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Synthesis of ^{11}C-Labeled Imipramine and Its Biodistribution in Mice: A Potential Tracer for Positron Emission Tomography

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A tricyclic antidepressant, ^{11}C-labeled imipramine was synthesized by N-methylation of desipramine with $^{11}CH_3I$ to assist in the imaging of the human imipramine receptor by positron emission tomography. The radiochemical yield after purification of ^{11}C-imipramine by high performance liquid chromatography was 28—63% at a specific activity of 26—53 Ci/mmol. The time required for synthesis, including purification was 30 min from the end of $^{11}CH_3I$ trapping. The organ distribution of ^{11}C-imipramine was investigated in mice at various times after i.v. injection. The main accumulation of radioactivity was in the kidney, followed by the lung and the heart. In the brain, the radioactivity levels in the hypothalamus and striatum were the highest and remained constant, differentiating them from other portions of the brain. Furthermore, the result of a binding assay with ^{3}H-labeled imipramine suggested that the regional distribution of ^{11}C-imipramine in the same mouse brain correlated to that of the high affinity imipramine binding site.

Keywords ^{11}C-labeled imipramine; mouse brain region; organ distribution; radiolabeled binding assay; receptor; imipramine pool

Imipramine, a commonly used tricyclic antidepressant, has a variety of pharmacological effects on the central nervous system. Recently, a specific high affinity imipramine binding site has been demonstrated in the human brain and platelets, and many studies have reported that the number of imipramine binding sites in depressed patients was less than that of healthy controls.[1-8] These reports suggest that the number of imipramine binding sites can be utilized as a biochemical marker of depression. Consequently there is great interest in the potential utilization of positron emission tomography (PET), in conjugation with a suitable radiotracer, for the non-invasive determination of regional concentrations of brain imipramine receptors *in vitro*.

^{11}C-Labeled imipramine has already been synthesized by two research groups using different ^{11}C precursors. This labeled pharmaceutical was obtained by N-amino methylation of desipramine using ^{11}C-formaldehyde by Berger *et al.*[9] and using an excess of desipramine with $^{11}CH_3I$ by Denutte *et al.*[10] However, no report has appeared on the distribution of ^{11}C-imipramine in the brain.

In this paper, we describe the synthesis of ^{11}C-imipramine and we present the result of a preliminary experiment on clinical application of ^{11}C-imipramine for PET.

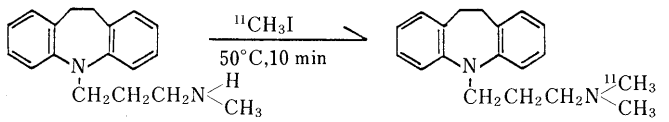
Materials and Methods

Chemicals Imipramine hydrochloride and desipramine hydrochloride were kindly supplied by Ciba-Geigy Limited (Takarazuka, Japan). ^{3}H-Imipramine hydrochloride (20.0 Ci/mmol) was purchased from Amersham Japan Co., Ltd. Other chemicals used were of reagent grade.

Animals All experiments used male ddY mice, maintained on a 12-h light-dark cycle, with free access to food and water. The mice were approximately 7—8 weeks old and weighed 28—32 g each.

Synthesis of $^{11}CH_3I$ $^{11}CH_3I$ was synthesized from $^{11}CO_2$ using an automated synthesis system.[11] $^{11}CO_2$ was produced from the proton bombardment of N_2 gas by the ^{14}N (p,α) ^{11}C nuclear reaction using the Tohoku University cyclotron.

Synthesis of ^{11}C-Imipramine (Chart 1) ^{11}C-Imipramine was prepared from the reaction of $^{11}CH_3I$ with desipramine by modifying the method of Denutte *et al.*[10] Desipramine in the free base form was prepared from an alkaline aqueous solution of the hydrochloride salt by extraction with diethyl ether. The ether layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. For the preparation of ^{11}C-imipramine, the $^{11}CH_3I$ was trapped in 1.5 ml of an acetone solution of desipramine (5 mg) in the free base form at −78 °C (cooled by dry ice–acetone) for 15 min. The reaction mixture was stirred at 50 °C for 10 min, then the reaction solvent was removed by evaporation, and the labeled product was purified by high performance liquid chromatography (HPLC) under the conditions described in Fig. 1. After HPLC separation, the ^{11}C-imipramine fraction containing 0.01 N ethanolic HCl (0.5 ml) was evaporated to dryness under reduced pressure. The residue was dissolved in saline and sterilized by filtration through a membrane filter (0.20 μm). The specific activity was determined by using reverse-phase liquid chromatography.



