Ginsenoside Rg1 attenuates structural disruption of the blood-brain barrier to protect the central nervous system in ischemia/reperfusion

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Available online 20 Jan. 2013

[ABSTRACT] AIM: Although ginsenoside Rg1 possesses potent neuroprotective effects, it cannot easily be transported to brain parenchyma because of the blood-brain barrier (BBB). This study was aimed to verify the hypothesis that the ginsenoside Rg1 neuroprotective effect might be mainly derived from its direct protective effects on BBB. METHODS: Male Sprague-Dawley rats were subjected to 2 h of middle cerebral artery occlusion (MCAO) by using the suture insertion method followed by 22 h of reperfusion. In the Rg1-treated group, Rg1 (45 mg·kg⁻¹) was administrated via tail venous (i.v.) 1 h before focal ischemia and 3 h after reperfusion. The integrity of the BBB was measured in vivo, MDA and SOD were estimated in vitro. The expression and activity of matrix metalloproteinases (MMPs), and the expression of tissue inhibitor of matrix metalloproteinases (TIMPs) mRNA, were determined to evaluate the protective effect of Rg1 on BBB structure both in vivo and in vitro. RESULTS: In ischemia/reperfusion rats, using the EB dye extravasation, the expression and activity of MMPs were increased as compared to sham rats, while in Rg1-treated rats, these increases were inhibited. The expression of TIMP-2 mRNA in the ischemia/reperfusion rats was decreased as compared to sham rats, while in Rg1-treated rats, these decreases were ameliorated. The results of in vitro models were consistent with those of in vivo models. CONCLUSION: Ginsenoside Rg1 may exert its protective effect of CNS indirectly by protecting the structure of the BBB, through protecting BMECs and reducing the expression and activity of MMPs in pathological conditions.

[KEY WORDS] Ginsenoside Rg1; Blood-brain barrier; Matrix metalloproteinases; Tissue inhibitor of matrix metalloproteinases; Extracellular matrix


1. Introduction

Ginsenoside Rg1 (Rg1, Fig. 1) is the main ingredient of Panax notoginseng saponins (PNS). A number of clinical reports and experiments have indicated that PNS has a wide variety of therapeutic values, including for some central nervous system (CNS) diseases[1-3]. In a previous study from these laboratories[4], it was found that Rg1 cannot easily be transported to the brain in ischemia/reperfusion rats, as well as in healthy rats. In order to explain the contradictory observation that ginsenoside Rg1 possesses potent neuroprotective effects, while it has very limited brain distribution, we hypothesized that the blood-brain barrier (BBB) might be the primary target of Rg1 and that its neuroprotective effects may be mainly derived from its direct protective effects on BBB. In this hypothesis, although Rg1 cannot be transported to brain parenchyma, it is easy to achieve a high concentration on the BBB, which is extremely important to the CNS because disruption of the BBB may cause brain exposure to a number of toxicities resulting in many brain disorders, and even secondary damage to neurons. In this study, the Rg1

Fig. 1  Structure of ginsenoside Rg1
protective effects on the structure of the BBB were evaluated to examine this hypothesis and derive an understanding of the mechanism of some CNS-targeted drugs, which possess neuroprotective effects, while having very limited brain distribution.

2. Materials and Methods

2.1 Reagents

Ginsenoside Rg1 standard (> 99% purity) was provided by the College of Chemistry of Jilin University (China); Evan’s Blue (EB) dye, N, N-dimethylformamide, methyl thiazoly tetrazolium (MTT), and the molecular weight standards were supplied by Sigma Chemical Co. (USA); Trypsin-nase was from Ameresco (USA); F12: DMEM was from Gibco (USA); Fetal calf serum was from Hyclone (USA); MDA and SOD ELISA Kit were from Jiancheng Bioengineering Institute (China); MMPs ELISA Kit was from AdiViteteram Diagnostic Laboratories (USA); MMP-2/MMP-9 standards were from Chemicon (USA); Superscript One-Step RT-PCR system were from Invitrogen (USA); Bio-Rad Protein Assay Kit was from Bio-Rad Laboratories (USA); Occludin and Claudin antibodies were from Santa Cruz Biotechnology (USA); α-actin antibody was from Boster (China); Tris-HCl, SDS, Triton X-100, glycocine was from Ameresco (USA); Gelatin Sepharose-4B was from Pharmacia (USA); Quantitative Gel and Western Blot Imaging System was from Alpha Innotech (USA); PowerWave 200 96-well Microplate Spectrophotometer was from BioTek Instruments (USA); Water was purified by Milli-Q Ultra-pure water system (Millipore, USA). Other chemicals were of analytical grade.

