The restorative effect of ascorbic acid on liver injury induced by asymmetric dimethylarginine

Ali OKUYUCU1,*, Osman ŞALIŞ2, Ömer ALICI3, Abdullah GÜVENLİ4, Yüksel TERZİ5, Muhammed Emin KELEŞ6, Fatih İLKAY A7, İbrahim GÖREN8, Hasan ALAÇAM6

1Department of Medical Biochemistry, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey
2Department of Nutrition and Dietetics, Samsun Health School, Ondokuz Mayıs University, Samsun, Turkey
3Department of Pathology, Faculty of Medicine, Gaziosmanpaşa University, Tokat, Turkey
4Samsun Public Health Laboratory, Ministry of Health of Turkey, Samsun, Turkey
5Department of Statistics, Faculty of Science and Arts, Ondokuz Mayıs University, Samsun, Turkey
6Department of Medical Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, Turkey
7Department of Medical Pharmacology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey
8Department of Gastroenterology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey

* Correspondence: okuyucuali@hotmail.com

Abstract: We aimed to determine whether increased levels of asymmetric dimethylarginine (ADMA) directly cause hepatic deterioration and whether it is decreased by ascorbic acid (AA) as an antioxidant. The study included three groups: Control (n = 10), ADMA (n = 10), and ADMA + AA (n = 10). ADMA was administered at 2 mg kg⁻¹ day⁻¹ and AA was given at 50 mg kg⁻¹ day⁻¹ for 10 days. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase, and alkaline phosphatase activities were analyzed. Histomorphological evaluation of the liver was performed based on the Ishak scoring system. When compared with the control, ALT and ALP levels of the ADMA group were significantly higher (P = 0.006 and P = 0.041, respectively). When compared with the control, interface hepatitis, confluent necrosis, focal necrosis, histological activity index, severity of hepatitis, and portal inflammation were significantly higher in the group administered ADMA. The mentioned biochemical/histopathological parameters were lower in the ADMA + AA group when compared with the ADMA group even if no significant difference was encountered (P > 0.05). The results indicate that ADMA is a factor in the formation of liver injury. Insufficient recovery in biochemical/histopathological parameters when AA was given make us consider that different factors other than prooxidant factors are effective in the formation of liver injury.

Key words: Asymmetric dimethylarginine, ascorbic acid, Ishak score, alanine aminotransferase, alkaline phosphatase

1. Introduction
Asymmetric dimethylarginine (ADMA) is a methylated arginine derivative. The first stage in the synthesis of ADMA is transfer of the methyl from S-adenosyl methionine (SAM) to the arginine residues in the proteins mediated by the protein arginine methyl transferase (PRMT1) enzyme. In the second stage, free ADMA forms after proteolytic breakdown (Paik and Kim, 1968; Tran et al., 2003; Vallance and Leiper, 2004; Zakrzewicz and Eickelberg, 2009). ADMA decreases intracellular nitric oxide (NO) level by inhibiting nitric oxide synthase (NOS) and also blocking intracellular penetration of L-arginine, from which NO is synthesized. Endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) are the NOS species that are predominantly inhibited by ADMA.

Inducible nitric oxide synthase (iNOS) is inhibited at lower levels (Forstermann et al., 1991; Leiper and Vallance, 1999; Ueda et al., 2003).

An adult subject produces daily 300 µmol (60 mg) of ADMA (Bode-Boger et al., 2007). The liver and kidneys are the most important organs in the synthesis and elimination of ADMA (Kimoto et al., 1993, 1995; Wilcken et al., 2007). Nijveldt et al. showed a hepatic uptake of ADMA in an amount of 0.89 nmol 100 g body weight⁻¹ min⁻¹. The liver eliminates ADMA at rates 700-fold higher than plasma (Nijveldt et al., 2003a). The liver metabolizes ADMA particularly by dimethylarginine dimethylaminohydrolase 1 (DDAH1). Beside this, ADMA may also be drained through the bile duct (Ferrigno et al., 2014) and broken down to α-keto-d-((N,N-dimethylguanidino)valeric acid
by alanine glyoxylate aminotransferase 2 (Vallance and Leiper, 2004; Rodionov et al., 2010). Changes in DDAH enzymatic activity due to reactive oxygen species (ROS), inflammatory mediators, ADMA itself with excessively increased levels, and nitrosative stress lead to pathophysiological changes in the body tissues by causing increased hepatic and plasma levels of ADMA (Leiper et al., 2002; Sydow and Munzel, 2003; Lee et al., 2005; Nakagami et al., 2009; Nakamura et al., 2009). The reduction in DDAH1 activity is the main cause of the elevation in the hepatic levels of ADMA (Jacobi et al., 2005; Mookerjee et al., 2015). Inflammatory mediators also contribute to elevated levels of ADMA by activating PRMT (Sydow and Munzel, 2003; Lee et al., 2005).

