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Research Article

Dopamine D2 Receptor Radiopharmaceutical: I-123-Epidepride Toxicology and Preclinical SPECT Image

Shih-Ying Lee, Yuan-Ruei Huang, Jun-Ming Shih, Yu-Lung Wu, Chia-Chieh Chen and Kang-Wei Chang*

Division of Isotope Application, Institute of Nuclear Energy Research, Atomic Energy Council, Taiwan

***Corresponding Author**: Kang-Wei Chang, Department of Biomedical Imaging and Radiological Sciences, Division of Isotope Application, Radiation Application Center, Institute of Nuclear Energy Research, Atomic Energy Council, Taiwan.

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Abstract

Dopamine plays an important role in the brain, and several diseases of the central nervous system are associated with dopamine system dysfunction. A significantly different density of dopamine D2 receptors has been reported in Parkinson's disease patients. Dopamine D2 receptor distribution in the central nervous system can be evaluated using the radiopharmaceutical [^{123}I]Epidepride, which shows great potential for use as an imaging agent. In the image analysis software showed that the binding ratios of [^{123}I]Epidepride significantly useful alterations of dopamine D2/D3 receptor binding sites in striatum, hypothalamus and midbrain. To investigate the toxicity of this radiopharmaceutical, we determined its effects in Sprague Dawley rats. We first synthesized Epidepride for the toxicity study, and then sought to determine the "No Observed Adverse Effect Level" (NOAEL) of Epidepride by analyzing acute single-dose effects. We injected unlabeled Epidepride (0 to 2000 μg/kg) into the tail vein of rats. This dose provided a safety margin of 100- to 10,000-fold over the maximum recommended human dose (200 μg/60 kg). After two weeks, we observed rat mortality, clinical situation (hypoactivity, body weight), and gross necropsy, and there were no changes. This lack of change showed that Epidepride exerted no adverse toxic effects in our rat model at dosage levels up to 2000 μg/kg. The results can achieve suitable be a reference method in toxicology and *in vivo* image for new drug studies of dopamine D2/D3 receptor related disease like schizophrenia and Parkinson's syndrome to helpful evaluate therapeutic effects in the future.

Keywords: *Epidepride; Toxicity; Parkinson's Syndrome; SPECT/CT*

Abbreviations

PD: Parkinson's disease; ADHD: Schizophrenia and Attention Deficit Hyperactivity Disorder; PET: Positron Emission Tomography; SPECT: Single-Photon Emission Computed Tomography

Introduction

Dopamine plays a major role in the brain system that is responsible for reward-driven learning. Several important diseases of the nervous system are associated with dysfunctions of the dopamine system. These diseases include movement disorders, Parkinson's disease (PD), schizophrenia, and memory and neurodegenerative disease [1]. PD, an age-related degenerative condition causing tremor and motor impairment, is caused by a loss of dopamine-secreting neurons in the substantia nigra [2,3]. Schizophrenia and attention deficit hyperactivity disorder (ADHD) are associated with changes in the levels of dopamine activity [4,5]. In clinical reports, it has been noted that more than one million Americans are affected with PD and the number of new PD patients has been extrapolated to be fifty thousand per year [6,7]. Lewy bodies, which can only be revealed by autopsy, are regarded as a hallmark of classical PD. Autopsies have uncovered Lewy bodies in a surprising number of older persons without diagnosed PD, affecting approximately 8% of those over 50, 13% of those over 70, and 16% of those over 80 years of age [4,7].

In vivo neuroimaging technology has become an important cornerstone of modern neurology [8]. In nuclear medicine, positron emission tomography (PET) and single-photon emission computed tomography (SPECT) have rapidly established themselves as important tools for the *in vivo* study of brain neurochemistry [9]. Brain imaging techniques such as using fluorodeoxy glucose (FDG) for PET imaging are not specific [10]. There are some [18F]FDG applications for clinical evaluations of PD patients [11,12]. These techniques measure the brain glucose utilization, which appears as "cold spots" in PD patients. However, it would be desirable to develop specific tracers and techniques to visualize brain differences in PD directly as "hot spots" [10]. These differences could be caused by a change in the number and distribution of dopamine receptors. In addition to exploring various types of mental illnesses, specific tracers could be used to predict related diseases and could act as therapeutic agents through their interaction with specific receptors [13]. Dopamine D2 receptor distribution determined using SPECT may have potential applications for the monitoring and prediction of antipsychotic treatment [1].

