Comparison of Standardized Uptake Values with Volume of Distribution for Quantitation of [11C]PBR28 Brain Uptake

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Abstract

Introduction—[11C]PBR28 is a high-affinity ligand for the Translocator Protein 18kDa (TSPO), which is considered to be a marker for microglial activation. Volume of distribution (V_T) estimated with an arterial plasma input function is the gold standard for quantitation of [11C]PBR28 binding. However, arterial sampling is impractical at many PET sites for multiple reasons. Reference region modeling approaches are not ideal for TSPO tracers, as the existence of a true reference region cannot be assumed. Given that it would be desirable to have a non-invasive index of [11C]PBR28 binding, we elected to study the utility of the semi-quantitative metric, standardized uptake value (SUV) for use in brain [11C]PBR PET studies. The primary goal of this study was to determine the relationship between SUV and V_T.

Methods—We performed a retrospective analysis of data from sixteen [11C]PBR28 PET scans acquired in baboons at baseline and at multiple time points after IV injection of lipopolysaccharide, an endotoxin that transiently induces neuroinflammation. For each scan, data from 14 brain regions of interest were studied. V_T was estimated with the Logan plot, using metabolite-corrected input functions. SUV was calculated with data from 30–60 minutes after [11C]PBR28 injection.

Results—Within individual PET studies, SUV tended to correlate well with V_T. Across studies, the relationship between SUV and V_T was variable.

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Conclusions—From study to study, there was variability in the degree of correlation between [^{11}C]PBR28 V_T and SUV. There are multiple physiological factors that may contribute to this variance.

Advances in Knowledge—As currently applied, the non-invasive measurement of SUV does not appear to be a reliable outcome variable for [^{11}C]PBR28. Additional work is needed to discover the source of the discrepancy in SUV between [^{11}C]PBR28 scans.

Implications for Patient Care—There is a need to develop alternatives to arterial plasma input functions for TSPO ligands in order to facilitate multi-center trials.

Keywords

PET; PBR28; TSPO; SUV; validity; inflammation

INTRODUCTION

[^{11}C]PBR28 is a high-affinity ligand for the Translocator Protein 18kDa (TSPO), which is expressed on the outer mitochondrial membrane [1, 2]. The first *in vivo* imaging studies with [^{11}C]PBR28 were published in 2007, and included brain imaging in rats [3] and the initial characterization of dosimetry and biodistribution of [^{11}C]PBR28 in non-human primates and humans [4]. Increased expression of TSPO has been documented in activated macrophages and microglia [5, 6], and thus is often considered as a marker for microglial activation. Volume of distribution (V_T) estimated with an arterial plasma input function is the gold standard for quantitation of radioligands, including [^{11}C]PBR28 binding [7, although see 8]. However, arterial sampling is impractical at many PET sites for multiple reasons: it is invasive, can be painful, and can be difficult in populations such as the elderly. In rodents, arterial sampling requires vascular cannulation of a femoral or carotid artery. While this may be an acceptable procedure for acute, single scan studies, it is problematic for longitudinal experimental designs because of the difficulty in maintaining patency of arterial catheters over the course of weeks to months. Because of this, and the volume of blood needed for accurate measurement of radiolabeled parent and metabolite species, arterial cannulation is prohibitive for longitudinal studies in rodents. "Reference region" approaches (which use information from a target-free region as a surrogate for a plasma input function) have been proposed for the prototypical TSPO ligand, [^{11}C]PK11195 [9–12]. These methods rely on the assumption that a true reference region exists for the TSPO (which may be problematic, given that the TSPO is a cholesterol binding site common to all mitochondria). These reference methods also have not been validated for [^{11}C]PBR28. Image-derived input functions are sometimes a viable alternative to arterial input functions, but this methodology is not recommended for [^{11}C]PBR28 [7]. Availability of a non-invasive index of [^{11}C]PBR28 binding would help facilitate the use of high-affinity TSPO ligands in both human and small animal PET studies. We elected to study the utility of the semi-quantitative index, standardized uptake value (SUV), for use in brain [^{11}C]PBR28 PET studies by comparing the performance of SUV to the accepted quantitative metric, V_T. To accomplish this objective, we performed a retrospective analysis of [^{11}C]PBR28 brain image data in non-human primates that were recently published [6].

