Synthesis and Biodistribution of N- [(2RS, 3RS)-1-Benzyl-2-Methyl-3-Pyrrolidinyl]-5-Chloro-2-Methoxy-4-[11C]Methylaminobenzamide, [11C]YM-09151-2: A New in Vivo Ligand for Dopamine D2 Receptors

著者	Sugahara M., Ishiwata K., Ido T., Iwata R., Ujiie A., Hatano K., Kanno Y., Kawashima K., Ito M.
journal or publication title	CYRIC annual report
volume	1986
page range	164-170
year	1986
URL	http://hdl.handle.net/10097/49355

III. 3 Synthesis and Biodistribution of N-[(2RS, 3RS)-1-Benzyl-2-Methyl-3-Pyrrolidinyl]-5-Chloro-2-Methoxy-4-[^{11}C]Methylaminobenzamide, [^{11}C]YM-09151-2: A New In Vivo Ligand for Dopamine D_2 Receptors

Sugahara M., Ishiwata K., Ido T., Iwata R., Ujiie A.*, Hatano K., Kanno Y., Kawashima K.**, Ito M.***
Cyclotron and Radioisotope Center, Tohoku University.
Department of Radiology, Tohoku University School of Medicine*
Department of Neurology, Institute of Brain Diseases, Tohoku University School of Medicine**
Department of Radiology and Nuclear Medicine, The Research institute for Tuberculosis and Cancer, Tohoku University***

Introduction

In vivo quantitative measurement of neuroreceptors in human by combination of positron-emitting radiopharmaceuticals and positron emission tomogoraphy (PET) is of great interest in the recent human brain studies. After the first demonstration of dopamine receptors by Wagner and co-workers who used ^{11}C-labeled N-methylspiperone as a tracer,[1] many efforts have been made to develop the promising tracers for measuring neuroreceptors. As in vivo ligands for dopamine D_2 receptors ^{18}F-labeled or radio-Br-labeled spiperone analouges, ^{11}C-labeled raclopride and ^{76}Br-bromolisuride are good candidates. N-[(2RS, 3RS)-1-benzyl-2-methyl-3-pyrrolidinyl]-5-chloro-2-methoxy-4-methylaminobenzamide, YM-09151-2, has been reported as the extremely high selective and potent blocker of the dopoamine D2 receptors,[2] which indicates that ^{11}C-labeled YM-09151-2 has potential as a tracer for measuring selectively dopamine D2 receptors in human with PET.

This paper describes the synthesis of ^{11}C-labeled YM-09151-2 and its tissue distribution in mice.

Materials and Methods

Synthesis of [^{11}C]YM-09151-2

Carbon-11-labeled YM-09151-2 was synthesized by the reaction of nor-YM-09151-2 and [^{11}C]CH_2I as shown in Scheme 1. [^{11}C]CH_3I was prepared from [^{11}C]CO_2. Nor-YM-09151-2 (2 mg, 5.7 μmol) was dissolved in 0.75 ml DMF and cooled in an ice bath. After NaH (1.3 mg, 57 μmol) suspended in 0.75 ml DMF was added, [^{11}C]CH_3I was bubbled in a helium carrier gas (30 ml/min) through this solution. The solution was stirred for 10 min at 22°-25°C. After removal of unreactive [^{11}C]CH_3I from the solution with a helium stream, 8 ml water was added. The solution was passed through a SEP-PAK C18 which was pre-washed with methanol and water. The SEP-PAK C18 was washed further with 20 ml water to remove DMF and NaOH, and the ^{11}C-labeled products were eluted

with 5 ml methanol from the SEP-PAK C18. The methanol fraction was evaporated to dryness. The residue was dissolved in a small amount of $CHCl_3$ and was applied to HPLC using UV and radioactivity monitors on a Radialpak Silica column (Waters, RCSS). Elution was performed with a $CHCl_3$/n-hexane/triethylamine (3:2:0.003, v/v/v) solution. The [^{11}C]YM-09151-2 fraction was collected and evaporated to dryness to remove triethylamine completely. The residue was dissolved in 1 ml 10 mM HCl in 90 % ethanol and the solution was evaporated to dryness. The [^{11}C]YM-09151-2 was dissolved in saline and was filtered through a membrane filter (0.22 μm) for animal studies.

