Binding kinetics of $^{123}$I[ADAM] in healthy controls: a selective SERT radioligand

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Abstract

$[^{123}]$IADAM (2-((2-((dimethylamino)methyl)phenyl)thio)-5-iodophenylamine) is a promising radioligand for in-vivo quantification of serotonin transporters (SERT) using single photon emission computed tomography (SPECT) in man. We performed tracer kinetic analysis in various brain regions to determine the optimum equilibrium time for SERT quantification with $[^{123}]$IADAM and SPECT. Radiosyntheses of $[^{123}]$IADAM were performed at MAP Medical Technologies Oy, Tikkakoski, Finland. Thirty healthy male volunteers (21–41 yr) received between 104 and 163 MBq $[^{123}]$IADAM intravenously as a bolus. Consecutively, multiple SPECT scans were performed between 14 and 420 min post-injection (p.i.) using a Siemens Multispect 3 camera. Reconstruction was performed applying filtered back projection with a Butterworth filter (cut-off 0.7, order 7) in 128 × 128 matrices. Regions of interest (ROI) were drawn manually on the individual T1-weighted magnetic resonance image (MRI) comprising midbrain/hypothalamus for specific binding to SERT, and the cerebellum as reference region. After re-orientation to the MRI, the ROI template was applied to SPECT studies. We generated time–activity curves for the ROI and calculated the ratio counts$_{target}$/counts$_{cerebellum}$ minus 1 ($V_3^a$) as a measure for specific SERT binding. Counts were corrected for applied activity, acquisition time and body-weight. Peak uptakes were observed between 14 and 50 min after bolus injection. Counts per voxel were highest in the midbrain/hypothalamus, 798 (max. 872, min. 728), whereas 462 counts per voxel (max. 599, min. 412) were measured in the cerebellum at a mean time of 31 min p.i. Stable values for $V_3^a$ reached 205–320 min p.i. Mean peak $V_3^a$ value was 1.43 (95% CI 171–230) for the midbrain/hypothalamus at 205 min p.i. $[^{123}]$IADAM is a useful ligand for in-vivo quantification of human SERT by means of SPECT, with a comparatively better signal-to-noise ratio compared to $[^{123}]$CIT. Our data suggest that the acquisition time for the SPECT scan is optimally, under pseudo-equilibrium conditions, between 205–320 min post-bolus injection of the tracer.

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Introduction

Abnormalities in the serotonergic system are involved in the aetiology of major depression, obsessive-compulsive disorders, anxiety, schizophrenia, as well as Alzheimer’s disease, drug addiction or eating disorders (Abi-Dargham et al., 1997; Hesse et al., 2004; Kasper et al., 1990, 2002; Neumeister et al., 2002; Tauscher et al., 2001). In addition to its association with pathophysiological processes, serotonin transporters (SERT) represent the main target for the widely prescribed selective serotonin reuptake inhibitors (SSRIs).

Single photon emission computed tomography (SPECT) and positron emission tomography (PET) are non-invasive methods of measuring protein molecules such as receptors, transporters or enzymes. The gathered information can provide a rationale for optimum dose-finding of various drugs (Tauscher and Kapur, 2001).
Still the most commonly used ligand for imaging the central nervous system 5-HTT in vivo is 2β-carbomethoxy-3β-(3-[[123]I]iodophenyl)tropane ([123]I]-β-CIT) (Laruelle et al., 1994a) and this radioligand has been very useful in demonstrating physiological (Pirker et al., 2000; van Dyck et al., 2004) as well as pathological neuropsychiatric conditions (Kasper et al., 2000; van Dyck et al., 2000). As [123]I]-β-CIT is not a very selective ligand and binds to both serotonin and dopamine transporters (DAT) the selectivity is not sufficient to distinguish between those two monoamine transporter sites. Therefore, there is an obvious need for more selective and easier to use radiotracers in the field of medical application.

In this SPECT study we used a highly selective SERT ligand, that was first synthesized by Oya et al. (2000): ADAM 2-((2-((dimethylamino)methyl)phenyl)thio)-5-iodophenylamine) provides a binding affinity >50000 times than that of DAT and noradrenaline transporters (NET). Labelling with [123]I]ADAM has proved to be a suitable SERT ligand for in-vitro autoradiographic studies (Lin et al., 2004; Ye et al., 2004a) as well as for in-vivo animal studies (Chalon et al., 2004; Lin et al., 2002; Ye et al., 2004b).

