Quantitative PET imaging of radioligands with slow kinetics in human brain

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Dear Sir,

We read with interest the commentary by Hirvonen et al. [1] on the quantification of radioligands with slow kinetics. Their critique of our work on the quantification of the type 1 cannabinoid receptor (CB1R) ligand [^{18}F] MK-9470 in the human brain [2] was of particular interest and triggered our response to support the methods that were used in the analysis of the behavior of this novel radioligand. Here we specifically address the vulnerabilities and pitfalls highlighted by Hirvonen et al. and argue that our assumptions can be reasonably justified even in the absence of a “gold standard” for radioligands with slow tissue kinetics.

The general approach for analyzing neuroreceptor PET studies is based on compartmental modeling approaches formulated by Mintun et al. [3]. Even though the mathematical formulation of this model relies on several assumptions (e.g., rate constants are not changing during the PET study and the tracer concentration in the compartments is homogeneous), brain PET imaging analyzed with this approach has been a powerful tool that has enabled the study of neuroreceptor systems in normal and pathological conditions as well as supporting drug development.

The compartmental model formulation is such that the parameters ($K_1, k_2, k_3 = f_{ND}k_{on}(B_{avail} - C_S)$ and $k_4 = k_{off}$, where C_S is the tracer concentration bound to the receptors

and f_{ND} is the tracer free fraction in tissue [4]) could be related to the biochemical and physiological characteristics of the tracer and the receptor system. The estimation of all five parameters (rate constants and B_{avail}) is however only possible when using multiple injection protocols [5–8]. Another approach is to perform a series of studies at different specific activities [9–11] and use an in vivo Scatchard analysis to estimate B_{avail} and K_d . It should be noted that very often, when performing single bolus or bolus plus constant infusion (B/I) tracer administration experiments, the individual model parameters cannot be identified (even when the number of parameters is reduced by using high specific activity tracer, so that $k_{on}(B_{avail} - C_S) \xrightarrow{\text{High SA}} k_{on}B_{avail}$). The reason that it is not possible to find a unique solution to the individual rate constants is because of the large correlation between parameters and the large coefficients of variation of the estimates. In general, the *macro parameters* (volume of distribution, binding potential, irreversible uptake rate constant) obtained from the combination of individual rate constants are more stable. As a consequence caution is required when interpreting any estimates of individual rate constants particularly when derived from single tracer injection experiments.

Hirvonen et al. [1] asserted that our assumption that k_4 is uniform across brain regions is invalid and that k_4 differs across brain regions in correlation with receptor density. In support of their argument they discussed [^{123}I]iomazenil, a SPECT ligand for benzodiazepine receptors that are widely distributed at high density throughout the brain. In nonhuman primates for [^{123}I]iomazenil, the in vivo k_{on} and k_{off} (estimated using a combination of parameters from in vivo Scatchard analysis [11] and from single bolus tracer studies [12]) were much slower than the in vitro measurements, whereas the ratio of the in vivo rates k_{on}/k_{off} was similar to the in vitro measured K_d values.

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Hirvonen et al. [1] invoked the re-binding hypothesis to explain the difference between in vivo and in vitro values by proposing that the in vivo dissociation rate constant is dependent on the density of receptors available for binding. However, it can be argued that the large differences in radioligand behavior and characteristics are expected because of the profound differences between in vivo and in vitro imaging techniques used to generate the data (see discussion and references in Inoue et al. [13]). Furthermore, in the [^{123}I]iomazenil studies [11], the in vivo k_4 estimates were actually similar between regions with different B_{avail} ([11], Table 4) and were reported to be “virtually identical across regions” when using a bolus tracer injection protocol ([12], Table 3). These data and interpretation are supported by the [^{123}I]iomazenil k_4 values estimated by Millet et al. [14] using a multi-injection protocol that were similar throughout the human brain with the exception of the k_4 value in the pons which was ~65% smaller relative to cortex and cerebellum regions. This difference was probably methodological and due to the small displacement observed during the cold tracer injection segment in this region which compromised accurate estimation of k_4 . In addition to these data from SPECT, PET studies using a multi-injection protocol with [^{11}C]flumazenil have also shown that k_4 is not dependent on B_{avail} and that it is uniform across regions in the human brain [6]. Moreover, the recent work of Vandehey et al. [8] suggests that k_4 is also uniform across all brain regions for the D2/D3 radioligands [^{18}F]fallypride and [^{11}C]FLB457. This is an important observation since any difference in k_4 across regions of high and low B_{avail} should be exaggerated as D2 receptor density in the postmortem human brain has been shown to be >15-fold lower in extrastriatal structures than in striatal structures [15].

