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Reproducibility of intraperitoneal 2-deoxy-2-[^{18}F]-fluoro-D-glucose cerebral uptake in rodents through time

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Abstract

Introduction: One strength of small animal imaging is the ability to obtain longitudinal measurements within the same animal, effectively reducing the number of animals needed and increasing statistical power. However, the variability of within-rodent brain glucose uptake after an intraperitoneal injection across an extended time has not been measured.
Methods: Small animal imaging with 2-deoxy-2-[^{18}F]-fluoro-D-glucose (^{18}FDG) was used to determine the variability of a 50-min brain ^{18}FDG uptake following an intraperitoneal injection over time in awake male and female Sprague–Dawley rodents.
Results: After determining the variability of an intraperitoneal injection in the awake rat, we found that normalization of brain ^{18}FDG uptake for (1) injected dose and body weight or (2) body weight, plasma glucose concentration and injected dose resulted in a coefficient of variation (CV) of 15%. However, if we normalized regional uptake to whole brain to compare relative regional changes, the CV was less than 5%. Normalized cerebral ^{18}FDG uptake values were reproducible for a 2-week period in young adult animals. After 1 year, both male and female animals had reduced whole-brain uptake, as well as reduced regional hippocampal and striatal ^{18}FDG uptake.
Conclusion: Overall, our results were similar to findings in previous rodent and human clinical populations; thus, using a high throughput study with intraperitoneal ^{18}FDG is a promising preclinical model for clinical populations. This is particularly relevant for measuring changes in brain function after experimental manipulation, such as long-term pharmacological administration.

Keywords: Age; Glucose utilization; Positron emission tomography; Small animal imaging; Sprague–Dawley; ^{18}FDG

1. Introduction

During the last decade, small animal imaging facilitated the synergy between studies of preclinical and clinical brain metabolism. For example, 2-deoxy-2-[^{18}F]-fluoro-D-glucose (^{18}FDG) is a radiotracer that has been used to determine cerebral glucose metabolic rates in humans [1], nonhuman primates [2] and more recently, rodents [3]. Small animal imaging [4] with ^{18}FDG represents an extension of the measurement of rodent local cerebral glucose utilization (lCGU) with 2-[^{14}C]-2-deoxyglucose coupled to ex vivo autoradiography with a time activity curve of arterial blood radioactivity [5] taken after an intravenous injection. With small animal imaging, longitudinal within-subject studies up to 10 days have now been carried out in unanesthetized rodents measuring lCGU [6]. However, this technique still requires an intravenous injection, blood sampling and handling of the animal during the uptake period, introducing several potential methodological and technical challenges.

One major technical difficulty in measuring lCGU is the need for arterial blood sampling during tracer uptake to obtain a plasma time activity curve. Sampling of the arterial blood entails the occlusion of the femoral, tibial or carotid artery for catheter placement. Though the occlusion of one carotid did not produce discernable cerebral brain lesion or

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edema, it did cause both loss in motor performance as well as peripheral tissue atrophy [7]. In addition, occluded femoral arteries caused ischemia in peripheral tissue [8], though no significant change in lCGU was observed [9]. Most importantly, the intrinsic difficulty of maintaining an arterial cannulation for several days, let alone a week or more, makes carrying out long-term studies more problematic.

In addition, blood collection during ^{18}FDG uptake frequently requires handling the animal, a known stressor. In one study, handling an animal increased plasma corticosterone levels that in turn physiologically decreased peripheral tissue and brain 2-[^{14}C]-deoxyglucose [10]. Handling also elevated cholinergic stimulation causing a decrease in acute tissue and regionally specific lCGU [11]. Lastly, animal handling increased neurotransmitter release in several brain regions [12–16], an effect that may also change ^{18}FDG uptake [17–19].

In order to circumvent these confounds, we proposed two methodological modifications of the ^{14}C-2-deoxyglucose method as a surrogate measure for lCGU, and we compared different strategies for normalizing ^{18}FDG uptake for inter- and intrasubject comparisons to assess brain ^{18}FDG uptake. First, a single blood sample was drawn at the end of the experimental scan in order to determine if a representative time activity curve based on a single time-point arterial blood sample would serve as a reproducible substitute for the arterial blood curve to determine cerebral glucose uptake. Second, ^{18}FDG was administered intraperitoneally [20], thereby reducing the confound caused by continued handling and restraint stress inherent in the intravenous injection and blood sampling required by lCGU measurement in awake animals.

It is important to note that an intraperitoneal injection without a plasma time activity curve does not allow us to extrapolate metabolic rates or utilization of glucose or deoxyglucose; however, if the time activity curve of the intraperitoneal injection has a low variability, it would enable the measurement of absolute values based on standard uptake values (SUVs) of ^{18}FDG in different brain regions. The alternative to an intraperitoneal injection of radiotracer is to use an intravenous injection; these two routes of administration produce similar autoradiograms suggesting a correlation between brain uptakes from intravenous and intraperitoneal injections [20] after arterial blood sampling was done. This correlation suggests that an intravenous injection is not necessary; however, it has been argued that intraperitoneal injection introduces a potential source of variability [21].

One benefit of an intravenous over an intraperitoneal injection is the apparent uniformity of uptake; however, in most studies involving a tail vein injection, the animal must be immobilized in order to cannulate the tail vein. This immobilization is a major stressor [22] and has been shown to confound radiotracer uptake [10,23].

In order to reduce these stressors, one could also use anesthesia. However, the use of anesthesia causes a reduction in lCGU [5,24] and changes the response to acute pharmacological stimulation [25]. In addition, clinical populations are almost always done in awake subjects, thereby making it difficult to extrapolate results between anesthetized rodents and awake humans.

A major strength of using ^{18}FDG small animal imaging is the ability to measure a change induced by drugs or other experimental manipulation in the same animal over time, particularly with a paradigm that would not be practical or ethical in humans. To this end, we investigated the feasibility and reproducibility of measuring long-term changes in intraperitoneal ^{18}FDG uptake and compared different normalization strategies in order to reduce variability across scans.

2. Methods

2.1. Animals

All animal procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the BNL Institutional Animal Care and Use Committee. Male and female Sprague–Dawley rats (14–64 weeks, Taconic Farms) were used throughout this study. Paired animals were housed in cages and were maintained on a-12:12 light/dark cycle with free access to food and water. A group of 10 animals, both male and female, were used to determine intersubject variability following intraperitoneal administration of ^{18}FDG. From this group, three animals (male and female) were randomly chosen to test the reproducibility of ^{18}FDG uptake over time. These animals were maintained on a standard diet and light cycle for 1 year prior to their final scan. An additional three animals were included in this 1-year study, bringing the sample size to six for each group.

A third group (n=4) of male animals were ordered with precannulated carotid arteries (Taconic Farms) and were used to determine the reproducibility of the integrated time activity curve for ^{18}F in arterial blood after intraperitoneal ^{18}FDG injection in the awake rodent.

2.2. Protocol

On the day of the study, animals were weighed, placed in a clean (home) cage with their cage mate and transported to the PET facility. Animals arrived at the facility approximately 3 h prior to scanning in order to habituate to their environment. Animals received free access to water and were not given food during this time in order to stabilize plasma glucose, as well as to prevent interference with anesthesia, which was administered after the uptake period but prior to scanning.

2.3. Plasma radioactivity measurement

Whole blood was collected from the tail vein at the end of each respective scanning period (~75 min after intraperitoneal injection). It was centrifuged 3 min at 10 000×g, and 20 μl of supernatant (plasma) was decanted. The radioactivity in the plasma was measured with a calibrated Picker well counter (NaI crystal), counts per minute were corrected