Parametric images of [11C]flumazenil binding

This document reviews the models that are used to compute parametric images representing the density of benzodiazepine receptors from dynamic [11C]flumazenil PET images.

Kinetics of [11C]flumazenil

[11C]Flumazenil has the properties of a good PET ligand: the metabolites formed in the liver are hydrophilic molecules that do not cross the blood-brain-barrier (Shinotoh et al. 1986; Debruyene et al. 1991), it is not metabolized in the brain (Shinotoh et al. 1986), its binding in the brain is reversible with fast kinetics, and its non-specific binding in brain is low (Abadie & Baron 1991, Hansen et al. 1991). After intravenous injection, [11C]flumazenil is initially distributed according to cerebral blood flow (Shinotoh et al. 1986).

Plasma protein binding is relatively low, about 40%, and blood-plasma ratio approaches unity (Klotz et al. 1984, Lassen et al. 1995). Red blood cells do not contain labelled metabolites.

In vivo autoradiography studies in rats suggest that non-specific binding of flumazenil is low, about 10% of the specific cortical binding, and volatile anaesthetics halothane and isoflurane did not induce changes in cortical non-specific binding (Hansen et al. 1991).

Suggested models

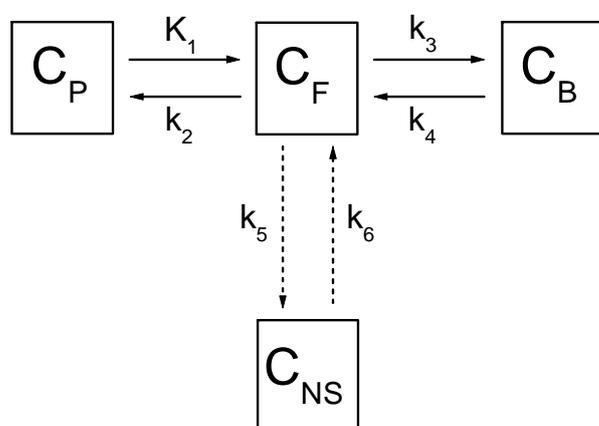### Theoretical three-tissue compartment model

Figure 1. Four-compartment model (three-tissue compartment model) for receptor studies. C_P= concentration of tracer in plasma, C_F= free concentration in tissue, C_{NS}= non-specifically bound tracer concentration in plasma, and C_B= receptor-bound (specifically) tracer concentration. For reference, see Koeppe et al. (1991).

Distribution volume of tracer in tissue can be represented with model rate constants:

$$DV_{ROI} = \frac{K_1}{k_2}\left(1 + \frac{k_3}{k_4} + \frac{k_5}{k_6}\right)$$

In a receptor-free (theoretical) reference region (rate constants with quotation marks) can be represented with:

$$DV_{REF} = \frac{K_1{}'}{k_2{}'}\left(1 + \frac{k_5{}'}{k_6{}'}\right)$$

Assuming that distribution volumes of free tracer and non-specifically bound tracer are similar in all brain regions ($K_1/k_2 = K_1'/k_2'$; $k_5/k_6 = k_5'/k_6'$), the specific binding can be described with two different distribution volumes (Laruelle 2000):

$$DV_3{}' = DV_{ROI} - DV_{REF} = \frac{K_1}{k_2} \times \frac{k_3}{k_4}$$

$$DV_3{}'' = \frac{DV_{ROI}}{DV_{REF}} - 1 = \frac{k_3}{k_4} \Big/ \left(1 + \frac{k_5}{k_6}\right)$$

DV_3'', which is commonly used in brain studies and often referred to as binding potential (BP), is vulnerable to changes of non-specific binding in tissue. Instead, DV_3' is vulnerable to changes in non-specific binding in plasma. Neither of these parameters is dependent on peripheral clearance or tissue perfusion, unless they change during the PET study (Laruelle 2000).

The fraction of [^{11}C]flumazenil bound to plasma proteins is about 0.4 (Klotz et al 1984), i.e. free fraction $f_1 \approx 0.6$. Lassen et al (1995) reported an average $f_1 = 0.64 \pm 0.03$ from five subjects, and Price et al. (1993) estimated $f_1 = 0.50 \pm 0.03$ also in five subjects. Fraction of free [^{11}C]flumazenil in brain, f_2, has been estimated (based on f_1 and K_1/k_2) to be 0.72 ± 0.03 (Price et al. 1993), or about 0.88 (Delforge et al. 1995).

