Comparative studies of D_2 receptors and brain perfusion in hemi-parkinsonism rats

LIN Yan-Song, LIN Xiang-Tong

(*Huashan hospital, Shanghai Medical University, Shanghai 200040*)

Abstract The relationship between dopamine D_2 receptors and brain perfusion in hemi-parkinsonism rats was studied. Hemi-parkinsonism rats were made by stereotaxic 6-hydroxy dopamine (6-OH-DA) lesions in substantia nigra(SN) and ventral tegmental area(VTA), apomorphine (Apo) which could induced the successful model rat rotates toward the intact side was used to select the rats, ^{125}I-IBZM *ex-vivo* autoradiography analysis and ^{99m}Tc-HM-PAO regional cerebral biodistribution were used to evaluate D_2 receptors and cerebral blood flow. The HPLC-ECD were used to measure striatum DA and its metabolites content. The lesioned side striatum DA and its metabolites HVA DOPAC reduced significantly than that of the intact side and seudo-operated group, striatum/cerebellum ^{125}I-IBZM uptake ratio was 8.04±0.71 in lesioned side of hemi-parkinsonism rats, significantly increased compared with the intact side and the seudo-operated group(p <0.05), 30.11±4.53% enhancement as compared to the intact side, and also show good correlation with 30 min Apo induced rotation numbers (r=0.98), the regional cerebral blood flow study didn't show significant difference between bilateral brain cortex area(p >0.05). These results indicated that in the 6-OH-DA lesioned side DA content decreased significantly and an up-regulation of striatum D_2 receptor binding sites was induced in hemi-parkinsonism rats, which showed good correlation with rotation behavior induced by Apo. Comparing with cerebral blood flow, D_2 receptor reflected by IBZM seems to be more specific and earlier to detect the cerebral functional impairment in experimental hemi-parkinsonism.

Keywords Parkinsonism, Receptors/dopamine D_2, Cerebral blood flow, Autoradiography, ^{125}I-IBZM, ^{99m}Tc-HM-PAO

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1 INTRODUCTION

Parkinson's disease(PD) is characterized by selective loss of dopaminergic cells in substantia nigra(SN) which project to striatum(ST), 14%~80% patients would accompanied with dementia during their later stage of PD according to various statistic data, studies revealed deficit of regional cerebral blood flow (rCBF) and glucose metabolism in frontal cortex etc. area in these patients[1]. The relationship between central dopamine(DA) transmitter system and brain perfusion in PD remains to be clarified. In the previous work[2], we established hemi-parkinsonism rats, explored their DA

Corresponding address: LIN Yan-Song, M.D., (Now is the Postdoc Fellow in the National Laboratory of Nuclear Medicine, 20 Qian Rong Road, Wuxi, Jiangsu 214063 E-mail:jsinm@public1.wx.js.cn

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D_2 receptors variation. To further investigate the relationship between brain perfusion and D_2 receptor, ^{125}I-IBZM autoradiography, and ^{99m}Tc-HM-PAO brain biodistribution were used in hemi-parkinsonism rats, meanwhile, HPLC-ECD was also used to detect *the contents of DA and its metabolites in ST of the models.*

2 MATERIALS AND METHOD

2.1 Instrument and reagents

Narishinge ST-79-28 stereo-taxic instrument (Kasuya, Japan); 865-model cryotate microtome (Buffalo,USA); HPLC-ECD (Salf Walters, USA). S(-)-3-iodine-2-hydroxy-6-methoxy-N[(1-ethyl-2-pyrrilidinyl) methyl] benzamide (IBZM(S)(-)), S(-)-2- hydroxy-6-methoxy-N[(1-ethyl-2-pyrrilidinyl) methyl] benzamide(BZM(S)(-)) were synthesized in National Laboratory of Nuclear Medicine. Hexamethyl-propylene amine oxime(HM-PAO, Amersham,UK); 6-hydroxy dopamine(6-OH-DA)(Sigma); apomorphine chloride (Shenyang First Pharmaceutical Factory, China).

2.2 Rat experimental hemi-parkinsonism model

Studies were carried out using male or female Sprague-Dawley rats weighted 197±21.3 g. Hemi-parkinsonism model rats were made by stereotaxic 6-OH-DA lesions in SN (A 2.0 mm, R 2.0 mm, H 7.8 mm) and ventral tegmental area(VTA)(A 2.0 mm, R 0.8 mm, H 7.2 mm), *animal model and seudo-operated control rats were established and screened out* as described previously[2,3]. Apomorphine(Apo)(0.2 mg/kg intraperitoneal injection i.p.) which could induced the successful model rotate toward the intact(left or unlesioned) *side was used to screen the model at* 1 week post-operation.

2.3 Experimental group

We chose 18 seudo-operated control rats and 18 hemi-parkinsonism model rats after 4 weeks 6-OH-DA lesioned, and the rats were randomly divided into 3 groups: group I for ^{125}I-IBZM autoradiography, group II for ^{99m}Tc-HM-PAO brain biodistribution and group III for HPLC-ECD detection of DA and its metabolite homovanillic acid(HVA),3,4-dihydroxyphenylacetic acid(DOPAC) in striatum.

