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Feasibility of ^{99m}Tc-TRODAT-1 Micro-SPECT imaging of dopamine transporter in animal retinas

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Abstract In this paper, ^{99m}Tc-TRODAT-1 Micro-SPECT (single-photon emission computed tomography) was used for imaging dopamine transporter (DAT) in retinas and to investigate the changes of DAT in retinas of guinea pigs with form deprivation myopia. Pigmented guinea pigs aged 3 weeks were devided into form deprivation myopia (FDM) group (n=6) and normal control group (n=6). The test group wore translucent goggles randomly for 4 weeks, and both groups underwent biometric measurement (refraction and axial length) before and after the experiment. Micro-SPECT retinas imaging was performed at the 4th week after injection of ^{99m}Tc-TRODAT-1. The retinas were clearly resolved in the images. The ratio of ^{99m}Tc-TRODAT-1 uptake in the myopic retinas (11.55±2.80) was 3.64±1.40 lower than that in the control eye (15.20±1.98), and 2.35±1.05 lower than that in the fellow eyes (13.90±2.04). The results showed that ^{99m}Tc-TRODAT-1 Micro-SPECT eye imaging can be used to trace the distribution and changes of DAT in retina, and DAT in the myopic retinas were lower than that in the normal control eyes and fellow eyes. Micro-SPECT may provide a new approach for further studies on the role of dopamine system in the experimental myopia.

Key words Micro-SPECT, ^{99m}Tc-TRODAT-1, Dopamine transporter, Form deprivation myopia, Guinea pig

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1 Introduction

The functional imaging of small animals, using Micro-PET and Micro-SPECT as a noninvasive imaging technique, is becoming an increasingly valuable tool for studying the animal models of human diseases. We have been making a joint effort to study the feasibility of using Micro-SPECT to detect dopamine transporter (DAT) in animal retinas and analyze the changes in myopic retinas. It is well-known that form deprivation or visual defocus can induce axial myopia in a variety of animals, including chickens, monkeys, tree-shrews and guinea pigs[1-4]. Moreover, biochemical and pharmacological studies demonstrated that myopic eyes had lower levels of retinal dopamine (DA) than the normal eyes[5]. This suggests that the DA may contribute to the development of form deprivation myopia (FDM) and len-induced myopia (LIM). DAT has been considered to be more sensitive and valuable than DA detected in early diagnosis of Parkinson's disease[6]. However, DAT has been seldom reported in *in vivo* studies of experimental myopia. As a novel technique, Micro-SPECT may provide a new approach for further studies on the dopamine system in experimental myopia.

2 Materials and methods

2.1 Animal models

The animal experiment in this study was approved by the Animal Care and Ethics Committee of

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Fudan University. Pigmented guinea pigs aged 3 weeks were randomly assigned into two groups: FDM group (n=6) and normal control group (n=6), all of which underwent biometric measurement (refraction and axial length) prior to the experiment. Those in the test group wore translucent goggles randomly for 4 weeks. The biometric measurement was made immediately after the goggles were removed. The same measurement went for the normal control group at the fourth week.

The goggles were daily examined to ensure that they were in proper place, with all the claw-nails trimmed in advance to minimize the chance of rupturing the goggles. All the animals were raised on a cycle of 12 h illumination and 12 h darkness on each day of the experiment, and their water and food were supplemented with Vitamin C.

2.2 Preparation of ^{99m}Tc-TRODAT-1

^{99m}Tc-TRODAT-1 was prepared from a freeze-dried kit (provided by Institute of National Atomic Energy Research, Wuxi, China) by adding 740 MBq(1mL) of freshly eluted ^{99m}Tc-pertechnetate (Yuan Pu Isotope Technology Co. Ltd, Shanghai, China), and was incubated at 100℃ for 30 min to complete the labeling[7]. Finally, the ^{99m}Tc-TRODAT-1 was cooled to room temperature. The ^{99m}Tc-TRODAT-1 was obtained in a neutral solution (pH 7.0~7.5) with radiochemical purity over 96%, using thin-layer chromatography eluted with methanol or saline.

2.3 Micro-SPECT imaging system

A desktop Micro-SPECT system (Fig.1) was used. This is a prototype system developed at Shanghai Institute of Applied Physics, Chinese Academy of Sciences. The system is based on a compact gamma camera consisting of a 5″ Hamamatsu R3292 position-sensitive photomultiplier tube (PSPMT) and a pixellated NaI(Tl) crystal array with 1.2 mm ×1.2 mm ×5 mm pixel elements, 0.2 mm inter-pixel gaps and 80×80 pixel arrays.

The camera has a useful field-of-view (FOV) of ~10.5 cm and can be fitted with interchangeable pinhole and parallel-hole collimators for imaging either whole body or regional activity uptakes of small animals. The system can achieve sub-millimeter spatial resolution with 0.5 mm pinhole aperture and 3 mm spatial resolution with the parallel-hole collimator.