A number of radiolabeled probes for PET and SPECT have been developed for detection of dopamine receptors. Biochemical studies in the 1970s, and subsequently, showed that dopamine receptors could be located postsynaptic (in postganglionic neurons) and function as auto receptors (in neurons). Through their different function and pharmacology, dopamine receptors could be sub classified into D1-like receptors (D1 and D5) and D2-like receptors (D2, D3, and D4) [13]. D1-like receptors can interact with G protein complexes, leading to the activation of adenine rings, and D2-like receptors will interact with Gi complexes to inhibit those kinds of enzyme [1,8]. For the D2 receptor, SPECT imaging may be potentially applied to motor neurodegenerative diseases, such as Parkinson's, Huntington's, and Wilson's diseases [1]. [3H]Spiperone was the first radiopharmaceutical developed for the D2 receptor [13], while [^{123}I]IBZM was the first radiopharmaceutical developed for SPECT imaging of D2 receptors [13]. Ligands based on a benzamide structure are a theme for the D2 receptor (for example, IBF, Epidepride, and IBZM) [1,13,14].

The use of nuclear medicine imaging techniques for noninvasive D2 receptor imaging cannot only be used to establish diagnostic credentials, but can also be used to assess the effectiveness of treatments. [^{123}I]Epidepride provides better imaging quality than other ligands. It is superior for visualization of pituitary adenomas [4] because extra striatal D2 receptor density is only 1% to 10% of that in striatum, and the Kd of 0.426 nM for IBZM is apparently unable to allow effective extra striatal imaging. Because of the high affinity of Epidepride for the D2 receptor (Kd = 0.024 nM), it is applicable for radiopharmaceutical imaging of the D2 receptor in striatal and extra striatal regions [15,16]. [^{123}I]Epidepride is the best choice for high imaging quality of dopamine D2 receptors in the striatum as well as in extra striatal regions [17,18].

Therefore, dopamine has revolutionized treatment in millions of patients with Parkinson's syndrome, and is now widely prescribed in the treatment of other neurological disorders [19,20]. In this study, we first synthesized Epidepride and obtained a high-purity preparation. We then used Sprague Dawley rats for an acute-toxicology assay and in vivo NanoSPECT/CT image. Finally high binding affinity with [^{123}I]Epidepride and D2/D3 receptors in brain by SPECT imaging, we also determined the safety of Epidepride from the toxicology. We suggest that these data could be applied to the clinical use of [^{123}I]Epidepride for the diagnosis of D2 receptor-related diseases in the future.

Materials and Methods

Animals

Crl:CD Sprague Dawley rats (BioLASCO Taiwan Co., Taipei, Taiwan), approximately six weeks old and 193-224 g (males) or 149 - 178g (females) at the time of dosing, were used in this study. All animal procedures and experiment protocols were approved by the Ethical Animal Use Committee of the Institute of Nuclear Energy Research Atomic Energy Council (INER) in Taiwan. The rats were maintained at 21 ± 2°C with 50 ± 20% relative humidity under an automatic 12h light and 12h dark cycle.

Chemicals

5-Iodo-2,3-dimethoxybenzoic acid and (S)-(-)-2-aminomethyl-1-ethylpyrrolidine (Sigma-Aldrich, St Louis, MO, USA). Thionyl chloride, dichloromethane, sodium hydroxide, sodium sulfate, silicon dioxide, chloroform, methanol, and other solvents were obtained from Merck & Co., Inc. (Whitehouse Station, NJ, USA).

Synthesis of Epidepride

5-Iodo-2,3-dimethoxybenzoic acid (1.0 g) was dissolved in $SOCl_2$ (10 mL) and the solution was heated for 2h. The solution was then filtered and washed with cold CH_2Cl_2 (20 mL). (S)-(-)-2-Aminomethyl-1-ethylpyrrolidine (0.41g) was added to the solution and reacted for 3h. After extraction using aqueous 1N NaOH (20 mL) the organic layer was dried using Na_2SO_4. The product was concentrated by liquid chromatography on SiO_2 using $CHCl_3$:CH_3OH (95:5) to obtain Epidepride (1.15 - 1.27g, 86 - 95%).

Epidepride administration

Epidepride was dissolved in vehicle (ethanol 20% and water for injection 80% (v/v)) to achieve the designated concentrations. The formulations were prepared on the dosing day and protected from light. A suitable disposable plastic syringe fitted with a 26-gauge needle was used. Epidepride solution or vehicle control was injected into rats through the tail vein. The volume administered was adjusted on the basis of individual body weight recorded before dosing. The dosing day was defined as D1 (Day 1).

