Analysis of [11C]L-deprenyl-D2 (DEP-D) brain PET studies

Introduction

L-deprenyl and monoamine oxidase B

Monoamine oxidase B (MAO B) is present in the outer mitochondrial membrane occurring in the brain predominantly in glial cells and in serotonergic neurons [Fowler et al., 2005]. It oxidizes amines (for example dopamine) from both endogenous and exogenous sources. MAO B is selectively and irreversibly inhibited by L-deprenyl (selegiline). When the enzyme-substrate complex is formed, the rate-limiting step of MAO B catalyzed oxidation creates a highly reactive intermediate, which forms a covalent bond with the enzyme, thus irreversibly inactivating it [Fowler et al., 2005]. When L-deprenyl is labelled with ^{11}C, the active enzyme MAO B becomes labelled, and it can be imaged *in vivo* with PET.

Three-compartment model

A three-compartment model can be applied to the time-activity curves (TACs) of labelled L-deprenyl in the brain and plasma to estimate MAO B activity in the brain. In the model, K_1 represents the plasma-to-organ transfer constant, k_2 is the transfer rate of radiotracer from organ back to plasma, and k_3 describes the rate of binding to MAO B. Under the PET study conditions, k_3 is proportional to the functionally active free enzyme concentration. Because binding is irreversible, it is assumed that $k_4=0$. From these model constants, K_1 and k_2 are dependent on blood flow, but k_3 is not. Due to the high extraction of L-deprenyl, K1 is dominated by blood flow instead of capillary permeability [Fowler et al., 1988, 1995]. Also the net influx rate K_i ($=K_1*k_3/(k_2+k_3)$) is dependent on blood flow [Lammertsma et al., 1991], as well as the more directly radiotracer uptake related parameters, like SUV.

The very high rate of binding of labelled L-deprenyl to MAO B creates difficulties in applying the model. The estimates of k_2 and k_3 tend to be highly correlated, and the rate of radiotracer binding (k_3) and delivery (K_1) are hard to separate [Fowler et al., 2005]. Especially in the brain regions of high MAO B concentrations (basal ganglia, thalamus and cingulate gyrus), and in the elderly people who have reduced blood flow, the rate limiting step is the radiotracer delivery instead of binding [Fowler et al., 1993].

To reduce the problem with correlating k_2 and k_3, a combination model parameter λk_3 has been introduced as an index of MAO B activity; λ is the K_1 over k_2 ratio [Fowler et al., 1993 and 1995]. K_1 and k_2 are both dependent on the blood flow, but λ and λk_3 are independent of blood flow. Because this index contains the ratio k_3/k_2, the effect of their (positive) correlation is expected to be smaller. Reproducibility in test/retest studies was improved by using λk_3 instead of k_3 [Logan et al., 2000].

Deuterium substitution

The rate-limiting step of MAO B catalyzed oxidation involves cleavage of a certain carbon-hydrogen bond in L-deprenyl. A carbon-deuterium bond is more difficult to cleave than the carbon-

hydrogen bond, which leads to reduced rate of reaction when this hydrogen is substituted with deuterium (so called deuterium isotope effect). Because the very high binding rate of [11C]L-deprenyl has been found to be problematic in quantification of MAO B activity, the deuterium-substituted L-deprenyl, [11C]L-deprenyl-D2 is therefore preferred as PET radiotracer [Fowler et al., 1988, 1995, 2004].

Confounding factors

Age

Unlike most enzymes, MAO B activity (λk_3) increases clearly with normal aging, which is accompanied by decreasing blood flow (K_1) [Fowler et al., 1997]. Therefore, the age must be controlled in the PET studies of MAO B. If the age effect is studied, the measured index of MAO B activity must not be flow dependent. The age-related and disease-associated increase of MAO B has been attributed to neuron loss and gliosis (increase in glial cells).

Blood flow

As described above, the tissue uptake of [11C]L-deprenyl-D2 and especially [11C]L-deprenyl is strongly dependent on blood flow. Therefore, to quantification of MAO B activity, the compartment model and a blood flow independent model parameter or index, like k_3 or λk_3 must be used. Otherwise, for example, the increase of MAO B activity in epileptogenic region might be underestimated because of subsequently reduced blood flow.

Smoking

MAO B is highly and variably inhibited in smokers [Fowler et al., 2003]. An overnight abstinence for smokers does not produce any recovery of MAO B activity. However, smoking a single cigarette does not produce a measurable decrease in MAO B activity in non-smokers [Fowler et al., 2003].

Decreased K_1 in later scans

In the test-retest setting, Logan et al. (2000) noticed a decrease of K_1 in the second scan (-7.7 $\pm$ 13.2%), although the decrease was not statistically significant (n=5). This may be caused by familiarization with the PET procedure and decreased anxiety [Logan et al., 2000].

