2-^{18}F-Fluoroacetate: A Useful Tool for Assessing Gliosis in the Central Nervous System?

Inflammation of the central nervous system (CNS) is a nearly ubiquitous consequence of neuropathologic processes involved in a spectrum of brain disorders, including brain trauma, ischemia, infection, and autoimmune and neurodegenerative diseases. CNS inflammation has seemingly paradoxical roles, responsible for both exacerbating and resolving tissue damage resulting from CNS insult. It is not only the primary mechanism by which tissue pathogens are sequestered and neutralized but also that which mitigates tissue repair and regeneration. CNS inflammation is characterized by

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the activation of intrinsic immune cells, such as microglia and astrocytes, and the induction of a plurality of inflammatory mediators including cytokines, cell necrosis factors, and prostaglandins (*1*). Infiltration of peripheral immune cells, particularly T lymphocytes, may be a consequence of blood–brain barrier (BBB) disruption. These same mechanisms may promote the destruction of intact or distressed neurons. In multiple sclerosis, T lymphocytes penetrate a compromised BBB and attack the CNS directly, resulting in a progressive loss of oligodendrocytes and subsequent axonal demyelination and degeneration. Similarly, brain infiltration of leukocytes after ischemic stroke may result in collateral neuron loss in the lesion penumbra through the secretion of the same proinflammatory mediators that promote a positive immune response. Conversely, inhibition of neutrophils and the adhesion molecules that facilitate their entry into the brain is associated with improved neurologic outcome after stroke (*2*). It is unclear under what conditions the immune response leads to these deleterious effects, although evidence suggests that the outcome of CNS injury may be related to familiar factors that include age, genetics, and the environment. Imaging technologies that enable the visualization of specific inflammatory processes in the brain could greatly promote a better understanding of their role in the pathophysiology of CNS diseases and guide the development of more effective therapies inhibiting specific inflammatory mediators, such as interleukin-1, cyclooxygenase, tumor necrosis factor-α, and complement (*1*).

Imaging CNS inflammation with acceptable specificity and sensitivity has proven to be a considerable challenge. Many PET and SPECT inflammation imaging agents with peripheral applications, including polyclonal immunoglobulins, monoclonal antibodies targeting leukocyte surface antigens, cytokines, and chemotactic peptides, are too large to achieve significant BBB penetration. Imaging approaches with potential applications for CNS inflammation include edema agents indicating BBB compromise (e.g., ^{99m}Tc-diethylenetriaminepentaacetic acid), direct labeling of leukocytes with either ^{111}In or ^{99m}Tc, or indirect labeling of immune cells using low-molecular-weight probes capable of brain entry through diffusion or facilitated transport (*3*). The specificity of edema agents for CNS inflammation remains undemonstrated, whereas the use of radiolabeled leukocytes for assessing CNS inflammation has been limited by the risk of sepsis, loss of the radiolabel in vivo, and the long half-life of ^{111}In (67 h). For these reasons, recent radiopharmaceutical development efforts have focused on methods to indirectly label immune cells in the CNS with greater specificity. Activated immune cells express a plurality of cell surface antigens and receptors that play a role in mediating the immune response. The peripheral benzodiazepine receptor, or translocater protein 18 kDa (TSPO), is one such receptor that has been widely investigated in a variety of CNS diseases (*4*). TSPO is located on the outer mitochondrial membrane and has many proposed functions that include transmembrane cholesterol transport, steroid and heme biosynthesis, and immunocompetent cell modulation (*5–7*). In the periphery, TSPO expression is rich in steroidogenic tissues, such as the adrenal glands. In the brain, TSPO sites are found predominantly in activated microglia. The utility of TSPO as a putative target for molecular imaging probes is based on the observations that constitutive expression of TSPO is low in the normal brain but selectively upregulated in activated microglia (*4,5,8*). The isoquinoline carboxamide derivative PK11195 is the most thoroughly characterized TSPO-specific ligand and also the first to be labeled with ^{11}C for PET studies. Although several neuroimaging studies have reported increases in *R*-[*N*-methyl-^{11}C]PK-11195 (^{11}C-PK-11195) binding in various CNS afflictions, a low specific binding signal has raised

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concerns regarding the sensitivity of ^{11}C-PK11195 (*3,4*). Nevertheless, TSPO continues to be an attractive target for radioligand development, with many new classes of TSPO-specific ligands identified (*9*).

