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Research Article

Therapeutic Effect of Salbutamol Enantiomer (R-Salbutamol) by Intranasal Administration on Rat Parkinsonian Model

Zhang R[1,2], Guo H[1], Deng L[1], Wen Tan[2,3]*

[1]Institute of Biomedical and Pharmaceutical Sciences, Guangdong University of Technology, China

[2]Post-Doctoral Innovation Site, Jinan University Affiliation, Yuanzhi Health Technology Co, Ltd, China

[3]Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Malaysia

***Corresponding author:** Wen Tan, Institute of Biomedical and Pharmaceutical Sciences, Guangdong University of Technology, Guangzhou 510006, China

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Abstract

The pathogenesis of Parkinson's Disease (PD) is complex. Recent research indicates that β2-adrenoreceptor agonist can reduce the risk of PD by inhibiting α-synuclein aggregation. Based on this, this study preliminarily explored the possibility of R-salbutamol (R-sal) nasal drops in the treatment of PD. After the rat PD model was established by rotenone, the grid, suspension and step-by-step experiments were used to investigate the effect of R-sal on the behavioral motor ability of PD rats. Immunohistochemical experiment was used to analyze GFAP, TH and α-Synuclein expression to further evaluate the effect from the perspective of pathology. We found that R-sal improved the motor ability of PD rats. In addition, the effect of nasal administration of R-sal was better than nebulization. Immunohistochemical results confirmed that R-sal could not only inhibit α-Synuclein expression, but also inhibited GFAP and promoted TH. Therefore, R-sal by intranasal administration has a therapeutic effect on PD rat and provides a reference for clinical PD treatment.

Keywords: Behavioral; Immunohistochemistry; Intranasal Administration; Parkinson; R-Salbutamol

Introduction

Parkinson's Disease (PD) is a neurodegenerative disease with complex causes. Its pathological feature is mainly the formation of a road body in substantia nigra [1]. At present, the treatment cost of PD is high [2,3], and levodopa is mainly administered clinically. However, the long-term use of this therapy will produce dose dependence and reduce the therapeutic effect [4]. The researchers found β2-adrenoceptor can inhibit the expression of α-synuclein (SNCA), thus inhibiting the formation of Lewy body and achieving the effect of treating PD [5]. This provides a new idea for the treatment of PD.

Salbutamol sulfate is β2 receptor agonist, which is a racemate of R - (levo-isomer) and S - (dextro-isomer) optical isomers. In vitro experiments showed that the affinity of R-Salbutamol for β2 receptor is twice that of racemate and 100 times that of dextrosome [6]. Nasal administration can bypass the blood-brain barrier and target the brain [7]. Glial fibrillary acidic protein (GFAP) is a marker protein in astrocytes [8]. Clinical studies have shown that GFAP is significantly elevated in PD patients [9]. Tyrosine Hydroxylase (TH) is the rate limiting enzyme of catecholamine synthesis, which catalyzes the hydroxylation of tyrosine to levodopa, thus affecting the exercise ability of PD patients [10].

In conclusion, this study investigated the therapeutic effect of R-sal on rat PD model through behavioral and immunohistochemical experiments, so as to provide experimental basis for PD research and new theory and method for clinical treatment.

Materials

Rotenone (≥95%, sigma Aldrich); R-sal (≥99%, self-made). PBS (Thermo Fisher Biotechnology Co., Ltd.); Th antibody (Biolegend, USA); α-Synuclein antibody (Biolegend, USA); GFAP antibody (biolegend, USA); Hematoxylin (GT100252, Merck millipore Technology Co., Ltd.); 4% paraformaldehyde (BL539A, biosharp Biological Reagent Co., Ltd.);

Animals

SPF grade male rats (Sprague Dawley, SD) weighing about 180-200 g were purchased from the experimental animal center of Southern Medical University. The culture conditions were controlled at room temperature, humidity about 60%, and alternating light time. There is an adequate supply of food and water. Replace the padding every three days.

Methods

Experimental animal group

The rats were randomly divided into blank group (without modeling) and model group. The model group was divided into inhalation administration group, nasal drip administration low, medium and high dose groups, anesthesia group (excluding the interference of isoflurane), inhalation normal saline group and nasal drip normal saline group, with 6 rats in each group.

