EFFECTS OF DIAMMONIUM GLYCRRHIZINATE ON INFLAMMATORY REACTIONS OF BASAL ARTERIAL WALL IN INTRACRANIAL ANEURYSM AND ITS MOLECULAR MECHANISM

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ABSTRACT

Objective: To analyze the effects of diammonium glycyrrhizinate (DG) on inflammatory reactions of the basilar arterial wall in intracranial aneurysms and its molecular mechanism.

Methods: Eighty white rabbits from New Zealand were randomly divided into 4 groups: control, IA (aneurysm modelling without drug intervention), DG (aneurysm modelling intervened by drugs) and NS (aneurysm modelling without normal saline intervention) groups, each with 20 cases marked. White modelling conditions were observed using Masson staining. Expressions of MMP-9 and NF-κB in the apex of the basilar artery were assayed using immunohistochemical staining; expressions of MCP-1 and TNF-α of tissue in the apex of the basal artery were detected using an ELISA method.

Results: Masson staining results showed injurious changes of endarterium and internal elastic force layer in the apex of the arterial wall in various modelling groups, and the animal modelling was successful. After modelling, the middle membrane of the basilar artery became thinner, and the differences between DG, IA and NS groups had statistical significance (P<0.05); ELISA detection methods showed that expressions of MCP-1 and TNF-α in the DG group were clearly lower than those of IA and NS groups (P<0.05); immunohistochemical staining methods showed that MMP-9 mostly gathered in middle and external layers, whereas NF-κB mostly gathered in the tunica intima of the artery. After modelling, expressions of MMP-9 and NF-κB in IA, DG and NS groups were all significantly higher than those of the control group (P<0.05 or 0.01); the expression percentage of MMP-9 and NF-κB in the DG group were clearly lower than those of IA and NS groups (P<0.05 or 0.01).

Conclusion: DG can lower the inflammatory reactions of intracranial aneurysm in white rabbits, inhibiting the occurrence and development of intracranial aneurysm-its mechanism may be related to inhibition of the NF-KB relevant inflammatory reaction pathway.

Keywords: Diammonium glycyrrhizinate, intracranial aneurysm, basal artery, inflammatory reactions.

DOI: 10.19193/0393-6384_2020_1_17

Received November 30, 2018; Accepted February 20, 2019

Introduction

Intracranial aneurysm refers to the tumorous prominence of the tumour wall caused by the abnormal enlargement of the internal cerebral artery lumen, representing one of the most common causes of cerebrovascular disease(1). Data show that intracranial aneurysms have become the leading cause of subarachnoid haemorrhage; they represent an acute fatal disease with an incidence rate of up to 10%, and the mortality rate of patients after tumour rupture is as high as 60%(2). Therefore, actively exploring new ways to prevent and treat intracranial aneurysms and determining the molecular mechanism of the occurrence and development of aneurysms has become a hot topic in the field of aneurysm research. However, due to conditions or technical limitations, the current research on prevention and treatment of intracranial aneurysms is still in its infancy, and its mechanism of occurrence and development has not yet been fully clarified. Some studies suggest that haemodynamic changes of local intracranial arteries may play a key role in the initiation of intracranial aneurysms. Local vascular wall inflammation has become a key cause of the development of intracranial aneurysms(3). Previous studies have demonstrated a close relationship between NF-KB-related signal transduction pathways and inflammatory responses, which may be involved in the inflammatory reactions of intracranial aneurysms.
Diammonium glycyrrhizinate (DG), a monomeric Chinese medicine ingredient extracted from liquorice, has significant efficacy on anti-non-specific inflammatory response, anti-viral, anti-oxidation and anti-fibrosis, considering that DG may have a certain regulatory role in inflammatory reactions of intracranial aneurysms in the basilar artery. However, the current clinical research on its application is mainly focused on the treatment of liver disease, and studies on intracranial aneurysms are still fewer. Therefore, this work aims to provide a scientific basis for finding prevention and treatment for new targets of the intracranial artery by ligating bilateral carotid arteries in rabbits to create a basilar aneurysm model and observe the effects of DG on inflammatory reactions of the basal artery in rabbit aneurysms.

Data and methods

Experimental animals

Eighty New Zealand white rabbits weighing 2.0~2.5kg were placed at 22 °C ± 2 °C and fed under 60% humidity conditions freely. The following experiments were conducted 1 w after feeding.

Main reagents and instruments of experiment

Slicer (provided by Leica, Germany), centrifuge (purchased from Ebend, Germany), Masson three-color composite dyeing solution, immunohistochemical staining kit (purchased from China Thermo Scientific), enzyme-linked immunosorbent kit (obtained from Shanghai Hengyuan Biotechnology Co., Ltd.), rabbit anti-rabbit NF-ΚB antibody (purchased from Amertech Technology Co., Ltd.), mouse anti-rabbit MMP-9 antibody (acquired from Aimei Technology Co., Ltd.).