Toxicology protocol

The groups, Epidepride dose concentration, dose volumes, and number of animals used are presented in table 1. Epidepride (14.6 mg) was dissolved in absolute alcohol to a concentration of 40 mg/mL, and then diluted with water for injection to a concentration of 2 mg/mL in 5% (v/v) alcohol solution for animals in the high-dose group. Based on the suggested clinical dose of (0.2 μg/kg), the high dose (2000 μg/kg) used is 10,000-fold greater than the clinical dose used in humans.

Group	Dose Level	Dose Concentration	Dose Volume	Number of rats	
No.	(μg/kg)	(μg/mL)	(mL/kg)	Male	Female
1	0[a]	0	1	6	6
2	20	20	1	6	6
3	200	200	1	6	6
4	2000	2000	1	6	6

Table 1: *Experimental design of the toxicity on Epidepride trial. Acute toxicity study in SD-rat experimental design of Epidepride: Group numbers (1~4), dose levels (0~2000* μg*/kg/day), dose volumes (1 mL/kg) and number of animals (n = 6, respectively).*

[a]Ethanol 5% (v/v) solution

Observation and examination

The rats were observed for mortality and clinical signs 0, 1, 2, 3 and 4h after dosing. On the following days, the animals were observed twice daily (at least 6h apart) for mortality and once for clinical signs for 14 days. Any mortality or clinical sign was documented. Body weight of all animals was recorded before dosing (D1) and then at one week (D8) and at the end of the study period (D15). The total body weight change (D15 - D1) was calculated. Gross necropsy was performed on surviving rats at the end of the study (D15). The surviving rats were first euthanized using carbon dioxide, followed by exsanguination and necropsy. The external surface of the body and all organs and tissues in the thoracic and abdominal cavities and injection site were examined. Any gross lesions observed at necropsy were recorded.

Radiolabeling of [^{123}I]Epidepride

[^{123}I]Epidepride was prepared from tin precursor by oxidative iododestannylation with [^{123}I]NH_4I. (S)-Bu3Sn-epidepride was first dissolved completely in methanol in a reaction vial and mixed well with [^{123}I]NH_4I. Five percent H_2O_2 mixture (injection water:freshly prepared 30% H_2O_2:1N acetic acid = 7:2:3) was then added into the above reaction vial and incubated at RT for 10 minutes. The reaction was quenched by adding 39% sodium hydrogen sulfite followed by injecting saturated Na_2HPO_4 immediately. Finally, [^{123}I]Epidepride was eluted by 100% ethanol through the C18 column. The radiochemical purity of the product, determined by TLC (TLC condition solvent, chloroform: methanol = 9:1) and analytical HPLC, ranged from 91% to 98%.

Ex vivo autoradiography

Rats were intraperitoneally injected 0.5 ml potassium iodide 10 minutes prior to the intravenous injection of 185 MBq of [^{123}I]Epidepride solution and sacrificed 30 minutes after tracer injection. Coronal brain slices (20-μm thickness) were then taken by a cryostat and mounted on silane-coated glass slides. Slices were kept in contact with imaging plates (BAS-SR2040, Fujifilm, Japan) for 5 days. The exposed plates were scanned with Fujifilm FLA-5100 Image Analyzer.

Small animal NanoSPECT/CT

Treatments of potassium iodide and tracer treatment were the same as described in ex vivo autoradiography. Small animal NanoSPECT/CT scans were performed on rats 30 minutes post-injection of [^{123}I]Epidepride (185 MBq) using a NanoSPECT/CT (Mediso, Inc., Budapest Hungary) previously calibrated for the ^{123}I isotope. The regions of interest (ROIs) were drawn onto striatum, hypothalamus, midbrain and cerebellum was served as the reference region. Specific binding ratio of [^{123}I]Epidepride was calculated from the average counts with the following equation: (target−reference)/reference.

Statistical analysis

Results are expressed as mean and standard deviation (Mean ± SD). Comparisons of all data collected for body weights for each dose group of either sex were performed using an ANOVA, followed by Dunnett's test (SigmaStat, version 3.0, 2003) when significance was found. $p < 0.05$ was used as the criterion of significance.

Results

Epidepride synthesis

Synthesis protocols are as per Scheme 1. 5-Iodo-2,3-dimethoxybenzoic acid as the starting compound was activated to its acyl chloride by thionyl chloride. An amidation reaction between 5-iodo-2,3-dimethoxybenzoyl chloride and (S)-(-)-2-aminomethyl-1-ethylpyrrolidine yielded Epidepride.

Body weight

The weekly and total body weight change is summarized in table 2. The mean body weight growth curves are presented in figure 1. No statistical differences for body weight or total body weight changes were found between treated and control rats.

