Cyclooxygenases as Potential PET Imaging Biomarkers to Explore Neuroinflammation in Dementia

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The most frequently studied target of neuroinflammation using PET is 18-kDa translocator protein, but its limitations have spurred the molecular imaging community to find more promising targets. This article reviews the development of PET radioligands for cyclooxygenase (COX) subtypes 1 and 2, enzymes that catalyze the production of inflammatory prostanoids in the periphery and brain. Although both isozymes produce the same precursor compound, prostaglandin H_2, they have distinct functions based on their differential cellular localization in the periphery and brain. For example, COX-1 is located primarily in microglia, a resident inflammatory cell in the brain whose role in producing inflammatory cytokines is well documented. In contrast, COX-2 is located primarily in neurons and can be markedly upregulated by inflammatory and excitatory stimuli, but its functions are poorly understood. This article reviews these 2 isozymes as biomarkers of neuroinflammation, as well as the radioligands that have recently been developed to image them in animals and humans. To place this work into context, the properties of COX-1 and COX-2 are compared with 18-kDa translocator protein, with special consideration of their application in Alzheimer disease as a representative neurodegenerative disorder.

Key Words: PET; COX-1; COX-2; biomarkers; neuroinflammation

J Nucl Med 2022; 63:53S–59S
DOI: 10.2967/jnumed.121.263199

PET is a powerful clinical and research tool with adequate sensitivity to measure specific proteins at low density in vivo (*1*). Some of these proteins can be used in clinical trials as biomarkers to stratify patients and facilitate therapeutic drug development. Pharmacokinetic biomarkers can be used to determine whether the therapeutic agent reaches its target by measuring target engagement or receptor occupancy. In addition, dynamic biomarkers can confirm the expected pharmacological action, which is distinct from clinical efficacy. For example, a dynamic biomarker of anti-inflammatory action could demonstrate that the drug had the expected pharmacological action shortly after administration, even though weeks or years may be required to show clinical efficacy.

Neuroinflammation is a significant contributor to the pathophysiology of several neurologic and psychiatric disorders, including Alzheimer disease (AD), multiple sclerosis, Huntington disease, and possibly major depressive disorder (*1,2*). The 18-kDa translocator protein (TSPO) has been extensively studied as a PET biomarker of neuroinflammation, and AD has been the most-studied disease (*1,2*). Although PET imaging of TSPO in AD has been shown to successfully reflect disease state and disease severity, it has several limitations as a biomarker of neuroinflammation, including its nonspecific localization in microglia, astrocytes, and vascular endothelium (*1*).

In the search for more useful targets of neuroinflammation, several new radioligands have been developed that target the cyclooxygenase (COX) system. Two isoforms of COX, subtypes 1 and 2 (*2*), catalyze the rate-limiting step in the production of proinflammatory mediators, which makes these enzymes potentially useful biomarkers of neuroinflammation. This article will review the development of PET radioligands selective for COX-1 and COX-2 as well as the potential utility of these 2 targets, in comparison to TSPO, as biomarkers of neuroinflammation in AD.

COX-1 AND COX-2: BACKGROUND

Given that both COX-1 and COX-2 convert arachidonic acid to prostaglandin H_2 (Fig. 1), they might be expected to have the same functions. However, the specificities of their function derive from the varying cellular locations of COX-1 and COX-2 (*3*). For example, platelets contain enzymes that convert prostaglandin H_2 to thromboxane A_2, which promotes platelet aggregation and clotting. Vascular endothelium contains enzymes that convert prostaglandin H_2 to prostaglandin I_2, which inhibits platelet aggregation and clotting. In addition, platelets contain primarily COX-1, whereas vascular endothelium contains primarily COX-2. Thus, a nonselective COX inhibitor will have not only different actions but opposing actions (e.g., inhibiting clotting in platelets but promoting clotting in the vascular endothelium). The differential effects of the COX isozymes have been revealed by studying selective, or at least preferential, inhibitors. For example, the preferential COX-1 inhibitor aspirin is commonly used to decrease clotting in patients at risk for or with a history of heart attack and stroke. Conversely, selective COX-2 inhibitors such as rofecoxib (Vioxx [Tremeau Pharmaceuticals, Inc.], effective as an antiinflammatory drug) increase clotting to the point that this agent was removed from use because it increased the risk of heart attacks (*4*). Thus, the differential effects of COX-1 and COX-2 derive from different cellular localizations and can be responsible for both therapeutic efficacy and unwanted side effects.

Cellular Location and Inducibility

Given the importance of cellular location for pharmacological effects, the issue of where COX-1 and COX-2 are located in the brain is critical. Although reports vary, COX-1 appears to be

Received Oct. 18, 2021; revision accepted Jan. 12, 2022.
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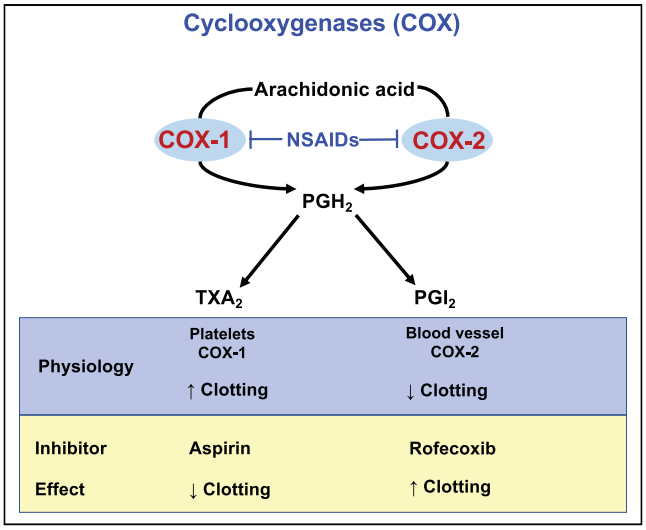

