The role of N-acetylcysteine in preventing hepatic injury associated with systemic oxidative stress after extracorporeal shock wave treatment

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Abstract

Background. Systemic oxidative stress may cause detrimental consequences for the liver, leading to hepatic fibrogenesis.

Objectives. To investigate histopathological changes in liver tissues due to the increased systemic oxidative stress associated with rat extracorporeal shock wave lithotripsy (SWL) model and to document the consequences of N-acetylcysteine (NAC) administration.

Material and methods. In this experimental SWL model, 18 Wistar albino rats were randomly assigned into 3 groups. The control group (group I) had no intervention. Group II underwent SWL treatment with intraperitoneal saline injection. Group III also had SWL with intraperitoneal NAC and was divided into short-term (group III-14 days) and long-term (group III-28 days) subgroup. Hepatectomy was performed for histopathological examinations. Histopathological alterations were evaluated with light microscopy. Immunohistological staining for p53 and myeloperoxidase was also performed.

Results. Blood samples revealed a significant increase in plasma oxidative stress index (OSI) after plasma total antioxidant status (TAS) and total oxidant status (TOS) had been measured. It was shown that this increased systemic oxidative stress adversely affected liver tissues. Predominantly, sinusoidal dilatation was remarkably observed in rats with significantly high OSI values ($p = 0.043$). Similarly, periportal necrosis significantly increased in rats with high OSI values ($p = 0.033$). p53 positivity was also remarkable in rats with systemic oxidative stress ($p = 0.049$). N-acetylcysteine administration provided a significant decrease in OSI. N-acetylcysteine also improved all these alterations, including p53 staining. Particularly, sinusoidal dilatation was significantly protected in the long-term NAC group (group III-28 days).

Conclusions. We demonstrated that SWL-induced systemic oxidative stress causes histological alterations in liver tissues. Increased p53 and myeloperoxidase staining as markers of oxidative damage were also detected. N-acetylcysteine may protect from these histological and ultra-structural alterations related to oxidative stress.

Key words: liver, oxidative stress, N-acetylcysteine, sinusoidal dilatation, p53

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Introduction

Systemic production of oxidative stress may produce detrimental consequences for several organs, particularly the liver. For instance, oxidative stress has long been considered a key driving factor of many obesity-related liver diseases. Non-alcoholic fatty liver disease is thought to be associated with obesity and oxidative stress; as a result, antioxidants seem to be one of the most promising pharmacologic treatment methods.[1] In a mouse model of non-alcoholic fatty liver disease and fibrosis, altered regulation of lipid metabolism, inflammation, fibrosis, and oxidation-associated expression, along with augmented lipoperoxidation have been observed.[2] Furthermore, administration of *Crepidiastrum denticulatum* extract and its active compound chicoric acid reduced oxidative stress by upregulating antioxidant enzymes and decreasing inflammation by inhibiting pro-inflammatory cytokines and nuclear factor kappa B (NF-κB) activation. Also, oxidative stress has been postulated to play a role in the development of bisphenol A-induced liver fibrosis.[3] It is clear that oxidative stress induces the activation of liver fibrogenic cells, thus promoting the expression of fibrosis-related genes, leading to hepatic fibrogenesis.[4] Therefore, the production of systemic oxidative stress markers may adversely affect liver tissue.

It was clearly observed that extracorporeal shock wave lithotripsy (SWL) used for the management of renal stones can cause systemic oxidative stress. An experimental SWL model on 69 rats clearly revealed a significant increase of malondialdehyde (MDA) levels and a decrease of superoxide dismutase activity (SOD) in blood as markers for oxidative stress.[5] In the previous report of this experimental SWL model, a significant increase in plasma oxidative stress index (OSI) was detected through measuring plasma total antioxidant status (TAS) and total oxidant status (TOS) in blood samples of rats.[6] Some histological alterations in renal tissues were reported. In this section of the study, the possible effects of systemic oxidative stress on liver tissues were investigated. Also, as a universal antioxidant, N-acetylcysteine (NAC) can prevent apoptosis and promote cell survival by activating the extracellular signal-regulated kinase pathway.[7] It is also proposed that NAC can modify DNA and may also have a preventive role for several processes, such as reducing endothelial dysfunction, inflammation, fibrosis, and invasion. The effects of NAC on liver tissue under systemic oxidative stress were also monitored in this experimental study.