Increased levels of ADMA lead to vasoconstriction, platelet aggregation, cell adhesion to the endothelium, increased vascular leakage, and increased vascular smooth muscle cell proliferation (Jeremy et al., 1999; Cerwinka et al., 2002). ADMA alters the body’s response against inflammation by causing changes in the inflammatory mediators (Avci et al., 2015). On the other hand, ADMA causes increased levels of malondialdehyde (MDA) as one of the oxidative stress indicators, whereas it results in reduced activities of antioxidant enzymes such as catalase, superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) (Alaçam et al., 2013). All these effects of ADMA on NO synthesis, oxidative stress, and the inflammatory process cause deterioration at the tissue level, primarily endothelial dysfunction (Iwakiri et al., 2008; Okuyucu et al., 2015).

ADMA, symmetric dimethylarginine (SDMA), and L-arginine compete to be carried by cationic amino acid transporters (CATs) on the cell membrane. When compared with tissues such as heart, kidney, and lung, higher rates of CAT expression and uptake of ADMA, primarily CAT-2A and CAT-2B, are found in the liver (Forstermann et al., 1991; Hattori et al., 1999; Ferrigno et al., 2015). Increased ADMA causes a reduction in levels of intracellular L-arginine by blocking transport of L-arginine through membranes. The reduction in the level of L-arginine causes formation of uncoupled eNOS that shows oxidase activity. Uncoupled eNOS causes formation of oxygen radicals by reacting with molecular oxygen instead of arginine (Sydow and Munzel, 2003). Therefore, increased ADMA causes tissue deterioration by a different mechanism.

It is found in the literature that the ADMA/DDAH/NO pathway is considered responsible in the pathophysiology of many diseases (Sheen et al., 2014). This pathway is also blamed for the pathophysiology of hepatic diseases. ADMA levels increase in liver cirrhosis (Lluch et al., 2004), alcoholic hepatitis (Mookerjee et al., 2007b), and acute liver failure (ALF) (Mookerjee et al., 2007a). Increased portal pressure observed with alcoholic hepatitis is associated with increased levels of ADMA (Mookerjee et al., 2007b). Some changes occur in the pathway of ADMA/DDAH also in the case of hepatic ischemia/reperfusion (IR). In the early period of reperfusion (1 h), DDAH1 mRNA and protein expressions show a reduction, whereas ADMA levels increase (Ferrigno et al., 2014). Increased ADMA under conditions of IR changes the balance between NO and endothelin and thereby causes vasoconstriction, which may lead to hepatic deterioration (Laleman et al., 2005).

Increased ADMA is noticed in the pathophysiology of many diseases in the literature. However, it is controversial whether increased levels of ADMA is the reason or the consequence. In this study, we have aimed to determine whether increased levels of ADMA directly cause hepatic deterioration and whether it is decreased by ascorbic acid as an antioxidant. For this purpose, ADMA was given to rats and subsequently deterioration and restoration were examined histopathologically and biochemically.

2. Materials and methods

The required approval was received from the local ethics committee of Ondokuz Mayis University. The experimental animals used in the study were purchased from the Ondokuz Mayis University Laboratory Animals Research and Application Center. Maintenance of the rats, administration of ADMA and ascorbic acid, euthanasia of the rats, sample collection, and obtaining of serum samples were all performed at the same center.

2.1. Experimental animals

Thirty Sprague Dawley rats were used in this study. Body weights of the rats ranged between 250 and 300 g. The rats were allowed to receive feed ad libitum prior to and during the experiment protocols. The rats were kept at a 12-h light/dark cycle. The temperature of the animal barn was 22 ± 1 °C.

2.2. Study groups

The study included three groups with 10 rats in each group:

1) Control group (n = 10): Administered only 0.2 mL of 0.9% NaCl intraperitoneally (i.p.).

