1-^{11}C-Methyl-4-piperidinyl *n*-butyrate

^{11}C-MP4B

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Created: October 20, 2009; Updated: December 14, 2009.

Chemical name:	1-^{11}C-Methyl-4-piperidinyl *n*-butyrate	
Abbreviated name:	^{11}C-MP4B or [^{11}C]BMP	
Synonym:	[^{11}C]nBMP	
Agent Category:	Compound	
Target:	Butyrylcholinesterase (BChE, BuChE)	
Target Category:	Enzyme	
Method of detection:	Positron emission tomography (PET)	
Source of signal / contrast:	^{11}C	
Activation:	No	
Studies:	• *In vitro* • Rodents • Non-human primates • Humans	No structure available.

Background

[PubMed]

Cholinesterase (ChE) is an enzyme that hydrolyzes the neurotransmitter acetylcholine into choline and acetic acid, and thus shuts off neural transmission (1, 2). There are two types of ChE: acetylcholinesterase (AChE, also known as erythrocyte cholinesterase or acetylcholine acetylhydrolase) and butyrylcholinesterase (BChE or BuChE, also known as plasma cholinesterase, pseudocholinesterase, or acylcholine acylhydrolase). Both enzymes are present in cholinergic and noncholinergic tissues as well as in plasma and other body fluids. They differ in substrate specificity, behavior in excess substrate, and susceptibility to inhibitors (1, 2).

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NLM Citation: Shan L. 1-^{11}C-Methyl-4-piperidinyl *n*-butyrate. 2009 Oct 20 [Updated 2009 Dec 14]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

BChE is encoded by the *BCHE* gene, which is located in humans on chromosome 3q26.1-q26.2 (3). Mutations of the *BCHE* gene result in various genotypes and phenotypes (4), and some *BCHE* gene variants, such as atypical, K, J, and H variants, cause reduced activity of BChE. The silent variants lead to total loss of the enzyme activity (0–2% of normal activity). On the other hand, some variants result in increased activity, such as the C5+ variant (combination of BChE with an unidentified protein), the Cynthiana variant (increased amount of BChE than normal level), and the Johannesburg variant (increased BChE activity with normal enzyme level). In the absence of muscle relaxants, there is no known disadvantage for individuals with these variants.

BChE is synthesized in many tissues, including the liver, lungs, heart, and brain. Similar to AChE, a single *BCHE* gene gives rise to different protein products by alternative splicing in the coding region of the original transcript. This provides a series of diverse but related molecular forms of BChE (G1, G2, and G4). G4 is the predominant isoform in the mature brain. These forms have similar catalytic properties, but they exhibit different cellular and extracellular distributions and non-catalytic activities.

BChE possesses three different enzymatic activities: esterase, aryl acylamidase, and peptidase (1). The esterase activity of BChE plays an important role in scavenging anti-AChE compounds such as cocaine, heroin, and organophosphate before they reach AChE at physiologically important sites. In the absence of AChE, BChE is believed to serve as a backup to AChE in supporting and regulating cholinergic transmission (5). BChE also inactivates some drugs, e.g., aspirin, amitriptyline, and bambuterol (1, 6). The aryl acylamidase activity of BChE may be involved in the crosstalk between serotonergic and cholinergic neurotransmission systems, but it is still poorly understood. The peptidase activity of BChE is related to the development and progress of Alzheimer's disease (AD) (7, 8), which is characterized by a loss of cholinergic neurons. In the brains of patients with AD, the level of the membrane-bound G4 form of AChE is selectively reduced by 90% or more in certain regions, while the level of the G1 form is largely unchanged. On the contrary, the G1 form of BChE shows a 30–60% increase, while the G4 form decreases or remains the same as in the normal brain. It has been indicated that BChE, which is found in the neuritic plaques and tangles, cleaves the amyloid precursor protein to the β-amyloid protein and helps β-amyloid diffusion to β-amyloid plaques (6). Abnormal expressions of BChE and AChE have also been observed in human tumors such as meningioma, glioma, acoustic neurinomas, and lung, colon, and ovarian cancers (9, 10). However, the relationship between altered BChE and AChE expressions and tumorigenesis is not clear, nor is the efficacy of specific inhibitors as chemotherapeutic agents.

Because of the potential diagnostic and therapeutic values, investigators have synthesized various radiolabeled acetylcholine and butyrylcholine analogs as positron emission tomography (PET) tracers (11-16). These tracers have been used to measure ChE activity, detect diseases with cholinergic deficits, and study the efficacy of ChE inhibitors. *N*-methylpiperidinyl esters are a group of synthetic AChE substrates; of them, 1-^{11}C-methyl-4-piperidinyl acetate (^{11}C-MP4A) and 1-^{11}C-methyl-4-piperidinyl propionate

(^{11}C-MP4P) have already been used in the clinic as PET tracers for *in vivo* assessment of AChE activity associated with AD. 1-^{11}C-Methyl-4-piperidinyl *n*-butyrate (^{11}C-MP4B or [^{11}C]BMP), a specific radiolabeled substrate of BChE, was developed for *in vivo* assessment of BChE activity with PET (15-18).