Dosage

Inhalation group: at present, the concentration of salbutamol inhalation solution sold in the market is 5mg/ml, 2ml per time. The same dose was used in this study. Nasal drip group: According to the dose conversion formula of rats and humans, the dosage of R-sal refers to the inhalation group, and the low dose group (0.45mg/kg/d), medium dose group (0.9mg/kg/d) and high dose group (1.8mg/kg/d)

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were continuously administered for 30 days.

Model Building

Rotenone suspension was injected subcutaneously into the neck at a dose of 1.5mg/kg/d [11]. The drug was administered for 30 days and stopped for 1 day a week. After the modeling, the behavioral experiment was carried out. If the rats have stiff movement, unstable gait, obvious reduction of autonomous movement and muscle tremor, it can be judged that the modeling was successful.

Behavioral Efficacy Evaluation [11-12]

Grid test: The degree of muscle stiffness in rats is mainly detected by grid experiment. First, make a grid with length 100cm and width 100cm, the spacing of grid holes is 1cm, and it can be placed vertically and stably with the horizontal plane. Place the rat in the middle of the grid to ensure that all its limbs grasp the grid, then release the rat, and start recording the time required for the rat to move from rest to any limb. This time interval is the movement latency.

Suspension test: It is mainly used to measure the muscle tension of rats. Place a metal wire (2mm in diameter and 50cm in length) horizontally, 50cm above the ground, make the rat's forelimb grasp the wire, and record the suspension time of the rat. If a paw is released, it was judged to have fallen. For suspension time score: 0 point for 0 to 4 seconds, 1 point for 5 to 9 seconds, 2 points for 10 to 14 seconds, 3 points for 15 to 19 seconds, 4 points for 20 to 24 seconds, 5 points for 25 to 29 seconds, and 6 points for greater than or equal to 30 seconds. The test was repeated 3 times for each rat.

Step test: Step test is mainly used to detect the motor function of rat forelimbs. The experimenter grasped the back of the rat's body with one hand to ensure that the hind limb was off the ground, and grasped one forelimb of the rat with the other hand to make the other forelimb fall to the ground, moving obliquely to one side, with a moving speed of about 18cm/s. At the same time, the number of strides of rat forelimbs on the ground was recorded, and the left and right forelimbs were detected alternately. Taking the total steps of the two forelimbs as the final result, each rat repeated the test three times.

Tissue Sampling

After the behavioral test, the rats were anesthetized, fixed via cardiac perfusion with 4% paraformaldehyde after flushing out the red blood cells 200ml normal saline and then took the brain.

Immunohistochemistry Test

Embedding, tissue section and dewaxing; Antigen repair; Hydrogen peroxide incubation; Goat serum blocking; Primary antibody incubation; Secondary antibody incubation; DAB color development; Hematoxylin counterstaining; Dehydration, transparency and sealing; Microscopic examination.

Results and Discussion

Behavioral Test

Grid test: Compared with the model group, there was a significant difference in the movement latency in the inhalation group and the nasal drip group, and there was a very significant difference in the medium dose and high dose nasal drip groups (Figure 1).

The grid experiment showed that the motor retardation was improved after the administration of R-sal. In addition, the effect of low-dose nasal drip group on improving bradykinesia was slightly better than that of inhalation group, about 1.3 times. This may be due to the fact that in atomization, more dosage will be lost with atomization, while nasal drops maximize the delivery of drugs to the nasal cavity. inhalation administration does not need anesthesia and has little irritation to the nasal cavity of animals. However, considering the cost of finished drugs, nasal drops have great advantages.

Suspension test: There was significant or extremely significant difference in the suspension score between the inhalation group and the nasal drip group compared with the modeling group (Figure 2). The results showed that nasal administration of R-sal could improve the muscle tone of PD rats. However, the inhalation group and nasal drip low-dose group did not show therapeutic effect, and the efficacy appeared only in the middle dose and high dose of nasal drip, which may be related to the large degree of rotenone modeling to reduce rat muscle tone and difficult to recover. In addition, the suspension test also showed that the effect of nasal drip was better than that of inhalation group. (Figure 2)

Stride test: There were significant or extremely significant differences in the number of steps between the inhalation group and the nasal drip group compared with the model group. There was a significant difference between the nasal drip group and the nasal drip group compared with the model group (Figure 3). The stride test showed that the forelimb motor function was improved after