desipramine imipramine

Chart 1. Synthesis of ^{11}C-Imipramine

Tissue Distribution of ^{11}C-Imipramine In preliminary *in vivo* studies, ^{11}C-imipramine hydrochloride (50 μCi/0.2 ml) was injected into the tail vein of mice. At 1, 5, 15, 30, and 60 min after the injection, the mice were killed by decapitation, and the brain, liver, kidney, lung, heart, spleen, stomach, small intestine, muscle, and blood were removed immediately. The ^{11}C activity in the tissues was assayed in a gamma well counter and the tissues were weighed. The distribution of radioactivity was expressed as the differential absorption ratio (*DAR*), a convenient means of studying the possible localization in a tissue. The *DAR* is defined as follows.

$$DAR = \frac{\text{observed tissue activity/tissue weight}}{\text{injected activity/animal weight}}$$

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

Regional Distribution of ^{11}C-Imipramine in the Brain ^{11}C-Imipramine hydrochloride (55 μCi/0.2 ml) was injected into the tail veins of the mice. At 15, 30, and 60 min after the injection, mice were killed by decapitation, and the brain was quickly removed. Eight brain areas (striatum, frontoparietal cortex, posterior cortex, hippocampus, hypothalamus, midbrain, cerebellum, medulla oblongata) were dissected according to the method of Glowinski and Iversen,[12] and then counted and weighed. The activity distribution was expressed as *DAR*.

^{3}H-Imipramine Binding Assay Mice were killed by decapitation and the brain was immediately removed. Eight brain areas were dissected according to the method of Glowinski and Iversen,[12] and homogenized in 50 volumes of ice-cold buffer (50 mM Tris–HCl pH 7.4; 100 mM NaCl, 5 mM KCl). The homogenate was centrifuged at 30000 × *g* for 10 min, and the

resulting pellet was resuspended in the same buffer at the same concentration and recentrifuged. This procedure was repeated three times and the pellet was finally resuspended in 30 volumes of the same buffer. Protein content was determined by the method of Lowry *et al.*[13] using bovine serum albumin as the standard.

The binding of ^{3}H-imipramine was determined by incubating 50 μl of the membrane suspension with ^{3}H-imipramine (0.2—10 nM) in a final volume of 250 μl for 60 min at 0 °C. After incubation, the incubation medium was supplemented with 5 ml of the same ice-cold buffer and rapidly filtered through Whatman GF/F glass-fiber filters. The filters were washed with 3 × 5 ml ice-cold buffer, then dried and the radioactivity was counted in toluene containing 2,5-diphenyloxazole (PPO) (7 g/l) and dimethyl 1,4-bis-2-(5-phenyloxazol-2-yl)benzene (DMPOPOP) (0.3 g/l) with a liquid scintillation counter.

The specific binding was defined as the binding inhibited in the presence of 100 μM desipramine hydrochloride. At 2 nM ^{3}H-imipramine, the specific binding was 65% of the total binding. B_{max} and K_d values were calculated by linear least-squares analysis of Scatchard plots.

Results

Synthesis of ^{11}C-Imipramine ^{11}C-Imipramine was synthesized with a radiochemical yield of 28—63% within 30 min from the end of $^{11}CH_3I$ trapping. Figure 1 shows the HPLC chromatogram of the reaction mixture. ^{11}C-Imipramine was retained for 5 min and desipramine for 9.5 min, so that the two are readily distinguishable. A small part of the radioactive fraction was used for identification and purity analysis by HPLC. After HPLC, the fraction corresponding to imipramine was collected and the solvent removed by evaporation. The mass spectra, and HPLC and thin layer chromatography (TLC) behavior were identical to those of authentic materials. The radiochemical purity of ^{11}C-imipramine was determined to be 99%. The specific activity of this pharmaceutical varied form was 26 to 53 Ci/mmol at the time of the injection.

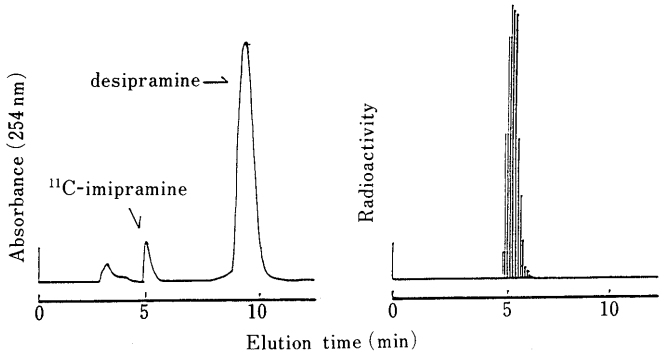


Fig. 1. Chromatogram of the Reaction Mixture by HPLC

HPLC conditions: column, Zorbax SIL (DuPoint Instruments) 4.6 mm × 15 cm; mobile phase, dichloromethane, methanol, water, diethylamine; (900 : 100 : 1.0 : 0.25 v/v); flow rate, 1.0 ml/min; column temperature, ambient.

