Investigation of the Role of GRP78 and the Potential Therapeutic use of Radix Astragali in Diabetic Complications

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ABSTRACT

Diabetes mellitus (DM) is one of the most prominent chronic diseases globally. It is characterized with high blood glucose level and the development of series life-threatening vascular complications such as the cardiovascular and cerebrovascular diseases. It imposed significant healthcare burden in every country. However, currently prescribed therapeutic drugs did poor work in prevention and management of diabetic complications and that the complicated regulatory networks involved in the pathology of diabetes are still unclear. Hence, unraveling the underlying mechanisms and new potential markers of DM and its complications is of great importance for the control of the disease. The study was aimed to (a) investigate novel pathways or markers involved in diabetic macrovasculopathy and diabetic cerebrovasculopathy. (b) examine the potential beneficial effects of Radix Astragali (RA) in diabetic cerebrovasculopathy.

For the investigations in diabetic macrovasculopathy, 2-DE analysis results showed a total of 8 dysregulated proteins found in high glucose treated vascular smooth muscle cell (VSMC) in comparison to normal glucose treated VSMC for 21 days. GRP78, which was found significantly downregulated, is the interested protein of this study for further investigations. This result was confirmed in later experiments that both mRNA and protein levels of GRP78 were depressed after 21 days high glucose treatment in VSMC. Time course study of the alternation of GRP78 expression revealed that GRP78 was transiently upregulated at day 3 of high glucose treatment in VSMC and later on decreased after 15 days of high glucose incubation. Additionally, the mRNA levels of GRP78 in the aorta of
STZ-induced type 1 diabetic rats were also shown to be attenuated at the 8th week and 12th week of diabetes induction.

For the study of diabetic cerebrovasculopathy, it was demonstrated that high glucose conditions promoted astrocytes reactivity in c6 glioma cells, primary rat astrocytes and in the hippocampus of type 1 diabetic mice. Both total methanol extract of RA (TRAE) and enriched saponins methanol extract of RA (ERAE) showed alleviating effects on high glucose-induced astrocytes activation on reducing the GFAP expression level. Additionally, GRP78 expression was downregulated by high glucose as shown in both in vitro and in vivo models. Moreover, longer incubation with high glucose significantly reduced phosphorylation of Akt in the c6 glioma cells. Apart from GRP78, GRP94, calreticulin and calnexin mRNA expression levels were also attenuated. Additionally, high glucose triggered ROS production may partly contributed to the decreased level of GRP78. Furthermore, it was revealed that GRP78 was related to the regulation of the inflammatory cytokines, IL-6 and TNF-α, for which these cytokines were found upregulated in activated astrocytes. Lastly, PCR array analysis results revealed significant downregulation of Igf2 in the hippocampus of STZ-induced type 1 diabetic rats which may be associated with AD pathology.

In conclusion, GRP78 is well demonstrated to participate in both diabetic macrovasculopathy and diabetic cerebrovasculopathy. More importantly the depleted GRP78 level may be connected with subsequent increased oxidative stress, dysregulated insulin signaling and activation of inflammatory process as indicated in the pathogenesis of
diabetic complications. Besides, current data may suggest RA as a potential therapeutic
drug in against astrocytes dysfunction.

(507 words)
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1.11.2 To examine the involvement of ER stress in c6 glioma cells treated with high glucose condition as the in vitro model and in hippocampus of STZ-induced type 1 diabetic mice as the in vivo model.
1.11.3 To evaluate the potential beneficial effects of the methanol extract of RA in against high glucose-induced astrocytes activation in c6 glioma cell as the *in vitro* model and in hippocampus of STZ-induced type 1 diabetic mice as the *in vivo* model.

1.11.4 To determine the correlation of the expression changes of major ER chaperone-GRP78 with the high glucose-induced astrocytes activation in primary rat astrocytes.

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