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Original article

Melatonin alleviates acute spinal cord injury in rats through promoting on progenitor cells proliferation

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ABSTRACT

The previous studies have shown that melatonin is beneficial for nervous system after spinal cord injury (SCI). After SCI, the endogenous neural stem/progenitor cells (eNSPCs) proliferate and differentiate into neurons and glial cells. In the present study, we examined the effect of melatonin on eNSPCs proliferation and differentiation in SCI rat model. SCI rat model was established by dropping a 10 g rod from the height of 25 mm. Then, the rats were randomly divided into the control group, the melatonin treated group, and the G3335 treated group. The Basso-Beattie-Bresnahan locomotor rating scale (BBB scale) was used to evaluate the recovery of locomotor function after SCI. Flow cytometry was used to evaluate eNSPCs proliferation and differentiation. The rats in the melatonin treated group demonstrated significantly faster locomotor function recovery and more eNSPCs proliferation and differentiation. However, these effects were abolished in the G3335 treated group. Melatonin can effectively promote locomotor function recovery via improving eNSPCs proliferation and differentiation after SCI.

1. Introduction

Spinal cord injury (SCI) is a serious central nervous system (CNS) injury resulting in a variety of complications including paralysis, sensory deficits and motor dysfunctions (Jones et al., 2005). In 1996, endogenous neural stem/progenitor cells (eNSPCs) have been found in adult human spinal cord (Weiss et al., 1996). In addition, the previous study has shown that eNSPCs transplantation may be potentially beneficial for functional recovery of patients after SCI (Madhavan et al., 2009).

Melatonin, as a neurohormone, is synthesized and secreted from the pineal gland (Vanecek, 1998). Recently, a variety of studies demonstrated that melatonin enhances adult stem cell viability, proliferation, and differentiation (Radio et al., 2006), suggesting that melatonin may play an important role in regulating neurogenesis of eNSPCs in CNS. In addition, Fu et al. (2011) have shown that melatonin stimulates eNSPCs proliferation and differentiation *in vitro*.

To the best of our knowledge, there is no study assessing the effects of melatonin on eNSPCs proliferation and differentiation after SCI *in vivo*. Therefore, we used SCI rat model to evaluate melatonin treatment effects.

2. Methods

2.1. Experimental animals

A total of 40 male 8-week-old Sprague-Dawley rats averagely weighting 250–270 g were enrolled in the present study. Ten of them developed health problems or died after SCI. The remaining 20 rats were used for further study. The 20 rats were randomly divided into three groups including the SCI group, the control group, and the melatonin treatment group.

2.2. SCI rat model

As described previously (Park et al., 2010), a contusive injury was performed. In brief, the T10 level was exposed with T9 laminectomy. Then, a rod weighting 10 g was lowered from 25 mm to the back surface of the spinal cord.

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2.3. Melatonin treatment

As described previously (Park et al., 2010), the melatonin treatment group rats were subcutaneously injected with melatonin (Sigma, St. Louis, MO, USA) twice a day at doses of 10 mg/kg body until the end of the experiment. Rats in the control group were injected subcutaneously with normal saline (Sigma, St. Louis, MO, USA) at the same dosage.

2.4. Assessment of motor function

Motor function was evaluated with the BBB scale (Basso et al., 1995) on postoperative days 1, 3, 7, 14, and 21.

2.5. Bromodeoxyuridine (BrdU) injection

Two days before the end of the experiment, rats were injected intraperitoneally with BrdU (Sigma, St. Louis, MO, USA) at doses of 50 mg/kg body weight once a day in order to label proliferating cells.

2.6. Tissue processing and immunohistochemistry

After assessment of motor function, rats were anesthetized and perfused using 4% PFA in 0.1 M PBS for 15 min. Then, mouse monoclonal anti-BrdU and rabbit polyclonal anti-Nestin were used to incubate with tissue at 4 °C. The slides were then incubated with goat anti-mouse antibody for 1 h.

2.7. Flow cytometry

Spinal cord tissue (T8-T10) was collected and dissociated in trypsin (0.5 mg/ml) and collagenase (0.5 mg/ml). The spinal cord cells were then incubated in 0.5 ml of 2 M HCl and 0.5% IFS for 20 min; 0.1 M Na2B4O7 for 2 min; TritonX-100 solution for 5 min; 100% normal rabbit and goat serum for 30 min; FITC conjugated BrdU antibody, Tritc conjugated NeuN or Tritc conjugated Nestin antibody for 1 h.