2) ADMA group (n = 10): Administered 2 mg kg⁻¹ day⁻¹ ADMA i.p. (Cat. No. D4268, Sigma-Aldrich, USA) (Alaçam et al., 2013).

3) ADMA + ascorbic acid (AA) group (n = 10): 2 mg kg⁻¹ day⁻¹ ADMA i.p. and 50 mg kg⁻¹ day⁻¹ ascorbic acid (Cat. No. 100468, Merck Millipore, Germany) via gavage (Donpunha et al., 2011; Nagda and Bhatt, 2011; Alaçam et al., 2013).

ADMA was dissolved in sterile 0.9% NaCl and each rat was given 0.2 mL. Ascorbic acid was dissolved in sterile distilled water and each rat was given 0.5 mL. Both ADMA and ascorbic acid were given together to the rats for 10 days.
2.3. Sample collection

After administration of ADMA and ascorbic acid for 10 days, the rats were fasted for one night and sample collection of blood and liver tissues was initiated. At the first stage, anesthesia was induced using 100 mg kg\(^{-1}\) ketamine hydrochloride and 10 mg kg\(^{-1}\) xylazine. Blood samples of rats were obtained by cardiac puncture under anesthesia and taken into tubes without an anticoagulant; subsequently, the rats were decapitated. The abdominal region was opened by incision, and liver tissue samples were taken and kept in 10% buffered neutral formalin solution for 24 h.

Blood samples taken into Eppendorf tubes were centrifuged at 3000 \(x\) g for 10 min and the obtained serum samples were put into Eppendorf tubes to be stored –80°C.

2.4. Biochemical analyses

After administration of ADMA and AA for 10 days, the liver enzyme levels were evaluated. Before biochemical analyses were performed, serum samples were kept at 4°C for one night to dissolve slowly. The samples were vortexed. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP) enzyme activities were tested spectrophotometrically with an autoanalyzer (Architect c16000, Abbott Laboratories, USA). Enzyme activities were expressed as U L\(^{-1}\).

2.5. Histopathological examination

After administration of ADMA and AA for 10 days, the liver histopathology were evaluated. After fixation, all the samples were divided into slices approximately 0.2 cm thick perpendicular to the long axis of the liver. Two slices were obtained from each liver and all the cases were sampled separately. Following the routine procedure, tissues were blocked in the paraffin. Three sections of 4 µm in thickness were obtained from each block. Each section was treated with hematoxylin and eosin (H&E), trichrome, and reticulin stains. Histomorphological evaluation was performed based on the Ishak scoring system (Ishak et al., 1995) (Table 1). Histological activity index (grade) and fibrosis assessment (stage) were determined separately for each case. Histological activity index was graded between 0 and 18. Scores of 1 to 3, 4 to 8, 9 to 12, and 13 to 18 were accepted to reveal minimal, mild, moderate, and severe degrees of hepatitis, respectively. Minimal, mild, moderate, and severe degrees of hepatitis were expressed as “1”, “2”, “3”, and “4”, respectively. Fibrosis assessment (stage) was evaluated separately.

2.6. Statistical analyses

In comparisons between more than two groups, the groups with normal distribution were tested by one-way ANOVA. Significant differences were tested by Tukey HSD test from the multiple comparison tests. The Kruskal–Wallis H test was used in comparison of the groups without normal distribution. Multiple comparisons were performed using the Bonferroni-corrected Nemenyi test. The correlations between variables were inspected by Spearman’s rho. P < 0.05 was accepted as statistically significant. The analyses were performed using SPSS 21.0 (IBM SPSS Statistics, version 21.0, Armonk, NY, USA; Ondokuz Mayis University Network License).

3. Results

3.1. Biochemical analyses

No significant difference was found between groups in terms of AST and GGT levels (P = 0.107 and P = 0.317, respectively). Although there was no significant difference, AST and GGT levels increased in the groups administered ADMA, whereas AST and GGT levels decreased in the groups administered ascorbic acid (Table 2).

When compared with the control group, ALT levels of the ADMA group were significantly higher (P = 0.006). A reduction was observed when ascorbic acid was administered, even though this reduction was insignificant (P > 0.05) (Table 2).

When compared with the control group, ALP levels of the ADMA group were significantly higher (P = 0.041). A reduction was observed when ascorbic acid was administered, even though this reduction was insignificant (P > 0.05) (Table 2).