FIGURE 1. Distinct functions of COX-1 and COX-2 derive from their cellular location. Both COX-1 and COX-2 convert arachidonic acid into prostaglandin H_2 (PGH_2), which is later enzymatically converted into several bioactive prostanoids with different and sometimes opposing functions. The specific prostanoid depends on the enzymes in a given cell. Platelets contain primarily COX-1 and produce thromboxane A_2 (TXA_2), which promotes clotting. Vascular endothelium primarily contains COX-2 and produces prostaglandin I_2 (PGI_2), which inhibits clotting. Nonsteroidal antiinflammatory drugs (NSAIDs) inhibit COX isomers, either nonselectively (e.g., naproxen) or selectively (e.g., aspirin for COX-1 and rofecoxib for COX-2). Thus, the pharmacological effect of inhibiting COX-1 is to inhibit clotting and that of inhibiting COX-2 is to promote clotting.

located primarily in microglia and COX-2 in neurons (Table 1) (*5–7*). This differential cellular localization is found both in animals after an inflammatory challenge and in humans with neurologic conditions, including AD.

The functional differences between COX-1 and COX-2 derive not only from their varying cellular localizations but also from a differential response to inflammatory stimuli between the periphery and the brain. In the periphery, COX-1 is generally regarded as a constitutive enzyme and not upregulated by inflammation. In contrast, COX-2 can be quickly upregulated severalfold, and the increase can be blocked or reversed (*8*).

In the brain, the response of the COX isoforms is less conclusive and may be species-dependent. Four studies using microglia cultured from mice reported that COX-1 was upregulated on exposure to stimulants such as lipopolysaccharide and β-amyloid (*9*). In contrast, a study using cultured human microglia found no upregulation (*5*). Additional studies may help resolve the extent to which this differential effect is species-dependent.

In contrast to COX-1, the response of COX-2 to inflammatory stimuli in the brain has been more consistently reported as an elevation (*5,10*). In fact, COX-2 reacts like an immediate early gene and is rapidly upregulated in neurons after activation. For example, COX-2—but not COX-1—was found to be elevated in monkeys 1 d after intracerebral injection of lipopolysaccharide, and a postmortem analysis showed that most COX-2 transcript was in neurons (*6*). Although not an inflammatory stimulus, electroconvulsive seizures markedly and rapidly (within 1 h) increased COX-2 in rat neurons (*11*); prednisone blocked the increase in COX-2, suggesting that this upregulation is likely related to inflammatory pathways.

Postmortem Studies in AD Brain

Immunohistochemical staining in the brains of individuals with AD found that COX-1 was present in microglia and was especially elevated surrounding amyloid plaques (Table 1) (*7*). However, the increased density of COX-1 surrounding amyloid plaques may simply reflect an increased number of microglia rather than an increased amount of COX-1 in individual microglia. A follow-up study using a large postmortem dataset found that the proportion of activated microglia strongly correlated with β-amyloid load, tau-related neuropathology, and rate of cognitive decline (*12*).

In postmortem brain studies, COX-2 was present predominantly in the neurons of individuals with AD compared with control tissue (*5,7*). Though clearly needed, quantitative measures of COX-2 in postmortem AD are likely to be confounded by the rapid turnover or degradation of this enzyme in healthy states and during the postmortem interval. For instance, the half-life of COX-2 messenger RNA in postmortem human brain is estimated to be less than 3.5 h (*13*), and the half-life of the protein in vivo varies from 2 to 7 h (*14*).

Microglia as Brain's Macrophages

Microglia—which both release cytokines and phagocytose foreign protein and cellular debris—are often described as the resident macrophages of the brain. However, the 2 cells have different embryonic lineages: microglia derive from the embryonic yolk sac, whereas most monocytes and macrophages derive from fetal liver or bone marrow (*15*). Both activated microglia and macrophages (i.e., the activated form of bone marrow monocytes) are virtually identical on histologic examination and can be distinguished only

TABLE 1
Properties of 3 Biomarkers of Neuroinflammation

Property	COX-1	COX-2	TSPO
Primary cell	Microglia	Neurons	Microglia, astroglia, vessels
Constitutive?	Yes	Yes	Yes
Induced?*	Species-dependent†	Yes	Uncertain‡
Remains elevated?	Days	Hours	Weeks/mo
AD brain	Microglia around amyloid plaques	Elevated in neurons, especially early disease	Microglia, astrocytes, vessels

*Defined as increased expression of protein per cell on exposure to inflammatory stimuli.
†Three positive reports in mouse microglia (*9*), and 1 negative report in human microglia (*5*).
‡May also be species-dependent (*63*).

by nonoverlapping transcriptomic or proteomic profiles (*15*). Because microglia express high concentrations of COX-1, COX-1 inhibitors may have antiinflammatory effects in the brain, analogous to the effects of COX-2 inhibitors in macrophages. In fact, evidence suggests that COX-1 inhibition may have antiinflammatory and beneficial effects in animal models, including the reduction of amyloid pathology and improved memory in a mouse model of AD (*16*).

PET IMAGING OF COX-1

Few radioligands have succeeded in imaging COX-1. Many early candidates were unsuccessful for various reasons, including poor entry into brain, high nonspecific binding, and inadequate affinity. The nonsteroidal antiinflammatory drug and COX-1 inhibitor ketoprofen has low brain uptake because of the extensive deprotonation of its carboxyl group at physiologic pH. Nonetheless, ^{11}C-ketoprofen-methyl ester (Supplemental Table 1; supplemental materials are available at http://jnm.snmjournals.org) enters brain and is rapidly hydrolyzed to ^{11}C-ketoprofen for binding to COX-1. Whereas studies with this prodrug radioligand showed uptake in inflamed rat brain regions, COX-1–specific binding could not be verified in blocking studies. Therefore, it remained unclear whether radioactivity in brain was due mainly to binding of ^{11}C-ketoprofen to COX-1 or to the inability of ^{11}C-ketoprofen to leave brain because of its negative charge (*17*).

