Monoamine Oxidase: Radiotracer Chemistry and Human Studies

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Abstract

Monoamine oxidase (MAO) oxidizes amines from both endogenous and exogenous sources thereby regulating the concentration of neurotransmitter amines such as serotonin, norepinephrine and dopamine as well as many xenobiotics. MAO inhibitor drugs are used in the treatment of Parkinson's disease and in depression stimulating the development of radiotracer tools to probe the role of MAO in normal human biology and in disease. Over the past 30 since the first radiotracers were developed and the first PET images of MAO in humans were carried out, PET studies of brain MAO in healthy volunteers and in patients have identified different variables which have contributed to different MAO levels in brain and in peripheral organs. MAO radiotracers and PET have also been used to study the current and developing MAO inhibitor drugs including the selection of doses for clinical trials. In this article, we describe (1) the development of MAO radiotracers; (2) human studies including the relationship of brain MAO levels to genotype, personality, neurological and psychiatric disorders; (3) examples of the use of MAO radiotracers in drug research and development. We will conclude with outstanding needs to improve the radiotracers which are currently used and possible new applications.

Introduction

The processes that control the concentrations of neurotransmitters and other physiologically active compounds such as dietary amines, drugs and other substances are of particular importance to human health because of their regulatory and protective effects. Monoamine oxidase (MAO) is a flavin-containing enzyme, which formally oxidizes neurotransmitter amines,

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other biogenic amines and xenobiotics thereby regulating biogenic amine tone.¹ The reaction requires oxygen and is accompanied by the stoichiometric production of a corresponding imine (which is usually oxidized further), an amine and hydrogen peroxide, which has been implicated in both oxidative stress and cell signaling (Figure 1).² The rate limiting step of the MAO reaction is stereoselective, abstraction of the α hydrogen from the substrate and MAO-catalyzed oxidation shows a robust deuterium isotope effect.³

MAO occurs in the outer mitochondrial membrane and is present in virtually all brain regions and in most peripheral organs⁴. There are two different isoforms, monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B), which are different gene products and have different substrate and inhibitor selectivities.^{1,5} MAO-A specifically breaks down serotonin and norepinephrine and tyramine and is irreversibly inhibited by clorgyline while MAO-B breaks down the trace amine, phenylethylamine, and is inhibited by L-deprenyl (selegiline). Both subtypes oxidize dopamine.

Medical interest in MAO dates back to the 1950's with the serendipitous discovery that iproniazide, a drug used to treat tuberculosis (TB), elevated the mood of depressed patients (for review see ⁶). It was later discovered that, in addition to inhibiting the replication of the TB bacillus, iproniazid also inhibited MAO.⁷ Though the MAO inhibitors (MAOIs) became the first effective drugs for the treatment of depression, the early MAOIs such as phenelzine, clorgyline and tranylcypromine were non-selective and irreversibly inhibited both MAO isoforms in the brain and also in the gut. This led to severe and potentially lethal hypertensive reactions in patients ingesting foods containing large amounts of the MAO-A substrate tyramine, which acts as a false neurotransmitter displacing norepinephrine from nerve terminals and producing hypertension.^{8, 9}

The biological and medical importance of MAO has stimulated a significant effort in developing radiotracers to investigate its activity in humans. In this article, we will highlight (1) the development of MAO radiotracers; (2) human studies including the relationship of brain MAO levels to genotype, personality, neurological and psychiatric disorders; (3) MAO and drug research. We note that there are earlier reviews on MAO radiotracers and PET^{10, 11} and recent reviews of MAO-A and MAO-B substrates and radiotracers^{12, 13}.

Development of MAO Radiotracers

Similar to all targeted radiotracers for PET imaging and quantification, the challenge to the development of radiotracers for MAO has been in the design of a chemical compound whose kinetics and distribution reflect a single biochemical process and whose distribution parallels the known regional concentration of the specific MAO isoform in different brain regions and in peripheral organs. To date many different radiotracers and approaches have been developed for imaging MAO. The first labeled compounds specifically investigated for measuring MAO activity *in vivo* were [¹¹C]octylamine and other labeled aliphatic amines of different chain lengths specifically for studies of lung function.^{14, 15, 16} These compounds are metabolized by MAO, eventually to ¹¹CO₂. Since the loss of ¹¹CO₂ reflects both MAO oxidation and other processes occurring in the various organs, other radiotracers were subsequently developed to achieve the specificity required for PET studies. To date only a handful of radiotracers have survived the validation process and been applied as investigative tools to study the neurobiology of MAO-A and MAO-B in humans and for evaluating drug action and dosing. We have classified MAO radiotracers into two groups: irreversibly trapped radiotracers, which include suicide enzyme inactivators and labeled substrates and reversibly binding radiotracers (Figure 2).