Corrections for the PET data

The brain concentrations of [11C]L-deprenyl-D2 peak at about 5 min after injection, and after a washout phase the concentrations reach a plateau about 30 min after injection [Fowler et al., 1995].

Blood volume correction

Fowler et al. (1995) subtracted from the PET data an approximate 4% blood volume before analyzing the data using the three-compartment model or graphical analysis for irreversible systems.

Lammertsma et al. (1991) included the vascular volume fraction in the model equations, noting that whole blood curve must be used instead of (total) plasma curve.

Plasma protein binding

Free fraction in plasma was 6.0% [Fowler et al., 2004]. Considering the high K_1 estimates, dissociation rate of the radiotracer from plasma protein may be high, so that most of protein bound radiotracer is also available for transport to the tissue.

Blood-to-plasma transformation

For [^{11}C]L-deprenyl, Lammertsma et al. measured the blood-to-plasma ratio from discrete samples between 5 and 90 min. Based on in vitro experiments, they assumed that the plasma-to-blood ratio is 1.126 at the time of the arrival of the tracer in the blood, taken to be the time where the blood curve increased above 1% of the peak value [Lammertsma et al., 1991]. They fitted a multi-exponential function to the ratios, determining the number of exponentials based on AIC and SC. The multi-exponential function was then used to calculate the total plasma curve from arterial blood curve which had been measured on-line.

Fowler and Logan et al. do not give details on this transformation in their publications.

Plasma metabolite correction

For [^{11}C]L-deprenyl, Lammertsma et al. measured the fraction of plasma metabolites from four samples at 5, 10, 15 and 20 min, and fitted a single exponential function to the fractions, assuming no metabolites at time 0. The exponential function was used to calculate the concentration of unchanged tracer in the plasma.

Fowler and Logan et al. do not give details on this correction in their publications.

Time delay correction

Lammertsma et al. (1991) corrected for the time delay between blood curve and whole brain PET data by including the delay as one of the fitted model parameters. The smaller ROIs were then fitted with the delay fixed to this value.

Graphical analysis

Fowler et al. (1995) have used graphical analysis for irreversible systems to calculate the net influx rate K_i. Ki were taken as an average of slopes between 6 and 45 min and 6 and 55 min [Fowler et al., 1995].

Estimation of K_1 and λk_3 using "linear method"

Nonlinear method

Fowler et al. (1988, 1995) and Logan et al. (2000) estimated the three-compartment model parameters using traditional nonlinear least squares approach.

Linear method

Fowler et al. (1997, 1999) and Logan et al. (2000) estimated the three-compartment model parameters K_1 and λk_3 in a procedure which involves also graphical analysis:

1) K_i is calculated using the average of graphical analysis slopes between 6-45 min and 7-55 min
2) K_1 (and k_2+k_3) are estimated with bilinear regression using a modification of the method of Blomqvist (Eq. 1); note that equations in the original articles from years 1997 and 1998 do not contain the necessary square brackets. Logan et al. (2000) describe this process in more detail: Several values for K_1 were estimated by successively increasing the maximum time T from 5 to 18 min, because K_1 is more sensitive to data at earlier time points; an average K_1 was used in the next step.
3) λk_3 is calculated by solving it from the equation that relates K_i to the three-compartment model parameters (Eq. 2).

$$ROI(T) = K_1 \int_0^T C_p(t)dt + (k_2 + k_3)\left[K_i \int_0^T \int_0^t Cp(t')dt'\,dt - \int_0^T ROI(t)dt \right] \qquad (1)$$

$$\lambda k_3 = \frac{K_1 K_i}{K_1 - K_i} \qquad (2)$$

The estimates of K_1 and λk_3 from this linear method correlated very well with the estimates from the nonlinear method [Logan et al., 2000]. No noise-induced bias was noticed in the linear method, and the repeatability was also similar.

K_1 and blood flow

Extraction of [^{11}C]L-deprenyl-D2 is high, and assuming that it is 1, then K_1 would equal plasma flow, that is about 40% of blood flow. Therefore, the K_1 estimates by Logan et al. (2000), ranging from 0.476 to 0.845, are quite high.

The step 2) may lead to an overestimation of K_1, if the blood volume in tissue is not considered: blood volume correction was not mentioned by Fowler et al. (1997, 1999) or Logan et al. (2000).

Graphical method without plasma sampling

Graphical method (Gjedde-Patlak plot) can be used to estimate the net MAO B uptake using either metabolite corrected plasma or reference tissue with no MAO B as model input. To compensate the significant amount of MAO B in cerebellum, the cerebellar time-activity curves can be multiplied by a mono-exponential function to correct the deviation from linearity [Bergström et al., 1998]. However, cerebellar MAO B activity is probably not constant between individuals, although in clinical studies this methods has been used [Kumlien et al., 2001], and even less so in MAO B inhibition studies.

Note that the results of graphical method are not independent from blood flow, although the flow effects may be smaller than with SUV.

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