

The effects of resveratrol on liver damage and ferroptosis in fructose-streptozotocin induced diabetic model

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Background

The liver is the main detoxification organ in the body and regulates normal glucose homeostasis. There is a relationship between diabetes and liver disease. Pathological changes such as hepatic steatosis, accumulation of fatty acids, and fibrosis have been demonstrated in the livers of diabetic patients. Due to the connection between Type 2 Diabetes Mellitus (T2DM) and progressive liver disease, further research is needed to understand the pathogenesis of diabetic liver disease (1). Studies on liver damage in diabetes have focused on inflammatory and insulin signaling pathways, as well as oxidative stress. Recent studies have found that ferroptosis plays a significant role in acute or chronic liver damage (2).

Ferroptosis is a form of non-apoptotic cell death characterized by excessive lipid peroxidation, iron dependence, and is associated with various pathological conditions in the liver. Increased interest has been shown in the role of ferroptosis in liver diseases because excessive iron overload and oxidative stress are major triggers for liver damage and disease progression in many liver diseases. Therefore, targeting ferroptosis could provide a promising new therapeutic strategy for the treatment of liver disease in affected patients (2,3).

A fructose-rich diet impairs aerobic capacity and leads to diabetes and fatty liver disease. It triggers various metabolic disorders, including hypertriglyceridemia, hyperglycemia, insulin resistance, and glucose intolerance. Fructose reduces antioxidant activity and harms the livers of animals (4).

Resveratrol (RSV) is a compound belonging to the stilbenes group, found in grape skins and leaves. It exhibits properties of phytoalexins, which are produced by plants in response to fungal or bacterial infections, and it prevents cellular damage caused by free radicals. In addition to its antioxidant and anti-inflammatory effects, resveratrol has protective effects against cancer, aging, obesity, diabetes, cardiovascular, and nervous system diseases. Resveratrol has various beneficial effects, such as normalizing the activities of antioxidant enzymes like catalase, superoxide dismutase, and glutathione S-transferase, as well as lowering blood sugar levels. Low doses of resveratrol can reduce blood sugar levels and improve insulin sensitivity in diabetic patients (4,5).

The aim of this study is to demonstrate the role of ferroptosis in the formation of liver damage in a diabetic model created with a high fructose diet and streptozotocin (STZ) and to investigate the potential effects of resveratrol treatment on this process.

Methods

In the study, 8-week-old Sprague-Dawley rats were divided into four groups: 1) Diabetic group (D), fed with a 10% fructose solution for two weeks, injected with STZ (40mg/kg) at the end of the second week, and then fed with a 10% fructose solution for three more weeks. 2) Diabetic + resveratrol group (D+RSV), treated with 1mg/kg/day resveratrol for four weeks. 3) Non-diabetic rats treated with resveratrol (C+RSV) (1mg/kg/day) for four weeks. 4) Control group (C). At the end of the experiment (9th week), all rats were sacrificed, and liver tissues

were collected. Throughout the experiment, calorie intake, body weight, and blood sugar levels were measured. The liver tissue sections were immunostained with GPX-4, COX-2, and H2AX antibodies. All values were analyzed using statistical methods.

Results

In the fasting blood glucose measurements of the rats that were administered STZ (40 mg/kg, i.p.) after 10% fructose for 2 weeks ($p < 0.001$), a highly significant difference was detected between the diabetic groups and the control groups. At the end of the experiment, there was a significant difference between the diabetic group and the control groups ($p < 0.001$). Additionally, a significant difference in blood glucose levels was detected between the control and D+RSV groups ($p < 0.05$). The calorie intake in the second and fifth weeks was significantly different in the D and D+RSV groups compared to the control groups ($p < 0.001$). However, at the end of the study, there were no differences between all groups.

H&E, Van Gieson, and Prussian blue staining of liver tissue sections revealed vacuolization of hepatocytes, an increase in collagen fibers in the portal areas and around the central vein, which is an indicator of fibrosis, and iron accumulation in the tissue in the diabetic group. In the D+RSV group, it was found that the damage caused by diabetes was recovered and iron accumulation in liver tissue was inhibited. Decreased GPX-4 protein expression and increased COX-2 expression were detected by immunohistochemistry staining in the diabetic group. Additionally, GPX-4 immune positive cell numbers were significantly higher in the D+RSV group. H2AX is used by many researchers as a tool to measure induced DNA damage. In the D group, an increase in the number of cells marked with phosphorylated H2AX antibody was observed, while there was a decrease in the D+RSV groups.

Conclusion

We suggest that the high-fructose diet and low-dose STZ administration increased blood glucose levels, caused liver fibrosis, and led to iron accumulation. Additionally, resveratrol treatment decreased blood glucose levels in diabetic rats, positively affected oxidative stress by increasing GPX-4 expression, the main purifier of lipid peroxides in cells, and prevented iron accumulation in hepatocytes, thereby protecting liver tissue from ferroptosis.

Keywords:

Ferroptosis, Streptozotocin, Fructose, Resveratrol, Liver

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