Recently published studies (e.g. Catarfau et al., 2005) point to the fact that [123]I]ADAM is a safe tracer for SPECT imaging, however, no large dataset has yet been obtained to verify the optimal time-period for the SPECT scan in relation to the tracer application. In this [123]I]ADAM SPECT study our aim was to investigate the kinetics of [123]I]ADAM in healthy individuals and to address the question of when to optimally perform the SPECT scan in a conventional medical setting.

Methods

[123]I]ADAM was synthesized at MAP Medical Technologies Oy, Tikkakoski, Finland as previously described (Oya et al., 2000). The amount of radioactivity in each syringe containing [123]I]ADAM was measured in a dose calibrator before and after injection.

Thirty healthy male volunteers with an average age of 25.75 yr (± 4.25 yr s.d.) who were not taking any medication were included in the study. In each of the volunteers, organic diseases and neurological and psychiatric disorders were ruled out by using clinical history, physical examination including blood chemical study and performing a structured clinical interview (M.I.N.I.; Sheehan et al., 1998) by an experienced psychiatrist. The study protocol and all other procedures involved were approved by the local ethics committee of the Medical University of Vienna (EK no. 165/2003). After receiving detailed information about the study, all subjects gave written informed consent to all procedures prior to inclusion in the study. One hour prior to the injection of the radiolabelled ligand all healthy individuals had been given 40 drops of sodium perchlorate p.o. in order to reduce [185] uptake in the thyroid, each subject received between 104 and 154 MBq [123]I]ADAM intravenously as a bolus.

SPECT studies were performed using a three-headed rotating scintillation camera (Siemens Multipuct 3) equipped with medium-energy collimators. The subjects' heads were adjusted in a constant position by means of a crossed laser beam system. For the first scans 14–50 min post-injection (p.i.) of [123]I]ADAM, image acquisition was started and images were obtained for 30–40 min (30 s per frame), 3–5 consecutive scans per individual were performed between 14 and 420 min p.i. (first scan 14–50 min p.i., second scan: 118–150 min p.i., third scan 180–266 min p.i., fourth scan 280–373 min p.i., fifth scan 407–420 min p.i.), indicated times reflect scan start. For each scan, a total of 180 frames were collected. Attenuation correction was performed assuming homogeneous attenuation (attenuation factor 0.12/cm) within an ellipse drawn around the head contour (Chang, 1978). Thereafter, 3.5-mm-thick cross-sections oriented parallel to the cantho-meatal plane were reconstructed by filtered back projection (Butterworth Filter, order 7, cut-off 0.7) in 128 × 128 matrices.

In each subject anatomical magnetic resonance image (T1-weighted, matrix 256 × 256) was co-registered to the SPECT image using RVView (Studholme, 1999). Regions of interest (ROIs) including the midbrain/hypothalamus and the cerebellum were drawn by using a template on co-registered MRIs by a single examiner blind to the clinical data. These ROIs were transferred to the corresponding SPECT images.

Data were analysed as the mean count rate in the ROI. Counts in midbrain/hypothalamic regions were calculated in three consecutive axial slices using the highest mean values to avoid tilting errors. The three midbrain/hypothalamic ROIs (three slices) and the six cerebellar ROIs (three slices, left + right) were each pooled together, then the average counts per pixel were calculated. Because of the very low density of SERT in the cerebellum the cerebellar ROIs are considered to represent non-specifically bound and free radioactivity, whereas the midbrain/hypothalamic ROIs indicate total bound plus free radioactivity (Backstrom et al., 1989).

Brain regional uptake was calculated as counts per minute (cpm)/MBq injected dose, corrected for
physical decay and body mass (cpm/pixel . MBq × kg). Time–activity curves were generated for each ROI. Subsequently the ratio of specific midbrain/hypothalamic uptake to non-specific uptake \( \left( V3^a = \frac{\text{ratio counts}_{\text{target}}}{\text{counts}_{\text{cerebellum}}} - 1 \right) \) was calculated as outcome measure. This equilibrium analysis has been established for other radiotracers that share a comparable half-life with \([^{123}\text{I}]\text{ADAM}\) (Laruelle et al., 1994b).