The objectives of this study were to explore the effects of SWL associated systemic oxidative stress on liver tissues and to detect the possible protective role of NAC administration.

Material and methods

Study design and groups

A total of 18 female Wistar albino rats all aged 12 weeks with a range of weight of 175–250 g were used. Animals were housed in cages with a maximum of 9 rats. The animals were kept at a temperature range of 18–20°C and a 12 h/12 h light/dark cycle.

The groups were randomly constructed among animals used in the previous study.[6] Animals were arbitrarily assigned to 3 groups stratified by weight. Group III was further divided into 2 subgroups – short-term (group III-14 days) and long-term (group III-28 days). Group I (3 rats) constituted the control animals without any SWL and NAC. Group II (5 rats) underwent SWL and received intraperitoneal saline at a dose of 1 mL/kg/day until hepatectomy as the placebo treatment. Group III (10 rats) also underwent SWL, and these rats received intraperitoneal NAC at a dose of 300 mg/kg/day for 14 (short-term subgroup, 5 rats) or 28 days (long-term subgroup, 5 rats).

The control group had no intervention at all. Rats in groups II and III received a total of 2000 shock waves that were applied to the left kidney with an amplitude of 18 kV and a rate of 60 SW/min (Stonelith-V5 Lithotripter; PCK Medical Systems, Ankara, Turkey) under general anesthesia with ketamine HCl (1 mg/kg) and xylazine HCl (10 mg/kg).

All the animals received humane care according to the criteria outlined in the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23 revised 1985; National Institutes of Health, Bethesda, USA). The study was approved by the Local Animal Ethics Committee. According to the strict restrictions of the ethical committee, the animal number in each group was restricted to a minimum enabling statistical analysis.

Procedure

Blood samples and liver tissues were obtained. The blood samples were centrifuged at 12,000 rpm at 4°C for 10 min and then stored at 80°C for biochemical tests. The OSI was calculated by measuring plasma TAS and TOS using a novel, colorimetric and fully automated method for measuring total antioxidant response against potent free radical reactions.[8] The tests were performed using an auto-analyzer (Beckman Coulter AU480; Beckman Coulter, Brea, USA) by using appropriate kits (Rel Assay Diagnostics, Gaziantep, Turkey). The TAS results were expressed as mmol Trolox Eqiv./L, while TOS values were expressed as μmol H_2O_2 Eqiv./L. The OSI was calculated using the formula as OSI [AU] = TOS [μmol H_2O_2 Eqiv./L]/TAS [μmol Trolox Eqiv./L].

The final procedure was performed on the 14th day in group I, group II, group III-14 days. The remaining rats

(group-III-28 days) underwent surgery on the 28th day. A midline laparotomy was performed, and blood samples were collected from the vena cava using syringe for biochemical analyses. Hepatectomy was performed, and the specimens were fixed in formalin solution, embedded in paraffin and stained with hematoxylin and eosin (H&E) for microscopic examination to document histological changes. Myeloperoxidase expression and p53 were assessed with immunohistochemistry.

The histological investigation was evaluated by a single pathologist. Five histological alterations were graded to score any hepatic injury. These parameters were sinusoidal dilatation, periportal necrosis, steatosis, cellular necrosis, and inflammatory cell infiltration. Furthermore, p53 and myeloperoxidase staining were also classified. A scoring system was separately used for all these parameters as 0 (normal morphology or no staining), 1 (mild), 2 (moderate), and 3 (severe). The mean score was calculated for each group.

