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Biological evaluation on ^{125}I-ADAM as serotonin transporter ligand

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Abstract ADAM (2-((2-((dimethylamino)methyl)phenyl)thio)-5-iodophenylamine) is suggested as a promising serotonin transporter (SERT) imaging agent for central nervous system. In this paper, biodistribution studies in rats showed that the initial uptake of ^{131}I-ADAM in the brain was high (1.087%ID at 2 min post-injection), and consistently displayed the highest binding (between 60~240 min post-injection) in hypothalamus, a region known with the highest density of SERT. The specific binding((T/CB)-1) of ^{131}I-ADAM in hypothalamus were 2.94, 3.03 and 3.09 at 60, 120 and 240 min post-injection, respectively. The (T/CB)-1 was significantly blocked by pretreatment with paroxetine, which is known as a serotonin site reuptake inhibitor, while another nonselective competing drug ($5HT_{2A}$ antagonist) Ketanserin, showed no block effect. The rat brain autoradiography and analysis showed that there was a high ^{131}I-ADAM uptake in hypothalamus, the ratio of hypothalamus/cerebellum was significantly reduced from 7.94±0.39 to 1.30±0.56 by pretreatment with paroxetine at 60 min post-injection. Blood clearance kinetics was performed in rats, and the initial half-life of 13.79 min and late half-life of 357.14 min were obtained. The kinetic equation is: $C=3.6147e^{-0.0725t} + 1.0413e^{-0.0028t}$. The thyroid uptake was 0.009% ID and 1.421% ID at 2 min and 120 min post-injection, respectively, suggesting that *in vivo* deiodination may be the major route of metabolism. Toxicity trial showed that the dose per kilogram administered to mice was 1000 times greater than that to humans, assuming a weight of 50kg. These data suggest that ^{131}I-ADAM may be useful for SPECT imaging of SERT binding sites in the brain.

Key words Depression, SERT, ADAM, Imaging agent, Radionuclide imaging, Biodistribution

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1 Introduction

Considerable evidence has been established in the last two decades to support the hypothesis that alterations in serotonergic neuronal function in the central nervous system (CNS) closely related to patients with major depression [1,2]. Neurotransmitters within serotonergic neuronal $5\text{-}HT_{1A}$ and serotonin transporter (SERT) are thought to be closely associated with the pathology of depression [3]. Since the presynaptically located SERT plays important roles in regulating the serotonin concentration and receptor-mediated action by a mechanism of reuptake, much of this work has focused on development of radioligands for *in vivo* mapping SERT. SERT could be non- invasively imaged and quantitated with a radiotracer that readily enters the brain and selectively binds to SERT. For this purpose, positron emission tomography (PET) and single photon emission computed tomography (SPECT) are used in conjunction with trace amounts of radiotracers for better understanding alterations of the serotonergic system and for providing useful in-

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formation in monitoring antidepressant therapy.

The most promising radioligand for mapping SERT is ^{11}C-McN5652 (6-(4-[^{11}C]- methylsulfanyl-phe-nyl)-1,2,3,5,6,10b-hexahydro-pyrrolo[2,1-a]isoqui-noline), though its clinical application as a PET radiotracer has been hindered by the low specific-to-nonspecific binding ratio [4,5]. ^{11}C-DSAB ([^{11}C]-3-amino-4-(2-dimethylaminomethylphenylsulfa-nyl)-benzonitrile) developed by Szabo et al in 2002 showed higher brain permeability and greater specific binding as compared with ^{11}C-McN5652 [6], however, detailed quantitative validation and pharmacological studies (pharmacokinetic (PK) and pharmacodynamic (PD) properties) of ^{11}C McN5652 in health and disease subjects has not been fully addressed yet [6,7].

On the other hand, radioligands for SPECT imaging are in greater demands due to the fact that SPECT is more practical, economic and available than PET. It has been demonstrated so far that [^{123}I] ADAM (2-((2-((dimethylamino)methyl)phenyl)thio)-5-iodophenylamine) has high *in vitro* selectivity and affinity in SPECT imaging of SERT[8]. It can readily penetrate BBB (brain –blood-barrier) and selectively accumulate in areas such as hypothalam, dorsal and median raphes, which were regarded as areas rich in SERT with higher signal-to-noise ratio [9-11]. It has also been used to evaluate the effect of fluoxetine and SSRI using microautoradiography on the same animal model [12].

We have synthesized the precursor and radioiodinated $^{125/131}$I-ADAM. In this paper, we report the biological evaluation and preclinical pharmacological studies on ^{125}I-ADAM as an SERT radioligand for SPECT imaging.

