Annals of Nuclear Medicine Vol. 17, No. 6, 451–456, 2003

Evaluation of radioiodinated (−)-*o*-iodovesamicol as a radiotracer for mapping the vesicular acetylcholine transporter

Kazuhiro SHIBA,* Hirofumi MORI* and Norihisa TONAMI**

**Radioisotope Center, and **Department of Nuclear Medicine, School of Medicine, Kanazawa University*

We evaluated the potencies of radioiodinated (−)-*o*-iodovesamicol [(−)-oIV] as a selective vesicular acetylcholine transporter (VAChT) mapping agent. (−)-[^{125}I]oIV exhibited significant accumulation (about 2.8% of the injected dose) in rat brain. The regional brain distribution of radioactivity was similar for both (−)-[^{125}I]oIV and (−)-[^{3}H]vesamicol. The accumulation of (−)-[^{125}I]oIV in the brain was significant reduced by post-administration of unlabeled vesamicol (0.5 μmol/kg^{-1}) and (−)-oIV (0.5 μmol/kg^{-1}). On the other hand, the post-administration of sigma ligands hardly affected the accumulation of (−)-[^{125}I]oIV in the brain. These studies showed that (−)-[^{125}I]oIV, as well as [^{3}H]vesamicol, bound to VAChT with high affinity in the rat brain. Furthermore, (−)-[^{125}I]oIV binding in the ipsilateral cortex to the lesion was significantly reduced by 17.0%, compared with that in the contralateral cortex in a unilateral NBM-lesioned rat. These results suggested that radioiodinated (−)-oIV may potentially be useful for the diagnosis of cholinergic neurodegenerative disorders.

Key words: (−)-o-iodovesamicol, vesamicol, radioligand, vescular acetylcholine transporter, cholinergic denervation

INTRODUCTION

SINCE VESAMICOL was found to bind to the vesicular acetylcholine transporter (VAChT) in presynaptic cholinergic neurons with high affinity,[1–3] many radiolabeled vesamicol analogs have been developed as radioligands for mapping presynaptic cholinergic neurons with single photon emission computed tomography (SPECT)[4–8] and positron emission tomography (PET).[9–12] Previously, we also reported the potency of (−)-*m*-iodovesamicol [(−)-mIV] as a VAChT mapping agent.[13,14] However, Custer et al. reported that many vesamicol analogs bound not only to VAChT, but also to sigma receptors (σ-1, σ-2) with high affinity.[15] We also investigated the affinity of iodovesamicol enantiomer analogs, which we prepared, for VAChT and sigma receptors *in vitro*.[16] These results showed that (−)-oIV bound to VAChT with high affinity and bound to sigma receptors with low affinity compared to the others *in vitro*. In the present study, part of our continuing effort to develop VAChT ligands with high-affinity and high-specificity, we evaluated the potential usefulness of (−)-[^{125}I]-*o*-iodovesamicol [(−)-[^{125}I]oIV]) as a vesicular acetylcholine transporter (VAChT) mapping agent for diagnosing cholinergic neurodegenerative disorder.

Received February 20, 2003, revision accepted May 8, 2003.

For reprint contact: Kazuhiro Shiba, M.D., Division of Tracer Kinetics, Advanced Science Research Center, Kanazawa University, 13–1 Takara-machi, Kanazawa 920–8640, Ishikawa, JAPAN.

E-mail: shiba@med.kanazawa-u.ac.jp

MATERIAL AND METHODS

(−)-[^{3}H]Vesamicol (1.30 TBq/mmol) and [^{125}I]-sodium iodide (644 GBq/mg) were purchased from Dupont-NEN products. Other drugs and reagents were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted.