Statistical analysis

Non-parametric Kruskal–Wallis and Dunn's statistical tests were used for analysis. The value of $p < 0.05$ was considered statistically significant. The statistical calculations were performed using SPSS Statistics v. 18 (SPPS Inc., Chicago, USA).

Results

Oxidative stress

The comparison of the groups clearly demonstrated that the application of renal SWL developed a systemic oxidative response (Fig. 1). Shock wave lithotripsy caused a significant rise in median TOS values from 11.12 μmol H_2O_2Eqiv./L in the control group (group I) up to 15.38 μmol H_2O_2Eqiv./L in rats which received SWL (group II) ($p < 0.05$). N-acetylcysteine administration for 14 days (group III-14 days) provided a remarkable decrease in TOS (8.58 μmol H_2O_2Eqiv./L) ($p = 0.027$). This improvement in TOS was maintained (10.92 H_2O_2Eqiv./L) on the 28th day (group III-28 days) without further decrease. As a similar trend, antioxidant status (TAS) showed a significant alteration after SWL from 1.19 μmolTroloxEqiv./L (group I) down to 0.96 μmolTroloxEqiv./L (group II) ($p = 0.006$). N-acetylcysteine administration provided an increase in TAS values on the 28th day ($p < 0.05$). As a result of these measurements, OSI demonstrated a significant rise with SWL procedure ($p < 0.05$). N-acetylcysteine administration provided a significant decrease in OSI on the 14th day ($p = 0.013$), which continued on the 28th day.

Histological findings

Sinusoidal dilatation was found to be prominent in rats receiving SWL (Table 1). While the rats in the control group had no sinusoidal dilatation at all, SWL group had a mean score of 1.17 for this parameter (Fig. 2, $p < 0.05$). This histological alteration was similarly observed in the short-term NAC group. However, the mean score decreased to 0.33 in the 28 days in the NAC group ($p = 0.043$). Similarly, periportal necrosis was prominent in group II with SWL. It was not observed in the control group (Fig. 3). The mean score was 1 in the SWL group, and this value was decreased to 0.33 both in group III-14 days and group III-28 days ($p = 0.033$). The other histological markers were similar to the control group.

With regard to the immunohistochemical staining, p53 positivity was prominent in the SWL group (Table 2, Fig. 3). The mean score was increased from 0 to 1 in control and SWL groups, respectively ($p = 0.049$). This staining return control values with NAC administration.

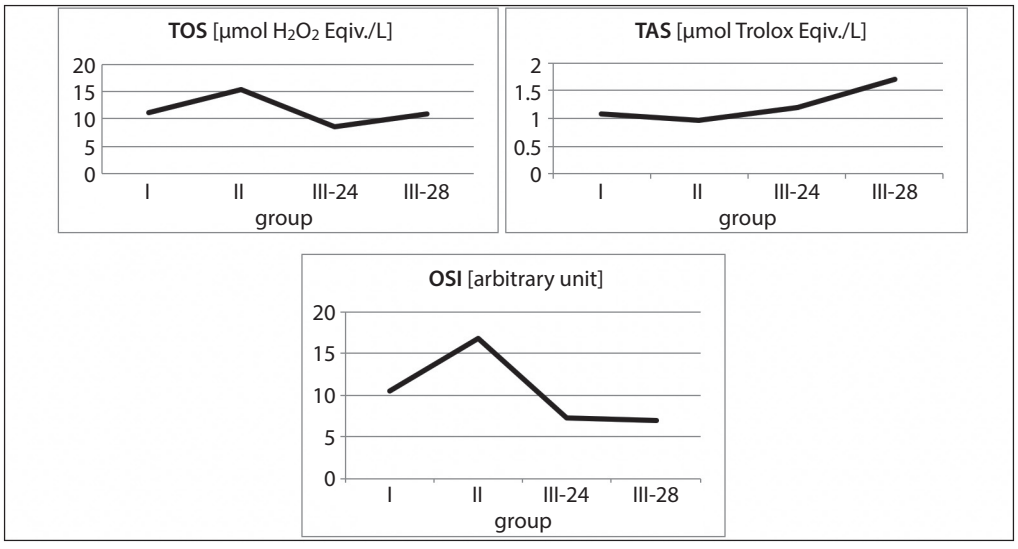

Fig. 1. The comparison of median systemic oxidative markers

group I – control group;
group II – SWL + saline;
group III – SWL + NAC 14-28 days;
TOS – total oxidant status;
TAS – total antioxidant status;
OSI – oxidative stress index;
SWL – extracorporeal shock wave lithotripsy;
NAC – N-acetylcysteine.

