In vivo quantification by SPECT of $^{[123]}I$ ADAM bound to serotonin transporters in the brains of rabbits

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Abstract

Background: A novel radioiodine ligand $^{[123]}I$ ADAM (2-((2-((dimethylamino)methyl)phenyl)thio)-5-iodophenylamine) has been suggested as a promising serotonin transporter (SERT) imaging agent for the central nervous system. In this study, the biodistribution of SERTs in the rabbit brain was investigated using $^{[123]}I$ ADAM and mapping images of the same animal produced by both single-photon emission computed tomography (SPECT) and microautoradiography. A semiquantification method was adopted to deduce the optimum time for SPECT imaging, whereas the input for a simple fully quantitative tracer kinetic model was provided from arterial blood sampling data.

Methods: SPECT imaging was performed on female rabbits postinjection of 185 MBq $^{[123]}I$ ADAM. The time-activity curve obtained from the SPECT images was used to quantify the SERTs, for which the binding potential was calculated from the kinetic modeling of $^{[123]}I$ ADAM. The kinetic data were analyzed by the nonlinear least squares method. The effects of the selective serotonin reuptake inhibitors fluoxetine and p-chloroamphetamine (PCA) on rabbits were also evaluated. After scanning, the same animal was sacrificed and the brain was removed for microautoradiography. Regions-of-interest were analyzed using both SPECT and microautoradiography images. The SPECT images were coregistered manually with the corresponding microautoradiography images for comparative study.

Results: During the time interval 90–100 min postinjection, the peak specific binding levels in different brain regions were compared and the brain stem was shown to have the highest activity. The target-to-background ratio was $1.89 \pm 0.02$. Similar studies with fluoxetine and PCA showed a background level for SERT occupation. Microautoradiography demonstrated a higher level of anatomical details of the $^{[123]}I$ ADAM distribution than that obtained by SPECT imaging of the rabbit brain.

Conclusion: SPECT imaging of the rabbit brain with $^{[123]}I$ ADAM showed high affinity, high specificity, and favorable kinetics. The time-activity curve showed that the accumulation of the $^{[123]}I$ ADAM in the brain stem reached a maximum between 90 and 100 min postinjection. The microautoradiography provides high-resolution images of the rabbit brain. Our results for the $^{[123]}I$ ADAM biodistribution in the rabbit brains demonstrate that this new radioligand is suitable as a selective SPECT imaging agent for SERTs.

Keywords: $^{[123]}I$ ADAM; SPECT; Microautoradiography; Serotonin transporters

1. Introduction

In recent years, depression has become a significant focus for the public. How to diagnose this disease early and correctly is an important objective in nuclear medicine [1]. The serotonergic system is implicated in many psychiatric and neurological disorders [2–6]. The concentration of synaptic serotonin is directly controlled by its reuptake into the presynaptic terminals and dysregulation of serotonin transporter (SERT) expression or function, leading to a decreased serotonergic neurotransmission, which has been proposed to play a key role...
Repeatability are the main advantages of this system [27–29]. It provides a real-time biodistribution of the radiotracer and the effect of fluoxetine and PCA. The information from the images of living brain can be compared with microautoradiography studies of the rabbit brain [1,30].

In this study, the in vivo binding properties of $^{123}$I ADAM using SPECT and the suitability of using $^{123}$I ADAM for the determination of PCA effect on SERT were evaluated in rabbits.

2. Materials and methods

2.1. Radiopharmaceuticals

The synthesis of $^{123}$I ADAM has been described in detail elsewhere [25,26].

2.2. Animals preparation

Rabbits (3.5–4 kg) were used in these studies. The animals were fed a complete pellet diet (Rabbit Chow Complete Blend, Purina Mills, Minneapolis MN) at a room temperature of 20°C with free access to water. The animal experiments were approved by the Laboratory Animal Care Panel of National Yang-Ming University.