Synthesis of oIV ((−)-oIV) enantiomers

The protocols for preparing oIV ((−)-oIV) enantiomers are shown in Figure 1. Racemic vesamicol was optically resolved by recrystallizing diasterioisomeric salts using (−)-di-*p*-toluoyl-L-tartaric acid monohydrate. The

enantiomers of oIV ((−)-oIV) were prepared by the method described previously, except for the use of the corresponding enantiomer of vesamicol.[17]

Characteristics of (−)-2-(4-(2-iodophenyl)piperidino) cyclohexanol [(−)-oIV]

Melting point 152–154°C. NMR ($CDCl_3$): δ 1.24 (4H, m), 1.81 (8H, m), 2.14 (1H, m), 2.29 (2H, m), 2.79 (2H, m), 2.98 (1H, m), 3.41 (1H, m), 6.92 (1H, ddd, J = 7.80 Hz, J = 7.80 Hz, J = 1.5 Hz), 7.24 (1H, dd, J = 7.80 Hz, J = 1.5 Hz), 7.33 (1H, ddd, J = 7.80 Hz, J = 7.80 Hz, J = 1.0 Hz), 7.84 (1H, dd), J = 7.80 Hz, J = 1.0 Hz). Mass Spectrum (m/e): 385 [M+]. Elemental analysis $C_{17}H_{24}NOI$; theory C: 53.00, H: 6.28, N: 3.64; found C: 52.89, H: 6.35, N: 3.50. Specific rotation: $[\alpha]_D^{23} = -34.0°$ (C = 0.543, $CHCl_3$).

Radiolabeling

(−)-oIV was radioiodinated by solid-phase exchange with [^{125}I]NaI in the presence of ammonium sulfate as a promoter as previously reported.[13]

Tissue distribution study

Animal experiments were carried out in compliance with the Guidelines for the Care and Use of Laboratory Animals at the Takara-machi Campus of Kanazawa University. Three groups of male Sprague-Dawley rats (n = 4 in each group), weighing 200–220 g, were anesthetized with ether and given an intravenous (i.v.) injection of (−)-[^{125}I]oIV (185–222 kBq) in 0.4 m*l* of 5% ethanol. At 30, 60 and 120 min post-injection, the animals were sacrificed immediately by decapitation. The organs of interest were dissected, weighed and the radioactivity levels were measured in an Aloka gamma scintillation counter. The accumulation of radiotracer was expressed as a percentage of the injected dose per gram of tissue (% dose/g).

Regional brain distribution studies

Male Sprague-Dawley rats (200–220 g) were anesthetized with ether and treated intravenously with dl-vesamicol (0.5 μmol/kg), (+)-3-PPP (0.5 (mol/kg), or the same volume (0.2 m*l*) of saline, 5 min before the radiotracer injection. Each radiotracer ((−)-[^{3}H]vesamicol or (−)-[^{125}I]oIV) was injected intravenously into the tail vein (185–222 kBq). At 45 min after injection of the radiotracer, the animal was immediately sacrificed by decapitation under ether anesthesia. The brains were quickly removed, and the cerebral cortices, striatum, and cerebellum were dissected, weighed and the radioactivity levels were measured in an Aloka gamma scintillation counter.

The samples dissected from the rats injected with (−)-[^{3}H] vesamicol were measured as previously reported.[6] The accumulation of radiotracer was expressed as a percentage of the injected dose per gram of tissue (% dose/g).

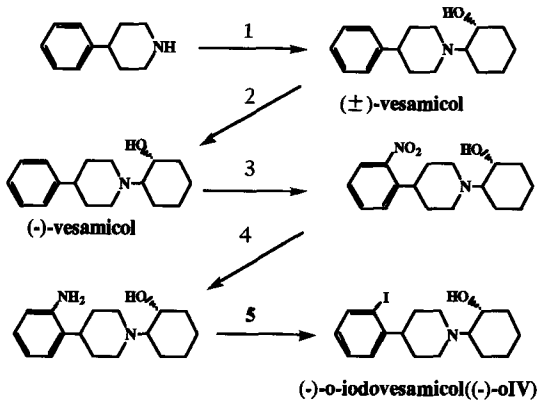

Fig. 1 Synthesis of (−)-*o*-iodovesamicol ((−)-oIV). 1, cyclohexane oxide; 2, (−)-di-*p*-toluoyl-L-tartaric acid; 3, HNO_3, H_2SO_4; 4, Fe, HCl; 5, $NaNO_2$, HCl, KI