2.2 Animals

The experimental protocol was approved by the local animal care and use committee of the College of Pharmacy, China Pharmaceutical University. Adult male Sprague-Dawley rats (Sino-British Sipper/BK Lab Animal Ltd. Shanghai, China) weighing between 250 and 280 g were used.

2.3 Surgical procedures

The animals were fasted overnight, and were allowed free access to water. Anesthesia was induced with ethyl carbamate (150 mg·kg⁻¹, i.p. injection). Temperature was maintained at 37 °C with a heating pad. Rats were intubated, and respiration was maintained with a small animal respirator. Rats were subjected to transient middle cerebral artery occlusion (tMCAO) as described by Koizumi et al. [5], with some modifications. Briefly, Sprague-Dawley rats (aged one week) were killed by decapitation. Immediately after death, craniotomy was performed and the choroid plexuses and diencephalon were removed to cold PBS. Then the cerebral cortices were dissected free of meninges, and digested with 0.05% trypsinase for 30 min at 37 °C. After digestion, the samples were filtered first through a 154 μm nylon mesh and then through a 74 μm nylon mesh. The pellets from the 74 μm nylon mesh were collected and centrifuged at 1 500 min⁻¹ for 10 min at 4 °C to remove fragments and impurities. The resulting pellets were washed with PBS and digested with dispase II (1 mg·mL⁻¹) for 30 min at 37 °C. After centrifugation at 1 500 min⁻¹ for 10 min at 4 °C, all cell fractions were collected and cultured in F12 : DMEM (1 : 1) with 20% bovine serum.

2.5 Treatment

In in vivo experiments, the tMCAO procedure was used. Briefly, the rats were divided randomly into three groups: sham, I/R (ischemia/reperfusion), and Rg1-treated group. The Rg1-treated group was injected with ginsenoside Rg1 (45 mg·kg⁻¹) via tail venous (i.v.) 1 h before focal ischemia and 3 h after reperfusion. The I/R group was injected with physiological saline (PS) at the same time before and after the operation. The sham group was treated with the same volume of , without the MCAO operation.

In in vitro experiments, the H₂O₂-induced hyperpermeability model was used to stimulate ischemia/reperfusion. Briefly, the Rg1-treated group was added with medium (F12: DMEM with 5% bovine serum) in the presence of Rg1 (1.6, 8 and 40 μmol·L⁻¹), while the control and H₂O₂ groups were treated with F12: DMEM (5% bovine serum). After incubation for 1 h at 37°C, the liquid from each well was removed and the medium in the presence of H₂O₂ (0.2 mmol·L⁻¹) was added to the H₂O₂ group and the Rg1-treated group, while the medium in the absence of H₂O₂ was added to the control group. Then the bBMEC monolayers were incubated 8 h at 37 °C.