The animals were fasted for 18 h before the scan and then anesthetized with an intramuscular injection of 0.125 ml/kg Rompun [2% w/v 2-(2,6-xylidino)-5,6-dihydro-4H-1,3-thiazine hydrochloride, Bayer, Leverkusen, Germany] and 50 mg/kg ketamine (50 mg/ml w/v ketamine hydrochloride, Sin-Ton Chemicals). During the study, anesthesia was maintained using an intramuscular injection of ketamine. An electric blanket was used to maintain the rabbit’s constant body temperature and was monitored carefully.

2.3. PCA-treated animals

The rabbits were given a high dose of PCA (catalogue no. C9635, Sigma, St. Louis, MO) of 10 mg/kg on 3 consecutive days. The rabbits’ temperature was maintained at 18±1°C for 4 h after each injection, and their behavior and response were evaluated for 5 days after each PCA administration.

2.4. SPECT imaging

SPECT imaging was carried out on a dual-head ADAC gamma camera (ADAC) with a low-energy ultrahigh-resolution parallel-hole collimator (VXGP). The center field of view was 25.4 cm$^2$ and a single energy centered window was used at 159 keV, with a width of 20%. A sequence of scans (22 min/frame×6) was obtained over a period of 150 min.

SPECT proceeded immediately after injections of 185 MBq $^{123}$I ADAM. Images were reconstructed in a 128×128 format from data with 32 projections distributed over 180° around the rabbit and a 40 s scan for each projection. The projections of each experiment were...
processed by reconstruction using filtered backprojection, with a low-pass Butterworth filter of order 22.4 and cutoff frequency of 0.43. The Chang’s first-order method with the precalculated attenuation ellipse was applied to correct the effect of photon attenuation. The summed images of the transverse slices were analyzed. Each transverse image was reconstructed in a $128^2$ array with a pixel size of 1.9 mm and a zoom of 2.0.

Four rabbits were studied with a total of 24 scans. Each rabbit had three control scans. Two rabbits were followed up with another three scans after treatment with fluoxetine (10 mg/kg). Another two rabbits were followed up with another three scans after treatment with neurotoxic PCA.

2.5. Magnetic resonance imaging

Each animal also had a single magnetic resonance scan to provide information on anatomic structure. The magnetic resonance scans were acquired on a 1.5 T instrument with a spoiled gradient echo acquisition in steady-state sequence that produces $0.7^2$ mm voxels, with a slice separation of 1.0 mm. The magnetic resonance scans were resized and resliced in planes parallel to the bregma.

2.6. Image analysis

The transverse slices of the SPECT images were used to draw regions-of-interest (ROIs) of the midbrain and cerebellum, with guidance from magnetic resonance images of a
rabbit and a rabbit atlas [32]. The time-activity curve from the SPECT image was used for the quantification of SERT bound \([^{123}\text{I}]\) ADAM to derive a signal-to-background ratio. The effects of the SSRI, fluoxetine, and neurotoxic, PCA, on the rabbits were also evaluated. After each scan, the animal was killed and its brain was removed for microautoradiography. ROIs were analyzed using both SPECT and microautoradiography images. SPECT images were compared side by side with corresponding microautoradiography images.

2.7. Tracer kinetic modeling

2.7.1. Plasma metabolite studies

Before the injection of \([^{123}\text{I}]\) ADAM, a control arterial blood sample was withdrawn and used as the baseline to measure the metabolism rate of the radioactive ligand. During the scan period, arterial blood samples of 1 ml were performed manually for the first 15 min, with individual samples taken every 1 min. After this time, arterial blood samples were withdrawn at 20, 30, 40, 50, 60, 70, 80, 90, 95, 100, 105, 110, 130, 140, and 150 min after injection of the tracer.

Blood samples were kept on ice and spun down in a centrifuge at 2500 rpm for 5 min. The plasma (500 µl) was counted in a gamma counter (1277 Gamma Master, Pharmacia, Uppsala, Sweden) to calculate the percentage of remaining radioactivity.