For [^{11}C]flumazenil, it seems that in a four-compartment model $K_1/k_2 \approx f_1$ and $k_5/k_6 \approx 1 - f_2$. In an inhibition study with five healthy subjects, Price et al. (1993) estimated that average grey matter K_1/k_2 is 0.68 ± 0.08 in a three-compartment model, which does not consider non-specific binding; also for white matter K_1/k_2 was similar, 0.68 ± 0.17. Millet et al. (1995) estimated using a multi-injection protocol and five-parameter model that $k_1/k_2 = 0.555 \pm 0.056$ in healthy control subjects. Calculating K_1/k_2 using the ratio of the water contents of the brain and plasma (77.4/94) divided by $(1 + (1 - f_1))$ gives an estimate 0.51. Lassen et al. (1995) estimated $DV_F + DV_{NS} = 0.79$ using cold flumazenil infusion. If we assume that $DV_F + DV_{NS} = (K_1/k_2) \ast (1 + k_5/k_6)$, and that f_1 and $1 - f_2$ are about 0.6 and 0.28, respectively, we can calculate that $DV_F + DV_{NS} = 0.8$. This is close to the estimate by Lassen et al (1995). Magata et al. (2003) estimated with Logan plot that DV in brain stem is 0.84 ± 0.33, suggesting that specific binding is very low in white matter.

One-tissue compartment model

The estimation of receptor density and dissociation constant requires different concentrations of bound ligand, which is usually obtained using at least two injections of the ligand with different specific radioactivity (Blomqvist et al. 1990) or using different multi-injection protocols (Millet et al. 1995).

A single tissue compartment (2-compartment model) has been found sufficient to describe the tissue dynamics of [^{11}C]flumazenil in several studies (Koeppe et al. 1991; Lassen et al. 1995; Millet et al. 1995; Millet et al. 2002). Altered ligand delivery does not affect distribution volume of [^{11}C]flumazenil even with this simple model (Holthoff et al. 1991). It must be noticed that iomazenil, which is widely used in SPET if labelled with Iodine-123, has different kinetics, and Buck et al. (1996) have recommended three-compartment model for [^{11}C]iomazenil and fitting simultaneously multiple brain regions with coupled k_4 or k_4 and K_1/k_2'. Recently, however, Klumpers et al. (2005) showed that two-tissue model was preferable (based on AIC and SC), providing $6 \pm 4\%$ higher DV than one-tissue compartment model; however, this result was based on regional analysis, but for pixel-by-pixel analysis one-tissue model may still be preferable.

Non-compartmental analysis

One analysis method used by Lassen et al. (1995) was to estimate distribution volumes using integrals of both the metabolite corrected plasma curves and the tissue curves. Both curves were extrapolated using a single exponential obtained from a fit over the interval from 40 to 120 min.

Model input

Reference region

In [^{11}C]flumazenil studies, pons is most often used as reference region, because it provides reasonable binding estimates with acceptable coefficients of variation and is easier to define anatomically than hemispheric white matter (Abadie et al., 1991; Abadie et al., 1992). In displacement studies the binding in pons was not changed but the binding in other regions was reduced to the level of pons (Persson et al., 1985). Yet, pons may contain a significant amount of benzodiazepine receptors (Braestrup et al. 1977; Alkire & Haier 2001; Millet et al. 2002). The values used by Alkire & Haier (2001) are listed in Appendix A. Hall et al. (1992) estimated that pons region has about 2% benzodiazepine receptors compared with frontal cortex. Delforge et al. (1995, 1997) estimated that B'_{max} in pons is 5-7 % of cortical values, and the *BP* derived from their results is about 1.1. *In vitro* benzodiazepine binding densities in cortical regions are about 3.5-5 times higher than in pons (Alkire & Haier 2001).

Delforge et al. (1996, 1997) have corrected the pons curve for bound tracer using previously estimated (Delforge et al., 1995) model parameter values and average

plasma curve. They estimated that the percent of bound ligand in pons is 52% with very small injected mass of flumazenil. However, because the receptor sites also affect the free concentration, the correction was simplified by subtracting the pons curve with the bound concentration estimated at 50 min after injection (Delforge et al., 1996).