TABLE I. Tissue Distribution of ^{11}C-Imipramine in ddY Mice

Tissue	DAR 1 min	5 min	15 min	30 min	60 min
Brain	0.91 ± 0.04[a]	1.01 ± 0.13	1.14 ± 0.19	0.95 ± 0.02	0.59 ± 0.06
Blood	0.23 ± 0.01	0.26 ± 0.02	0.30 ± 0.07	0.26 ± 0.02	0.18 ± 0.03
Heart	3.05 ± 0.54	1.16 ± 0.15	0.89 ± 0.18	0.70 ± 0.28	0.36 ± 0.03
Liver	0.48 ± 0.06	1.07 ± 0.06	1.17 ± 0.08	1.45 ± 0.26	1.43 ± 0.14
Kidney	3.41 ± 0.52	4.08 ± 0.18	4.40 ± 0.46	4.12 ± 0.49	2.26 ± 0.09
Lung	12.98 ± 2.01	7.21 ± 1.21	4.43 ± 0.49	2.55 ± 0.86	1.90 ± 0.40
Spleen	0.24 ± 0.05	0.94 ± 0.01	1.86 ± 0.18	1.62 ± 0.05	1.00 ± 0.10
Stomach	0.44 ± 0.12	0.96 ± 0.28	1.33 ± 0.14	1.79 ± 0.16	2.27 ± 0.01
Small intestine	0.88 ± 0.37	1.03 ± 0.07	1.11 ± 0.25	1.22 ± 0.54	—
Muscle	0.75 ± 0.14	0.56 ± 0.16	0.60 ± 0.07	0.42 ± 0.04	0.33 ± 0.08

a) Values represent mean ± S.D. (n = 3).

Organ Distribution of ^{11}C-Imipramine in Mice Table I shows the tissue distribution of ^{11}C-imipramine in mice. Blood radioactivity fell to a low level within 1 min after the injection, and high brain-to-blood ratios were found. The labeled pharmaceutical was rapidly accumulated in the heart, kidney, and especially in the lung at 1 min after the injection, but the brain uptake was not so high. In the heart, lung, and muscle, the uptake decreased in a time dependent manner. On the other hand, in the liver, stomach and small intestine, the uptake increased with time. The brain, kidney and spleen showed the highest accumulation at 15 min after the injection.

Figure 2 shows the effect of the loading dose (8—

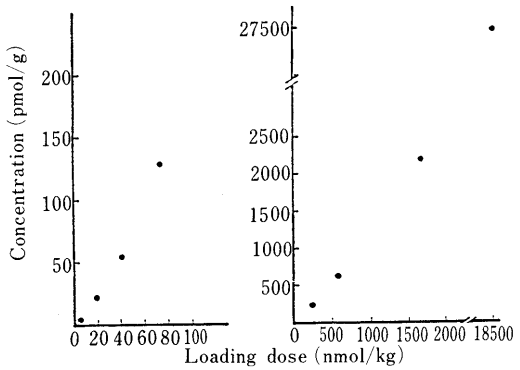


Fig. 2. The Effect of Administered Dose on the BrainUptake of ^{11}C-Imipramine

The distribution of ^{11}C-impramine at 15 min after i.v. injection was assayed as described in Materials and Methods. Each point represent the mean of triplicate determinations.

TABLE II. Regional Distribution of ^{11}C-Imipramine in Mouse Brain

Region	DAR 15 min	30 min	60 min
Striatum	1.97 ± 0.45[a]	1.70 ± 0.36	1.74 ± 0.69
Frontoparietal cortex	1.51 ± 0.21	1.25 ± 0.22	0.95 ± 0.11
Posterior cortex	1.46 ± 0.25	1.28 ± 0.12	0.91 ± 0.12
Hippocampus	1.59 ± 0.28	1.31 ± 0.09	1.27 ± 0.57
Hypothalamus	1.86 ± 0.16	1.81 ± 0.01	1.93 ± 0.04
Midbrain	1.54 ± 0.37	1.06 ± 0.04	1.15 ± 0.39
Cerebellum	1.34 ± 0.19	1.01 ± 0.19	0.69 ± 0.03
Medulla oblongata	1.47 ± 0.34	1.00 ± 0.27	0.97 ± 0.06

a) Values represent mean ± S.D. (n = 3).

TABLE III. Regional Distribution of ^{3}H-Imipramine Binding in Mice Brain

Region	B_{max} (fmol/g orinal tissue weight)
Striatum	10.7 ± 1.5[a]
Frontoparietal cortex	12.1 ± 0.1
Posterior cortex	5.3 ± 0.9
Hippocampus	9.3 ± 0.8
Hypothalamus	21.4 ± 2.2
Midbrain	13.2 ± 0.4
Cerebellum	4.1 ± 0.7
Medulla oblongata	8.5 ± 0.8

a) Values represent mean ± S.D. (n = 3).

