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LETTER TO THE EDITOR

The validity of ^{18}F-GE180 as a TSPO imaging agent

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Dear Sir,

To image the 18-kDa mitochondrial translocator protein (TSPO), a putative biomarker for inflammation, dozens of PET radioligands have been synthesized over the years [1]. None of them, however, presents optimal imaging characteristics. A drawback common to most, if not all, TSPO ligands is that they are sensitive to a single-aminoacid polymorphism of the target protein [2]. This polymorphism differentiates subjects into high- (HAB), mixed- (MAB), and low- (LAB) affinity binders.

Therefore, a TSPO ligand providing better imaging quality is still a much sought-after goal. ^{18}F-GE180 is a novel tricyclic indole compound that binds to TSPO with high affinity [3], and has been used with satisfactory results in a variety of preclinical models [4–6]. Head-to-head comparisons in animal models of neuroinflammation showed ^{18}F-GE180 to have better imaging properties than ^{11}C-(*R*)-PK11195 [4] and ^{18}F-DPA-714 [6], although it seemed to be less sensitive than ^{11}C-DPA-713 [5].

When injected in humans, however, ^{18}F-GE180 revealed poor imaging characteristics: the brain uptake is very low, the tissue time-activity curves are almost flat, kinetic modeling is difficult because of the noise and the presence of high uptake in the blood vessels—about 20% of the activity in brain regions comes from blood—and the outcome parameters are often poorly identified [7–9]. Indeed, the total volume of distribution (V_T) of ^{18}F-GE180 is about 20 times smaller than that of ^{11}C-PBR28, despite a greater concentration of ^{18}F-GE180 in plasma due to its slower metabolism [9]. These should have been disqualifying characteristics for any PET tracer, especially when so many valid alternatives are already available [1]. Nevertheless, ^{18}F-GE180 has moved forward to be employed in several human trials, including subjects with glioblastoma [10, 11] and multiple sclerosis [12, 13].

Two advantages are commonly attributed to ^{18}F-GE180: (1) it is insensitive to TSPO polymorphism and (2) its lesion-to-background ratio is higher than that of the other TSPO tracers. This letter explains why, in our opinion, these are only illusory advantages.

^{18}F-GE180 appears to be insensitive to genotype only because of its poor imaging quality

^{18}F-GE180 has very low uptake in the healthy brain parenchyma and high activity in the blood vessels. The confinement of activity to the vascular compartment has often been attributed to a high binding to blood proteins [7, 11, 13]. However, this hypothesis is disproven by the measurements of the plasma free fraction of ^{18}F-GE180 in humans, which show that about 3–4% of the parent concentration is in free form, a percentage similar or greater than that of other TSPO tracers with excellent brain uptake, such as ^{11}C-PBR28 [9], ^{18}F-PBR06 [14], or ^{11}C-ER176 [15]. By consequence, the most plausible reason for a low uptake is because ^{18}F-GE180 cannot cross the BBB. This explanation is supported by the results of kinetic modeling: the rate constant K_1, which is related to the transfer of the tracer across the blood brain barrier, is much smaller for ^{18}F-GE180 than for ^{11}C-PBR28 [9].

In the healthy human brain, all in vivo studies, except one [7], did not find any genotype-related difference despite a 15:1 binding affinity difference between HABs and LABs measured in vitro [8]. Thus, ^{18}F-GE180 has been likened to ^{11}C-(*R*)-PK11195, which is often (incorrectly [16]) claimed to be

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insensitive to genotype on the basis of a perceived in vitro – in vivo discrepancy [1]. A similar discrepancy is found with ^{11}C-ER176, a quinazoline analog of ^{11}C-(*R*)-PK11195 [17], which displays an obvious in vivo genotype sensitivity that had not been expected from in vitro studies [15]. Although ^{11}C-(*R*)-PK11195, ^{11}C-ER176, and ^{18}F-GE180 all display a discrepancy between in vitro and in vivo affinity data, the *direction* of this discrepancy is important. If an in vitro assay does not detect a genotypic sensitivity that is apparent in vivo, as in the case of ^{11}C-(*R*)-PK11195 and ^{11}C-ER176, the most plausible explanation is that binding assays may not always be a good model for the complex protein-to-protein interactions that happen in vivo. But if an important affinity difference that is present in vitro (15:1 between HABs and LABs, which is greater than that of many TSPO tracers [18]) disappears when ^{18}F-GE180 is injected in vivo, the most plausible explanation is that the image quality of ^{18}F-GE180 is so poor that even this large difference cannot be reliably detected in vivo. Nor the lack of apparent genotype sensitivity in vivo for ^{18}F-GE180 can be explained by the small number of subjects present in each study [10–12]. LABs may be recognizable on an individual basis. For instance, the existence of LABs has been discovered from the images of a single subject [19]. In general, tracers with good imaging properties are able to differentiate the three different populations even with two to three individuals per group [15].