Despite this uncertainty, ^{11}C-ketoprofen-methyl ester was studied in healthy human volunteers, individuals with mild cognitive impairment, and individuals with AD (*18*). No differences in washout of radioactivity from brain were observed among these 3 groups. These results might reflect insufficient COX-1 expression, insufficient binding of ^{11}C-ketoprofen to COX-1, or inability of the radioligand or its radiometabolites to leave the brain. In our opinion, these results are also uninterpretable given that radioactivity reflects both the prodrug (^{11}C-ketoprofen-methyl ester) and the product of hydrolysis (i.e., ^{11}C-ketoprofen) trapped in the brain. ^{11}C-ketoprofen-methyl ester underscores that prodrug-type radioligands are usually difficult to quantify because PET cannot distinguish the prodrug from its radiometabolites. ^{18}F-FDG provides a notable contrast. Its ability to quantify the rate of glucose metabolism is based on the irreversible trapping of its radiometabolite in brain. Such is not the case for reversibly binding radioligands, for which both uptake and washout of the active component must be measured.

The direct-acting radioligand ^{11}C-1,5-bis(4-methoxyphenyl)-3-(2,2,2-trifluoroethoxy)-1H-1,2,4-triazole (^{11}C-PS13) was developed to overcome the issues associated with prodrugs (*19*). ^{11}C-PS13 was found to be potent (half-maximal inhibitory concentration, ~1 nM) and selective (>1,000 fold) for COX-1 compared with COX-2 (*20*). High affinities—particularly those in the nanomolar or subnanomolar range—are desired because they increase specific binding to the target of interest. In contrast, nonspecific binding is determined by lipophilicity, as measured by the experimental distribution coefficient, logD. LogD values of 2.0–3.5 indicate moderate lipophilicity and are optimal for brain entry. Ligands with values that are too low risk not crossing the blood–brain barrier, whereas those with values that are too high may have high nonspecific binding to brain tissue and plasma proteins (*21,22*). Despite its very high logD of 4.26, ^{11}C-PS13 crossed the blood–brain barrier and bound to COX-1. ^{18}F-PS13 has also been prepared, thus providing another avenue to synthesize the radiotracer and extend its use, given the longer half-life associated with ^{18}F (*23*).

Both animal and human studies indicate that ^{11}C-PS13 is promising for the in vivo imaging of COX-1. In whole-body scans of rhesus monkeys, ^{11}C-PS13 showed significant uptake in organs where COX-1 was expected, including the gastrointestinal tract, spleen, kidneys, and brain, indicating appropriate distribution (Fig. 2) (*24*). This uptake was blocked after administration of ketoprofen, a COX-1–specific inhibitor, but not celecoxib, a preferential COX-2 inhibitor, indicating good in vivo pharmacological specificity (*24*). Whole-body scans in healthy human volunteers found ^{11}C-PS13 uptake in most major organs and subsequent blockade by ketoprofen but not celecoxib, reproducing the appropriate distribution and pharmacological specificity seen in animals (*25*). Furthermore, in the brains of healthy human volunteers, ^{11}C-PS13 uptake was highest in the hippocampus, occipital cortex, and pericentral cortex (Fig. 3) (*19*). This distribution appears to be appropriate given the significant correlation with COX-1 gene transcript levels, as obtained from the Allen Human Brain Atlas (*24*). ^{11}C-PS13 also demonstrated good absolute test–retest variability (range, 6.0%–8.5%) and reliability (intraclass correlation coefficient range, 0.74–0.87), with no radiometabolite accumulation and excellent time stability (*19*).

PET IMAGING OF COX-2

Numerous radioligands have been tested to image COX-2, but most have either failed or not progressed to human studies. Extensive reviews detail the synthesis, in vitro results, and in vivo results for these radioligands (*26,27*). Arachidonic acid is the substrate for both COX-1 and COX-2 (Fig. 1), and ^{11}C-arachidonic acid is the only radiotracer mentioned in these reviews that has moved to human studies (*28,29*). Both these early radioligands and more recently developed ones (*30–35*) were primarily unsuccessful because of high nonspecific binding. However, in some cases, radiodefluorination (*36,37*), rapid metabolism (*36*), and poor brain entry (*38–40*) were also reasons for failure. A few radioligands appeared successful in small animals, but no ensuing human studies were published (Supplemental Table 1) (*41–43*).

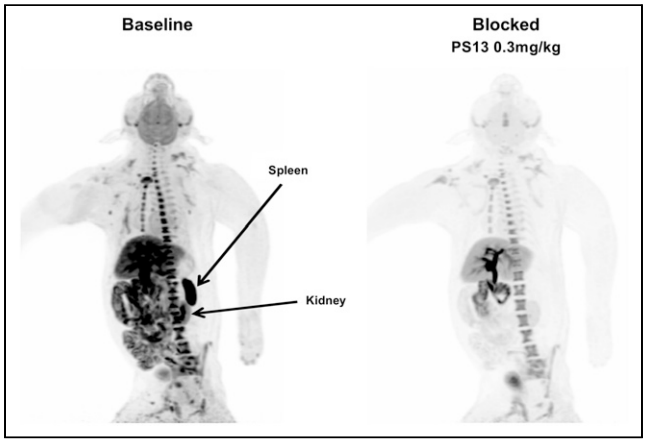

FIGURE 2. Imaging of COX-1 with ^{11}C-PS13 in monkey at baseline and after blocking with nonradioactive PS13, which is highly selective for COX-1. High specific binding (i.e., blockable) was shown in brain (percentage blockade, 35%), spleen (86%), gastrointestinal tract (61%), and kidney (~75%). (Reprinted from (*24*).)