MATERIALS AND METHODS

General

This study utilized data from a recently published report that demonstrated the effects of peripheral endotoxin administration on neuroinflammation [6]. All procedures were carried out in accordance with the Animal Welfare Act, other federal regulations governing the care and responsible use of animals for research, and under the recommended principles set forth in the Guide for Care and Use of Laboratory Animals [13]. The protocol was approved by the Yale University Institutional Animal Care and Use Committee. Details regarding the prior study of six of the animals can be found in Hannestad et al. [6]. The present work also includes a baseline scan from a seventh baboon. Briefly, animals received a bolus injection of 172 ± 12.4 MBq of [^{11}C]PBR28, and dynamic data were acquired for 120 minutes on an ECAT EXACT HR+ (Siemens, Knoxville TN). Animals were scanned at baseline ($n = 7$), and at 1 hr ($n = 4$), 4 hr ($n = 3$), and 22 hr ($n = 2$) after IV administration of 0.1 mg/kg lipopolysaccharide (LPS). Arterial sampling was conducted during PET scanning for analysis of parent [^{11}C]PBR28 and radiolabeled metabolites. Image processing was as described previously [6]. A total of sixteen [^{11}C]PBR28 PET studies and results from the corresponding metabolite assays were available for this analysis.

Estimation of V_T and Calculation of SUV

For each baboon, time-activity curves (TACs) of [^{11}C] radioactivity in the brain were extracted from 14 standard brain regions of interest (ROIs; brainstem, caudate, centrum semiovale, cerebellum, cerebellum without vermis, cingulate cortex, frontal cortex, pallidum, insula, accumbens, occipital cortex, pons, putamen, and thalamus). For each ROI, total volume of distribution (V_T) was estimated with Logan graphical analysis with the metabolite-corrected plasma input function [14], using 0–60 min of data, and $t^* = 10$.

Calculation of SUV

Standardized uptake value (SUV) is defined as:

$$SUV = \frac{Q_{ROI}/M_{ROI}}{ID/M_{Subject}}$$

where Q_{ROI} is the radionuclide quantity in an ROI, M_{ROI} is the mass of the ROI, ID is the injected dose of the radiopharmaceutical, and $M_{Subject}$ is the mass of the subject. A single SUV was calculated from each brain time-activity curve (TAC) using 30–60 min of data. To generate a single representative number for the whole brain for each study, the SUV values from the 14 ROIs were averaged.

LPS Effects on SUV

Paired *t*-tests were used to test for effects of LPS between baseline and 1-hour post- LPS ($n = 4$) and between baseline and 4-hours post-LPS ($n = 3$).

Comparison of SUV to V_T

Linear regression analysis was used to determine the correspondence of SUV to V_T within each [^{11}C]PBR28 PET study.

RESULTS

Power of SUV to detect effects of LPS on [^{11}C]PBR28 uptake

Figure 1 shows the average whole brain [^{11}C]PBR28 SUV for the two groups of baboons scanned at baseline and at 1hr and 4hr post-LPS. There were no statistical differences between the baseline and LPS conditions. The average baseline SUV was 2.2 ± 0.72 ($n = 7$).

SUV vs V_T

In fifteen of the sixteen [^{11}C]PBR28 PET studies, regional SUV was highly correlated with regional V_T (Figure 2). The r-value range was 0.78 to 0.96, and the p-value range was 0.001 – $1 \times 10{-}06$. However, the slope of the relationship between SUV and V_T varied across studies, even within subjects. In general, the robustness of agreement between SUV and V_T appeared to decrease substantially with higher values of V_T.

Figure 3 illustrates the relationship of SUV to V_T for three ROIs with high, medium, and low [^{11}C]PBR28 uptake.

