

Biodistribution, toxicity and radiation dosimetry studies of the serotonin transporter radioligand 4-[¹⁸F]-ADAM in rats and monkeys

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Received: 30 June 2009 / Accepted: 4 September 2009 / Published online: 10 October 2009
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Abstract

Purpose 4-[¹⁸F]-ADAM is a potent serotonin transport imaging agent. We studied its toxicity in rats and radiation dosimetry in monkeys before human studies are undertaken. **Methods** Single and multiple-dosage toxicity studies were conducted in Sprague-Dawley rats. Male and female rats were injected intravenously with 4-F-ADAM as a single dose of 1,023.7 µg/kg (1,000 times the human dose) or as five consecutive daily doses of 102.37 µg/kg (100 times the human dose). PET/CT scans were performed in seven Formosa Rock monkeys (four males and three females) using a Siemens Biograph scanner. After injection of

4-[¹⁸F]-ADAM (182±8 MBq), a low dose CT scan and a series of eight whole-body PET scans were performed. Whole-body images were acquired in 3-D mode. Time-activity data of source organs were used to calculate the residence times and estimate the absorbed radiation dose using OLINDA/EXM software.

Results In the rats neither the single dose nor the five daily doses of 4-F-ADAM produced overt adverse effects clinically. In the monkeys the radiation doses received by most organs ranged between 7.1 and 35.7 µGy/MBq, and the urinary bladder was considered to be the critical organ. The effective doses extrapolated to male and

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female adult humans were 17.4 and 21.8 $\mu\text{Sv}/\text{MBq}$, respectively.

Conclusion Toxicity studies in Sprague-Dawley rats and radiation dosimetry studies in Formosa Rock monkeys suggested that 4- ^{18}F -ADAM is safe for use in human PET imaging studies.

Keywords 4- ^{18}F -ADAM · Serotonin transporter imaging agent · Toxicity · Radiation dosimetry

Introduction

Abnormalities in the serotonin transporter (SERT) have been implicated in several neurological and psychiatric disorders [1, 2]. It is also the target for selective serotonin reuptake inhibitors [3, 4]. In vivo SERT imaging in humans would assist in the early diagnosis and follow-up of treatment in these diseases [5]. In the 1990s, [^{11}C]-(+)-McN5652 was the most promising PET agent for studying SERT in humans [6–8]. However, this agent has high nonspecific binding and has only moderate signal contrast in human PET studies [9]. Its ^{18}F analogues, [^{18}F]FET-McN [10] and [^{18}F]FMe-McN [11] have shown some utility as SERT imaging agents in animals [12–14]. Recently, *N,N*-dimethyl-2-(arylthio)benzylamines (403U76) have been reported to possess very high selectivity and affinity for SERT binding sites [15]. Their analogues, [^{11}C]N,N-dimethyl-2-(2-amino-4-cyano-phenylthio)benzylamine ([^{11}C]DASB) [16], [^{11}C]N,N-dimethyl-2-(2-amino-4-fluoromethylphenylthio)benzylamine ([^{11}C]AFM) [17], [^{11}C]N,N-dimethyl-2-(2-amino-4-methylphenylthio)benzylamine ([^{11}C]MADAM) [18] and [^{11}C]N,N-dimethyl-2-(2'-amino-4'-hydroxymethyl-phenylthio)benzylamine ([^{11}C]HOMADAM) [19] were synthesized and found to be potential radioligands for studying SERT in humans [20–23]. Nevertheless, ^{18}F has some advantages over ^{11}C . Thus, ^{18}F -labelled 403U76 analogues such as (2-[2-amino-4-chloro-5- ^{18}F]fluorophenylthio)-*N,N*-dimethyl-benzenemethanamine ([^{18}F]ACF) [24], *N,N*-dimethyl-2-(2-amino-4- ^{18}F]fluorophenylthio)benzylamine (4- ^{18}F -ADAM) [25, 26], 2-[2-(dimethylaminomethyl)phenylthio]-5- ^{18}F]fluoromethylphenylamine ([^{18}F]AFM) [27], and 2-(2'-((dimethylamino)methyl)-4'-(3- ^{18}F]fluoropropoxy)phenylthio)benzeneamine [28] have been synthesized and evaluated in animals [24, 27–30] as potent SERT imaging agents. Preliminary studies in rats and baboons have shown that 4- ^{18}F -ADAM is suitable for studying SERT in the living brain using PET [29]. Thus, we studied the toxicity and radiation dosimetry of 4- ^{18}F -ADAM in rats and monkeys before human studies are undertaken. We report here the acute toxicity and radiation dosimetry of 4- ^{18}F -ADAM in rats and monkeys.

Material and methods

Acute toxicity studies of 4-F-ADAM in rats

Synthesis of 4-F-ADAM

The 4-F-ADAM was synthesized by Chemistry and Life Sciences Division, Research Triangle Institute, Research Triangle Park, NC, using a method reported previously [25] under contract with the National Institute of Mental Health (NIMH, contract number N01-MH-32005). The identity and chemical purity of this compound was confirmed by ^1H NMR, mass spectral elemental analysis, HPLC and TLC.

Acute toxicity studies

The toxicity studies were carried out in male and female Sprague-Dawley rats by SRI International Toxicology Laboratory, Menlo Park, CA, and were fully supported by NIMH (M422-05) under the Molecular Pharmacology Research Program.