2.6 Quantitative measurements of Evans Blue dye

Disruption of the BBB was analyzed 24 h after the MCAO (n = 5 for each group) using Evan’s Blue (EB) dye as reported previously. but with some modifications [7]. Briefly, EB dye (4%, 4.0 mL·kg⁻¹) was injected over 2 min into the left femoral vein and allowed to circulate for 1 h. Rats were deeply anesthetized and transcardially perfused with PBS until a colorless perfusion fluid was obtained from the right atrium. For quantitative measurements, the brain hemispheres were surgically removed, and the left hemisphere was rapidly frozen in a cryostat. The right hemisphere was dissected and fixed in a cryostat. The brain hemispheres were then sectioned into 10 mm slices, and each slice was incubated in PBS containing 0.05% trypsin for 1 h at 37 °C. After digestion, the samples were filtered first through a 154 μm nylon mesh and then through a 74 μm nylon mesh. The resulting pellets were removed by centrifugation at 1 500 min⁻¹ for 10 min at 4 °C to remove fragments and impurities. The resulting pellets were washed with PBS and digested with dispase II (1 mg·mL⁻¹) for 30 min at 37 °C. After centrifugation at 1 500 min⁻¹ for 10 min at 4 °C, all cell fractions were collected and cultured in F12 : DMEM (1 : 1) with 20% bovine serum.

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(ischemia side) were homogenized in N,N-dimethylformamide (1:10), incubated for 24 h at 25 °C and centrifuged. The supernatants were analyzed at 630 nm by Microplate Spectrophotometer.

2.7 Lipid peroxidation and SOD activity assay

To test the protective effect of Rg1 on rat brain microvascular endothelial cell (rBMECs) viability, a lipid peroxidation and SOD activity assay was used. Briefly, rBMECs were seeded on to plates at a density 1×10⁵·cm⁻² and cultured in the cell growth medium until a tight monolayer was formed. After treatment with H₂O₂ or Rg1 as mentioned in “Treatment”, an estimate of lipid peroxidation was performed by measuring the formed malondialdehyde (MDA). The MDA level and the SOD activity was evaluated by the MDA ELISA Kit and the SOD ELISA Kit according to the manufacturer’s instructions.

2.8 Enzyme-linked immunosorbent assay (ELISA)

ELISA performed on samples of rat brain tissue was used to measure MMPs concentration (n = 5). Samples of brain tissue (1 cm³) were snap frozen in liquid nitrogen and stored at −80 °C until analysis. The brain tissue was homogenized in a lysis buffer (1 g: 10 mL) consisting of a cocktail of 50 mmol·L⁻¹ Tris, pH 7.4, 150 mmol·L⁻¹ NaCl, 5 mmol·L⁻¹ CaCl₂, NP-40 1% and 0.1% protease inhibitors. After centrifugation at 10 000 g for 10 min at 4 °C, the supernatant was collected. The concentration of MMPs in rat brain was measured by the ELISA kit in accordance with the manufacturer’s instructions. 100 μL of supernatant was added to 96-well microtiter plates, and incubated at room temperature for 2 h. After being washed four times, 100 μL antibodies was added to each well, and incubated at room temperature for 1 h. Following washing, samples were incubated at room temperature for 30 min in 100 μL of detecting antibody. The color evolved with the addition of 100 μL coloration solution. After incubation away from light for 30 min, the reaction was stopped with 100 μL of termination solution. The plates were read on a Microplate Spectrophotometer at 450 nm.

2.9 Zymography

Animals were euthanized 22 h after MCAO or Rg1 treatment for zymography (n = 5 for each group). Briefly, samples were homogenized in lysis buffer containing 50 mmol·L⁻¹ tris-HCl pH 7.6, 150 mmol·L⁻¹ NaCl, 5 mmol·L⁻¹ CaCl₂, 0.05% Brij-35, 1% Triton X-100, and 0.02% NaN₃. Protein concentrations in the homogenate were determined using the Bio-Rad Protein Assay Kit. MMP-2 and MMP-9 present in the homogenates were concentrated with Gelatin-Sepharose 4B beads. 800 μg of the total protein in 500 μL was incubated with 80 μL of Gelatin-Sepharose 4B beads for 2 h at 4 °C with gentle agitation. The beads were collected and the gelatinases were eluted by incubating with 80 μL of elution buffer (10% DMSO in phosphate buffered saline) for 1 h at 25 °C with shaking. Equal amounts of samples (20 μL) were mixed in a loading buffer and electrophoresed on each lane of 10% SDS gels containing 1% gelatin. After separation by electrophoresis, the gel was renatured and then incubated with development buffer at 37 °C for 48 h. After development, the gel was stained with 0.5% Coomassie brilliant blue R-250 for 30 min and then destained appropriately. Detection was performed by the Quantitative Gel and Western Blot Imaging System and quantified by Image-Pro Plus (Version 6.0). Enzymatic activity was visualized, quantified and normalized by MMP-2/MMP-9 standards.