Sex	Dose (μg/kg)	Body Weight (g)			Total Body Weight Changes (g)
		D1	D8	D15	D15 - D1
Male	0[a]	219.7 ± 8.8	279.8 ± 11.1	336.7 ± 17.0	117.0 ± 12.9
	20	221.0 ± 6.5	277.7 ± 11.6	335.0 ± 14.8	114.0 ± 10.5
	200	216.7 ± 12.8	275.3 ± 17.6	330.2 ± 23.4	113.5 ± 16.4
	2000	219.8 ± 13.2	271.5 ± 20.5	324.3 ± 19.1	104.5 ± 6.7
Female	0[a]	162.2 ± 5.1	180.3 ± 10.2	197.2 ± 14.7	35.0 ± 11.9
	20	164.8 ± 9.9	187.5 ± 12.5	203.0 ± 9.5	38.2 ± 6.1
	200	164.3 ± 10.9	183.5 ± 9.5	202.2 ± 10.8	37.8 ± 6.4
	2000	161.3 ± 6.3	183.7 ± 11.6	200.7 ± 13.2	39.3 ± 9.6

***Table 2**: Body weight on male and female rats after treat with Epidepride. Recorded on all animals prior to the start of dosing (D1) and then at weekly interval (D8) to the end of the study period (D15). (n = 5, mean ± SD).*

D15 - D1: Body weight on D15 minus body weight on D1.

[a]: 5% (v/v) alcohol solution.

