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Preventive and therapeutic effect of simvastatin on secondary inflammatory damage of rats with cerebral hemorrhage

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ABSTRACT

Objective: To investigate the preventive and therapeutic effect and mechanism of simvastatin on secondary inflammatory damage of rats with cerebral hemorrhage.

Methods: Sixty SD rat aged 9–12 weeks were chosen and divided into the control group, model group and simvastatin-treated group randomly with 20 rats in each group. Rats in the model group and simvastatin-treated group were infused with autologous fresh uncoagulated blood to the right brain tissue of the basal ganglia to build the cerebral hemorrhage model, while rats in the control group were treated with the same amount of normal saline. Then, rats in the simvastatin-treated group were given a gavage of 3 mg/kg of simvastatin once a day after modeling. Rats in the three groups were given nerve dysfunction score (NDS) and wet-dry weighting method was used to detect the brain water content (BWC) of brain tissues around the lesion of the rats. Then Nissl staining was conducted and the undamaged neurons were counted. Immunohistochemical SP method was applied to count the number of NF-κB, TLR4 and IL-1β positive cells in brain tissues around the lesions, and the immuno fluorescence method was employed to determine the expression levels of NF-κB, TLR4 and IL-1β proteins.

Results: The NDS results of the simvastatin-treated group at all time points were all significantly higher than those of the model group ($P < 0.05$); the BWC values of the simvastatin-treated group at all time points were all significantly lower than those of the model group at the same periods ($P < 0.05$); the number of the undamaged neurons around the lesions of the simvastatin-treated group at all time points were all significantly higher than those of the model group ($P < 0.05$); seven days after treatment, the number of the NF-κB, TLR4 and IL-1β positive cells in brain tissues around the lesions of the simvastatin-treated group were all significantly lower than those of the model group ($P < 0.05$), and its expression levels of NF-κB, TLR4 and IL-1β protein were also significantly lower than those of the model group ($P < 0.05$).

Conclusions: Simvastatin can inhibit the expressions of NF-κB, TLR4 and IL-1β proteins in rats with cerebral hemorrhage, and protect neurons and reduce secondary inflammatory damages by down-regulating the above protein-mediated inflammatory responses.

1. Introduction

Cerebral hemorrhage is a common clinical disease which refers to non-traumatic parenchymal hemorrhage caused by cerebrovascular rupture. With the aggravation of aging population, the occurrence rate of the disease tends to increase year by year [1]. The occurrence of cerebral hemorrhage is related to cerebral amyloid angiopathy. Most survivors would be left general sequelae of different levels, which influence their life quality seriously [2]. There are studies claiming that after the onset of cerebral hemorrhage, endothelial cells, inflammatory cells, gliocytes and neurons of local brain tissues of the lesions were stimulated and NF-κB and irritable inflammatory responses were activated due to ischemia and hypoxia, which, as a result, leads to secondary inflammatory damages [3–5]. Therefore, it is more and more attractive in clinic to prevent and treat secondary inflammatory damages and inflammatory

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responses around lesions after cerebral hemorrhage. Simvastatin is a kind of cholesterol inhibitors which possesses certain neuroprotective effect and is widely used in cerebrovascular accidents [6]. In this study, in order to observe the preventive and therapeutic effect and mechanism of simvastatin on secondary inflammatory damage of rats with cerebral hemorrhage, SD rats aged 9–12 weeks were selected to establish the cerebral hemorrhage model and given simvastatin for intervention to observe its preventive and therapeutic effect and mechanism on secondary inflammatory damage of rats with cerebral hemorrhage, which aimed to provide theoretical basis for the clinical prevention and treatment of secondary inflammatory damage after cerebral hemorrhage.

2. Materials and methods

2.1. Animals

Sixty male SD rats [9–12 weeks, (300 ± 20) g] were purchased from Beijing Vital River Laboratories were selected. They belonged to the II-level raised animals. They were fed at room temperature (25 ± 2) °C, and they could take water freely. The experiment was carried out in two weeks.

