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# Effect of Nitric Oxide Pathway Regulation on Water/Sodium Balance and Renal Function in a Rodent Model of Acute Liver and Renal Failure

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Data Interpretation D  
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**Background:** The pathomechanism of acute hepatorenal syndrome (HRS), a particular form of acute renal failure that occurs in the course of acute liver injury, is still poorly understood. The aim of our study was to estimate the influence of the activation and inhibition of the nitric oxide pathway on the water/sodium balance and development of acute renal failure in the course of HRS.

**Material/Methods:** We used male Sprague-Dawley rats in the acute galactosamine (Ga1N) model of HRS. The nitric oxide synthase (NOS) inhibitors L-NAME and L-arginine were administered intraperitoneally before and after liver damage.

**Results:** HRS developed in all tested groups. L-NAME increased osmotic clearance and urine volume more effectively before liver injury. Furthermore, administration of L-NAME increased creatinine clearance both before and after Ga1N injection. A double dose of L-NAME did not yield further improvement before Ga1N injection, but improved creatinine clearance after Ga1N intoxication. Injection of L-arginine increased sodium excretion and urine volume, but only after liver injury. Moreover, L-arginine injected after Ga1N caused significant improvement of the creatinine clearance in a dose-dependent manner.

**Conclusions:** Our study shows that inhibition of the nitric oxide pathway improves parameters of water and sodium balance and prevents development of acute renal failure in the course of acute liver injury and liver failure. Activation of the nitric oxide system also has a favorable influence on water/sodium balance and renal failure, but only after liver injury.

**MeSH Keywords:** **Hepatorenal Syndrome • Liver Failure, Acute • Nitric Oxide • Renal Insufficiency • Water-Electrolyte Balance**

**Full-text PDF:** <http://www.medscimonit.com/abstract/index/idArt/890757>

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## Background

Hepatorenal syndrome (HRS) is defined as functional renal failure in the course of an acute liver injury and liver failure [1–3]. Liver disease is the only etiological factor in this syndrome, and a peculiar form of renal failure characterized by oliguria, hyperazotemia, and hyponatremia develops without clinical, laboratory, and histopathological features of any known kidney disease causing its failure [4,5]. In clinical practice, HRS often develops in patients with alcoholic cirrhosis, ascites, and portal hypertension, and rarely in the course of acute viral hepatitis or primary biliary cirrhosis [6,7]. This syndrome usually has a difficult clinical course, negative prognosis, and high mortality rate. Among patients with cirrhosis and ascites, HRS occurs after 1 year in approximately 20% and after 5 years in 40% of the patients [8]. The median survival time is approximately 2 weeks in patients with the rapidly progressive type 1 HRS and approximately 4–6 months in patients with the slower progressive type 2 HRS [9]. This dramatic prognosis results from a lack of efficient and specific treatment, due to the still poorly understood pathogenesis of the disease and its basic mechanisms.

In 1863, Austin Flint first described the relationship between the prevalence of oliguria and cirrhosis, and ascites, underlying a lack of proteinuria and histopathological changes in kidneys, which suggested the functional character of HRS [10]. Although many previous studies have clarified several issues regarding HRS, the exact mechanism underlying the development of renal failure due to liver injury is not completely known. A recent study reported that hemodynamic disturbances are a major pathomechanism of HRS [11]. Chronic liver disease and the subsequent portal hypertension cause a dilatation of the splanchnic vascular bed, which leads to a reduction in the effective plasma volume in systemic circulation, and the stimulation of vascular baroreceptors activates the renin-angiotensin-aldosterone system, sympathetic nervous system, or arginine vasopressin system. These mechanisms lead to vasoconstriction and renal hypoperfusion [12,13]. Some studies have shown that the above-mentioned vasocontractile factors only slightly affect the splanchnic vascular bed. This happens because of an increased production of local splanchnic vasodilatory substances [14]. Some other studies have confirmed that endogenous nitric oxide (NO) plays a key role in this process, although prostaglandins or vascular peptides may also be important [15,16].

However, the pathomechanism of acute renal failure due to liver injury and liver failure is not yet well known [17]. A few studies have suggested that acute liver injury leads to the rapid development of portal hypertension, opening of arteriovenous anastomoses, and increase in the portal venous flow. As a result of dilatation of the splanchnic vascular bed, there is a decrease in the effective plasma volume in systemic circulation, and hyperdynamic circulation develops [18,19]. These

mechanisms might be responsible for the above-described phenomena, leading to hydro-electrolyte disorders and renal failure.

The significance of the endogenous NO pathway in models of chronic liver diseases, especially cirrhosis, has been relatively well documented [19–21]. To our knowledge, no previous study has examined the role of NO in the development of HRS in acute liver disease models. Our previous studies showed that the congenital factors (i.e., the specific strain of experimental animal) are reliable for development of acute renal failure in the course of an acute liver injury experimental model of HRS induced by galactosamine (Ga1N) [22]. The purpose of the present study was to determine the effect of stimulation and inhibition of the endogenous NO pathway on 2 factors: (1) the water and sodium balance and (2) the development and degree of renal failure in an animal model of acute HRS induced by Ga1N.

## Material and Methods

This study was approved by the local Bioethics Committee for Experimental Studies in Animals. In this study, we used 96 randomly selected male Sprague-Dawley rats, body weight 200–250 g, obtained from the Department of Experimental Animals, Polish Mother's Memorial Hospital, Lodz. The animals were housed in standard group cages, fed a standard diet, with free access to water and food, with a natural day/night cycle of 12 h, at a temperature  $22\pm 2^\circ\text{C}$  and humidity of 45–50%. The experiments were performed between 10 a.m. and 6 p.m. in awake, moving animals. This study was performed in accordance with the guidelines of the *Animals in Scientific Procedures Act*. During the course of the experiments, the rats were housed in individual glass metabolic cages with free access to water and food.