Irreversible inhibitors and substrates: [^{11}C]clorgyline (**1**) and [^{11}C]L-deprenyl (**3**) and their deuterium labeled counterparts [^{11}C]clorgyline-D2 (**2**) and [^{11}C]L-deprenyl-D2 (**4**) were first radiolabeled through a collaboration with Professor Bengt Langstrom who had developed a rapid method for labeled methyl iodide and introduced the method to Brookhaven National Laboratory.^{17, 18} These molecules are suicide enzyme inactivators which forms a covalent attachment to the enzyme (or cofactor) during the normal catalytic step which involves the cleavage of the C-H bond on the methylene carbon of the propargyl group (Figure 3). Crystal structures of clorgyline and L-deprenyl covalently bound to recombinant human MAO-A and MAO-B were recently reported.¹⁹

In principle, a PET isotope labeled suicide inactivator will result in the covalent labeling of the catalytically active enzyme molecules and reflect the concentration of these molecules when administered *in vivo* and imaged with PET (Figure 3). Initial studies in mice with [^{11}C]clorgyline and [^{11}C]L-deprenyl showed their specificity for MAO-A and MAO-B respectively and that the major fraction of the C-11 became bound to protein of MAO 58,000 (corresponding to the MW of MAO).²⁰ PET studies in the baboon with [^{11}C]L-deprenyl followed showing that the radiolabel rapidly accumulated in the brain, could be blocked by the administration of unlabeled L-deprenyl and showed a robust deuterium isotope effect with [^{11}C]L-deprenyl-D2.²¹ Interestingly, even though [^{11}C]clorgyline showed selectivity for brain MAO A in the mouse²⁰ it did not bind to MAO A in the baboon or rhesus monkey brain suggesting that the characteristics of the clorgyline binding site in the MAO-A protein differ for the two species.^{22, 23}

The first human studies with [^{11}C]clorgyline and [^{11}C]L-deprenyl showed that the binding of both [^{11}C]clorgyline and [^{11}C]L-deprenyl could be blocked by the administration of the non-selective MAO inhibitors phenelzine and L-deprenyl and that [^{11}C]L-deprenyl binding was stereoselective.²⁴ Subsequently [^{11}C]L-deprenyl and [^{11}C]L-deprenyl- α,α -D2 were compared in healthy human volunteers and showed a robust deuterium isotope effect and provided a means of selectively controlling the rate of trapping of tracer in brain and enhancing sensitivity of binding to changes in MAO (Figure 4).²⁵ Even though [^{11}C]clorgyline also showed a robust deuterium isotope effect in humans, [^{11}C]clorgyline-D2 proved to be a poor PET radiotracer because deuterium substitution reduced the binding in MAO-B containing regions revealing high non-specific binding of this lipophilic molecule in white matter regions and reducing signal to noise.^{26, 27}

PET studies with [^{11}C]clorgyline and [^{11}C]L-deprenyl paired with their deuterium labeled counterparts proved to be very useful for mapping and validating the distribution of MAO-A and MAO-B in peripheral organs in humans (Figure 5).^{28, 29} MAO-A and MAO-B were visualized in those organs showing a reduction in binding with deuterium substitution thus avoiding the use of MAO inhibitor drugs to determine binding specificity. The selectivity [^{11}C]clorgyline for brain MAO-A vs MAO-B was also shown in humans by the lack of an effect of L-deprenyl treatment on [^{11}C]clorgyline binding.³⁰

For quantification of MAO-A or MAO-B activity, PET time-activity data for [^{11}C]clorgyline or [^{11}C]L-deprenyl-D2 from different brain regions and time-activity data in arterial plasma are used to calculate the model term λk_3 which is related to the concentration of catalytically active MAO-A molecules.³¹ [^{11}C]Clorgyline and [^{11}C]L-deprenyl-D2 outcome measures (λk_3) in human brain were recently shown to be proportional to MAO-A protein content.³² Thus, suicide enzyme inactivators labeled with positron emitters could be used to quantitate the distribution and kinetic characteristics of MAO in human brain structures. These

studies served as a springboard for the first measurements of the regional distribution of MAO-A and B in brain and in peripheral organs in many clinical studies and to measure target engagement by drugs and xenobiotics (*vide infra*).

MAO-B has also been imaged with PET using carbon-11 labeled N,N-[¹¹C]dimethylphenylethylamine (DMPEA, Figure 2).³³ DMPEA is oxidized by MAO-B to produce [¹¹C]dimethylamine which is charged at physiological pH and trapped within the brain. Mechanistic studies including the demonstration of a deuterium isotope effect and PET studies in the monkey with DMPEA labeled in different positions validated its use as a MAO tracer and showed that carbon-11 was trapped in MAO-B rich regions in the human brain.^{34, 35} To our knowledge, no human studies with DMPEA have been reported following these initial studies.

Reversible subtype-selective radiotracers: While the irreversibly binding radiotracers have provided good correlations with MAO protein content³², they are subject to flow limitations when they bind very avidly to the enzyme and when blood flow is low. In the case of [¹¹C]L-deprenyl, this problem was mitigated by deuterium substitution which significantly reduced the rate of binding. However, another approach was to develop *reversible* inhibitors of MAO A.³⁶

Harmine, a harmala alkaloid belonging to the beta-carboline family of compounds, is a potent and selective MAO-A inhibitor with an apparent K_D of 2.0 nM. It served as a template for development of the reversibly binding subtype selective radiotracer [¹¹C]harmine (**7**) whose binding was shown to be inhibited by nanomolar concentrations of the MAO-A inhibitors clorgyline, esuprone, brofaromine and Ro41-1049.³⁷ Preclinical studies in monkeys showed reversible binding and MAO-A selectivity supporting its development for human studies.³⁷ Initial human studies with [¹¹C]harmine in healthy volunteers showed blockade of binding by the MAO-A inhibitor drugs esuprone and moclobemide.³⁸ Kinetic modeling of [¹¹C]harmine binding to MAO-A sites in the human brain with placebo and after treatment with clinical doses of the MAO-A inhibitor moclobemide showed that estimates of specifically bound radioligand distribution volume (DV) using an unconstrained 2-compartment model were stable and correlated well with the known regional distribution of MAO A.³⁹ [¹¹C]Harmine binding in human brain has also been shown to be proportional to MAO-A protein content.³² Interestingly brain MAO-A availability as measured with [¹¹C]harmine, changes with tryptophan depletion (a precursor for serotonin) suggesting that MAO-A maintains monoamine neurotransmitter homeostasis by rapidly compensating for fluctuating monoamine levels.⁴⁰