DISCUSSION

This was retrospective analysis of sixteen [^{11}C]PBR28 brain imaging datasets acquired in baboons at baseline, and at several time points after lipopolysaccharide (LPS) administration to induce an inflammatory state. We examined the relationship between the quantitative volume of distribution, V_T, and the semi-quantitative standardized uptake values (SUV) for characterization of brain uptake of [^{11}C]PBR28. The primary observation was that, within a single PET study, there was a consistent proportional relationship between SUV and V_T sampled at different locations. Unfortunately, the proportionality between SUV and V_T was not consistent from scan to scan. Additionally, with V_T as an endpoint, Hannestad et al. [6] found a significant effect of 1hr and 4hr LPS; however, using SUV, no LPS effect was detected at either timepoint. This is likely due, in part, to the sample size ($n = 4$, $n = 3$). Small group numbers are unlikely to provide enough power for detecting effects with SUV, especially under conditions of high variance across studies. The degree of variance of the SUV values observed here (~30%) is similar to variability observed with estimations of brain V_T for [^{11}C]PBR28 in both humans [15–18] and the data from the baboons in the present study [6]. Given the SUV data above, a basic power analysis for a paired t-test (with $\alpha = 0.05$ and power = 0.80) indicates that $n = 24$ would be needed to detect a 20% difference in SUV. Sample sizes would decrease to 12 and 6 for 30% and 50% effects, respectively. In the case of a between-groups design, a power analysis for an independent t-test results in $n = 44$ to detect a 20% effect, and 20 and 8, respectively, for 30% and 50% differences between groups [19]. Although [^{11}C]PBR28 SUV has proven sensitive to the human TSPO binding affinity phenotype [20], the TSPO genotype effect is relatively large relative to what may be expected in disease states.

In order to discern likely sources of variability in SUV, it is helpful to consider the definition of SUV presented above. Conceptually, SUV represents the ratio of the radionuclide quantity observed in a tissue region to the quantity that would be observed in this region if the radionuclide was uniformly distributed throughout the body of the subject. In order for the SUV to serve as a reliable surrogate marker of TSPO expression within a tissue of interest, two criteria should be met: (1) the temporal characteristics of the concentration of the radiopharmaceutical in the arterial blood after intravenous injection follow a consistent pattern from study to study, and (2) the area under the arterial blood curve within the tissue of interest must be proportional to the ratio of the injected radiopharmaceutical dose to the mass of the subject. Violation of either/both of these two assumptions will lead to increased variability and/or bias in SUV. Breakdown of these assumptions may occur in several ways. For example, if TSPO expression varies from study to study, the shape of the arterial blood curve of the parent radiotracer will differ across studies [21], which would violate the first assumption (or possibly both), and could result in increased variability in the SUV measurement. Indeed, in this cohort, lower plasma parent fraction was observed after LPS administration, presumably as a function of increased TSPO expression via microglial activation [6; although see below]. It also possible that variable levels of isoflurane anesthesia across scans may contribute to differential TSPO binding via alterations of microglial activation [22, 23]. Another potential situation is that organs which constitutively express high levels of TSPO (e.g., lung, heart, kidney, endothelial tissue) have undergone changes in e.g., post-translational modification or mitochondrial morphology that result in apparent altered levels of TSPO. Yet another possible source of variance is differential metabolism of parent [11C]PBR28, which may significantly alter how much [11C]PBR28 is present in the arterial blood. In fact, the lower parent fraction observed post-LPS in the present cohort was attributed to faster metabolic rate [6]. However, in the present dataset, accounting for the rate of metabolism of parent within the SUV calculation did not improve the relationship of SUV to V_T (data not shown). Regardless of the cause, changes in the temporal behavior of the parent radiopharmaceutical concentration in the blood between studies would lead to violation of one, or both, of the criteria listed above. Finally, another consideration is that, for radiopharmaceuticals like [11C]PBR28, which have a high first-pass extraction fraction and tissue retention, the delivery of tracer to a tissue of interest is dependent on blood flow to the tissue. This dependence on blood flow means that tracer delivery will have a very weak relationship, if any, to body mass, which would violate the second assumption above. In this circumstance, body mass becomes a source of variability to SUV.

CONCLUSION

In baboons, at baseline and under conditions of LPS-induced TSPO expression, SUV is not consistently representative of specific binding of [11C]PBR28 in the brain as defined by V_T. It is unclear how these results would translate into human disease conditions that involve more subtle levels of neuroinflammation. Additional work is needed to determine the source of discrepancy in SUV across [11C]PBR28 scans, and to develop alternatives to arterial plasma input functions (e.g., image-derived methods), for quantitation of [11C]PBR28.