2 Materials and methods

2.1 Instruments

PACKARD-COBRA γ-counter (Parkard Instrument Co. USA), 80-2 centrifuger (Y2, Shanghai), MNT cryostat microtome (SLEE TECHNIK GMBH, Germany), GS-250 Phosphor Imaging Screen-BI, GS-250 Molecular Imager (Bio-Rad, USA).

2.2 Reagents

$^{125/131}$I-ADAM was prepared in our lab, with over 98% of radiochemical purity (RCP). Freezing compound O.C.T for embedding was from Miles Scientific (Elkhart, IN, USA).

2.3 Animals

Rats (Sprague-Dawley) were purchased from the Center of Experimental Animals of East China.

2.4 Biodistribution and brain uptake of ^{131}I-ADAM in normal control rats

^{131}I-ADAM (0.2mL, 0.37MBq) was injected through tail veins into fasted rats, which were 180~200g and divided into eight groups (three per group). The rats were killed under anaesthesia (diethyl ether) at regular intervals post-injection. The organs of interest (heart, liver, spleen, lung, kidney, thyroid, etc.) were removed, weighed, and prepared for counting. The brains were further dissected into different regions such as cerebellum (CB), striatum (ST), cortex (Ctx), hippocampus (HP), and hypothalam (TH). The uptake of each organ and brain region was expressed in fraction of injection dosage per gram organ.

2.5 *In vivo* selectivity of ^{131}I-ADAM in brain

In vivo selectivity of ^{131}I-ADAM in brain was investigated by pretreating (5 min prior to injection of radiotracer) three groups of the fasted rats (180~200g, three in each group) with thigh vein injections with paroxetine (2.0mg/kg，0.1mL), ketanserin (2.0mg/kg，0.1mL) and saline (0.2mL), respectively, and followed by tail vein injection of ^{131}I-ADAM (0.2mL, 0.74MBq). The rats were sacrificed 1 h post-injection of ^{131}I-ADAM under anaesthesia, similar regional brain tissues were removed, weighed and counted. The uptake of each organ and brain region was expressed in fraction of injection dosage of per gram organ. The specific binding ration in each region was obtained by comparing counts different between regions with that in the CB, the CB was regarded as reference because it contains no SERT.

2.6 Blood-drug clearance in rats

^{125}I-ADAM (0.2mL, 7.4MBq) was injected through thigh vein into four rats (200~250g). Blood samples (20μL) were obtained from tail vein at regular intervals (2, 5, 10, 15, 30, 45, 60 and 120min post-injection), prepared for counting and expressed as percentage of injected dose per milliliter (%ID/mL blood) of blood. Blood radioactivity-time curve of

^{125}I-ADAM in rats was plotted.

2.7 *Ex vivo* autoradiography in rat brains

The specific and selective binding of [^{131}I]ADAM to SERT-rich brain regions of rats were evaluated by pretreatment with the potent and selective SERT ligand paroxetine (for specificity study) and $5HT_2$ ligand ketanserin (for selectivity study). Doses of paroxetine (2mg/kg) and ketanserine (2.0mg/kg) were administered respectively at 5 min prior to injection of ^{131}I-ADAM (0.2mL, 3.7MBq). The rats were sacrificed under anaesthesia and perfused transcardially with 4% paraformaldehyde (PFA) in PBS (pH 7.3). The brains were removed from the calvarium, immersed post-fixed in the same fixative solution, dehydrated in 20% sucrose, and embedded in freezing compound (O.C.T.; Miles Scientific, Elkhart, IN, USA) and cryostat sectioned at 20μm and mounted on superfrost slides. The slides containing the brain sections were exposed to GS-250 Phosphor Imaging Screen-BI for 2 h before being imaged in GS-250 Molecular Imager, and the OD (optical densities) were determined with an image analysis system.

2.8 Toxicity test

Under the Regulations of Pharmacopoeia of P.R.China (2000), the toxicity of [^{131}I]ADAM was determined by observing the death and survival of mice (18~20g) within a period of 48 h after an injection of 0.4 mL ^{131}I-ADAM (4/10 of the human dose).

2.9 Statistics

Student test was employed in this study, and all data were shown as $\bar{x} \pm s$.