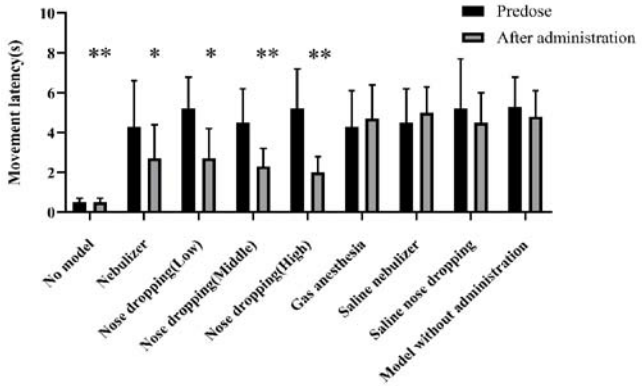

Figure 1: Grid test. *p <0.05, **p <0.01 t-test (compared with model group without administration).

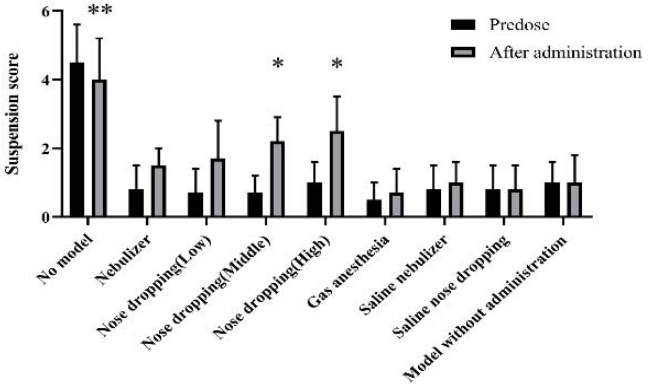

Figure 2: Suspension test. *p<0.05, **p<0.01 t-test (compared with model group without administration).

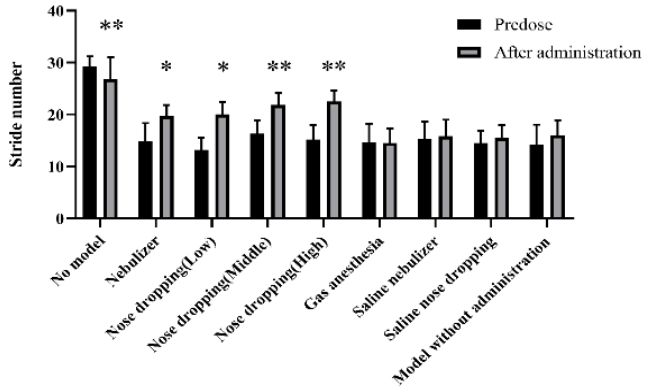

Figure 3: Step experiment. *p<0.05, **p<0.01 t-test (compared with model group without administration).

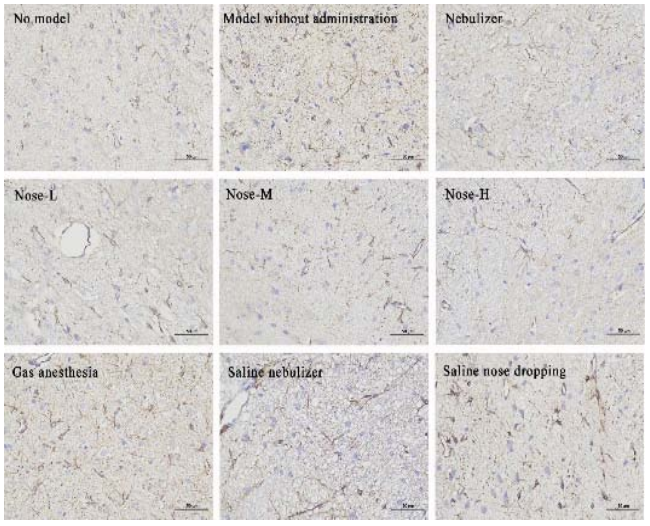

Figure 4: Immunohistochemical section of GFAP in rat midbrain.