Table 1. Histological alterations in liver tissue

Variable	Group	n	Mean	Median	SD	Min	Max	p-value
Sinusoidal dilatation	I	3	0.00	0.00	0.37	0	1	0.043
	II	5	1.17	1.00	0.408	1	2	
	III-14 days	5	1.17	1.00	0.753	0	2	
	III-28 days	5	0.33	0.00	0.516	0	1	
Periportal necrosis	I	3	0.00	0.00	0.37	0	1	0.033
	II	5	1.00	1.00[a]	0.000	1	1	
	III-14 days	5	0.33	0.00[b]	0.516	0	1	
	III-28 days	5	0.33	0.00[b]	0.516	0	1	
Steatosis	I	3	0.00	0.00	0.000	0	0	1.000
	II	5	0.00	0.00	0.000	0	0	
	III-14 days	5	0.00	0.00	0.000	0	0	
	III-28 days	5	0.00	0.00	0.000	0	0	
Cellular necrosis	I	3	0.00	0.50	0.548	0	1	0.072
	II	5	0.50	0.50	0.548	0	1	
	III-14 days	5	1.17	1.00	0.408	1	2	
	III-28 days	5	0.50	0.50	0.548	0	1	
Inflammatory cell infiltration	I	3	0.00	0.00	0.00	0	00	0.084
	II	5	0.83	1.00	0.753	0	2	
	III-14 days	5	1.50	1.50	0.548	1	2	
	III-28 days	5	0.67	1.00	0.516	0	1	

group I – control group; group II – SWL + saline; group III – SWL + NAC; SD – standard deviation; SWL – extracorporeal shock wave lithotripsy; NAC – N-acetylcysteine; [a], [b], [ab] – different index letters indicate statistically significance for each column.

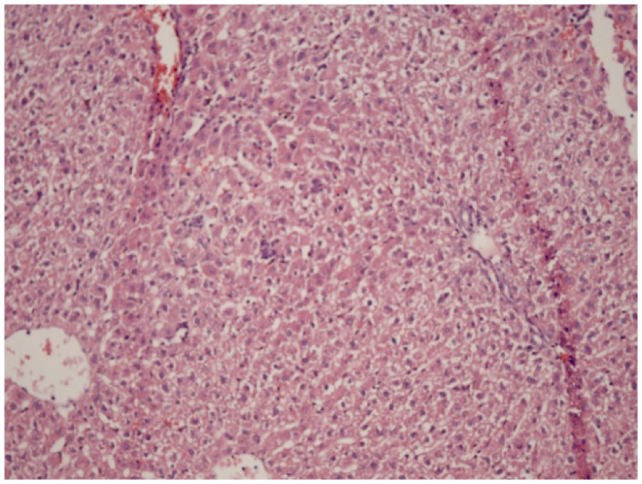

Fig. 2. Mild (grade 1) sinusoidal dilatation and lymphocyte infiltration (H&E staining, ×200 magnification)