The animals were given saline, Ga1N, and an NO synthase inhibitor – N-nitro-L-arginine methyl ester (L-NAME) – or L-arginine (L-ARG), as an NO donor, to examine the role of NO in this HRS model. The rats were divided into 12 groups, with 8 individuals in each group: Group 1 (sham group) received an intraperitoneal (i.p.) injection of 1 ml of 0.9% saline solution; Group 2 received 1.1 g/kg body weight (b.w.) of Ga1N (Sigma Aldrich Poland) via an i.p. injection of 200 mg/ml of 0.9% saline solution; Group 3 (control L-NAME group) received an i.p. injection of 100 mg/kg of L-NAME (Sigma Aldrich Poland); Group 4 received the same dose of L-NAME at 48 h and 24 h before Ga1N injection; Group 5 received 200 mg/kg (double dose) of L-NAME at 48 h and 24 h before Ga1N injection; Group 6 received 100 mg/kg of L-NAME at 24 h and 48 h after Ga1N injection; Group 7 received 200 mg/kg (double dose) of L-NAME at 24 h and 48 h after Ga1N injection; Group 8 (control L-ARG group) received an i.p. injection of 150 mg/kg b.w. of L-ARG (Sigma Aldrich Poland); Group 9 received the same dose of L-ARG at 48 h and 24 h before Ga1N injection; Group 10

received 300 mg/kg b.w. (double dose) of L-ARG at 48 h and 24 h before Ga1N injection; Group 11 received an i.p. injection of 150 mg/kg b.w. of L-ARG at 24 h and 48 h after Ga1N injection; Group 12 received 300 mg/kg b.w. (double dose) of L-ARG at 24 h and 48 h after Ga1N injection.

Twenty-four-hour urine samples were collected for a 24-h period between the 24th and 48th h after saline or Ga1N injection and were evaluated 48 h after saline or Ga1N injection. Blood samples (6 ml) were also collected from the beating hearts of the deeply anaesthetized animals 48 h after saline or Ga1N injection. Biochemical parameters, except ammonium concentration, were determined in serum or urine by using an Integra 700 autoanalyzer (Roche, USA) and bilirubin, alanine aminotransferase (ALT), creatinine, urea, and sodium reagents (Roche, Germany); the ammonium concentration in plasma was measured using the EDTA-K<sub>3</sub> anticoagulant with an autoanalyzer. Urine osmolality was measured using an automatic osmometer (Osmometer Automatic, Knauer, Germany). Creatinine clearance, as a parameter of the glomerular filtration rate (GFR), was calculated according to the following equation:

$$\text{Creatinine clearance (ml/min)} = (\text{Creat}_u \times V_{U_{24h}}) / (\text{Creat}_s \times 1440)$$

Here, Creat<sub>u</sub> represents urine creatinine, Creat<sub>s</sub> represents serum creatinine, and V<sub>U<sub>24h</sub></sub> represents the 24-h urine volume. Osmolality clearance was calculated according to the following equation:

$$\text{Osmolality clearance (ml/24 h)} = (U \text{ osm} \times V_{U_{24h}}) / P \text{ osm}$$

Here, U osm represents urine osmolality, V<sub>U<sub>24h</sub></sub> represents the 24-h urine volume, and P osm represents plasma osmolality, which was calculated using the following equation:

$$2 \times (\text{Na}_s + \text{K}_s + \text{Urea}_s)$$

Here, Na<sub>s</sub> represents serum sodium, K<sub>s</sub> represents serum potassium, and Urea<sub>s</sub> represents serum urea [23].

After animals were killed by exsanguination, liver and kidney tissues were collected for histopathological examination: the liver and kidney sections were fixed in formalin, paraffin embedded, stained with hematoxylin and eosin, and examined under a light microscope.

### Statistical analysis

Statistical analysis was performed using the *t*-test and analysis of variance when multiple comparisons were required. Where appropriate, the Mann-Whitney *U* test was used to analyze non-parametric data. The limit of significance was set at *p*<0.05. All data are expressed as means ±SE.

## Results

### Experimental HRS

The biochemical parameters for the development of acute HRS in the experimental animals are presented in Tables 1 and 2. Ga1N administration at a dose of 1.1 g/kg of b.w. caused serious liver injury leading to liver failure, and a statistically significant increase in the blood serum concentrations of bilirubin (*p*<0.004), ALT (*p*<0.001), and ammonia (*p*<0.005) was observed in Group 2, in comparison with the control group (Group 2 vs. Group 1). In the same study Group 2, besides liver injury, the acute renal failure occurred with a significant increase in the serum concentrations of creatinine (*p*<0.001) and urea (*p*<0.001) and a significant decrease in creatinine clearance (*p*<0.0012) (Tables 1 and 2; Figures 1 and 2) and the 24-h urine volume (*p*<0.003) in Group 2 (Group 2 vs. Group 1). A significant decrease in the hydro-electrolyte balance parameters typical for HRS such as osmotic clearance (*p*<0.001) (Tables 1 and 2; Figures 3 and 4), fraction of ejected sodium (*p*<0.016) (data not shown), and 24-h diuresis (*p*<0.003) was observed in Group 2, in comparison with Group 1. Furthermore, a decrease in 24-h natriuresis and increase in urine osmolality were observed in Group 2; however, these differences were not statistically significant (Group 2 vs. Group 1).

