Commentary on "PET imaging of microglia by targeting macrophage colony-stimulating factor 1 receptor (CSF1R)"

SRSTT Radiopharmaconnect[1]

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Horti, A. G. *et al.* **PET imaging of microglia by targeting macrophage colony-stimulating factor 1 receptor (CSF1R).** Proc Natl Acad Sci U S A 116, 1686-1691, doi:10.1073/pnas.1812155116 (2019).

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1. Abstract:

Neuroinflammation is involved in several brain neurodegenerative diseases, such as Alzheimer's Disease (AD) and Multiple Sclerosis (MS)[1,2]. Horti, A. G. *et al.* (2019) developed [^{11}C]CPPC, a Positron Emission Tomography (PET) radiotracer with high specificity for the macrophage colony-stimulating factor 1 receptor (CSF1R), essentially expressed in microglia. In addition, the authors discussed [^{11}C]CPPC application in models of neuroinflammation, AD and MS. Here, this commentary will be focused on reviewing the steps for [^{11}C]CPPC radiosynthesis, and discussing its application in animal models of neuroinflammation and a mouse model of AD.

2. Synthesis

The precursor (Pre-CPPC) was prepared in a four-step reaction. Radiosynthesis (Figure 1) of [^{11}C]CPPC was obtained by using [^{11}C]CH_3I, with a radiochemical yield of 21 ± 8% and high radiochemical purity (> 95%).

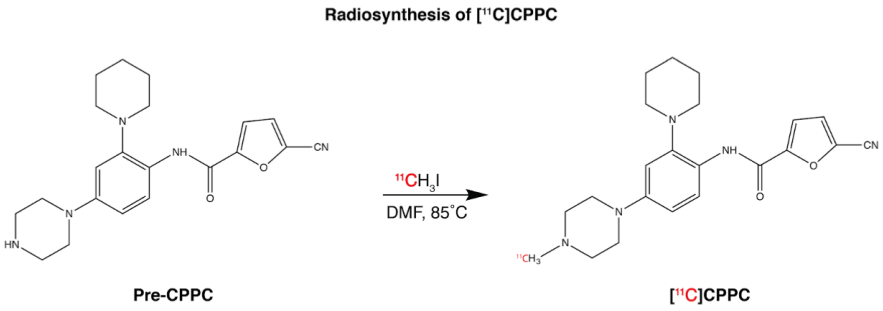

Figure 1: Radiosynthesis of [^{11}C]CPPC.

3. Results:

The brain regional distribution of [^{11}C]CPPC was evaluated using healthy C57BL/6J mice. Animals were sacrificed at 5, 15, 30 and 60 min and %SUV calculated.

· Major [^{11}C]CPPC uptake was seen in the frontal cortex with 150% SUV, between 5-15 min after radiotracer injection;

· Blocking studies demonstrated the specific binding of [^{11}C]CPPC only when correcting brain uptake for radioactivity concentration in blood (SUVR);

· Results indicated a 20% decrease on radioactivity in mice treated with high doses of CPPC blocker;

· The [^{11}C]CPPC brain uptake has a small significant reduction in microglia-depleted mice;

The [^{11}C]CPPC brain

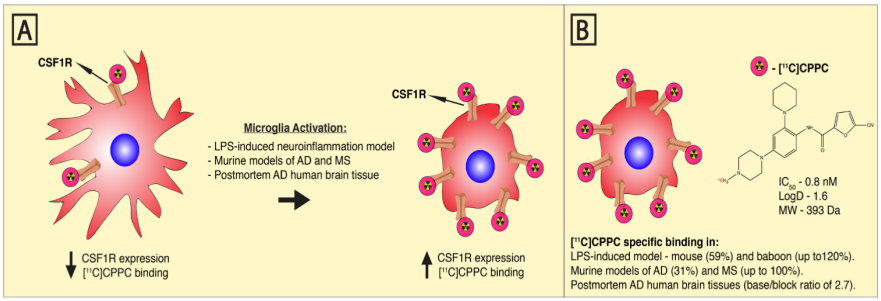

Figure 2: **CSF1R expression and [^{11}C]CPPC binding in healthy and disease. A** – CSF1R expression and [^{11}C]CPPC binding are increased in LPS-induced or disease-related neuroinflammatory conditions. **B** - Physicochemical properties and specific binding in animal models suggest great potential for [^{11}C]CPPC clinical translation.

