J. Radiat. Res., 52, 450–455 (2011)

Regular Paper

Biological Safety of Nasal Thallium-201 Administration: A Preclinical Study for Olfacto-scintigraphy

Kohshin WASHIYAMA[1], Hideaki SHIGA[1,2]*, Kyoko HIROTA[3], Asuka TSUCHIDA[3], Junpei YAMAMOTO[2], Sayaka YAGI[3], Tomokazu YOSHIZAKI[3], Mitsuru FURUKAWA[4], Ryohei AMANO[1] and Takaki MIWA[2]

Thallium-201/Nasal administration/Biological safety/Preclinical study.

Nasal administration of thallium-201 (^{201}Tl) has previously been shown to be useful for the assessment of olfactory nerve connectivity *in vivo*. We assessed the biological effects of nasal ^{201}Tl administration in mice to determine its safety before conducting clinical trials on humans. ^{201}Tl uptake was evaluated in normal mice (n = 5) *in vivo* by using a high-resolution gamma camera and radiography 15 min, 1, 2 and 9 d after administration of ^{201}TlCl to the right side of the nasal cavity (10 µl ^{201}TlCl per nostril, 74 MBq/ml). Murine olfactory epithelial thickness (n = 5) was measured 9 d following nasal administration of ^{201}TlCl. We assessed the odor detection ability of normal mice (n = 8) following nasal administration of ^{201}TlCl to both sides of the nasal cavity, by observing cycloheximide solution avoidance behavior. We subsequently administrated ^{201}TlCl (n = 4) or saline (n = 4) to both nostrils to assess the odor detection ability of mice following bilateral olfactory nerve transection. ^{201}Tl uptake by the nasal cavity decreased immediately following nasal administration of ^{201}Tl in normal mice. Nasal administration of ^{201}Tl did not affect the olfactory epithelial thickness or the odor detection ability of normal mice. Recovery of odor detection ability following olfactory nerve transection was not significantly different between mice nasally administered with ^{201}Tl, and mice administered with saline. Thus, nasal administration of ^{201}Tl for the diagnosis of traumatic olfactory impairment did not produce harmful biological effects *in vivo*.

INTRODUCTION

Olfaction — the sense of smell — is an essential function of human life. However, some individuals lose olfaction, for example, due to olfactory dysfunction secondary to olfactory nerve injury. At present, it is difficult to visualize olfactory nerve damage *in vivo*. Pathological lesions of the olfactory fila, olfactory bulb and olfactory tract are typically inadequately visualized by CT or MRI.[1)] Although olfactory bulb volume visualized with MRI in patients with cerebral damage has been shown to correlate with posttraumatic olfactory function,[2)] MRI could hardly detect the location of olfactory nerve lesions.

We have demonstrated the transport of nasally administered ^{201}Tl-thallium chloride (^{201}TlCl) to the olfactory bulb in rodents.[3)] Moreover, we have previously shown that, in mice, transport of nasally administered ^{201}Tl to the olfactory bulb is significantly decreased by transection of the olfactory nerve fibers,[4)] and that odor detection ability was correlated with the rate of ^{201}Tl transport to the olfactory nerve.[5)] Recently, we have shown that olfactory nerve damage can be diagnosed non-invasively by administering ^{201}TlCl nasally using scintigraphy combined with radiography (olfacto-scintigraphy) in rats.[6)] If we could adapt thallium imaging for patients with traumatic olfactory dysfunction by a simple intranasal administration of ^{201}Tl, it may be possible to better diagnosis injuries to the olfactory nerves.

^{201}TlCl is a myocardial and tumor-scanning radiopharmaceutical.[7)] ^{201}Tl has a physical half-life of 3.08 days, and a whole-body biological half-life of about 10 days when administered intravenously.[8)] Intravenous administration of 74 MBq of ^{201}TlCl to patients for diagnostic purposes did

*Corresponding author: Phone: +81-76-218-8146,
Fax: +81-76-286-5566,
E-mail: shigah@kanazawa-med.ac.jp
[1]Department of Quantum Medical Technology, Graduate School of Medical Science, Kanazawa University, Ishikawa 920-0942, Japan; [2]Department of Otorhinolaryngology-Head and Neck Surgery, Kanazawa Medical University, Ishikawa 920-0293, Japan; [3]Department of Otorhinolaryngology-Head and Neck Surgery, Graduate School of Medical Science, Kanazawa University, Ishikawa 920-8640, Japan; [4]Kanazawa University, Ishikawa 920-1192, Japan.
doi:10.1269/jrr.10153

not result in toxicity. It is therefore safe for clinical imaging.[8–10] However, nasally administered ^{201}TlCl has not been used for routine clinical imaging and its biological safety has not been evaluated.

