ORIGINAL CONTRIBUTION

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Use of thallium transport to visualize functional olfactory nerve regeneration *in vivo**

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SUMMARY

Objective: *To image olfactory nerve regeneration in vivo using a high-resolution gamma camera and radiography after nasal administration of thallium-201 (olfacto-scintigraphy).*
Methods: *Six Wistar rats were trained to avoid the smell of cycloheximide as a test of olfactory function. The olfactory nerve fibers of 3 rats were then carefully transected bilaterally with a Teflon knife, avoiding damage to the olfactory bulbs. The remaining 3 rats underwent sham operations and were used as controls. Steel wires were implanted in the left olfactory bulb of each rat for locating the bulbs with plain X-rays. The rats were assessed 2, 14, 28, and 42 d after the olfactory nerve transection or sham operation for their ability to detect odours and for transport of ^{201}Tl to the olfactory bulb area 8 h after nasal administration of ^{201}Tl.*
Results: *Both transport of ^{201}Tl to the olfactory bulb area ($p < 0.04$) and ability to detect odours ($p < 0.04$) significantly increased with a time course after olfactory nerve transection.*
Conclusion: *^{201}Tl transport to the olfactory bulb may be useful to visually assess olfactory ability in vivo. We plan to test olfacto-scintigraphy clinically by nasal administration of ^{201}Tl in patients with posttraumatic olfactory loss.*

Key words: olfactory nerve regeneration, thallium, nasal administration, gamma camera, posttraumatic olfactory loss

INTRODUCTION

Traumatic olfactory impairment can occur as a result of sinonasal contusions or nasal fractures, tearing or shearing of olfactory nerve fibers, or intracranial contusion and hemorrhage in olfactory brain regions [(1)]. It is difficult to predict the prognosis of patients with head injuries resulting in olfactory impairment, in part because the pathological lesions of the olfactory fila, olfactory bulb, or olfactory tract typically cannot be well visualized with computed tomography (CT) or magnetic resonance imaging (MRI) [(2)]. Injuries to the olfactory bulb and tract have been visualized with MRI in patients with posttraumatic olfactory loss; however, the injuries did not correlate well with olfactory test scores [(3)].

The radioisotope thallium-201 is transported within the olfactory neural tract after nasal administration in rodents [(4)], and this transport is significantly decreased after the transection of olfactory nerve fibers in mice [(5)]. We have also shown a correlation between odour detection ability (ODA) and the rate of ^{201}Tl transport in the olfactory nerve in mice dissected [(6)]. Regeneration of the olfactory nerve has also been assessed by autoradiography of mouse head sections after nasal administration of ^{201}Tl [(6)]. However, it remains to be seen whether olfactory nerve regeneration after transection can be detected by using an *in vivo* imaging system that could potentially be applied to patients with posttraumatic olfactory impairment.

In this study, we bilaterally transected the olfactory nerve as a model of traumatic olfactory injury in rats. Rats with bilateral olfactory nerve transection (BNTX) and sham-operated controls were administered ^{201}Tl nasally, and transport of the ^{201}Tl in the olfactory nerve was examined by using an *in vivo* imaging system with a high-resolution gamma camera and plain X-ray (olfacto-scintigraphy).

MATERIALS AND METHODS

Materials

Female Wistar rats aged 8 wk (Japan SLC, Shizuoka, Japan) were housed in a 22°C air-conditioned room with a 12-hour light:12-hour dark cycle and were freely provided food (CLEA Japan, Tokyo, Japan) and water. The Kanazawa University animal experiment committee approved all animal experimental procedures in advance.

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Bilateral olfactory nerve transection and olfactory bulb marker

The olfactory nerve fibers of rats were transected according to a previously described method [5-6]. We exposed both the right and left olfactory bulbs by cutting the frontal bones of the rats under anesthesia (ether inhalation followed by intraperitoneal administration of pentobarbital sodium, 0.05 mg/g body weight). The olfactory nerve fibers of 3 rats were carefully transected bilaterally with a Teflon knife under a microscope, avoiding damage to the olfactory bulbs. The skin incision was closed with nylon sutures. Sham-operated rats had their olfactory bulbs exposed, but the olfactory nerves were not transected (Sham controls, n = 3).