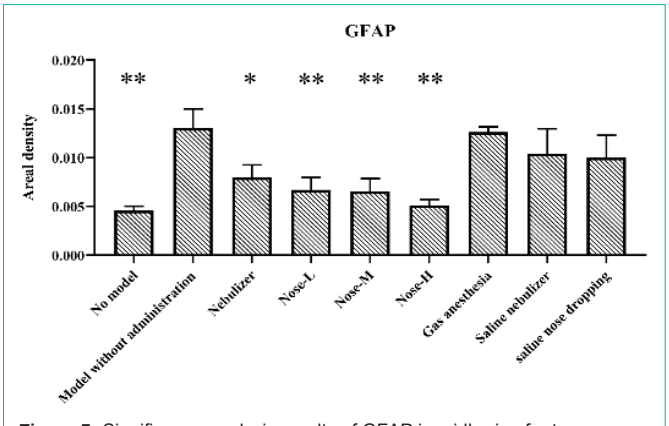

Figure 5: Significance analysis results of GFAP in midbrain of rats.

the administration of R-sal. At the same time, the conclusion that the effect of nasal drip is better than that of inhalation group is also proved again from the stride test.

Immunohistochemistry Test

GFAP: The immunohistochemical results of glial fibrillary acidic protein (GFAP), a marker of astrocytes, showed that GFAP was significantly increased in the model group compared with the non-model group. After administration of R-sal, the increase of GFAP was inhibited in both inhalation group and nasal drip group. Compared with the low-dose nasal drip group and inhalation group, the expression of GFAP in the low-dose nasal drip group decreased slightly. The expression of GFAP decreased with the increase of nasal drip dose. R-sal has strong inhibition on GFAP, and each administration group has therapeutic effect, which is significantly or extremely significant compared with the model group (Figure 4 and Figure 5); The above results show that R-sal can reduce the expression of GFAP. The therapeutic effect of nasal drip group was better than that of inhalation group.

TH: The immunohistochemical results of Tyrosine Hydroxylase (TH) showed that TH decreased significantly in the model group compared with the non-model group. After administration of R-sal, TH improved, but not particularly obvious, while in the high-dose nasal drip group, TH increased significantly. The promoting effect of R-sal on the expression of TH was relatively weak, and there was only a very significant difference in the high-dose nasal drip group (Figure 6 and Figure 7).

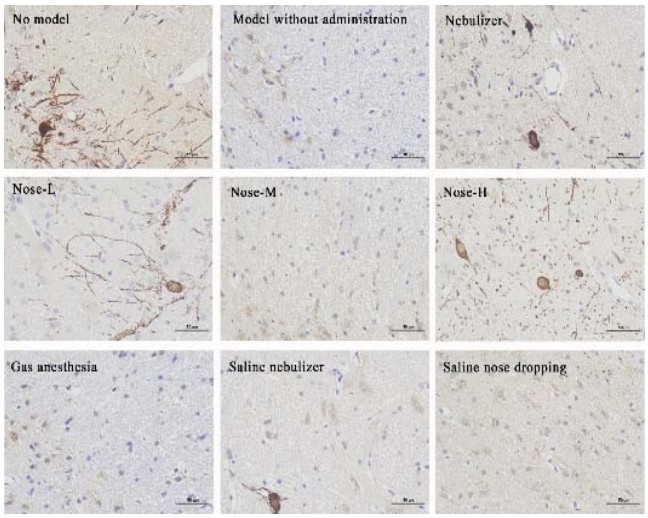

Figure 6: Immunohistochemical section of TH in rat midbrain.

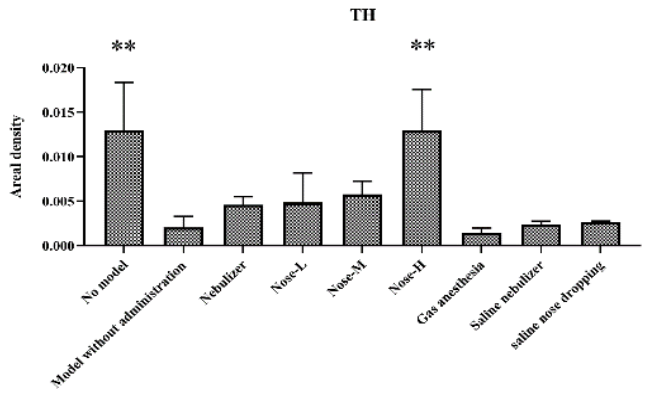

Figure 7: Significance analysis results of TH immunohistochemical surface density in midbrain of rats.