Nasal administration of drugs has been used for the treatment of local diseases. It also serves as an alternative route to enable systemic delivery of drugs.[11] In addition, radioisotopes are nasally administered in the clinical setting. For example, nasal administration of ^{99m}Tc-DTPA (diethylenetriamine-pentaacetic acid) or ^{99m}Tc-MAA (macroaggregated albumin) has been shown to be safe and reliable for the evaluation of nasal mucociliary clearance function (rhinoscintigraphy).[12,13] Similarly, nasal administration of carbon-11-triamcinolone acetonide is used for pharmacoscintigraphy.[14] Therefore, nasal administration of ^{201}TlCl for the diagnosis of olfactory dysfunction (olfacto-scintigraphy) appears to be a promising modality, provided its safety is demonstrated.

The purpose of this study was to determine the safety of ^{201}TlCl administered nasally before clinical trials are conducted in olfactory-impaired human patients. We assessed the pharmacokinetics of ^{201}Tl in mice following nasal ^{201}TlCl administration by using olfacto-scintigraphy technique, and subsequently evaluated its effects on olfactory epithelial thickness. In addition, we also assessed the effects on odor detection ability. To evaluate the effects of ^{201}Tl administered nasally during recovery of olfaction in mice, olfactory nerve transection was performed and odor detection ability was assessed.

MATERIALS AND METHODS

Materials

Male ICR mice (Japan SLC, Shizuoka, Japan) at 8 weeks of age were housed in an air-conditioned room maintained at 22°C under a 12:12 h light-dark cycle, and fed *ad libitum*. The Kanazawa University Animal Experiment Committee approved all animal experimental procedures in advance.

Pharmacokinetics of nasally administered ^{201}Tl in normal mice

We carefully instilled 10 µl of ^{201}TlCl saline solution (^{201}TlCl, 74 MBq/ml; Nihon Medi-Physics, Kobe, Japan) into the right nasal cavity of normal mice (n = 5) with a microinjection pipette under anesthesia (ether inhalation) to prevent sneezing. In addition, 10 µl of saline was administered to the left side of the nasal cavity. Pharmacokinetics of ^{201}Tl was assessed by gamma scintigraphy at 15 min (0 d), 1, 2 and 9 d following ^{201}TlCl nasal administration.

The mice were kept in the lateral decubitus or prone position under anesthesia (intraperitoneal administration of pentobarbital sodium at 0.05 mg/g). Each mouse was laid on a small, upward-facing CdTe semiconductor gamma camera with high resolution collimator (MGC1500, Acrorad, Tokyo, Japan; effective field 44.6 × 44.6 mm). ^{201}Tl static image acquisition was performed to the head and body for 5 min in each section. After image acquisition, each mouse was moved to an X-ray irradiation system (M60; SOFTEX, Kanagawa, Japan) without change in position. Subsequently, a plain radiograph was taken at 26 kVp, 20 mAs. The ^{201}Tl image and radiograph were overlapped by three positional markers (^{201}Tl deposit on aluminum disc 10 mm in diameter: Top disc 74 kBq; Middle disc 7.4 kBq and Bottom disc 0.74 kBq, which are correspond to 10%, 1%, 0.1% dose of the initial administered activity, respectively) (Fig. 1A). Regions of interest (ROIs) for ^{201}Tl activity were set in each positional marker and each organ from the lateral decubitus or prone position images treated with MGC software 2 (Acrorad, Tokyo, Japan). The organs were identified by using an overlapping plain radiograph. The ROIs of both kidneys was combined. The 10%, 1%, and 0.1% dose of the initial administered radioactivity were determined on ROIs of three positional markers, respectively. The organ ROIs were divided by appropriate positional marker ROI, and consequently the isotope uptake (% dose) in each organ was obtained as the

