Long-term improvement by pramipexole in restless leg syndrome animal model

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ABSTRACT

Objective: Using an animal model of restless leg syndrome (RLS) we have developed recently to evaluate the dose and efficacy of pramipexole and to determine the acute and chronic efficacy of pramipexole's anti-RLS effect. **Methods:** C57BL/6 mice aged 4 wk were used. One month after dietary manipulation, all animals were lesioned with 6-OHDA in the bilateral A11 nuclei. Locomotive activities were tested one day before and 21days after the 6-OHDA lesion. In order to determine the acute anti-RLS effects of parmipexole, 21 days after A11 nuclei lesion, pramipexole was given by i.p at three different concentrations (0.1mg/kg, 0.5mg/kg, and 2.5mg/kg) once a day for 3 days. Administration of the same volume of saline served as control. Locomotive activities were measured before and 30 minutes after drug administrations everyday. Then, for chronic effects' evaluation, pramipexole was continuously injected once a day for 1 month. Locomotive activities were tested 28 days after the initial administration of prampexole. **Results:** Locomotive activities at 21 days after 6-OHDA lesion (total distance traveled and moving time) were significantly increased compared to control. Acute administration of pramipexole, the RLS-like symptoms were significantly alleviated. After chronic use of pramipexole, we measured the locomotive activities and found pramipexole significant improved the RLS-like symptoms even at the 28th day. There were no statistically significant difference between the three different doses of pramipexole used for these animals groups for acute response and chronic efficacy of this drug. **Conclusions:** Collectively, our results suggest that pramipexole is an effective treatment in RLS. Low dose of pramipexole is as effective as high dose of this drug.

BACKGROUND

Restless legs syndrome (RLS) is a sensory-motor disorder which is characterized by an urge to move the limbs, especially at rest and in the evening, whereas could be relieved with movement.
Although a complete pathophysiologic understanding for PLS remains illusive, dopaminergic dysfunction and iron deficiency (ID) are believed to play key roles. Thus we developed an animal model of RLS by bilateral 6-OHDA lesions in A11 nuclei plus iron deprivation in C57BL/6 mice. Using this animal model we tested the long-term improvement of pramipexole on animal locomotor activity. .

METHODS

Forty C57BL/6 mice aged 4 wk were used. They were divided into 5 groups: Control, RLS, Low Dose, Medium Dose, High Dose. Each group consisted of 8 mice, 4 male, 4 female. Mice in control group were fed with normal diet with 35mg iron/kg, others were given iron-deficit diet (ID) (TD 80396, Harlan Teklad, Madison, WI) with 3.5 mg iron/kg. One month after dietary manipulation, all animals were lesioned with 6-OHDA in the bilateral A11 nuclei. Locomotive activities were tested one day before and 21days after the 6-OHDA lesion. In order to determine the acute anti-RLS effects of parmipexole, 21 days after A11 nuclei lesion, intraperitoneal (i.p.) administration of pramipexole at three different concentrations (0.1mg/kg, 0.5mg/kg, 2.5mg/kg) were given once a day respectively in Low Dose, Medium Dose and High Dose groups for 3 days. Administration of the same volume of saline served as control. Locomotive activities (Moving Time and Total Distance Traveled determined for 60 min each time using VersaMax Monitor) were measured before and 30 minutes after drug administrations everyday. To evaluate the chronic effects, pramipexole was intraperitoneal injected at three doses for 1 month. Locomotive activities were tested 28 days after the initial administration of prampexole.

RESULTS

We determined that iron-deficit diet plus 6-OHDA lesion in A11 nuclei successfully developed the RLS model, while single A11 nuclei lesion didn't show the same effect. Locomotive activities at 21 days after 6-OHDA lesion (Moving Time and Total Distance Traveled) were significantly increased compared to one day before (Fig.1a, b) and the changes in locomotive activities lasted at least one month (Fig. 2 a and 2b) Thirty minutes after the administration of pramipexole, we measured the locomotive activities in all mice and it showed that the RLS-like symptom were significantly alleviated (Fig. 2a and 2b). After one month of administration of pramipexole we evaluated the chronic effect of pramipexole on the locomotive activities in the RLS animal model. Our result showed that chronic use of pramipexole at three different doses can significantly improve the animal's behaviors (Fig. 3a and 3b). There were no statistically significant difference among the mice treated with three different doses of pramipexole for acute response and chronic efficacy. Collectively, our results suggest that pramipexole administration is an effective treatment in the animal model of RLS. Low dose of pramipexole is as effective as high dose.