Methods

Animal grouping:

Eighty rabbits were randomly divided into 4 groups: control, IA (aneurysm modelling without drug intervention), DG (aneurysm modelling intervened by drugs) and NS (aneurysm modelling without normal saline intervention) groups, each with 20 cases marked. Half of the rabbits in each group were detected using ELISA, and the remaining rabbits were used for other detection methods. Modelling and material acquisition: basic anaesthesia was performed on rabbits with 2.5% pentobarbital. After routine disinfection, the bilateral common carotid arteries were carefully separated. Bilateral common carotid arteries of rabbits in the IA, DG and NS groups were ligated with aseptic lines. The control group was given separation of bilateral common carotid arteries without ligation. The soft tissue and skin were sutured layer by layer and bandaged. The vein of ear margin of the white group in the DG group was injected with DG (20 mg/kg) every day, and the vein of ear margin of the white group in the NS group was injected with the same amount of normal saline every day. Seven days after modelling, white rabbits in various groups were injected with air into the ear for killing purposes, and the intracranial basilar artery tissues were treated with the ELISA method, extracted from the relevant literature.

Tissue treatment:

- The tissue was prepared for staining and processed into paraffin tissue sections and sectioned. The modelling of white rabbits was observed by Masson staining. The expressions of MMP-9 and NF-KB were detected by immunohistochemical staining;
- Tissue stored in liquid nitrogen was extracted using ELISA, mixed fully in a homogenizer and centrifuged at 3000 r/min for 20 min at 4 oC. The supernatant liquid was collected into an EP tube. The expressions of MCP-1 and TNF-α in the basilar artery apex tissue were detected by ELISA.

Statistical methods

The relevant data were analyzed using the SPSS0.0 software package. GraphPad 7.0 software was used for statistical mapping. Measurement data were represented by Mean ± standard deviation (X±s). EVG staining, Masson staining, immunohistochemical staining and ELISA were used for one-way ANOVA examinations. Comparison between groups was with independent samples or a paired data t-test, and the variance was then compared using the LSD method. When the variance was not uniform, the rank-sum test was performed to compare the differences between groups. The relevant enumeration data were expressed as a percentage, and the difference was compared between the groups using the χ² test. P<0.05 was considered statistically significant.

Results

Results of intracranial basal aneurysm

Masson staining showed that after the bilateral common carotid artery ligation in the white rabbits, the partial tube wall of the basilar artery showed a prominent change, and injurious changes of
endarterium and internal elastic force layer can be seen in the apex of arterial wall in various modelling group; hence, the animal modelling was successful. After successful modelling, the middle membrane of the basilar artery became thinner, and differences between DG, IA and NS groups had statistical significance (P<0.05); there was no statistical significance between IA and NS groups (P>0.05), showing that aneurysmal destructive remodelling was formed in the apex of the rabbit basilar artery (Figure 1).

Expressions of MCP-1 and TNF-α in apex tissue of basilar artery in various groups
ELISA method detection results showed that after modelling, expressions of MCP-1 and TNF-α in various groups improved compared to those of the control group. Expressions of MCP-1 and TNF-α in DG group were clearly lower than those of IA and NS groups, and the differences had statistical significance (P<0.05); differences between IA and NS groups had no statistical significance (P>0.05) (see Figure 2).

Expressions of MMP-9 and NF-KB in basal artery apex
Immunohistochemical staining showed that MMP-9 mostly gathered in the middle and outer layers of the artery, and NF-KB mostly concentrated in the tunica intima of the artery. After modelling, expressions of MMP-9 and NF-KB in IA, DG and NS groups were all significantly higher than those of the control group; the differences had statistical significance (P<0.05 or 0.01); positive expressions of MMP-9 and NF-KB in DG group were clearly lower than those of IA and NS groups, and the differences had statistical significance (P<0.05 or 0.01- differences between IA and NS groups had no statistical significance (P>0.05) (see Figures 3 and 4).

Discussion
Intracranial aneurysm is an intracranial localized arterial wall weakness and local abnormal bulging caused by various reasons and characterized by arterial wall injury such as internal elastic layer defect, middle membrane injury and local abnormal wall expansion(5). Tumour rupture and bleeding are usually the first symptoms of intracranial aneurysm.
There is evidence that approximately 35% of patients with rupture and bleeding may die, 20% of ruptured patients may have re-rupture within 2w, and the death rate of re-rupture patients may be as high as 80%(6). Therefore, an intracranial aneurysm is a disease that poses a significant threat to human health and life. Currently, for the treatment of unruptured aneurysms, vascular intervention or craniotomy is the main treatment method. Although this treatment significantly reduces the incidence rate of aneurysm rupture and bleeding, it has its own limitations(7). Current studies suggest that hemodynamic changes, heredity, and arterial wall inflammatory reaction are closely related to the occurrence and development of aneurysms, but the current clinical mechanisms for their occurrence are not completely clear(8). Therefore, in-depth understanding and analysis of the aetiology and pathogenesis of intracranial aneurysms has become an urgent problem for the prevention and treatment of intracranial aneurysms in clinics.