Acknowledgments

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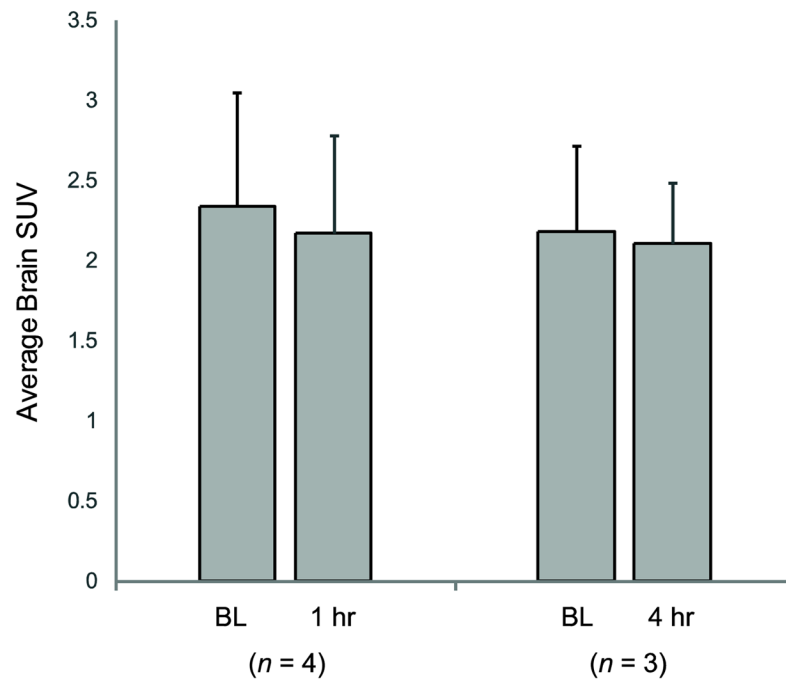
Figure 1.
Mean ± s.d. [^{11}C]PBR28 standardized uptake values (SUV) for the whole brain at baseline (BL) and one and four hours after LPS injection (1 hr, 4 hr).
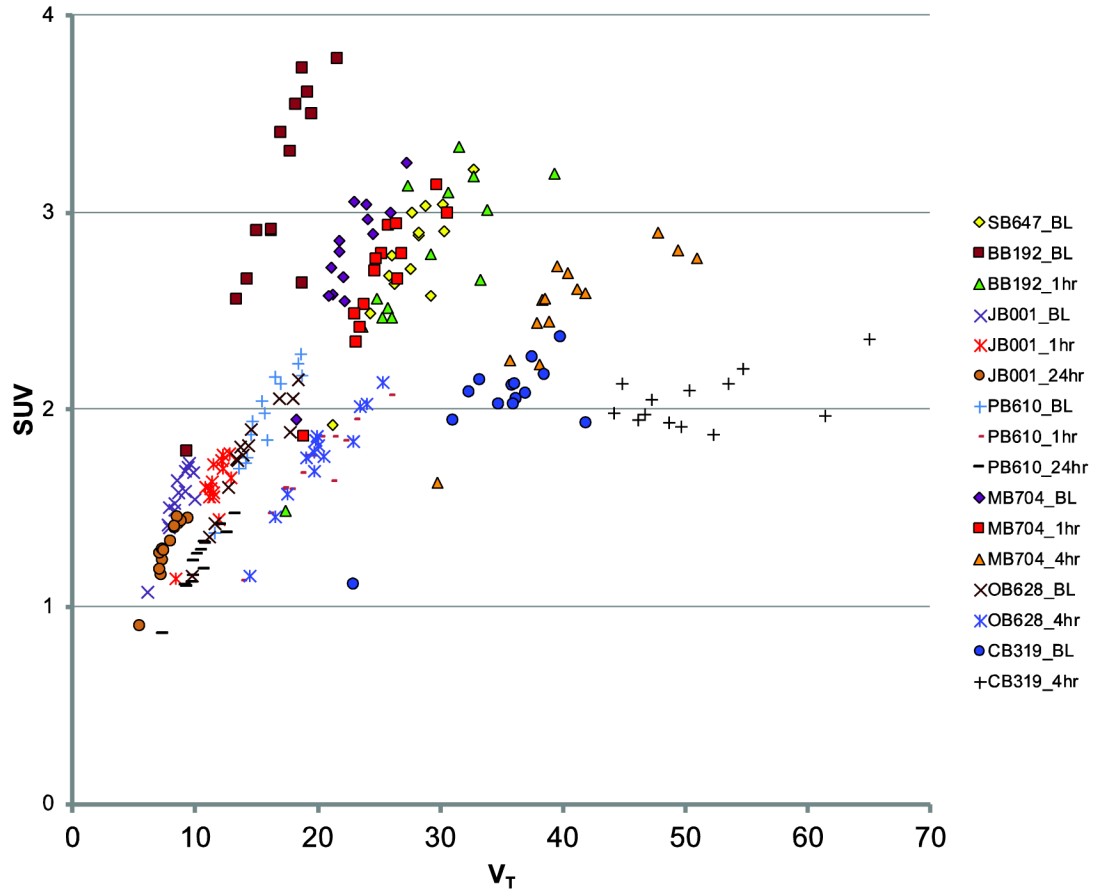
Figure 2.
Relationships between $[^{11}C]$PBR28 standardized uptake value (SUV) and volume of distribution (V_T). Each set of symbols corresponds to a single $[^{11}C]$PBR28 PET study; individual points within each set are from 14 different brain regions of interest (see text for details). Individual baboon IDs are two letter, three number designations, and are followed by the type of study, identified