Other approaches that are used to indirectly label activated immune cells in the CNS have focused on metabolic pathways that are relatively suppressed in normal brain tissue but locally enhanced in areas of immune cell activation. Immune cells, like cancer cells, have the ability to concentrate and rapidly proliferate in response to CNS insult, resulting in locally increased metabolic demands. Elevated glycolysis in both proliferating cancer cells and activated immune cells has been observed, supporting the use of the radiolabeled glucose analog ^{18}F-FDG as an index of energy metabolism in either case. However, because of the high levels of ^{18}F-FDG uptake in the normal brain and an activation-dependent distribution, the application of ^{18}F-FDG in CNS inflammation is limited. Consequently, radiolabeled substrates for other metabolic pathways that are relatively suppressed in normal brain tissue, such as protein and lipid synthesis, have been explored as more sensitive and specific indicators of disease. Methionine, for example, is preferentially taken up by glial cell tumors through the action of the *L*-type amino acid transporter family and is incorporated into proteins or converted to *S*-adenosylmethionine, a substrate for transmethylation reactions. The uptake of ^{11}C-methionine in the brain has been shown to reflect cellular proliferation activity, and ^{11}C-methionine may be a useful agent for grading glial cell neoplasms (*10*).

Acetate has been radiolabeled with ^{11}C for PET studies of oxidative metabolism in the myocardium and prostate tumor imaging. Acetate is transported into the cell via the monocarboxylic acid transporter where it is converted in mitochondria to acetyl-coenzyme A (acetyl-CoA). Acetyl-CoA is a substrate for several biochemical pathways, most notably the tricarboxylic acid (TCA) cycle and in lipid synthesis. In the first stage of the TCA cycle, acetyl-CoA condenses with oxaloacetate to form citrate. α-Ketoglutarate is another TCA cycle intermediate that may be transaminated to form glutamate, a precursor to the neurotransmitter glutamine. Although glucose and acetate are both precursors to acetyl-CoA, earlier studies have established that acetate is preferentially taken up by astrocytes (rather than neurons) and used for the biosynthesis of glutamine (*11–13*). Additional studies have demonstrated that the preferential use of acetate by astrocytes is mediated by transport, although the exact mechanism is not fully understood (*14*).

The utility of ^{14}C-acetate and ^{13}C-acetate as tools to study glial metabolism using autoradiography and nuclear magnetic resonance spectroscopy is well established and, consequently, acetate labeled with a positron-emitting radionuclide such as ^{11}C may be similarly useful as a noninvasive indicator of reactive astrocytosis for PET. Indeed, recent PET studies of 1-^{11}C-acetate in glioma have demonstrated its potential for differentiating tumor grades, and it may be superior to ^{11}C-methionine in this regard (*15,16*). The analog 2-fluoroacetate (FAC) is a metabolic toxin that has been proposed as a PET agent on the basis of its shared mechanism of uptake with acetate. FAC is metabolized by acetate thiokinase to fluoroacetyl-CoA and then to fluorocitrate after a reaction with oxaloacetate catalyzed by citrate synthase. Unlike citrate, which is achiral, there are 4 isomers of fluorocitrate. (−)-*erythro*-2-fluorocitrate is transformed to fluoro-*cis*-aconitate by aconitase, a key component of the TCA cycle. Subsequent release of fluoride ion and hydroxylation form 4-hydroxy-*trans*-aconitate (HTA). HTA has been shown to be a potent inhibitor of aconitase, and the resulting impairment of TCA cycle–mediated oxidative metabolism is the direct mechanism of fluoroacetate toxicity (*17*). FAC, like acetate, is preferentially taken up by astrocytes relative to neurons (*18*), and it follows that 2-^{18}F-fluoroacetate (^{18}F-FAC) could be a potentially useful noninvasive indicator of glial cell metabolism.