Animal procedures conducted at SRI were approved by SRI's Institutional Animal Care and Use Committees (IACUC). SRI's animal facilities have been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). SRI's facilities are registered with the US Department of Agriculture as a Research Facility. SRI files assurances with the Public Health Service (PHS) Office of Laboratory Animal Welfare (OLAW) and adheres to PHS standards and practices.

Animals

Thirty male (6–8 weeks old, weighing 175–225 g) and 30 female (7–9 weeks old, weighing 150–200 g) Sprague-Dawley rats (Charles River Laboratories) were used in this study. Upon arrival, the rats were placed in quarantine for 3 days. The general appearance of the animals was evaluated by the attending veterinarian, who documented that the animals were healthy before releasing them from quarantine. The rats were fed with Purina Certified Rodent Chow (#5002; Richmond, IN) ad libitum. Water (purified, reverse osmosis) was provided ad libitum during quarantine and study periods. The rats were housed (five per cage) in stainless steel cages in an animal room that was monitored for temperature (65–79°F, 18–26°C) and humidity (30–70%) with a light cycle of 12 h light/12 h dark.

Preparation of dose formulation

The test article, 4-F-ADAM, was formulated in sterile phosphate-buffered saline (Gibco Invitrogen, Grand Island,

NY). Dose formulations were prepared by dissolving the appropriate amount of test article in the vehicle to achieve target concentrations using a sterile stir bar and sonication. Dose formulations were stored refrigerated at 2–8°C until the day of use. Formulations were brought to room temperature prior to administration to the animals. Drugs were administered via intravenous (i.v.) injection into a lateral tail vein. The i.v. route is intended for human clinical trials. Therefore, i.v. injection was selected to model the intended route of administration to humans.

Verification of formulation

All 4-F-ADAM formulations used for the toxicity studies were analysed by SRI staff using reversed-phase HPLC methods. The conditions were as follows: Luna C-18 analytical column (4.6×250 mm, 5 µm particle size); mobile phase linear gradient (solvent A/solvent B 85/15 for 10 min followed by 50/50 for 1 min and then 85/15 for 4 min); flow rate 1.0 ml/min; UV detector 245 nm; ambient temperature. Solvent A comprised 0.1% TFA in water, solvent B 0.1% TFA in CH₃CN. The HPLC system used was a Hewlett-Packard Model 1100 Series liquid chromatography system. Data were analysed using HP Chemstation Software; version A.06. Concentration, homogeneity, and stability of the formulation were confirmed before testing and were found to be within 10% of target concentrations for all treatment groups.

Dosage calculation

The procedures used to determine the 4-F-ADAM dosage to be administered to rats required that all dosages were converted from units of milligrams per kilogram body weight to units of milligrams per square metre body surface area. Basing the dosage on body surface area allows determination of an equivalent dosage in another species. The equation used was:

$$\text{dosage}(\text{mg}/\text{kg}) \times F = \text{dosage}(\text{mg}/\text{m}^2)$$

where F is a constant based on the species of animal being tested (a value of 37 for humans). Dosages were based on the body weight recorded on day 1 before dosing (and converted to milligrams per square metre), and preset multiples of the maximum proposed dose of 8.3 µg to be administered to a 50-kg person were administered to the test dosage groups (Table 1). The dosages used in these studies, expressed in both milligrams per kilogram and milligrams per square metre, as well as individual species F values are presented in Table 1.

Dosing procedure

Male and female rats (ten males and ten females per group) were given 4-F-ADAM i.v. as a single dose of 1,023.7 µg/kg (1,000 times the human dose) on day 1 or as five daily doses of 102.37 µg/kg per day (100 times the human dose) on days 1–5. Control rats (ten males, ten females) were given a single i.v. dose of vehicle (sterile phosphate-buffered saline) at an equivalent volume (5 ml/kg) on day 1. Dosing of the 5-day dosing group was initiated 4 days prior to dosing of the single-dose animals.

Observation protocol

All animals in this study were observed twice-daily on weekdays and once daily at weekends for signs of mortality, morbidity, injury, and availability of food and water. Detailed clinical observations were recorded daily throughout the study. Individual body weights were measured and recorded for each animal on day 1 prior to dosing and at necropsy (days 3 and 15 for groups 1 and 2, and days 7 and 19 for group 3). Food consumption was measured for a period of about 24 hours twice weekly throughout the study. Blood for clinical pathology evaluation was collected on days 3, 7, 15 and 19. Animals were killed on days 3 and 15 (interim and terminal necropsy of groups 1 and 2) or days 7 and 19 (interim and terminal necropsy of group 3) by overdose of sodium pentobarbital (150 mg/kg) administered by i.p. injection.

Clinical pathology

After collection from SRI, the clinical pathology samples were sent to Quality Clinical Labs (haematology and clinical chemistry parameters; Mountain View, CA) and AniLytics (coagulation parameters; Gaithersburg, MD). Blood samples were collected into tubes containing EDTA (haematology samples), sodium citrate (coagulation samples) or no anticoagulant (clinical chemistry samples).

Gross necropsy

On days 3, 7, 15 and 19, after recording body weight and collection of clinical pathology blood samples, animals were killed and given a complete post-mortem examination, which included a thorough inspection of all body orifices and surfaces and an examination of all cranial, thoracic and abdominal organs. The weights of the following organs were recorded: adrenal glands, brain, heart, kidneys, liver, spleen, thymus, testes and ovaries. All of the organs and tissues were retained and fixed in phosphate-buffered 10% formalin.