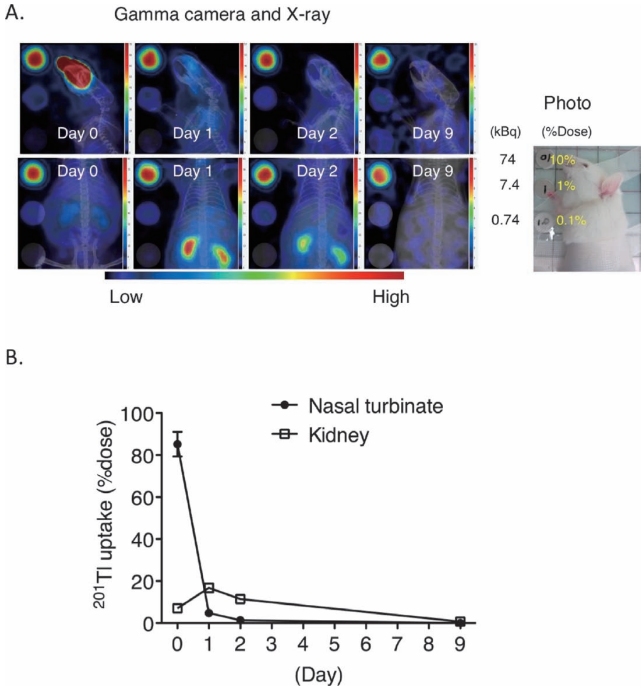

Fig. 1. ^{201}Tl uptake change after nasal administration of ^{201}Tl in normal mice. A) ^{201}Tl uptake images overlapped with radiograph show changes of radioactivity in the head and body of mice by three positional markers (^{201}Tl deposit on aluminum disc 10 mm in diameter: Top disc 74 kBq; Middle disc 7.4 kBq and Bottom disc 0.74 kBq, which are correspond to 10%, 1%, 0.1% dose of the initial administered activity, respectively). The maximum of color range of each 8 image was normalized by 10% dose (10 µL of 7.4 MBq/mL) ROI count. B) ^{201}Tl uptake (% dose) in the nasal turbinate decreased after nasal administration of ^{201}Tl (n = 5, Mean ± S.D.).

percentage of radioactivity in each organ relative to the initial administered radioactivity.

Measurement of the olfactory epithelial thickness and immunohistochemistry (olfactory marker protein)

For the five normal mice nasally administered with ^{201}TlCl (right nasal cavity) and saline (left nasal cavity) we then calculated olfactory epithelial thickness of as the mean distance from the basal to the superficial membrane of the olfactory epithelium in three fields of each side, at 100× under a Nikon Eclipse E1000 microscope (Nikon, Tokyo, Japan).

We determined expression of olfactory marker protein (OMP) to ascertain the location of the olfactory epithelium by immunohistochemical staining, because OMP is a cell-specific marker of mature olfactory chemosensory neurons.[15] Normal mice (n = 5) were perfused with physiological saline and fixed with 4% paraformaldehyde under ether anesthesia after image acquisition on day 9. The head was dissected and the facial bones were removed. After fixation with 4% paraformaldehyde overnight, the head was decalcified in K-CX solution (Falma, Osaka, Japan) and embedded in paraffin. The samples were cut into 3-μm-thick sections and mounted on slides for immunohistochemical staining. The sections were deparaffinized with xylene and rehydrated through a graded alcohol series. After blocking and antigen retrieval, the sections were incubated with anti-OMP antibodies (1:6000 dilution; Wako, Osaka, Japan) in an antibody diluent (Dako Cytomation, Glostrup, Denmark) for 1 h at room temperature. After washing with PBS (Phosphate Buffered Saline), each section was incubated for 30 min with biotinylated secondary antibody (Dako Cytomation, Glostrup, Denmark), again washed in PBS, and then incubated for 30 min with an avidin-biotin complex reagent (Dako Cytomation, Glostrup, Denmark). The reaction products were then washed with PBS. After development, the sections were lightly counterstained and mounted. As a negative control, an antibody diluent was applied instead of the primary antibody solution.

Measurement of odor detection ability in normal mice

The assay of odor detection ability is described elsewhere.[5,6] Briefly, cycloheximide has a peculiar odor and unpleasant taste. Normal mice (n = 8) were first deprived of water for 2 days, then trained to avoid cycloheximide solution. The mice were conditioned in two training sessions, each consisting of 10 trials. In each trial, the mice were allowed to choose between 0.01% cycloheximide solution and tap water. The positions of a bottle containing cycloheximide solution and another containing tap water were randomized according to a uniform random number (the cycloheximide bottle was placed on the right side of the cage if the number was odd, and the water bottle was placed on the right if the number was even). After the training sessions, we examined the odor detection ability of normal mice by observing their cycloheximide solution avoidance behavior 2 and 9 days following nasal administration of ^{201}Tl to both nasal cavities.