The strategy of simultaneously fitting data from several brain regions while coupling parameters that are expected to be similar across the brain has been used by many investigators to increase the identifiability of the kinetic parameters. In the specific case of k_4 , several examples can be found in the literature for a variety of PET ligands for different targets where tracer binding quantification has been made more robust by assuming a global k_4 value [5, 14, 16–21]. We cannot therefore find any empirical or theoretical reason that would argue against our simplifying assumption that [^{18}F]MK-9470 k_4 values are uniform throughout the human brain.

Hirvonen et al. [1] appear to have overlooked that our analyses of [^{18}F]MK-9470 made use of the known pharmacological characteristics of [^{18}F]MK-9470 to determine the tracer modeling approach and to quantify the tracer-specific binding in the human brain. Indeed, despite the brain kinetics of [^{18}F]MK-9470 being relatively slow, studies in nonhuman primates have clearly shown that the radioligand can be displaced from the receptors [22] supporting the use of a

reversible binding model. In our studies, the use of a global k_4 estimated using simultaneous fittings of the brain data to a two-compartment model provided reliable estimates of V_T , $V_{ND}k_3$, and k_4 . Furthermore, the results from our early studies using this reversible approach showed that the scan time could be reduced to 180 min ([2], Fig. 4) improving the practicality of data acquisition during the conduct of the imaging studies. Whilst we have described [^{18}F]MK-9470 tracer kinetics using a reversible model, the tracer clearance during the initial 180-min interval required to estimate the tracer binding to the receptors is negligible ([2], Fig. 1), consistent with the small k_4 value ($0.0041 \pm 0.0010 \text{ min}^{-1}$, $n=6$ studies). As a result of the small k_4 the use of an irreversible model may also be justified even though the tracer binding is known to be reversible. This is analogous to the way [^{18}F]FDG brain data are handled, where although it is possible to measure a small k_4 value, the net irreversible uptake rate constant K_i is the parameter generally used to estimate the glucose consumption throughout the brain [23]. We argue that such a macro parameter approach is also applicable for receptor binding studies and that [^{18}F]MK-9470 can also be simply analyzed in this way. We therefore used K_i and the fractional uptake rate (FUR) as indexes of tracer binding to CB1 receptors. A clear linear relationship between the reversible and irreversible parameters ([2], Fig. 7) demonstrated that K_i and FUR indeed convey receptor availability information. Since the integrals of the plasma curve (0–180 min) and the brain curves (120–180 min) are used for the calculation of FUR values, a reduction in the bias from noise in the measurements is also observed. The use of a simple irreversible model to analyze [^{18}F]MK-9470 significantly improved the test-retest reproducibility of tracer binding indexes (see Table 3 in [2] for a comparison of reproducibility for all studied parameters including brain uptake as discussed in isolation by Hirvonen et al.).

We therefore can conclude that the kinetics of radioligands that slowly wash out from the brain can successfully be characterized with macro parameters that convey receptor availability information. Therefore, we reiterate that for the CB1R inverse agonist [^{18}F]MK-9470-specific binding in the brain can be quantified accurately using a simplified method and that imaging of the CB1 receptors in the human brain using this radioligand can reliably support the development of potential therapeutics.

Conflicts of interest SMSB is an employee of Merck Inc., Co.

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