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Antidiabetic drug metformin is effective on the metabolism of asymmetric dimethylarginine in experimental liver injury

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ABSTRACT

Aims: We aimed to investigate the pharmacological efficiency of metformin on asymmetric dimethylarginine (ADMA) metabolism in inflammation caused by the lipopolysaccharide (LPS)/D-galactosamine (D-GalN) treatment.
Methods: Adult Sprague-Dawley rats were injected LPS/D-GalN intraperitoneally. One half of the animals was injected metformin (250 mg kg^{-1} body mass for one week) prior to LPS/D-GalN treatment. Six hours after the LPS/D-GalN injection, livers were removed, and used for the measurements of dimethylarginine dimethylaminohydrolase (DDAH) and myeloperoxidase (MPO) activities, glutathione (GSH), ADMA and arginine levels. Liver tissues were examined histopathologically. The Kruskal–Wallis (*posthoc* Mann–Whitney *U*) test was used for the statistics.

LPS/D-GalN injections caused liver injury as evidenced by the activities of aminotransferases and arginase. GSH level and DDAH activity were decreased in the liver. Metformin pretreatment alleviated the activity of serum enzymes, and attenuated histopathological lesions caused by LPS/D-GalN injections. LPS/D-GalN-induced inflammation, as confirmed by the increased MPO activity, created an asymmetrical distribution of arginine and ADMA between the tissue and plasma. Metformin decreased tissue ADMA level while it restored the DDAH activity and GSH.
Conclusion: Our findings showed that metformin administration for one week has a potency to protect liver through regulating ADMA metabolism in LPS/D-GalN-induced injury.

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1. Introduction

Asymmetric dimethylarginine (ADMA) is an endogenous nitric oxide synthase (NOS) inhibitor which competes with arginine for the binding site in the active center of NOS, thereby limiting the generation of nitric oxide (NO). ADMA is endogenously formed by a class of enzymes termed protein arginine methyltransferases (PRMT), which specifically methylate protein-incorporated arginine residues. ADMA is released within cells upon proteolytic cleavage of arginine-methylated proteins and secreted into the extracellular space

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including plasma. PRMT activity prior to the intracellular degradation of proteins is an important determinant of ADMA generation. ADMA is mainly degraded in the liver by dimethylarginine dimethylaminohydrolase (DDAH), and only a small fraction is excreted by the kidneys. Impaired liver function has been suggested to result in the accumulation of ADMA.

Elevated ADMA levels have been observed in various pathologic conditions. It is well documented that plasma ADMA concentrations are elevated in patients with type 2 diabetes. Studies have indicated a direct correlation between ADMA and glucose concentrations in the subjects with or without diabetes [1–3]. A significant correlation has also been demonstrated between plasma ADMA levels and the degree of hepatic dysfunction in patients suffering from liver diseases with varying etiologies [4–6]. All these studies claimed that plasma ADMA levels are closely associated with hepatic dysfunction.

The mechanism underlying the rise in ADMA has been the focus of several studies [7–9]. In diabetes mellitus, hyperglycemia can activate proinflammatory cytokines such as tumor necrosis factor (TNF)-α that could impair endothelial function [10]. It has been shown that TNF-α inhibited DDAH activity in cultured human endothelial cells and promoted the accumulation of ADMA [11]. Furthermore, TNF-α was defined as the major mediator leading to liver injury following LPS/D-GalN injections [12]. Several other studies suggested that the arginine-nitric oxide (NO) pathway is also involved in the inflammation and organ injury and high ADMA concentrations is thought to play an important role in these processes [13,14].