Befloxatone, a competitive reversible inhibitor of MAO-A has been labeled with carbon-11 and evaluated for brain PET imaging.⁴¹ The reversibility and MAO-A selectivity of [¹¹C]befloxatone (**8**) was demonstrated in the baboon by blockade with moclobemide but not the MAO-B-selective inhibitor lazabemide and by displacement of carbon-11 in brain by moclobemide.⁴² A recent kinetic analysis of PET data for [¹¹C]befloxatone in healthy volunteers showed that binding of [¹¹C]befloxatone to MAO-A can be quantified using an arterial input function and a two-compartment model.⁴³

Efforts to develop C-11 labeled MAO-A inhibitors continue. For example, (R)-(-)- and (S)-(+)-1-(1-methyl-1 H-pyrrol-2-yl)-2-phenyl-2-(1-pyrrolidiny)ethanone (ROMAO) were radiolabeled with carbon-11 based on reports that they have high selectivity and high potency for MAO-A (3.5 and 9.5 nM respectively).^{44, 45} Images from pig brain were qualitatively similar to those of [¹¹C]harmine.

In addition to the reversible MAO-A radiotracer which have been evaluated in humans, reversible carbamate MAO-B inhibitor [¹¹C]SL25.1188 (**9**) was synthesized via [¹¹C]phosgene as a C-11 precursor and although its in vivo binding properties were promising for imaging brain

MAO B, the requirement for [^{11}C]phosgene as a labeling precursor limited its utility.^{46, 47} However, this limitation was recently overcome by developing a direct $^{11}\text{CO}_2$ fixation method thus avoiding the need for [^{11}C]phosgene (Figure 6).⁴⁸ Kinetic modeling of [^{11}C]SL25.1188 in human brain with high resolution PET showed high brain uptake and the regional V_T values correlated with postmortem measures of brain MAO B protein levels.⁴⁹ This represents the addition of a reversibly binding MAO-B radiotracer to the armamentarium of radiotracers for human MAO-B studies.

An important new study reported the measurement of the distribution of MAO proteins by immunoblotting in autopsied human brain sections and examined the correlation of these values with PET outcome measures for [^{11}C]clorgyline (MAO-A), [^{11}C]L-deprenyl-D2 (MAO-B), [^{11}C]harmine (MAO-A) and [^{11}C]befloxatone (MAO-A).³² They reported good correlations ($R > 0.8$) for all except [^{11}C]befloxatone. However, when the correlation was reinvestigated using regional MAO-A values from a recent modeling study,⁴³ [^{11}C]befloxatone distribution was well correlated to monoamine oxidase A protein levels in the human brain.⁵⁰ It is important to note that, even though the correlations were good, these tracers all underestimated contrast by ~ 2-fold. Nonetheless, though imperfect, these radiotracers have proven to provide some remarkable new insights on human neurobiology and have emerged as important new tools in understanding drug mechanisms.

Fluorine-18 Labeled MAO Radiotracers

Although the 20.4 minute half-life of carbon-11 holds the advantage of enabling PET studies at 2 hour intervals in the same day in the same individual, fluorine-18 labeled MAO tracers have been under development for many years to extend the scanning period and potentially to distribute the radiotracer to distant user sites (Figure 7). For example, in 1990, (R)-(-) and (S)-(+)-4-fluorodeprenyl were labeled with carbon-11 and baboon studies showed the expected stereoselective binding of the R-(-)-enantiomer demonstrating that fluorine-substitution in the 4-position did not compromise the activity of the parent compound.⁵¹ Subsequent F-18 labeling via multistep nucleophilic aromatic substitution produced D,L-4-[^{18}F]fluorodeprenyl (**10**). However, the multi-step synthesis and requirement for chiral resolution detracted from the approach.⁵² Seeking an F-18 labeled MAO-B radiotracer that was easier to synthesize, Mukherjee reported the radiosynthesis of N-(6- ^{18}F -fluorohexyl)-N-methylpropargylamine (**11**).⁵³ Although *in vitro* and *in vivo* studies in mice showed high affinity for MAO-B (6.8 nM) and high selectivity for MAO-B vs MAO-A, a more recent study shows the presence of a radiolabeled metabolite which enters the brain.⁵⁴

The radiofluorination of L-deprenyl by incorporating the fluorine-18 in different positions on the side chain rather than the ring was recently revisited. N-[(2S)-1-[^{18}F]fluoro-3-phenylpropan-2-yl]-N-methylprop-2-yn-1-amine (**12**), proved to have MAO-B selectivity and high affinity (IC_{50} : 171 nM).⁵⁵ PET studies in monkeys showed appropriate regional distribution selectivity for MAO-B.⁵⁶ Continuing on this path, this group also developed and evaluated a F-18 labeled derivative rasagiline (1S,2S)-2-fluoro-N-(prop-2-yn-1-yl)indan-1-amine (rasagiline) (**13**, **14**), labeled on the indane ring.⁵⁷ Monkey studies indicated the brain entry of a labeled metabolite at later times, a problem that was addressed by deuterium substitution in the methylene carbon of the propargyl group, which reduced the rate of metabolism.⁵⁸ In another example, (S)-1-[^{18}F]fluoro-N,4-dimethyl-N-(prop-2-ynyl)pentan-2-amine (**15**) was synthesized and showed appropriate selectivity for MAO-B, regional distribution

in post-mortem human brain and brain uptake and regional distribution in monkey brain in vivo.⁵⁹