2.4 Preparation of ^{125}I-IBZM

^{125}I-IBZM was synthesized by the kit process reported by Kung MP[4], the radiochemical purity was up to 92%, special activity 2.22 PBq/mol.

2.5 *Ex-vivo* autoradiography in rats brain

^{125}I-IBZM 7.4 MBq(0.3mL) was injected into the femoral vein of each hemi-parkinsonism model and control rats. The rats were sacrificed under anesthesia(diethyl ether)by decapitation 2 hours after injection. The brains were rapidly removed and placed in OCT

embedding medium, and frozen at -15°C. After reaching equilibrium at that temperature, consecutive 20μm coronal sections were cut with a cryostat microtome, thaw-mounted on gelatin-coated microscope slides, and air dried at room temperature. The slides contains the brain sections were exposed to GS-250 Phosphor Imaging Screen-BI for 2h and imaged in GS-250 Molecular Imager. The optical density volume which showed good correlationship with the radioactivity of each brain ROI was determined with an image analysis system.

2.6 ^{99m}Tc-HM-PAO biodistribution in rats

^{99m}Tc-HM-PAO 7.4 MBq(0.3mL) was injected into the femoral vein of each rat. The rats were sacrificed under anesthesia(diethyl ether) by decapitation 20 min postinjection, brains were quickly removed on the iced plate, the interested brain region such as frontal cortex, occipital cortex, parietal cortex and ST were dissected, weighed, then the radioactivity of aliquot sample were measured using a γ-counter. The percent of injected dose(%ID) per gram of sample was calculated by comparing the sample counts with the 1% count of the diluted initial dose. The uptake ratios of each region to cerebellum (CB) were then recorded.

2.7 HPLC-ECD detection of DA and HVA DOPAC in striatum

The rats were sacrificed under anesthesia(diethyl ether) by decapitation, brains were quickly removed on the iced plate, the interested brain region such as ST was dissected, weighed, put into 1 mL H_3ClO_4(0.005mol/L), homogenized under iced bath, then centrifuged at 15000×g for 30min, the supernatants were taken for further HPLC-ECD detection. The content of DA and its metabolites HVA DOPAC in striatum were measured by HPLC-ECD detection described previously[3].

2.8 Statistical analysis

Data were expressed as mean standard deviation, statistical analysis were carried out by t test.

3 RESULTS

3.1 Behavioral change induced by Apo in hemi-parkinsonism rats

2~5 min after Apo injection, the successful hemi-parkinsonism model rats showed its characteristic rotation toward the intact side accompanied by sniffing and seeking behavior, the mean rotation number induced by Apo was above 7 times/minute, show significantly differrence compare with the pseudo-operated group($p < 0.05$).

3.2 Increase of ^{125}I-IBZM D_2 receptor binding sites

Autoradiography and analysis (Fig.1) showed that the lesioned (right) side ST/CB ^{125}I-IBZM uptake ratio was 8.04±0.71, higher than the left (intact) side (6.23±0.47) and pseudo-operated group (6.23±0.32 and 6.22±0.63 for left and right side respectively) (p <0.05), and 30.11±4.53% enhancement as compared with the intact side (Fig.2), which showed good correlationship with the 30min rotation number induced by DA receptor agonist Apo(r=0.9802) Fig.3.

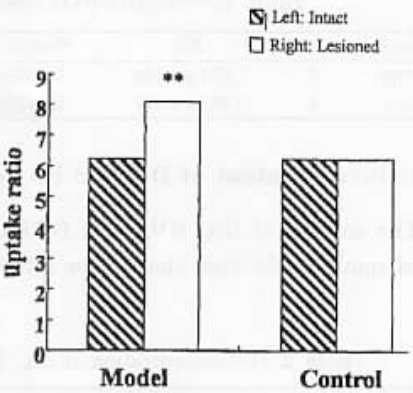

Fig.1 ^{125}I-IBZM D_2 receptor autoradiography and analysis (n=6). **Significantly different compared with the control group. (t >2.571, p <0.05)

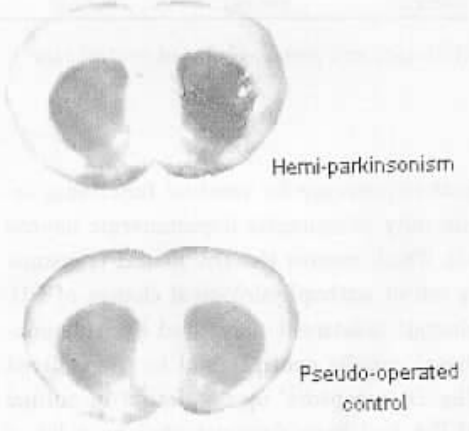

Fig.2 Distribution of ^{125}I-IBZM in coronal brain slices measured by *ex-vivo* autoradiography in hemi-parkinsonism model and control rat