Steel wire fragments were implanted in the left olfactory bulb of each rat to enable the olfactory bulb to be located with plain X-rays.

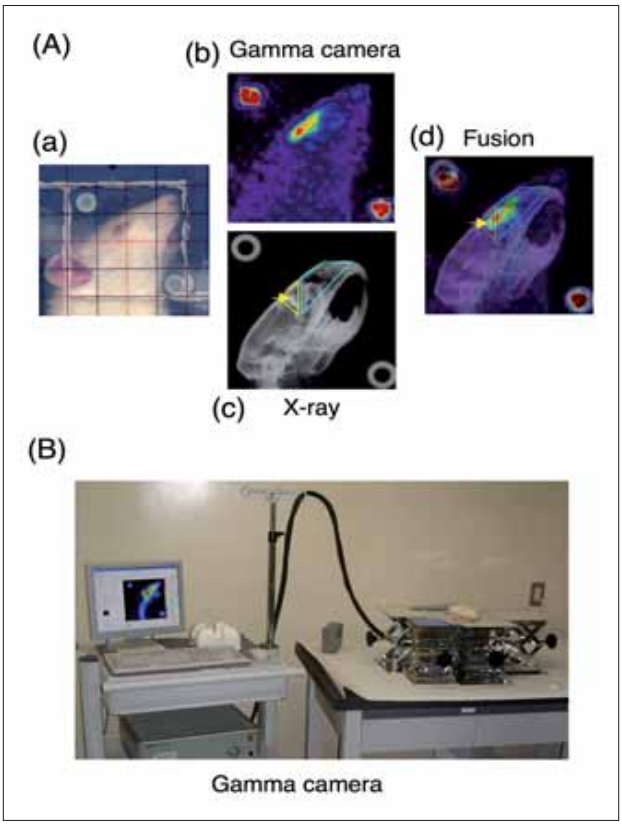

Figure 1. (A) (a) Photograph of a rat on the imaging plate. Each rat was implanted with steel wire fragments in the left olfactory bulb, and a ^{201}TlCl solution (60 l; 74 MBq/ml) was instilled into the right nasal cavity of each rat. Two positional markers (^{201}TlCl solution in the center of a steel ring) can be seen. (b) Eight hours after nasal administration of ^{201}Tl, each rat was laid with its head on the gamma camera, and a sagittal image of the head was acquired. (c) After ^{201}Tl image acquisition, each rat was moved to an X-ray irradiation system without any change in position and a plain radiograph was taken to locate the olfactory bulb. Yellow outline and arrow indicate the olfactory bulb area; blue outline indicates the nasal cavity. (d) The ^{201}Tl image and radiograph were overlapped by aligning the 2 positional markers. (B) The gamma camera imaging setup.

^{201}Tl scintigraphy

^{201}TlCl saline solution (74 MBq/ml) was obtained from Nihon Medi-Physics (Tokyo, Japan). A ^{201}TlCl solution (60μl) was instilled into the right nasal cavity of each rat with a microinjection pipette. The rats were anesthetized by ether inhalation followed by intraperitoneal administration of pentobarbital sodium (0.05 mg/g) to prevent sneezing. The rats were kept lateral decubitus position under anesthesia for eight hours after nasal administration of ^{201}Tl. Eight hours after nasal administration of ^{201}Tl, each rat was laid laterally with its head on an upward-facing gamma camera (MGC1500, Acrorad, Tokyo, Japan), and a sagittal image of the head was obtained. After ^{201}Tl image acquisition, each rat was moved to an X-ray irradiation system (M60; SOFTEX, Kanagawa, Japan) without any change in position and a plain radiograph was taken. The ^{201}Tl image and radiograph were overlapped by aligning two positional markers (^{201}TlCl solution in the center of a steel ring), and the olfactory bulb area and the nasal cavity area were marked as regions of interest (Figure 1).

^{201}Tl transport from the nasal cavity area to the olfactory bulb area was determined as the percentage of the mean ^{201}Tl intensity in the olfactory bulb area divided by that in the nasal cavity area. ^{201}Tl intensity was assessed with MGC software 2 (Acrorad, Tokyo, Japan).

