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VI. 6.   Measurement of Free Fraction in Plasma for Biomathematical Prediction of *SUVR* of Amyloid PET Radiotracers

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Introduction

Alzheimer's Disease (AD) is a form of dementia, defined by histopathological features like senile plaques and neurofibrillary tangles, with clinical symptoms such as loss of memory & executive functions, which may only be apparent many years later[1]. As there is no known treatment for AD once dementia set in, and with the increasing cost of care for AD, there is a growing interest in diagnosing subjects for possible AD conversion before clinical symptoms appear. Amyloid imaging using Positron Emission Tomography (PET) provides a non-invasive, in-vivo diagnosis of subjects based on cerebral amyloid load. In developing successful amyloid radiotracers to diagnose the amyloid burden in subjects, many challenges to consider (e.g. poor in-vitro to in-vivo conversion, different Aβ and tau binding etc.). To facilitate decision making in moving candidate amyloid radiotracers to clinical application, a screening methodology of amyloid PET radiotracers based on in-silico data and a biomathematical model was developed by the authors.

The biomathematical model developed was based on a 1-tissue compartment model developed by Guo et al. for CNS tracers[2]. Two in-vitro pharmacological parameters, free fraction in plasma (f_P) and free fraction in tissue (f_{ND}) are required to generate kinetic parameters for *SUVR* simulations. However, f_P values of only three amyloid radiotracers were reported in literatures and were measured using either rat or monkey plasma (Table 1). We proposed a methodology based on in-silico lipophilicity values and a relational model[3] to derive in-silico f_P and f_{ND} values. The purpose of this project was to validate the in-silico f_P values with in-vitro f_P values measured by means of ultrafiltration for 3 available amyloid

radiotracers in CYRIC, Tohoku University – [11C]BF227, [11C]PIB and [18F]florbetapir.

Methods

Ultrafiltration

One tube of frozen human plasma samples (4 mL, with Heparin) was defrost at 37°C for 30 min in a pre-warmed incubator. Presence of triglycerides and plasma pH were checked[4]. 4 mL of PBS were pipetted into another storage tube and kept in the incubator for 30 min. For each tracer, 1% (F-18) or 5% (C-11) of the total volume of plasma sample (40 μL and 200 μL respectively), of radioactive compounds were pipetted into plasma and PBS storage tubes respectively. Both tubes were vortexed and incubated for 30 min at 37°C, with side-to-side tiling motion to ensure continuous mixing.

Radioactive plasma and PBS were pipetted into 3 Centrifree tubes (1 mL, 10 kDa MWCO, Millipore) each and centrifuged with sliding buckets at 2000 x g for 20 min at 37°C, using a temperature-controlled centrifugal machine (Kubota 2800, Japan)[5]. The Centrifree tubes of both plasma and PBS each, were weighted as a whole with their respective ultrafiltrate containers, before and after centrifugation to obtain the weight of the top plasma ($W_{\mathrm{p}ti}$) and bottom ultrafiltrate ($W_{\mathrm{pf}i}$).

Fifteen empty gamma counter tubes were weighed. 100 μL of the plasma in the original storage tubes ($C_{\mathrm{p}i}$), plasma in the top part of the Centrifree tubes ($C_{\mathrm{pt}i}$) and the respective ultrafiltrate ($C_{\mathrm{pf}i}$) were pipetted into gamma counter tubes and radioactivity in each tube was measured using WIZARD2 (2480, Perkin Elmer) in three aliquots. The same procedures were repeated for PBS to obtain $C_{\mathrm{b}i}$, and $C_{\mathrm{bf}i}$ only. For each tracer, f_{P} was measured using three aliquots to determine variability within each measurement and measurements were carried thrice to determine reproducibility of measurements.

Calculation of recovery, non-specific binding (NSB) & free fraction in plasma (f_{P})

Due to NSB in ultrafiltration, a few methods were proposed to calculate f_{P} from ultrafiltration measurements, with basic method used as a standard[4]. However, it does not correct for NSB and hence a "reference" method was introduced to correct for NSB[6]. However, correcting f_{P} measurements using PBS was said to be inappropriate as PBS has different viscosity properties from plasma[7]. Moreover, ultrafiltration measurements were dependent on volume ratio of ultrafiltrate, hence a mass-balanced method[7] was introduced to correct for possible differences in measurements due to differences in volume ratio. The

various methods of determining f_P values were explored to compare with reported f_P values (Table 1).

A. Based on Mass-Balanced Method[7]:

Protein binding, recovery were calculated by mass balance as follows:

$$\%\text{Recovery} = \sum_{i}^{n}\left\{\frac{\left(C_{\text{pf}_i} \times W_{\text{pf}_i}\right) + \left(C_{\text{pt}_i} \times W_{\text{pt}_i}\right)}{\left(C_{\text{p}_i} \times W_{\text{p}_i}\right)}\right\} \times \frac{100\%}{n} \tag{1}$$

$$\%\text{NBS} = \sum_{i}^{n}\left\{1 - \frac{C_{\text{bf}_i}}{C_{\text{b}_i}}\right\} \times \frac{\mathbf{100\%}}{n} \tag{2}$$

$$f_P = \sum_{i}^{n}\left\{\frac{C_{\text{pf}_i} \times V_{\text{pf}_i}}{\left(C_{\text{pt}_i} \times V_{\text{pt}_i}\right) + \left(C_{\text{pf}_i} \times V_{\text{pf}_i}\right)}\right\} \times \frac{100\%}{n} \tag{3}$$