In earlier studies, N-[3-(2',4'-dichlorophenoxy)-2-¹⁸F-fluoropropyl]-N-methylpropargylamine (¹⁸F-fluoroclogyline) was developed as a potential F-18 labeled radiotracer from MAO-A. In vitro measures showed high affinity for MAO-A (39 nM) and selectivity for MAO-A vs MAO-B.⁶⁰ Other ring labeled F-18 derivatives of clogyline have been developed by replacing either the 2-chloro or the 4-chloro substituent with F-18 using [¹⁸F]fluorodestannylation with [¹⁸F]acetylhypofluorite to produce low specific activity labeled compounds.^{61, 62}

To our knowledge, no human studies with the F-18 labeled MAO inhibitors have been reported though we note that there are promising candidates for translation.

Human Studies

Brain MAO-A, MAOA genotype and personality

The genetic deletion of MAO-A produces an aggressive phenotype across species.^{63,64, 65} Interest in the relationship between the MAOA gene and behavior and aggression intensified by the identification of a common polymorphism in the promoter region of the MAOA gene that has two common alleles (4-repeat and 3-repeat) which are commonly referred to as high and low MAOA genotypes defined by their significantly different transcriptional activities.⁶⁶ One study, in particular, reported that maltreated male children with the low MAO A genotype were more likely to develop antisocial behavior than those with the high MAO A genotype.⁶⁷ This led to the hypothesis that MAOA genotype may serve as a marker of MAOA gene function and to many studies of the association between high and low MAOA genotype and vulnerability to environmental stressors in humans.⁶⁸ *We note that in all PET studies of brain MAO-A levels, smokers were excluded due to the MAO-A inhibitory actions of tobacco smoke (vide infra).*

PET and specific radiotracers for MAO-A provided the opportunity to determine whether high and low MAO A genotypes are associated with high and low levels of brain MAO-A, thereby potentially influencing the balance of neurotransmitters in the brain and influencing behavior. However PET studies with [¹¹C]clogyline in 38 healthy, adult non-smoking male volunteers who were genotyped for the MAOA polymorphism revealed that, even though the baseline MAO-A level varies by a factor of two, the polymorphism itself does not contribute to differences in baseline brain MAO A activity (Figure 8).⁶⁹ The absence of a relationship between MAOA genotype and brain MAO-A levels has been replicated in two more independent cohorts (E. Shumay, personal communication).

Though there was no difference in brain MAO-A levels for the high and low MAOA genotypes, the rather large inter-subject variability in brain MAO A activity, even in a group well matched for age, education and intelligence, stimulated the investigation of other factors which might contribute to individual differences in brain MAO A activity. Since DNA methylation silences gene expression, the hypothesis that site-specific methylation of the core MAOA promoter may be associated with different baseline brain MAO-A levels was tested in 33 non-smokers who were also genotyped. The study revealed a robust association of the regional and CpG site-specific methylation of the core MAOA promoter in DNA extracted from lymphocytes with brain MAO A levels as measured with [¹¹C]clogyline. This suggests that methylation of the MAOA promoter, which might be imprinted early on development, contributes to inter-individual differences in brain MAO-A (Figure 9).⁷⁰ Since the genotype

itself may modulate levels of DNA methylation⁷¹ this might be a mechanism by which environmental factors differentially influence MAOA gene expression.

Because the genetic deletion of MAO-A produces aggressive phenotypes across species, the prediction that MAO-A activity in the brain, rather than MAOA genotype, would be associated with measures of trait aggression was tested. PET measures with [¹¹C]clorgyline in 27 healthy male volunteers revealed that brain MAO-A levels were inversely associated with a trait measure of aggression (but not with other personality traits) such that the lower the MAO A activity in cortical and subcortical brain regions, the higher the self-reported aggression (in both MAOA genotype groups) (Figure 10).⁷² These findings highlight the involvement of brain MAO-A level as a neurochemical target with clinical implications for the treatment of aberrant aggression. They were also confirmed in another study with [¹¹C]harmine which reported a correlation between angry-hostility and brain MAO A and also extended the results by reporting a positive correlation between brain MAO-A levels and deliberation.⁷³ These authors propose a continuum describing the relationship between brain MAO A levels and personality with deliberate/thoughtful characteristics contrasting with aggressive/impulsive characteristics.