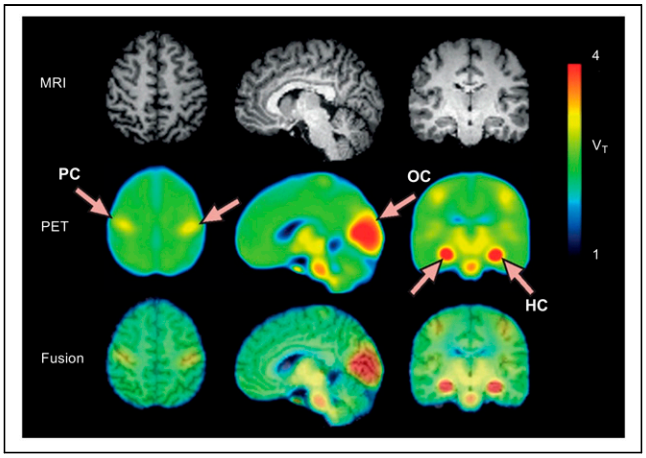

FIGURE 3. Distribution of COX-1 in healthy human brain. After ^{11}C-PS13 injection, enzyme density was calculated on the pixel level as distribution volume (V_T). The MRI is from a representative participant, and PET images of COX-1 are an average from 10 participants. Notable ^{11}C-PS13 binding (arrows) was detected in hippocampus (HC), occipital cortex (OC), and pericentral cortex (PC). The third row shows images fused from MRI and PET scans. (Reprinted from (*19*).)

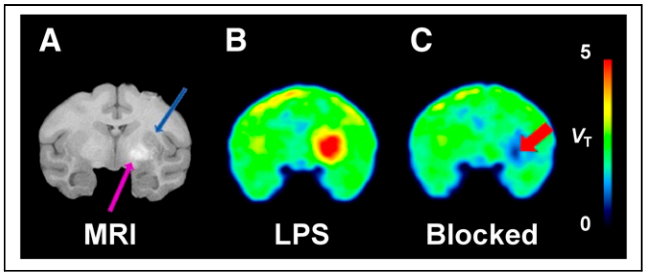

FIGURE 4. COX-2 was increased after a lesion in the brain of a rhesus macaque was found. The inflammatory agent lipopolysaccharide (LPS) was injected into the right putamen and initially caused edema and, later, a hemorrhage. (A) The coronal T2-weighted magnetic resonance image (MRI) scan of a rhesus macaque showing poorly visualized edema (blue arrow) and hematoma (purple arrow) around the injection site. (B) The PET image after ^{11}C-MC1 injection showed markedly elevated COX-2, especially overlying the hematoma. (C) The COX-2 selective compound MC1 (1 mg/kg intravenously) blocked uptake in the lesion area, confirming the existence of both specific (i.e., blockable) and non-specific (i.e., residual) binding of the radioligand. (Adapted from (*6*).)

Other radioligands are still being investigated and have not yet moved to in vitro or in vivo studies (*44–46*).

Currently, 3 radioligands exist to image COX-2, but only one—^{11}C-6-methoxy-2-(4-(methylsulfonyl)phenyl)-*N*-(thiophen-2-ylmethyl)pyrimidin-4-amine (^{11}C-MC1)—has moved on to human studies. ^{11}C-celecoxib and ^{11}C-3-(4-methylsulfonylphenyl)-4-phenyl-5-trifluoromethyl isoxazole (^{11}C-TMI) (Supplemental Table 1) have been studied in baboons and have been shown to penetrate the blood–brain barrier, accumulate in brain, and gradually wash out (*47*). Both ^{11}C-celecoxib and ^{11}C-TMI produced a heterogeneous distribution in the brain that aligned with known COX-2 distribution (*48,49*). ^{11}C-celecoxib also had uptake in organs known to express COX-2. However, no animal models of inflammation and no human studies have yet been published for either ^{11}C-celecoxib or ^{11}C-TMI.

^{11}C-MC1 is the most recently developed radioligand with the potential to image COX-2 in human neuroinflammation. On the basis of a 2-(4-methylsulfonylphenyl)pyrimidine scaffold, ^{11}C-MC1 was found to be potent (half-maximal inhibitory concentration, ~1 nM) and selective (>1,000 fold) for COX-2 over COX-1. ^{11}C-MC1, which has a slightly high logD of 3.74, crossed the blood–brain barrier and bound to COX-2 (*20,50*). ^{11}C-MC1 lacked the sensitivity needed to measure low baseline concentrations of COX-2 in the brains of healthy rhesus macaques (*24*). In addition, the radioligand had minimal specific uptake in major organs except the ovaries and possibly the kidneys, both of which have high COX-2 expression (*24*). ^{11}C-MC1 uptake in the ovaries was blocked by inhibitors of COX-2 but not COX-1, thereby affirming its pharmacological specificity.

Because COX-2 can be rapidly upregulated by inflammation, Shrestha et al. (*6*) subsequently tested whether ^{11}C-MC1 could image upregulated COX-2 in monkey brain after intracerebral injection of the inflammagen lipopolysaccharide. In this PET study, 2 monkeys received a single lipopolysaccharide injection, and 2 monkeys received a second lipopolysaccharide injection. COX-2 binding of ^{11}C-MC1 increased after 1 and 2 lipopolysaccharide injections, and postmortem brain analysis at the gene transcript or protein level confirmed these in vivo PET results (*6*). Interestingly, the monkeys that received 2 lipopolysaccharide injections developed delayed intracerebral hemorrhages after the first lipopolysaccharide injection, and the increased uptake of ^{11}C-MC1 overlaid the hemorrhage rather than the injection site (Fig. 4). Thus, the increased uptake of ^{11}C-MC1 likely resulted from a delayed effect associated with the first lipopolysaccharide injection rather than an acute effect associated with the second lipopolysaccharide injection.