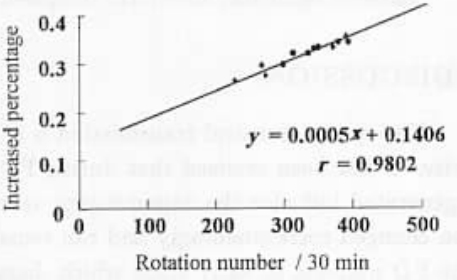

Fig.3 The increased percentage of lesioned to intact side uptake ratio (ST/CB) of ^{125}I-IBZM in hemi-parkinsonism (n=6) versus apomorphine induced rotation number

3.3 ^{99m}Tc-HM-PAO cerebral perfusion study

^{99m}Tc-HM-PAO regional brain biodistribution didn't show any difference in brain region between hemi-parkinsonism model and control rats(t <2.571, p >0.05) (Table 1).

Table 1 ^{99m}Tc-HM-PAO regional brain perfusion study (n=6)

Group	n	ST	Frontal cortex	Parietal cortex	Occipital cortex
control	6	1.021±0.104	0.969±0.015	1.013±0.015	1.003±0.017
Model	6	0.983±0.017	0.972±0.021	1.102±0.032	0.992±0.029

3.4 Reduced content of DA and its metabolites

The content of DA, HVA and DOPAC in lesioned side of hemi-parkinsonism decreased significantly than that of the intact side and pseudo-operated group rats(Table 2).

Table 2 The concentration of DA, HVA and DOPAC in bilateral striatum

Group	n	Side	DA	HVA	DOPAC
Model	6	Lesioned*	1647±31*	257±13*	237±17*
		Intact	9463±76	987±33	853±26
Control	6	Right	9421±74	979±39	861±44
		Left	9389±23	983±51	869±67

*Showing significant diffenrence compared with intact and pseudo-operated control rats

4 DISCUSSION

The synaptic neural transmission is of great importance for cerebral functional activity. It has been realized that during PD, not only presynaptic dopaminergic neuron degenerated but also the dopaminergic receptor which receive the DA neural transmission changed correspondingly and can sensibly reflect pathophysiological change of PD. The PD patients in early stage which dopaminergic treatment naive had been demonstrated the up-regulation of D_2 receptors in basal ganglia contralateral to the suffered limb by ^{125}I-IBZM SPECT D_2 imaging[5]. The D_2 receptors' up-regulation in animal after SN-ST neural circuit destroyed by 6-OH-DA had been demonstrated by a lot of *in-vitro* experiments, but the degree of up-regulation are variant, which might has something to do with the lesioned degree, measured techniques and time. When dopaminergic neuron of SN lesioned by 6-OH-DA, the release of DA reduced markedly, accordingly a series compensation mechanism in SN-ST dopaminergic circuit would occur: (1) Increased DA synthesis and release by the survived DA neurons, (2) Increased post-synaptic DA receptors to compensate the deficiency of DA, the compensatory role changed correspondingly with the amount of lesioned DA neurons and DA content until the compensation mechanism exhaustion occurred, the process implicated with a series of complicated mechanism. In our study, increased ^{125}I-IBZM uptake in lesioned side ST was observed in hemi-parkinsonism, suggesting an up-regulation which showed good correlation with Apo induced rotation behavior[3]. All these indicated that our prepared radiolabelled IBZM can objectively reflect the change of cerebral D_2 receptor and behavioral changes induced by dopaminergic lesions.

It is suggested that the blood flow changes of PD is due to the degeneration of SN-cortex dopaminergic projection which terminate in interior of frontal cortex, thereafter caused the atrophy and hypofunction of frontal cortex[6], thus induced hypoperfusion of frontal cortex. Whereas this hypothesis is disagreed by other people who insist that the degeneration of acetylcholinergic projection which terminate in frontal cortex may be responsible for brain blood change of PD[7]. There are also some disagreement of cerebral blood flow reflected by ^{99m}Tc-HM-PAO SPECT in PD which not accompanied by dementia, some reported that no significant difference between PD and controls[8], whereas results of our department indicated the diverse hypoperfusion in cerebral cortex, basal ganglia and cerebellum which has no significant correlation with the clinical manifestation, we ascribe it to PD accompanied by cerebral arteriosclerosis[9]. In this study we found there was no significant change in brain perfusion during unilateral ST-SN DA transmitter exhaustion in hemi-parkinsonism. It suggests that D_2 receptor is superior to brain perfusion for reflecting the change of brain function, the D_2 receptor change revealed by radiolabelled IBZM seems more specific and ealier to reflect the hypofunction of SN-ST neural pathway in early period expeimental parkinsonism, this was confirmed by decreased DA and its metabolites in lesioned side, and also showed good correlationship with apomorphine induced behavioral change.

It is worthwhile to mention that we just chose small animal model in this study, and we could not observe detailed perfusion change in regional brain area such as putamen, caudate nucleus etc., this is different from the monkey model studies reported by Dr Chen Shengdi[10]. Some further studies about the relationship between D_2 receptors and brain perfusion remains to be further investigated.

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