3.1 Reverse transcriptase-PCR (RT-PCR)

Total RNA from rat pericytes was extracted using TRIzol reagent (Invitrogen). The primer pair used in the reverse transcription-polymerase chain reaction (RT-PCR) was designed based on the nucleotide sequence of the rat TIMP-1, TIMP-2, and rat GAPDH. The sequences of primers were shown in Table 1. A SuperScript One-Step RT-PCR system (Invitrogen) was used for reverse transcription of RNA, and cDNA was amplified by PCR. Amplification was performed in a DNA thermal cycler according to the following protocol: The PCR for GAPDH was performed with specific primers through 1 cycle of 94 °C for 90 s, and 32 cycles of 94 °C for 35 s, 58 °C for 30 s, 72 °C for 30 s and finally 72 °C for a further 10 min. The PCR for TIMP-2 was performed with specific primers through 1 cycle of 94 °C for 90 s, and 32 cycles of 94 °C for 35 s, 57 °C for 30 s, 72 °C for 40 s and finally 72 °C for a further 10 min. Each 7 μL of PCR product was separated by electrophoresis on an agarose gel and visualized using a Quantitative Gel and Western Blot Imaging System and quantified by Image-Pro Plus (Version 6.0).

Table 1 Primer characteristics of TIMP-1, TIMP-2 and GAPDH

<table>
<thead>
<tr>
<th>Gene</th>
<th>Amplicon</th>
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<th>Sequence reverse</th>
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<tr>
<td>TIMP-1</td>
<td>216 bp</td>
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<td>5’-CAGGAGGCTGAGGAGTGAT-3’</td>
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<td>5’-AAGGTCGGAGTCAACCGATTT-3’</td>
<td>5’-AGATGATGACCCCTTTGGCTC-3’</td>
</tr>
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3. Results

3.1 Blood brain barrier integrity evaluation

In order to evaluate the protective effect on blood brain barrier integrity of ginsenoside Rg1, following focal cerebral ischemia and reperfusion, EB dye extravasation evaluation was performed between the sham, I/R, and Rg1-treated groups. As shown in Fig. 2, the concentration of EB in the ipsilateral hemisphere of the sham group was only (2.7 ± 1.9) ng·mg⁻¹, whereas it was significantly increased 4.25-fold compared to those of the I/R group (P < 0.05). However,
after treatment with Rg1, the concentration increase was decreased to 2.97-fold compared to the sham group \((P < 0.05)\), which is 69.9% of the I/R group \((P < 0.05)\).

3.2 Estimation of lipid peroxidation

As shown in Fig. 3, the MDA level of rBMECs was found to be elevated after being treated with \(H_2O_2\) \((0.2 \, \text{mmol} \cdot \text{L}^{-1})\), and about a 4.75-fold increase in the MDA was observed in the \(H_2O_2\)-treated group compared to the control group. Rg1-treatment \((40 \, \text{µmol} \cdot \text{L}^{-1})\) reduced lipid peroxidation to 2.29- and 1.49-fold of the control group, respectively. The effect was found to be statistically significant \((P < 0.05)\).

![Fig. 3](image)

3.3 Estimation of superoxide dismutase (SOD) activity

As shown in Fig. 4, there was a 68% decrease in the SOD activity in the \(H_2O_2\)-treated group compared to the control group. However, the \(H_2O_2\)-induced decrease in the SOD activity was reversed by Rg1-treatment, and the reversal was statistically significant at the dose of 40 \(\text{µmol} \cdot \text{L}^{-1}\).

