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Research Paper

Visualization of drug translocation in the nasal cavity and pharmacokinetic analysis on nasal drug absorption using positron emission tomography in the rat

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ABSTRACT

We performed positron emission tomography (PET) using 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) to evaluate the pharmacokinetics of nasal drug absorption in the rat. The dosing solution of [^{18}F]FDG was varied in volume (ranging from 5 to 25 μl) and viscosity (using 0% to 3% concentrations of hydroxypropylcellulose). We modeled the pharmacokinetic parameters regarding the nasal cavity and pharynx using mass balance equations, and evaluated the values that were obtained by fitting concentration–time profiles using WinNonlin® software. The regional nasal permeability was also estimated using the active surface area derived from the PET images. The translocation of [^{18}F]FDG from the nasal cavity was visualized using PET. Analysis of the PET imaging data revealed that the pharmacokinetic parameters were independent of the dosing solution volume; however, the viscosity increased the absorption rate constant and decreased the mucociliary clearance rate constant. Nasal permeability was initially higher but subsequently decreased until the end of the study, indicating regional differences in permeability in the nasal cavity. We concluded that the visualization of drug translocation in the nasal cavity in the rat using PET enables quantitative analysis of nasal drug absorption, thereby facilitating the development of nasal formulations for human use.

1. Introduction

When considering alternative drug administration routes and product discrimination, nasal drug administration is being re-evaluated from the viewpoint of pharmaceutical product lifecycle management [1]. Because of the presence of a highly developed vasculature with wide fenestrae under the nasal epithelia, nasally administered drugs, especially lipophilic nature, are well absorbed into the systemic circulation. Moreover, they can avoid the hepatic and intestinal first-pass effect [2] inherent in oral drug administration; thus, nasal administration is suitable for drugs such as peptides [3] with the combination of absorption enhancers. It has been reported that nasal administration can be used for direct drug transport to the brain [4–6] and the cervical lymph nodes. In addition, nasal administration has attracted attention as an alternative drug administration method because it is easier and less invasive than oral delivery, not only for the elderly but also for long-term care patients with swallowing difficulties. Therefore, this method has considerable clinical potential for improving the quality of life of both patients and care givers.

Most studies on nasal drug absorption have focused on evaluating the amount of drug absorption and the associated pharmacological effects. Despite hundreds of clinical trials worldwide, not so many pharmacokinetics of nasal drug absorption in human is

Abbreviations: PET, positron emission tomography; [^{18}F]FDG, 2-deoxy-2-[^{18}F]fluoro-D-glucose; HPC, hydroxypropylcellulose, H-grade; MRI, magnetic resonance image; VOIs, volumes of interest; %IA, the percent of injected activity; MC, mucociliary clearance; AUC, the area under the curve; ROIs, regions of interest; BA, bioavailability.

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reported so far. We previously reported on the pharmacokinetic analysis of nasal drug absorption [4,5,7–10] and its pharmacological effects as a result of direct drug delivery to the brain [6]. However, to better understand the nasal drug absorption process, we require a quantitative methodology, which enables the direct measurement of the distribution, regional absorption, and transit of drugs in the nasal cavity. The establishment of a valid evaluation methodology will facilitate the use of nasal drug administration in human.

Positron emission tomography (PET) allows for a minimally invasive analysis of drug concentrations in tissues with high sensitivity and good spatiotemporal resolution. We introduced PET as a powerful tool for investigating drug distribution in living systems, and clarified its usefulness in pharmacokinetics and drug–drug interaction preclinical and clinical studies involving membrane transporters [11–18]. In the latest approach, PET imaging technology has been applied to the study of intestinal absorption to analyze fluid and drug distribution along the gastrointestinal tract, in both animals and humans, as a tool to develop oral dosage protocols [19–22]. Because PET imaging analysis is applicable to all of the stages of drug development, from early drug discovery to the clinical phase, we expect it to be applicable to pharmacokinetic model analysis of nasal drug absorption as well.

In the present study, we conducted a pharmacokinetic investigation using PET imaging to evaluate nasal drug absorption and transit in the nasal cavity in rats. We selected 2-deoxy-2-[^{18}F] fluoro-D-glucose ([^{18}F]FDG) as a model PET probe because we can (a) easily obtain the tracer for our daily use in clinical tumor diagnosis, and (b) access a considerable amount information about its physiological characteristics. A PET scan was performed to visualize the drug distribution profiles in the nasal cavity and to determine the pharmacokinetic parameters and regional change of nasal permeability of [^{18}F]FDG facilitating the development of nasal formulations for human use.

2. Materials and methods

2.1. Materials

[^{18}F]FDG was provided as saline solution by the Division of Molecular Imaging at the Institute of Biomedical Research and Innovation (Kobe, Hyogo, Japan). Hydroxypropylcellulose, H-grade, (HPC) was kindly supplied by Nippon Soda Co., Ltd. (Tokyo, Japan). Urethane was purchased from Wako Pure Chemical Industries (Osaka, Japan). Head holders for the rats (SGP-3, MRI-compatible) were purchased from Narishige Group (Tokyo, Japan). [^{18}F]FDG was diluted to approximately 200 MBq/ml with saline or saline solution of HPC (1–3%).

