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The Efficacy of N-acetylcysteine Against Renal Oxidative Stress After Extracorporeal Shock Wave Treatment: An Experimental Rat Model

N-asetilsisteinin Ekstrakorporeal Şok Dalga Tedavisi Sonrasında Oluşan Renal Oksidatif Stres Hasarına Karşı Etkinliği: Deneysel Rat Modeli

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What's known on the subject? and What does the study add?

Current study confirmed that shock wave lithotripsy (SWL) causes oxidative stress as expressed by a remarkably increased plasma oxidative stress index in blood samples of SWL-treated rats as the first time in literature. SWL also produced acute renal damage as tubular damage and interstitial inflammation. N-acetylcysteine (NAC) was found to be effective in decreasing SWL-induced oxidative stress and also may protect to some extend certain histological alterations. This experimental model provides an important background for subsequent clinical trials on protective role of NAC in patients receiving SWL.

Abstract

Objective: To evaluate effects of renal extracorporeal shock wave lithotripsy (SWL) on plasma Oxidative Stress index (OSI) and to observe histopathological alterations in an experimental model. Secondly, protective role of N-acetylcysteine (NAC) was investigated.

Materials and Methods: A total of 24 rats were randomly divided into 3 groups as control (group 1), SWL + saline (group 2), and SWL + NAC (group 3). Study groups were further divided into two subgroups as short-term and long-term. In groups 2 and 3, 2000 shock waves were applied. Intraperitoneal saline was administered in group 2, and intraperitoneal NAC was given to group 3. No treatment was administered to group 1. Blood samples and nephrectomy specimens were obtained for biochemical and histopathological examinations, respectively. OSI was calculated by measuring plasma total antioxidant status (TAS) and total oxidant status (TOS). Acute and chronic histopathological damage were evaluated by light microscopy.

Results: SWL caused a remarkable increase in oxidative stress. Strikingly, TOS levels were significantly lower (p=0.027) and TAS levels were significantly higher (p=0.006) in rats with SWL + NAC (group 3). As a result, OSI was lower (p=0.013). This effect was particularly significant in the short-term subgroup. It was also concluded that tubular damage and interstitial inflammation were higher in the SWL group (p=0.022). These acute histological alterations were slighter in rats with NAC.

Conclusion: The current study demonstrated that SWL can cause severe oxidative stress and acute renal damage by increasing free oxygen radical production. NAC was effective in decreasing SWL-induced oxidative stress and preventing certain histological alterations to some extent.

Keywords: Extracorporeal shock wave lithotripsy, Kidney, Oxidative stress, Rat

Öz

Amaç: Bu çalışmanın amacı deneysel bir modelde renal ekstrakorporeal şok dalga litotripsisinin (ESWL) plazma Oksidatif Stres indeksi (OSİ) üzerindeki etkilerini değerlendirmek, histopatolojik değişiklikleri gözlemlemek ve N-asetilsisteinin (NAC) koruyucu rolünü araştırmaktır.

Gereç ve Yöntem: Toplam 24 rat rastgele 3 gruba; kontrol (grup 1), SWL + salin (grup 2) ve SWL + NAC (grup 3) olarak ayrıldı. Çalışma grupları ayrıca kısa ve uzun süreli olarak iki alt gruba ayrıldı. Grup 2 ve 3'te 2000 şok dalga ESWL uygulandı. Grup 2'de intraperitoneal salin ve grup 3'e

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intraperitoneal NAC verildi. Grup 1'e tedavi uygulanmadı. Biyokimyasal ve histopatolojik incelemeler için sırasıyla kan örnekleri ve nefrektomi örnekleri alındı. OSİ, plazma toplam antioksidan seviyesi (TAS) ve toplam oksidan seviyesi (TOS) ölçülerek hesaplandı. Akut ve kronik histopatolojik hasar ışık mikroskobu ile değerlendirildi.