3.2. Histopathological examination

Interface hepatitis was significantly higher in the group administered ADMA (P = 0.040), whereas, the ADMA + AA group revealed significant reduction only when compared with the group administered ADMA (P = 0.040) (Table 3; Figures 1 and Figure 2).

When compared with the control group, confluent necrosis, focal necrosis, and histological activity index were found significantly higher in the group administered ADMA (P = 0.001). When ascorbic acid was administered, a reduction was monitored, even though this reduction was insignificant (P > 0.05) (Table 3; Figures 1 and 2).

Portal inflammation was found higher in the group administered ADMA when compared with the control group (P = 0.030). It was found lower in the ADMA + AA group when compared with the ADMA group, even if no significant difference was encountered (P > 0.05) (Table 3; Figures 1 and 2).

Severity of hepatitis was found significantly higher in the group administered ADMA when compared with the control group (P = 0.003). A reduction was observed in the ADMA + AA group when compared with the group administered only ADMA, even though this difference was insignificant (P > 0.05) (Table 3; Figure 1).
No significant difference was encountered between the groups when compared with respect to fibrosis ($P = 0.342$) (Table 3).

When the ADMA-administered group was compared to the control group, histopathological parameters including interface hepatitis, confluent necrosis, focal necrosis, histological activity index, and portal inflammation were significantly higher in the ADMA group than the control group.

4. Discussion
In this study, liver injury was induced by ADMA in rats. The injury was showed by using biochemical and histopathological parameters. Ascorbic acid restored the injury, but this was not sufficient.

NO is a molecule that is synthesized from L-arginine mediated by NOS and has important functions in many pathophysiological events. There are mainly three kinds of NOS that mediate NO synthesis: endothelial NOS (eNOS,
Table 2. AST, ALT, ALP, and GGT levels after 10 days of ADMA and AA administration. It is clearly seen that they were increased in the ADMA-administrated group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (mean ± SD)</th>
<th>ADMA (mean ± SD)</th>
<th>ADMA + AA (mean ± SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U L⁻¹)</td>
<td>66.60 ± 17.44</td>
<td>85.80 ± 17.24</td>
<td>74.70 ± 23.34</td>
<td>0.107</td>
</tr>
<tr>
<td>ALT (U L⁻¹)</td>
<td>27.20 ± 8.84</td>
<td>42.30 ± 12.36</td>
<td>33.70 ± 6.55</td>
<td>0.006*</td>
</tr>
<tr>
<td>ALP (U L⁻¹)</td>
<td>49.30 ± 19.00</td>
<td>91.70 ± 56.99</td>
<td>65.20 ± 14.13</td>
<td>0.041*</td>
</tr>
<tr>
<td>GGT (U L⁻¹)</td>
<td>0.40 ± 0.70</td>
<td>6.90 ± 9.78</td>
<td>2.70 ± 5.27</td>
<td>0.317</td>
</tr>
</tbody>
</table>

*Obtained by comparison between the control and ADMA groups. AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; ADMA: asymmetric dimethylarginine; AA: ascorbic acid.

Figure 1. Light micrographs of the histological view of the liver after 10 days of ADMA and AA administration.  A) There is mild-moderate inflammation in all portal areas in the group administered ADMA. Piecemeal necrosis in a portal area (thin arrow) and confluent necrosis in the parenchyma (thick arrow) are noteworthy (H&E 100×). B) Although the basic histological structure of the liver is very largely protected in the group administered ADMA + AA (H&E 100×), there is slight inflammation in a portal area (arrow). C) Liver section of control group. There is no inflammation in portal areas and parenchyma (H&E 100×). PT: Portal area (tract); CV: central vein; ADMA: asymmetric dimethylarginine; AA: ascorbic acid.
type 3), neuronal NOS (nNOS, type 1), and inflammatory NOS (iNOS, type 2) (Tsutsui et al., 2009, 2010). As well as NOS-mediated synthesis, NO is also generated from proteins such as S-nitrosothiols, S-nitroproteins, and nitrosyl hemoglobin (Kelm, 1999). Certain amounts of NO are quite important for endothelial functions and tissue homeostasis. Its insufficient secretion causes dysfunction in the endothelium and various systems while excessive levels lead to tissue deterioration. Excessive levels of NO produced by iNOS form toxic peroxynitrite radicals by reacting with superoxide radicals (Hierholzer et al., 1998; Fan et al., 1999). Peroxynitrite, beside many other harmful effects, causes reduction in DDAH activity and increased levels of ADMA (Leiper et al., 2002).