Brain MAO, cigarette smoking, depression and alcohol dependence

Several early clinical studies analyzing platelet MAO B and peripheral markers of MAO-A reported evidence that smokers had reduced MAO-B and MAO-A. At the time it was not known if this was due to MAO inhibition by substances in tobacco smoke, or to low rates of MAO synthesis in smokers or whether low MAO individuals were more vulnerable to smoking (for review see⁷⁴). Since these reports were based on peripheral measures of MAO-A activity, it was also not known if brain MAO was also lower in smokers. PET studies with [¹¹C]clorgyline and [¹¹C]L-deprenyl-D2 were the first to document low brain MAO A and B in smokers relative to nonsmokers and former smokers with average reductions of brain MAO-A and MAO-B of 28% and 40% respectively (Figure 11).^{75, 76} Later PET studies confirmed early reports that nicotine does not inhibit brain MAO-B⁷⁷, that smoking a single cigarette does not produce a measurable change in brain MAO-B in nonsmokers and that an overnight cigarette abstinence for smokers does not produce a measurable recovery of brain MAO-B activity.^{78, 79} Widespread inhibition of cortical MAO-A in smokers was recently replicated with [¹¹C]befloxatone and PET and this study reported an even greater average inhibition of MAO A (~ 60%) in a group of 7 smokers and 6 healthy volunteers.⁸⁰

A comparison of MAO-A activity in non-smokers and smokers in heart, lungs, kidneys, revealed significantly lower lung MAO-A in smokers using PET and the [¹¹C]clorgyline/[¹¹C]clorgyline-D2 pair.⁸¹ Contrasting with the limited effect of smoking on MAO-A in peripheral organs, paired studies with [¹¹C]L-deprenyl and [¹¹C]L-deprenyl-D2 revealed that smokers have significantly reduced MAO-B in the heart, lungs, spleen, and kidneys (Figure 12).⁸² Because MAO breaks down catecholamines and other physiologically active amines, including those released by nicotine, MAO inhibition by cigarette smoke may alter sympathetic tone as well as central neurotransmitter activity, thereby contributing to some of the central and peripheral effects of smoking.

Following these studies the hypothesis was tested that brain MAO-A levels would increase during acute withdrawal from cigarettes and that this would be related to plasma clearance of the potent MAO-A inhibitor, Harman, an alkaloid which is present in tobacco smoke.⁸³ Brain MAO-A V_T as measured with [¹¹C]harmine increased between active smoking and acute withdrawal and the percent change correlated with plasma levels of harman. As

plasma harman levels dropped during the withdrawal period, MAO-A levels increased along with depression symptoms supporting the suggestion that the elevation of MAO-A during the acute withdrawal phase may account for depressed mood during the withdrawal from cigarettes. Contrasting with results with [^{11}C]clorgyline⁷⁵ and [^{11}C]befloxatone⁸⁰, this study with [^{11}C]harmine showed no significant differences in baseline brain MAO-A levels. Possible variables could include smoking dose or time between last cigarette and PET scan.

The monoamine hypothesis of depression posits that the pathophysiological basis for depression is an underlying deficit in norepinephrine, serotonin and dopamine which are neurotransmitters involved in arousal, mood and reward respectively. However, no specific molecular deficiency had been identified, until a PET study with [^{11}C]harmine revealed that individuals with major depressive disorder have significantly elevated levels of brain MAO-A.⁸⁴ This led to the suggestion that elevated MAO-A density is the primary monoamine lowering process contributing to the monoamine imbalance during major depression.

PET and [^{11}C]harmine have also been used to measure brain MAO-A in non-smoking alcohol dependent subjects relative to non-smoking healthy controls and revealed a significant elevation in brain MAO-A in alcohol dependent subjects.⁸⁵ Greater duration of alcohol use was positively correlated with higher levels of brain MAO-A and represents a new neurochemical marker in alcoholism that is therapeutically targetable.

Brain MAO-B in Aging and Neurodegenerative Disorders

MAO-B is highly localized in glia cells, which human post-mortem brain studies have shown increase with normal aging.⁸⁶ Many neurodegenerative disorders are also associated with activated microglia and gliosis as shown by [^3H]L-deprenyl autoradiography in combination with histochemical methods.⁸⁷ Consistent with these post-mortem studies, PET studies with [^{11}C]L-deprenyl-D2 in a group of 21 normal healthy human subjects (age range 23–86) showed the expected increase in [^{11}C]L-deprenyl-D2 binding with age in all brain regions.⁸⁸ In contrast, subjects showed the expected regional age-related *decreases* in cerebral blood flow and metabolism in subjects in whom both MAO-B and brain glucose metabolism (measured with ^{18}F FDG) were measured.

In principle, regions of brain injury or degeneration should show *increased* binding of [^{11}C]L-deprenyl-D2 and *decreased* brain metabolism as measured with ^{18}F FDG. This proved to be the case in patients with temporal lobe epilepsy (TLE) for which the most common histopathological abnormality is gliosis. In patients with TLE, the [^{11}C]L-deprenyl-D2 images showed significantly increased uptake in the amygdala on the affected side of the brain whereas there was a significant reduction in brain glucose metabolism in all TLE patients in temporal regions (Figure 13).^{89, 90}

A retrospective study with both ^{18}F FDG and [^{11}C]L-deprenyl-D2 was performed in 7 patients with traumatic brain injury (TBI) who suffered from seizures and memory loss was done to determine whether hypometabolic regions would show correspondingly elevated MAO-B.⁹¹ Contrary to prediction and to PET studies in TLE, no consistent inverse relationship between brain metabolism and MAO-B activity were found, indicating that prospective studies are needed to determine the pathophysiology of hypometabolic lesions in TBI.

Autoradiography with [^3H]L-deprenyl in amyotrophic lateral sclerosis (ALS) has revealed that motor neuron loss is accompanied by astrocytosis which is likely to play an active role in the neurodegenerative process.⁹² [^{11}C]L-deprenyl-D2 provided an opportunity to localize astrocytosis in vivo in the brain of patients with ALS compared with healthy controls.