Histopathological examination of the liver (Figures 5 and 6) showed generalized necrosis of hepatocytes in all study groups that received Ga1N, as compared with the sham group, which received saline and in which no changes were observed (Groups 2, 4–7, 9–12 vs. Group 1). On the other hand, on histopathological examination of the kidneys (Figures 7 and 8), no changes were observed in any study groups or in the sham group (Groups 2, 4–7, 9–12 vs. Group 1).

### Inhibition of the endogenous NO pathway and water-electrolyte balance and renal failure

No significant differences in the hepatic and renal parameters following only L-NAME administration were observed between Group 3 and the control group (Group 3 vs. Group 1). Administration of the single dose of L-NAME before Ga1N injection caused a significant decrease in the concentrations of creatinine (*p*<0.001) and urea (*p*<0.001) and a significant increase in creatinine clearance (*p*<0.0017) in Group 4, as compared with Group 2 (Table 1; Figure 1) (Group 4 vs. Group 2). Furthermore, a significant decrease in urine osmolality (*p*<0.004) and significant increase in osmotic clearance (*p*<0.023) and 24-h diuresis (*p*<0.0005) were observed in Group 4 (Table 1; Figure 3). Administration of the double dose of L-NAME did not cause any further improvement in GFR, although the blood serum concentration of creatinine significantly decreased (*p*<0.002) in Group 5 (Group 5 vs. Group 4). Furthermore, urine osmolality

**Table 1.** Biochemical profile of liver and renal parameters in rats with HRS after L-NAME injection.

Group (n)	Bil, mg/dl	ALT, IU/L	Ammon, $\mu$ mol/l	Creat, mg/dl	Urea, mg/dl	Creat Cl ml/min	Urine osmol mosmol/kg	Urine sodium mmol/l	Osmol Cl ml/24h	24 h urine ml/24h
1 (8) sham	0.4 $\pm 0.26$	56.2 $\pm 9.9$	52.8 $\pm 38.1$	0.47 $\pm 0.04$	33.7 $\pm 3.8$	0.89 $\pm 0.4$	1480.7 $\pm 69.3$	52 $\pm 15.79$	57.3 $\pm 13.9$	11.9 $\pm 2.9$
2 (8) Ga1N	3.43 $\pm 1.35^*$	2098.6 $\pm 886.1^*$	275.7 $\pm 73.7^*$	0.76 $\pm 0.09^*$	80.1 $\pm 10.1^*$	0.18 $\pm 0.12^*$	1528.6 $\pm 95.5$	46.25 $\pm 12.73$	24.09 $\pm 10.7^*$	5.05 $\pm 2.1^*$
3 (8) sham L-NAME	0.28 $\pm 0.17$	82.2 $\pm 16.2^*$	26.9 $\pm 7.8$	0.46 $\pm 0.04$	34.2 $\pm 2.27$	0.81 $\pm 0.29$	940.9 $\pm 189.1^*$	57.75 $\pm 11.15$	33.8 $\pm 10.3^*$	11.6 $\pm 4.3$
4 (8) L-NAME/Ga1N	3.27 $\pm 0.52$	1343.75 $\pm 451.92$	210 $\pm 48.18$	0.41 $\pm 0.03^*$	28.1 $\pm 5.3^*$	0.69 $\pm 0.23^*$	941.8 $\pm 187.6^*$	46.87 $\pm 18.27$	51.6 $\pm 26.4^*$	16.5 $\pm 6.4^*$
5 (8) 2xL-NAME/Ga1N	2.67 $\pm 0.75$	1590.12 $\pm 504.26$	193.87 $\pm 80.92$	0.21 $\pm 0.05^*$	38.3 $\pm 5.8^*$	0.63 $\pm 0.32^*$	425.7 $\pm 93.8^*$	28.62 $\pm 4.71^*$	18.3 $\pm 9.3$	14.03 $\pm 7.8^*$
6 (8) Ga1N/L-NAME	2.58 $\pm 0.46$	1425 $\pm 475.61$	206.37 $\pm 51.11$	0.45 $\pm 0.05^*$	37 $\pm 7.3^*$	0.47 $\pm 0.22^*$	1225.8 $\pm 160.2^*$	43.62 $\pm 13.53$	31.01 $\pm 10.6$	8.2 $\pm 3.1^*$
7 (8) G Ga1N/2xL-NAME	3.1 $\pm 0.75$	1707.12 $\pm 448.87$	201.5 $\pm 42.33^*$	0.31 $\pm 0.05^*$	50.7 $\pm 13.9^*$	0.53 $\pm 0.35^*$	979.2 $\pm 243.2^*$	63 $\pm 20.19$	22.5 $\pm 14$	7.7 $\pm 5.2$
Gr. 2 v Gr. 1 - p	p<0.004	p<0.001	p<0.005	p<0.001	p<0.001	p<0.0012	p<0.30	p<0.46	p<0.001	p<0.003
Gr. 3 v Gr. 1 - p	p<0.37	p<0.03	p<0.089	p<0.61	p<0.77	p<0.67	p<0.0005	p<0.44	p<0.03	p<0.89
Gr. 4 v Gr. 2 - p	p<0.77	p<0.064	p<0.068	p<0.001	p<0.001	p<0.0017	p<0.004	p<0.94	p<0.023	p<0.0005
Gr. 5 v Gr. 2 - p	p<0.21	p<0.064	p<0.067	p<0.001	p<0.001	p<0.0042	p<0.001	p<0.004	p<0.3	p<0.011
Gr. 6 v Gr. 2 - p	p<0.13	p<0.098	p<0.06	p<0.003	p<0.001	p<0.0095	p<0.0074	p<0.71	p<0.24	p<0.046
Gr. 7 v Gr. 2 - p	p<0.57	p<0.31	p<0.036	p<0.01	p<0.005	p<0.028	p<0.007	p<0.084	p<0.81	p<0.23