Vascular endothelial cell dysfunction is a common pathological change in the occurrence and development of intracranial aneurysms. Vascular endothelial cell dysfunction can express a large number of cytokines, including MCP-1 and NF-KB in order to promote inflammatory reactions (9). The results of this study showed that 7 days after modelling in each model group, the expression of NF-KB in the apex of the intracranial basilar artery was significantly higher than that of the control group and was mainly expressed in the tunica intima of the artery. This observation is consistent with previous studies, demonstrating that a large number of pro-inflammatory factors such as NF-KB and p50 occur in aneurysm at the early stage (10). Over-activation of the MAPK pathway and COX-2-NF-KB positive feedback may be involved in the activation of NF-KB. NF-KB is the core of multiple inflammatory response signalling pathways, and its nuclear transfer or activation has a significant effect on the activation of inflammatory cytokines such as IL-1β and MCP-1, which can promote aggregation and infiltration of inflammatory cells including lymphocytes and monocytes into the arterial wall, thus inducing inflammatory reactions of the local wall.

MCP-1 is a downstream receptor for NF-KB, and NF-KB regulates its expression level (11). This study found that 7 days after modelling in each model group, the expression of NF-KB in the apex of the intracranial basilar artery was significantly higher than that of the control group, suggesting that MCP-1 is associated with the occurrence and development of aneurysms. Some scholars have used the mouse as an experimental model to knock out the MCP-1 gene, and the incidence rate of aneurysm and MMP-9 are significantly decreased, showing that MCP-1 may promote the occurrence and development of aneurysms by over-activation of MMPs and other factors. Furthermore, MCP-1 has a strong ability to promote the aggregation and infiltration of inflammatory cells such as monocytes into the tube wall. Inflammatory factors such as TNF-α secreted by inflammatory cells can maintain and aggravate the degree of inflammation on the arterial wall, a process that is extremely important in the development of intracranial aneurysms(12).

TNF-α plays an important role in maintaining the inflammatory reactions. Some scholars have found that the incidence of intracranial aneurysms and the incidence rate of rupture in mice after knocking out the TNF-α gene are significantly reduced(13). The results of this study showed that 7 days after aneurysm modelling, the level of TNF-α of white rabbits in the model group was significantly higher than that of the control group, suggesting that TNF-α may be involved in the occurrence of aneurysms. TNF-α is mainly secreted by dysfunctional endothelial cells, and overproduction of TNF-α can further aggravate endothelial dysfunction and even apoptosis. In addition, TNF-α can act on cytokines such as selectin E and vascular endothelial cell adhesion molecule 1 to enhance the adhesion of inflammatory cells and endothelial cells and activate the inflammatory reaction. TNF-α produced by inflammatory cells can further aggregate more inflammatory cells to aggravate this process. Furthermore, TNF-α also can induce vascular smooth muscle cell phenotype transformation and even apoptosis(14).

This study found that the expression of MMP-9 in the modelled white rabbits was also significantly higher than that of rabbits in the control group. The expression level of MMP-9 in normal tissues was particularly low, and when intracranial aneurysms occurred, transformed smooth muscle cells and macrophages can produce excessive MMP-9. Generally, the body MMPs maintain a dynamic balance with the inhibitory component TIMPs in vivo. When the expression of MMP-9 increased, the balance was broken, thus resulting in extensive degradation of the extracellular matrix, destruction of the wall structure and weakening of the wall to promote the formation of intracranial aneurysms(15).

Diammonium glycyrrhizinate (DG), a monomeric Chinese medicine ingredient extracted from
liquorice, has significant efficacy on anti-non-specific inflammatory response, anti-viral, -oxidation, -fibrosis and is mainly used in the treatment field of liver disease. DG can inhibit the transcription, translation and activation of NF-KB through a variety of signalling pathways\(^{16}\). This study found that the percentage of positive expression of MMP-9 and NF-KB protein in the DG group was significantly lower than that of IA and NS groups, indicating that DG inhibits the expression of NF-KB and MMP-9 proteins. Based on this observation, we considered that DG can reduce the expression of MMP-9 protein and reduce the level of basal artery inflammation in intracranial aneurysms by inhibiting the NF-KB-mediated inflammatory reaction signalling pathway.

In conclusion, DG can reduce the inflammatory reaction and injury degree of the basilar artery in intracranial aneurysms, inhibit the occurrence and development of aneurysms, and its mechanism may be related to inhibition of the NF-KB-mediated inflammatory reaction signalling pathway and decrease of the downstream MMP-9, MCP-1 and TNF-α protein expressions.

References


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