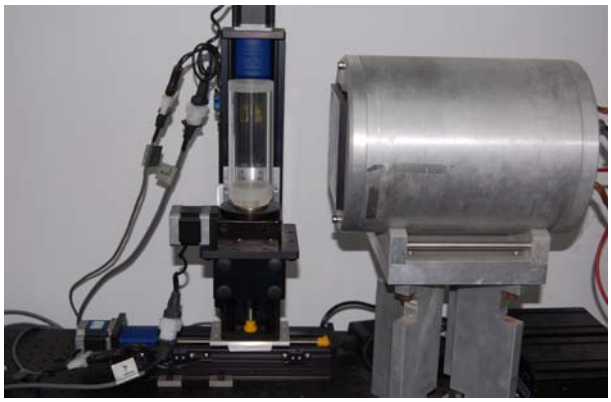

Fig.1 The desktop Micro-SPECT system based on a mini gamma camera using pixellated NaI(Tl) crystal array and 5″ Hamamatsu R3292 PSPMT.

2.4 Data acquisition and image reconstruction

The guinea pigs received 740 MBq ^{99m}Tc-TRODAT-1 in 1 mL via intramuscular injection from their thighs. One hour after administration of ^{99m}Tc-TRODAT-1, the guinea pigs were anesthetized using 1% pentobarbital (0.1 mL per 100 g), fixed on a plastic plate and placed vertically on a rotational stage for SPECT imaging. A high-resolution parallel-hole collimator was used for imaging the heads of the guinea pigs. Sixty emission projections over 360° were acquired with 30 seconds per view. The radius-of-rotation was about 3 cm, where the Micro-SPECT system with the parallel-hole collimator has a spatial resolution of about 3.5 mm.

The projection data were reconstructed using an iterative 3D OS-EM algorithm. The reconstructed image size was 80×80×80 with pixel width and slice thickness of 1.4 mm. The analysis of region-of-interests (ROIs) on retinas of the guinea pigs was performed by the Micro-CT software developed by Division of Medical Imagining Physics, John Hopkins Medical Institution.

2.5 Statistical analysis

All the data were presented as the means ±SD (standard deviation). The results were not only statistically compared between deprived eyes and fellow eyes within the same group (paired sample

t-test, SPSS Version 15.0), but also compared between the FDM and normal control groups (independent samples *t*-test, SPSS Version 15.0). Both the intra-group difference and inter-group difference were defined as significant at $P<0.05$.

3 Results

3.1 Form deprivation

There was no significant difference in refraction and axial length between the two eyes in each guinea pig prior to form deprivation. Therefore, only the results from one random eye of the normal control were analyzed and compared with those from the FDM. The deprived eyes were approximately 5D more myopic than either the fellow eyes or the normal control ones following 4 weeks of form deprivation (deprived vs. fellow or normal control: $P<0.05$ at age 7 weeks) (Table 1). Following 4 weeks of treatment, all the deprived eyes showed excessive growth and become myopic: axial length was longer than that of the fellow and normal control eyes.

Table 1 Comparison of refraction and axial length of eyes after form deprivation

	Refractive state / D	Axial length / mm
Deprived	-3.17±0.98$^{*,\triangle}$	7.90±0.19$^{*,\triangle}$
Fellow	2.79±0.78	7.52±0.19
Normal	2.96±0.40	7.51±0.15

$^{*}P<0.05$, Deprived *vs.* Fellow, $^{\triangle}P<0.05$, Deprived *vs.* Normal.

3.2 Micro-SPECT study

The retina was clearly visible with the tracer. DAT mainly existed in the retina of eye, and the annular specific uptake in the slice was the DAT of the retina. The ratio of ^{99m}Tc-TRODAT-1 uptake in retina to background was achieved via the statistical software (Fig.2). Transverse and sagittal images of the retina uptake of ^{99m}Tc-TRODAT-1 in FDM were acquired via Micro-SPECT (Fig.3). Transverse slices were acquired from bottom to top of the animal, while sagittal slices were acquired from left to right. It suggested that ^{99m}Tc-TRODAT-1 might be well combined with the DAT in retina. ROI was placed on the retina, which was annular uptake in the slice, and background was placed on the brain, which had hardly any uptake and always 5 mm ×5 mm. The average quantities of retinal uptake were 69.11, 82.96 and 95.87 in the deprived, fellow and normal control eyes, respectively, while the background values were 8.29 and 6.37 in FDM and normal control group, respectively. Statistical results showed that the ratio of ^{99m}Tc-TRODAT-1 uptake in retina to background in the FDM group was 11.55±2.80. Repeated measurements revealed that the ratio of ^{99m}Tc-TRODAT-1 uptake in the experimental retinas was 3.64±1.40 lower than that in the normal control eyes (15.20±1.98, $P=0.026$, $F=2.94$, $t=2.605$), and 2.35±1.05 lower than that in the fellow eyes (13.90±2.04, $P=0.003$, $t=5.476$). And both differences were statistically significant. Obviously, the uptake ratio in the deprived eyes was lower than those in fellow and normal control as shown in Fig.2.