![Fig. 4](image)

3.4 Evaluation of MMPs expression

To determine the effect of Rg1 treatment on MMPs expression, an ELISA kit was used. As shown in Fig. 5, the expression of MMP-2 and MMP-9 in healthy rats were \((169.5 \pm 13.9)\) and \((136.9 \pm 7.1)\) ng \(\text{g}^{-1}\), respectively. After ischemia/reperfusion, they were significantly increased to \((342.5 \pm 92.5)\) and \((260.0 \pm 59.3)\) ng \(\text{g}^{-1}\), respectively. However, Rg1 treatment reduced the expression of MMP-2 and MMP-9 to \((204.0 \pm 23.8)\) and \((144.8 \pm 38.1)\) ng \(\text{g}^{-1}\) respectively.

![Fig. 5](image)
3.5 Evaluation of MMPs activity

Zymography was used to determine the effect of Rg1 treatment on MMPs activity. As shown in Fig. 6, the in vitro model, MMP-2 and MMP-9 activities were increased to (123.8 ± 11.2)% and (138.8 ± 22.1)%, in the H2O2-treated group as compared to the control group, respectively. However, Rg1 reduced MMP-2 and MMP-9 activities to (95.6 ± 26.3)% and (106.1 ± 16.4)%, as compared to the control group, respectively. In the in vivo model, as shown in Fig. 7, both MMP-2 and MMP-9 showed increased activity after ischemia/reperfusion. It was increased to (178.2 ± 21.0)% and (273.7 ± 22.1)% as compared to the sham group, respectively. However, Rg1 can reduce MMP-2 and MMP-9 activity to (118.6 ± 23.6)% and (129.4 ± 27.7)% as compared to the sham group, respectively.

Fig. 6  Effect of ginsenoside Rg1 on MMPs activity in rat BMECs after induction with H2O2 (Model, H2O2 200 µmol·L⁻¹; Rg1-H, H2O2 200 µmol·L⁻¹ + Rg1 40.0 µmol·L⁻¹; *P < 0.05 vs control, #P < 0.05 vs model)

Fig. 7  Effect of ginsenoside Rg1 on MMPs activity in rat brain after focal cerebral ischemia (*P < 0.05 vs sham group, **P < 0.01 vs sham group, ##P < 0.05 vs I/R group)

3.6 Expression of TIMP-1 and TIMP-2 mRNA

TIMP-1 and TIMP-2 mRNA expression was determined by reverse transcriptase-PCR. As shown in Fig. 8, in the in vitro model, the expression of TIMP-1 and TIMP-2 mRNA were decreased to (28.4 ± 14.6)% and (58.0 ± 3.3)% in the H2O2-treated group as compared to the control group, respectively. However, Rg1 increased the expression of TIMP-1 and TIMP-2 mRNA to (110.8 ± 23.0)% and (70.4 ± 19.6)% as compared to the control group, respectively. In the in vivo model, as shown in Fig. 9, the expression of TIMP-2 mRNA was dramatically decreased after ischemia/reperfusion as compared to the sham group. It was found to be only (7.8 ± 2.7)% of the sham group. However, in the Rg1-treated group, the expression of TIMP-2 mRNA was increased to (55.3 ± 12.5)% of the sham group.

4. Discussion

Stroke is the third most common cause of death after heart attack and cancer, following negative social and economic effects. Current treatment is focused on reversing
Fig. 8 Effect of ginsenoside Rg1 on TIMPs expression in rat BMECs after induction with H2O2 (Model, H2O2 200 µmol·L⁻¹; Rg1-H, H2O2 200 µmol·L⁻¹ + Rg1 40.0 µmol·L⁻¹; **P < 0.01 vs control, # P < 0.05 vs model, ## P < 0.01 vs model)

Fig. 9 Effect of ginsenoside Rg1 on TIMP-2 activity in rat brain after focal cerebral ischemia (***P < 0.01 vs sham group, ##P < 0.01 vs I/R group)

neurodegeneration and restoring premorbid function.

Ginseng, the root of Panax ginseng has been used in several Asian countries for over 2000 years as a tonic in traditional Chinese medicine. Ginsenoside Rg1, the main ingredient of ginseng, has been proven to have a number of pharmacological effects, such as stimulation of the central nervous system, increasing of initial learning performance and anti-fatigue activity, the promotion of DNA, protein and lipid synthesis in animal bone marrow cells, and so on[8-9].