NO is one of the essential regulators of intrahepatic vascular tone and decreased production of NO results in portal hypertension (Iwakiri et al., 2008; Sanyal et al., 2008). NOS inhibitors such as caveolin-1 (Shah et al., 1999), ADMA (Mookerjee et al., 2007b), and NOSTRIN (Mookerjee et al., 2007c) play roles in the occurrence of portal hypertension. Severity of portal hypertension is correlated with levels of ADMA (Mookerjee et al., 2007b), ADMA: asymmetric dimethylarginine; AA: ascorbic acid.

Table 3. Comparison of the groups with respect to histopathological parameters after 10 days of ADMA and AA administration. The parameters showed that there was liver injury after administration of ADMA.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control, median (min–max)</th>
<th>ADMA, median (min–max)</th>
<th>ADMA + AA, median (min–max)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interface hepatitis</td>
<td>0 (0–0)</td>
<td>0 (0–1)</td>
<td>0 (0–0)</td>
<td>0.040&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Confluent necrosis</td>
<td>0 (0–1)</td>
<td>1.5 (0–3)</td>
<td>1 (0–2)</td>
<td>0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Focal necrosis</td>
<td>1 (0–1)</td>
<td>2 (0–3)</td>
<td>1.5 (1–2)</td>
<td>0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Portal inflammation</td>
<td>1 (0–1)</td>
<td>1 (0–3)</td>
<td>1 (0–1)</td>
<td>0.030&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Histologic activity index</td>
<td>1 (0–3)</td>
<td>5 (0–10)</td>
<td>3.5 (2–5)</td>
<td>0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Severity of hepatitis</td>
<td>1 (0–1)</td>
<td>2 (0–3)</td>
<td>1.5 (1–2)</td>
<td>0.003&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>0 (0–0)</td>
<td>0 (0–1)</td>
<td>0 (0–1)</td>
<td>&gt;0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Obtained by comparison between control and ADMA groups.
<sup>b</sup>Obtained by comparison between ADMA and ADMA + AA groups.
<sup>c</sup>Obtained by Kruskal–Wallis H test.
ADMA: asymmetric dimethylarginine; AA: ascorbic acid.
Cardiovascular risk factors such as hyperglycemia and hyperhomocysteinemia cause a reduction in DDAH activity by ROS-mediated ways whereas antioxidants lead to an elevation in DDAH activity (Nakagami et al., 2009; Nakamura et al., 2009). Inflammatory genes cause increased ADMA levels by activating PRMT and inhibiting DDAH (Sydow and Munzel, 2003; Lee et al., 2005). ADMA itself also causes increased levels of MDA, which is an indicator of oxidative stress, while it leads to reduction in the activities of antioxidant enzymes and DDAH (Jeremy et al., 1999; Cerwinka et al., 2002). ADMA administered to the rats in our study may have caused a reduction in DDAH activity and consequently there may have been excessive amounts of ADMA in the liver. This possibility is not unlikely when the oxidative stress-increasing effect of ADMA is considered (Alaçam et al., 2013). However, antioxidant ascorbic acid administered to the rats did not provide sufficient recovery of either biochemical or histopathological parameters. These results make us consider that the deteriorative effect of ADMA may result from different factors other than oxidative stress.

