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

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

Nasal administration of thallium-201 (201Tl) has previously been shown to be useful for the assessment of olfactory nerve connectivity in vivo. We assessed the biological effects of nasal 201Tl administration in mice to determine its safety before conducting clinical trials on humans. 201Tl 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 201TlCl to the right side of the nasal cavity (10 μl 201TlCl per nostril, 74 MBq/ml). Murine olfactory epithelial thickness (n = 5) was measured 9 d following nasal administration of 201TlCl. We assessed the odor detection ability of normal mice (n = 8) following nasal administration of 201TlCl to both sides of the nasal cavity, by observing cycloheximide solution avoidance behavior. We subsequently administrated 201TlCl (n = 4) or saline (n = 4) to both nostrils to assess the odor detection ability of mice following bilateral olfactory nerve transection. 201Tl uptake by the nasal cavity decreased immediately following nasal administration of 201TlCl. Nasal administration of 201TlCl 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 201TlCl and mice administered with saline. Thus, nasal administration of 201Tl 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 201Tl-thallium chloride (201TlCl) to the olfactory bulb in rodents.3 Moreover, we have previously shown that, in mice, transport of nasally administered 201Tl 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 201Tl transport to the olfactory nerve.5 Recently, we have shown that olfactory nerve damage can be diagnosed non-invasively by administering 201TlCl 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 201TlCl, it may be possible to better diagnosis injuries to the olfactory nerves.

201TlCl is a myocardial and tumor-scanning radiopharmaceutical.7 201Tl 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 201TlCl to patients for diagnostic purposes did

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doi:10.1269/jrr.10153
Safety of Nasal Thallium-201 Administration

Not result in toxicity. It is therefore safe for clinical imaging. However, nasally administered 201TlCl 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. In addition, radioisotopes are nasally administered in the clinical setting. For example, nasal administration of Tc-DTPA (diethylenetriamine-pentaacetic acid) or Tc-MAA (macroaggregated albumin) has been shown to be safe and reliable for the evaluation of nasal mucociliary clearance function (rhinoscintigraphy). Similarly, nasal administration of carbon-11-labeled tritiated acetonide is used for olfato-scintigraphy. Therefore, nasal administration of 201Tl 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 201TlCl administered nasally before clinical trials are conducted in olfactory-impaired human patients. We assessed the pharmacokinetics of 201Tl in mice following nasal 201TlCl 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 201Tl 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 201Tl in normal mice

We carefully instilled 10 μl of 201TlCl saline solution (201TlCl, 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 201Tl was assessed by gamma scintigraphy at 15 min (0 d), 1, 2 and 9 d following 201TlCl 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). 201Tl 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 201Tl image and radiograph were overlapped by three positional markers (201Tl 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 201Tl 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...
Measurement of the olfactory epithelial thickness and immunohistochemistry (olfactory marker protein)

For the five normal mice nasally administered with 201TlCl (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. 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), again washed in PBS, and then incubated with biotinylated secondary antibody (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. 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, 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 201Tl to both nasal cavities.

Bilateral olfactory nerve transection

The olfactory nerve fibers were transected according to a method previously described. 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 201Tl to BNTX mice

On 2 day following olfactory nerve transection, 10 μl of 201TlCl solution or saline was carefully instilled into each nostril in each BNTX mouse (n = 8; four mice received 201Tl, 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 201Tl in mice after nasal administration

To determine the pharmacokinetics of 201Tl after nasal administration in vivo, we assessed 201Tl uptake in normal mice at 4 time points during the 9-day period with a gamma camera and a plain radiograph (Fig. 1A). 201Tl retained in the nasal cavity decreased after administration (Fig. 1B; n = 5). In the kidneys, 201Tl uptakes increased after administration, and increased at day 1, and then subsequently decreased. At 9 days, 201Tl uptake in the kidneys was less than 1% of the dose administered. 201Tl 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 201TlC

Olfactory epithelium thickness was not significantly dif-
No significant change in olfactory epithelial thickness was observed after nasal administration of 201Tl 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 201Tl (n = 5, Mean ± S.D.; p = 1.0, paired t-test).

Fig. 2. No significant change in olfactory epithelial thickness was observed after nasal administration of 201Tl 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 201Tl (n = 5, Mean ± S.D.; 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 201Tl administration of 201TlCl or saline (Fig. 2; p = 1.0, paired t-test).

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

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 201TlCl (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 201Tl, and controls administered with saline (Fig. 4).

DISCUSSION
Accurate evaluation of olfactory function remains difficult clinically. Many studies have explored the use of newly developed imaging methods, such as functional MRI, magnetic encephalography, PET, olfactory event-related potential, and near-infrared spectroscopy 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.

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

The pharmacokinetics of 201Tl showed that radioactivity in the nasal cavity of normal mice began to decrease immediately after nasal administration of 201Tl, and that 201Tl accumulated mainly in the kidneys; there was no notable uptake in other organs. In nuclear medicine, 201TlCl is used for myocardial scintigraphy. 201TlCl is injected intravenously into patients and first accumulated in cardiac muscle within 5 min, and then accumulated mainly in kidney. Our results showed that 201Tl uptake in the other organs including car-
diac muscle was not high at the value of $0.72 \pm 0.41 \% \text{dose}$ compared to the nasal cavity ($80.3 \pm 13.1 \% \text{dose}$) and kidneys ($6.86 \pm 1.14 \% \text{dose}$) at 15 min in vivo. We have already finished another study about biodistribution measurements during 14 days using two groups of mice administered with 201Tl intranasally or intravenously for radiation dose estimation. The results showed that the uptakes of intranasally administered 201Tl in all tissue except olfactory bulb and nasal cavity were comparable or less to that of intravenously administered 201Tl during 14 days (unpublished data). Therefore, cardiac toxicity may be tolerable in patients intranasally administered with 201Tl.

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

201TlCl 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.5 Nasally administered 201Tl may be imported into the olfactory nerve cells as a substitute for potassium.

Except for 201Tl, 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 201Tl transport to the olfactory nerve. However, during the olfactory nerve regeneration process, whether 201Tl administration for functional evaluation affects olfaction recovery remains unknown. To determine the effect of 201Tl 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 201Tl to that of mice administration with saline. We showed nasal administration of 201Tl to BNTX mice did not significantly affect the recovery of olfaction after transection of the olfactory nerve. Our results show that 201Tl administered nasally to mice with surgically induced olfactory impairment produced no harmful effect.

We are now planning further investigations of nasally administered 201TlC1 for the detection of olfactory nerve damage in a clinical trial with olfactory-impaired patients. 201Tl 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. 201Tl scintigraphy with nasal administration of 201Tl 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 201Tl produce no biologically harmful effects when used for the diagnosis of traumatic olfactory impairment in vivo. Nasal administration of 201Tl 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.

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