In a previous study[1], the protection effect of ginsenoside Rg1 in ischemia/reperfusion rats was examined. The results suggested that Rg1 had a very marked central nervous system (CNS) protection effect in ischemia/reperfusion rats. These results were coincident with previous reports[10]. However, following studies showed that Rg1 cannot be transported to the brain in ischemia/reperfusion rats as well as in healthy rats.

It seemed contradictory that ginsenoside Rg1 possesses potent neuroprotective effects while it has very limited brain distribution. Something which is blocking Rg1 distribution from the blood to the brain, and yet also has an influence with CNS, attracted our attention. That is the blood brain barrier (BBB), a well-differentiated network of brain microvessels. BBB is a functional and structural component of the CNS and maintains homeostasis of the neural microenvironment, which is extremely important for the CNS because disruption of the BBB may cause brain exposure to a variety of toxic influences resulting in many brain disorders, and even secondary damage to neurons[11-12]. It was hypothesized that the BBB might be the primary target of Rg1 and that its neuroprotective effects might be mainly derived from its direct protective effects on the BBB.

Although the exact mechanism of BBB breakdown, and its role in the progression of many CNS diseases, is currently unknown, it was accepted that the expression levels of a number of inflammatory cytokines were increased in association with an increase in the permeability of the BBB. In this study, whether Rg1 has a protective effect on BBB integrity was examined. A variety of methods have been used to evaluate the function of the BBB, for example, leakage of some tracer agents into the CNS have been used to assess BBB permeability. Among this tracer agents, Evan’s Blue dye has been widely used for assessing disruption of the BBB owing to its property of binding to serum albumin in vivo and in vitro by quantitating extravasation of EB dye into the brain[13]. Quantitative measurements of EB dye revealed that I/R rats had a significantly (P < 0.05) higher concentration than sham rats. But the concentration of EB dye of Rg1 treated I/R rats was lower than I/R rats. This result revealed that Rg1 can protect the integrity of the BBB from ischemia damage in tMCAO rats.

The BBB is built up from three cellular elements of the brain microvasculature compose: brain microvascularendo-
thelial cells (BMEC), astrocyte end-feet, and pericytes. BMECs are closely interconnected by continuous tight junctions (TJs), which prevent the passage of toxic and xenobiotic blood-borne substances into the brain parenchyma [14]. Astrocytic end-feet tightly ensheathe the vessel wall and appear to be critical for the induction and maintenance of the TJ barrier, but astrocytes are not believed to have a barrier function in the mammalian brain [15]. The specialized extracellular matrix (ECM) of the basement membrane connects endothelial cells with neighboring neuroglial cells, like astrocytes and pericytes, to form a neurovascular unit [16]. BMECs and ECM are the structural components of BBB, so these components were studied separately to reveal the mechanism of the Rg1 protective effects on BBB integrity.

In a previous study, it was confirmed in this laboratory that Rg1 has a protective effect on rBMEC viability by the MTT test [4]. Here, the malondialdehyde (MDA) and superoxide dismutase (SOD) were examined. Lipid peroxidation injury of tissue induced by oxygen free radicals is one of the important factors in cerebral ischemia/reperfusion injury. Many experiments have proved that cerebral ischemia/reperfusion of the rat at the acute stage can produce a large number of free radicals, increasing the content of MDA and consuming SOD to decrease its activity. MDA is one of the main products of lipid peroxidation which can reflect the degree of lipid peroxidation and indirectly reflects the degree of oxidative stress in cells. SOD can clean out superoxide radical anions and protect cells from damage. It plays a vital role in maintaining the balance between oxidation and antioxidation. SOD activity can indirectly reflect the antioxidant capacity of cells [17-18]. In these experiments, the results showed that the level of MDA was remarkably raised and the level of SOD was dramatically reduced after injury by H2O2. However, after treatment with Rg1, especially at 40 µmol·L−1, the level of MDA was reduced and the level of SOD was increased, close to that of the control group. It is suggested that Rg1 also has an effect against lipid peroxidation injury in ischemia/reperfusion to protect BMECs.