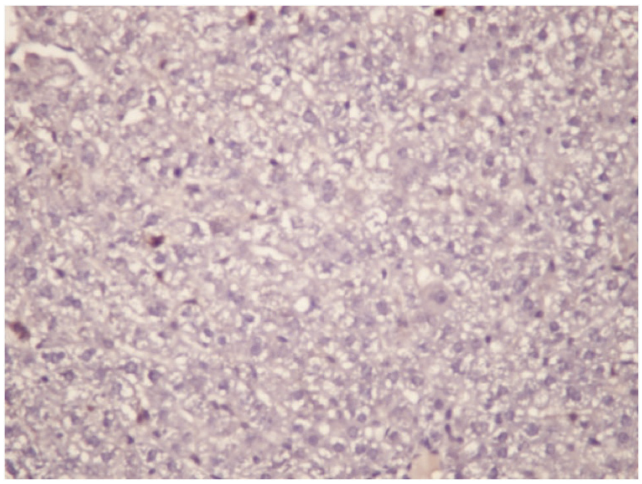

Fig. 3. Moderate (grade 2) periportal necrosis and nuclear p53 staining (H&E staining, ×400 magnification)

Although the expression of myeloperoxidase was higher in the SWL-treated rats (group II and III), the difference was not significant.

Discussion

The liver is first of all the principal detoxifying organ, acting in the clearance of waste products and toxic chemicals from the body. It also participates in almost all physiologic systems to maintain homeostasis.[9] Continuous exposure of the liver to adverse agents or conditions can cause hepatic injury. These events can then lead to inflammation and liver degeneration.

Shock wave lithotripsy remains a widely applied treatment modality for the majority of patients with renal stones.[10] However, several clinical and experimental models have clearly demonstrated that SWL is associated with increased systemic oxidative stress due to renal ischemia/reperfusion injury. A remarkable increase of MDA

Table 2. Results of immunostaining for p53 and myeloperoxidase in liver tissues

Variable	Group	n	Mean	Median	SD	Min	Max	p-value
p53	I	3	0.00	0.00[b]	0.00	0	1	0.049
	II	5	1.00	1.00[a]	0.632	0	2	
	III-14 days	5	0.33	0.00[b]	0.516	0	1	
	III-28 days	5	0.17	0.00[b]	0.408	0	1	
Myeloperoxidase	I	3	0.00	0.00[b]	0.00	0	1	0.533
	II	5	0.50	0.50	0.548	0	1	
	III-14 days	5	0.33	0.00	0.516	0	1	
	III-28 days	5	0.67	1.00	0.516	0	1	

group I – control group; group II – SWL + saline; group III – SWL + NAC; SD – standard deviation; SWL – extracorporeal shock wave lithotripsy; NAC – N-acetylcysteine; [a], [b], [ab] – different index letters indicate statistically significance for each column.

levels and a decrease of SOD in blood as markers for oxidative stress after SWL was documented in the rabbit SWL model.[5] Furthermore, it was postulated that astragalosides as a novel antioxidant agent can prevent shock wave-induced renal oxidative injury.[5] A well-designed clinical trial on 120 patients receiving SWL showed that oral antioxidant administration is associated with reduced mean serum concentration of MDA, higher levels of serum ascorbic acid and serum albumin, lower alpha-tocopherol/cholesterol ratio, and lower urinary albumin and β2 microglobulin levels.[11] The authors suggested that SWL generates free radicals through ischemia/reperfusion injury mechanism, and oral administration of antioxidants may have a protective role.[11] Similarly, SWL caused a significant rise in MDA, urine N-acetyl-beta-glucosaminidase (NAG) activity, and uric acid and white cell counts as systemic markers of oxidative stress.[12] A clinical observation also showed elevated plasma and urinary nitric oxide (NO) levels after SWL.[13] Plasma and urinary MDA levels were also remarkably elevated after SWL. Another clinical trial concluded that lipid peroxidation might be induced, and antioxidative defense mechanisms may be transiently impaired in erythrocytes after SWL, as a model of systemic consequences of SWL-induced oxidative stress.[14] All these studies indicate a systemic oxidative process after SWL. The initial results of this study also showed that SWL caused a decrease in TOS levels with subsequently higher TAS levels, indicating a remarkable increase in systemic oxidative stress index in blood samples.[6] This study also showed that NAC administration for 14 days provided a remarkable decrease in TOS, indicating a protective efficacy against oxidative stress.