2.8. RNA isolation and RT-PCR analysis

In order to isolate RNA, the spinal cord tissue (T8-T10) was harvested and homogenized with 1 mL Tri-reagent (Sigma, St. Louis, MO, USA). The RNA was reverse transcribed with oligo (dT) 15 primers and SuperScript II reverse transcriptase. The reaction mixture was used as a previously reported PCR templates (Pierpaoli and Maestroni, 1987). Oct4 Forward: Δ-17 FAD gene sequences has been retrieved from NCBI (FW362214.1) as follows 5-ATGGCGAC TAAGCAGCCGTAC-3 Reverse: 3-CAGTTCCCGACCCTGACGGAG-5; GFAP Forward: 5-ATCAAGCGGTCGCTGCCCAGC-3; Reverse: 3-GA GTGCTTTGAGGCCTCGGTG-5; MAP2 Forward: 5-CCGCTGTCGCTC TACTACACG-3; Reverse: 3-GTGCGCATCGTGGCCATCGCC-5.

2.9. Statistics

The SPSS software was used to perform statistics analysis. Data were presented as mean ± SEM. One-way ANOVA was performed to analyze statistical significance. When p values were <0.05, the differences were considered statistically significant.

3. Results

3.1. The effects of melatonin on motor function recovery

All rats showed complete flaccid paralysis within the acute phase of SCI. Then, all groups improved until the end of experiment.

On 14th and 21st days after SCI, compared with those in the SCI and control groups (4.5 ± 1.4, 7.7 ± 1.7 and 4.5 ± 1.5, 7.8 ± 1.8, respectively), BBB score in the melatonin treatment group was significantly higher (8.7 ± 1.7, 11.7 ± 1.5, respectively) (Fig. 1).

3.2. The effects of melatonin on eNSPCs proliferation

We further assessed the effects of melatonin on eNSPCs proliferation using flow cytometry. On day 1, 7, 14, and 21, rats in the melatonin group showed significantly higher values of BrdU-positive expression indicating significantly higher eNSPCs proliferation. However, on day 3, there was no statistically significant differences observed between groups (Fig. 2).

3.3. The effects of melatonin on Nestin and NeuN expression

In addition, we used immunohistochemistry to assess the effects of melatonin on Nestin and NeuN expression. The results

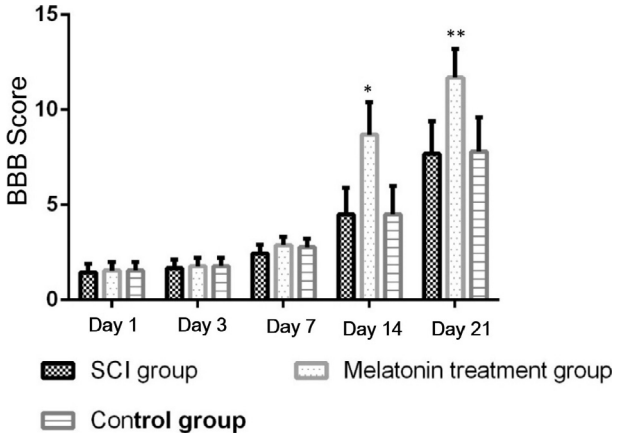

Fig. 1. The effects of melatonin on motor function recovery. Data were expressed as means ± S.D. (n = 4). *P < 0.05, compared with the SCI and control groups, **P < 0.01, compared with the SCI and control groups.

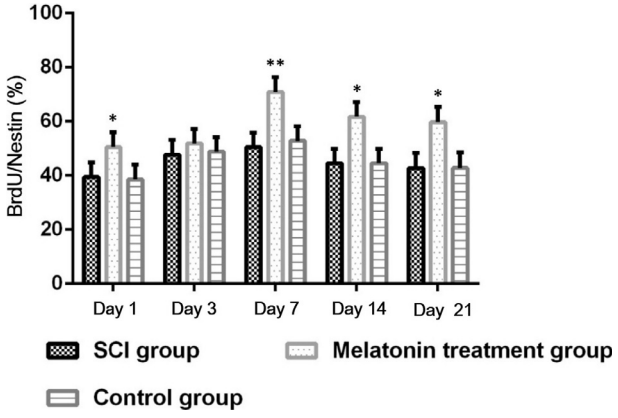

Fig. 2. The effects of melatonin on eNSPCs proliferation. Data were expressed as means ± S.D. (n = 4). *P < 0.05, compared with the SCI and control groups, **P < 0.01, compared with the SCI and control groups.