Table 1 Biodistribution of (−)-[^{125}I]-oIV in rats

Tissue	% dose/g of tissue		
	30 min	60 min	120 min
Blood	0.07 ± 0.01	0.07 ± 0.00	0.07 ± 0.00
Muscle	0.31 ± 0.05	0.22 ± 0.02	0.12 ± 0.01
Liver	1.54 ± 0.08	1.52 ± 0.10	1.28 ± 0.02
Lung	2.11 ± 0.21	1.03 ± 0.23	0.55 ± 0.09
Kidney	4.03 ± 0.42	3.23 ± 0.15	2.31 ± 0.17
Heart	0.39 ± 0.05	0.24 ± 0.03	0.13 ± 0.01
Brain	1.50 ± 0.10	1.27 ± 0.11	0.76 ± 0.04

Values are mean ± S.E.M. (n = 4 per group)

Ligand displacement studies

Six groups of male Sprague-Dawley rats (n = 4) weighing 200–220 g received an intravenous injection of (−)-[^{125}I]oIV (185–222 kBq). After 30 min, each animal received an i.v. dose of one of the following unlabeled blocking drugs (0.5 μmol/kg): vesamicol, (−)-oIV, spiperone, ketanserin QNB, (+)-3-PPP, pentazocine or the same volume (200 μ*l*) of the vehicle (saline). All animals were sacrificed 60 min after injection with the radiotracer. The brains were quickly removed and weighed. The radioactivity levels in the brain samples were measured in an Aloka gamma scintillation counter. The data were analyzed as described above.

Ibotenic acid lesions of the NBM area

Ibotenic acid lesions in the nucleus basalis magnocellularis (NBM) were induced in five male Sprague-Dawley rats using a method described previously.[14] In brief, in the right NBM area (0.8 mm before the bregma, 2.5 mm to the right of the midline and 7.5 mm below the dura) of the male Sprague-Dawley rat brain (250–350 g), and 0.7 μ*l* of a 10 μg/μ*l* solution of ibotenic acid in 0.9% NaCl were implanted for five minutes. An equal number of rats given a saline injection were considered controls. Postsurgically, rats were individually housed with free access to food and

Table 2 Effect of vesamicol and (+)-3-PPP on the accumulation of (−)-[^{125}I]-oIV and [^{3}H]vesamicol in rat brains

Drugs	% dose/g of tissue			
	region	control	vesamicol (0.5 μmol/kg)	(+)-3-PPP (0.5 μmol/kg)
(−)-[^{125}I]oIV	cortex	1.55 ± 0.06 (100)	0.54 ± 0.04 (35)	1.50 ± 0.07 (97)
	striatum	1.57 ± 0.08 (100)	0.58 ± 0.05 (37)	1.46 ± 0.06 (95)
	cerebellum	1.19 ± 0.07 (100)	0.49 ± 0.02 (40)	1.16 ± 0.06 (94)
(−)-[^{3}H]vesamicol	cortex	0.68 ± 0.04 (100)	0.23 ± 0.04 (32)	0.69 ± 0.08 (101)
	striatum	0.74 ± 0.05 (100)	0.25 ± 0.04 (34)	0.73 ± 0.06 (99)
	cerebellum	0.47 ± 0.03 (100)	0.18 ± 0.03 (38)	0.47 ± 0.02 (100)

Values are mean ± S.E.M (n = 4 per group)

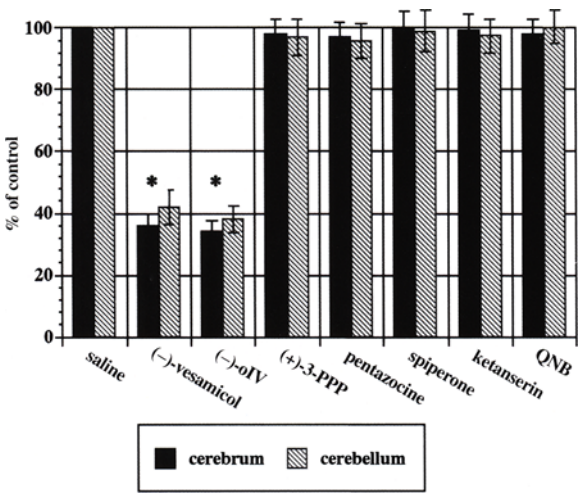

Fig. 2 Inhibition of (−)-[^{125}I]oIV accumulation in the rat brain by post-administration of various receptor ligands.
*$p < 0.01$ vs. control, by one-way ANOVA followed by Student's t-test

