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Effects of silymarin on angiogenesis and oxidative stress in streptozotocin-induced diabetes in mice

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A R T I C L E I N F O

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A B S T R A C T

The present study evaluated the effects of acute treatment with silymarin, an extract that is obtained from *Silybum marianum*, on angiogenesis, oxidative stress, and inflammation in normoglycemic and diabetic mice. Diabetes was induced by streptozotocin (80 mg/kg, intraperitoneal) in male Swiss mice, 6 weeks of age. A polyether-polyurethane sponge was surgically implanted in the back of the mice as a model of healing in both diabetic and normoglycemic animals that were treated with oral silymarin or water for 10 days. The pancreas, liver, kidneys, blood, and sponges were collected and analyzed. Diabetes led to impairments of antioxidant defenses, reflected by a reduction of pancreatic superoxide dismutase and hepatic and renal catalase and an increase in pancreatic lipoperoxidation. An inflammatory process was observed in diabetic mice, reflected by an increase in pancreatic tumor necrosis factor α (TNF-α) and the infiltration of inflammatory cells in islets. The number of vessels was lower in the implanted sponges in diabetic mice. Silymarin treatment attenuated this damage, restoring antioxidant enzymes and reducing pancreatic TNF-α and inflammatory infiltration. However, silymarin treatment did not restore angiogenesis or glycemia. In conclusion, treatment with silymarin red uced oxidative stress and inflammation that were induced in the model of streptozotocin-induced diabetes in several organs, without apparent toxicity. Silymarin may be a promising drug for controlling diabetic complications.

1. Introduction

Diabetes is a disease that is characterized by metabolic disturbances, including chronic hyperglycemia [1,2]. It is associated with a high risk of death and a lower life expectancy [3]. The number of adults with diabetes more than doubled in the last three decades [4]. Its complications are triggered by several disturbances in homeostasis, including vascular complications that are associated with impairments of angiogenesis [5] and poor wound healing [6], which can sometimes lead to limb amputations [7].

Angiogenesis is the process that is responsible for the formation and stabilization of vessel sprouts. The regulation of angiogenesis is complex and involves several growth factors. Alterations of these processes can lead to malignant, ischemic, and inflammatory disorders. An increase in angiogenesis is related to several diseases, including cancer, asthma, and obesity. Conversely, a decrease in angiogenesis can also trigger illness, such as osteoporosis, Alzheimer's disease, and hair loss [8]. In diabetes, complications may develop because of either increases or decreases in angiogenesis. Diabetic complications that are related to disturbances in angiogenesis include retinopathy, nephropathy, neuropathy, cardiovascular disease, and impairments of wound healing [9]. Disturbances that are related to microvascular and macrovascular complications evolve during diabetes progression. In diabetic retinopathy, for example, hyperglycemia leads to structural and physiological

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alterations of retinal capillaries. In the early stages, blood and fluids extravasate from retinal capillaries, causing edema (non-proliferative diabetic retinopathy). In proliferative diabetic retinopathy, abnormal blood vessels are formed in the retina and optic disc in response to ischemia [9]. These new vessels are fragile and highly permeable. Moreover, diabetic nephropathy is associated with glomerular hypertrophy, with increases in the length and number of capillaries. Impairments in wound healing are additional diabetic complications that are a consequence of deficits in inflammation and cell proliferation stages [9,10].

Chronic hyperglycemia leads to an increase in reactive oxygen species (ROS) production, and impairments of angiogenesis are associated with the mitochondrial overproduction of ROS [2]. Recent evidence shows that fluctuations of blood glucose can lead to microvascular damage through oxidative stress, reflected by higher levels of malondialdehyde and glutathione peroxidase activity and alterations of 3-nitrotyrosine expression. Proinflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin-6, and intercellular adhesion molecule-1, are also involved in this damage [11]. Thus, diabetic complications might be treatable with antioxidant substances, such as silymarin [12].

