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Effect of rosiglitazone on asymmetric dimethylarginine metabolism in thioacetamide-induced acute liver injury

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ABSTRACT

Asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, is metabolized in the liver by dimethylarginine dimethylaminohydrolase (DDAH). We aimed to investigate the effect of rosiglitazone, a peroxysome proliferator-activated receptor-gamma (PPAR-γ) agonist, on ADMA metabolism in acute liver injury. Male Sprague Dawley rats were injected thioacetamide (TAA; 500 mg kg^{-1}) intraperitoneally in order to induce acute liver injury. ADMA, SDMA and arginine levels were determined in plasma by the HPLC. Liver DDAH activity and malondialdehyde (MDA) levels were measured by spectrophotometric procedures. TAA injection caused marked increases in ALT and AST activities. Plasma ADMA levels were increased, while arginine levels and arginine/ADMA ratio were decreased. Liver DDAH activity was significantly diminished and MDA levels were elevated. In another group of animals which were treated with a PPAR-γ agonist (rosiglitazone, 5 mg kg^{-1}) daily via gastric intubation for a week prior to TAA injection, significant recoveries in DDAH activity and antioxidant status were observed when compared with solely TAA-injected animals. Rosiglitazone pretreatment improved the plasma arginine/ADMA ratio.

Our findings indicated that PPAR-γ agonist rosiglitazone beneficially influenced hepatic metabolism of ADMA in TAA-induced acute liver damage.

1. Introduction

Peroxisome proliferator-activated receptor-gamma (PPAR-γ), a member of the nuclear receptor superfamily, is widely expressed in adipocytes and other tissues, including the liver. Several lines of evidence have demonstrated that PPAR-γ agonists not only improve insulin resistance, but also exert antioxidant and anti-inflammatory properties, leading to enhanced endothelial function and NO production [1,2]. In experimental animals, a PPAR-γ agonist rosiglitazone ameliorated endotoxin-induced organ damage, decreased the release of proinflammatory cytokines [3], and protected liver against ischemia/reperfusion injury [4].

The arginine-nitric oxide (NO) pathway is crucially involved in inflammation, infection and organ injury as well as the regulation of vascular function. Arginine is the only known substrate for nitric oxide synthases (NOS). The generation of NO can be regulated by the bioavailability of arginine and endogenous NOS inhibitors. Asymmetric dimethylarginine (ADMA), a competitive inhibitor of NOS, is released within cells upon proteolysis of proteins that have been post-translationally methylated at arginine residues by the protein-arginine methyltransferases (PRMTs). The proteolytic cleavage following methylation liberates two distinct residues of arginine, ADMA and symmetric dimethylarginine (SDMA). The major ADMA-clearing organ is liver that contributes to ADMA clearance through dimethylarginine dimethylaminohydrolase (DDAH) [5]. Contrary to ADMA, SDMA, does not inhibit NOS, and is excreted via kidneys [6]. High DDAH activity in transgenic mice decreases plasma ADMA concentrations and increases NOS activity [7]. Both production and degradation of ADMA, as well as ADMA-mediated inhibition of NOS occur intracellularly. The intracellular concentrations of arginine and ADMA are the major determinants of NO synthesis. The ratio of arginine to ADMA rather than ADMA alone has been accepted as a strong indicator of NO bioavailability [8]. Impaired liver function has been suggested to result in accumulation of ADMA. Seriously elevated ADMA concentrations have been observed in various pathological conditions [9–12]. All these studies claimed that ADMA levels are significantly associated with hepatic dysfunction.

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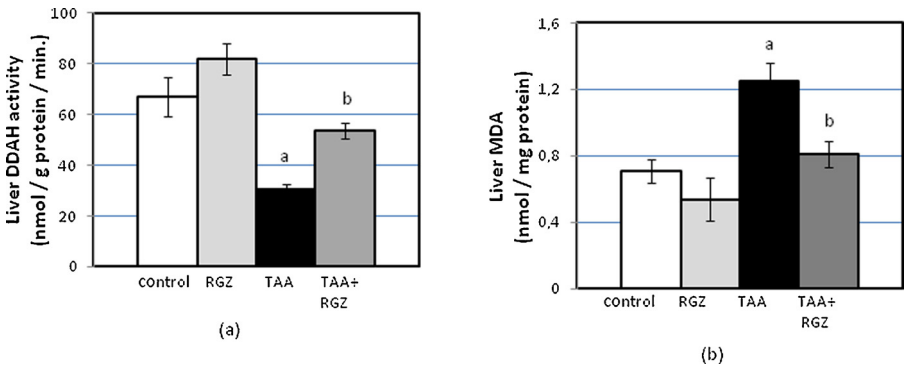

Fig. 1. Effect of rosiglitazone (RGZ) treatment on hepatic DDAH activity (a) and MDA levels (b) in rats. Liver homogenates were collected at 24 h following TAA injection. RGZ was administered by gavage for 7 consecutive days. One hour after the last gavage, TAA was injected. Data are presented as means ± SEM ($n = 8$). DDAH: Dimethylarginine dimethylaminohydrolase; MDA: Malondialdehyde.
[a] $p = 0.000$ for DDAH; $p = 0.002$ for MDA, in comparison with the control group.
[b] $p = 0.000$ for DDAH; $p = 0.007$ for MDA, in comparison with the TAA group.