As an alternative, Magata et al. (2003) have used white matter, when calculating regional binding potential from *DV* values derived from plasma-input Logan plots. Their estimate of DV in white matter (brain stem) was 0.84 ± 0.33. Hammers et al. (2003) have found differences also in white matter DV in certain forms of epilepsy. Klumpers et al. (2005) have used white matter and cerebellum as reference tissue with simplified reference tissue model (SRTM), but did not yet report the results from reference region comparison. Benzodiazepine binding in white matter (corpus callosum) may be about 70% of pontial values (Alkire & Haier 2001). Previously, Lassen et al. (1995) have considered white matter not suitable as a reference region, because it cannot a priori be assumed to have the same non-specific distribution volume (in this case $DV_F + DV_{NS}$) of the tracer as the grey matter. They estimated that BP in white matter is about 0.2 (Lassen et al. 1995).

Reference region can be used in analysis if the distribution volume of free tracer, or free tracer and non-specifically bound tracer, is assumed to be the same in all brain regions, as suggested by Lassen et al. (1995). Millet et al. (1995) measured with a multi-injection protocol and five-rate constant model in healthy subjects, that k_1/k_2 did not show any correlation with B_{max}'.

Arterial plasma

Sanabria-Bohórquez et al. (2000) have suggested omitting the measurement of plasma metabolite fractions, but instead using a mathematical metabolite correction by fitting several regions simultaneously, assuming that [^{11}C]flumazenil fraction in plasma can be modelled by a mono-exponential function plus a constant. The method was validated in a three-injection imaging protocol, but for single-injection protocol the validation was based on simulated data only (Sanabria-Bohórquez et al., 2000).

Suggested computation methods

Parametric *BP* images were produced and compared with several different methods in the studies of Millet et al. (2000a, 2000b, 2002). Parametric DV images were computed using weighted integration method by Koeppe et al. (1991) and using linearized one-tissue compartment model by Salmi et al. (2004).

Graphical analysis (Logan plot) with plasma input

Graphical analysis is independent on the compartment model structure or tissue homogeneity inside the defined region of interest. In the graphical analysis the measured dynamic data is converted into a linear plot, the slope of which represents the DV. However, the effects of plasma protein binding and non-specific binding in tissue are inherent in the DV, in addition to the receptor density and the affinity. Either of these binding effects can be corrected by dividing or subtracting the DV

values by DV from a receptor-free reference region (Laruelle 2000), if data from such a brain region are available.

Magata et al. (2003) used Logan plot with metabolite corrected plasma input to calculate DV values, and then calculated BP=DV_{ROI}/DV_{REF}-1, using white matter as reference region.

DV images have been computed using Logan plot and used as such in SPM analysis by Ihara et al. (2004).

Logan plot with reference region input

Millet et al (2002) have studied several reference input, including the model with two-compartments for reference region, and found a underestimation of BP values especially in regions with low receptor densities, when compared to plasma input methods.

Simplified reference tissue model (SRTM)

Klumpers et al. (2005) have used regional SRTM analysis with pons or white matter as reference region, and found a good correlation (Pearson correlation 0.997 and 0.988, respectively) between BP from SRTM and DV from the two-tissue compartment model with arterial plasma input. They suggest using SRTM with pons as reference region to be used in clinical studies.

Lucignani et al. (2004) have calculated BP images using SRTM with pons as reference region.

Gyulai et al.

Gyulai et al. (2001) estimated regional *DV* by fitting a two-compartment model (K_1 and k_2') to the tissue and metabolite corrected plasma data. Because *DV* incorporates both receptor specific and non-specific binding, they calculated DV_{RATIO} as DV_{ROI}/$DV_{NONSPECIFIC}$. This is the same as DV_3''+1 in the terms of Laruelle (2000). They obtained the *DV* for non-specific binding by correlating the measured DV_{ROI} values with the $GABA_A$-benzodiazepine site density data of Braestrup et al. (1977), and using the *Y* intercepts of the regression lines as $DV_{NONSPECIFIC}$. For unknown reason, Gyulai et al. (2001) did not use individual values of $DV_{NONSPECIFIC}$ in calculation of DV_{RATIO}, but instead averaged it across subjects for each experimental condition. Partial volume correction was not applied in their study, which may hamper the correlation of *DV* values and receptor density literature values. In addition, this method assumes that literature values of benzodiazepine receptor densities (B_{max}) are directly comparable to binding potentials measured with PET, which by definition are dependent on the density of *available* receptors (B_{max}') and the *affinity* of the receptor to the ligand (K_d). It is possible that affinity is different in different brain regions and with different B_{max}' (Delforge et al. 1995; Lassen et al., 1995; Millet et al. 1995, 2000), and it is even probable that GABA-benzodiazepine receptor affinity is changed by anaesthetics, or in epilepsy (Nagy et al., 1999).