B. Based on Reference Method[6]:

$$f_P = \sum_{i}^{n}\left\{\frac{C_{\text{pf}_i}/C_{\text{p}_i}}{C_{\text{bf}_i}/C_{\text{b}_i}}\right\} \times \frac{100\%}{n} \tag{4}$$

C. Based on Basic Method[4]:

$$f_P = \frac{C_{\text{pf}_i}}{C_{\text{p}_i}} \times 100\% \tag{5}$$

where i refers to the no. of samples measured (n = 1 ~ 3), p refers to plasma and b refers to PBS (buffer), t refers to top part of Centrifree tube, f refers to the ultrafiltrate part of the Centrifree tube, without t or f means the total of both top and filtrate part of Centrifree tube. C refers to the radioactive concentration measured using WIZARD and W refers to the weight of the sample. For example, $C_{\text{p}i}$ is radioactive concentration in plasma, $W_{\text{p}i}$ is the weight of total weight of the samples in the top of the Centrifree tube and in the ultrafiltrate container, C_{ti} and W_{ti} are the radioactive concentration and weight of sample in the top of the Centrifree tube, C_{fi} and W_{fi} are the radioactive concentration and weight of the ultrafiltrate in the filtrate container.

Results

Table 2 shows the calculated recovery, NSB and f_P calculated by the 3 methods mentioned. The in-silico f_P values are also shown in Table 2.

Discussions

Up to date, very few literatures have reported the values of plasma free fraction (f_p),

(Table 1), and the equations used to calculate f_P were not discussed. f_P values were measured by means of thin layer chromatography and ultrafiltration and no f_{ND} values were reported in the literatures. f_P values measured using animals' (rat and monkey) plasma samples were used for measurements (Table 1), instead of human plasma samples, which made comparison of reported and measured f_P values (Table 2) difficult.

Equilibrium Dialysis is the gold standard used to measure both f_P and f_{ND}, but was not carried out due to limitations and long time required for measurement. Ultrafiltration was applied instead but measurements could only be used reliably if verified with ultrafilitration. However, non-specific binding (NSB) should be kept low (<5%)[4], and volume of ultrafiltrate should be kept controlled within 40% of total volume[4,5]. The average volume ratio of the ultrafiltrate is kept less than 20%, with an overall mean of 18.4% and standard deviation of 0.5%. The variabilities within experiment and between the experiments, were less than 5% regardless of the tracers used and the calculation methods applied (Table 2). Hence, the procedure parameters were well-controlled for f_P measurements.

[11C]PIB had the highest NSB to filter membrane, followed by [11C]BF227 then [18F]florbetapir, with the same order for f_P values calculated using mass-balanced and basic methods. [11C]BF227 has the highest referenced f_P values, followed by [11C]PIB then [18F]florbetapir. In-silico f_P values showed similar trend with reference f_P values.

Due to the binding nature of all three amyloid tracers measured, NSB values measured were always greater than 50% and %Recovery values measured were also less than 90%, hence ultrafiltration was not a suitable method for measuring f_P. Moreover, only three clinical amyloid radiotracers were available for f_P measurements hence it was difficult to use the f_P values for validating in-silico f_P values or for correlating with clinical outputs or for use in in-silico/in-vitro model prediction.

Conclusions

The results showed that ultrafiltration was not a suitable method for measuring f_P values. Although only three radiotracers were evaluated, the measured results showed a similar trend in terms of clinical tracer evaluation, whereby [11C]PIB showed better amyloid binding then [11C]BF227 and [18F]florbetapir. If more clinical radiotracers were available, further evaluation on the possible co-relationships could be carried out.

References

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Table 1.   Free fraction in plasma (f_P) reported in Literatures.

Tracer	f_P (%)	Method of Measurement	Species	References
[^{11}C]PIB	14	Thin-layer Chromatography at 60 min	Rat	(8)
[^{18}F]Flutemetamol	1 (0.9–1.3)$^{\#}$	Ultrafiltration	Rat	(8)
[^{11}C]MeS-IMPY	0.83±0.17*	Ultrafiltration	Monkey	(9)

$^{\#}$Range of f_P values

*Mean ± Standard deviation

Table 2.   Recovery, NSB, Plasma Free Fraction (f_P) and ultrafiltrate volume ratio measured using ultrafiltration (Mean ± Standard deviation) and in-silico f_p values (Right) for [^{11}C]PIB, [^{18}F]Florbetapir and [^{11}C]BF227.

Tracers	Recovery (%)	NSB (%)	Volume ratio (%)	Mass balanced f_P (%)	Referenced f_P (%)	Basic f_P (%)	In-Silico f_P (%)
[^{11}C]PIB	82.1±1.3	99.0±0.2	18.7±0.4	0.04±0.02	15.0±4.4	0.13±0.11	30.3
[^{18}F]Florbetapir	83.3±0.5	65.6±2.3	16.7±0.8	0.64±0.07	9.28±0.62	3.17±0.19	27.0
[^{11}C]BF227	81.7±1.5	94.7±0.3	18.1±0.5	0.19±0.01	16.2±0.7	0.84±0.03	30.4