Bulgular: ESWL, oksidatif streste belirgin bir artışa neden oldu. Grup 3'teki ratlarda TOS düzeyleri anlamlı olarak düşük (p=0,027), TAS seviyeleri anlamlı derecede yüksek saptandı (p=0,006). Sonuç olarak, OSİ daha düşük saptandı (p=0,013). Bu etki kısa süreli alt grupta daha anlamlıydı. Ayrıca SWL grubunda tübüler hasar ve interstisyel enflamasyonun daha yüksek olduğu sonucuna varıldı (p=0,022). Bu akut histolojik değişiklikler NAC verilen ratlarda daha az saptandı.

Sonuç: Mevcut çalışma, ESWL'nin serbest oksijen radikal üretimini artırarak şiddetli oksidatif stres ve akut böbrek hasarına neden olabileceğini göstermiştir. NAC, ESWL'nin neden olduğu oksidatif stresi azaltmada ve bazı histolojik değişikliklerin oluşumunu engellemede etkili olduğu görülmüştür.

Anahtar Kelimeler: Ekstrakorporeal şok dalga litotripsisi, Böbrek, Oksidatif stres, Sıçan

Introduction

Urinary Stone disease represents a major common health problem all over the world. There have been remarkable changes in the indications for surgical treatment as a result of the technological development in endourologic instruments (1). Nevertheless, extracorporeal shock wave lithotripsy (SWL) remains the initial treatment modality for the majority of patients with renal stones. Moreover, current guidelines suggest that SWL remains the least-invasive procedure for stone management in children (2). Therefore, long-term tissue effects of SWL are needed to be investigated despite its long-term proven efficacy. There have been several reports proposing that SWL causes oxidative stress due to renal ischemia-reperfusion injury (3,4,5). Furthermore, some agents have been tested to prevent SWL-induced renal oxidative stress (6,7).

N-acetylcysteine (NAC) is a well known antioxidant (8). NAC inhibits activation of c-Jun N-terminal kinase, p38 MAP kinase and redox-sensitive activating protein-1 and nuclear factor kappa B transcription factor activities regulating expression of numerous genes. Furthermore, NAC can prevent apoptosis and promote cell survival by activating extracellular signal-regulated kinase pathway (8). It is also proposed that NAC can modify DNA. Thereby, NAC has a preventive role in several processes as reducing endothelial dysfunction, inflammation, fibrosis, and invasion.

The objective of this experimental study was to investigate the effects of SWL on plasma oxidative stress, and to observe possible histopathological alterations in rat kidneys. Moreover, we aimed to detect the protective role of NAC administration during SWL treatment.

Materials and Methods

Animals

All experiments in this study were performed in accordance with the Universal Declaration of Animal Rights (Paris, 1978) and were approved by the Local Animal Ethics Committee (10-01/03). Twenty-four female Wistar-Albino rats all aged 12 weeks with a range of weight of 175-250 g were used in the present study. The animals were housed in cages with 9 rats at maximum. The animals lived at a temperature range from 18°C to 20°C and a 12 h light and dark cycle. Sample size estimates were based on data from previous studies (9).

Experimental Groups and Procedures

The animals were randomly assigned to three groups according to weight by using simple randomization method (Figure 1). Study groups (2 and 3) were further randomly divided into two subgroups as short-term (14 days) and long-term (28 days). Group 1 (6 rats) constituted control animals without any SWL and NAC. Group 2 (9 rats) underwent SWL and received intraperitoneal saline at a dose of 1 mL/kg/day for 14 (short-term) or 28 days (long-term) as placebo treatment. Group 3 (9 rats) also underwent SWL, and these rats received intraperitoneal NAC (Asist®, Husnu Arsan, Turkiye) at a dose of 300 mg/kg/day for 14 (short-term) or 28 days (long-term).