DISCUSSION

Increasing evidence indicates that the diencephalospinal dopaminergic pathway may contribute to RLS, and iron deficiency may also play a role in the development of the condition (Ondo et al, 2000; Connor et al, 2003; Allen 2004). According to this hypothesis, we have established a model of 6-OHDA lesioned mice in diencephalic-spinal (A11) dopaminergic nucleus plus dietary iron deprivation (Qu et al, 2007; Zhao et al, 2007). Our recent study confirm that iron plays a key role in the RLS pathophysiology as our data showed single A11 nuclei lesion without dietary iron deprivation didn't establish RLS model.

Dopaminergic (DA) agents are presently the treatment of choice for RLS. Levodopa was the first medication of this class of drugs to be used for treating RLS. However, because of the morning rebound and daytime augmentation of symptoms with levodopa, DA agonists progressively replaced levodopa as the first choice treatment for RLS (Hening et al, 2004; Montagna P, 2007). Bromocriptine and pergolide, which are ergot alkaloids agonists at the D1 and D2 dopamine receptors, have been used to treat RLS (Walters et al, 1988; Winkelmann et al, 1998). Pramipexole, which is a nonergoline derivative selectively stimulate the D2 and D3 dopamine receptors, is also supposed to have the same efficacy.

Our present study demonstrated that pramipexole significantly improved the RLS-like symptoms in our animal model of RLS and low doses of pramipexole will have both acute and chronic efficacy. Higher doses do not show more advantages. It indicated that pramipexole may be an effective treatment for RLS. Low dosage will be enough to improve symptoms of RLS. Our results are consistent with some former researches (Merlino et al. 2006; Partinen et al. 2006).

FIGURES

Figure 1

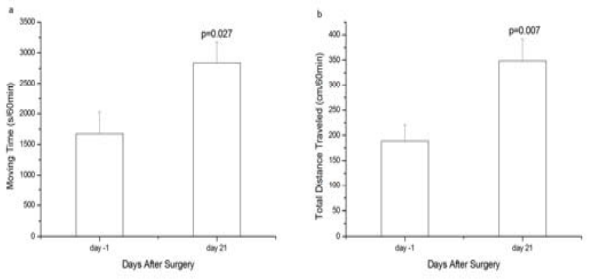


Figure 2

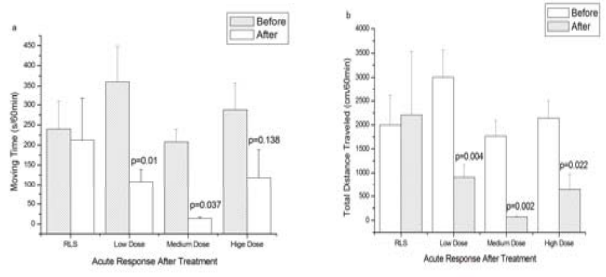
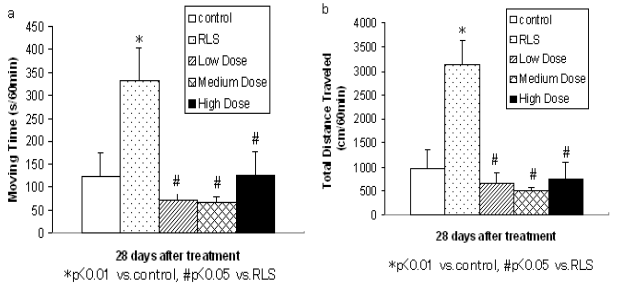


Figure 3



LEGENDS

Fig. 1 6-OHDA were stereotactic injected into A11 nuclei after one months' dietary manipulation. Locomotive activities were tested one day before and 21 days after the A11 nuclei lesion. Animals' behaviors were significantly changed which indicated that the model is successfully established.(a) the moving time increased by 41.08%; (b) the total distance travelled increased by 45.89%.The results were expressed as means ± SEM.

Fig. 2 Acute efficacy of Pramipexole for RLS treatment. Locomotive activities were performed before and 30min after the drug administration. (a) the moving time decreased respectively by 70.01%, 93.26%, 59.01% in Low Dose, Medium Dose and High Dose Group; (b) the total distance travelled decreased respectively by 69.73%, 95.70%, 70.02% in Low Dose, Medium Dose and High Dose Group. The results were expressed as means ± SEM.

Fig. 3 Chronic efficacy of parmipexole for RLS treatment. Locomotive activities were measured 28 days after the administration of pramipexole: Compared to the RLS Group, (a) animals' moving time decreased respectively by 78.31%, 19.88% and 62.17% in Low Dose, Medium Dose and High Dose Group; (b) the total distance travelled decreased respectively by 78.97%, 83.96% and 75.99% in Low Dose, Medium Dose and High Dose Group. The results were expressed as means ± SEM.

CONCLUSIONS

Our present study demonstrates that pramipexole significantly improves the RLS-like symptoms in our animal model of RLS and low dosage of pramipexole has both acute and chronic efficacy. It indicates that pramipexole may be an effective treatment for RLS. Low dose of pramipexole is as effective as higher doses to improve symptoms of RLS.

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