Bilateral olfactory nerve transection

The olfactory nerve fibers were transected according to a method previously described.[4,5] Briefly, we exposed the right and left olfactory bulbs, cutting the frontal bones of the mice under anesthesia (ether inhalation, followed by intraperitoneal administration of pentobarbital sodium, 0.05 mg/g). The olfactory nerve fibers of mice (n = 8) conditioned to recognize cycloheximide solution were carefully transected bilaterally with a Teflon knife while avoiding damage to the olfactory bulbs. For convenience, the mice that underwent bilateral olfactory nerve transection are referred to as BNTX mice.

Nasal administration of ^{201}Tl to BNTX mice

On 2 day following olfactory nerve transection, 10 μl of ^{201}TlCl solution or saline was carefully instilled into each nostril in each BNTX mouse (n = 8; four mice received ^{201}Tl, and four saline) with a microinjection pipette under anesthesia (ether inhalation) to prevent sneezing.

Measurement of odor detection ability in BNTX mice

BNTX mice were assessed on days 2, 7, 14, 21, and 28 after olfactory nerve transection for their odor detection ability.

Statistical analysis

We statistically compared mean values with the paired t-test or Mann-Whitney U-test (Prism 5, GraphPad, San Diego, CA, USA). All p values were two tailed. A p value of < 0.05 was considered statistically significant.

RESULTS

Pharmacokinetics of ^{201}Tl in mice after nasal administration

To determine the pharmacokinetics of ^{201}Tl after nasal administration *in vivo*, we assessed ^{201}Tl uptake in normal mice at 4 time points during the 9-day period with a gamma camera and a plain radiograph (Fig. 1A). ^{201}Tl retained in the nasal cavity decreased after administration (Fig. 1B; n = 5). In the kidneys, ^{201}Tl uptakes increased after administration, and increased at day 1, and then subsequently decreased. At 9 days, ^{201}Tl uptake in the kidneys was less than 1% of the dose administered. ^{201}Tl uptake in the other organs was not high compared to the nasal cavity and kidneys (Fig. 1A). These trends were the same in each assessed mouse.

Olfactory epithelial thickness after nasal administration of ^{201}TlCl

Olfactory epithelium thickness was not significantly dif-

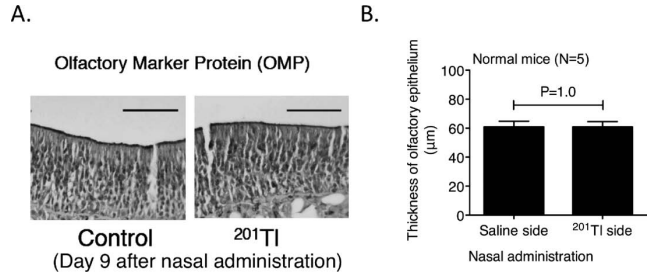

Fig. 2. No significant change in olfactory epithelial thickness was observed after nasal administration of ^{201}Tl to normal mice. (A) The location of the olfactory epithelium was confirmed by olfactory marker protein (a cell-specific marker of mature olfactory chemosensory neurons) expression after immunohistochemical staining. Scales indicate 50 μm. (B) Olfactory epithelial thickness was not significantly different between either side of the nasal cavity 9 d after nasal administration of ^{201}Tl (n = 5, Mean ± S.D.; p = 1.0, paired t-test).

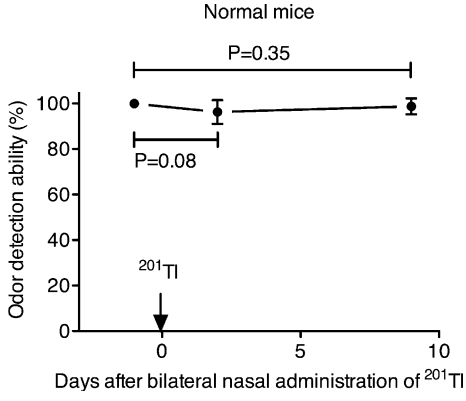

Fig. 3. Odor detection ability in normal mice after nasal administration of ^{201}Tl. Odor detection ability was not significantly decreased on days 2 and 9 after administration of ^{201}Tl, compared to that before ^{201}Tl administration (n = 8, Mean ± S.D.; p = 0.08 (day 2), p = 0.35 (day 9), paired t-test).

ferent between the two sides of the nasal cavity 9 d after nasal administration of ^{201}TlCl or saline (Fig. 2; p = 1.0, paired t-test).