Table 1 Experimental dosage regimens and summary of mortality/morbidity and clinical observations for toxicity study of 4-F-ADAM in male and female rats

	Dosage regimen						
	Male rats			Female rats			
	Vehicle	1,000 × human dosage ^a	100 × human dosage ^a	Vehicle	1,000 × human dosage ^a	100 × human dosage ^a	
No. of animals	10	10	10	10	10	10	
Dose per day	By surface area (µg/m ²)	0	6,142	614.2	0	6,142	614.2
	By body weight (µg/kg)	0	1,023.7	102.37	0	1,023.7	102.37
No. of doses	1	1	5	1	1	5	
Total dose (µg/kg)	0	204.74	20.474	0	204.74	20.474	
Dose volume (ml/kg)	5	5	5	5	5	5	
Rats killed ^b							
Interim	Day	3	3	7	3	3	7
	No. killed	5	5	5	5	5	5
Terminal	Day	15	15	19	15	15	19
	No. killed	5	5	5	5	5	5
Appearance							
Normal	9	10	6	10	10	10	
Soft stool	1 (1)	–	4 (4, 5)	–	–	–	

^a Maximum human dose is 8.3 µg per 50 kg person, i.e. 0.166 µg/kg; $0.166 \times 37 = 6.142 \text{ g/m}^2$. Scaling for the rat gives $6.142/6 = 1.0237 \text{ µg/kg}$ as an equivalent human dosage. $1,000 \times 1.0237 = 1,023.7 \text{ µg/kg}$ as the high dosage.

^b Five rats in each group and of each sex were killed on day 3 (2 days following single dosing) and five on day 15 (14 days following single dosing). Five rats in each group and of each sex were killed on day 7 (2 days following 5-day dosing) and five on day 19 (14 days following 5-day dosing). Dosing of the 5-day dosing animals was initiated 4 days prior to dosing of the single-dose animals so that necropsies occurred on the same calendar day for all groups, thus allowing sharing of control clinical pathology and necropsy data between the two dosage regimens

– No observation.

Numbers in parentheses indicate the day(s) of first and last occurrence. If an animal was observed to be other than normal at any time during the study, it does not appear as normal in the table

Histopathologic examination

Sections of the retained tissues were embedded in paraffin, cut at a thickness of approximately 5 µm, and stained with haematoxylin and eosin by Environmental Pathology Laboratories (Herndon, VA). All tissues from animals in the control and high dosage groups were examined by a board-certified veterinary pathologist. If a tissue had gross findings at the time of necropsy, the same tissue from the low and mid dosage groups was also examined.

Statistical analyses

One-way ANOVA was performed for body weight, food consumption, organ weights and clinical pathology data (LABCAT In-Life v. 6.2). When appropriate, a post-hoc analysis (Dunnett's *t*-test) was carried out. In all cases, statistical significance was defined as $p \leq 0.05$.

Dosimetry studies of 4-[¹⁸F]-ADAM in monkeys

Synthesis of 4-[¹⁸F]-ADAM

The radioligand 4-[¹⁸F]-ADAM was synthesized in an automated synthesizer by fluorination of the corresponding nitro precursor with $\text{K}[\text{¹⁸F}]/\text{K}_{2.2.2}$ followed by reduction with $\text{NaBH}_4/\text{Cu}(\text{OAc})_2$ and purification by HPLC and solid phase extraction [25, 26]. The radiochemical yield of 4-[¹⁸F]-ADAM was $1.5 \pm 0.3\%$ ($n=13$, EOS) and the mean specific radioactivity was $64.8 \pm 28.5 \text{ GBq}/\mu\text{mol}$ ($n=13$, EOS) at the time of injection. The radiochemical and chemical purities were >95% and 98%, respectively, as determined by HPLC and TLC.

Animals

The animal study protocol used for this study was approved by the IACUC of the National Defense Medical College.

Seven fasted Formosan Rock monkeys (*Macaca cyclopis*) comprising three females (5.3 ± 1.5 kg) and four males (5.6 ± 0.8 kg) were immobilized with ketamine (2 mg/kg), anaesthetized with 2% isoflurane via an endotracheal tube, and given atropine sulphate (2 mg i.m.) to minimize secretions during the course of the experiment. Body temperature was kept constant at 37°C with an additional blanket. The heart rate, pO_2 and pCO_2 were checked every 10 min and kept in the normal range throughout the imaging sessions.

PET imaging protocol

Whole-body transmission and emission scans were acquired with a Biograph PET/CT scanner (Biograph Duo, Siemens, USA) which has a 58.5-cm transverse and a 15.5-cm axial field of view, and 4.8 mm FWHM spatial resolution. After injection of 4-[^{18}F]-ADAM (182±8 MBq), a low-dose CT scan (130 kVp, 50 mAs) and a series of eight whole-body PET scans were performed (15, 55, 95, 110, 155, 170, 215 and 230 min, respectively, after injection). Total acquisition time was 240 min. Each scan covered the monkey's body from the head to the thigh and consisted of five or six bed positions depending on the size of the monkey. In each bed position, data were acquired for 2 min in 3-D mode. The data were then reconstructed by OS-EM (ordered subsets expectation maximization) on a 128×128 matrix (slice thickness 5 mm), two iterations eight subsets, 3-mm FWHM gaussian filter, and corrected by photon attenuation using the CT scan.