Building on this work, another study evaluated the ability of ^{11}C-MC1 to measure COX-2 concentrations in humans when concentrations were adequately elevated by peripheral inflammation. This first-in-human study (*6*) examined 2 individuals with rheumatoid arthritis (RA) and 2 healthy volunteers who were imaged with both ^{11}C-MC1 and the TSPO radioligand ^{11}C-ER176. Patients with RA were chosen because COX-2 is known to be upregulated in the affected joints of RA patients and because COX-2 gene expression is known to be upregulated in synoviocytes (*51*) and macrophages (*52*) in response to cytokine triggers. In individuals with RA, the symptomatic joints had increased ^{11}C-MC1 and ^{11}C-ER176 uptake, but ^{11}C-ER176 also showed uptake in asymptomatic joints (Fig. 5), reflecting past evidence of inflammation. ^{11}C-MC1 uptake was partially blocked by 400 mg of the preferential COX-2 inhibitor celecoxib (*6*), confirming that ^{11}C-MC1 can be used to image elevated COX-2 levels in humans. Higher oral doses of celecoxib would likely have caused complete blockade in the symptomatic joints because higher intravenous doses of celecoxib or more potent inhibitors completely blocked elevated COX-2 in monkey brain (*6*).

A preliminary study then explored whether ^{11}C-MC1 could measure COX-2 in 10 healthy human brains. In 9 of the 10 healthy participants, ^{11}C-MC1 detected specific binding in brain that could be displaced by 600 mg of celecoxib, and the binding distribution correlated with that of the gene transcript in the Allen Brain Atlas (*53*). On the basis of the Lassen plot, celecoxib occupied 72% of available COX-2 in the brain, but this specific binding was only about 20% of total uptake. This finding suggests that ^{11}C-MC1 has adequate sensitivity to measure low-density COX-2 in healthy human brain. However, because of the low specific binding in the normal brain, additional studies of individuals with neuroinflammatory disorders—who presumably would have higher densities of COX-2—are needed.

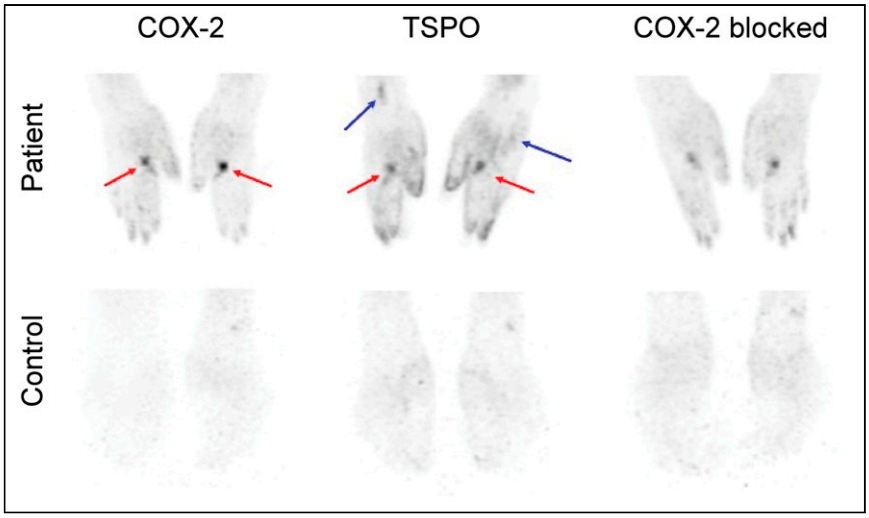

FIGURE 5. PET images of COX-2 and TSPO in a patient with RA and a healthy volunteer. The red arrows indicate symptomatic joints, and the blue arrows indicate asymptomatic joints. Increased COX-2 in the hands reflected currently symptomatic joints, whereas increased TSPO binding reflected both currently symptomatic and previously symptomatic joints. Celecoxib (400 mg orally) blocked only ~25% of the ^{11}C-MC1 uptake in the joints of the patient, confirming uptake selectivity for COX-2 compared to COX-1. Animal studies suggest that higher doses of celecoxib are required for complete blockade. Adapted from (*6*).

POTENTIAL USE OF COX-1 AND COX-2 AS BIOMARKERS

The most extensively studied biomarker of neuroinflammation in neurologic (*1*) and psychiatric (*2*) disorders has, to date, been TSPO. Thus, the potential use of COX-1 and COX-2 as biomarkers of neuroinflammation in AD and other neuropsychiatric disorders will inevitably be compared with the success and limitations of TSPO (Tables 1 and 2). As background, TSPO is concentrated in microglia and astrocytes that have been activated by various inflammatory stimuli (Table 1). AD, which generates a significant inflammatory response in the brain, is the disorder most frequently studied with TSPO radioligands. One large metaanalysis of 28 studies comprising 755 individuals (318 healthy volunteers, 168 individuals with mild cognitive impairment, and 269 with AD) found that elevated TSPO was a biomarker of disease state and disease severity (*54*), and another study found that TSPO was a biomarker of disease progression (*55*). Although these findings seem quite promising, TSPO has at least 3 clear limitations: it is not specific to a single cell type, it remains elevated for long periods, and the TSPO gene has a codominantly expressed polymorphism that affects the binding affinity of all known PET radioligands. In this context, COX imaging of neuroinflammation would have several advantages.

First, TSPO is not specific to a single cell type but, rather, is present in microglia, astroglia, and vascular endothelium. In contrast, COX-1 is located almost exclusively in microglia. Specifically, COX-1 is elevated surrounding amyloid plaques, and the elevation may reflect the induction of the amount of COX-1 in microglia. Most researchers and pharmaceutical companies are interested in biomarkers selective to microglia because that is the current target for several neuroinflammatory therapies.

Second, pharmaceutical companies seek a dynamic biomarker of pharmacological effects, and TSPO is unlikely to fulfill that role. With regard to AD drugs in particular, a novel antiinflammatory drug would require months to years to show a beneficial effect on cognition. Such long studies would benefit from identifying a biomarker—preferably in the brain but possibly in plasma—that could confirm antiinflammatory activity while assessing therapeutic efficacy. In this context, TSPO is unlikely to fulfill the role of a dynamic biomarker because TSPO levels remain elevated long after inflammation has resolved, limiting its utility as a biomarker of active inflammation (*1,2*); ultimately, however, it may prove to be a good reflection of the cumulative effects of neuroinflammation over time (*2*). In contrast, because COX-2 is rapidly upregulated and degraded, it could potentially be used as a biomarker of active neuroinflammation. In the aforementioned study of RA patients imaged for COX-2 and TSPO, COX-2 was elevated only in symptomatic joints, whereas TSPO was elevated in both symptomatic and asymptomatic joints (*6*). If replicated, these results suggest that COX-2 may be useful as a dynamic biomarker of current inflammation and be decreased by some antiinflammatory medications.