The use of ^{18}F-FAC as an indicator of glial cell metabolism may have several advantages over 1-^{11}C-acetate. One obvious advantage is the longer half-life, which would make a radiofluorinated analog of acetate more practical for clinical use. A second advantage is that although much of the radiolabel associated with 1-^{11}C-acetate is released as ^{11}C-CO_2 by oxidative metabolism in the TCA cycle, TCA cycle metabolism of ^{18}F-FAC is incomplete and results in the accumulation of radioactivity in tissue. However, it is not entirely clear whether ^{18}F-FAC or some metabolite is responsible for the observed accumulation of radioactivity in the brain. In rodents, it is likely that some of the label exists as intracellular ^{18}F-F^- released in the transformation of ^{18}F-fluoro-*cis*-aconitate to HTA and in the less active isomers of ^{18}F-fluorocitrate. Earlier ^{18}F-FAC studies reported significant uptake of the ^{18}F-F^- ion in rodent bone, suggesting that some aconitase inactivation had occurred. However, baboon studies did not exhibit detectible bone uptake of ^{18}F-F^- and suggest that the accumulation of radioactivity in tissue is the result of a slower rate of ^{18}F-FAC metabolism before aconitase inactivation, although it is not clear which is the rate-limiting step (*19*). This is a potentially important detail, as the slow transformation of ^{18}F-FAC to 2-^{18}F-fluoroacetyl-CoA would emphasize the influence of ^{18}F-FAC transport and this initial metabolic step on the overall radiotracer kinetics rather than oxidative metabolism by the TCA cycle. It is also possible that some of the radiolabel associated with the injection of ^{18}F-FAC is associated with lipid synthesis and not oxidative metabolism. In tumor cells, the mechanism of increased uptake of 1-^{11}C-acetate (and presumably ^{18}F-FAC) has been shown to be related to enhanced lipid synthesis (*20*). It is also well established that lipid synthesis is one of the major roles of astrocytes, supplying essential cholesterol to neurons and fatty acids (e.g., palmitate and oleate) required for the synthesis of brain phospho-

lipids (*21*). Evidence suggests that other types of glial cells and some neurons are involved in fatty acid biosynthesis, particularly myelin-producing oligodendrocytes, so it is likely that uptake of ^{18}F-FAC may not be limited to astrocytes (*22,23*). Indeed, it has been demonstrated that glioma tumors express high levels of fatty acid synthase, an enzyme that catalyzes the synthesis of palmitate from acetyl-CoA and malonyl-CoA (*24*). Therefore, it is possible that the relative amounts of radioactivity after the injection of ^{18}F-FAC associated with oxidative metabolism and lipid biosynthesis differs between brain regions and pathologic substrates.

The work of Marik et al. (*25*), presented in this issue of *The Journal of Nuclear Medicine,* is the first study, to our knowledge, to examine the utility of ^{18}F-FAC as a noninvasive index of glial cell metabolism. The study provides an intriguing first look at what might represent a novel and potentially useful application for imaging CNS inflammation. What must follow is a careful validation of the proposed technique. A critical first step should be to verify that the cellular source of the signal is indeed reactive astrocytes and to characterize the potential importance of other cell types, such as microglia and T lymphocytes, which are tightly associated with astrocytes. A validation must also examine the metabolic fate of the tracer and establish the relative importance of the pathways of oxidative metabolism and lipid biosynthesis across CNS pathologies. This information will guide the development of a model of ^{18}F-FAC brain kinetics in support of future quantitative analyses in human subjects. It will also be important to establish the sensitivity of the method, as presumably it will be of interest to apply this technique to study CNS inflammation in neurodegenerative diseases such as Alzheimer disease, with a modest presentation but possibly significant role in this disease pathology. The validation of an astrocyte-specific probe for noninvasive imaging could significantly enhance the understanding of CNS inflammation in disease and would complement similar investigations of microglial activation using TSPO-specific radioligands such as ^{11}C-PK11195.

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