Odor detection ability in normal mice

There was no significant difference in the odor detection ability between the days before and after ^{201}TlCl administration in normal mice (Fig. 3).

Recovery of odor detection ability in BNTX mice

Two days after nerve transection, the odor detection ability of all BNTX mice was less than 60%. After bilateral nasal administration of ^{201}TlCl (n = 4) and saline (n = 4), the mice recovered their odor detection ability; this recovery did not significantly differ at any time points between mice nasally administered with ^{201}Tl, and controls administered with saline (Fig. 4).

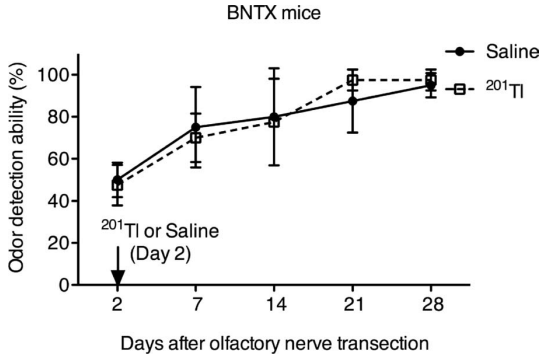

Fig. 4. Nasal administration of ^{201}Tl had no significant effect on the recovery of odor detection ability of mice with surgical olfactory impairment (BNTX mice). Recovery of odor detection ability after olfactory nerve transection was not significantly different between BNTX mice nasally administered with ^{201}Tl, and that of control mice administered with saline (n = 4 for each group, Mean ± S.D.; p = 0.76 (day 2), p = 0.87 (day 7), p = 0.87 (day 14), p = 0.41 (day 21), p = 0.61 (day 28), Mann-Whitney U-test).

DISCUSSION

Accurate evaluation of olfactory function remains difficult clinically. Many studies have explored the use of newly developed imaging methods, such as functional MRI,[16,17] magnetic encephalography,[18] PET,[19] olfactory event-related potential,[20] and near-infrared spectroscopy[21] to enable objective assessment of olfactory function. However, most of these imaging modalities do not adequately locate olfactory nerve lesions, and imaging methods for the olfactory nerves remain to be developed.

Recently, small semiconductor gamma cameras with high resolution and sensitivity have been developed. In these cameras, their high resolution and sensitivity with linearity of wide range of count rate could make useful in imaging for small animal experiments.[22]

We previously developed a new imaging method based on nasal administration of ^{201}Tl in animals to assess visually the olfactory nerves.[4–6] It has been shown that intravenous administration of ^{201}Tl is safe for clinical imaging,[8] but nasally administered ^{201}Tl has not been used in routine clinical imaging and its biological safety has not been demonstrated.

The pharmacokinetics of ^{201}Tl showed that radioactivity in the nasal cavity of normal mice began to decrease immediately after nasal administration of ^{201}Tl, and that ^{201}Tl accumulated mainly in the kidneys; there was no notable uptake in other organs. In nuclear medicine, ^{201}TlCl is used for myocardial scintigraphy. ^{201}TlCl is injected intravenously into patients and first accumulated in cardiac muscle within 5 min, and then accumulated mainly in kidney. Our results showed that ^{201}Tl uptake in the other organs including car-

diac muscle was not high at the value of 0.72 ± 0.41 % dose compared to the nasal cavity (80.3 ± 13.1 %dose) and kidneys (6.86 ± 1.14 %dose) at 15 min *in vivo*. We have already finished another study about biodistribution measurements during 14 days using two groups of mice administered with ^{201}Tl intranasally or intravenously for radiation dose estimation. The results showed that the uptakes of intranasally administered ^{201}Tl in all tissue except olfactory bulb and nasal cavity were comparable or less to that of intravenously administered ^{201}Tl during 14 days (unpublished data). Therefore, cardiac toxicity may be tolerable in patients intranasally administered with ^{201}Tl.