These studies revealed increased binding in white matter and in pons though prospective studies are needed to determine whether [^{11}C]-L-deprenyl-D2 tracks disease progression and reflects gliosis.⁹³

A multi-tracer PET study with [^{15}O]water, [^{11}C]-L-deprenyl-D2 and with ^{18}F FDG was also performed in patients who fulfilled criteria of probable Creutzfeld-Jacob Disease (CJD) to measure cerebral blood flow and to detect characteristics of astrogliosis and neuronal death respectively.⁹⁴ These patients showed simultaneous high [^{11}C]-L-deprenyl-D2 and low ^{18}F FDG uptake affecting several regions of the brain and cerebellum but not the temporal lobes. The PET imaging findings were consistent with neuronal dysfunction and astrogliosis, which characterize neuropathology in CJD patients.

A study that used whole hemisphere autoradiography with [^3H]-L-deprenyl recently showed elevated MAO-B which co-localized with fibrillary $\alpha\beta$ -plaques in post-mortem brain sections of patients with Alzheimer's disease.⁹⁵ Results of a multi-tracer study with [^{11}C]-L-deprenyl-D2, [^{11}C]PIB (a radiotracer for A β protein) and ^{18}F FDG in healthy controls and in patients with mild cognitive impairment (MCI) who were either [^{11}C]PIB positive or [^{11}C]PIB negative suggested that astrogliosis is an early phenomenon in the development of Alzheimer's disease but found no correlations between [^{11}C]-L-deprenyl-D2 binding and brain glucose metabolism as measured with ^{18}F FDG.⁹⁶

MAO and Drug Research

PET imaging, with highly selective radiotracers, is a relatively non-invasive method for determining CNS penetration and distribution, as well as, targeted activity at molecular brain sites of new chemical entities, as well as approved drugs. (^{97, 98, 99}) Because of its short half-life (20.4 min), carbon-11 labeled radiotracers enable serial scans in the same individual, thus allowing an individual to serve as his/her own control and reducing the impact of inter- and intra-subject variability. In addition, drug plasma concentrations obtained at the time of PET imaging can serve as a means of demonstrating the relationship between plasma drug levels and pharmacodynamic effects in the brain and clinical efficacy. Several PET studies with different carbon-11 labeled subtype-selective MAO radiotracers have demonstrated their usefulness to measure the degree, duration, and specificity of brain MAO inhibition by various drugs and other substances in humans (Figure 14).

Phenelzine (**16**) and tranylcypromine (**17**) are non-selective MAO inhibitors, which irreversibly inhibit MAO. Phenelzine inhibits both [¹¹C]clorgyline and [¹¹C]L-deprenyl binding in the human brain²⁴ and tranylcypromine has been shown to inhibit 58% of brain MAO-A after a 3-day treatment with 10 mg/day⁷⁵.

Two early studies focused on drug dosing for a new reversible MAO B inhibitor, lazabemide (**18**, Ro19-6327) using PET and [¹¹C]L-deprenyl in healthy subjects¹⁰⁰ and in patients with Parkinson's disease for whom the drug was developed.¹⁰¹ In the study in Parkinson's patients, each patient has 3 scans with [¹¹C]L-deprenyl on day 0 to assess baseline MAO-B activity, on day 7 (12 hours after the last dose of lazabemide) to assess degree of MAO-B inhibition and on day 8 to assess reversibility. The study showed that a 50-mg dose was sufficient to block >90% of the enzyme, whereas the 25-mg dose was inadequate and also confirmed the reversibility of Ro19-6327 thereby determining both the dose and the frequency of administration needed for clinical trials.

The reversibility of lazabemide contrasts sharply with the irreversible inhibition of MAO-B by L-deprenyl (**19**). Serial PET studies with [¹¹C]L-deprenyl, revealed that the half time for recovery of MAO-B to baseline levels after withdrawal from L-deprenyl treatment is 40 days.¹⁰² Since the binding of L-deprenyl to MAO-B involves covalent modification of the enzyme, the 40-day half-life for recovery of brain MAO-B activity is a measure of the half-life for the synthesis of new MAO-B molecules. Moreover, the slow turnover of brain MAO B suggests that reduced dosing of L-deprenyl should be evaluated and may have an impact on reducing the side effects and the costs arising from excessive drug use.

Rasagiline (**20**) is a new selective MAO-B irreversible inhibitor, which has been developed for the treatment of Parkinson's disease. Its structural difference relative to L-deprenyl precludes the formation of amphetamine metabolites. [¹¹C]L-Deprenyl-D2 was used to demonstrate the MAO-B inhibitory effect of a 10-day treatment with a therapeutic dose of rasagiline and to monitor the recovery of MAO-B activity after drug withdrawal. Only one blood sample was taken during the scan precluding the complete quantification of tracer binding. However, the authors report a gradual recovery of MAO-B activity similar to prior studies in baboons¹⁰³, pigs¹⁰⁴ and in humans¹⁰².