Conflicting results exist with regard to the effect of rosiglitazone on ADMA metabolism in humans. A significant decrease in plasma ADMA levels following the administration of this drug has been reported [13,14]. Rosiglitazone improved endothelial function in diabetic rats, and diminished inflammatory response induced by ADMA in endothelial cells [15]. However, in critically ill patients, no significant ADMA-lowering effect of rosiglitazone was noticed [11].

In this study, we wanted to investigate whether ADMA handling by the liver is affected in thioacetamide (TAA)-induced acute liver injury and whether the administration of the PPAR-γ agonist rosiglitazone has any effect on this function of the liver.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats obtained from the Experimental and Medical Research Institute, Istanbul University, were fed a standard laboratory chow and had a free access to water. They were kept in wire-bottomed stainless steel cages. The experimental procedure used in this study met the guidelines of the Committee for Animal Care and Use, Istanbul University.

2.2. Treatments

32 rats were equally divided into control and rosiglitazone-treated groups. Rosiglitazone (Sigma–Aldrich) was dissolved in 0.9% NaCl. 16 animals received rosiglitazone (5 mg/kg/day) by gastric intubation for one week, while the remaining was kept as control and received 0.9% NaCl. On the 7th day, 1 h after the gastric intubation, half of the animals in each group were injected thioacetamide (TAA, Sigma, 500 mg kg^{-1}) intraperitoneally in order to induce acute liver injury, while the other half were injected 0.9% NaCl to equalize the conditions. 24 h after TAA injection, rats were anesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.) and blood was obtained by cardiac venipuncture.

2.3. Biochemical analyses

Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities and creatinine levels were determined using Roche autoanalyzer. Plasma ADMA, SDMA and arginine levels were determined by high-performance liquid chromatography (HPLC)-fluorometric method after samples had been treated with *o*-phthalaldehyde to convert methyl arginines to a fluorescent compounds [16].

Liver was rapidly removed, washed in ice-cold saline, and homogenized in ice-cold 0.1 M phosphate buffer (20%; w/v) and centrifuged at 1000 × *g* for 10 min. DDAH activity and malondialdehyde (MDA) levels were determined in the supernatant.

Tissue DDAH activity was measured spectrophotometrically by DDAH-dependent citrulline generation from ADMA, and expressed as nmol citrulline per gram protein per minute [17]. Tissue MDA levels were determined by the thiobarbituric acid test, and calculated using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} [18].

Tissue protein levels were determined by the bicinchoninic acid method [19].

2.4. Statistical analysis

The results were expressed as means ± SEM. Experimental groups were compared using Kruskal–Wallis test, and post-hoc Mann–Whitney *U*-test was performed when appropriate. Pearson's test was computed for the correlation between variables. *P* values less than 0.05 were considered significant.

3. Results

TAA treatment caused significant increases in plasma ALT and AST activities in rats (Table 1). Following TAA treatment, a significant inhibition in hepatic DDAH activity with a profound increase in MDA content was observed (Fig 1a,b). TAA treatment caused increased ADMA levels while SDMA levels remained unaffected (Fig 2a,b). Plasma arginine/ADMA ratio was decreased (Fig 2c).

Rosiglitazone-treated rats without a subsequent injection of TAA gave results similar to the control animals.

Pretreatment by rosiglitazone did not prevent TAA-induced increases in the activities of ALT and AST (Table 1), but hepatic DDAH activity was significantly recovered and MDA content was decreased (Fig 1a,b). Rosiglitazone pretreatment did not have an effect on increased ADMA levels, while it caused an increment in

Table 1
Effect of rosiglitazone (RGZ) treatment on plasma ALT, AST activities.

	Control	RGZ	TAA	TAA + RGZ
ALT (U/L)	67.3 ± 3.93	68.6 ± 5.46	690 ± 93.5 [a]	660 ± 111 [a]
AST (U/L)	147 ± 16.8	181 ± 13.9	1843 ± 215 [a]	1775 ± 328 [a]

Data are presented as means ± SEM ($n = 8$).
[a] $p < 0.05$, significantly different from control.