Biodistribution of [^{11}C]CPPC in LPS-induced murine models of neuroinflammation

* Two LPS-induced neuroinflammation models were performed: administration via intraperitoneal (i.p.) and intracranial (i.c.);

* The %SUV of [^{11}C]CPPC uptake was increased in both LPS models compared to control mice;

* In blocking studies %SUV was only significant in i.c. LPS model;

* For i.p. LPS model, brain %SUV had to be corrected by the radioactivity concentration in blood.

A previous work published by Illig, C. R. et al. [3], describes the pharmacological properties of CPPC and other CSF1R specific inhibitors. The best molecules were tested in arthritis mouse model, showing efficacy by reducing bone erosion, cartilage damage and inflammation. These data indicate that the CSF1R inhibitors may interact throughout the body before reaching the brain. Indeed, this may explain the need for blood correction to obtain significant results in blocking studies involving a systemic inflammation mouse model (i.p. LPS).

A Murine model of Alzheimer's Disease:

* [^{11}C]CPPC was tested in a mouse model of AD, using mice overexpressing the human amyloid precursor protein (APP) with Swedish and Indiana APP mutations;

* [^{11}C]CPPC uptake was increased in AD mice compared to wild-type (WT) littermates controls mice;

* Highest [^{11}C]CPPC uptake was in cortex and hippocampus, two brain areas affected in AD [4].

Herein, aged APP-mice (16 months) were used to test [^{11}C]CPPC uptake. At this age, these animals present high insoluble amyloid-beta (Aβ) content widely distributed in the brain [5]. In fact, recent evidence suggests microglial activation as an early stage in AD pathology, which occurs even before the formation of mature insoluble Aβ plaques [6,7]. In keeping with this, [^{11}C]CPPC could be differentially taken up in different stages of amyloidosis. A PET longitudinal study with APP-mice and [^{11}C]CPPC, would be a very important step to elucidate initial inflammatory changes in AD[8].

A nonhuman primate LPS model of neuroinflammation:

* A systemic administration of LPS increased the distribution volume in the whole brain compared to control baboons;

* The increase on distribution volume in the brain was fully blocked by injecting non-radioactive CPPC;

* Blood sample analysis detected the presence of two hydrophilic metabolites, which were shown to minimally penetrate the brain;

4. Conclusions:

It is well known that most PET radiotracers aiming the CNS fail due to its inability to cross the blood-brain barrier. The innovative PET radiotracer [^{11}C]CPPC for imaging CSF1R discussed in this commentary, presented good brain penetrance, a moderate heterogenous distribution and good clearance. [^{11}C]CPPC is obtained with sufficient radiochemical yield, purity and specific activity, essential features for clinical translation. Of note, [^{11}C]CPPC has high specificity in mouse and baboon models of neuroinflammation. Additionally, in murine models of AD, there was significant increase on [^{11}C]CPPC binding compared to controls. Together these results indicate [^{11}C]CPPC as a potential tool to help on the diagnosis of brain diseases involving neuroinflammation, such as AD and MS. However, an important question remains: would [^{11}C]CPPC be useful for detecting microglia activation and neuroinflammation in the early stages of AD, before the symptomatic phase?

Take home message:

What would I like to see on the follow up of this paper?

The application of [^{11}C]CPPC targeting CSF1R in neuroinflammation-linked diseases has great potential for clinical translation. Currently, the inaccuracy of diagnosis and treatment for neurodegenerative diseases is a great matter of discussion. In AD, it seems that neuroinflammatory changes may emerge even earlier than detectable canonical Aβ and tau pathology. If [^{11}C]CPPC could detect this initial neuroinflammatory alterations with topographical resolution could help to detect the earliest AD-related changes, opening a new window for therapeutic intervention.

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