MAO-B is elevated in activated microglia (gliosis), which accompanies neurodegenerative diseases such as Alzheimers.^{87, 95, 105} This has stimulated the development and evaluation of a new MAO B inhibitor drugs in part to mitigate the increased oxidative stress

arising from elevated MAO-B. One of these inhibitors is (N-[4-fluoro-3-benzyloxy)-phenyl]-malonamide (**21**: EVT-301), a reversible inhibitor of MAO B. In a recent dose-finding study with [¹¹C]L-deprenyl-D2 in a group of Alzheimer's patients and a group of healthy controls, daily doses of 75-150 mg of EVT-301 resulted in near complete occupancy of brain MAO-B.¹⁰⁶

The antidepressant properties of the non-selective, irreversibly binding MAO inhibitors has been shown to reside in their inhibition of the MAO-A isoform.¹ The desire to take advantage of the antidepressant properties of MAO-A inhibitors without the need for dietary restrictions associated with the non-selective irreversibly binding MAO-A inhibitors has stimulated the development of reversibly binding MAO-A inhibitor drugs.¹ PET and [¹¹C]harmine were used to examine the binding properties of the reversible MAO-A inhibitors, esuprone (**22**) and moclobemide (**23**), revealing a high degree of target engagement with both of these drugs and a slight recovery of MAO-A activity after 23 and 11 hour drug free intervals.³⁸ Later studies of moclobemide with [¹¹C]harmine showed that a 7-day treatment with clinical doses of moclobemide leads to a 64% to 79% MAO-A blockade across most brain regions.³⁹ MAO-A occupancy was also measured after 6 weeks of treatment in depressed patients with a *clinically effective* dose of moclobemide and after repeated administration of St. John's wort, an herb purported to have MAO-A inhibitor properties, and which is used by millions of people for the treatment of depressive episodes.¹⁰⁷ The study showed that for new MAO-A inhibitors, about 74% occupancy at steady-state dosing is desirable as this exceeds the MAO-A elevation in major depression (*vide supra*). Consistent with this, St. John's wort, which inhibits MAO-A by <5% should not be classified as a MAO-A inhibitor and thus patients who do not respond to St. John's wort should not be classified as being non-responders to MAO inhibitor therapy.

Another alternative medicine, extracts of Ginkgo biloba, have been reported to reversibly inhibit both MAO-A and MAO-B in rat brain¹⁰⁸ in vitro leading to the speculation that MAO inhibition may contribute to some of the central nervous system effects in humans including improvement of cognitive function in patients suffering from dementia. However, a PET study using [¹¹C]clorgyline and [¹¹C]L-deprenyl-D2 to measure MAO A and B respectively in 10 subjects treated for 1 month with Ginkgo biloba extract EGb 761 determined that Ginkgo biloba administration did not produce significant changes in brain MAO-A or MAO-B. This suggests that mechanisms other than MAO inhibition need to be considered as mediating some of Ginkgo biloba's CNS effects.

In another example of the development of reversible MAO-A inhibitors to avoid side effects of irreversible drugs, [¹¹C]clorgyline and PET were used to investigate the brain penetration, MAO-A inhibition and reversibility of CX-157 (**24**), an investigational compound currently being developed for the treatment of major depressive disorder.¹⁰⁹ PET studies in healthy human volunteers showed that CX157 entered the brain rapidly after oral dosing producing a dose- and time-related inhibition of brain MAO-A. MAO-A inhibition peaked early then declined over the 24 hours after a single-dose (**Figure 15**). Multiple twice-daily dosing with CX-157 produced a more sustained inhibition of MAO-A over a 12 hour period, suggesting that repeated dosing could achieve continuous therapeutic levels of MAO-A inhibition. Brain MAO-A inhibition was directly correlated with plasma levels indicating the usefulness of CX157 plasma levels as a biomarker for brain MAO-A inhibition for efficacy studies.

There is growing evidence that different formulations and different routes of administration of the same drug molecule can have a profound effect on drug bioavailability, pharmacokinetics and pharmacodynamics.¹¹⁰ PET studies with [¹¹C]clorgyline have shown that conventional doses of 10 mg/day (oral) L-deprenyl is selective for MAO-B and does not inhibit

brain MAO-A.³⁰ However, controlled studies have demonstrated antidepressant activity for high doses of oral L-deprenyl and for transdermal L-deprenyl (which bypasses first-pass metabolism) suggesting that when plasma levels of L-deprenyl are elevated, brain MAO-A might also be inhibited producing an antidepressant effect. Zydys selegiline (Zelapar®) is an orally disintegrating formulation of L-deprenyl, which is absorbed through the buccal mucosa, limiting first pass metabolism and producing higher plasma levels of selegiline. Recently, PET and [¹¹C]clorgyline were used to show that a 28-day treatment with transdermal selegiline or high dose Zydys selegiline (10 mg) lose their selectivity for MAO-B and also inhibit brain MAO-A.¹¹¹ This represents the first direct evidence that targeted inhibition of brain MAO by formulations of L-deprenyl that bypass the gut, also inhibit brain MAO-A. By sparing gut MAO, these formulations of selegiline also reduce the need for dietary restrictions and re-open the use of MAO inhibitors for depression treatment.

Summary and Outlook

PET studies of brain MAO in healthy volunteers and in patients have evolved over the past 30 years since the first radiotracers were developed and the first PET images of MAO in humans were carried out. Different variables such as age, smoking status, depressive mood, impulsive and aggressive personality, alcohol dependence and DNA methylation state have been discovered to contribute to different brain MAO levels. These studies have also contributed to a mechanistic understanding of the relationship between brain chemistry and behavior and disease. MAO radiotracers and PET have also been used to study the current and developing MAO inhibitor drugs including the selection of doses for clinical trials based on PET measurement of target engagement.