Third, the TSPO gene has a codominantly expressed polymorphism that affects the binding affinity of all known PET radioligands (*1*); thus, TSPO studies must correct or control for this polymorphism in all participants. Although no polymorphism is currently known to affect radioligand binding to COX-1 and COX-2, future studies should be alert to this possibility. Indeed,

TABLE 2
Potential Role of 3 Proteins as Biomarkers of Neuroinflammation in AD*

Biomarker	COX-1	COX-2	TSPO
Disease state	Yes, numerous microglia	Visual increase in neurons, no quantitation	Yes
Disease severity	?	?	Yes
Disease progression	?	?	Possibly
Dynamic, reflecting pharmacological action	?	Periphery, yes; brain, possibly	Unlikely: remains elevated for long time

*Because COXs have not yet been imaged in AD, their biomarker potential is estimated from postmortem studies. The utility of TSPO is based on numerous PET studies.

both COX-1 and COX-2 genes have several encoding polymorphisms (*56*) that may contribute to functional differences (*57,58*). For instance, COX-1 polymorphisms may modulate the response of platelets to aspirin (*59,60*), and COX-2 polymorphisms may be associated with interindividual variability in gene expression and response to COX-2 inhibitors (*61,62*).

CONCLUSION

Neuropathological studies in AD show elevated COX-1 in microglia surrounding amyloid plaques as well as elevated COX-2 in neurons. Although these results are based largely on the semiquantitative method of immunohistochemistry, both isozymes may be biomarkers of disease state—that is, the presence of neuroinflammation. Steroids block or reverse the elevation of COX-2 in peripheral inflammation and in neurons after electroconvulsive shock. Thus, in addition to being a biomarker of disease state, COX-2 may also be a dynamic biomarker of pharmacological action (i.e., of antiinflammatory action).

Recently developed radioligands show promise in animal models and healthy humans to selectively image both COX-1 and COX-2. ^{11}C-PS13 can quantify the distribution of COX-1 in the periphery and brain and is pharmacologically specific, based on blockade in humans by COX-1 versus COX-2 preferential inhibitors. ^{11}C-MC1 can quantify COX-2 upregulation in monkey brain after lipopolysaccharide injection and has the sensitivity to measure the low density of this target in healthy human brain. In our opinion, both radioligands are well suited to study as biomarkers of disease state or as dynamic biomarkers of pharmacological action in neurodegenerative diseases such as AD.

DISCLOSURE

This study was funded by the Intramural Research Program of the National Institute of Mental Health, National Institutes of Health (ZIAMH002795, ZIAMH002852, and ZIAMH002793). No other potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENT

We thank Paul Parcon, MD, for helpful insights on the use of nonsteroidal antiinflammatory drugs in AD.