2.7.2. Parametric images

The two-tissue, three-compartment model was adopted. The tissue concentration \(C_1\) is related to the metabolite-corrected plasma concentration \(C_{ad}\) and the specifically bound receptor compartment concentration \(C_2\) for the transporter containing region as shown in Fig. 1A. We assumed that nonspecifically bound radioactivity in the second compartment \((C_1)\) equilibrates rapidly with free tissue radioactivity and then merged the free and nonspecific binding compartment with specific binding compartment into a single compartment \((C_1')\) as shown in Fig. 1B. The kinetic equations of the radiosubstance concentration in different compartments were calculated as shown below (Eqs. 1–4).

This in vivo system consists of back and forth delivery rate constants \((K_1, k_2)\) and the forward \((k_3)\) and
dissociation ($k_4$) rate constants of $[^{123}I]$ ADAM as shown in Fig. 1.

$$
\frac{dC_{ad}}{dt} = -K_1 C_{ad} + k_2 C_1
$$

$$
\frac{dC_1}{dt} = K_1 C_{ad} - (k_2 + k_3)C_1 + k_4 C_2
$$

$$
\frac{dC_2}{dt} = k_3 C_1 - k_4 C_2
$$

and

$$
\frac{dC'_1}{dt} = K'_1 C_{ad} - k'_2 C'_1 (t)
$$

where $K_1$ and $K'_1$ are delivery rate constants and $k_2$, $k'_2$, $k_3$, and $k_4$ are the first-order kinetic rate constants, and binding potential (BP) is defined in below:

$$
BP = \frac{k_3}{k_4}
$$

All parameters were determined by the nonlinear least squares method implemented in composed MTLAB 6.1 (MathWork, Natick, MA) to estimate the value of $k_3/k_4$ (BP).

2.8. Microautoradiography

Each rabbit was injected with 2 ml saline solution containing 185 MBq $[^{123}I]$ ADAM via the ear vein. Animals were sacrificed with chloroform at 100 min postinjection, and brains were immediately removed and frozen for 15 min with dry ice. The frozen brain was further embedded on a cryostat holder (5×5 cm) with OCT (optical cutting temperature, catalogue no. 4583, Sakura Finetechical, Tokyo, Japan). The embedded brain was set on the quick freezing stage (−30°C) for 30 min in a cryomicrotome (CM3050, Leica, Bensheim, Germany). Consecutive frontal sections were made with a thickness of 20 μm. The sectioned slices were transferred onto the imaging plate (BAS-SR2040, Fuji Photo Film, Tokyo, Japan) and were exposed for 15–20 h. The imaging plates were then scanned with a high-resolution imaging plate reader (FLA5000, Fuji Photo Film) at the following settings: a resolution of 25, a gradation of 16 bits, a dynamic range of L5, and a sensitivity of 10,000. Imaging contrast was adjusted with a tone curve using the bioimaging analyzer MacBAS v2.5 (Fuji Photo Film).

2.9. Statistical analysis

The ratio of relative specific binding at different regions was recorded and tested for statistically significant differences between normal subjects and drug-treated rabbits using paired Student’s $t$ test. Summaries of study variables were reported as mean±SD.

3. Results

3.1. SPECT studies in rabbits

After the injection of $[^{123}I]$ ADAM, there was rapid and high uptake of the ligand in the brain. The regions that accumulated $[^{123}I]$ ADAM are known to contain a high

![Fig. 5. This serum time-activity curve was used as input function for the 150 min scan. This shows an activity of about 15% of injected dose that remained in arterial blood at 15 min.](image)
density of SERTs (Fig. 2). The highest counts were in midbrain. The specific binding curve reached a peak between 90 and 100 min with a slow washout thereafter averaged over the three control scans. All specific binding counts were subtracted from cerebellum. The specific binding ratio curve of the midbrain is shown in Fig. 4. At 100 min postinjection, the peak in specific binding in the different brain regions are compared and the midbrain shows the highest activity, whereas the cerebellum shows the lowest. The ratio of midbrain to cerebellum increased with time until a peak ratio of 1.66:1 at 100 min postinjection was found.