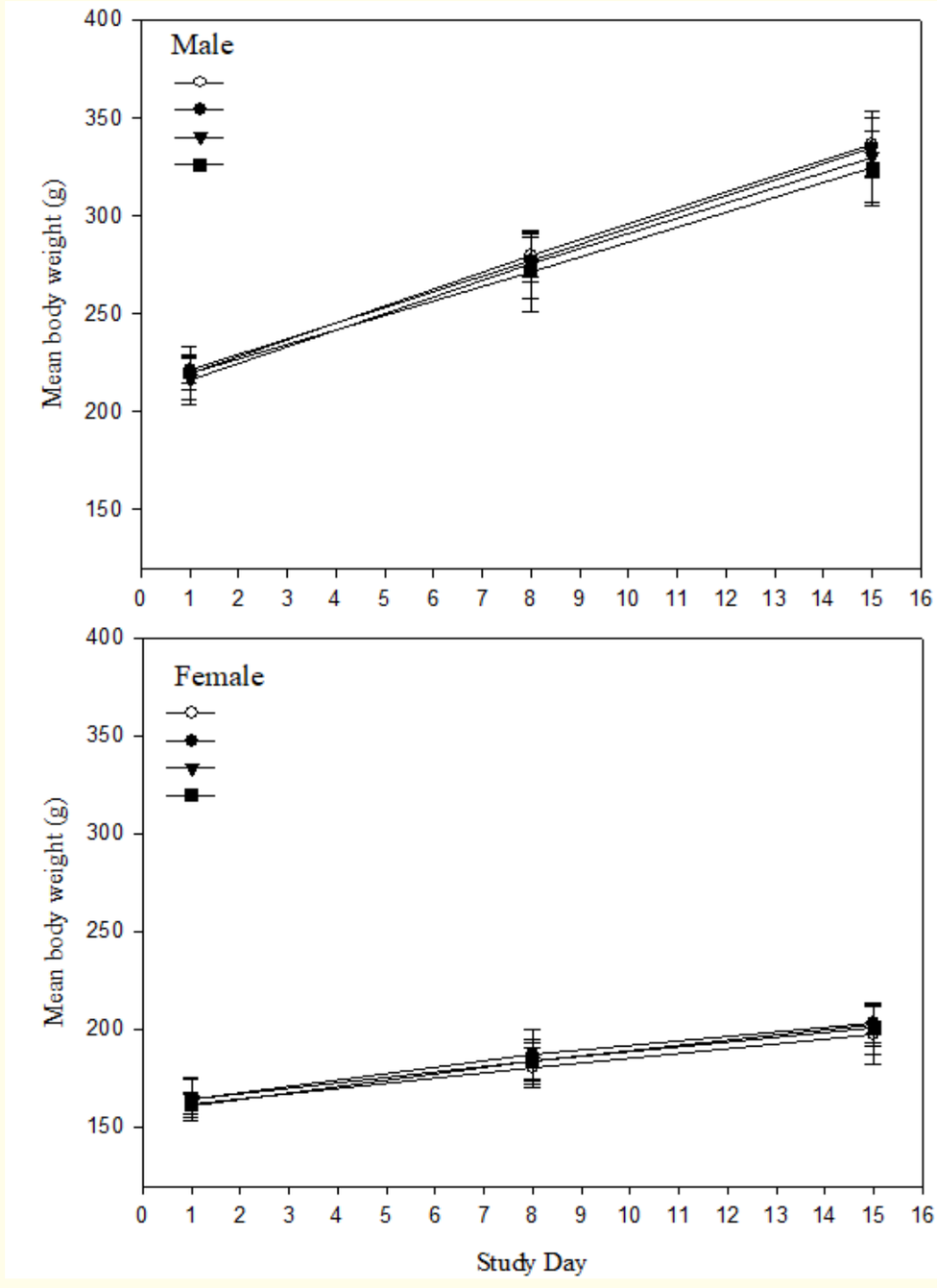

Figure 1: *Body weight growth curve of male (A) and female (B) after treat with Epidepride in toxicity study in rat. Recorded on all animals prior to the start of dosing (D1) and till to the end of the study period. (n = 6, mean ± SD).*

Mortality and clinical observations

The mortality and clinical signs are summarized in table 3-5. Whatever in male and female rat there are no animal death occurred. Wounds on the dorsal back area were observed in one male animal from the 200 μg/kg group from D10 to D14. This is a sign of fighting between rats in the same cage and was not related to the treatment. No clinical signs were observed in other male or female rats.

Sex	Dose (μg/kg)	Results
Male	0[a]	No animal deaths were observed. Wounds on the dorsal back area were observed in one mid-dose rat from D10 to D14.
	20	
	200	
	2000	
Female	0[a]	No animal deaths and no clinical signs were observed.
	20	
	200	
	2000	

Table 3: *Clinical signs in male and female rats after treatment with Epidepride. Recorded on all animals prior to the start of dosing (D1) to the end of the study period (D15). Dose from 0 to 2000 μg/kg of dosing were summarized. (n = 6, mean ± SD).*

[a]Ethanol 5% (v/v) solution.

Items	Dose	Incidence Occurring on Study Day (n'/n')					Total Incidence (n/n)
		1	2	3	4 - 5	6 - 14	
Mortality	0	0/6	0/6	0/6	0/6	0/6	0/6
	20	0/6	0/6	0/6	0/6	0/6	0/6
	200	0/6	0/6	0/6	0/6	0/6	0/6
	2000	0/6	0/6	0/6	0/6	0/6	0/6
Hypoactivity	0	0/6	0/6	0/6	0/6	0/6	0/6
	20	0/6	0/6	0/6	1/6	0/6	1/6
	200	0/6	0/6	0/6	0/6	1/6	1/6
	2000	0/6	1/6	1/6	1/6	1/6	1/6
Poor appetite	0	0/6	0/6	0/6	0/6	0/6	0/6
	20	0/6	0/6	0/6	0/6	0/6	0/6
	200	0/6	0/6	0/6	0/6	0/6	0/6
	2000	0/6	0/6	0/6	0/6	0/6	0/6
Moderate dehydration	0	0/6	0/6	1/6	0/6	0/6	0/6
	20	0/6	1/6	0/6	1/6	0/6	1/6
	200	0/6	0/6	0/6	1/6	1/6	1/6
	2000	0/6	1/6	1/6	1/6	1/6	1/6
Hunched posture	0	0/6	0/6	0/6	0/6	0/6	0/6
	20	0/6	0/6	0/6	0/6	0/6	0/6
	200	0/6	0/6	0/6	0/6	0/6	0/6
	2000	0/6	0/6	0/6	0/6	0/6	0/6
Vocalization	0	0/6	0/6	0/6	0/6	0/6	0/6
	20	0/6	0/6	1/6	0/6	1/6	1/6
	200	0/6	0/6	0/6	1/6	1/6	1/6
	2000	0/6	0/6	1/6	0/6	1/6	1/6

Table 4: *The observations of mortality and clinical sign of male rats after treat with Epidepride. Mortality, hypoactivity, poor appetite, moderate dehydration, hunched posture, and vocalization as the signification behavior of observed. Recorded were at dosing D1, D2, D3, D4-5 and the end of the study period (D6-14). (n = 6, mean ± SD).*

n/n: Total number of abnormal animals observed/Total number of animals examined.

n'/n': No. of abnormal animals observed/No. of animals survived on study day.