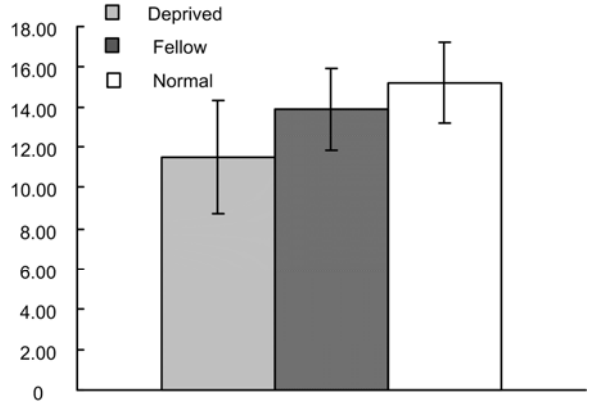

Fig.2 Ratio of ^{99m}Tc-TRODAT-1 uptake in retina to background in groups of FDM and normal control (x±s).

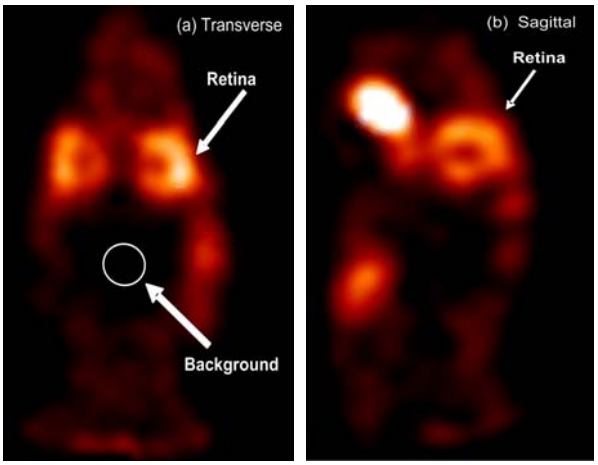

Fig.3 ^{99m}Tc-TRODAT-1 Micro-SPECT retina images, the transverse (a) and the sagittal (b). The images were taken with 1.5 mm parallel hole collimators 1h after injection,showing the uptake of ^{99m}Tc-TRODAT-1 in the guinea pig retina (FDM).

4 Discussion

Micro-SPECT is a useful tool to study animal models of human diseases. Its advantage of functional imaging provides possibilities to perform real-time dynamic observation and conduct further study on myopia mechanism. Andrew *et al* indicated that Micro-SPECT was a good tradeoff between resolution and sensitivity[8]. Paul *et al* demonstrated that ultra-high resolution pinhole SPECT imaging was capable of quantitative, accurate and repeatable measurements of dopamine D_2 receptors and DAT binding sites in mouse brain, using [^{123}I]IBF and ^{99m}Tc-TRODAT-1 respectively as radionuclide tracing agents[9,10]. And it revealed that Micro-SPECT, as in the case of small animal PET [11], could be applied to small animal research to generate useful, quantitative data.

Other investigations suggested that ^{99m}Tc-TRODAT-1 SPECT or ^{131}I-FP-CIT SPECT might serve as a sensitive and objective *in vivo* marker for detecting DAT as a reflection of the severity of PD, and the former was more accurate and distinct than the latter[12]. ^{99m}Tc-TRODAT-1, as a specific radioactive ligand of DAT, hardly binding with other transporters, has already applied in clinical study of early diagnosis of Parkinson's disease, and is considered to be safe and credible[6,13]. Therefore, it is feasible for us to use ^{99m}Tc-TRODAT-1 in the study of human myopia in the future.

Xi *et al* detected changes of chicken retinal DAT using ^{125}I-β-CIT[14]. Wang *et al* used [^{11}C]CFT to map DAT in rabbit eyes by small animal PET, but the images were less distinct than those of the brain. And *ex vivo* autoradiography in their study clearly demonstrated the ocular distribution of DAT[15]. Obviously, the images acquired in our study were much more distinct than theirs, and the retinas could be clearly resolved, which were the annular uptakes in the slices. The uptakes of ^{99m}Tc-TRODAT-1 in the background of our study were very low, which contributed to our clear images. Guinea pig is the most popular animal for experimental myopia at present, but the presence of DAT in the guinea pig's retinas has not been confirmed by radionuclide tracing to our knowledge. In the present study, guinea pigs were chosen as the animal model, thus our results were useful to the future study of experimental myopia.

5 Conclusions

This study demonstrated that ^{99m}Tc-TRODAT-1 imaging using Micro-SPECT can be used to trace the distributions and changes of DAT in retina. DAT in the myopic retinas were lower than that in the normal control eyes and fellow eyes, and DAT in the retina may be involved in the formation of form deprivation myopia. Radionuclide tracing might well be directly performed on human beings' myopia in the future. In conclusion, the application of radionuclide tracing may provide a new approach to further study on the role of dopamine system in the myopia.

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