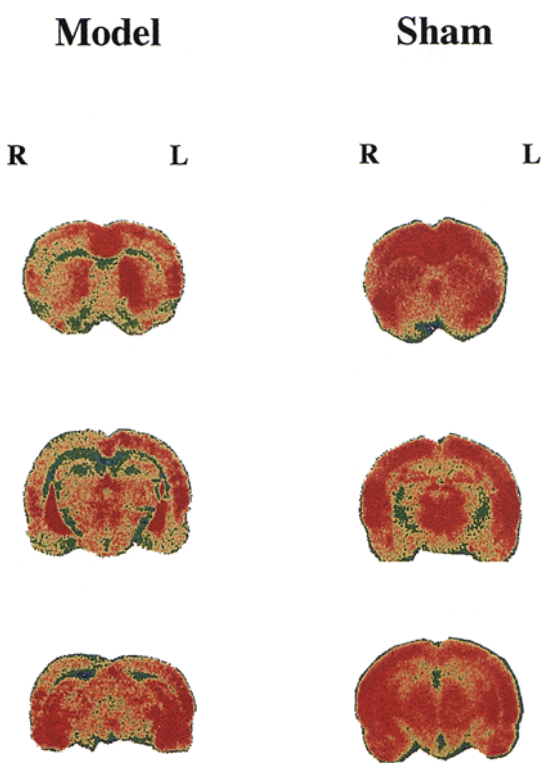

Fig. 3 *Ex vivo* autoradiograms of (−)-[^{125}I]oIV in the unilateral NBM-lesioned rat brain. R = Affected, L = Unaffected.

water for two weeks. The unilateral NBM lesioned rats were verified to show memory impairment by the same memory test using a step-through apparatus for the evaluation of passive avoidance performance as in a previous report[18] before being used for *in vivo* study.

Ex vivo *autoradiography of (−)-[^{125}I]oIV*

Two-weeks after the operation, the five NBM-lesioned rats and control rats were injected intravenously with (−)-[^{125}I]oIV (1.11 MBq). The rats were sacrificed by decapitation 60 min after (−)-[^{125}I]oIV injection. The brains were removed, frozen in an embedding medium at −78°C and cut into sections 20 μm thick at −30°C using a cryostat microtome. The sections were mounted onto gelatine-chromealum coated glass slides. Then, they were placed on an imaging plate BAS-III 2040 (Fuji Photo Film, Tokyo) and exposed for four weeks in a lead box.

Analysis of autoradiograms

The radioactive images recorded on the imaging plate were read with a Fuji Bio-Imaging Analyzer BAS2000 (Fuji Photo Film, Tokyo, Japan). The imaging data were

recorded as digital values, photo-stimulated luminescence (PSL), in an analyzing unit. Radioligand concentrations (PSL/mm^2) in rat brain regions were determined bilaterally using elliptical regions of interest (ROIs) on the autoradiographic images. The percentage ratio values of the regional brain accumulation of (−)-[^{125}I]oIV in the lesioned hemisphere to that in the contralateral hemisphere in all rats (LC percent ratio) were calculated. A comparison of differences in the LC ratio between each group was performed with analysis of variance followed by Mann-Whitney U-test and Wilcoxon signed rank test.

RESULTS

Synthesis and radiolabeling

Preparation of (−)-*o*-iodovesamicol ((−)-oIV) from 4-phenylpiperidine via the five-step reaction produced overall yields of 7.7–14.5% (Fig. 1).

Solid-state isotopic exchange catalyzed by $(NH_4)_2SO_4$ produced (−)-[^{125}I]oIV at a radiochemical yield of 45–75%. The radiochemical purity of (−)-[^{125}I]oIV following HPLC purification exceeded 95% when determined by radio-HPLC (Aloka, RLC-700). The specific activity of (−)-[^{125}I]oIV was 600–1100 GBq/mmol.

Tissue and regional brain distribution studies

Table 1 shows the distribution of (−)-[^{125}I]oIV in the rat. There was a high level of radioactivity of (−)-[^{125}I]oIV in the rat brain, whereas the blood level of radioactivity remained consistently low. (−)-[^{125}I]oIV showed a high brain to blood ratio.