REFERENCES

1. Kreisl WC, Kim M-J, Coughlin JM, Henter ID, Owen DR, Innis RB. PET imaging of neuroinflammation in neurological disorders. *Lancet Neurol.* 2020;19:940–950.
2. Meyer JH, Cervenka S, Kim M-J, Kreisl WC, Henter ID, Innis RB. Neuroinflammation in psychiatric disorders: PET imaging and promising new targets. *Lancet Psychiatry.* 2020;7:1064–1074.
3. Schrör K. Aspirin and platelets: the antiplatelet action of aspirin and its role in thrombosis treatment and prophylaxis. *Semin Thromb Hemost.* 1997;23:349–356.
4. Yu Y, Ricciotti E, Scalia R, et al. Vascular COX-2 modulates blood pressure and thrombosis in mice. *Sci Transl Med.* 2012;4:132ra54.
5. Hoozemans JJ, Rozemuller AJ, Janssen I, De Groot CJ, Veerhuis R, Eikelenboom P. Cyclooxygenase expression in microglia and neurons in Alzheimer's disease and control brain. *Acta Neuropathol (Berl).* 2001;101:2–8.
6. Shrestha S, Kim MJ, Eldridge M, et al. PET measurement of cyclooxygenase-2 using a novel radioligand: upregulation in primate neuroinflammation and first-in-human study. *J Neuroinflammation.* 2020;17:140.
7. Yasojima K, Schwab C, McGeer EG, McGeer PL. Distribution of cyclooxygenase-1 and cyclooxygenase-2 mRNAs and proteins in human brain and peripheral organs. *Brain Res.* 1999;830:226–236.
8. Murakami M, Kudo I. Recent advances in molecular biology and physiology of the prostaglandin E2-biosynthetic pathway. *Prog Lipid Res.* 2004;43:3–35.
9. Ghazanfari N, van Waarde A, Dierckx RAJO, Doorduin J, de Vries EFJ. Is cyclooxygenase-1 involved in neuroinflammation? *J Neurosci Res.* 2021;99:2976–2998.
10. Fiebich BL, Mueksch B, Boehringer M, Hüll M. Interleukin-1beta induces cyclooxygenase-2 and prostaglandin E_2 synthesis in human neuroblastoma cells: involvement of p38 mitogen-activated protein kinase and nuclear factor-kappaB. *J Neurochem.* 2000;75:2020–2028.
11. Yamagata K, Andreasson KI, Kaufmann WE, Barnes CA, Worley PF. Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. *Neuron.* 1993;11:371–386.
12. Felsky D, Roostaei T, Nho K, et al. Neuropathological correlates and genetic architecture of microglial activation in elderly human brain. *Nat Commun.* 2019;10:409.
13. Lukiw WJ, Bazan NG. Cyclooxygenase 2 RNA message abundance, stability, and hypervariability in sporadic Alzheimer neocortex. *J Neurosci Res.* 1997;50:937–945.
14. Kang Y-J, Mbonye UR, DeLong CJ, Wada M, Smith WL. Regulation of intracellular cyclooxygenase levels by gene transcription and protein degradation. *Prog Lipid Res.* 2007;46:108–125.
15. Li Q, Barres BA. Microglia and macrophages in brain homeostasis and disease. *Nat Rev Immunol.* 2018;18:225–242.
16. Choi S-H, Aid S, Caracciolo L, et al. Cyclooxygenase-1 inhibition reduces amyloid pathology and improves memory deficits in a mouse model of Alzheimer's disease. *J Neurochem.* 2013;124:59–68.
17. Takashima-Hirano M, Shukuri M, Takashima T, et al. General method for the ^{11}C-labeling of 2-arylpropionic acids and their esters: construction of a PET tracer library for a study of biological events involved in COXs expression. *Chemistry.* 2010;16:4250–4258.
18. Ohnishi A, Senda M, Yamane T, et al. Exploratory human PET study of the effectiveness of ^{11}C-ketoprofen methyl ester, a potential biomarker of neuroinflammatory processes in Alzheimer's disease. *Nucl Med Biol.* 2016;43:438–444.
19. Kim M-J, Lee JH, Juarez Anaya F, et al. First-in-human evaluation of [^{11}C]PS13, a novel PET radioligand, to quantify cyclooxygenase-1 in the brain. *Eur J Nucl Med Mol Imaging.* 2020;47:3143–3151.
20. Singh P, Shrestha S, Cortes-Salva MY, et al. 3-substituted 1,5-diaryl-1*H*-1,2,4-triazoles as prospective PET radioligands for imaging brain COX-1 in monkey. Part 1: synthesis and pharmacology. *ACS Chem Neurosci.* 2018;9:2610–2619.
21. Pike VW. PET radiotracers: crossing the blood-brain barrier and surviving metabolism. *Trends Pharmacol Sci.* 2009;30:431–440.
22. Pike VW. Considerations in the development of reversibly binding PET radioligands for brain imaging. *Curr Med Chem.* 2016;23:1818–1869.
23. Taddei C, Morse CL, Kim M-J, et al. Synthesis of [^{18}F]PS13 and evaluation as a PET radioligand for cyclooxygenase-1 in monkey. *ACS Chem Neurosci.* 2021;12:517–530.
24. Kim MJ, Shrestha SS, Cortes M, et al. Evaluation of two potent and selective PET radioligands to image COX-1 and COX-2 in rhesus monkeys. *J Nucl Med.* 2018;59:1907–1912.
25. Manly L, Kim M-J, Montero Santamaria J, et al. Aspirin blockade of COX-1 in human organs is reduced by plasma as shown by PET imaging and blood assays with ^{11}C-PS13 [abstract]. *J Nucl Med.* 2021;62(suppl 1):115.
26. Laube M, Kniess T, Pietzsch J. Radiolabeled COX-2 inhibitors for non-invasive visualization of COX-2 expression and activity: a critical update. *Molecules.* 2013;18:6311–6355.
27. Pacelli A, Greenman J, Cawthorne C, Smith G. Imaging COX-2 expression in cancer using PET/SPECT radioligands: current status and future directions. *J Labelled Comp Radiopharm.* 2014;57:317–322.
28. Giovacchini G, Chang MC, Channing MA, et al. Brain incorporation of [^{11}C]arachidonic acid in young healthy humans measured with positron emission tomography. *J Cereb Blood Flow Metab.* 2002;22:1453–1462.
29. Rapoport SI. In vivo approaches to quantifying and imaging brain arachidonic and docosahexaenoic acid metabolism. *J Pediatr.* 2003;143(suppl):S26–S34.
30. Nolting DD, Nickels M, Tantawy MN, et al. Convergent synthesis and evaluation of ^{18}F-labeled azulenic COX2 probes for cancer imaging. *Front Oncol.* 2013;2:207.
31. Lebedev A, Jiao J, Lee J, et al. Radiochemistry on electrodes: synthesis of an ^{18}F-labelled and in vivo stable COX-2 inhibitor. *PLoS One.* 2017;12:e0176606.
32. Chang CW, Yeh CN, Chung YH, et al. Synthesis and evaluation of ortho-[^{18}F] fluorocelecoxib for COX-2 cholangiocarcinoma imaging. *Drug Des Devel Ther.* 2018;12:1467–1478.
33. Prabhakaran J, Underwood M, Zanderigo F, et al. Radiosynthesis and in vivo evaluation of [^{11}C]MOV as a PET imaging agent for COX-2. *Bioorg Med Chem Lett.* 2018;28:2432–2435.
34. Carpinelli A, Rainone P, Belloli S, et al. Radiosynthesis and preclinical evaluation of ^{11}C-VA426, a cyclooxygenase-2 selective ligand. *Contrast Media Mol Imaging.* 2019;2019:5823261.
35. Elie J, Vercouillie J, Arlicot N, et al. Design of selective COX-2 inhibitors in the (aza)indazole series: chemistry, in vitro studies, radiochemistry and evaluations in rats of a [^{18}F] PET tracer. *J Enzyme Inhib Med Chem.* 2019;34:1–7.