The high ^{18}F-GE180 in brain lesions is mostly driven by the breakdown of the blood brain barrier

At odds with its behavior in the healthy brain, ^{18}F-GE180 unquestionably displays a high lesion-to-background ratio, notably in brain tumors [10, 11] and in multiple sclerosis plaques [12, 13]. But these lesions are associated with BBB damage, and the high ratio can be explained on one side by the aspecific accumulation of both ^{18}F-GE180 and its radiometabolites in the areas of leakage, and on the other side by the inability of ^{18}F-GE180 to cross the healthy BBB.

The ^{18}F-GE180 uptake generally exceeds that of contrast-enhanced MRI in both tumors and multiple sclerosis lesions [10–13]. Although contrast-enhanced MRI is commonly defined as the gold standard for tumor delineation, subtle BBB changes are extremely challenging to study. PET can detect radioactivity at picomolar concentrations, whereas MRI using classical gadolinium-based contrast agents has a sensitivity in the micromolar range [20]. Some PET studies with labeled amino acids have successfully correlated the PET uptake to histopathological findings, and therefore shown that the specific uptake was robust enough to be practically independent from the integrity of the BBB [21, 22]. However, no histological comparison has been done for ^{18}F-GE180, and any uptake beyond that of gadolinium is uncritically attributed to specific inflammatory uptake in the tumor. Thus, a positive signal with PET in an area that does not show any contrast enhancement with MRI can simply be due mainly to the higher sensitivity of PET to detect subtle alterations of the BBB.

Indeed, the pattern of ^{18}F-GE180 uptake is compatible with that of BBB breakdown. For instance, the lesion-to-background ratio was higher in the more aggressive IDH-wildtype gliomas compared to IDH-mutant gliomas, which have better prognosis [11]. Also, WHO grade IV gliomas had higher uptake with ^{18}F-GE180 than with ^{18}F-FET, while the uptake was comparable between the two tracers with grade III gliomas [11]. While both these findings could be compatible with a higher expression of TSPO in more aggressive tumors, the greater BBB breakdown associated with more aggressive tumors is also a plausible explanation.

In malignant gliomas, characterized by a mixture of necrotic and viable tissue, the relationship between gadolinium and ^{18}F-GE180 is predictably not perfect. More often, areas with little or no gadolinium uptake show strong ^{18}F-GE180 uptake. Sometimes, small areas within the lesion show higher gadolinium than ^{18}F-GE180 uptake [10]. But even in the latter case, the relative contribution of BBB breakdown and specific binding cannot be ascertained. It should be noted that ^{18}F-GE180 uptake in tumoral lesions does not show the genotype-related differences predicted by in vitro studies [10, 11], which is compatible with a signal largely dominated by aspecific accumulation of radioactivity.

Similarly, multiple sclerosis lesions presented either gadolinium +^{18}F-GE180 uptake or only ^{18}F-GE180 uptake (the only lesions with isolated gadolinium uptake, which would directly suggest a dissociation of ^{18}F-GE180 uptake from BBB integrity, were too small to be imaged with PET) [12]. Also, tracer uptake was higher in the lesions with gadolinium enhancement than in those without, which again suggests that ^{18}F-GE180 is correlated with the degree of disruption of the BBB. Finally, also in the lesions of these patients with multiple sclerosis, there was no genotype-related difference of uptake among HABs, MABs, and LABs, which is again compatible with a predominantly nonspecific uptake.

Brain tumors and multiple sclerosis are diseases with large permeability leaks, but subtler disruptions in the neurovascular unit are also present in chronic vascular disease and dementia. For instance, numerous imaging and postmortem studies have shown that Alzheimer's disease is associated with BBB leakage [23, 24]. In particular, PET studies with labeled substrates found widespread differences in BBB permeability between AD and controls, independently of blood flow [25, 26]. It would not be surprising if subtle alterations of the BBB contributed to some extent to significant group differences if the baseline uptake is very low.

In summary, the imaging characteristics of ^{18}F-GE180 are so poor and its alleged advantages so plausibly explained by an alternative hypothesis (i.e. aspecific accumulation through

a broken BBB) that, if its use is to be continued, we suggest that first it should be better characterized in at least one of the following ways:

1) By comparing ^{18}F-GE180 uptake to histopathological analysis using stereotactic brain biopsies.
2) By assessing the amount of specific binding in brain lesions with a pharmacological challenge, for example, by using the blocking agent XBD173.
3) By comparing the area of ^{18}F-GE180 uptake to that of a PET tracer specific for the integrity of the BBB, such as ^{11}C-Verapamil or ^{11}C-N-desmethyl-loperamide.

Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

This article does not contain any studies with human participants or animals performed by any of the authors.

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