\* p<0.05 – significant. Bil – serum bilirubin; ALT – serum alanine aminotransferase; Ammon – serum ammonium; Creat – serum creatinine; Creat Cl – creatinine clearance; Urine osmol – urine osmolality; Osmol Cl – osmolar clearance; p – value of p. Biochemical parameters were evaluated 48 h after saline or Ga1N injection. Twenty-four-hour urine samples were collected during the 24 h from the 24<sup>th</sup> to the 48<sup>th</sup> h after saline or Ga1N injection and were evaluated 48 h after saline or Ga1N injection. Values are means  $\pm$ SE, significance – p<0.05. Gr. 1 – sham; Gr. 2 – given 1.1 g/kg Ga1N; Gr. 3 – sham L-NAME, given 100 mg/kg L-NAME; Gr. 4 – given 100 mg/kg L-NAME 48 h and 24 h and before Ga1N; Gr. 5 – given 200 mg/kg L-NAME 48 h and 24 h and before Ga1N; Gr. 6 – given 100 mg/kg L-NAME 24 h and 48 h after Ga1N; Gr. 7 – given 200 mg/kg L-NAME 24 h and 48 h after Ga1N.

significantly decreased (p<0.001), whereas the 24-h urine volume did not change significantly.

Inhibition of the endogenous NO pathway after Ga1N injection showed other effects. Administration of a smaller dose of L-NAME after Ga1N injection also improved the parameters of kidney function; the concentrations of creatinine (p<0.0003) and urea (p<0.001) significantly decreased, whereas the creatinine clearance (p<0.0095) significantly increased in Group 6 (Group

6 vs. Group 2) (Table 1; Figure 1). Moreover, L-NAME administration after Ga1N injection significantly decreased urine osmolality (p<0.0074) and increased the 24-h urine volume (p<0.046), free water clearance (p<0.032) (data not shown), and osmotic clearance (albeit in an insignificant manner); however, it did not show any effect on 24-h natriuresis (Table 1; Figure 3). Administration of a double dose of L-NAME insignificantly increased GFR, and significantly decreased creatinine concentration (p<0.0036) (Group 7 vs. Group 6). Moreover, as compared to the single dose,

**Table 2.** Biochemical profile of liver and renal parameters in rats with acute HRS after L-ARG injection.

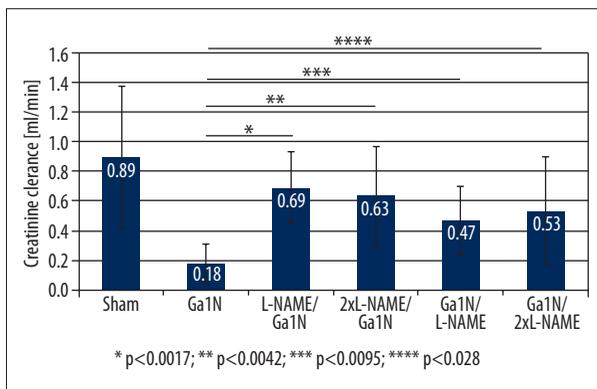
Group (n)	Bil, mg/dl	ALT, IU/L	Ammon, µmol/l	Creat, mg/dl	Urea, mg/dl	Creat Cl ml/min	Urine osmol mosmol/kg	Urine sodium mmol/l	Osmol Cl ml/24h	24 h urine ml/24h
1 (8) sham	0.4 ±0.26	56.2 ±9.9	52.8 ±38.1	0.47 ±0.04*	33.7 ±3.8*	0.89 ±0.4	1480.7 ±69.3	52 ±15.79	57.3 ±13.9	11.9 ±2.9
2 (8) Ga1N	3.43 ±1.35*	2098.6 ±886.1*	275.7 ±73.7*	0.76 ±0.09	80.1 ±10.1*	0.18 ±0.12*	1528.6 ±95.5	46.25 ±12.73	24.09 ±10.7*	5.05 ±2.1*
8 (8) sham L-ARG	0.38 ±0.21	53.75 ±7.37	50.7 ±46.2	0.43 ±0.08	25.7 ±4.17	1.05 ±0.35	1249.1 ±168.06*	55.4 ±18.64	48.2 ±14.1	11.6 ±2.7
9 (8) L-ARG/ Ga1N	2.96 ±0.67	1546.37 ±349.75	199.12 ±42.8*	0.72 ±0.11	87.1 ±7.5	0.17 ±0.12	1286.8 ±135.4*	92.37 ±27.73	19.2 ±8.1	4.76 ±2.02
10 (8) 2xL-ARG/ Ga1N	2.92 ±0.89	1603.75 ±364.47	193.87 ±50.49*	0.8 ±0.07	85.6 ±4.9	0.19 ±0.08	1132.3 ±126.5*	68.87 ±27.92	20.1 ±7.2	5.3 ±1.9
11 (8) Ga1N/ L-ARG	2.86 ±0.94	1486.25 ±450.83	195.25 ±41.69*	0.75 ±0.08	79.6 ±5.5	0.41 ±0.25	1180.3 ±132.5*	58.17 ±18.34	33.2 ±11.06	9.11 ±2.63*
12 (8) Ga1N/ 2xL-ARG	2.96 ±0.98	1435.62 ±281.33	180.5 ±45.80*	0.73 ±0.09	82.1 ±4.7	0.39 ±0.15*	1138.7 ±227.1*	52.5 ±16.28*	29.12 ±9.68	8.21 ±2.12*
Gr. 2 v Gr. 1 - p	p<0.004	p<0.001	p<0.005	p<0.001	p<0.001	p<0.0012	p<0.30	p<0.46	p<0.001	p<0.003
Gr. 8 v Gr. 1 - p	p<0.11	p<0.60	p<0.34	p<0.31	p<0.0022	p<0.49	p<0.005	p<0.63	p<0.24	p<0.86
Gr. 9 v Gr. 2 - p	p<0.41	p<0.14	p<0.032	p<0.53	p<0.17	p<0.91	p<0.0017	p<0.067	p<0.37	p<0.79
Gr. 10 v Gr. 2 - p	p<0.41	p<0.19	p<0.029	p<0.42	p<0.2	p<0.83	p<0.001	p<0.071	p<0.23	p<0.78
Gr. 11 v Gr. 2 - p	p<0.37	p<0.12	p<0.024	p<0.80	p<0.91	p<0.058	p<0.003	p<0.056	p<0.13	p<0.006
Gr. 12 v Gr. 2 - p	p<0.46	p<0.08	p<0.011	p<0.64	p<0.64	p<0.018	p<0.009	p<0.002	p<0.35	p<0.014