AChE- and BChE-Related Resource Links

- Chapters in MICAD
- Gene information in NCBI (AChE and BChE).
- Articles in OMIM
- Clinical trials (AChE, BChE)
- Substances and Compounds in PubChem
- Drug information in FDA

Synthesis

[PubMed]
The ^{11}C-MP4B was synthesized by the reaction of 4-butyryl-piperidine, generated *in situ* from its hydrochloride salt using 1,2,2,6,6-pentamethyl-piperidine, and ^{11}C-methyl triflate in methanol-acetonitrile for 1 min at 60°C (15-18). The crude product was purified with high-performance liquid chromatography. After addition of sterile propylene glycol-ethanol (7:3 v/v) and HCl, the fraction containing the product was evaporated, re-dissolved in physiological phosphate buffer (0.1 mol/L, pH 7.4), and filtered through a 0.2-µm sterile filter. The radiochemical yield of ^{11}C-MP4B was 5–7 GBq (0.135–0.189 Ci) with a 40-min target irradiation with 17-MeV protons at 10 µA. The specific radioactivity was 60 ± 10 GBq/µmol (1.62 ± 0.27 Ci/µmol). The radiochemical purity was >99%, and the tracer was radiochemically stable for more than 90 min.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Snyder et al. analyzed the *in vitro* enzyme-mediated cleavage rate with purified enzymes of AChE (electric eel) and BChE (horse serum), as well as in whole mouse blood (n = 3 animals) (17). One unit (U) of AChE was defined as the amount of enzyme necessary to hydrolyze 1.0 µmol ACh/min at 25°C and pH 7.4. One U of BChE was similarly defined but was based on the hydrolysis of its specific substrate BCh. Analysis showed that both BCh and MP4B were specific for BChE. The BChE-mediated cleavage rate of MP4B was -0.045 ± 0.002 ΔA/min per U per L (n = 3 assays) at the BChE concentration of 20 U/ml. The AChE-mediated cleavage rates of BCh and MP4B were both well below the accurate detection limit of 0.004 ΔA/min per U per L, even at an enzyme concentration of 100 U/ml. In the whole mouse blood, ^{11}C-MP4B was completely metabolized within 30 min at room temperature.

Animal Studies

Rodents

[PubMed]

Snyder et al. investigated the *in vivo* distribution in brains of ^{11}C-MP4B in female CD-1 mice (n = 28) after tail vein injection (17). ^{11}C-MP4B exhibited high initial brain uptake at 1 min after injection. It washed out rapidly with <2% injected dose per gram wet weight (ID/g) trapped in tissues at 30 min after injection. After 30 min, the amount of radioactivity remained constant in all brain regions, indicating complete metabolism of ^{11}C-MP4B in both the central nervous system and periphery, and irreversible trapping of the radiolabeled metabolite in brain tissue. The retention fraction in each brain region was calculated as the ratio of radioactivity retained at 30 min to the initial uptake of radioactivity at 1 min after injection. Because studies using [^{14}C]methylpiperidinol have shown that the labeled metabolite formed by the peripheral ChE activity is unable to enter the brain, both the retention fraction and the hydrolysis rate constant (k_3) (see below) depend on complete *in vivo* metabolism of the radiotracer to form exclusively 1-[^{11}C]methyl-4-piperidinol, and these values are indices of the ChE activity in each brain region. The retention fraction of ^{11}C-MP4B in mice was 0.11 ± 0.03 for striatum, 0.08 ± 0.02 for cortex, 0.10 ± 0.02 for cerebellum, 0.10 ± 0.02 for hippocampus, and 0.12 ± 0.04 for thalamus, which was consistent with the previous histochemical results of strong BChE reactivity in striatum and thalamic nuclei, and of mild BChE reactivity in neocortex and hippocampus. The detailed data were not provided regarding the identity of the ^{11}C-MP4B metabolite and the percentage of the metabolite based on the radioactivity in the tissues.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

Snyder et al. performed a PET imaging study in a female pigtail monkey (*M. nemistrina*) after vein injection of ^{11}C-MP4B (17). The k_3 value, which reflects both the actual rate of enzymatic hydrolysis and the local concentration of BChE, was calculated on the basis of the PET data. In contrast to the rodent data, the initial uptake in primate brain was much less. Approximately 50% of the ^{11}C-MP4B entering the brain was rapidly metabolized and trapped in brain tissue. The k_3 values were 0.054 min^{-1} and 0.047 min^{-1} for striatum (caudate and putamen) and cerebral cortex, respectively. This value of k_3 is in a range in which it can be estimated with good precision and should be sensitive to both increases and decreases in BChE in disease states.

Human Studies

[PubMed]

Investigators from several groups analyzed the biodistribution of ^{11}C-MP4B and its ability to measure the BChE activity with PET in healthy people as well as in patients with AD (15-18).