3.2. Pharmacological blocking study

When the studies were repeated after pretreatment with either high-dose fluoxetine (10 mg/kg) or PCA (10 mg/kg), the occupation of SERTs by the ligand was reduced to the background level. (Fig. 3) The known SERT ligand, fluoxetine, completely inhibited binding of [123I] ADAM. A comparison between the control and the fluoxetine studies showed a specific binding ratio of 2.27. Systemic administration of neurotoxic PCA destroyed most serotonergic neurons. The lesioned rabbits showed a decreased appetite with lower activity.

3.3. Tracer kinetic modeling

The metabolism of [123I] ADAM was relatively fast. Within 10 min after injection of [123I] ADAM, activity in arterial blood was at only about 4% of the injected dose activity (Fig. 5), which fell to 3% at 40 min and remained at this low level for up to 150 min. For kinetic analysis, we used SPECT images by drawing the ROIs of midbrain and cerebellum to derive the specific binding rate curve and to calculate the $K_1$, $k_2$, $k_3$, $k_4$, and BP ($k_3/k_4$; Table 1). The BP value was compared between the control and the drug-treated rabbits. The control (1.89 ± 0.02) has a more than 2-fold higher BP value compared with fluoxetine-treated (0.83 ± 0.84) and PCA-treated (0.72 ± 0.17) animals. The result indicated a high concentration of SERTs in the specific areas and a high specificity for SERTs. This simple and modified method of quantifying SPECT images on small animals may provide more information on biodistribution and the time to reach the equilibrium state of the radiotracer.

3.4. Autoradiography of rabbit brains

The microautoradiography demonstrated greater anatomical details of the [123I] ADAM distribution than the
SPECT imaging. The autoradiograms of rabbit brain section showed specific intensities in several regions such as the olfactory tubercle, lateral septal nucleus, hypothalamus, thalamic nuclei, globus pallidus, central gray matter, substantia nigra, interpeduncular nucleus, and dorsal and median raphes (Fig. 6). These areas are known to have a high density of SERT sites. The frontal cortex, caudate putamen, and hippocampus were found to have a much lower accumulation of $^{[123]}$I ADAM.

In the midbrain region, the SPECT images of both the control and the drug-treated animals were compared by autoradiography (Fig. 7). A good one-to-one correlation was found among the images.

4. Discussion

In this study on a rabbit model, we confirmed a previous study [22] of baboon images that demonstrated selective, high-affinity binding of the SERT ligand $^{[123]}$I ADAM. After the injection of $^{[123]}$I ADAM, the results indicated that large amounts of ligand entered the midbrain, reached a peak value between 90 and 100 min postinjection and then gradually decreased [23,33]. Although the activity in the cerebellum was similar to that in midbrain initially (Fig. 4), the former rapidly dropped to background values later. As the specific binding rate rose continuously, the activity difference between midbrain and cerebellum was seen at about 50 min postinjection and reached a peak value after 100 min. From then on, it remained at about 2:1 ratio, comparing untreated with fluoxetine-treated rabbits.

Our rabbit brain model studies indicate that $^{[123]}$I ADAM can quickly pass the blood brain barrier to the target area, the midbrain. After the radiotracer is in equilibrium, it maintains a stable contrast value of 1.66±0.02 until the study was completed. This result is similar to previous studies in baboons stated below. A peak specific uptake of $^{[123]}$I IDAM in the midbrain occurs at around 120 min, with a mean ratio to cerebellum in baboons of 1.80±0.13 from 120 to 140 min [22]. The PET tracer $^{11}$C McN5652 showed an average midbrain to cerebellum ratio in baboons of 1.5 at 95–125 min [15]. After injection, the radioactivity value in the arterial blood of rabbit increases quickly up to 10 min and does not change significantly afterward. This indicates that $^{[123]}$I ADAM is quickly absorbed and stayed in equilibrium. Radioactivity level in cerebellum, a brain region devoid of SERT sites [34], was unaffected by fluoxetine and PCA pretreatment, confirming that cerebellum can be used to estimate the nonspecific binding of $^{[123]}$I ADAM.