Radiochemical purity was determined by HPLC and thin layer chromatography. A μBondapak C18 column (Waters, RCSS) was used with a mixture of 3 % triethylamine-H_3PO_4, pH 2.0, aqueous solution, and CH_3CN (7/3, v/v) at a flow rate of 1.5 ml/min. The retention times for YM-09151-2 and nor-YM-09151-2 were 8.8 and 5.2 min, respectively. A silica gel plate was used with a solution of $CHCl_3$/CH_3OH/triethylamine (20/2/0.1, v/v/v). The Rf values for YM-09151-2 and nor-YM-09151-2 were 0.69 and 0.58, respectively.

The specific activity was determined by HPLC on a μBondapak C18 column with the same condition as described above.

Tissue Distribution Study

Mal ddY mice weighing 30-35 g were injected with [^{11}C]YM-09151-2 through a lateral tail vein. The mice were sacrificed by cervical dislocation at 1, 5, 10, 30 and 60 min after injection. The tissue uptake was expressed as the differential absorption ratio, DAR, (counts/g tissue) × (g body weight/total injected counts).

Other five groups of mice (n=5) were also injected with [^{11}C]YM-09151-2 with different doses and sacrificed at 30 min after injection. The tissue distribution as well as the regional distribution in the brain was also measured.

Results and Discussion

Synthesis of [^{11}C]YM-09151-2

Table 1 summarizes the results of nine experiments. Figure 1 shows the profile of radio-HPLC for the separation of [^{11}C]YM-09151-2. Methylation of nor-derivative was carried out in the presence of NaH. By the methylation at room temperature the [^{11}C]YM-09151-2 for injection was prepared with a radiochemical yield of 10-37 % within 60 min from the end of irradiation. The specific activity was 1.2-2.0 Ci/μmol at the end of irradiation. When the reaction temperature increased, the yield of total methylation increased slightly. However, the yield of [^{11}C]YM-09151-2 rather decreased, which suggests the degradation of [^{11}C]YM-09151-2 in the presence of NaH at higher temperature.

When the methylation using [^{11}C]CH_3I was carried out in the presence of tetra butylammonium hydroxide at 40° to 60°C, a radiochemical yield of [^{11}C] YM-09151-2 for injection was less than 1 %.

Tissue distribution studies

The results of tissue distribution study are presented in Table 2. Blood clearance of ^{11}C radioactivity after injection of [^{11}C]YM-09151-2 was very rapid. The lung, kidney, heart and muscle showed the rapid clearance of ^{11}C. The ^{11}C level in brain was rather high and decreased gradually after 10 min. In other tissues after 10 min the ^{11}C levels decreased.

The effect of loading doses on tissue distribution at 30 min after injection is presented in Table 3. In the brain, heart, pancreas, spleen and kidney the uptakes decreased at high doses (more than 11 nmol). The effect in the liver and muscle was small. The levels in the blood at the higher doses of YM-09151-2 were slightly higher than those at the lower doses.

Regional distribution in brain was measured at 30 min after injection of different amount of YM-09151-2 (Table 4). In the experiments with low loading doses the highest regional accumulation was found in the striatum which is rich in dopamine receptors and the ^{11}C level was the lowest in the region of cerebellum. The uptake ratio of striatum to cerebellum was more than 2. In the experiments with high doses (11 or 71 nmol) the uptake was decreased significantly in the striatum. This decrease was also found in other four regions including the cerebellum in which the level of dopamine recerptors is very low. The dose-dependent uptake suggest the specific uptake correlated with dopamine receptors.

Clinical studies of dopamine receptors in human using N-[^{11}C]methylspeperone have presented the interesting new information. Tissue distribution pattern of [^{11}C]YM-09151-2 are very similar as those of N-[^{11}C]methylspiperone in rats[3]. Although N-[^{11}C]methylspiperone has a high affinity for dopamine receptors, the affinity for serotonin S_2 receptor is also relatively high. From the standpoint of selectivity for dopamine D_2 receptors, the YM-09151-2 has better chatacteristics in the in vitro studies than the spiperone.[2] The affinity of YM-09151-2 for both the dopamine D_2 and serotonin S_2 receptors is by more than three orders of magnitude than that of spiperone. As in vivo ligand for receptor assay, the lipophilicity of compound is also important factor. Because of the high lipophilicity of the [^{11}C]YM-09151-2, the high brain uptake was observed. However, the uptake ratio of striatum to cerebellum is relatively low compared to the N-[^{11}C]methylspiperone[3]. Two reasons explain these results. The first explanation is that in the sample of the striatum some tissue which is poor in dopamine receptors is included. As the second possibility, because of the high lipophilicity of YM-09151-2 the non-specific binding of [^{11}C]YM-09151-2

is not negligible even in the experiment with low loading dose. Further in vivo investigation will make clearer the selectivity of [^{11}C]YM-09151-2 for dopamine D_2 receptors.