Table 2 shows the regional distribution of (−)-[^{125}I]oIV and (−)-[^{3}H]vesamicol in the rat brain and the effect of cold vesamicol and (+)-3-PPP. The regional distribution of (−)-[^{125}I]oIV and (−)-[^{3}H]vesamicol was qualitatively similar. (−)-[^{125}I]oIV showed higher accumulation in the rat brain than (−)-[^{3}H]vesamicol. The accumulation of (−)-[^{125}I]oIV in the cortex, striatum, and cerebellum of the rat brain was blocked by the prior administration of vesamicol (0.5 μmol/kg), which reduced accumulation by 65, 63 and 60%, respectively.

Ligand displacement studies

To estimate the characteristics of radiolabeled (−)-oIV binding *in vivo*, we studied the effects of 7 receptor ligands on the regional brain uptake of (−)-[^{125}I]oIV. The effects of post-administration of (−)-vesamicol, (−)-oIV, (+)-3-PPP, pentazocine, spiperone, ketanserin and QNB on the accumulation of (−)-[^{125}I]oIV in the rat brain are shown in Figure 2. The post-administration of vesamicol and (−)-oIV resulted in statistically significant ($p < 0.05$) changes in the concentrations of (−)-[^{125}I]oIV in the rat brain. On the other hand, the post-administration of sigma, dopamine, serotonin, or acetylcholine receptor ligands barely affected the accumulation of (−)-[^{125}I]oIV in the brain. These results showed that the characteristics of (−)-[^{125}I]oIV for binding sites in the rat brain are similar *in vivo* and *in vitro*, and that (−)-[^{125}I]oIV bound specifically to VAChT *in vivo*.

Table 3 Percentage ratio values of (−)-[^{125}I]oIV accumulation in the lesioned hemisphere to that in the contralateral hemisphere in unilateral NBM-lesioned and sham rats

Structure	Model	Sham
Cortex	83.0 ± 5.3*	98.7 ± 2.5
Striatum	96.5 ± 3.9	98.8 ± 2.2
Hippocampus	98.7 ± 3.4	98.9 ± 1.9
Thalamus	98.4 ± 2.6	99.2 ± 1.8

Values are mean ± S.E.M.; n = 5

*$p < 0.01$; Significant difference in (−)-[^{125}I]oIV accumulation between model group and sham group, by Mann-Whitney U-test and Wilcoxon signed rank test

Analysis of autoradiograms of (−)-[^{125}I]oIV in cholinergic denervation rats

To evaluate the potential benefit of (−)-[^{125}I]oIV, a VAChT mapping agent, for diagnosing cholinergic deficit dementia, we investigated changes in the brain distribution of (−)-[^{125}I]oIV in cholinergic denervation rats produced by a unilateral lesion of the nucleus basalis magnocellularis (NBM). Figure 3 shows radioluminographic images of (−)-[^{125}I]oIV in an NBM-lesioned rat brain. The accumulation of (−)-[^{125}I]oIV in the projection cortices' ipsilateral cortex to the lesion was significantly lower than that in the contralateral cortex. Table 3 shows the LC percent ratio values of (−)-[^{125}I]oIV in each region from the two groups. The LC percent ratio values of (−)-[^{125}I]oIV in the NBM lesioned group (83.0%) were significantly lower than those in the sham-operated group (98.5%) ($p < 0.01$).

DISCUSSION

The (−)-enantiomer of oIV [(−)-oIV] was prepared from racemic vesamicol via four-step reactions, and was identified by HPLC and specific rotation as reported previously.[13] In the present study, a solid-state isotopic exchange method was used for radioiodination of (−)-[^{125}I]oIV. A radiotracer prepared by this radiosynthetic method is suitable for *in vitro* and *in vivo* animal study. However, for a clinical study, it is necessary that a no-carrier-added radioiodinated (−)-[^{125}I]oIV be prepared by iododestannylation of the corresponding (−)-ortho-(tri-*n*-butyltin) vesamicol precursor. Now, we are studying a new method for preparation of (−)-ortho-(tri-*n*-butyltin) vesamicol precursor, because this was not prepared by a common method due to steric hindrance.