An interesting finding in this trial is that systemic oxidative response after SWL is associated with some histological alterations in liver tissues. A major observation revealed that systemic oxidative stress caused a significant sinusoidal dilatation in rats receiving SWL. In the control group, no sinusoidal dilatation at all was seen. N-acetylcysteine administration has a protective role in terms of preventing sinusoidal dilatation in rats having NAC, particularly with longer administration. Alterations in hepatic sinusoids are regarded as a critical point in the development of certain liver diseases, including cirrhosis and portal hypertension.[15] It was shown that the hepatic sinusoids are the essential component of intrahepatic microcirculatory unit, where cells are intimately associated with one another and communicate through paracrine and autocrine effects.[16] Therefore, alterations in this microenvironment are related to the early steps of fibrogenesis and include sinusoidal remodeling, vasoconstriction, endothelial dysfunction, and angiogenesis.[17] Therefore, documentation of sinusoidal dilatation associated with increased oxidative status should be regarded as an important finding in this experimental study. Moreover, improvement in the sinusoidal dilatation with NAC administration indicates a possible relationship between oxidative stress and sinusoidal dilatation. It is postulated that oxidative stress plays an important role in the pathogenesis of non-alcoholic steatohepatitis and is likely involved in the progression of the disease from steatosis to non-alcoholic steatohepatitis and potentially cirrhosis.[17] In an animal model, the levels of total glutathione (GSH) and hepatic MDA were found to be increased significantly in rats with non-alcoholic steatohepatitis group.[18] It was reported that the administration of 20 mg/kg/day of oral NAC improved the level of GSH as attenuation of oxidative stress. Authors also observed a decrease in fat deposition and necroinflammation, indicating healing in liver histology caused by NAC. Similarly, periportal necrosis was also prominent in rats with increased oxidative stress after SWL. This was not observed in the control group. N-acetylcysteine also has a protective effect against developing periportal fibrosis. Periportal fibrosis is a recognized histological step in non-alcoholic steatohepatitis.[19,20]

With regard to the immunohistochemical staining, p53 positivity was prominent in the SWL group. This staining return control values with NAC administration. Silver nanoparticles administration caused a remarkable increase in the levels of MDA and total glutathione in adult

zebrafish.[21] Moreover, the mRNA levels of the oxyradical-scavenging enzymes catalase and glutathione peroxidase 1a were reduced. They also showed DNA damage by the expression of p53 protein in liver tissues. Similarly, lead significantly increased the levels of reactive oxygen species (ROS) and MDA in mice.[22] Also, severe DNA damage was obviously observed as an increased expression of p53. Therefore, all these studies including the current trial confirm that increased p53 staining may indicate oxidative stress related to ultrastructural changes in the liver. Although the expression of myeloperoxidase was higher in the SWL treated rats, the difference was not significant. The activity of this enzyme is also known to be a marker for oxidative stress.[23] The increased expression of this marker also suggests oxidative stress-related alterations in the liver.

A recent review clearly states that NAC, due to its antioxidant and anti-inflammatory roles, has important functions for liver diseases.[24]

It was shown that NAC can attenuate markers of inflammation and oxidative stress in hepatic damage. The results of both experimental and clinical trials show that supplementation of NAC in any form of administration and type of study is mostly satisfactory with promising endpoints. The current trial also confirms the protective role of NAC in liver tissue related to oxidative damage. However, clinical studies are urgently required to have a routine clinical utilization of NAC to prevent oxidative damage in liver tissue.

Conclusions

The current study showed that SWL causes systemic oxidative stress as expressed by a remarkably increased plasma oxidative stress index in blood samples of rats. The increased oxidative stress was shown to be associated with sinusoidal dilatation and periportal fibrosis in liver tissues. The expression of p53 and myeloperoxidase as immune stained markers of oxidative damage were also increased in the liver. Moreover, in this study, NAC was found to be effective in decreasing oxidative stress and in improving these histological and ultrastructural alterations. This experimental model provides important background for subsequent clinical trials on the protective role of NAC for liver diseases.

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