Items	Dose	Incidence Occurring on Study Day (n'/n')				Total Incidence (n/n)
		1	2	3 - 5	6 - 14	
Mortality	0	0/6	0/6	0/6	0/6	0/6
	20	0/6	0/6	0/6	0/6	0/6
	200	0/6	0/6	0/6	0/6	0/6
	2000	0/6	0/6	0/6	0/6	0/6
Hypoactivity	0	0/6	0/6	0/6	0/6	0/6
	20	0/6	0/6	0/6	1/6	1/6
	200	0/6	0/6	0/6	0/6	0/6
	2000	0/6	1/6	1/6	1/6	1/6
Poor appetite	0	0/6	0/6	0/6	0/6	0/6
	20	0/6	0/6	0/6	0/6	0/6
	200	0/6	0/6	0/6	0/6	0/6
	2000	0/6	0/6	0/6	0/6	0/6
Moderate dehydration	0	0/6	0/6	1/6	0/6	1/6
	20	0/6	1/6	0/6	1/6	1/6
	200	0/6	0/6	0/6	1/6	1/6
	2000	0/6	1/6	1/6	1/6	1/6
Hunched posture	0	0/6	0/6	0/6	0/6	0/6
	20	0/6	0/6	0/6	0/6	0/6
	200	0/6	0/6	0/6	0/6	0/6
	2000	0/6	0/6	0/6	0/6	0/6
Vocalization	0	0/6	0/6	0/6	0/6	0/6
	20	0/6	0/6	1/6	0/6	1/6
	200	0/6	0/6	0/6	1/6	1/6
	2000	0/6	0/6	1/6	0/6	1/6

Table 5: *The observations of mortality and clinical sign of female rats after treat with Epidepride. Mortality, hypoactivity, poor appetite, moderate dehydration, hunched posture, and vocalization as the signification behavior of observed. Recorded were at dosing D1, D2, D3, D4-5 and the end of the study period (D6-14). (n = 6, mean ± SD).*

n/n: Total number of abnormal animals observed/Total number of animals examined.

n'/n': No. of abnormal animals observed/No. of animals survived on study day.

Gross necropsy

The gross necropsy findings are summarized in table 6. Wounds caused by animal fighting were observed in a male rat from the mid-dose group and did not appear to be related to the treatment. No gross lesions were found in other animals. Based on the data gathered from this study, a single intravenous injection of Epidepride of up to 2000μg/kg (10,000-fold greater than the suggested human dose) did not cause any adverse effect in rats in clinical observation, body weight, or gross necropsy.

Ex vivo autoradiography

The specificity of the probe was determined by *ex vivo* autoradiography. The study was intravenous injection of 185 MBq of [^{123}I] Epidepride for 30min distribution in SD-rat. The images represent [^{123}I]Epidepride not only binding in highest level of dopamine D2/D3 receptors (striatum) and also in low D2/D3 receptor region: hypothalamus and midbrain represent in figure 2. The specific binding ratio of [^{123}I]Epidepride showed radioactivity retention in striatum (28.37 ± 2.98), hypothalamus (4.05 ± 0.76) and midbrain (4.45 ± 0.54). The significant radioactivity retention in the dopamine D2/D3 receptor regions, indicated the specificity of [^{123}I]Epidepride.

	Incidence Occurring (n/n)	
Dose (mg/kg)	0	2000 μg/kg
N	12	12
Brain		
The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.	-	-
Lung		
The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection.	-	-
The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. In one focus, numerous red blood cells are seeing in the airway and in alveolar spaces. No other abnormalities are associated with this one focus. Most likely, anesthesia and removal of the lung and heart en bloc from the thoracic cavity	1/12	
Liver		
The lobule structure is normal; there is neither inflammation nor vacuoles within the parenchymal cells of the liver.	-	-
Heart		
The myocardium, endocardium and coronary vessels are normal	-	-
Kidney		
The cortex, medulla and urinary space exhibit a normal histology. In one cross-section of a tubule, located in the medulla, there is necrosis and calcification of the cells.	-	1/12
The cortex, medulla and urinary space exhibit a normal histology.		
The cortex, medulla and urinary space exhibit a normal histology. In several focal areas of the medulla, some cross sections of tubules have eosinophilic casts and one of the tubules shows calcification		1/12
The cortex, medulla and urinary space exhibit a normal histology. Several cross sections of kidney tubules in the medulla show eosinophilic casts.		1/12
The cortex, medulla and urinary space exhibit a normal histology. In a few tubules, there are a few eosinophilic casts.	1/12	
Spleen		
White and red pulp have normal structure	-	-
Testis		
The testis is immature and there are no spermatozoa in the seminiferous tubules.	-	-

Table 6: *The observations of gross lesions of male and female rats after treat with Epidepride (0 and 2000*μg/kg*) in the end of the study (D15). Brain, Lung, Liver, Heart, Kidney, Spleen and Testis as the target organ of interested. (n = 12, mean ± SD).*

n/n: Total number of abnormal animals observed/Total number of found dead animals examined.