We recently reported that (−)-[^{125}I]oIV, as well as (−)-vesamicol, had high affinity for VAChT but had relatively low affinity for sigma receptor *in vitro*.[16] To evaluate the characteristics of radiolabeled (−)-oIV binding *in vivo* and the potential of (−)-[^{125}I]oIV, a VAChT mapping

agent, for diagnosing cholinergic deficit dementia, *in vivo* animal studies were carried out. (−)-[^{125}I]oIV showed a high level of accumulation in the rat brain (about 2.8% of the injected dose) and a high level brain to blood uptake ratio (about 23.1) at 30 min post-injection. The regional brain distribution of (−)-[^{125}I]oIV was similar to that of (−)-vesamicol. Especially, (−)-[^{125}I]oIV as well as (−)-vesamicol had a higher accumulation of radioactivity in striatum than in cortex. On the other hand, a previous report[13] showed that (−)-[^{125}I]mIV had a lower accumulation of radioactivity in striatum than in cortex.

The results of a ligand displacement study showed that the characteristics of (−)-[^{125}I]oIV for the binding sites in the rat brain were similar *in vivo* and *in vitro*,[16] namely (−)-[^{125}I]oIV bound specifically to VAChT *in vivo*.

The unilateral NBM-lesioned rats used in these studies were prepared by the same method as previously reported[14] and were verified to show memory impairment by the same memory test as previously reported.[14] The reproducibility of the preparation method for the unilateral NBM-lesioned rats is thought to be very high.

The changes in the brain accumulation of (−)-[^{125}I]oIV in the NBM lesioned rats (83.0%) were significantly larger than those in the sham-operated group (98.5%) ($p < 0.01$). These results suggested that radioiodinated (−)-oIV labeled with I-123 may be able to detect changes in VAChT density *in vivo* using single photon emission tomography (SPECT).

Finally, we tried comparing (−)-[^{125}I]oIV with (−)-[^{125}I]mIV by both the results of the present study and those of a previous report.[14] The results of the *in vivo* regional brain distribution studies suggested that the *in vivo* binding characteristics of (−)-[^{125}I]oIV in rat brain were more similar to those of (−)-[^{3}H]vesamicol than those of (−)-[^{125}I]mIV. In the regional brain distribution studies using unilateral NBM-lesioned rats, the rate of changes in brain accumulation of (−)-[^{125}I]oIV (17.0%) was larger than that of (−)-[^{125}I]mIV (10%)[14] to VAChT ($p < 0.01$; by Mann-Whitney U-test). The difference between (−)-[^{125}I]oIV and (−)-[^{125}I]mIV could be due to (−)-[^{125}I]oIV's showing a lower affinity for sigma receptors (σ-1, σ-2) than (−)-[^{125}I]mIV as reported previously.[16] The higher affinity of (−)-[^{125}I]mIV to sigma receptors is thought to mask the rate of changes in the accumulation of (−)-[^{125}I]mIV to VAChT in unilateral NBM-lesioned rats. However, these results do not exclude a possibility of a change of sigma receptors in Alzheimer's disease, because unilateral NBM-lesioned rats are not necessarily sufficient as an animal model of Alzheimer's disease.

In conclusion, radioiodinated (−)-oIV bound to VAChT with high affinity and specificity and may be suitable for the study of dementia characterized by degeneration of the cholinergic neurotransmitter system; it may be better than the previously reported radioiodinated (−)-mIV.

ACKNOWLEDGMENT

This study was supported by a Grant-in-Aid Scientific Research (C-14570845) from the Japanese Ministry of Education, Science, Sports and Culture.

