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Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research http://dx.doi.org/10.1016/j.apjtm.2017.01.003

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

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ARTICLE INFO

Article history:
Received 16 Nov 2016
Received in revised form 14 Dec 2016
Accepted 17 Jan 2017
Available online 19 Jan 2017

Keywords:
Simvastatin
Cerebral hemorrhage
NF-κB
TLR4
IL-1β
Secondary inflammatory damage

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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Peer review under responsibility of Hainan Medical University.
Foundation project: This study is supported by Hebei Social Science Fund Project in 2016 (Grant No. HB16LJ006), the Dr. Start-up Fund of North China University of Science and Technology (2015).

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

Table 1
Comparison of NDS results at all time points after modeling.

Group	12 h	24 h	48 h	72 h	7 d
Model group	8.2 ± 1.1*	8.1 ± 1.5*	7.9 ± 1.4*	7.7 ± 1.4*	10.9 ± 1.1*
Simvastatin-treated group	10.2 ± 1.7*#	10.0 ± 1.5*#	9.7 ± 1.1*#	9.1 ± 1.6*#	12.1 ± 1.4*#
Control group	18.2 ± 1.1	18.2 ± 1.2	18.3 ± 1.4	17.9 ± 0.6	18.5 ± 1.2

Compared with the control group, *$P < 0.05$; compared with the model group, #$P < 0.05$.

Table 2
Comparison of BWC (%) around lesions at all time points after modeling.

Group	12 h	24 h	48 h	72 h	7 d
Model group	81.0 ± 0.3*	81.9 ± 1.6*	83.0 ± 1.8*	83.6 ± 2.1*	79.4 ± 1.5*
Simvastatin-treated group	78.7 ± 1.4*#	79.6 ± 0.9*#	79.3 ± 2.1*#	81.5 ± 2.5*#	78.6 ± 1.6*#
Control group	77.1 ± 2.1	77.0 ± 1.8	73.3 ± 2.1	76.5 ± 3.8	75.8 ± 1.8

Compared with the control group, *$P < 0.05$; compared with the model group, #$P < 0.05$.

of the control group at the same periods ($P < 0.05$); and the number of undamaged neurons around lesions 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 3).

3.4. Comparison of number of counted NF-κB, TLR4 and IL-1β positive cells in brain tissues around lesions in rats of each group after 7 d for modeling

The number of the counted NF-κB, TLR4 and IL-1β positive cells in brain tissues around lesions after 7 d for modeling of the model group and simvastatin-treated group were all significantly higher than those of the control group at the same periods ($P < 0.05$), and the number of counted NF-κB, TLR4 and IL-1β positive cells of the simvastatin-treated group after modeling were all significantly lower than those of the model group at the same periods ($P < 0.05$) (Table 4).

3.5. Comparison of expression quantity of NF-κB, TLR4 and IL-1β proteins in brain tissues around lesions in rats of each group after 7 d for modeling

The number of the expression quantity of NF-κB, TLR4 and IL-1β protein in brain tissues around lesions after 7 d for modeling of the model group and simvastatin-treated group were all significantly higher than those of the control group ($P < 0.05$), and the expression quantity of NF-κB, TLR4 and IL-1β protein of the simvastatin-treated group after 7 d for meddling were all significantly lower than those of the model group at the same periods ($P < 0.05$) (Table 4).

4. Discussion

Cerebral hemorrhage is a common type of strokes accounting for about 20%–30% of the total prevalence rate of strokes. With the increasing aging populations, its incidence trends to rise year by year, which has been a life-threatening public health problem [7–10]. The pathological change of the pathogenesis of cerebral hemorrhage is a complicated process involving a lot of factors. Some researchers demonstrated that cerebral hemorrhage is caused by arteriole damages and rupture resulted from chronic hypertension or vascular amyloidosis [11]. Other researches confirmed that inflammatory cell infiltration of brain tissues in lesions, edema and cell apoptosis are all important predisposing factors for secondary cerebral injury after cerebral hemorrhage [12–15]. Among them, theories of inflammatory responses and cell apoptosis have attracted much interest in clinical research [16].

Table 3
Comparison of number of counted undamaged neurons around lesions at all time points after modeling.

Group	12 h	24 h	48 h	72 h	7 d
Model group	44.2 ± 3.1*	42.1 ± 3.2*	40.6 ± 3.7*	33.4 ± 3.6*	43.4 ± 3.4*
Simvastatin-treated group	48.3 ± 3.3*#	45.6 ± 3.0*#	43.5 ± 3.0*#	41.5 ± 3.5*#	46.3 ± 3.4*#
Control group	53.8 ± 2.3	52.5 ± 2.9	52.1 ± 2.7	51.6 ± 3.0	52.7 ± 3.4

Neurons were counted as the number per Hp. Compared with the control group, *$P < 0.05$; compared with the model group, #$P < 0.05$.