Metformin, a structural analog of ADMA, is a commonly used antidiabetic drug [15]. Since metformin therapy appears to decrease the risk of diabetes-related endpoints, it is suggested as the first-line pharmacological therapy of choice in type 2 diabetes [16,17]. Metformin has beneficial effects on various abnormalities associated with insulin resistance. Besides its hypoglycemic activity, a body of evidence also revealed the anti-inflammatory properties of metformin. Metformin inhibited the production of pro-inflammatory cytokines in LPS-treated mouse macrophages and rat primary microglial cells in a dose-dependent manner [18,19]. In the patients with type 2 diabetes, metformin treatment lowered plasma concentrations of ADMA without any change in L-arginine, thereby causing a favorable increase in plasma L-arginine/ADMA ratio [20].

Lipopolysaccharide (LPS) is a widely used endotoxin to activate the pro-inflammatory cascade and to result in an experimental liver injury [21,22]. While liver is the primary target of LPS-induced injury, it also has a major role in detoxifying LPS [23]. LPS injections to animals caused increased ADMA levels and decreased DDAH activity in endothelial cells [24]. In a recent study, it has been shown that metformin treatment alleviated LPS-induced fulminant liver injury in mice [25]. Additionally, in patients with impaired glucose tolerance, metformin produced monocyte-suppressing and systemic anti-inflammatory effects in combination with fibrate [26]. Metformin reversed obesity-associated insulin resistance (IR) and inhibited different types of inflammatory responses [27].

It seemed of interest to search whether metformin, as an analog of ADMA, has any effect on the arginine-nitric oxide (NO) pathway in inflammatory liver injury. In this study, attempts were made to examine the metabolism of ADMA together with some inflammatory and oxidative stress parameters in acute liver injury induced by the LPS/D-GalN treatment, and to search for the possible effects of metformin.

2. Materials and methods

2.1. Animals and treatments

Male Sprague-Dawley rats weighing 220–270 g obtained from the Experimental Medical Research Institute, Istanbul University were used in the study. All rats were kept in wire-bottomed stainless steel cages, housed in a room that was maintained at a constant temperature (25 °C), with free access to food and water, and with a 12 h (light) −12 h (dark) cycle. All of the protocols used in this study met the guidelines of the Committee for Animal Care and Use, Istanbul University.

32 rats were equally divided into *control* and *metformin-treated* groups. 16 animals received daily metformin (PHR 1084, Sigma, Aldrich) injections (250 mg kg^{-1} body mass, dissolved in saline, i.p.) for a week, while the remaining was kept as control and received 0.9% NaCl. At the end of one week, one hour after the last metformin injection, half of the animals in each group were injected intraperitoneally with *Escherichia coli* LPS (100 μg kg^{-1}, serotype 011: B4; Sigma, Aldrich), plus D-GalN (Sigma, Aldrich; 700 mg kg^{-1}) in order to induce acute liver injury, while the other half were injected 0.9% NaCl to equalize the conditions. Six hours after the LPS/D-GalN injections, the animals were sacrificed under sodium thiopental anesthesia (50 mg kg^{-1}) and blood samples were taken by the cardiac venipuncture.

2.2. Assays in plasma

Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using Roche autoanalyzer (Mannheim, Germany). The arginase activity was determined in serum by measuring the rate of urea production using α-isonitrosopropiophenone (9% in absolute ethanol) [28]. In brief, the diluted serum was incubated with L-arginine (0.5 mol L^{-1} pH 9.7) at 37 °C for 60 min. The hydrolysis of L-arginine by arginase was stopped by adding 800 μL of an acid solution mixture (H_2SO_4:H_3PO_4:H_2O, 1:3:7). Enzyme activity was calculated in micromoles of urea per minute per milliliter of serum.

Plasma ADMA and arginine levels were determined by the high-performance liquid chromatography (HPLC)-fluorometric method after samples had been treated with o-phthalaldehyde to convert methyl arginines to a fluorescent compound [29].

2.3. Assays in liver tissue

Livers were removed and homogenates were prepared as appropriate for the measurements of dimethylarginine dimethylaminohydrolase (DDAH), myeloperoxidase (MPO) activities and glutathione (GSH), ADMA and arginine levels.

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