Image data analysis

The image data analysis and residence time calculation were performed by methods reported previously, with some modifications [31–33]. Each CT and PET whole-body image was successively loaded to the PMOD 2.5 software (PMOD Technologies, Switzerland; www.pmod.com) to generate the fusion images. Organs with high uptake of 4-[^{18}F]-ADAM, such as brain, lungs, heart, liver, gallbladder, kidneys, spleen and bladder were easily identified and outlined. Organs with low uptake of 4-[^{18}F]-ADAM or complicated anatomy, such as stomach, intestine and thyroid were processed manually. The regions of interest of these organs were manually drawn as precisely as possible on the organ itself on each horizontal slice. The activity in each organ was non-decay-corrected and expressed as percent injected dose per organ ($n=7$, mean±SD).

Residence times and absorbed dose calculations

To calculate the absorbed radiation dose of each organ as well as the effective dose, the injected dose of 4-[^{18}F]-

ADAM and the non-decay-corrected time–activity curves (TACs) of the brain, lungs, heart, liver, gallbladder, spleen, kidneys, and bladder of seven monkeys were individually entered into an Excel spreadsheet and the data processed individually for each animal. The difference between the injected dose and the sum of the whole-body radioactivity (assuming that there was no excretion) plus the radioactivities of the above-mentioned organs was taken as the TAC of the remainder.

The residence time (hours) of the selected organ was calculated as the area under the TAC of the source organ from time zero to infinity over the initial total body activity (about five half-lives of ^{18}F , i.e. about 550 min). The area under the TAC of each organ (brain, lungs, heart, liver, kidneys, spleen and bladder) was generated using the following strategy including trapezoidal integration of the first four TAC data points through the origin and exponential decline of the four remaining TAC data points to infinity such that the residence times of these organs were obtained [31].

The human dosimetry was estimated from both the male and female monkey biodistribution data. The organ weight and body mass were used for allometric scaling [33]. That is, the residence time in each organ was converted to the corresponding human value by multiplication by a factor to scale organ and body weights (in kilograms) as $(w_{m,b}/w_{m,o})$ ($w_{h,o}/w_{h,b}$), where $w_{m,b}$ is the monkey body weight, $w_{m,o}$ is the monkey organ weight, $w_{h,b}$ is the human body weight and $w_{h,o}$ is the human organ weight. However, the $w_{m,o}$ values for the Formosan Rock monkey were unavailable, so instead we used the $w_{m,o}$ values for the rhesus monkey (*Macaca mulatta*) in our calculations. Both monkey organ weights [34, 35] and human organ weights [36] were obtained from the literature. The residence time of 4-[^{18}F]-ADAM in the rest of the body was obtained by subtracting the total organ residence time from the reciprocal of the ^{18}F decay constant.

Absorbed radiation doses were calculated from the residence times in all source organs for each monkey by entering the information into the Java-based OLINDA 1.0/EXM computer program [37] using the model for a 70-kg adult male and female phantom.

Results

Acute toxicity of 4-F-ADAM in rats

The acute toxicity of 4-F-ADAM was studied in rats using single dosing and multiple dosing paradigms. The following parameters were evaluated: mortality/morbidity, clinical observations, body weight, food consumption, clinical pathology (haematology and serum chemistry), organ