Because ^{201}Tl activity in the nasal cavity was high following nasal administration of ^{201}Tl, we assessed olfactory epithelial thickness in five normal mice 9 d after ^{201}TlCl administration into one side of the nasal cavity and saline into the other. We confirmed histologically that olfactory epithelial thickness did not change significantly after nasal administration of ^{201}Tl in normal mice after 9 d. We also demonstrated that nasally administered ^{201}Tl did not cause any change to the odor detection ability in normal mice. The radioactivity of ^{201}Tl in the nasal cavity does not remain high following *in vivo* nasal administration. Thus, the effect of ^{201}Tl on the olfactory nervous system may be tolerable during clinical trials. As such, ^{201}Tl may be a safe candidate as a bio-tracer for olfactory nerve damage in clinical imaging.

^{201}TlCl is used for myocardial scintigraphy due to its chemical similarity to potassium.[7] Thallium has been shown to readily substitute for potassium at the (Na+/K+)-membrane ATPase activation sites, and it does not leak out of tissue as rapidly as potassium.[7] Nasally administered ^{201}Tl may be imported into the olfactory nerve cells as a substitute for potassium.

Except for ^{201}Tl, certain metal ions such as manganese (Mn), zinc, and rubidium are known to be transported from the nasal cavity to the olfactory bulb.[3,23,24] In particular, Mn has been used in MRI imaging owing to its paramagnetic nature. Transport of Mn from the nasal cavity to the olfactory nerve has been shown by MRI in live animals.[25] However, inhalation of high doses of Mn is associated with neurotoxicity in animals, including humans.[26] Therefore, nasal administration of Mn is not used clinically for objective assessment of olfactory nerve damage.

In our previous study, we showed that odor detection ability is correlated with the rate of ^{201}Tl transport to the olfactory nerve. However, during the olfactory nerve regeneration process, whether ^{201}Tl administration for functional evaluation affects olfaction recovery remains unknown. To determine the effect of ^{201}Tl on olfaction recovery, BNTX mice were assessed on 2, 7, 14, 21, and 28 d after olfactory nerve transection. Because saline has not been thought to physiologically affect odor detection ability, we compared the odor detection ability of BNTX mice administered with ^{201}Tl to that of mice administration with saline. We showed nasal administration of ^{201}Tl to BNTX mice did not significantly affect the recovery of olfaction after transection of the olfactory nerve. Our results show that ^{201}Tl administered nasally to mice with surgically induced olfactory impairment produced no harmful effect.

We are now planning further investigations of nasally administered ^{201}TlCl for the detection of olfactory nerve damage in a clinical trial with olfactory-impaired patients. ^{201}Tl may be a candidate for the first safe bio-tracer to monitor olfaction during treatment for olfactory disorders. Patients with intact olfactory nerve fibers may be well selected by means of a new isotope imaging technique for the long-term treatment of olfactory dysfunction after head trauma. ^{201}Tl scintigraphy with nasal administration of ^{201}Tl may be also useful for the analysis of treatment efficacy with new medicines especially for patients with posttraumatic olfactory impairment.

In conclusion, nasal administration of ^{201}Tl produce no biologically harmful effects when used for the diagnosis of traumatic olfactory impairment *in vivo*. Nasal administration of ^{201}Tl may thus be adapted for visual diagnosis of olfactory nerve damage in clinical trials in patients with olfactory disorders.

ACKNOWLEDGEMENTS

We would like to extend out sincere gratitude to the Division of Radioisotopes, Kanazawa University Hospital, and to Daisuke Ogawa and Yuko Oya for their technical support and assistance. This research was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (C21592174 to H.S.; C20591997 to T.M.), and a research grant from the Tanabe Mitsubishi Pharma Corporation (T.M., H.S., K.H.).

Authors' Disclosures of Potential Conflicts of Interest: The authors indicate no potential conflicts of interest.