\* p<0.05 – significant. Bil – serum bilirubin; ALT – serum alanine aminotransferase; Ammon – serum ammonium; Creat – serum creatinine; Creat Cl – creatinine clearance; Urine osmol – urine osmolality; Osmol Cl – osmolar clearance; p – value of p. Biochemical parameters were evaluated 48 h after saline or Ga1N injection. Twenty-four-hour urine samples were collected during the 24 h from the 24<sup>th</sup> to the 48<sup>th</sup> h after saline or Ga1N injection and were evaluated 48 h after saline or Ga1N injection. Values are means ±SE, significance – p<0.05. Gr. 1 – sham; Gr. 2 – given 1.1 g/kg Ga1N; Gr. 8 – sham L-ARG group, given 150 mg/kg L-ARG; Gr. 9 – given 150 mg/kg L-ARG 48 h and 24 h and before Ga1N; Gr. 10 – given 300 mg/kg L-ARG 48 h and 24 h and before Ga1N; Gr. 11 – given 150 mg/kg L-ARG 24 h and 48 h after Ga1N; Gr. 12 – given 300 mg/kg L-ARG 24 h and 48 h before Ga1N.

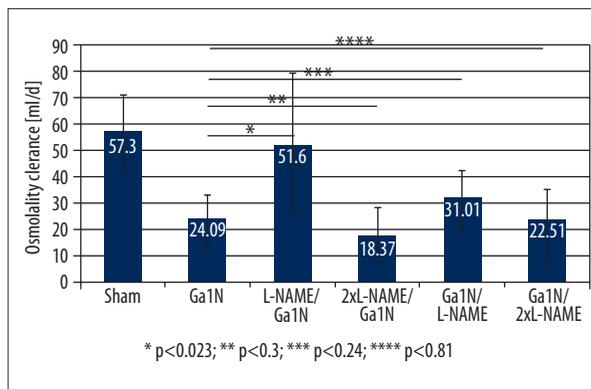
administration of the double dose of L-NAME caused a further significant decrease in urine osmolality (p<0.041) and an insignificant increase in 24-h natriuresis (p<0.053). It had no effect on osmotic clearance or 24-h urine volume.

It should be noted that as compared to L-NAME administration after Ga1N injection, L-NAME administration before Ga1N injection, at both the smaller as well as the larger doses, more effectively improved the parameters of kidney function and

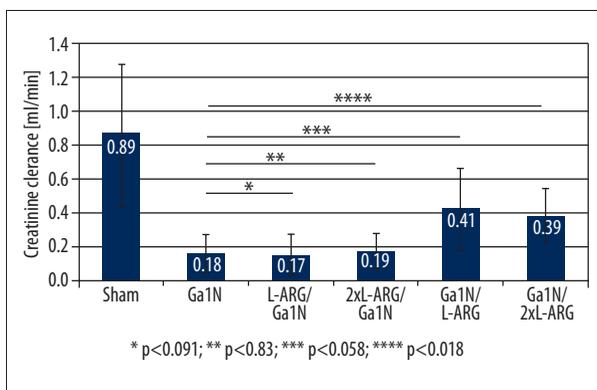
hydro-electrolyte balance. In the smaller dose, the urea concentrations were significantly lower (p<0.022), the creatinine concentrations were lower, and GFR was higher, but not significantly (Group 4 vs. Group 6) (Table 1; Figure 1). Urine osmolality was significantly lower (p<0.0087) and osmotic clearance was higher, but this difference was not significant (Table 1; Figure 3). In the larger dose of L-NAME, the creatinine and urea concentrations were significantly lower (p<0.0075 and p<0.048, respectively) and the GFR was insignificantly higher



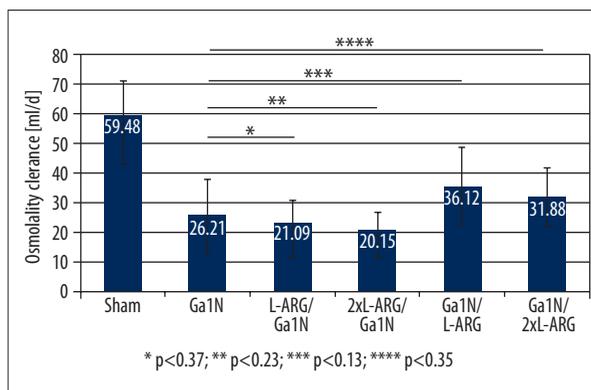
**Figure 1.** Influence of L-NAME injection on creatinine clearance before and after liver injury. Inhibition of the NO system before Ga1N intoxication significantly increased GFR. A double dose of L-NAME did not yield further improvement. Inhibition of NO after Ga1N injection also improved renal efficiency, and a double dose of L-NAME delivered further improvement. Treatment before turned out better than treatment after Ga1N intoxication. Values are means ±SE, significance – p<0.05.