Odour detection ability (ODA)

ODA was assessed as described previously by using avoidance of cycloheximide solution [6]. Cycloheximide has a peculiar odour and unpleasant taste and has been used as a mouse poison. After being deprived of water for 36 h, rats were trained to avoid the cycloheximide solution. The cycloheximide solution avoidance behavior was examined by testing the ability to distinguish a 0.01% cycloheximide solution bottle from tap water bottle (the counts how many times the rats visited each bottle were assessed). The positions of the cycloheximide solution bottle and tap water bottle were randomized according to a uniform random number system: the cycloheximide bottle was placed on the right side of the cage if the random number was odd, the water bottle was placed on the right if it was even. Each rat was examined twice a day, with 10 trials each time, for conditioning to the odour of cycloheximide before the transection of olfactory nerve or sham operation. After the training, the rats avoided the scent of cycloheximide solution.

Statistical analysis

Statistical comparison of median values was performed for the Kruskal-Wallis test with Prism 5 software (GraphPad, San Diego, CA, USA). All tests were two-tailed and $p < 0.05$ was considered to indicate significance.

RESULTS

Measurement of thallium transport using a gamma camera and plain X-ray

^{201}Tl transport from the nasal cavity area to the olfactory bulb

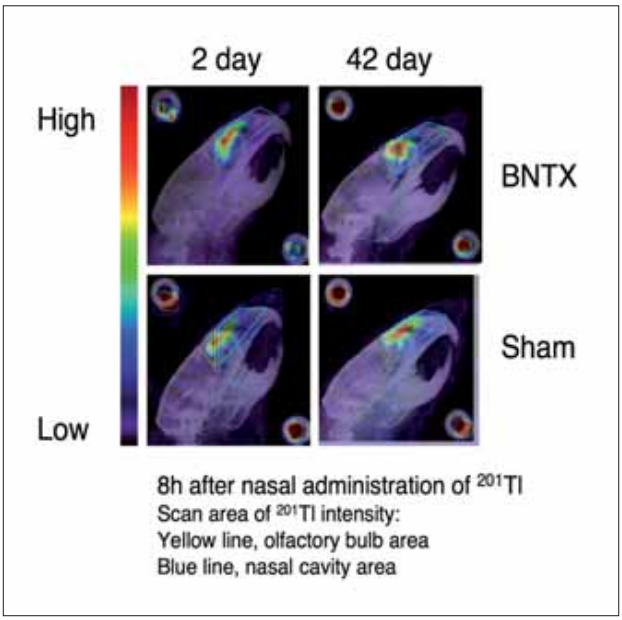

Figure 2. Analysis of olfactory nerve regeneration with a gamma camera and radiography in rats with traumatic olfactory injuries (BNTX) and sham-operated controls. ^{201}Tl transport from the nasal cavity area (outlined in blue) to the olfactory bulb area (outlined in yellow) was decreased 2 d after the transection of the olfactory nerve, but increased again by 42 d after the transection. ^{201}Tl was transported in the olfactory nerve at both 2 d and 42 d after the sham operation in the controls. The images were obtained 8 h after nasal administration of ^{201}Tl. Representative images are shown.

was assessed in 3 BNTX rats and 3 sham controls 2, 14, 28, and 42 d after the transection. A ^{201}TlCl solution was instilled into the right nasal cavity of each rat on each examination day. Transport of ^{201}Tl in the olfactory nerve was lower in the 3 BNTX rats than in the sham-operated controls 2 d after the operation, but ^{201}Tl transport to the olfactory bulb area was