2.2. Instruments and reagents

Olympus light microscope, PM-10AD Olympus photomicrographic device (Olympus, Japan), CCD gel imaging system (BLO-RAD), PHS-3C acidimeter (Xiaoshan Analytical Instrument Factory), Leitz1512 histotome (Leitz, German), CMIAS image analysis system (Beihang University), 722 spectrophotometer (Shanghai Medical Instrument Factory) and AXiovert200 inverted fluorescence microscope (ZEISS, German) were employed. Simvastatin (Zhejiang Nanyang Pharmaceutical Co. Ltd, approved by 20073719), TLR4 primary antibody (Wuhan Boster Biotechnology Co. Ltd.), NF-κBP65 primary antibody (Santa Cruz Biotechnology Inc, USA), SP kit (Fuzhou Maixin Biotech. Co., Ltd.), TNF-α primary antibody and IL-1β primary antibody (Bioss Antibodies, Beijing, China) were used in this study.

2.3. Model preparation and grouping

Sixty SD rats were divided into the control group, model group and simvastatin-treated group randomly with 20 rats in each group. Rats in the model group and simvastatin-treated group were infused with fresh autologous uncoagulated blood to the right brain tissue of the basal ganglia to build the cerebral hemorrhage model. Modeling method: rats were administered with 0.03 mL/kg of chloralic hydras by intraperitoneally injection for anesthesia. After the right autogenous femoral arterial blood was collected, the rats were fixed to open up their skins. In order to inject 50 μL autoblood to the caudate nucleus basal ganglia of the rats, a hole at 3.0 mm beside the right of the midline at 0.2 mm in front of the bregma was drilled, and a needle was inserted to about 6.0 mm using microinjector. The injection took 5 min. Sterile bone wax pore was used for suture and disinfection. Rats in the control group did not receive autoblood injection. Instead, they were treated with the same amount of normal saline to the right brain tissue of the basal ganglia. After modeling, rats in the simvastatin-treated group were given a gavage of 3 mg/kg of simvastatin once a day after they were awake from anesthesia.

2.4. Observation

Twelve hours after totally reviving from anesthesia, the nerve dysfunction score (NDS) of rats in the three groups was assessed. Besides, at the 12, 24, 48, and 72 h and the 7th day after modeling, four rats of each group were executed. Wet-dry weighting method was used to detect the brain water content (BWC) of brains tissues around the lesion of the rats. Detection methods: 100 mg brain tissue around the lesions in rats was collected and its wet weight was weighed. Then, it was roasted in an electrothermostat at 100 °C for 48 h to measure its dried weight. BWC (%) = [(Wet weight − dried weight)/Wet weight] × 100%. After that, the brain tissues around the lesions of rats in each group were fixed conventionally to prepare sections for Nissl staining. The undamaged neurons were calculated. Immunohistochemical SP method was applied to count the NF-κB, TLR4 and IL-1β positive cells in brain tissues around the lesions, and the immunofluorescence method was employed to determine the expression levels of NF-κB, TLR4 and IL-1β proteins.

2.5. Statistical methods

Measurement data were analyzed by SPSS13.0. Means of the measurement data between two groups were compared by independent-sample *t*-test and expressed by mean ± SD. Comparisons among multiple groups were analyzed by One-way ANOVA. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Results of NDS after modeling

The NDS results of the model group and simvastatin-treated group at all time points after modeling were all significantly lower than those of the control group at the same periods ($P < 0.05$). Besides, the results of NDS of the simvastatin-treated group at all time points after modeling were all significantly higher than those of the model group ($P < 0.05$) (Table 1).

3.2. Comparison of BWC around lesions at all time points after modeling

The BWC values of the model group and simvastatin-treated group at all time points after modeling were all significantly higher than those of the control group at the same periods ($P < 0.05$); the BWC values of the simvastatin-treated group at all time points after modeling were all significantly lower than those of the model group at the same periods ($P < 0.05$) (Table 2).

3.3. Comparison of counted undamaged neurons around lesions at all time points after modeling

The number of undamaged neurons around lesions in brain tissues of the model group and simvastatin-treated group at all time points after modeling were all significantly lower than those

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