by: BL, baseline; 1hr, one hour post-lipopolysaccharide (LPS); 4hr, four hours post-LPS; 24hr, twenty-four hours post-LPS. In general, SUV correlates with V_T within a given study (with the exception of CB319_4hr). However, the wide range of slopes across studies (even within animals) indicates that this relationship is not equivalent for each study.
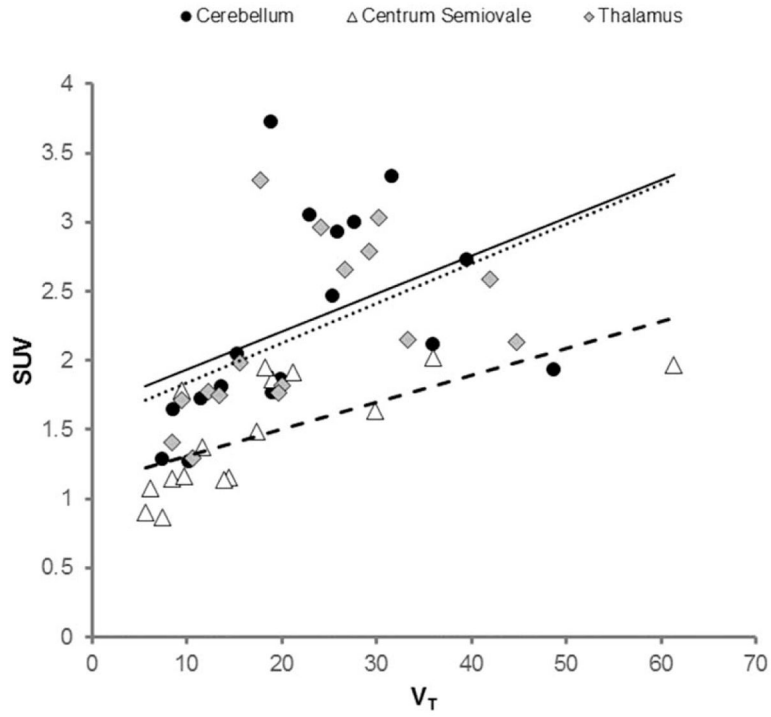
Figure 3.
Relationships between [11C]PBR28 SUV and V_T for regions of high (thalamus; gray diamonds, dotted line), medium (cerebellum; black circles, solid line) and low (centrum semiovale; white triangles, dashed line) [11C]PBR28 uptake. Data points are from the sixteen [11C]PBR28 PET studies described in the text.