18500 nmol/kg) on the brain uptake of ^{11}C-imipramine at 15 min after the injection. As the dose of imipramine was increased from 8 nmol/kg (2.5 μg/kg) to 18.5 μmol/kg (5.8 mg/kg) the relative concentration of ^{11}C-imipramine increased in a dose-dependent manner.

We next examined the regional distribution in the brain (Table II). At 15 min after the injection, the radioactivity in brain was almost evenly located, except in the striatum and hypothalamus. The radioactivity in the hypothalamus remained high, though the radioactivities in other regions decreased in a time-dependent manner.

Regional Distribution of the Imipramine Binding Site in the Brain To elucidate the relationship between the receptor site of imipramine and the regional distribution of ^{11}C-imipramine in the brain, the binding assay of ^{3}H-imipramine was examined using the eight sectional homogenates separated from the brains of mice (Table III). In this experiment, we found that ^{11}C-imipramine had the greatest number of receptors in the hypothalamus, followed by midbrain and frontoparietal cortex, and the lowest number was found in the cerebellum. The dissociation constant (K_d) values of ^{3}H-imipramine did not vary significantly among the eight regions of the brain and the average value was about 4.8 ± 0.8 nM.

Discussion

PET, an *in vivo* autoradiographic technique, can provide cross sectional distribution images of radiolabeled pharmaceuticals administered to animals or man. The dopamine,[14-18] benzodiazepine,[19,20] and opiate receptors[21] in the human brain have been characterized by PET. This technique used short-lived positron-emitting radioisotopes such as ^{11}C ($t_{1/2}=20.4$ min), ^{13}N ($t_{1/2}=9.96$ min), ^{15}O ($t_{1/2}=2.07$ min), and ^{18}F ($t_{1/2}=109.7$ min). Therefore the synthesis of positron labeled pharmaceuticals must be accomplished within three times the half life following the completion of cyclotron bombardment. The entire synthesis was accomplished, with material ready for injection, within 45 min after the end of cyclotron bombardment in these experiments. The specific activity of ^{11}C-imipramine was low (26—53 Ci/mmol) compared with the theoretical specific activity (9.3×10^6 Ci/mmol) due to contamination with unlabeled carbon dioxide.

The current study presents evidence for the specific labeling *in vitro* of neuroleptic binding sites in mice brains by ^{3}H-imipramine. The clearance of ^{11}C-imipramine in the blood was rapid, while the uptake in the brain was not so high (Table I). Similar results have been obtained using ^{14}C-imipramine by Bickel *et al.*[22]

Radioactivity was higher in the striatum and hypothalamus than in other regions and the ratio of radioactivity clearance was negligible after 60 min (Table II). In other brain regions, radioactivity decreased in a time-dependent manner. In view of the high capacity for ^{11}C-imipramine (Fig. 2) in mouse brain and the selective distribution for a long time after injection of low-specific-activity imipramine, the striatum and hypothalamus may represent the "imipramine pool" in mouse brain.

To elucidate the relationship between the receptor site of imipramine and ^{11}C-imipramine distribution, the binding assay of ^{3}H-imipramine was carried out (Table III). When the density of imipramine receptor was compared with the distribution of ^{11}C-imipramine at 60 min after the injection, a significant correlation was found between these two parameters ($r=0.78$, $p<0.05$). In contrast, a comparison between imipramine receptor and ^{11}C-imipramine distribution at 15 and 30 min after the injection showed no correlation. These results suggest that the initial distribution of imipramine is a nonspecific distribution and the elimination of imipramine from high-capacity imipramine pools is delayed. The imipramine pools are distributed in receptor-containing regions in mouse brain. As the binding constant between imipramine and its receptors is not so high,[2] the imipramine pool near the receptor may stimulate it by constant release of imipramine. Furthermore, imipramine is considered to pass the tight junction of blood vessels in the brain freely because of its high lipophilicity and should therefore be useful for the diagnosis of blood-brain barrier changes in cerebral infarction.

Our knowledge of imipramine demethylation, which represents an important process of degradation of imipramine, in mouse brain is poor in mice. The half time of imipramine in rat brain was reported to be 3 h after i.v. injection, and at that time desipramine amounted to a half of it.[23] Thus, the conversion of imipramine to desipramine is a prolonged phenomenon compared to the 20 min ^{11}C half-life.

In conclusion, these results indicate that an imipramine pool is localized near imipramine receptors, and positron emission tomography using low-specific-activity ^{11}C-imipramine appears to have some potential for identifying imipramine pools.

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