterior cortex, hippocampus, medulla oblongata, hypothalamus, midbrain and cerebellum), according to the method of Glowinski and Iversen\textsuperscript{10}. After the radioactivity was counted, the brain sections were weighed.

**Results and Discussion**

**Synthesis of $^{11}$C-cocaine**

After purification of $^{11}$C-cocaine, the radiochemical yield and the radiochemical purity of $^{11}$C-cocaine synthesized were 47-58 % and >99 % at specific activity of 22 $\mu$Ci/mmol, respectively. The HPLC chromatogram of the reaction mixture are shown in Fig.1. $^{11}$C-cocaine retained for 2.8 min and norcocaine for 5.2 min. A small part of the radioactive fraction was used subsequent studies. The fraction corresponding to cocaine was collected and the solvent was removed by evaporation. The mass spectra, HPLC and TLC behavior were identical to those of authentic materials. The specific activity of $^{11}$C-cocaine was low (22 $\mu$Ci/mmol) in comparison with Fowler et al\textsuperscript{5} and that was due to contamination with unlabeled carbon dioxide.

**Organs distribution in mice**

The distribution of $^{11}$C-cocaine in organs of mice are shown in Fig.2. The radioactivity of blood showed the lowest level until 60 min. High accumulation of carbon-11 was observed in the lung (DAR=3.5), kidney (DAR=1.75), but the brain was not so high (DAR=1.5) with 1 min after injection. The uptake of carbon-11 by the lung, kidney, brain decreased in a time dependent manner from 5 min after injection. On the other hand, in the liver and small intestine, the uptake of carbon-11 increased in a time dependency. The liver and small intestine showed the highest accumulation (DAR=2.0-2.75) at 30 min after injection of $^{11}$C-cocaine. The high radioactivity of carbon-11 at 30 min after injection might be ascribed to its labeled metabolites (e.g o-benzoyl ecgonine, ecgonine, ecgonine methyl ester\textsuperscript{11}). $\gamma$-counter measure total radioactivity in a region of organs and it does not directly identify the chemical form of the radioactive products.

The relationship between the loading dose of $^{11}$C-cocaine and its uptake in kidney, brain and blood was determined at 15min after injection. The results are shown in Fig.4. The relative concentration of $^{11}$C-cocaine in those organs increased in a dose-dependent manner, when the dose of cocaine was used from 0.4 ng/head to 4 $\mu$g/head.

The distribution of $^{11}$C-cocaine in brain of mice after intravenous injection of $^{11}$C-cocaine are shown in Fig.3. The uptake of carbon-11 by each section of brain was very fast and reached maximum at 5 min. Its clearance of radioactivity in brain was rapid. We reported previously the distribution of $^{11}$C-methamphetamine in the brain of mice. The radioactivity of carbon-11 at 30 min after injection of it still remained very high level (DAR=2.5-3.0)\textsuperscript{6}. In contrast, the radioactivity of $^{11}$C-cocaine at the same time after injection was low level (DAR=1.0 -1.5). We found out that the cocaine uptake and egress from the mice brain were rapid in comparison with methamphetamine. Such the difference in
time course of distribution of brain between cocaine and methamphetamine could influence to pharmacological effect to drug abusers. The frequent dependency of cocaine may be related to the rapid clearance of cocaine from the brain. The dependency of cocaine was at least recognized to stronger than that of methamphetamine under drug abusers.

The radioactivity and radiochemical yield obtained by this synthetic method were comparatively lower than those obtained by the method described by Fowler et al\cite{5}. The improvement of the synthetic method and detailed brain distribution of $^{11}$C-cocaine are now under investigation.

Summary

$[\text{N}-^{11}\text{C-methyl}]-\text{cocaine}$ ($^{11}\text{C-cocaine}$) was synthesized by $\text{N}$-methylation of norcocaine with $^{11}\text{CH}_3\text{I}$ to assist in imaging the variety of local distribution by positron emission tomography (PET). The radiochemical yield and the radiochemical purity after purification of $^{11}\text{C-cocaine}$ by high performance liquid chromatography (HPLC) were 47 - 58 \% and $>99$ \% at a specific activity of 22 $\mu$Ci/mmole, respectively. The time required for synthesis, including purification was 45 minutes from the end of $^{11}\text{CH}_3\text{I}$ trapping. The organ distribution of $^{11}\text{C-cocaine}$ was investigated in mice at various time after i.v. injection. The main accumulation of radioactivity was in the lung, the kidney and the brain at 1 min after injection. In the brain, the difference of radioactivity in each section was not appeared. The radioactivity levels in each section increased until 5 minutes and the clearance of radioactivity was rapid.

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References

Scheme 1. Synthetic of $^{11}$C-Cocaine

Norocaine

Norocaine (7 mg) in THF (2 ml)
  cooled by dry ice-acetone
  bubbled by $^{11}$CH$_3$I (5 min)
  heated at 50 °C for 5 min
  evaporated to dryness under reduced pressure

Residue
  dissolved in 100 µl mobile phase
  injected onto a HPLC-column
  collected $^{11}$C-Cocaine fraction

$^{11}$C-Cocaine
  supplemented with 0.01N ethanolic HCl (0.05 ml)
  evaporated to dryness under reduced pressure
  dissolved in saline

$^{11}$C-Cocaine Hydrochloride
Fig. 1. Separation of the Reaction Mixture by HPLC

HPLC condition
Column: Zorbax SIL (du pont Instruments) 4.6 mm × 15 cm
Mobile phase: Hexane : AcOEt (1:1 v/v), 25% NH₄OH (saturated)
Flow rate: 1.0 ml/min

Fig. 2. Tissue distribution of ¹¹C-Cocaine in mice. Data present an average (n = 3)
Fig. 3. Regional distribution of $^{11}$C-Cocaine in the mouse brain. Data present an average ($n = 3$)

Fig. 4. The Effect of Administered Dose on the Organ Uptake of $^{11}$C-cocaine
The distribution of $^{11}$C-cocaine at 15 min after i.v. injection was assayed as described in Materials and Methods. Data present an average ($n = 3$)