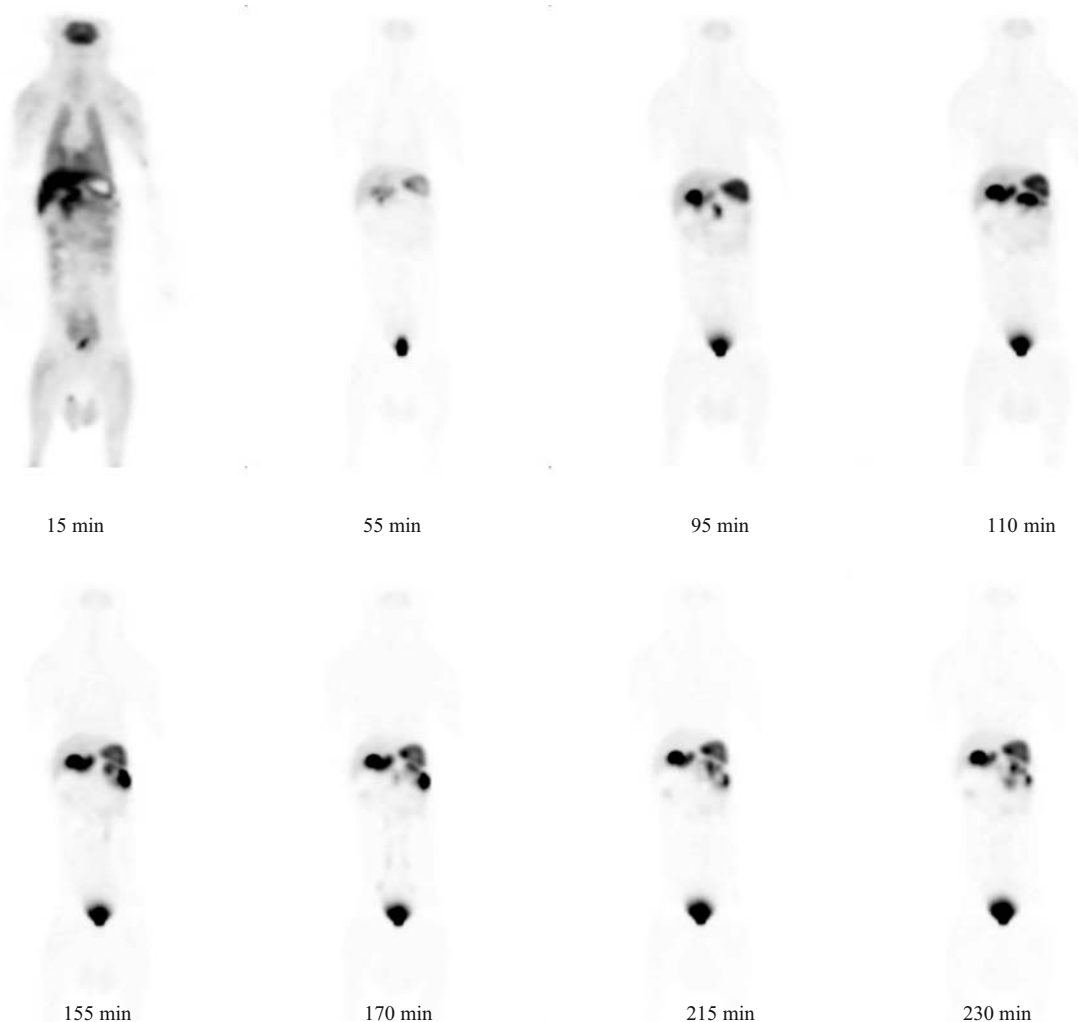


Fig. 1 Typical 4-[^{18}F]-ADAM whole-body images of a male monkey at different time points

weights, necropsy macroscopic observation and histopathology. All animals survived until their scheduled necropsy. Soft stool was observed in four of ten male animals in the repeat-dosing group versus one of ten males in the control group. No other drug-related clinical adverse effects were observed, such as hyperactive, agitated or otherwise changed behaviour and myoclonus were observed. In addition, no adverse clinical pathological effects were observed, such as hyponatraemia and increased bleeding time, body weight, food consumption or organ weights, and necropsy findings (Table 1)

Protocol-specified tissues from all animals were evaluated microscopically. Minimal to mild inflammation and myofibre necrosis/degeneration were present in skeletal muscle from two of five single-dosing males and one of five repeat-dosing males at the interim time of death, and from one of five single-dosing males at the terminal time of death. Minimal inflammation was also present in skeletal muscle from one of five control group females at the terminal time of death.

Biodistribution of 4-[^{18}F]-ADAM in monkeys

Typical whole-body biodistribution of 4-[^{18}F]-ADAM in male and female monkeys are shown in Figs. 1 and 2, respectively. The uptake and the TACs of 4-[^{18}F]-ADAM in selected organs are shown in Table 2 and Fig. 3, and are expressed as percent injected dose per organ (non-decay-corrected). The uptake of 4-[^{18}F]-ADAM in the liver, brain, lungs and kidneys were high in the early time points and declined slowly, whereas the uptake in the gallbladder and bladder were low in the beginning and gradually increased throughout the experiments.

Absorbed radiation dose estimates

The residence times of selected organs in monkeys are shown in Table 2. Among these organs, the bladder, liver and lungs had long residence times. Using the above residence times, the individual organ doses in humans