3 Results and discussion

3.1 Biodistribution of ^{131}I-ADAM in rats (Table 1)

A biodistribution study provides critical information about brain penetration. Generally, a small and freely diffusible compound with an optimal log PC (a parameter of partition coefficient) value of 2~3 will have an initial brain uptake of 1%~3% dose/whole brain. The log PC of ^{131}I-ADAM was 2.5 (data will be published elsewhere). As expected, ^{131}I-ADAM displayed high brain uptake (1.087±0.091 ID% at 2 min post-injection), indicating a level sufficient enough for brain imaging. ^{131}I-ADAM displayed good clearance from the normal brain. As one can see, the brain uptakes were 0.598±0.059, 0.296±0.065, 0.273±0.034, 0.112±0.012 and 0.098±0.007 ID% at 30, 60, 120, 240 and 360 min post-injection, respectively. In the meantime, [^{125}I]ADAM showed a high initial biodistribution in liver, lung and muscles following the blood flow, and the radioactivity washed out of these organs at later time points. The uptakes of ^{131}I-ADAM in liver, lung and muscles were 11.87±2.767, 3.995±0.671 and 37.21±6.928 ID%, respectively, and decreased rapidly to 5.207, 2.269, and 5.909 ID% at 240 min post-injection. The thyroid uptake increased with time (0.009% ID and 1.421%ID at 2 min and 120 min, respectively), suggesting that *in vivo* deiodination reaction of the parent compound might be the main metabolic route, leading to the release of free iodide ion taken up by the thyroid, which is in consistent with Ref. [4].

Table 1 Biodistribution of ^{131}I-ADAM in rats (ID%/g, $\bar{x} \pm s$, n=4)

Organ	Time/min					
	2	30	60	120	240	360
Heart	0.681±0.133	0.181±0.026	0.122±0.022	0.091±0.012	0.060±0.005	0.055±0.007
Liver	11.87±2.767	13.39±1.284	8.817±1.418	7.410±1.094	5.207±1.055	3231±0.478
Spleen	0.198±0.064	0.232±0.025	0.169±0.038	0.103±0.013	0.075±0.001	0.075±0.008
Lung	3.995±0.671	3.555±1.639	3.445±0.981	2.451±0.416	2.269±0.778	0.317±0.038
Kidney	2.158±0.293	2.232±0.111	1.391±0.478	1.245±0.110	0.572±0.061	0.452±0.057
Thyroid	0.009±0.006	0.043±0.030	0.122±0.128	1.421±0.123	2.124±0.312	2.365±0.399
Muscle	37.21±6.928	14.41±1.511	10.66±1.308	8.410±0.859	5.909±0.359	4.810±0.914
Blood	14.54±2.674	4.549±1.675	1.981±0.621	0.945±0.231	0.878±0.436	0.676±0.112
Brain	1.087±0.091	0.598±0.059	0.296±0.065	0.273±0.034	0.112±0.012	0.098±0.007

3.2 Uptake of ^{125}I-ADAM in rats' brain regions

Table 2 shows the highest uptakes and retention in hypothalamus region, a SERT rich area of the brain (0.989, 0.699, 0.387, 0.234, 0.131 and 0.043 ID%/g at 2, 30, 60, 120, 240 and 360 min post-injection, respectively). The lowest uptakes were observed in cerebellum (0.687, 0.098 and 0.032ID%/g at 2, 60 and 240 min post-injection) so that it was regarded as a reference region. The specific binding (Target/ cerebellum – 1, i.e. (T/CB – 1), Table 3) of ^{125}I-ADAM in hypothalamus region was the highest (0.43, 1.24, 2.94. 3.03, 3.09 and 1.26 at 2, 30, 60, 120, 240, and 120 min post-injection, respectively). Furthermore, the specific binding of ^{125}I-ADAM in other regions containing SERT, such as cortex, striatum and hippocampus also show relatively high target-to-non-target (T/NT) ratio, which was in consistent with the known distribution of SERT in the brain [10].

Table 2 Biodistribution of ^{131}I-ADAM in rats (ID%/g, $\bar{X}$ ±*s*, *n*=4)

Region	Time/min					
	2	30	60	120	240	360
Cerebellum	0.687±0.198	0.312±0.097	0.098±0.043	0.058±0.019	0.032±0.011	0.019±0.008
Cortex	1.009±0.213	0.534±0.086	0.289±0.034	0.187±0.025	0.018±0.003	0.019±0.004
Striatum	0.767±0.145	0.491±0.087	0.274±0.056	0.199±0.031	0.058±0.003	0.021±0.009
Hippocampus	0.668±0.112	0.499±0.087	0.266±0.076	0.189±0.061	0.089±0.023	0.037±0.006
Hypothalamus	0.989±0.176	0.699±0.076	0.387±0.079	0.234±0.012	0.131±0.006	0.043±0.001

Table 3 Specific binding of ^{131}I-ADAM in rats' brain ((T/CB)-1, *n*=4)

