Chapter 3.1

Radiation dose of the novel P-gp tracer $^{[11}C]laniquidar

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ABSTRACT

Resistance to current drug therapy is an important issue in the treatment of epilepsy. Inadequate access of central nervous system drugs to their targets in the brain may be caused by overexpression or overactivity of multidrug transporters, such as P-glycoprotein (P-gp), at the blood-brain barrier. Laniquidar, an inhibitor of P-gp, has been labeled with carbon-11 for the use in PET studies of P-gp expression in humans. Given potential interspecies differences in biodistribution, the purpose of this study was to ensure safe use of [11C]laniquidar by determining the dosimetry of [11C]laniquidar using whole body positron emission tomography (PET) studies.

Methods
Six healthy volunteers were each subjected to a series of 10 whole body PET scans within a period of ~70 minutes. Five blood samples were taken during the entire PET study.

Results
High uptake of [11C]laniquidar was seen in liver, spleen, kidneys and lung, whereas brain uptake was low. The effective dose for [11C]laniquidar was 4.76 ± 0.13 and 3.69 ± 0.01 μSv·MBq⁻¹ for females and males, respectively.

Conclusions
Biodistribution and measured effective dose indicate that [11C]laniquidar is a safe tracer for PET imaging with a total dose of ~2 mSv for a brain PET-CT protocol.
INTRODUCTION

Resistance to drug therapy affects approximately 30% of all patients who suffer from epilepsy. This could, at least partly, be due to decreased passage of anti-epileptic drugs across the blood-brain barrier (BBB). Uptake and efflux drug transporters play a major functional role in regulating drug entry into the brain. Two large and important drug transporter families are the organic anion-transporting polypeptide (OATP) family and the ATP-binding cassette (ABC) transporter superfamily (ABC transporters). Several members of both families are expressed at the human BBB, including OATP1A2, OATP1C1 and OATP3A1, which are members of the OATP family, as well as P-glycoprotein (P-gp), breast-cancer resistance protein and multidrug resistance protein 4, which are members of the ABC transporter superfamily. In this paper the focus is on the most widely studied efflux transporter P-gp. It has been proposed that changes in P-glycoprotein (P-gp) expression and/or function at the blood-brain barrier play an important role in pharmacoresistance in epilepsy. The multidrug transporter P-gp and other efflux transporters actively transport substrates, including many central nervous system (CNS) drugs, against a concentration gradient from brain to blood and cerebrospinal fluid. Hence, overexpression and/or increased activity of the transporter system may result in reduced tissue concentrations of CNS drugs in the brain, thereby greatly limiting their therapeutic efficacy. There are two case reports suggesting that inhibiting P-gp in medically refractory epilepsy patients decreases seizure frequency, at least temporarily. P-gp functionality can be assessed in vivo by means of (R)-[11C]verapamil and [11C]N-desmethyl-loperamide PET scans. However, at present overexpression of P-gp in refractory patients can only be confirmed by examining brain tissue post-mortem or from surgically removed brain tissue. Therefore, availability of non-invasive imaging techniques that would allow assessment of the distribution of P-gp in the brain is of vital importance. Laniquidar is an inhibitor of P-gp and therefore it should bind proportionally to P-gp density. Recently, this compound was labeled with carbon-11 and its biodistribution in rats was evaluated. Previously, however, it has been shown that metabolic profiles for the P-gp substrate tracer (R)-[11C]verapamil are substantial different between species, and this could also be the case for [11C]laniquidar. Therefore, the purpose of the present study was to determine the radiation dosimetry of [11C]laniquidar based on biodistribution studies in humans.
MATERIALS AND METHODS

Subjects
Six healthy subjects without any evidence of renal or hepatic dysfunction (4 males and 2 females) with a mean age (± SD) of 43 ± 18 years and a mean body weight of 86 ± 23 kg were included. Prior to inclusion, each participant signed a protocol-specific informed consent. The study was approved by the Medical Ethics Review Committee of the VU University Medical Center.

Synthesis of \([^{11}C]laniquidar\)
\([^{11}C]laniquidar\) was synthesized according to Good Manufacturing Practice (GMP) standards as described previously\(^{10}\) with some modifications to enable human use. Briefly, \(2.1 \pm 0.6 \text{ GBq}\) of \([^{11}C]laniquidar\) was obtained via alkylation of \(O\)-desmethyl laniquidar (R102207) with \([^{11}C]\)methyl triflate and, after HPLC purification, reformulated in a sterile aqueous solution of 0.9% NaCl, 2.5% polysorbate 80 and 8.5% ethanol. Radiochemical purity was higher than 98% and no chemical impurities were observed as assessed by radio/UV-HPLC. The identity of the product was confirmed by comparing its retention time with that of authentic laniquidar. The mean specific activity at the time of injection was \(70 \pm 24 \text{ GBq} \cdot \mu\text{mol}\).\(^{1}\)

Scan protocol
Each subject received two venous cannulas. One cannula is used for tracer administration and the other for blood sampling (the use of two separate cannulas excludes the possibility of contamination of blood samples with radioactivity traces left from the i.v. administration). Subsequently the subjects were positioned on the bed of a Gemini–TF64 PET/CT scanner (Philips Medical Systems, Best, The Netherlands). The mean and standard deviation of the administered mass of \([^{11}C]laniquidar\) was \(3.5 \pm 1.4 \mu\text{g}\) (range, 2.2–5.9 \mu g). The mean administered activity was \(347 \pm 63 \text{ MBq}\) (range, 222-396 MBq). After a 35 mAs low dose whole body (WB) CT scan a series of 10 WB sweeps was started (30 seconds per bed position, typically 11 bed positions), covering the time interval between 0 and ~70 minutes post tracer injection. Five blood samples per subject were taken manually, one after each odd numbered WB injection. Five blood samples per subject were taken manually, one after each odd numbered WB injection.