REFERENCES

1. Gottfried JA, *et al* (2002) Functional heterogeneity in human olfactory cortex: an event-related functional magnetic resonance imaging study. J Neurosci **22**: 10819–10828.
2. Rombaux P, *et al* (2006) Retronasal and orthonasal olfactory function in relation to olfactory bulb volume in patients with posttraumatic loss of smell. Laryngoscope **116**: 901–905.
3. Kanayama Y, *et al* (2005) Axonal transport of rubidium and thallium in the olfactory nerve of mice. Nucl Med Biol **32**: 505–512.
4. Kinoshita Y, *et al* (2008) Thallium transport and the evaluation of olfactory nerve connectivity between the nasal cavity and olfactory bulb. Chem Senses **33**: 73–78.
5. Shiga H, *et al* (2008) Odor detection ability and thallium-201 transport in the olfactory nerve of traumatic olfactory-impaired mice. Chem Senses **33**: 633–637.

6. Shiga H, *et al* (2009) Use of thallium transport to visualize functional olfactory nerve regeneration in vivo. Rhinology **47**: 460–464.
7. Ando A, *et al* (1988) Biodistributions of radioactive alkaline metals in tumor bearing animals: comparison with 201Tl. Eur J Nucl Med **14**: 352–357.
8. Saha GB (1984) Fundamentals of Nuclear Pharmacy, 5th ed., pp. 293–294. Springer-Verlag, New York.
9. Iida H and Eberl S (1998) Quantitative assessment of regional myocardial blood flow with thallium-201 and SPECT. J Nucl Cardiol **5**: 313–331.
10. Higuchi T, *et al* (2005) Characterization of cartilaginous tumors with ^{201}Tl scintigraphy. Ann Nucl Med **19**: 95–99.
11. Illum L (2003) Nasal drug delivery--possibilities, problems and solutions. J Control Release **87**: 187–198.
12. Di Giuda D, *et al* (2000) Rhinoscintigraphy: A simple radioisotope technique to study the mucociliary system. Clin Nucl Med **21**: 1029–1032.
13. Sun SS, *et al* (2002) Evaluation of nasal mucociliary clearance function in allergic rhinitis patients with technetium 99m-labeled macroaggregated albumin rhinoscintigraphy. Ann Otol Rhinol Laryngol **111**: 77–79.
14. Berridge MS, *et al* (1998) Biodistribution and kinetics of nasal corbon-11-triamcinolone acetonide. J Nucl Med **39**: 1972–1977.
15. Kream RM and Margolis FL (1984) Olfactory marker protein: turnover and transport in normal and regenerating neurons. J Neuroscience **4**: 868–879.
16. Koizuka I, *et al* (1994) Functional imaging of the human olfactory cortex by magnetic resonance imaging. ORL J Otorhinolaryngol Relat Spec **56**: 273–275.
17. Sobel N, *et al* (1998) Sniffing and smelling: Separate subsystems in the human olfactory cortex. Nature **392**: 282–286.
18. Tonoike M, *et al* (1998) Ipsilateral dominance of human olfactory activated centers estimated from event-related magnetic fields measured by 122-channel whole-head neuromagnetometer using odorant stimuli synchronized with respirations. Ann N Y Acad Sci **855**: 579–590.
19. Dade LA, *et al* (1998) Human brain function during odor encoding and recognition. A PET activation study. Ann N Y Acad Sci **855**: 572–574.
20. Olofsson JK, *et al* (2006) Laterality of the olfactory event-related potential response. Chem Senses **31**: 699–704.
21. Kobayashi E, *et al* (2007) Functional optical hemodynamic imaging of the olfactory cortex. Laryngoscope **117**: 541–546.
22. Kiyono Y, *et al* (2007) Applicability of a high-resolution small semiconductor gamma camera to small animal imaging. Nulc Med Commun **28**: 736–741.
23. Persson E, *et al* (2003) Transport and subcellular distribution of intranasally administered zinc in the olfactory system of rats and pikes. Toxicology **191**: 97–108.
24. Brenneman KA, *et al* (2000) Direct olfactory transport of inhaled manganese ($^{54}MnCl_2$) to the rat brain: toxicokinetic investigations in a unilateral nasal occlusion model. Toxicol Appl Pharmacol **169**: 238–248.
25. Drobyshevsky A, *et al* (2006) Sensory deficits and olfactory system injury detected by novel application of MEMRI in newborn rabbit after antenatal hypoxia-ischemia. Neuroimage **32**: 1106–1112.
26. Aschner M, *et al* (2007) Manganese: Recent advances in understanding its transport and neurotoxicity. Toxicol Appl Pharmacol **221**: 131–147.

Received on October 21, 2010
Revision received on January 8, 2011
Accepted on March 4, 2011
J-STAGE Advance Publication Date: May 13, 2011