**Figure 3.** Influence of L-NAME injection on osmolality clearance before and after liver injury. Inhibition of the NO system before Ga1N intoxication significantly increased osmolality clearance the smaller dose of L-NAME. Inhibition of NOS after Ga1N did not significantly change the value of osmolality clearance. Values are means ±SE, significance – p<0.05.



**Figure 2.** Influence of L-ARG injection on creatinine clearance before and after liver injury. Activation of NO before liver injury did not change significantly level of GFR. However, L-ARG injection after Ga1N intoxication brought insignificant improvement of GFR in the smaller dose, and significant improvement in the larger dose of L-ARG. Values are means ±SE, significance – p<0.05.



**Figure 4.** Influence of L-ARG injection on osmolality clearance before and after liver injury. Activation of NO system before liver injury did not change the value of osmolality clearance. L-ARG injection after Ga1N intoxication increased osmolality clearance, but not significantly. Values are means ±SE, significance – p<0.05.

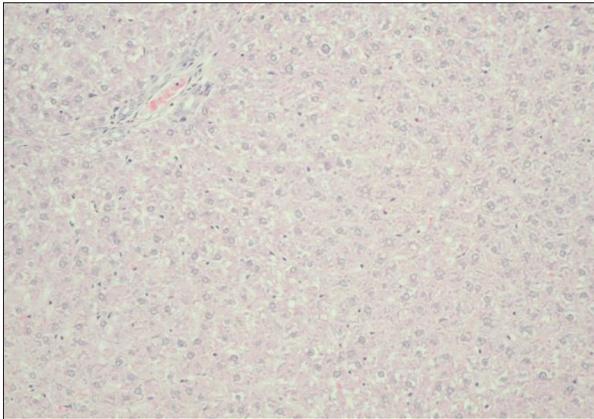
(Group 5 vs. Group 7). Urine osmolality was also significantly lower (p < 0.001) and 24-h diuresis was higher (not significantly). No difference was observed in osmolality clearance (Group 5 vs. Group 7, respectively).

**Stimulation of the NO pathway and water-electrolyte balance and renal failure**

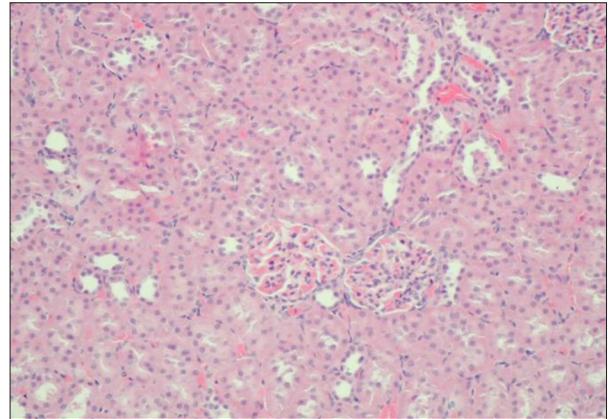
In comparison with the sham animals, L-ARG administration did not cause any significant changes in the hepatic or renal parameters. L-ARG administration at the smaller dose before

Ga1N injection did not affect any of the renal efficiency parameters and did not affect most of the hydro-electrolyte balance parameters. It did not affect the creatinine and urea concentrations, GFR, 24-h natriuresis, diuresis, or osmolality clearance; it only decreased urine osmolality (p<0.0017) (Group 9 vs. Group 2) (Table 2; Figures 2 and 4). Similarly, a double dose of L-ARG did not cause changes in the renal efficiency or water/sodium balance parameters (Group 10 vs. Group 2).

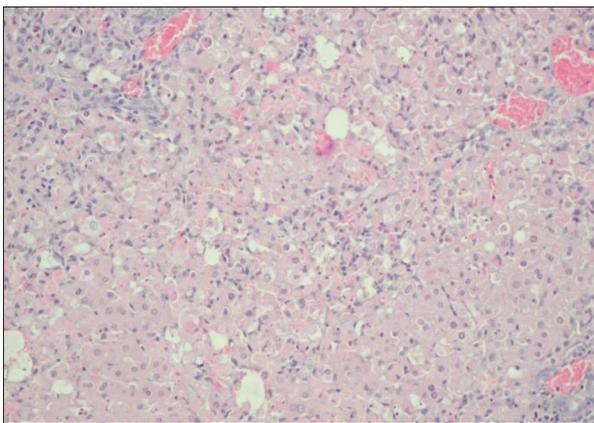
Stimulation of the endogenous NO pathway after Ga1N injection showed other effects. Administration of a smaller dose of L-ARG after Ga1N injection did not cause changes in the creatinine and urea concentrations; however, the creatinine increased 2-fold, although insignificantly (p<0.058) (Group 11 vs. Group 2)