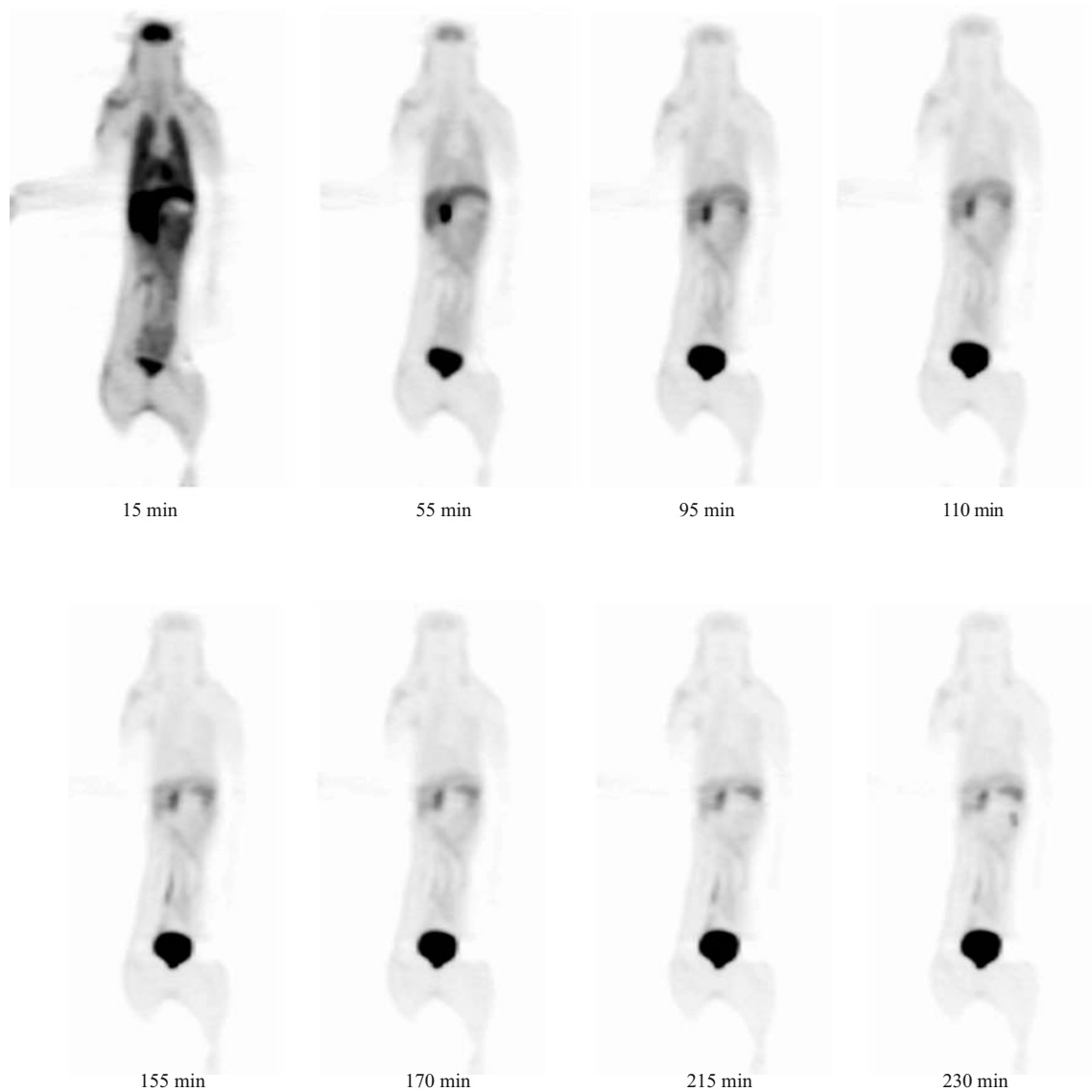


Fig. 2 Typical 4-[¹⁸F]-ADAM whole-body images of a female monkey at different time points

Table 2 Biodistribution and residence times of 4-[¹⁸F]-ADAM in monkeys. Values are percent injected dose (mean±SD, *n*=7; non-decay-corrected)

Time after injection (min)	Brain	Lungs	Heart	Liver	Gallbladder	Spleen	Kidneys	Bladder
15	3.09±0.69	6.08±1.58	0.88±0.23	5.31±1.15	0.51±0.28	0.20±0.08	1.59±0.45	0.55±0.54
55	1.61±0.30	2.64±0.77	0.41±0.06	3.76±0.52	1.78±1.36	0.08±0.03	1.21±0.86	2.87±1.31
95	0.88±0.12	1.56±0.42	0.25±0.04	2.38±0.51	2.35±1.98	0.06±0.02	0.61±0.29	3.98±1.46
110	0.72±0.09	1.29±0.35	0.22±0.03	2.04±0.36	2.12±1.81	0.06±0.02	0.52±0.18	4.36±1.38
155	0.44±0.05	0.89±0.20	0.15±0.02	1.48±0.28	1.93±1.60	0.05±0.02	0.43±0.20	3.74±1.26
170	0.38±0.04	0.79±0.19	0.13±0.02	1.36±0.21	2.11±1.49	0.04±0.02	0.39±0.21	3.69±1.29
215	0.24±0.02	0.60±0.14	0.09±0.01	0.97±0.16	1.56±1.22	0.02±0.01	0.20±0.06	3.24±1.05
230	0.21±0.02	0.55±0.14	0.08±0.01	0.89±0.14	1.62±1.10	0.02±0.01	0.17±0.06	3.08±1.09
Residence time (h)	0.050±0.015	0.093±0.050	0.010±0.002	0.094±0.023	–	0.008±0.006	0.023±0.009	0.153±0.055

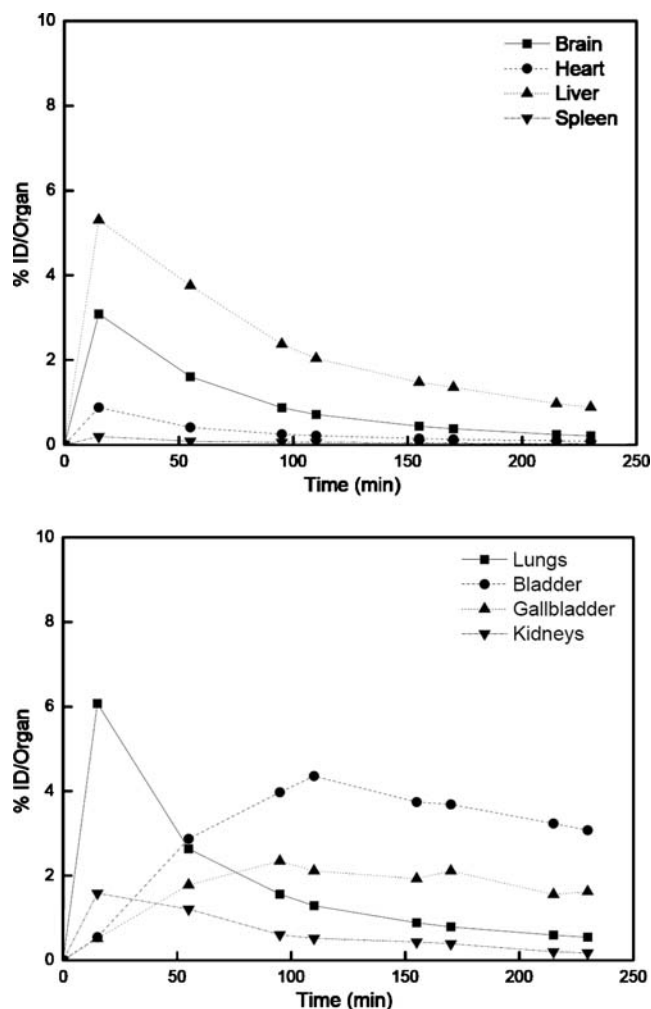


Fig. 3 Non-decay-corrected time-activity curves of 4-[¹⁸F]-ADAM in various organs of monkeys (average of seven monkeys)