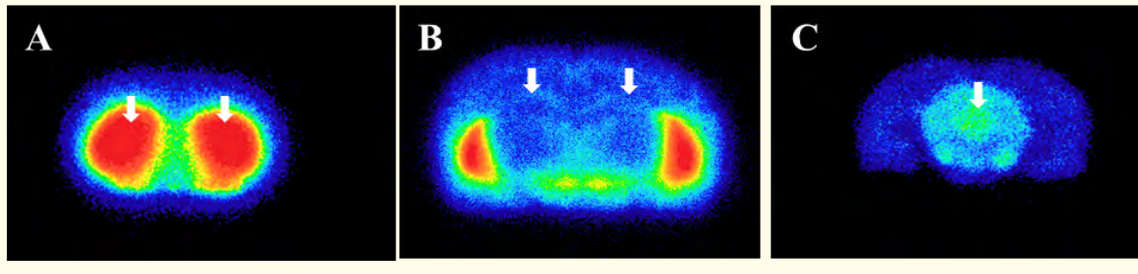

Figure 2: *Brain autoradiograms of the SD-rat after incubation with I-123-Epidepride (185MBq/200μL). A-C represent the region of striatum, Hippocampus and midbrain the binding ratio of I-123-Epidepride in dopamine D2/D3 receptor used cerebellum as reference region (n = 3, respectively).*

Small animal NanoSPECT/CT

On the [^{123}I]Epidepride/NanoSPECT/CT images, the radioactivity accumulated with the dopamine D2/D3 receptors in brain regions showed in figure 3. We used the cerebellum as reference region (no D2/D3 receptor), different sections of the brain were detected in the NanoSPECT/CT scan (striatum, midbrain and cerebellum). The signal quantified in the ROIs in these coronal sections compare with the reference baseline region indicated ratios were 9.23 ± 2.88 in striatum and 1.12 ± 0.44 in midbrain.

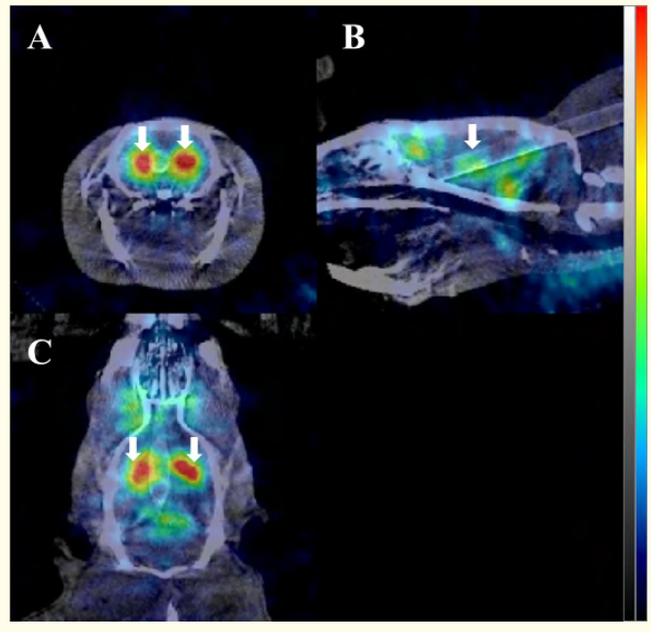

Figure 3: *In vivo NanoSPECT/CT imaging assay of the accumulation of I-123-Epidepride in brain sections 30 min after its injection (185 MBq/200 µL) into SD-rat. A to C represent the coronal, sagittal and transverse section of brain region. The arrow indicates the relative dopamine D2/D3 receptor position of the brain (striatum) (n=3).*

Discussion

PD is the most common neurodegenerative disorder in the world after Alzheimer's disease [21]. PD, cancer, and AIDS are considered by the World Health Organization (WHO) to have the three most important effects on social psychology. PD belongs to a group of conditions called motor system disorders because of its involvement in both production and inhibition of biological drivers, which is the result of the loss of dopamine-producing brain cells [5,22]. Epidemiological studies of the world found that PD affects about 0.3% of people over the age of 50 in industrialized countries, rising to 5% of the population over 80 years of age [5].

The dopamine system modulates cognitive function and enhances the efficiency of certain forms of thinking and working memory [4,16,23]. Dopamine is an important brain neurotransmitter and plays a significant role in major psychiatric and neurological disorders such as schizophrenia and PD. Dopamine acts on membrane-bound protein receptors, and a significant reduction in the density of these receptors in the basal ganglia region has been reported following the post-mortems of people with PD [7]. Currently, the clinical requirement for diagnosis of PD is based on medical history and neurological examination, without any laboratory tests [24]. Further tests, including brain scans, are required to rule out the presence of other diseases [5].