In conclusion these results and biochemical properties indicate that the [^{11}C]YM-09151-2 has potential for measuring dopamine D_2 receptors with PET.

References

1) Wagner H. N. Jr., Burns H. D., Dannals R. F. et al., Science. 221 (1983) 1264.
2) Terai M., Usuda S., Kuroiwa I. et al., Jpn. J. Pharmacol. 33 (1983) 749.
3) Yanai K., Ido T., Ishiwata K. et al., Eur. J. Nucl. Med. 11 (1986) 438.

Table 1. Synthesis of [^{11}C]YM-09151-2

Experiment*	Run	Methylation		Overall preparation of [^{11}C]YM-09151-2		
		Total yield (%)	Percent of [^{11}C]YM-09151-2	Radiochemical yield (%)	Radiochemical purity (%)	Specific activity** (C/μmol)
1. 22°-25°C	5	40.1 (18.8-56.9)	79.2*** (52.3-95.3)	17.7 (10.1-35.8)	99.2 (> 96.8)	1.6 (1.2-2.0)
2. 32°C	1	46.6	22.7			
3. 40°C	1	50.7	8.5			
4. 60°C	2	48.9	trace			

Data indicate an average and range (in parentheses).
*Methylation was carried out in 1.5 ml DMF containing $^{11}CH_3I$, 2.0 mg nor-YM-09151-2 and 1.3 mg NaH at indicated temperature.
**Specific activity is calculated at the end of irrdiation.
***Data are calculated from three runs.

Table 2. Tissue distribution of ^{11}C after intravenous injection [^{11}C]YM-09151-2 in ddY mice.

	Uptake, DAR				
	1 min	5 min	10 min	30 min	60 min
Blood	0.44 ± 0.12	0.28 ± 0.00	0.22 ± 0.01	0.14 ± 0.00	0.12 ± 0.02
Brain	1.10 ± 0.20	0.87 ± 0.24	1.07 ± 0.07	0.71 ± 0.10	0.51 ± 0.12
Heart	1.94 ± 0.08	1.21 ± 0.14	0.86 ± 0.24	0.49 ± 0.10	0.30 ± 0.06
Lung	8.41 ± 0.72	3.45 ± 0.85	3.36 ± 0.21	1.18 ± 0.17	0.81 ± 0.02
Liver	1.17 ± 0.12	2.03 ± 0.15	1.94 ± 0.07	1.60 ± 0.09	1.43 ± 0.03
Pancreas	3.90 ± 0.18	3.72 ± 0.80	5.06 ± 0.27	2.80 ± 0.68	2.24 ± 0.34
Spleen	1.36 ± 0.26	1.99 ± 0.27	1.81 ± 0.28	1.27 ± 0.27	1.00 ± 0.28
S. intestine	1.47 ± 0.64	3.13 ± 0.16	2.55 ± 0.28	1.84 ± 0.45	1.31 ± 0.32
Kidney	5.85 ± 0.55	4.03 ± 0.07	3.05 ± 0.24	1.19 ± 0.13	1.02 ± 0.08
Muscle	0.89 ± 0.26	0.66 ± 0.10	0.56 ± 0.08	0.34 ± 0.01	0.24 ± 0.01

Data present an average s. d. (n=3).
Dose: 0.26 nmol

Table 3. Effect of loading dose on tissue distribution of ^{11}C at 30 min after injection of [^{11}C]YM-09151-2 in ddY mice.