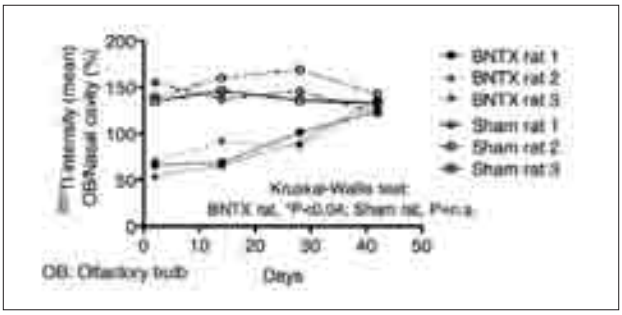

Figure 3. ^{201}Tl transport from the nasal cavity area to the olfactory bulb area significantly increased with a time course after transection of the olfactory nerve fibers in BNTX rats ($P < 0.04$, Kruskal-Wallis test, N = 3), but not did not change significantly with time after a sham operation (P = n.s., Kruskal-Wallis test, N = 3). ^{201}Tl transport in the olfactory nerve was determined as the percentage of the mean ^{201}Tl intensity in the olfactory bulb area divided by that in the nasal cavity area (see the areas marked in Figures 1 and 2).

restored by 42 d after transection of the olfactory nerve fibers (Figure 2). Transport of ^{201}Tl to the olfactory bulb area was not affected by the sham operation (Figure 2).

Recovery of ^{201}Tl transport with a time course after transection of the olfactory nerve fibers in BNTX rats

Transport of ^{201}Tl from the nasal cavity area to the olfactory bulb area significantly increased with a time course after transection of the olfactory nerve fibers (Figure 3; $p < 0.04$, Kruskal-Wallis test, n = 3). Transport of ^{201}Tl from the nasal cavity area to the olfactory bulb area did not change significantly after the sham operation (Figure 3; p = n.s., Kruskal-Wallis test, n = 3).

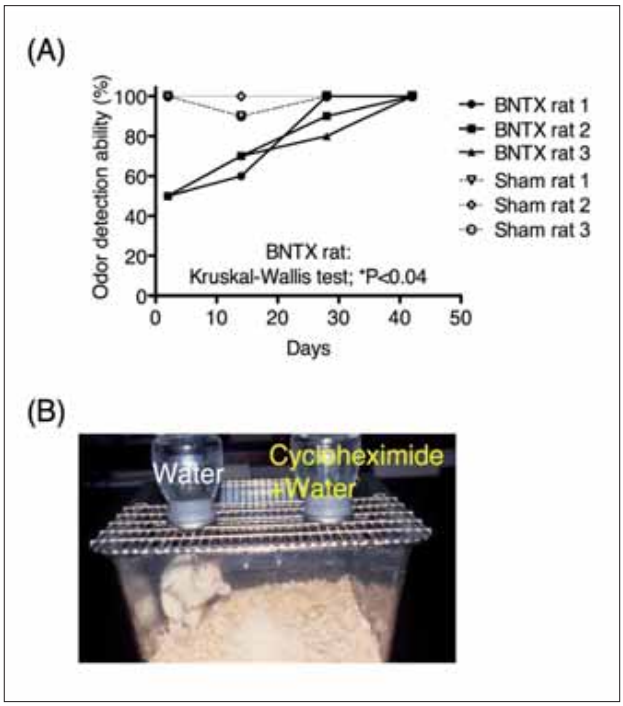

Figure 4. (A) Odour detection ability (ODA) significantly increased with a time course after transection of the olfactory nerve fibers in BNTX rats ($P < 0.04$, Kruskal-Wallis test, N = 3). ODA was 90% or more on each examination day in three sham-operated controls. (B) The positions of the cycloheximide solution bottle and tap water bottle were randomized by generating uniform random numbers and assigning bottle position on the basis of even and odd number.

Recovery of ODA after traumatic olfactory injury

To assess ODA in the BNTX rats imaged with ^{201}Tl olfacto-scintigraphy, 6 rats were trained to avoid the smell of cycloheximide solution before transection of the olfactory nerve fibers or sham operation. The ODA of the rats was assessed 2, 14, 28, and 42 d after transection of the olfactory nerve fibers, before the ^{201}Tl olfacto-scintigraphy on each day. ODA significantly increased with a time course after transection of the olfactory nerve fibers (Figure 4; $p < 0.04$, Kruskal-Wallis test, n = 3),

consistent with the imaging data that the olfactory nerves had regenerated. ODA was 90% or more on each examination day in three sham-operated controls.

DISCUSSION

Using olfacto-scintigraphy in BNTX rats, we showed that ^{201}Tl transport from the nasal cavity area to the olfactory bulb area increased with a time course after olfactory nerve transection. The recovery of ODA in the same rats also increased with a time course after the olfactory nerve transection. ^{201}Tl transport in the olfactory nerve fibers of sham controls did not significantly change over 42 d.