Data analysis
Radioactivity in 0.5 ml blood samples was measured with a gamma counter (Perkin Elmer, Turku, Finland). All WB PET scans were reconstructed using the standard whole body time of flight reconstruction algorithm including normalization of the data as well as scatter, attenuation, decay and dead time corrections.\(^{13}\) In particular, decay correction was performed within each scan (in order to account for the time difference between
the various bed positions), but not for each scan with respect to the time of injection (since residence time calculations should be performed on non-decay corrected source organ time-activity data). Regions of interest (ROIs) were defined for organs that showed positive image contrast on a PET image: myocardium, liver, kidneys, spleen, and lungs. With exception of the lungs these regions were delineated on an early PET scan. Organs that did not have a positive contrast in the PET scan were regarded as a part of the remainder of the body term in the subsequent calculation of the radiation absorbed dose. In addition, the brain was delineated on the CT scan as brain imaging is the intended use of the tracer. Both CT regions were automatically segmented based on a Hounsfield unit threshold. The lung ROI was manually edited if the location on the respiratory averaged PET scan differed from that on the CT scan. All ROIs were copied onto all PET scans in order to generate mean time activity concentration curves per organ. At the end of the PET studies activity remained in various organs. To account for this residual activity, each individual time activity curve was extrapolated to infinity by assuming only physical decay from the end of the last WB scan onwards. In this extrapolation biological clearance is ignored, resulting in a worst case estimate of residual activity and thus an upper limit of the estimated radiation dose. The red marrow activity concentration was assumed to be one third of the whole blood activity concentration. The area under each time activity curve was integrated and the residence time for each organ was obtained through multiplication of this area by the scaled organ mass from reference man / woman and division by the injected dose. To calculate the residence time for the remainder of the body, residence times of all source organs were summed and subtracted from the fixed theoretical value in the absence of excretion. The software package Olinda was used to calculate the effective dose in μSv·MBq⁻¹ according to ICRP 60 (1990) tissue weighting factors.

**RESULTS**

There were no adverse or clinically detectable pharmacologic effects in any of the 6 subjects. No significant changes in vital signs were observed. No patient motion between CT and PET scans was observed. A typical coronal whole body slice of [¹¹C]laniquidar uptake as function of time is shown in Figure 1. Urinary bladder and testes did not show significant tracer accumulation and therefore were not included in dose calculations. Figure 2 shows time activity curves for individual organs as well as the blood sampling averaged over all subjects. Organ, red marrow and effective doses are given in table 1. Data for individual subjects are presented to illustrate inter-individual variability. An average effective dose of 4.76 ± 0.13 and 3.69 ± 0.01 μSv·MBq⁻¹ was obtained for females and males, respectively.
Figure 1. Coronal views showing the biodistribution of $[^{11}C]$laniquidar as a function of time (the numbers in the panels indicate the start time of the scan post injection (in minutes)) for subject F-1. Note, that no decay correction was performed between images, i.e. images illustrate the sum of biological clearance and physical decay. For the upper row the image scale runs between a SUV of 0 (white) and 16 (black). For the lower row these numbers are 0 and 4, respectively.

Figure 2. Time activity curves of individual organs and whole blood averaged over all patients.
DISCUSSION

The measurement protocol allowed for a good visualization of the kinetics of the tracer in the body. There was no indication of patient motion either between the CT and the PET or during the PET scans. The biodistribution of $^{11}$C]laniquidar showed highest uptake in liver, followed by spleen, kidneys and lung. In rats, highest uptake was seen in the lung, followed by liver, spleen and kidney.\(^{10}\) It is not known why lung uptake seems to be species dependent. All time activity curves were descending, allowing for a reasonable estimate of the residence time for the time interval after the scanning period. The absence of contrast in the lumbar vertebrae at the end of the scanning period suggests no active uptake in the bone marrow, allowing for an estimate of the bone marrow residence time from the blood samples. Organ and effective doses reproduced well despite the small number of female (n=4) and male (n=2) subjects. The measured average effective dose of \(4.40 \pm 0.56 \, \mu Sv\cdot MBq^{-1}\) is within the range observed for other C-11 based tracers.\(^{16}\) The liver receives the highest organ dose of \(\approx 25 \, \mu Sv\cdot MBq^{-1}\). A typical injection of 370 MBq would lead to a total dose of \(\approx 2\) mSv for a brain PET-CT protocol (including a low dose CT scan).

CONCLUSION

In this dosimetry study the average effective dose derived from $^{11}$C]laniquidar was \(4.40 \pm 0.56 \, \mu Sv\cdot MBq^{-1}\). Therefore, $^{11}$C]laniquidar is safe for PET imaging of P-gp expression on the current generation of PET-CT scanners.
REFERENCES