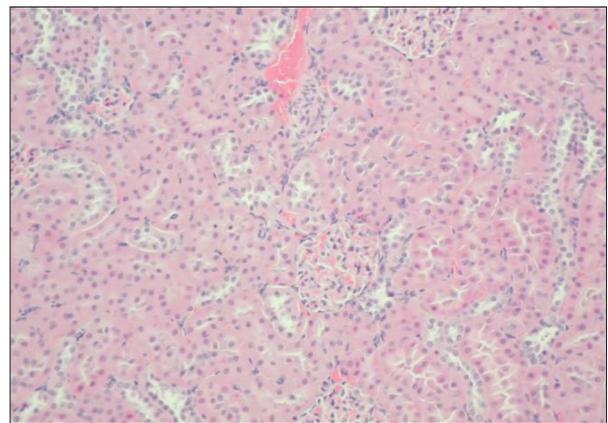
**Figure 5.** Histopathological picture of the liver in sham rats. Lack of necrosis of hepatocytes in the liver from sham SDR rats (Group 1) at 48 h after saline injection. Hematoxylin and eosin staining, light microscope, magnification  $\times 20$ .



**Figure 7.** Histopathological picture of the kidney in sham rats. Lack of morphological changes in the kidney from sham SDR rats (Group 1) at 48 h after saline injection. Hematoxylin and eosin staining, light microscope, magnification  $\times 20$ .



**Figure 6.** Histopathological picture of the liver in tested rats. Diffuse necrosis of hepatocytes in the liver from tested SDR rats (Group 2) at 48 h after galactosamine injection. Hematoxylin and eosin staining, light microscope, magnification  $\times 20$ .



**Figure 8.** Histopathological picture of the kidney in tested rats. Lack of morphological changes in the kidney from tested SDR rats (Gr. 2) at 48 h after galactosamine injection. Hematoxylin and eosin staining, light microscope, magnification  $\times 20$ .

(Table 2; Figure 2). Furthermore, there was a significant decrease in urine osmolality ( $p < 0.003$ ) and an increase in the 24-h diuresis ( $p < 0.006$ ), fraction of ejected sodium ( $p < 0.027$ ) (data not shown), and natriuresis ( $p < 0.056$ ), whereas there was no change in the osmotic clearance rates (Table 2; Figure 4). The double dose of L-ARG improved the creatinine clearance and 24-h sodium excretion ( $p < 0.018$  and  $p < 0.002$ , respectively) (Group 12 vs. Group 2). No differences in other renal function parameters or hydro-electrolyte balance parameters were observed.

## Discussion

Many different experimental HRS models were used in experimental studies; these included models of toxic liver injury

induced by  $\text{CCl}_4$ , ligation of bile tract, partial ligation of portal vein, and partial hepatectomy and galactosamine-intoxicated experimental animals [24–27]. Some of these were chronic HRS models in which liver failure and consequent renal failure develop within a few weeks or months. The remaining models were acute models in which the HRS symptoms occurred within a few hours or days. An example of such models is the galactosamine intoxication model, in which Ga1N has been intraperitoneally administered at a dose of 1.1 g/kg b.w. Ga1N inhibits the hepatic RNA synthesis through the formation of UDP hexosamines, thus leading to hepatocyte necrosis. The kidneys are intact both in histological and electron microscopy examination and a renal tubular cell line incubated with Ga1N did not show any nephrotoxic effect [23]. Within 24–48 h of Ga1N administration, acute liver injury, acute liver failure,

and the resultant acute functional renal failure develop in the experimental animals [22]. Liver injury and liver failure manifest by accumulation of bilirubin, aminotransferase, and ammonia and decrease in albumin levels. Renal failure characterizes an increase of urea and creatinine and a decrease of renal blood flow (RBF) and glomerular filtration rate (GFR). Decrease of RBF and GFR resulted from contraction of renal vessels, especially within the cortical vessels. Arterial blood pressure, effective plasma volume, and peripheral vascular resistance are lowered, thereby increasing cardiac output and leading to hyperkinetic circulation [28].

In the present study, acute HRS developed typically. Within 48 h of liver injury, the animals exhibited clinical and biochemical characteristics of acute liver failure and the resultant acute functional renal failure accompanied by decreases in 24-h diuresis, natriuresis, osmotic clearance, and urine concentration. Anand et al. [23] presented a model of acute liver and renal failure after Ga1N administration to Sprague-Dawley rats in a standard and repeated manner. The findings of our previous study [22] also showed that acute functional renal failure can occur as a result of acute liver injury and liver failure after Ga1N administration, despite the absence of any histopathological features of renal injuries. We showed there that the development and the grade of renal failure in experimental HRS models may depend on the genetics of the experimental animals [22].

The role of endogenous NO in HRS pathogenesis is well studied, but the majority of these studies were conducted using chronic HRS models, in particular, cirrhosis models [29–31]. Increased cGMP concentrations by the secondary messenger NO was reported in the mesenteric vessels of rats with portal hypertension and in cell lines incubated with the splanchnic vessels of these animals [32,33]. NO exerts a relaxing effect directly on vessels via an alternative pathway such as potassium channel [34]. Pizcuet et al. [35] and Casadevall et al. [36] confirmed that fast inhibition of NO synthesis in splanchnic vessels caused near total normalization of hemodynamic disorders (i.e., decrease of venous blood flow from portal vein and increase of resistance of splanchnic vessels). They proved that extended splanchnic vessels do not respond to the constrictive activity of any known vasoconstrictors; however, prior inhibition of endogenous NO synthesis in animals with portal hypertension restores the original constrictive property of the splanchnic vessels [37,38]. Total removal of vascular endothelium (a source of NO biosynthesis) repairs impaired vascular reactivity to the above-mentioned vasoconstrictors [39]. In a recent study, Sharma et al. [40] showed that the total NO production in the first hours of acute liver ischaemia in large experimental animals is similar to that in the sham group. However, this finding was based on models of acute liver failure, not typical HRS, observed only during the first 6 h after induction of liver

injury; moreover, the animal species used in that study was different from those used in our study [40].