Experience with SPECT imaging of SERTs in depression patients is steadily increasing. The first study used well-known $^{123}$I β-CIT and its BP can be simply quantified using the ratio between specific and nonspecific activities during the plateau phase of tracer uptake [13]. However, the prolonged wait for highest striatal uptake before SPECT can be performed is not easy to apply in clinical practice. Semiquantification was not enough to provide better information about density of the SERTs. In this study, a tracer kinetic model was identified using a different method from the previous study [35]. It should be noted that distribution volume measurements of $^{[123]}$I ADAM were calculated using a revised TLC method for analysis of plasma $^{[123]}$I ADAM and its metabolites, the results of which are used as the input function [35]. With this method, there is no need to remove any systematic error from the arterial blood measurements [35]. In this study, we reported that the control (1.89±0.02) has a more than 2-fold higher BP value compared with fluoxetine-treated (0.83±0.84) and PCA-treated (0.72±0.17) animals in rabbit brain. In contrast to our finding, Ginovart et al. reported that the BP of $^{11}$C DSAB in cats was 2.2 and reduced up to 90% following pretreatment with fluoxetine [19]. $^{123}$I nor-β-CIT (2.04±0.10) showed lower BP values than $^{123}$I β-CIT (2.15±0.28) in rat brain [14]. A relatively low BP of $^{[123]}$I ADAM to SERT observed in the present study as compared with above-mentioned studies may be due to a species difference. Another possibility is that the lower observed BP derives from the tracer kinetic modeling used to estimate kinetic parameters.

Using quantification analysis of the physical phantom with different concentrations in the cylinders, we are able to verify the standard curve to validate the correlation between the standard curve of the phantom and that from the mean values in ROIs of the rabbit brains. Using the combination of the specific binding rate in the rabbit brain and its integration with the blood parameters, we are able to calculate the kinetic rate constants. An analysis using an increased rate of blood sampling over the initial 15 min postinjection should provide improved information on the $^{[123]}$I ADAM time-activity curve [35,36]. Sampling and analysis of the parent and metabolized compounds in the blood will help clarify the uptake equilibrium and to develop a more accurate pharmacokinetic model.

The biodistribution of $^{[123]}$I ADAM in the rabbit brain was compared with previous published articles for other SERT radioligands [11,12,16]. In various animal models, the best absorption time curve was from 90 to 100 min for mice and rabbits and they both showed high contrast in midbrain [22,23,25]. For best contrast in a drug-treated animal model, we chose a mouse model to build up the normal distribution and to quantify the effect in fluoxetine and PCA pretreated mice. $^{[123]}$I ADAM BP values in midbrain were reduced up to 56% and 62% following pretreatment with fluoxetine and PCA, respectively. This indicates that a major proportion of $^{[123]}$I ADAM binding in this region represents specific binding to SERTs. PCA-induced SERT BP reduction of $^{[123]}$I ADAM was higher (~62%) than pretreated with fluoxetine (56%). The 56% to 62% decrease in SERT specific binding rate during treatment is best explained by the high affinity of fluoxetine and PCA for SERT. Using $^{[123]}$I ADAM, a previous article reported 80% blockade of SERT in midbrain following a 0.3
mg/kg iv dose of fluoxetine in mice [26]. As a result of drug binding to SERTs, fewer SERT sites are available for [123I] ADAM binding, thus decreasing the detected SERT BP. Another reason for the SERT BP to decrease may be down-regulation so that SERT mRNA is reduced in animals after administration of fluoxetine [37]. Fluoxetine (Prozac) was reported as the most used antidepressant but has significant side effects, which were shown in a rabbit model of drug treatment. In this competitive study, the low count ratios (or the residual uptake) in the drug-treated cases could be due to limitations in the precise drawing of the ROIs. Mental patients are not eager to take these drugs and this causes certain difficulty in therapy. In this research, a high dose of fluoxetine (10 mg/kg) was used and this gave a very strong inhibitory effect. However, the physiological loading at such a level is a concern, especially to the kidney and liver, due to the drug’s long half-life; thus, the side effects of fluoxetine cannot be ignored [8]. The behavior of the rabbits was therefore monitored for at least 1 month. In the later period, two of the five rabbits started to lose their appetite and weight. It is a doubtful point whether it would be possible to observe any effect on the SERTs after long-term treatment with fluoxetine or other SSRIs. The long half-life of fluoxetine and its main active metabolite in plasma has been regarded as an advantage in the poorly compliant patient and might explain why fluoxetine treatment is less frequently associated with withdrawal symptoms and relapse on abrupt discontinuation when compared with shorter half-life SSRIs. Conversely, it also needs a long period of washout before introducing other drugs (especially monoamine oxidase inhibitors); thus, there can be an interaction with serotonin function and can lead to the serotoninergic syndrome [19].