Region	Time/min					
	2	30	60	120	240	360
Striatum	0.12	0.57	1.79	2.43	0.81	0.11
Hippocampus	-0.02	0.60	1.71	2.26	1.78	0.94
Cortex	0.47	0.71	1.94	2.22	-0.43	0.0
Hypothalamus	0.43	1.24	2.94	3.03	3.09	1.26

3.3 Pharmacological blocking studies

In Table 4, the specific binding of ^{131}I-ADAM in hypothalamus decreased significantly (2.94 *vs* 0.148) by pretreatment with a selective SERT ligands and paroxetine. On the other hand, the specific binding of ^{131}I-ADAM in hypothalamus did not decrease (2.94 *vs* 2.76) by pretreatment with a selective 5-HT_{2A} antagonist, ketanserin, indicating the *in vivo* specific and selective binding to SERT. To evaluate the effect of ^{131}I-ADAM on SERT, *ex vivo* autoradiography of ^{131}I-ADAM on rat brain sections was also performed. As shown in Fig.1, high OD of ^{131}I-ADAM in control hypothalamus was detected, and the OD ratio of hypothalamus to cerebellum was found 7.94±0.39. However, the OD ratio was significantly decreased to 1.30±0.56 (p<0.05) in the presence of paroxetine, and no significant decrease was found by pretreatment with ketanserine (6.59±0.95, p>0.05). These results strongly suggested that ^{131}I-ADAM binds to SERT specifically and selectively.

Table 4 Blocking trial of ^{131}I-ADAM in rats' brains ((T/CB) – 1, *n*=3)

Region	Control	Paroxetine	Ketanserin
Hypothalamus	2.94	0.148*	2.76

*: p<0.01

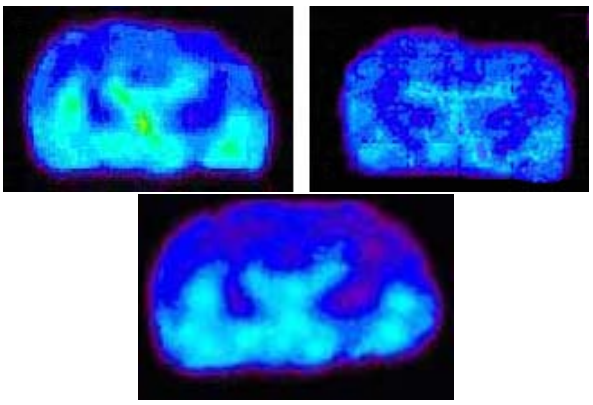

Fig.1 Autoradiography of ^{131}I-ADAM in rats' brain (upper left: control; upper right: pretreated with paroxetine; bottom: pretreated with ketanserin).

3.4 Blood radioactivity—time curve

The curve in rats was shown in Fig. 2. Clearance of ^{125}I-ADAM from blood was rapid within about 40 min post-injection. $T_{1/2}\alpha$ was 13.79 min, $T_{1/2\beta}$=357.14 min, K_{12}=0.046min^{-1}, K_{21}=0.018min^{-1}, K_{10}=0.011min^{-1}, V_d=0.22L, and AUC=421.75. The equation was C=3.6147e$^{-0.0725t}$ + 1.0413e$^{-0.0028t}$.

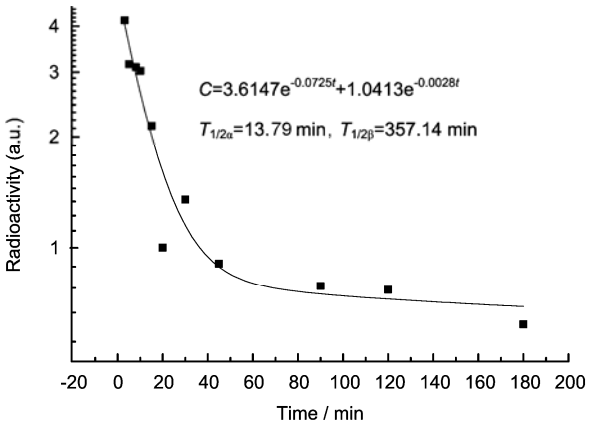

Fig.2 Blood radioactivity—time curve of ^{125}I-ADAM in rats.

3.5 Toxicity test

Each mouse was injected (i.v.) with 0.40 times the human's dose of ^{131}I-ADAM. None of the mice died in two days of normal feeding, even though they had received a dose per kg of 1000 times as high as a patients' dose (assuming he/she weighs 50 kg).

4 Conclusion

The study proved that ^{131}I-ADAM, which displayed excellent specific and selective binds to SERT, was a safe and effective radiotracer for monitoring changes in SERT binding sites in the brain in conjunction with SPECT.

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