REFERENCES

1. Alter CA, Marien MR. [^{3}H]Vesamicol binding in brain: autoradiographic distribution, pharmacology, and effects of cholinergic lesions. *Synapse* 1988; 2: 486–493.
2. Bar BA, Parsons SM. Demonstration of a receptor in Torpedo synaptic vesicles for the acetylcholine storage blocker L-trans-2-(4-phenyl[3,4-^{3}H]-piperidino)cyclohexanol. *Proc Natl Acad Sci USA* 1986; 83: 2267–2270.
3. Marien MR, Persons SM, Altar CA. Quantitative autoradiography of brain binding sites for the vesicular acetylcholine transport blocker 2-(4-phenylpiperidino)cyclohexanol (AH5183). *Proc Natl Acad Sci USA* 1987; 84: 876–880.
4. Jung YW, Van Dort ME, Gildersleeve DL, Wieland DM. A radiotracer for mapping cholinergic neurons of the brain. *J Med Chem* 1990; 33: 2065–2068.
5. Efange SMN, Michelson RH, Khare AB, Thomas JR. Synthesis and tissue distribution of (*m*-[^{125}I]iodobenzyl) trozamicol ([^{125}I]MIBT): potential radioligand for mapping central cholinergic innervation. *J Med Chem* 1993; 36: 1754–1760.
6. Shiba K, Mori H, Matsuda H, Tsuji S, Tonami N, Hisada K. *In Vivo* Characterization of Radioiodinated 2-(4-Phenylpiperidino)cyclohexanol (Vesamicol) Analogs: Potential Radioligand for Mapping Presynaptic Cholinergic Neurons. *Nucl Med Biol* 1995; 22: 823–828.
7. Khare AB, Langason RB, Parsons SM, Mach RH, Efange SMN. *N*-(3-Iodophenyl)trozamicol (IPHT) and related inhibitors of vesicular acetylcholine transporter: Synthesis and preliminary biological characterization. *Nucl Med Biol* 1999; 26: 609–617.
8. Bando K, Taguchi K, Cinoza Y, Naganuma T, Tanaka Y, Koike K, et al. Synthesis and evaluation of radiolabeled piperazine derivatives of vesamicol as SPECT agents for cholinergic neurons. *Nucl Med Biol* 2001; 28: 251–260.
9. Widen L, Eriksson L, Ingvar M, Parsons SM, Rogers GA, et al. Positron emission tomographic studies of central cholinergic nerve terminals. *Neuroscience Letters* 1992; 136: 1–4.
10. Efange SMN, Mach RH, Kharea AB, Michelson RH, Nowak PA, Evora PH. *p*-[^{18}F]Fluorobenzyltrozamicol ([^{18}F]FBT): molecular decomposition-reconstitution approach to vesamicol receptor radioligands for positron emission tomography. *Appl Radiat Isot* 1994; 45: 465–472.
11. Mulholland GK, Wieland DM, Kilbourn MR, Frey KA, Sherman PS, Carey JE, et al. [^{18}F]Fluororthoxy-benzovesamicol, a PET radiotracer for the vesicular acetylcholine transporter and cholinergic synapses. *SYNAPSE* 1998; 30: 263–274.
12. Efange SMN, Nader MA, Ehrenkaufer RLE, Kharea AB, Smith CR, Morton TE, et al. (+)-*p*-([^{18}F]fluorobenzyl) spirotrozamicol {(+)-[^{18}F]Spiro-FBT}: synthesis and biological evaluation of a high-affinity ligand for the vesicular acetylcholine transporter (VAChT). *Nucl Med Biol* 1999;

26: 189–192.
13. Shiba K, Mori H, Matsuda H, Ichikawa A, Tonami N. Radioiodinated (−)-2-[4-(3-iodophenyl)piperidino]cyclohexanol: A potential radioligand for mapping presynaptic cholinergic neurons. *Nucl Med Commun* 1996; 17: 485–492.
14. Shiba K, Mori H, Ikeda E, Sumiya H, Tonami N. The potential of radioiodinated (−)-*m*-iodovesamicol for diagnosing cholinergic deficit dementia. *Nucl Med Biol* 2001; 28: 261–264.
15. Custer FGJ, Leysen JE, Stoof JC, Herscheid JDM. Vesamicol and some of its derivatives: Questionable ligands for selectively labelling acetylcholine transporters in rat brain. *Eur J Pharmacol* 1997; 338: 177–183.
16. Shiba K, Yano T, Sato W, Mori H, Tonami N. Characterization of radioiodinated (−)-ortho-iodovesamicol binding in rat brain preparations. *Life Sciences* 2002; 71: 1591–1598.
17. Shiba K, Mori H, Matsuda H, Tsuji S, Kuji S, Sumiya H, et al. Synthesis of radioiodinated analogs of 2-(4-phenylpiperidino)cyclo-hexanol (vesamicol) as vesamicol-like agent. *Nucl Med Biol* 1995; 22: 205–210.
18. Matsuda H, Kinuya K, Tsuji S, Terada K, Shiba K, Kojima K, et al. Preparation and Assessment of Animal Model of Alzheimer's Disease. *KAKU IGAKU (Jpn J Nucl Med)* 1990; 27: 883–891.