Our results show that the use of the clinical SPECT after acute administration of PCA can detect a decrease in [123I] ADAM binding in normal rabbit SERT. Neurotoxic PCA has been shown to destroy 90% of the serotonergic neurons [9]. This irreversible effect causes a gross loss of accumulation of [123I] ADAM. The finding suggested a rapid modulation of SERT by [123I] ADAM, compatible with the general hypothesis that SERTs can play a neuromodulatory role in the central nervous system. The frequency of PCA treatment at a specific dosage and time interval may be a decisive factor in determining the level of damage to serotonin system. The PCA group was the most difficult to control and thereby the most difficult part of this study. The PCA lesion had to be made by continuous injection to induce the destruction of the serotonergic neurons [8,9,38]. Due to variation in dosage tolerance by different individuals, the mortality rate is very high. It is necessary to balance the repression effect against the fatal dose as shown with the animal model.

With this methodology, we have demonstrated that specific binding for [123I] ADAM in rabbit model can be shown by time-activity curves generated from SPECT images and can be detected by microautoradiography also. Based on the specific binding curve, it should be possible to predict the time point to process the autoradiograph of the rabbit brain and this will help us to find the ideal contrast value and avoid sacrificing a large number of animals. In autoradiography of rabbit brain, we assessed the biodistribution of SERTs and compared it with those in the mouse model, and a very high correlation was shown with the rabbit model. In terms of anatomy, the higher density areas of SERTs detected by [123I] ADAM are similar. Autoradiography was used because it gave high-resolution images and this helped in the comparison with the SPECT images. We used the same rabbit for the control, for the drug-treated sample, and for autoradiography, so that the influence of individual physiological differences is minimized. At the same time, with the use of complementary and multi-modality image information (SPECT/magnetic resonance imaging/autoradiography), we can draw an inference that these images provide not only evidence on how to use [123I] ADAM but also more complete information.

Although we are able to demonstrate that SERT can be detected semiquantitatively to an extent in regions with higher SERT density (at least in midbrain regions), the spatial resolution is certainly very low for true quantification of SERT number due to partial volume effect. However, this research will help us to establish a clear framework for future studies when we have a high-resolution small animal imager.

5. Conclusion

These results on a rabbit model showed that [123I] ADAM is highly selective for SPECT imaging of SERTs in the nonhuman primate brain. The time-activity curve showed that the accumulation of the [123I] ADAM in the brain stem reached a maximum at 90–100 min postinjection. The microautoradiography provided high-resolution images of each rabbit brain. The multiple modality imaging provided more information to evaluate the radiotracer.

In conclusion, these results suggested that [123I] ADAM has potential as a SERT ligand for SPECT imaging in rabbit brains. Future studies will aim at obtaining high-resolution images for drawing ROIs for use in calculation for tracer kinetic modeling.

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