PET and SPECT are important tools for the *in vivo* imaging of brain neurochemistry [9]. Measurement of dopamine neuronal loss or increase would be a useful indicator for PD [4]. Nuclear medical studies have identified that some diseases involving central nervous system lesions, such as schizophrenia, Parkinson's disease, and Huntington's disease have a close relationship with the density of brain dopamine receptors, especially the dopamine D2-type (D2) receptor [25,26]. Large numbers of D2 receptor radiopharmaceuticals had been derived by modification of D2 receptor ligands and have been reported as useful in PET and SPECT imaging.

Benzamide has a high affinity for the D2 receptor, and ligands based on this structure have been developed. Three candidates based on this structure show good binding characteristics for the D2 receptor, IBF ((S)-5-iodo-7-N-(1-ethyl-2-pyrrolidinyl)methyl carboxamido-2, 3-dihydrobenzofuran), IBZM ((S)-3-iodo-N-[(1-ethyl-2-pyrro-lidinyl)] methyl-2-hydroxy-6-methoxybenzamide), and Epidepride ((S)-(-)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2,3-dimethoxybenzamide) (32-37). A similar lipophilicity is found for IBF (2.05), IBZM (2.75), and Epidepride (2.32) (32-34). However, Epidepride (Kd 0.024 nM) showed a better occupancy ratio for the D2 receptor than IBF (Kd 0.09 nM) and IBZM (Kd 0.43 nM) (36-37). For imaging, ^{123}I integrated with Epidepride is also superior in visualization of quantitative studies of dopamine receptors [13,16]. [^{123}I]Epidepride provides good contrast not only in the high-density with D2 receptor regions in the striatum but also in the low-density extrastriatal regions (about 20 - 80 times less dense than the striatum) [26]. Therefore, [^{123}I]Epidepride is the best choice for its imaging quality of dopamine D2 receptors in the striatum as well as in extrastriatal regions [25,26].

In this study, we modified a synthesis protocol and successfully synthesized unlabeled Epidepride at high purity, as shown in scheme 1. The different dose levels (20, 200, and 2000 μg/kg) for this study, based on the clinical dose of about 10 mCi (equivalent to 42 pmole) determined from the relative molecular mass of 416.27 and human body weight (60 kg), provided safety margins of 100-, 1000-, and 10 000-fold over the maximal recommended human dose. We present the results of Epidepride toxicology in figure 1 and table 2-3. There were no statistically significant differences in the body weights of the treated and control rats. Consideration of mortality, clinical signs, and gross lesions indicated that Epidepride had no adverse effects on rats at doses of up to 2000 μg/kg (Table 4-5). No clinical signs or observable gross lesions were found in post-mortem examinations of rats killed on schedule (Table 6). Thus, no adverse effects of Epidepride were seen in rats at doses of up to 2000 μg/kg.

In *ex vivo* autoradiography and *in vivo* NanoSPECT/CT image assay (Figure 2-3), [^{123}I]Epidepride binding in D2/D3 rich region: striatum and extrastriatal regions (hypothalamus and midbrain) in SD-rat. The present study suggests that [^{123}I]Epidepride has excellent potential utility in dopamine related syndromes, like Parkinson's syndrome and schizophrenia useful in evaluating therapeutic effects in the future.

The results of this study may be used as a reference point for the safety margin of Epidepride in humans. These data could be applied to the future clinical diagnosis of dopamine related disease in humans.

Conclusion

Dopamine is an important brain neurotransmitter which plays a significant role in the major psychiatric and neurological disorders such as schizophrenia and Parkinson's disease. The selectivity for the dopamine D2/D3 receptors that [^{123}I]Epidepride may be yet another useful imaging agent for noninvasively visualizing radiopharmaceutical in the brain and the peripheral nervous system.

In the toxicity assay, the Epidepride in rat model established in the study is 2000 μg/kg. The dose selected in this study provided safety margins of 10,000 folds over the recommended human dose, based on the conversion of body weights. Corresponding safety margins of 51,660 folds were also reached based on the conversion of surface area. It is also consistent with the toxicology analysis in studies of the rodents that showed Epidepride to be relatively stable *in vivo* and appears to be pharmacologically safe in healthy volunteers.

In vivo SPECT imaging quantity, [^{123}I]Epidepride also superior in visualization of pituitary adenomas. [^{123}I]Epidepride is the best choice for its high imaging quality of dopamine D2/D3 receptors in striatum as well as extrastriatal region. In the future we plan to assess the distribution and pharmacologic analysis of [^{123}I]Epidepride in rat model, wish to produce a successful platform for understanding the pathogenic process of PD and provide a gold-standard method for identifying promising treatments.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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