Roivainen et al. evaluated the distribution and kinetics of ^{11}C-MP4B uptake (15). Kinetic analysis (10 patients with AD and 18 healthy controls) showed that the plasma level of intact ^{11}C-MP4B decreased rapidly from 28 ± 14% ID at 0.5 min to 7 ± 6% ID at 15 min after injection. The half-life ($t_{1/2}$) in patients with AD and in normal subjects was 1.65 ± 0.73 min and 1.40 ± 0.52 min (P = 0.28), respectively, and the metabolite-corrected $t_{1/2}$ was 5.03 ± 2.19 min and 3.95 ± 1.18 min (P = 0.18), respectively. Large individual variation was observed in the rate of plasma ^{11}C-MP4B hydrolysis, but there was no significant difference in the degradation of ^{11}C-MP4B either between male and female or between healthy subjects and AD patients ($P > 0.05$). Approximately 17% of the ^{11}C-MP4B radioactivity was bound to human plasma proteins. In the brains of patients with AD, the ^{11}C-MP4B exhibited high initial uptake followed by gradual decrease. At ~30 min after injection, the amount of radioactivity reached a plateau in the cerebellum, striatum, and cortex. The highest ^{11}C-MP4B activity was detected in the cerebellum, followed by striatum, pons, and thalamus. Lower ^{11}C-MP4B activity was seen in cortical areas. Roivainen et al. concluded that ^{11}C-MP4B was excreted rapidly through the renal system. The biodistribution of ^{11}C-MP4B in the brains of patients with AD appeared to be in accordance with the distribution of BChE observed in postmortem studies of human brains, except for the observed higher activity in striatum than in cortex. However, no comparisons were made between AD patients and control subjects.

Virta et al. analyzed the biodistribution and residence time of ^{11}C-MP4B (16). Up to 60% of the injected dose was excreted *via* the urinary pathway, and the clearance was rapid as 30% of the radioactivity was excreted within 60 min after injection. The organs with the highest radiation-absorbed dose (mGy/MBq) at 2 h after injection in a 70-kg reference man were the urinary bladder (0.019), kidneys (renal cortex, 0.014), liver (0.008), upper large intestine (0.007), trabecular bone (0.005), parotid glands (0.007), and heart wall (0.003). The residence times were 0.113 h for the muscle, 0.068 h for the cortical bone, 0.041 h for the liver, 0.025 h for the urinary bladder content, 0.018 h for the brain, 0.015 h for the red bone marrow, 0.014 h for the kidneys, 0.008 h for the upper large intestine, 0.007 h for the trabecular bone, and 0.006 h for the lung. With a 2-h voiding interval, the effective dose was 4.2 μSv/MBq. The investigators concluded that ^{11}C-MP4B caused less radiation burden than previously studied ^{11}C-labeled PET tracers. No intolerably high absorbed doses were observed in critical organs. With 740 MBq (20 mCi) of injected radioactivity, the radiation burden was equivalent to 3.11 mSv. This would allow multiple PET examinations per year to be performed on the same subject.

Kuhl et al. investigated and compared the ^{11}C-MP4B PET scan data of the brains between healthy control subjects and patients with AD (18). The *in vivo* k_3 in control cases agreed well with the regional BChE activity from the biochemical assay of postmortem human brains. BChE activity was 2.5 times lower in cerebral cortex than in cerebellum. The total cerebral cortical k_3 increased with age, showing 25% higher k_3 in elderly (62 ± 7 years old; $n = 12$) than in young (30 ± 9 years old; $n = 13$) subjects ($P = 0.009$). Neither sex effect ($P = 0.15$) nor age by sex interaction ($P = 0.17$) were significant. Relative to the parietal cortex distribution, the distributions of ^{11}C-MP4B in different cerebral cortex regions (frontal, temporal, occipital, visual, posterior cingulate) did not differ significantly ($P > 0.1$) between young and elderly subjects. Administration of physostigmine (inhibits both BChE and AChE) led to 49 ± 6% inhibition of BChE activity ($P = 0.001$) as measured with ^{11}C-BMP ($n = 4$ subjects) and 52 ± 9% inhibition of AChE activity ($P = 0.0002$) as measured with *N*-[^{11}C]methylpiperidin-4-yl propionate ([^{11}C]PMP, a substrate of AChE) ($n = 5$ subjects). The inhibition levels of the two ChEs did not differ significantly ($P = 0.6$). After treatment with the selective AChE inhibitor donepezil ($n = 4$ subjects), AChE was significantly inhibited (27 ± 5%, $P = 0.003$) but BChE was not (4 ± 1.2%, $P = 0.5$). Comparison between elderly control subjects ($n = 12$) and AD patients ($n = 15$) showed that the activities of both AChE (75 ± 13% of normal, $P = 0.00001$) and BChE activities (82 ± 14% of normal, $P = 0.001$) were decreased in AD cerebral cortex. Higher BChE/AChE ratio was not observed in AD patients than in control people. The BChE activity also did not increase in human brains after months of selective AChE inhibition. Their findings do not support the concept that BChE-targeted inhibition will benefit patients with AD.

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