While the current generation of radiotracers have served the scientific community well as tools to probe human neurobiology, chemists continue to develop and test new radiotracers. One area for improvement has been suggested by the recent correlation analyses between the known concentration of brain MAO-A and MAO-B and PET outcome measures.³² Though correlations were good, PET outcome measures underestimated the dynamic range of MAO-A concentrations by a factor of 2 suggesting that improvements in radiotracer characteristics would enable investigations with greater sensitivity to MAO changes. Additionally, the availability of well-validated F-18 labeled MAO-A radiotracers could enable studies of MAO activity in sites remote from a cyclotron.

Finally, certain cancers have been reported that MAO-A mediates prostate tumorigenesis and cancer metastasis and that MAO-B is elevated in human gliomas when compared with meningiomas or non-tumoral tissue.^{112, 113} These studies suggest that the quantification of MAO activity with PET may be useful in characterizing tumor biology and for tailoring cancer treatment to the biology of the tumor.

Figure Captions

Figure 1. Monoamine oxidase (MAO) oxidizes amines to initially produce an imine and an amine and hydrogen peroxide.

Figure 2. MAO radiotracers which have been validated and used in human studies. Parentheses indicated the MAO subtype.

- Figure 3. Proposed adduct between a MAO suicide inactivator and the covalent adduct formed via catalysis (from ¹¹⁴). The arrow indicates the bond which is cleaved in the rate-limiting step.
- Figure 4. Comparison of [¹¹C]L-deprenyl and [¹¹C]L-deprenyl-D2 binding in the human brain showing a decrease in binding with deuterium substitution and demonstrating that the C-H bond in the propargyl group is broken in the rate limiting step (from ²⁵).
- Figure 5. Whole body images showing the distribution of C-11 after the administration of [¹¹C]clorgyline and [¹¹C]L-deprenyl (from ¹¹⁵).
- Figure 6. Radiosynthesis of the reversibly binding MAO-B radiotracer [¹¹C]SL 25.1188 via direct [¹¹C]CO₂ fixation.⁴⁸
- Figure 7. Fluorine-18 labeled MAO radiotracers.
- Figure 8. Individual values of MAO-A activity in high and low MAOA genotype men measured with [¹¹C]clorgyline. There was no statistically significant difference between the high and low MAOA genotype groups (data from ⁶⁹).
- Figure 9. Correlation plot of Brain MAO-A levels as measured by [¹¹C]clorgyline vs DNA methylation status of the MAOA promoter (data from ⁷⁰). The R and p values represent the correlation value and significance for the whole population.
- Figure 10. Brain MAO-A activity as measured with [¹¹C]clorgyline vs aggression score on the Multidimensional Personality Questionnaire (MPQ, data from ^{Error! Bookmark not defined.}). The R and p values represent the correlation value and significance for the whole population.
- Figure 11. Comparison of brain MAO-A and MAO B of non-smokers and smokers using PET and [¹¹C]clorgyline and [¹¹C]L-deprenyl-D2 (data from ^{75,76}).
- Figure 12. Whole body PET scans of MAO-B comparing a non-smoker and a smoker. Images were made with [¹¹C]L-deprenyl and are scaled so that they can be compared directly (data from ⁸²).
- Figure 13. Coronal PET images with ¹⁸FDG and [¹¹C]L-deprenyl-D2 in a patient with right temporal lobe epilepsy showing decreased uptake of ¹⁸FDG and increased uptake of [¹¹C]L-deprenyl-D2 in the medial right temporal lobe (images from ⁹⁰ with permission from Wiley).
- Figure 14. Structures of MAO inhibitor drugs which have been studied in humans with PET.
- Figure 15. PET images with [¹¹C]clorgyline in a human volunteer at baseline (top), 2 hours after receiving an oral dose of 60 mg of CX-157 (middle row) and 12 hours later (bottom row), showing significant blockade and reversibility of this new MAO-A inhibitor drug (data from ¹⁰⁹).

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Figure 1 (Fowler)

Figure 2 (Fowler)

1: [^{11}C]clorgyline (A), R = H
2: [^{11}C]clorgyline-D2 (A) R = D

3: [^{11}C]L-deprenyl, (B) R = H
4: [^{11}C]L-deprenyl-D2, (B) R = D

5: [^{11}C]DMPEA (B), R = H
6: [^{11}C]DMPEA (B), R = D

7: [^{11}C]harmine (A)

8: [^{11}C]befloxacine (A)

9: [^{11}C]SL 25.1188 (B)

Figure 3 (Fowler)

Figure 4 (Fowler)

[¹¹C]L-deprenyl

[¹¹C]L-deprenyl-D₂

Figure 5 (Fowler)

Figure 6 (Fowler)

a, $^{11}\text{CO}_2$ + BEMP; b, POCl_3 , CH_3CN ; c, HPLC purification * = ^{11}C

Figure 7 (Fowler)

Figure 8 (Fowler)

Figure 9(Fowler)

Figure 10 (Fowler)

MAO-A

non-smoker

MAO-B

non-smoker

smoker

smoker

Figure 12 (Fowler)

FDG

DED intercept

DED slope

Figure 15 (Fowler)

16: phenelzine (A/B)

17: tranlycypromine (A/B)

18: lazabemide (B)

19: L-deprenyl (B)

20: rasagiline (B)

21: EVT-301 (B)

22: esuprone (A)

23: moclobemide (A)

24: CX-157 (B)

Figure 16 (Fowler)