A smoothed curve describing the dependence of \( V3^a \) on time was obtained by linear regression including the predictors time, time² and time³. For corrected (corr.) \( \text{counts}_{\text{target}} \) and corr. \( \text{counts}_{\text{cerebellum}} \) only time and time² were used as predictors. Corr. \( \text{counts}_{\text{cerebellum}} \) was log-transformed before analysis because of its skew distribution. Confidence intervals (CIs) for the smoothed curves were found by resampling (Davison, 1997), i.e. by analysis of 2000 random samples of 30 probands each, drawn with replacement from the original sample of 30 probands. For each time-point between 15 and 420 min p.i. a 95% CI for the smoothed curve was obtained from the 2000 resamples using the quantile method (Davison, 1997). A 95% CI for the time of maximum ratio was similarly obtained by using the maxima of the smoothed curves in each of the 2000 resamples.

Test–retest reliability was assessed by obtaining two independent series of measurements of \( V3^a \) from four individuals. The mean, minimum and maximum differences were calculated. Additionally, the intra-class correlation coefficient (Cronbach’s \( \alpha \)) and an associated 95% CI was calculated, as well as the percentage in differences:

\[
\text{% differences} = 100 \times \frac{\text{retest} - \text{test}}{\text{test}},
\]

and the percentage in variability (S.D. of % differences).

To compare differences between counts/pixel in midbrain and cerebellum a paired test was performed with \( p \) values <0.05 indicating statistical significance.

The statistical software packages R (www.r-project.org) and SPSS (SPSS Inc. Chicago, IL, USA) were used for statistical analysis.

**Results**

Initially, within the first 20 min p.i., the images seemed to display similar pattern as cerebral blood flow. Subsequently, accumulation of \([^{123}\text{I}]\text{ADAM}\) uptake mainly concentrated in regions that dispose high SERT densities such as the midbrain/hypothalamic areas whereas the cerebellum (the structure we used as a reference region), showed low levels of activity (Figure 1).

Averaged midbrain/hypothalamic uptake (counts per pixel, corrected for body mass, time and physical decay) showed subsequently higher uptake than the other structures in the brain, which became more prominent throughout the time scan. After peak
uptake of $^{123}$IADAM within the first 20 min p.i., midbrain/hypothalamus uptake declined quite steeply in the first 205 min, followed by a steady decrease thereafter that remained constant for up to 320 min. Counts per voxel reached 798 (max. 872, min. 728) in midbrain/hypothalamus for the first pooled time-point (31 min p.i.), whereas the peak uptake of cerebellum ranged at 462 (max. 599, min. 412) counts per voxel.

Cerebellar uptake (Figures 2 and 4b) paralleled the midbrain/hypothalamus uptake curve after a slightly steeper washout during the first 200 min. A statistically significant difference ($p<0.001$) between uptake in midbrain/hypothalamus and cerebellum could be shown for the period of peak equilibrium that was established.

Whereas the average changes in midbrain/hypothalamus and cerebellar uptake between 205 and 320 min were low, washout in the first 200 min in the cerebellum was slightly more pronounced. Consequently, the average ratio of specific midbrain/hypothalamic uptake to non-specific uptake (V3 = ratio counts$_{target}$/counts$_{cerebellum}$ - 1) increased significantly ($p=0.027$) by 0.55 from the first scan (14–50 min p.i.) to the third one (190–288 min p.i.) and reached its maximum value at 205 min p.i. at 1.43 (95% CI 171–230), this peak was subsequently followed by a decline of 7.7% until 315 min p.i. (V3 = 1.32). For that period of time, a peak equilibrium of $^{123}$IADAM uptake between target and reference region could be observed (Figures 3 and 4).

A test–retest study in four healthy subjects scanned showed a mean change in ratio of $-0.04$ (min. $-0.23$, max. 0.13), which corresponds to an intra-class correlation coefficient of 0.95 (95% CI 0.22–1.0); mean per cent difference $-2.6\%$ (s.d. = $\pm10.1\%$).

Discussion

Our dataset was obtained in 30 healthy human subjects. We could demonstrate that the optimal time-period to perform an $^{123}$IADAM SPECT in a clinical setting lies in the range of 205–320 min p.i. During this period of time a peak equilibrium is established that allows the estimation and quantification of the specific
binding potential of the tracer. The highest specific binding was detected in the midbrain/hypothalamus and the optimum scan duration was 30 min (Figure 3). This period of time is both acceptable for a patient suffering from a neuropsychiatric disorder to be situated in a restraint position in the SPECT camera and guarantees enough cpm/pixel to quantitatively analyse the SPECT study.