extrapolated from the male and female monkeys were calculated with OLINDA/EXM software and the results are shown in Table 3. The absorbed radiation dose data showed that the bladder would receive the highest dose followed by the lungs, kidneys and liver. The effective doses in male and female humans, according to ICRP 60 [38], extrapolated from the data in male and female monkeys were 17.4 and 21.8 μ Sv/MBq, respectively.

Discussion

4-[¹⁸F]-ADAM has been demonstrated to be a potent serotonin transport imaging agent in rats and baboon [29, 30]. In order to evaluate it as a promising SERT imaging agent in human, it is necessary to estimate its mass dosage associated with no observable adverse effect level (NOAEL) and the organ radiation dose burden in animal models before human PET studies are undertaken.

The acute toxicity studies of 4-F-ADAM were carried out in rats to determine potential toxic effects, identify potential target organs of toxicity, and determine its NOAEL. Since some patients may need to receive 4-[¹⁸F]-ADAM twice within 1 or 2 weeks, we used both single dosing and multiple dosing paradigms for the rat toxicity studies. The dosages we administered to the rats were 100 to 1,000 times more than will be administered to humans. Intravenous administration of 4-F-ADAM to male and female rats for a single day at 1,023.7 μ g/kg (6,142.2 μ g/m²) or for 5 days at 102.37 μ g/kg per day (614.22 μ g/m² per day) did not produce overt adverse effects clinically. Additionally, we found no clear evidence that skeletal muscle lesions were related to the treatment with 4-F-ADAM. Given the fact that this microscopic finding in the skeletal muscle of the treated males occurred with a low incidence and with minor severity and that a similar lesion was observed in a control female, it is considered to be of minimal toxicological significance. Soft stool observed in four out of ten repeat-dosing animals

Table 3 Radiation dosimetry estimates (μ Gy/MBq) for 4-[¹⁸F]-ADAM using the mean of four male and three female monkeys, extrapolated to adult male and female humans

Organ	Male	Female
Adrenals	13.3±0.2	17.5±0.3
Brain	9.3±0.8	14.8±3.9
Breasts	9.4±0.1	12.2±0.5
Gallbladder	14.1±0.3	17.1±0.2
Lower large intestine wall	15.4±0.4	17.9±1.2
Small Intestine	14.3±0.2	16.0±0.6
Stomach	12.8±0.1	16.1±0.5
Upper large intestine wall	13.9±0.2	17.0±0.5
Heart wall	13.4±1.3	16.4±2.5
Kidneys	18.3±3.6	27.3±7.1
Liver	15.5±3.0	23.7±4.2
Lungs	16.8±4.7	35.7±12.4
Muscle	11.6±0.1	14.1±0.3
Ovaries	15.7±0.4	18.1±1.2
Pancreas	13.8±0.1	17.7±0.5
Red marrow	11.0±0.1	13.6±0.3
Osteogenic cells	17.4±0.3	22.4±0.8
Skin	8.9±0.1	10.9±0.4
Spleen	11.8±0.1	17.0±5.1
Testes	12.4±0.3	-
Thymus	11.7±0.1	15.2±0.6
Thyroid	11.4±0.2	13.3±0.6
Urinary bladder	96.6±9.2	90.4±54.7
Uterus	18.7±0.6	19.9±2.6
Total body	12.0±0.1	15.0±0.2
Effective dose equivalent (μ Sv/MBq)	19.4±0.8	24.2±3.2
Effective dose (μ Sv/MBq)	17.4±0.6	21.8±2.3

Table 4 Comparisons of effective dose of 4-[¹⁸F]-ADAM and other radiopharmaceuticals

Radiopharmaceuticals	Target site	Subject	Critical organ	Effective dose (μSv/MBq)	Reference
4-[¹⁸ F]-ADAM	SERT	Monkey	Bladder	19.3	This study
[¹¹ C]DASB	SERT	Rat	Bladder	5.5	[43]
		Monkey	Bladder	6.4	[33]
		Baboon	Lungs	9.0	[31]
		Human	Lungs	7.0	[41]
		Human	Lungs	7.0	[41]
[¹²³ I]ADAM	SERT	Human	Large intestine	21.6	[39]
		Human	Large intestine	36.0	[42]
		Human	Bladder	26.0	[40]
[¹⁸ F]FECNT	DAT	Monkey	Kidney	22.1	[44]
[¹⁸ F]FPCIT	DAT	Human	Bladder	12.0	[56]
(S,S)-[¹⁸ F]FMeNER-D ₂	NET	Monkey	Bladder	35.5	[45]
		Human	Bladder	17.0	[46]

may have reflected a serotonergic effect and is also considered to be of minor toxicological significance and is not expected to be a dose-limiting effect in clinical trials. Thus, the NOAEL is considered to be greater than 1,023.7 μg/kg for a single i.v. dose and greater than 102.37 μg/kg per day for a 5-day i.v. dosage regimen.