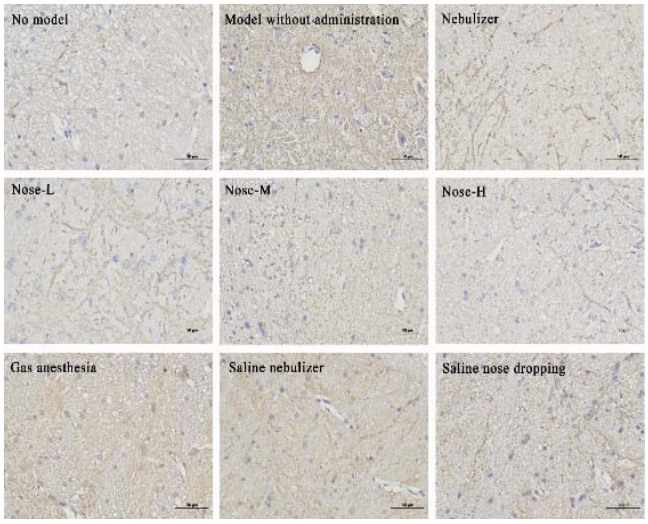

Figure 8: Immunohistochemical sections of α - syn in rat midbrain.

The above results show that R-sal can promote the expression of TH. The therapeutic effect of nasal drip group was better than that of inhalation group. However, in the inhalation group, low and medium doses of nasal drops did not show obvious pharmacodynamic effect, which may be due to the serious damage of rotenone to TH in SD rats and difficult to recover.

α- Synuclein: α-Synuclein test results show that compared with the non-model group, the α-Synuclein of model group was increased significantly. After administration of R-sal, nasal drip administration, the middle and high dose group had significant decreased of α-Synuclein. However, the decrease in inhalation group and nasal drip low-dose group was not significant. (Figure 8, Figure 9).

The above results show that R-sal can reduce α-Synuclein. The therapeutic effect of nasal drip group was better than that of inhalation group. This result is consistent with the research results of Mittal et al [5] (Figure 8).

Conclusion

The rat Parkinson's model induced by rotenone used in this study has obvious behavioral differences between the model and the non-model, and the symptoms of dyskinesia in the successfully modeled rats basically simulate the clinical characteristics of PD. After administration of R-sal, it showed therapeutic effect on PD rats. And we found that R-sal can not only inhibit the expression of α-Synuclein to achieve the effect of treating PD, but also inhibit GFAP and promote TH to treat PD. Compared with the inhalation group, the efficacy of nasal administration group is better, indicating that nasal administration of R-sal has higher brain targeting. The results can provide reference for clinical PD treatment.

Author Contributions

Conceptualization, Rui Zhang; Data analysis, Rui Zhang, Haihua Guo; Project administration, Rui Zhang; Funding acquisition, Wen Tan; Methodology, Haihua Guo; Supervision, Wen Tan; Writing-original draft, Rui Zhang; Writing-review and editing, Rui Zhang, Liangjun Deng and Wen Tan.

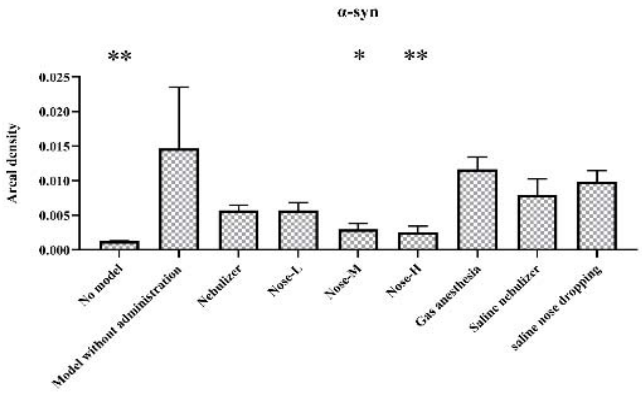

Figure 9: Significance analysis results of α-syn immunohistochemical surface density in midbrain of rats.

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Conflicts of Interest

The authors declare no conflicts of interest.

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