We could confirm findings of animal studies (Chalon et al., 2004; Lin et al., 2002; Ye et al., 2004b) that this recently established SERT tracer has proved to be a safe ligand. The findings stand in line with previous in-vitro kinetic studies that showed the high affinity of ADAM for the SERT. $^{[123]}$IADAM has been shown to bind highly selectively to SERT: saturation binding to rat frontal cortical membrane gave a $K_d$ value of 0.15 nM, and a $B_{max}$ of 194 fmol/mg (Choi et al., 2000). In another investigation, the affinity of ADAM to membrane preparations expressing SERT displayed a $K_d$ of 0.013 nM. The selectivity against SERT was 1000-fold higher than for NET or DAT – $K_i$ = 699 and 840 nM for NET and DAT respectively (Oya et al., 2000). $^{[123]}$IADAM also exceeded the $K_i$ values of $\beta$-CIT that reach 6.3 nM for the inhibition of $[^3H]$dopamine at DAT, 29.2 nM of 5-HT at SERT and 33.0 nM for norepinephrine at NET (Okada et al., 1998).

To obtain the specific/non-specific binding ratios we performed calculations based on a simple ratio method (Pirker et al., 2000). Carson et al. (1993) demonstrated that, if the plasma concentration of a tracer is constant for a sufficiently long period of time, the regional activity ratios will become equal to the ratio of the regional equilibrium distribution volumes. Under these conditions, if a region of reference devoid of receptors can be used as a measure of the non-specific binding, the specific/non-specific ratio is numerically equal to $V_3^{a}$. Laruelle et al. (1994b) showed that the error associated with this equilibrium analysis was acceptable for plasma tracer terminal half-lives $>$10 h. As $^{[123]}$IADAM provides a half-life of $\sim$13–14 h (Eersels et al., 2005) we could also choose this model that has been established for $^{[123]}$I$\beta$-CIT (Laruelle et al., 1994b). Furthermore this model has been previously verified for a derivate of $^{[123]}$IADAM (Acton et al., 1999).

It can be argued that invasive kinetic models have several advantages over non-invasive ones. However, it has to be considered that if arterial sampling is required this not only results in considerable discomfort for the patient, but also in substantially more technical demand. Apart from the need of accurate determination of plasma metabolites as well as of the free fraction of plasma unmetabolized parent radioligand, arterial sample measurements often appear to be less reproducible (Ichise et al., 2001). In a preliminary SPECT study Frokjaer et al. (2004) demonstrated good correlation between full kinetic modelling and a simplified reference tissue model in humans.

Animal studies that have been performed on $^{[123]}$IADAM showed specific binding of the tracer in the regions of midbrain and hypothalamus, where the...
peak of specific binding was observed (120–240 min p.i.) (Choi et al., 2000). Kung et al. (2004) found a midbrain/cerebellum ratio of 1.66 ± 0.02 after the radiotracer reached its equilibrium, this result is similar to previous studies in baboons (Acton et al., 2001). Data obtained from healthy human volunteers are available on the radiation dosimetry as well as the biodistribution of [123I]ADAM based on whole-body scans (Kauppinen et al., 2003; Newberg et al., 2004). Newberg et al. found a midbrain/cerebellum ratio of 1.95 ± 0.13, Catafau et al. (2005) measured a lower midbrain/cerebellum ratio of 1.21 ± 0.13. These figures stand in line with our findings as the midbrain/cerebellum ratio of even 2.43 (95% CI 171–230) could be reached while the peak equilibrium was being established.

In summary our findings show that [123I]ADAM is a specific ligand for quantification of SERT by means of SPECT in vivo, and a valuable neuroimaging tool in clinical routine. However, compared with PET ligands such as [11C]DASB, [123I]ADAM may be limited in imaging SERT in high SERT density areas (Ichise et al., 2003). Although this ligand does not seem a suitable choice to visualize limbic cortical and subcortical regions such as amygdala and hippocampus, the field of application for [123I]ADAM SPECT seems wide considering the number of psychiatric disorders including abnormalities in the serotonergic system.

Since preliminary data of studying the density of SERT in patients suffering from major depressive disorder has been obtained (Newberg et al., 2005; Uebelhack et al., 2005), our kinetic study provides a sounder base for additional in-vivo studies (Catafau et al., 2005; Erlandsson et al., 2005) investigating SERT occupancy under SSRI treatment in healthy individuals as well as in patients undergoing anti-depressive pharmacotherapy.

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Statement of Interest

None.

References