The organ radiation absorbed dose studies of 4-[¹⁸F]-ADAM were carried out in Formosan Rock monkeys. Following 4-[¹⁸F]-ADAM injection, the uptakes of radioactivity in the brain and liver were high in the beginning and then declined gradually thereafter. The radioactivity in the gallbladder and bladder increased with time suggesting the 4-[¹⁸F]-ADAM may be eliminated via the hepatobiliary and renal systems as are [¹¹C]DASB and [¹²³I]ADAM [31, 33, 39–43]. The uptake of 4-[¹⁸F]-ADAM in the lungs was high (6.1 %ID/organ at 15 min after injection) which has also been observed for other monoamine transporter radioligands, such as [¹¹C]cyanoimipramine, [¹¹C](+)-McN5652, [¹¹C]DASB, (S,S)-[¹⁸F]FMeNER-D₂, [¹⁸F]FECNT, [¹²³I]ADAM and [¹²³I]β-CIT [33, 40, 41, 44–47]. The exact reason(s) for the high uptake of these radioligands in the lungs is unclear. However, it has been suggested that this may be due to the specific binding of the circulating 5-HT on pulmonary membranes, nonspecific binding of amine by macrophages, and the large blood volume in the lungs [41, 46, 48–51].

The guidelines on radiation exposure for human subjects involved in research studies varies internationally. Radiation risk estimates were recently issued by the National Institutes of Health (NIH) in the United States and the effective dose estimates are considered to be the most accurate measure of radiation risks. Based on the NIH guidelines, the maximum exposure is 50 mSv of effective dose per year for a research subject [52]. On the other hand, under the guidelines of the European Commission, the

intermediate risk levels in adults is equivalent to an effective dose range of 1–10 mSv per annum [53]. To estimate the human dosimetry from the Formosan Rock monkey whole-body PET image data, the organ weight and body mass were used for allometric scaling after obtaining the residence time of each organ for each subject. However, the organ weights of Formosan Rock monkey are unavailable and the rhesus monkeys (*Macaca mulatta*) are genetically similar to the Formosan Rock monkeys (*Macaca cyclopis*) [54, 55]. We therefore used organ weights of the rhesus monkey to calculate the human dosimetry. The results (Table 3) showed that the human estimated dosimetry of 4-[¹⁸F]-ADAM in most organs ranged between 7.1 and 35.7 μGy/MBq, and were comparable with those of other SERT [31, 33, 39–43], DAT or NET imaging agents [44–46, 56] (Table 4). The critical organ of 4-[¹⁸F]-ADAM was the urinary bladder that has also been reported for other monoamine transporter radioligands [33, 40, 43, 45, 46, 56]. However, compared to other widely used ¹⁸F radiopharmaceuticals, such as [¹⁸F]FDG (119 μGy/MBq) [57] and [¹⁸F]FDOPA (159 μGy/MBq) [58], the radiation dose of 4-[¹⁸F]-ADAM in the urinary bladder was relatively low. Increasing both the bladder volume and frequency of voiding will reduce the radiation dose in the urinary bladder [59–61].

In addition, in this study almost all organs, except the urinary bladder, had higher 4-[¹⁸F]-ADAM uptakes in females ($n=3$) than in males ($n=4$), particularly the liver, lungs and kidneys. The gender differences in radiation dosimetry of this SERT imaging agent have also been observed in a small group of adult females ($n=4$) and adult males ($n=3$) using ¹²³I-ADAM as an imaging agent [42]. However, a recent study in humans has shown that, compared to males, females have significantly higher 5-HT_{1A} receptor and lower SERT binding potentials in a

wide array of cortical and subcortical brain regions [62]. The exact reason(s) for this discrepancy and gender differences are unclear. A systematic study with a large population may be warranted.

Conclusion

The toxicity studies in rats showed that i.v. administration of 4-F-ADAM as a single dose of 1,023.7 $\mu\text{g}/\text{kg}$ (1,000 times the human dose) or as five daily doses of 102.37 $\mu\text{g}/\text{kg}$ per day (100 times the human dose) did not produce overt adverse effects clinically. The radiation dosimetry estimates obtained in Formosa Rock monkeys extrapolated to humans suggested that the received doses in most organs in humans would range between 7.1 and 35.7 $\mu\text{Gy}/\text{MBq}$. The urinary bladder was considered to be the critical organ. It received around 96.6 and 90.4 $\mu\text{Gy}/\text{MBq}$ for male and female human adults, respectively, which are below NIH guidelines. These results suggest that 4-[^{18}F]-ADAM is safe for human studies from both pharmacological and radiation perspectives.

Acknowledgments We thank the Institute of Nuclear Energy Research of Taiwan for providing the OLINDA 1.0/EXM computer program. The synthesis of 4-F-ADAM was funded by the National Institute of Mental Health (NIMH) under contract no. NO1-MH-32005. Toxicology studies were also funded by NIMH, under contract no. M422-05. This work was supported by the National Science Council of Taiwan (grants NSC 95-2811-B-016-002, 95-2321-B-016-001-MY2 and 96-2811-B-016-004).

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