Many pathways are accepted as being responsible for the pathophysiology of acute and chronic liver diseases. The ADMA/DDAH/NO pathway is also one of the affected pathways (Mookerjee et al., 2007a). Increased levels of ADMA observed in hepatic dysfunctions are due to increased levels of protein breakdown or decreased DDAH activity (Mookerjee et al., 2007b; Pilz et al., 2015). The reduction in DDAH activity may originate from local inflammation, from increased oxidative stress, or directly from the reduction in the amount of DDAH protein (Nijveldt et al., 2003b). In the study of Bal et al., hepatic injury was induced by administering LPS/D-GalN in rats. In the group administered LPS/D-GalN, reduced DDAH activity and increased levels of arginine and ADMA were encountered. When the rats were administered metformin, DDAH activity increased while levels of arginine and ADMA regressed to the levels of control group. In the same study, a reduction was found in the hepatic glutathione levels and increased prooxidant activity was considered as the reason for this reduction. It was reported that increased myeloperoxidase activity in the liver tissue reveals inflammation and reduced DDAH activity resulted from this inflammation (Bal et al., 2014). Pilz et al. analyzed the correlation between Child–Pugh and Model for End-Stage Liver Disease (MELD) scores that indicate liver dysfunction and arginine metabolites. Homoarginine, SDMA, and DAS (ADMA + SDMA) were found correlated with only MELD score, whereas ADMA was detected to be correlated with both Child–Pugh and MELD scores. Mortality rate was found correlated with ADMA, SDMA, and DAS values over median values and homoarginine levels below median value. It was
stated that ADMA is one of the indicators of hepatic dysfunction and that ADMA is one of the responsible factors for poor outcome in the patients with cirrhosis (Pilz et al., 2015). In a study conducted in an intensive care unit, baseline ADMA levels of the patients with liver failure that developed due to high-dose administration of acetaminophen at admission to the intensive care unit were found higher than those of the control group. In the same study, ADMA levels showed elevation in the anhepatic phase during transplantation. Providing reperfusion of the portal vein and hepatic artery reduced ADMA levels and this reduction in ADMA levels continued in the period following reperfusion. In the same study, a positive correlation was detected between proinflammatory mediators such as TNF-α, IL-1β, and IL-6 and ADMA while the highest ADMA levels were encountered in patients with the highest systemic inflammatory response syndrome score (Mookerjee et al., 2007a). As can be seen, prooxidant activity and inflammation that locally increased in the liver tissue contributed to the liver injury caused by ADMA. In our study, an elevation was also observed in portal inflammation when ADMA was administered, and it probably contributed to the injury induced by ADMA.

The suggested ADMA-multiorgan failure (MOF) hypothesis states that ADMA plays an important role in MOF (Lopez et al., 2004). Increased hepatic ADMA levels lead to deterioration of hepatic perfusion and functions. This deterioration causes an excessive elevation in serum ADMA levels and results in further hepatic dysfunction by a vicious circle. Increased ADMA levels cause not only hepatic dysfunction but also lead to impaired functions of other organs, and this elevation in ADMA levels is very important in the pathophysiology of MOF (Harbrecht et al., 2001). High levels of ADMA were encountered in MOF that developed in patients with sepsis and that accompanied hepatic dysfunction; it was an independent prognostic predictor (Nijveldt et al., 2003b). Milewski et al. found higher plasma and brain cortex ADMA levels than those in the control group in rats with thioacetamide-induced acute liver failure. In the same study, a reduction was encountered in brain and liver DDAH activity and also in brain NOS activity in rats with acute liver failure (Milewski et al., 2015). ADMA administered in our study may have caused deterioration in other tissues by leading to a reduction in NO levels. New studies may be conducted to clarify this issue.

There are publications that suggest that plasma ADMA levels may be reduced by pharmacotherapy and dialysis (Beltowski and Kedra, 2006). High plasma levels of ADMA have been successfully reduced using renin-angiotensin system inhibitors (Yilmaz et al., 2007). Since ADMA (~202 Da) has a low molecular weight similar to urea (60 Da), dialysis treatment appears to be a treatment selection to reduce ADMA levels (Anderstam et al., 1997; Schmidt et al., 1999). DDAH activity can be increased by several signal pathways. GW4064, being an agonist of farnesoid X receptor, causes increased DDAH1 gene expression and a reduction in ADMA levels (Hu et al., 2006). The effects of flavonoids and antioxidants on ADMA/DDAH levels are currently being studied. In the study of Trocha et al., a reduced hepatic ADMA level and an increased DDAH activity were encountered when quercetin as a flavonoid was administered in a low dosage (Trocha et al., 2012). ADMA-reducing treatments may be an approach in treatment of advanced-stage liver diseases. For this purpose, ascorbic acid was administered as an antioxidant in our study and it was tested whether it reduced the harmful effects of ADMA. Ascorbic acid decreased in vivo oxidative load and injury induced by ADMA thanks to its antioxidant properties even if this reduction was not significant. This may be due to an insufficient dosage of ascorbic acid and lack of time. Ascorbic acid might be given for more than 10 days and at higher dosages. We chose the time and dosage for this study based on the literature. (Alaçam et al., 2013; Okuyucu et al., 2015). Different doses and durations may be tested in future studies.

In conclusion, occurrence of liver injury when rats were administered ADMA indicates that ADMA is a factor in the formation of liver injury. Insufficient recovery when an antioxidant was administered makes us consider that different factors besides prooxidant factors are effective in the formation of liver injury.

References


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