In our study, in an acute model of HRS, after Ga1N intoxication of rats, the kidneys preserved the capability to concentrate urine and to retain sodium and other osmotically active substances. Inhibition of endogenous NO synthesis increased 24-h sodium excretion and 24-h diuresis significantly after Ga1N administration of the NOS inhibitor in a dose-dependent manner. Furthermore, inhibition of the NO system led to improvement in renal efficiency (also in dose-independent manner); this improvement was more pronounced when L-NAME was administered before liver injury.

Disorders of water-electrolyte balance, especially water and sodium excretion, are typical for HRS [41]. Enhanced sodium and water retention mediated by activation of the sympathetic, renin-angiotensin, and arginine-vasopressin systems results mainly from decreased effective plasma volume in systemic circulation [42]. Excessive sodium retention is a result of increased tubule re-absorption due to stimulation of angiotensin, aldosterone, and  $\alpha_1$ -adrenergic receptors [43]. Decreased excretion of water results from increased secretion of antidiuretic hormone and increased renal synthesis of prostaglandin [44]. Atucha et al. [45] showed that inhibition of NO synthesis in a cirrhosis model increased 24-h diuresis and 24-h natriuresis. However, Martin et al. [46] showed that chronic inhibition of NOS led to increase in 24-h sodium excretion and 24-h urine volume and normalization of decreased blood sodium concentration and serum osmolality. Our findings are in concordance with those of Martin et al., but they are probably the first in the available literature to consider the influence of the NO system on hydro-electrolyte balance in an acute HRS model. Inhibition of NO synthesis increased 24-h natriuresis, 24-h diuresis, and the fractional excretion of sodium and free water clearance. Therefore, a change in the hemodynamic conditions as a result of inhibition of endogenous NO system led to improvement of water and sodium balance in this experimental model.

The role of endogenous NO in renal failure in HRS has been determined only in cirrhosis models [47,48]. Ros et al. studied rats with cirrhosis and showed that inhibition of the NO system influenced RBF and increased GFR [49]. In contrast, Atucha et al. [45] and Martin et al. [50] did not observe any significant changes in GFR following NO inhibition in cirrhosis models. In addition, Atucha et al. showed that the inhibitory effect is dependent on the time of administration of the NOS inhibitor, but not on the dose. We believe our study is probably the first to use a model of acute liver injury. We showed that although the improvement in renal function was dose-independent, inhibition of NO synthesis significantly improved glomerular filtration, and administration of the NOS inhibitor

before liver injury results in better improvement of GFR than treatment after.

On the other hand, activation of the endogenous NO system increased 24-h sodium excretion and 24-h diuresis in Ga1N-intoxicated animals independently of the dose of the NO system donor. Furthermore, NO system activation after liver injury by using a small dose of the donor slightly increased renal efficiency, while administration of twice the original dose significantly increased the GFR. Importantly, L-ARG administered at the same time did not change the concentration of bilirubin and ALT; therefore, it had no influence on degree of liver injury.

All previous studies on the use of an NO donor and its influence on renal function in HRS were conducted using models of cirrhosis. *In vitro* studies by Zhang et al. [51] and Kawada et al. [52] showed that the activation mechanism of the NO system completely restored the reactivity of intrahepatic vessels to the constrictive activity of endothelin-1 and activated stellate cells. However, in models of portal hypertension and cirrhosis, no clear results were obtained [45,53]. This is probably associated with decreased number as well as change in the structure of endothelium cells of the sinusoid vessels of cirrhotic liver, where endothelial NO is synthesized. As a result of the above-mentioned changes, especially because of reduction in endothelial cell numbers and sinusoid vessel stenosis by the regeneration nodes of cirrhotic liver, the endothelial cells of the sinusoid vessels cannot effectively synthesize NO [54]. In our model of acute liver injury induced by Ga1N administration, activation of NO synthesis using L-ARG improved renal efficiency in a dose-dependent manner. In our model of acute liver injury, rapid hepatocyte necrosis did not alter the structure of hepatic tissue, which provided NO synthesis in intact endothelium cells of sinusoid vessels. Therefore, by using a NO donor, NO synthesis could be restored within the sinusoid vessels, which resulted in decreased total intrahepatic vascular resistance, decreased pressure in the splanchnic vascular

bed, peripheral hemodynamic changes, and improvement of glomerular filtration. Further studies are required to fully understand the mechanism underlying these findings. However, our results prove that L-ARG may improve renal function in a model of acute HRS.

## Conclusions

Our study showed that inhibition of the endogenous NO system increased osmotic clearance, 24-h diuresis, and natriuresis and this effect was stronger when the NOS inhibitor was administered before liver injury. Furthermore, administration of the NOS inhibitor improved GFR in a dose-independent manner. In this case, pre-treatment also turned out to be better than treatment after liver injury.

On the other hand, activation of the NO system increased 24-h diuresis and 24-h natriuresis independently of the dose of the NO system donor, but this effect was better when the donor was administered after liver injury. Finally, activation of the NO system only after liver injury improved GFR in dose-independent manner.

The nitric oxide pathway plays a significant role in development of water-electrolyte disorders and acute renal failure in the course of acute liver injury and liver failure.

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## Conflicts of interest

The authors declare no conflict of interest.

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