The ECM connecting BMEC with the surrounding brain resident cells is an essential part of the BBB. Neuroglia-secreted matrix metalloproteinases (MMPs) are a family of zinc-binding proteolytic enzymes mediating the ECM composition, and assume an important role in the integrity and function of the BBB during BBB breakdown after ischemia, and some other CNS diseases. It is reported that upregulation of MMP-2 and MMP-9 triggers apoptosis in human cerebral endothelial cells after hypoxia reoxygenation by interrupting cell matrix [19]. In addition, MMP levels have been shown to be elevated in the plasma and brains of stroke patients, associated with hemorrhagic transformation [20-21]. The presence and activity of MMPs are dependent on their interaction with their endogenous inhibitors, the tissue inhibitors of matrix metalloproteinases (TIMPs) [22]. Four distinct TIMPs have been identified to date [23]. Both MMP-2 and MMP-9 exist in ‘pro’ inactivated forms. While most TIMPs react with all activated MMPs, TIMP-2 binds to pro-MMP-2 and TIMP-1 inhibits specifically pro-MMP-9 [24]. In this study, the activity and expression MMP-2 and MMP-9 were measured by both zymography and an ELISA assay. As shown in Fig. 5 and Fig. 6, after ischemia/reperfusion the expression and the activity of MMP-2 and MMP-9 were both upregulated, and Rg1 can downregulate this upregulation. Although the quantitative measurement of TIMP-1 failed, TIMP-2 mRNA expression was determined by reverse transcriptase-PCR, in sham, I/R and Rg1-treated rat brains. From the results, the expression of TIMP-2 mRNA was dramatically decreased after ischemia/reperfusion, while Rg1 could restore its expression. The results in the in vivo model were consistent with those in the in vitro model. These results are important for understanding the Rg1 protective effect on BBB permeability by restoring the balance between MMPs and TIMPs, which is dynamically regulating the ECM composition, and finally affects BBB integrity.

In conclusion, this study showed that ginsenoside Rg1 can protect both BMECs and ECM, which are the most important ingredients of the BBB structure. Although Rg1 cannot easily be transported to the neurons, it can exert its protective effect of CNS disease indirectly by protecting the structure of BBB. This evidence is important for understanding the mechanism of some CNS-targeted drugs, which possess neuroprotective effects while having very limited brain distribution, and has implications for the development of a new strategy for the treatment of CNS diseases.

References

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人参皂甙Rg1减轻缺血/再灌注引起的血脑屏障结构破坏以保护中枢神经系统

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【摘要】目的: 尽管人参皂甙Rg1有较强的神经保护作用, 但前期研究表明Rg1难以透过血脑屏障到达脑实质, 血脑屏障能否维持中枢神经系统内环境的稳定, 对中枢神经系统至关重要。本研究旨在验证人参皂甙Rg1可能是通过保护血脑屏障而发挥神经保护作用的观点。方法: 雄性SD大鼠用大脑中动脉闭塞(MCAO)2 h后再灌2 h, Rg1(45 mg·kg−1)于缺血后1 h和再灌注3 h通关尾静脉给药。动物模型测定血脑屏障的致密性, 体外测定基质金属蛋白酶的表达量和活性。同时在体内外试验中测定基质金属蛋白酶的表达量和活性以及基质金属蛋白酶组织抑制剂mRNA的表达量以评估Rg1对血脑屏障结构的保护作用。结果: 在缺血/再灌注模型大鼠中, 依文思蓝的渗透率、基质金属蛋白酶的表达量和活性明显升高, 而继以Rg1的模型大鼠中这种升高得到明显抑制。结论: 人参皂甙Rg1可能是通过保护血脑屏障而发挥保护中枢神经系统的作用, 从而间接发挥了中枢神经系统保护作用。

【关键词】 人参皂甙Rg1; 血脑屏障; 基质金属蛋白酶; 基质金属蛋白酶组织抑制剂; 细胞外基质

【基金项目】 江苏省药代动力学重点实验室提升项目(No. BM2012012)资助