36. Prabhakaran J, Underwood MD, Parsey RV, et al. Synthesis and in vivo evaluation of [^{18}F]-4-[5-(4-methylphenyl)-3-(trifluoromethyl)-^{1}H-pyrazol-1-yl]benzenesulfonamide as a PET imaging probe for COX-2 expression. *Bioorg Med Chem.* 2007;15:1802–1807.
37. Toyokuni T, Kumar JS, Walsh JC, et al. Synthesis of 4-(5-[^{18}F]fluoromethyl-3-phenylisoxazol-4-yl)benzenesulfonamide, a new [^{18}F]fluorinated analogue of valdecoxib, as a potential radiotracer for imaging cyclooxygenase-2 with positron emission tomography. *Bioorg Med Chem Lett.* 2005;15:4699–4702.
38. Fujisaki Y, Kawamura K, Wang WF, et al. Radiosynthesis and in vivo evaluation of ^{11}C-labeled 1,5-diarylpyrazole derivatives for mapping cyclooxygenases. *Ann Nucl Med.* 2005;19:617–625.
39. Tanaka M, Fujisaki Y, Kawamura K, et al. Radiosynthesis and evaluation of ^{11}C-labeled diaryl-substituted imidazole and indole derivatives for mapping cyclooxygenase-2. *Biol Pharm Bull.* 2006;29:2087–2094.
40. Yamamoto Y, Toyohara J, Ishiwata K, et al. ^{11}C-labeled analogs of indomethacin esters and amides for brain cyclooxygenase-2 imaging: radiosynthesis, in vitro evaluation and in vivo characteristics in mice. *Chem Pharm Bull (Tokyo).* 2011;59:938–946.
41. Uddin MJ, Crews BC, Ghebreselasie K, et al. Fluorinated COX-2 inhibitors as agents in PET imaging of inflammation and cancer. *Cancer Prev Res (Phila).* 2011;4:1536–1545.
42. Litchfield M, Wuest M, Glubrecht D, Wuest F. Radiosynthesis and biological evaluation of [(^{18})F]triacoxib: a new radiotracer for PET imaging of COX-2. *Mol Pharm.* 2020;17:251–261.
43. Tietz O, Wuest M, Marshall A, et al. PET imaging of cyclooxygenase-2 (COX-2) in a pre-clinical colorectal cancer model. *EJNMMI Res.* 2016;6:37.
44. Tian H, Lee Z. Synthesis of ^{18}F-labelled cyclooxygenase-2 (COX-2) inhibitor as a potential PET imaging agent. *J Labelled Comp Radiopharm.* 2006;49:583–593.
45. Majo VJ, Prabhakaran J, Simpson NR, Van Heertum RL, Mann JJ, Kumar JSD. A general method for the synthesis of aryl [^{11}C]methylsulfones: potential PET probes for imaging cyclooxygenase-2 expression. *Bioorg Med Chem Lett.* 2005;15:4268–4271.
46. Wüst FR, Höhne A, Metz P. Synthesis of ^{18}F-labelled cyclooxygenase-2 (COX-2) inhibitors via Stille reaction with 4-[^{18}F]fluoroiodobenzene as radiotracers for positron emission tomography (PET). *Org Biomol Chem.* 2005;3:503–507.
47. Kumar JSD, Zanderigo F, Prabhakaran J, Rubin-Falcone H, Parsey RV, Mann JJ. In vivo evaluation of [^{11}C]TMI, a COX-2 selective PET tracer, in baboons. *Bioorg Med Chem Lett.* 2018;28:3592–3595.
48. Kaufmann WE, Andreasson KI, Isakson PC, Worley PF. Cyclooxygenases and the central nervous system. *Prostaglandins.* 1997;54:601–624.
49. Breder CD, Dewitt D, Kraig RP. Characterization of inducible cyclooxygenase in rat brain. *J Comp Neurol.* 1995;355:296–315.
50. Cortes-Salva MY, Shrestha S, Singh P, et al. 2-(4-methylsulfonylphenyl)pyrimidines as prospective radioligands for imaging cyclooxygenase-2 with PET-synthesis, triage, and radiolabeling. *Molecules.* 2018;23:2850.
51. Crofford LJ. The role of COX-2 in rheumatoid arthritis synovial tissues [abstract]. *Arthritis Res.* 2000;1(suppl 1):S30.
52. Simon LS. Role and regulation of cyclooxygenase-2 during inflammation. *Am J Med.* 1999;106(suppl):37S–42S.
53. Yan X, Zhang A, Zoghbi S, et al. ^{11}C-MC1 has adequate sensitivity to measure low density cyclooxygenase 2 (COX-2) in healthy human brain [abstract]. *J Nucl Med.* 2021;62(suppl 1):103.
54. Bradburn S, Murgatroyd C, Ray N. Neuroinflammation in mild cognitive impairment and Alzheimer's disease: a meta-analysis. *Ageing Res Rev.* 2019;50:1–8.
55. Kreisl WC, Lyoo CH, Liow JS, et al. ^{11}C-PBR28 binding to translocator protein increases with progression of Alzheimer's disease. *Neurobiol Aging.* 2016;44:53–61.
56. Ulrich CM, Bigler J, Potter JD. Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils and pharmacogenetics. *Nat Rev Cancer.* 2006;6:130–140.
57. Lee CR, Bottone FG, Krahn JM, et al. Identification and functional characterization of polymorphisms in human cyclooxygenase-1 (PTGS1). *Pharmacogenet Genomics.* 2007;17:145–160.
58. Bruno A, Tacconelli S, Patrignani P. Variability in the response to non-steroidal anti-inflammatory drugs: mechanisms and perspectives. *Basic Clin Pharmacol Toxicol.* 2014;114:56–63.
59. Halushka MK, Walker LP, Halushka PV. Genetic variation in cyclooxygenase 1: effects on response to aspirin. *Clin Pharmacol Ther.* 2003;73:122–130.
60. Maree AO, Curtin RJ, Chubb A, et al. Cyclooxygenase-1 haplotype modulates platelet response to aspirin. *J Thromb Haemost.* 2005;3:2340–2345.
61. Fries S, Grosser T, Price TS, et al. Marked interindividual variability in the response to selective inhibitors of cyclooxygenase-2. *Gastroenterology.* 2006;130:55–64.
62. Lee Y-S, Kim H, Wu T-X, Wang X-M, Dionne RA. Genetically mediated interindividual variation in analgesic responses to cyclooxygenase inhibitory drugs. *Clin Pharmacol Ther.* 2006;79:407–418.
63. Owen DR, Narayan N, Wells L, et al. Pro-inflammatory activation of primary microglia and macrophages increases 18 kDa translocator protein expression in rodents but not humans. *J Cereb Blood Flow Metab.* 2017;37:2679–2690.