Silymarin is an extract that is obtained from *Silybum marianum* fruits. It is known for its hepatoprotective effects and is capable of preventing liver cirrhosis that is induced by carbon tetrachloride in rats. In this animal model, liver cirrhosis is reflected by significant increases in liver triglycerides, collagen, bilirubin content, and lipoperoxidation and elevated serum activity of alkaline phosphatase, γ-glutamyl transpeptidase, glutamic pyruvic transaminase, and glucose-6-phosphatase. Silymarin treatment significantly attenuated all of these changes [13]. Several studies suggest the potential application of silymarin for the treatment of diabetic complications in both diabetic patients and animal models [14–19]. Silymarin has also been reported to have antioxidant [20] and healing [21] properties and is able to influence angiogenesis [22]. However, the influence of silymarin on diabetic conditions, characterized by deficits in angiogenesis and oxidative stress, has not been well established. Therefore, the present study evaluated the effects of silymarin on angiogenesis, oxidative stress, and inflammation in organs of interest in type I diabetic and normoglycemic mice. The pancreas was investigated because it is the main organ that is impaired in diabetes, especially in the model of streptozotocin (STZ)-induced diabetes, which destroys pancreatic β cells. The liver was investigated because is the organ that integrates the metabolism, and hepatic function can be affected in diabetes. Up to 44% of adults with type 1 diabetes mellitus have elevated liver tests or have been diagnosed with non-alcoholic fatty liver disease (NAFLD) [23–25]. Finally, the kidneys were investigated because renal function can also be severely impaired under diabetic conditions, referred to as diabetic kidney disease [26].

2. Material and methods

2.1. Animals

All of the experimental protocols were approved by the Ethical Committee for Animal Use (CEUA) of the Biological Science Sector of the Federal University of Paraná (certificate no. 876). Six-week-old male Swiss mice (*Mus musculus*) were housed at 22 °C ± 2 °C and maintained under a 12 h/12 h light/dark cycle. The animals had *ad libitum* access to water and standard laboratory chow during the experiments.

2.2. Diabetes induction

After 12 h of starvation, the mice received an intraperitoneal injection of streptozotocin (STZ; 80 mg/kg) or vehicle (10 mM citrate buffer, pH 4.5) [22]. Glycemia was measured 72 h and 7 days after diabetes induction using a glucometer (Accu-Chek® Active, Roche Diagnostics, Mannheim, Germany) by tail puncture. Animals that received STZ and had blood glucose > 250 mg/dl were considered diabetic.

Table 1
Experimental groups of mice and treatments.

Group no.	Mice/group (n^*)	Group name	Streptozotocin injection	Treatment by oral gavage
1	11	Normoglycemic/Water	No	Water
2	11	Normoglycemic/Silymarin	No	Silymarin at allometric dose (10.41 mg/kg)
3	11	Normoglycemic/Silymarin 10	No	Silymarin at 10-times higher dose (104.1 mg/kg)
4	8	Diabetic/Water	80 mg/kg	Water
5	10	Diabetic/Silymarin	80 mg/kg	Silymarin at allometric dose (10.41 mg/kg)
6	9	Diabetic/Silymarin 10	80 mg/kg	Silymarin at 10-times higher dose (104.1 mg/kg)

* The different numbers of animals among groups are attributable to loss of the animals in the diabetic groups.

2.3. Surgical sponge implantation

Polyether-polyurethane sponges (Vitafoam, Manchester, UK) were immersed in 70% ethanol overnight and boiled in distilled water before implantation. The mice were anesthetized with ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (10 mg/kg). The dorsal hair was shaved, and the skin was wiped with 70% ethanol. A 1 cm incision was made for implantation of the sponge in subcutaneous tissue. The skin incision was sutured with 5-0 mononylon.

2.4. Animal treatments

The animals were divided into six groups as described in Table 1. The mice were treated with silymarin (Pharma Nostra, Rio de Janeiro, Brazil) or water by gavage once daily for 10 consecutive days. The doses were determined by allometric extrapolation [27] based on doses that are used for the treatment of liver disease in humans (210 mg). A 10-times higher dose was also tested as a safety factor. Glycemia and body weight were recorded during treatment.

2.5. Sample collection

Twenty-four hours after the end of treatment, the animals were weighed and anesthetized as described in Section 2.3. The sponges were collected, dissected, weighed, and homogenized as described in Section 2.6 or stored in 4% buffered formalin for histological analysis. Blood was drawn from the abdominal cava vein using heparinized syringes. Plasma was separated by centrifugation and stored at −20 °C for further analysis. The liver, kidney, and pancreas were rapidly harvested, weighed, frozen in liquid nitrogen, and stored at −80 °C for the determination of oxidative stress, protein concentrations in mitochondrial homogenates, and TNF-α. An organ section was also kept in 4% buffered formalin for histological analysis. The organ mass and mouse mass were used to calculate the organ index (*organ mass / body mass × 100*). After sample collection, the animals were euthanized by deep anesthesia. The experimental design is shown in Fig. 1.

2.6. Sponge assessments

For the determination of hemoglobin, the sponge implants were

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