2.2. Animals

For *in vivo* PET studies, male Sprague–Dawley rats, aged 6–9 weeks (n = 3 for each set of experiments) and weighing 211–279 g, were purchased from Japan SLC Inc. (Hamamatsu, Shizuoka, Japan). The animals were maintained in a temperature- and light-controlled environment and provided with standard food and tap water, *ad libitum*. All experimental protocols were approved by the Ethics Committee on Animal Care and Use of the RIKEN Center for Life Science Technologies, and were performed in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised in 1985).

2.3. PET scans

All of the PET scans were performed using a microPET Focus220 scanner (Siemens, Knoxville, TN, USA) designed for laboratory animals. One day before the PET study, the rats (n = 3 for each set of experiments) were anesthetized and maintained using 1.5% isoflurane (Mylan, Tokyo, Japan) with an air flow rate of 2 l/min. The femoral vein was cannulated using Micro-Renathane® tubing (MRE040, Braintree Scientific Inc., Braintree, MA, USA) for the collection of blood samples. Before emission scanning, the rats were anesthetized with a saline solution containing 60% urethane (2 ml/kg), which was selected due to the least effect on mucociliary clearance (MC) [8]. The nasopalatine duct was closed using instant glue to ensure the accuracy of pharmacokinetic prediction in the disposition studies involving [^{18}F]FDG in the nasal cavity. The rats were placed prone at the center of the PET gantry (8 cm) and their heads were secured using a head holder. A 25-min transmission scan was performed using a rotating ^{68}Ge/^{68}Ga point source for positioning and attenuation correction. At the start of the emission scan, a saline solution containing [^{18}F]FDG was instilled nasally via the right frontal nostril of the rat in the prone position. For the dosing volume comparison, the tip of microsyringe was inserted at approximately 8 mm depth and then 5 μl (1.22–1.63 MBq/rat), 15 μl (1.22–1.69 MBq/rat) or 25 μl (2.07–4.27 MBq/rat) of solution was instilled. For the comparison of formulation viscosity, with MICROMAN® pipette (Model M10, Gilson, Middleton, WI, USA), 5 μl of 1% (0.85–0.93 MBq/rat), 2% (0.91–1.02 MBq/rat) or 3% HPC (0.96–1.00 MBq/rat) solution was applied in the same manner. The viscosity of each HPC solution measured using a viscometer (Viscomate, CBC Materials Co. Ltd., Tokyo, Japan) was 38.6 ± 0.2 mPa s, 137.0 ± 5.0 mPa s, and 375.3 ± 2.3 mPa s for 1%, 2% and 3% HPC, respectively. An emission scan in 3D list-mode was performed for 30 min and sorted into 58 dynamic sinograms according to the following sequence: 24 × 5 s, 9 × 20 s and 25 × 60 s. In another emission scan, we administered approximately 100 MBq of [^{18}F]FDG via the tail vein for 30 min to obtain the individual brain coregistration to the magnetic resonance image (MRI). Venous blood was sampled via the cannulated femoral vein six times for 30 min at the following time points: 2.5, 5, 10, 15, 20 and 30 min after the administration of [^{18}F]FDG. To estimate the fraction of nasally absorbed [^{18}F]FDG using deconvolution theory, we intravenously administered [^{18}F]FDG (26.1 ± 1.2 MBq/200 μl) via the tail vein, and sampled venous blood from the jugular vein at the following time points: 2.5, 5, 10, 15, 20, 30, 45, 60, 75, 90 and 120 min. The total blood volume sampled from a single rat did not exceed 1.6 ml, which was approximately 10% of the total circulating blood volume per rat. Blood radioactivity was measured using a 1470 Wizard Automatic Gamma Counter (PerkinElmer Life & Analytical Sciences, Waltham, MA, USA). The radioactivity in each sample was corrected for time decay from the point of [^{18}F]FDG administration.

2.4. Analysis of PET imaging data

PET images were reconstructed using a Siemens microPET Manager 2.5.0.0 by means of Fourier Rebinning and standard 2D filtered back projection using a Ramp filter, cutoff at the Nyquist frequency. The three dimensional position of individual PET images was coregistered to another rat's MRI brain template, obtained using a 3T MRI scanner (MAGNETOM Allegra, Siemens, Erlangen, Germany) with a custom 4-channel phased array receiving coil and a transmitter coil (Takashima Seisakusho Co., Ltd., Hino, Tokyo, Japan). In further detail, the MRI image was obtained using a magnetization-prepared rapid acquisition scan sequence with gradient echo (MPRAGE) set to the following parameters: repetition time (TR) = 2200 ms; echo time (TE) = 6.06 ms; inversion time (TI) = 1250 ms; flip angle = 8 degrees; field of view (FoV) = 64 mm; matrix = 320 × 320; and slice thickness = 0.2 mm. Volumes of interest (VOIs) were placed on the nasal cavity and pharynx of MRI image as indicated in Fig. 1 to analyze the three dimensional