The "$DV_{NONSPECIFIC}$" was used by Gyulai et al (2001) to divide the regional DV values. Contrary to their intention, this method normalizes the DV for binding to plasma proteins but leaves it vulnerable to changes in non-specific binding in brain (Laruelle 2000). To correct for non-specific binding in the brain, they should have calculated DV3' instead as $DV_{ROI}-DV_{NONSPECIFIC}$ (Laruelle 2000).

However, the principal idea in the method of Gyulai et al (2001) is correct, although not applicable as presented: If both DV and B_{max}'/K_d (close to or equal to BP, depending on the definition) are estimated, the DV can be plotted as a linear function of B_{max}'/K_d, which has slope DV_F and an intercept of DV_F+DV_{NS} (Koeppe et al. 1991). This method can only be applied to tracers like [^{11}C]flumazenil with very low non-specific binding (Koeppe et al. 1991).

Late time imaging

When two-compartment model is used, the study length may be set to 60 min, or even shorter in regional analysis. Results at 60 and 90 min were very similar to those at 120 min, and DV was underestimated only slightly when the analysis period was shortened to 30 min (Lassen et al. 1995).

PET image at a later time point (24-39 min from injection) and DV and B_{max}' images show close correlation (Millet et al. 1995). These "delayed activity maps" correspond only to relative indices of receptor density, and do not allow inter-subject or inter-group comparisons.

Comparisons of tissue [^{11}C]flumazenil concentrations, even when corrected for the injected dose or dose and subject weight, may be very misleading; for example Samson and Bernuau (1987) reported a uptake difference in hepatic encephalopathy, but the difference may well be explained by slower metabolism of [^{11}C]flumazenil in liver leading to higher concentration in plasma and thus in the brain.

Spectral analysis

Richardson et al. 1997 and Koepp et al. 1997 have applied spectral analysis and SPM to analyze differences in DV images in epilepsy patients. Spectral analysis has since then been widely used.

Wavelet filtering

Millet et al. (2000a, 2000b) have applied wavelet-based filter to produce parametric images of binding parameters (B_{max}', k_1, k_2, k_{on}/V_R, k_{off}) from multi-injection protocol studies.

Partial-saturation injection studies

Delforge et al. have developed a injection protocol suitable for producing quantitative images of receptor binding and affinity (Delforge et al., 1995, 1996, 1997). In this method the injected ligand dose is between a tracer dose and a saturation dose.

Analysis of displacement studies

The use of $[^{11}C]$flumazenil in benzodiazepine receptor occupancy studies was validated in a primate model by Brouillet et al. (1991). For regional analysis, two separate PET studies with plasma sampling in normal state and after displacement dosage of a receptor agonist, were analyzed assuming that only k_2'' was changed in the latter study (Malizia et al. 1996).

Friston et al. (1997) have suggested a preliminary model for analyzing dynamic $[^{11}C]$flumazenil displacement studies to statistical images.

Scatchard analysis

Before the development of above mentioned methods, B_{max}' and K_d were usually estimated separately using protocol which included at least two $[^{11}C]$flumazenil studies, one with high specific activity (low dose of cold ligand) and one with low specific activity (saturating dose of cold ligand) (Iyo et al., 1991). Time-activity curve of specifically bound tracer (bound) was calculated by subtracting pons curve (free) from other regional curves. Bound/free ratios were estimated either at the maximum of bound/free curve (e.g. Litton et al., 1993) or at certain time range, usually 15-40 min (e.g. Abadie et al., 1992). Usually a function, e.g. sum of four exponentials, was first fitted to the regional time-activity curves before subtraction (Litton et al., 1993). Bound/free ratios are then plotted against bound values (divided by specific activity) from the high and low specific activity studies, and the data points are connected with a line. The x axis intercept represents the B_{max}' value. This method is not easily applicable to producing parametric B_{max}' images, because it would require an exact realigning of the two PET studies (which look very different).

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