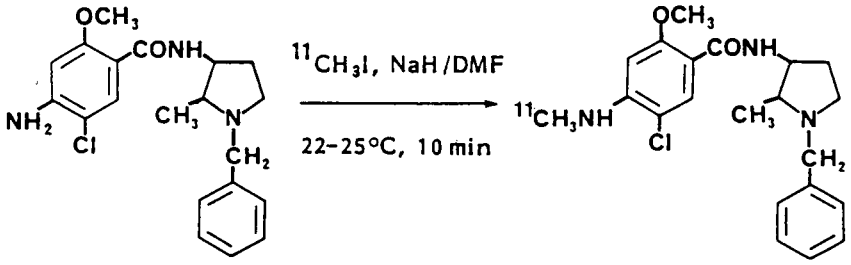
	Uptake, DAR				
	0.034 nmol	0.13 nmol	0.48 nmol	11 nmol	71 nmol
Blood	0.13 ± 0.01	0.11 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01
Brain	1.07 ± 0.05	0.92 ± 0.09	0.88 ± 0.05	0.41 ± 0.02	0.33 ± 0.02
Heart	0.63 ± 0.05	0.67 ± 0.02	0.75 ± 0.08	0.48 ± 0.01	0.34 ± 0.03
Liver	2.08 ± 0.16	1.72 ± 0.13	2.15 ± 0.06	1.79 ± 0.08	1.88 ± 0.08
Pancreas	3.92 ± 0.27	3.89 ± 0.21	3.86 ± 0.20	2.17 ± 0.12	1.56 ± 0.09
Spleen	1.86 ± 0.11	1.72 ± 0.08	1.66 ± 0.19	1.03 ± 0.06	0.68 ± 0.03
Kidney	1.98 ± 0.22	1.73 ± 0.04	2.20 ± 0.43	1.26 ± 0.06	0.94 ± 0.05
Muscle	0.32 ± 0.01	0.32 ± 0.02	0.72 ± 0.05	0.34 ± 0.04	0.26 ± 0.02

Data present an average s. d. (n=5).

Table 4. Effect of loading dose on regional distribution in the mouse brain of ^{11}C at 30 min after injection of [^{11}C]YM-09151-2.

	Uptake, DAR tissue-to-cerebellum ratio				
	0.034 nmol	0.13 nmol	0.48 nmol	11 nmol	71 nmol
Striatum	1.82 ± 0.16	1.53 ± 0.09	1.79 ± 0.23	0.51 ± 0.04	0.33 ± 0.03
	2.21	2.06	2.26	1.19	0.89
Cerebral	1.01 ± 0.03	0.90 ± 0.14	0.78 ± 0.03	0.35 ± 0.01	0.30 ± 0.01
hemisphere	1.22	1.22	0.99	0.81	0.81
Thalamus	1.01 ± 0.11	0.84 ± 0.04	0.86 ± 0.03	0.42 ± 0.04	0.30 ± 0.02
	1.23	1.13	1.09	0.98	0.81
Brain stem	1.10 ± 0.07	0.87 ± 0.04	0.81 ± 0.03	0.49 ± 0.02	0.38 ± 0.02
	1.34	1.17	1.03	1.14	1.03
Cerebellum	0.82 ± 0.03	0.74 ± 0.05	0.79 ± 0.08	0.43 ± 0.02	0.37 ± 0.02
	1.00	1.00	1.00	1.00	1.00

Data present an average s. d. (n=5).



Scheme 1. Synthesis of [^{11}C]YM-09151-2.

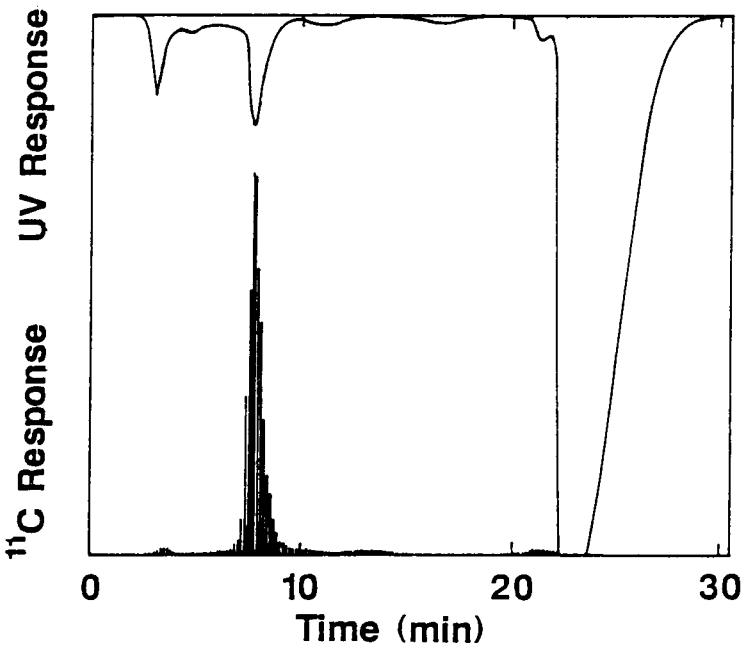


Fig. 1. Separation of [^{11}C]YM-09151-2 by radio-high-performance liquid chromatography.