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Protective effects of Schisandrin on high glucose-induced changes of RhoA and eNOS activity in human umbilical vein endothelial cells

Running title: Protective effects of Schisandrin on RhoA and Enos

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Abstract: Schisandrin, derived from the Chinese medicinal herb *Schisandra chinensis*, has been found to confer protective effects on circulation systems. But the underlying molecular mechanisms remain unclear. The aim of this study was to investigate the effects of a high level of glucose on RhoA and eNOS activity in human umbilical vein endothelial cells (HUVECs) and how Schisandrin plays a role in mediating these effects. To find the optimal treatment time, HUVECs were cultured at a high glucose concentration (30 mM) for different lengths of time (0, 12, 24, and 48 hours). Subsequently, the cells were randomized into five groups: a normal group, a high glucose group, and three high glucose groups that were given different doses (5, 10, and 20 μ M) of Schisandrin. The cells were pretreated with Schisandrin for 24 hours before stimulation with high glucose. The morphology of HUVECs in the various groups was assessed under a light microscope. Immunocytochemical staining was used to detect the level of p-MYPT1 expression. The levels of RhoA activity were determined using the RhoA Activation Assay Biochem Kit. The levels of eNOS activity were examined using a nitrate reduction test. The results showed that in the high glucose group, the activity of RhoA was increased and the activity of eNOS was reduced, thus decreasing the secretion of NO. However, after pretreatment with Schisandrin (10, 20 μ M), the activity of RhoA was inhibited and the activity of eNOS increased, which led to an increase in NO production compared with the high glucose group. There was no evident difference between the 5 μ M Schisandrin group and the high glucose group. Taken together, these findings indicate that Schisandrin can improve the function of endothelial cells by lowering the activity of RhoA/Rho kinase and raising both the activity of eNOS and the production of NO.

Keywords: Human umbilical vein endothelial cells, Schisandrin, RhoA, eNOS, NO, p-MYPT1

1. Introduction

In the past 30 years, the number of people suffering from diabetes worldwide has quadrupled. As this figure increases day by day, more attention should be paid to the patients who suffer from diabetic complications. Diabetic vascular disease is one of the most common complications and seriously affects the quality of life of patients with diabetes mellitus. Vascular lesions associated with glycolipid metabolism disorders and hypercoagulability caused by hyperglycemia can significantly accelerate the development of atherosclerosis. Considerable cardiovascular and cerebrovascular diseases occur owing to atherosclerosis [1].

Previous findings have demonstrated that the activity of endothelial NO synthase (eNOS) plays a crucial role in the process of atherosclerosis in diabetes [2]. eNOS can catalyze the L-arginine

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