In a previous study in BNTX mice [5], a decrease in ^{201}Tl transport in the olfactory nerve 2 d after transection of the olfactory nerve fibers was confirmed by nasal administration of ^{54}Mn and a fluorescent neural tracer. We also showed previously that ODA is significantly correlated with ^{201}Tl transport in the olfactory nerve of BNTX mice and controls [6]. Thus, the transport of ^{201}Tl from the nasal cavity to the olfactory bulb can be interpreted as ^{201}Tl transport in the olfactory nerve in rodents.

To exclude the possibility that the change of ^{201}Tl uptake intensity in the heads of the rats was a surgical artifact, we assessed ^{201}Tl olfacto-scintigraphy in sham controls for 6 weeks after the sham operation. Transport of ^{201}Tl from the nasal cavity area to the olfactory bulb area did not change over 6 weeks in these controls. Therefore, surgery exposing the olfactory bulbs did not affect ^{201}Tl uptake intensity in the heads of the BNTX rats.

^{201}Tl uptake may be increased in the inflammatory area because blood circulation is good in the inflammatory area. However, if the transport of ^{201}Tl from the nasal cavity area to the olfactory bulb area were increased by inflammation of the transected olfactory nerve, rather than by olfactory nerve regeneration, ODA would not have recovered. Therefore, our results showing the recovery of both ODA and transport of ^{201}Tl to the olfactory bulb area support the hypothesis that recovery of ^{201}Tl transport to the olfactory bulb area reflects olfactory nerve regeneration in the BNTX rats; ^{201}Tl olfacto-scintigraphy may therefore be useful for the precise determination of the degree of connectivity in the olfactory nerve in patients with posttraumatic olfactory impairment.

Nasal administration of ^{201}TlCl solution has the potential to be used clinically for the objective assessment of damage to olfactory nerves, because ^{201}Tl is already widely administered systemically for diagnostic tests [7-8]. The safety of nasal administration of ^{201}Tl is under investigation in animals.

CT and single photon emission computed tomography (SPECT) would be possible techniques that could be utilized with ^{201}Tl. CT produces 2-dimensional X-ray images. SPECT is a nuclear medicine tomography imaging method that uses a gamma camera and is able to construct 3-dimensional images by collecting planar images at multiple angles around the object [9]. SPECT/CT analysis might provide more accurate assessments of the uptake of ^{201}Tl in the olfactory bulb than SPECT alone, because CT can be used to correlate anatomical information with the functional information from SPECT [10]; for example, SPECT/CT is superior to SPECT alone for the detection of nasal sinus cancers [11]. Anatomical localization of ^{201}Tl uptake sites without SPECT/CT imaging might be difficult for lesions in the base of the skull. SPECT/CT analysis with nasal administration of ^{201}Tl is under investigation in animals.

^{201}Tl may be used to validate newer techniques, such as fMRI [12-15], assess olfactory function without requiring patients to voluntarily respond to odours; however, the possibility of diagnosing a lesion in olfactory nerve fibers with fMRI is unconfirmed, and the imaging the olfactory nerves presents significant technical challenges. Our technique of olfactory nerve scintigraphy may be useful for validating decreases in odour-evoked signaling in fMRI in patients with post-traumatic olfactory impairment.

Olfactory disturbance is very common in patients with nasal polyposis [16]. It has been shown that 21.6% of total patients with nasal polyposis remained anosmic 6 months after endonasal sinus surgery [17]. ^{201}Tl may be useful for assessing the olfactory nerve damage in the patients with nasal polyposis after surgery as well as in the patients with head trauma.

CONCLUSION

We created an *in vivo* imaging system using nasal administration of ^{201}Tl and imaging with a gamma camera and radiography (^{201}Tl olfacto-scintigraphy) to visualize the regeneration of olfactory nerves in BNTX rats. We plan to test ^{201}Tl olfacto-scintigraphy in a clinical trial to assess its usefulness in healthy volunteers and patients with olfactory injuries.

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Competing interests statement: The authors declare that they have no competing financial interests.

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