Table 4
Comparison of positive NF-κB, TLR4 and IL-1β cell counts and their expression quantity in brain tissues around the lesions in rats of each group after 7 d for modeling.

Group	Positive cell counts			Expression quantity		
	TLR4	NF-κB	IL-1β	TLR4	NF-κB	IL-1β
Model group	30.10 ± 3.09*	27.10 ± 3.17*	20.20 ± 3.38*	0.57 ± 0.04	0.52 ± 0.03	0.61 ± 0.08
Simvastatin-treated group	13.99 ± 2.65*#	13.72 ± 3.34*#	12.02 ± 1.92*#	0.46 ± 0.05*#	0.39 ± 0.03*#	0.40 ± 0.06
Control group	8.65 ± 1.53	6.47 ± 1.98	5.21 ± 1.19	0.15 ± 0.02	0.20 ± 0.02	0.23 ± 0.03

Cells were counted as the number per Hp. Compared with the control group, *$P < 0.05$; compared with the model group, #$P < 0.05$.

Simvastatin is a kind of semisynthetic lipid-lowering statins synthetized by *Aspergillus terreus*-degraded products. It is often used for lipid-lowering therapy in clinic to reduce the synthesis and content of cholesterol [17]. Pharmacological studies revealed that besides the lipid-lowering effect, simvastatin also plays a unique role in treating nerve system diseases, and it possesses a fairly strong anti-inflammatory property as well which can protect the neurological function of primary and secondary cerebral injury remarkably [5,17]. Some researchers [18] used simvastatin for intervention therapy on rat models with cerebral injury and found that simvastatin could decrease the death rate of neurons, strengthen the regeneration of cerebral blood tissues synapse, and then improve the neurological and cognitive functions for rats. In this study, the NDS results of the simvastatin-treated group at all time points after modeling were all significantly higher than those of the model group ($P < 0.05$), and the number of undamaged neurons around lesions of the simvastatin-treated group at all time points after modeling were all significantly higher than those of the model group at the same periods ($P < 0.05$). Those also manifested that simvastatin could effectively improve the neurological function of rats with cerebral hemorrhage. After cerebral hemorrhage, the degree of encephaledema is a major mark for secondary cerebral injury and an important factor of deterioration as well [19]. There are other researches holding the idea that simvastatin could down-regulate the expression of Bax protein and up-regulate the expressions of HSP70 and Bcl-2 proteins so as to inhibit the apoptosis of nerve cells around the brain lesions of rats and relieve the degree of encephaledema for rats [20]. In this study, 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 ($P < 0.05$), which coincided well with the above references.

Some overseas researches showed that endothelial cells, inflammatory cells, gliocytes and neurons of brain tissues were stimulated and NF-κB and irritable inflammatory responses were activated due to ischemia and hypoxia in the course of cerebral hemorrhage [21]. It could be concluded that NF-κB plays a key role in the process of secondary inflammation of cerebral hemorrhage. Toll-like receptor can regulate the protective effect of animals and plants against microorganisms and induce the occurrence of inflammatory responses, which has an important significance in the pathological damaging process of inflammatory responses for bodies [22,23]. Toll-like receptor is an important member of TLRs family which can activate NF-κB and launch and magnify inflammatory responses [24]. There are researches demonstrating that statins can decline the expression of inflammatory factors mediated by TLR4 pathway effectively [25]. In this study, the number of counted NF-κB, TLR4 and IL-1β positive cells of the simvastatin-treated group after modeling were all significantly lower than those of the model group at the same periods ($P < 0.05$), and the expression quantity of NF-κB, TLR4 and IL-1β protein of the simvastatin-treated group after 7 days for meddling were all lower than those of the model group at the same periods ($P < 0.05$). Those indicated that simvastatin could relieve secondary inflammatory injury of brain tissues in rats with cerebral hemorrhage by down-regulating expression of NF-κB signal pathway mediated by TLR4.

The results of this study showed that simvastatin can inhibit the effect of the expressions of NF-κB, TLR4 and IL-1β proteins in rats with cerebral hemorrhage, and protect neurons and reduce secondary inflammatory injuries by down-regulating the above protein-mediated inflammatory responses.

Conflict of interest statement

We declare that we have no conflict of interest.

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