Control group had no intervention at all. In groups 2 and 3, contrast material was administered through an intravenous catheter that was placed in the rat tail vein, and a collecting system was visualized under fluoroscopy with general anesthesia by administrating 1mg/kg intramuscular injection of ketamine HCl (Rompun®, Bayer, Germany) and xylazine HCl (Ketalar®, Eczacibasi, Turkiye) (10 mg/kg). The anesthetized rats were then fixed at the thorax and hip in the supine position on the platform of the lithotripter. This maneuver allowed direct entry of the shock waves through the abdominal wall into the left

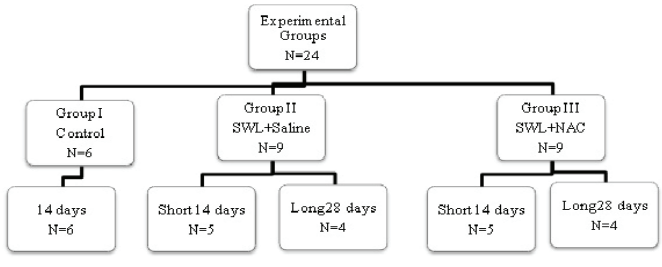

Figure 1. Study flowchart

kidney tissue. Then, the lower pole of the left kidney was treated after it was localizated using fluoroscopy. A total of 200 shock waves were applied to the left kidney with an amplitude of 18 kV and a rate of 60 SW/minute (PCK Stonelith-V5 Lithotripter, Ankara, Turkiye) at a single setting.

The final procedure was performed on the 14th day following SWL in the short-term subgroups and on the 28th day following SWL in the long-term subgroups, respectively. The rats in the group 1 were also operated on the 14th day. A midline laparotomy was performed, and blood samples were collected from the vena cava by syringe for biochemical analyses. After the kidneys had been excised, they were longitudinally bisected; one hemisection was fixed in formalin solution, embedded in paraffin and stained with hematoxylin-eosin (H&E) for microscopic examination.

The blood samples were centrifuged at 12000 rpm at 4°C for 10 min., and then stored at 80°C for biochemical tests. The Oxidative Stress index (OSI) was calculated by measuring plasma total antioxidant status (TAS) and total oxidant status (TOS) by using a novel, colorimetric and fully automated method for measuring total antioxidant response against potent free radical reactions as described by Erel (10,11). The tests were performed by an auto-analyzer (Beckman Coulter AU480, Japan) by using appropriate kits (Rel Assay, Turkiye). TAS results were expressed as mmol Trolox Eqiv./L, while TOS values were expressed as µmol H2O2 Eqiv./L. OSI was calculated by using the formula OSI (arbitrary unit) = TOS (µmol H2O2 Eqiv./L)/TAS (µmol Trolox Eqiv./L).

Histological investigation that was evaluated by the same pathologist involved acute and chronic damage. Acute renal damage was evaluated by 4 parameters: 1. tubular damage, 2. interstitial inflammation and hemorrhage, 3. dilatation-congestion in the glomerular and vascular structures, and 4. increase in inflammatory cells. A scoring system was separately used for these histological parameters as "0 (normal morphology)", "1 (mild)", "2 (moderate)" and "3 (severe)". The evaluation was made semiquantitatively. In each preparation, 30 sites were evaluated and rated on average (12). Renal glomeruli, tubule, interstitium and arteries were evaluated histopathologically regarding the parameters mentioned above. The evaluation was done as follows; score 1=mild (6-25%); score 2=moderate (26-75%); score 3=heavy (76-100%) (13). The mean score was calculated for each subgroup. On the other hand, chronic renal damage was examined by using 2 parameters: tubular atrophy and interstitial fibrosis (12). Tubular atrophy was graded as "0 (normal morphology)", "1 (<10%)", "2 (10-25%)", "3 (25-50%)", "4 (50-75%)", and "5 (>75%)". Interstitial fibrosis was scored similarly with acute damage parameters as from 0 to 3.

Statistical Analysis

Statistical analysis was done using the Statistical Package for Social Sciences, ver. 15.0 for Windows, (SPSS, Chicago, IL). The Kruskal-Wallis test was used for comparison of the groups and significant differences were determined using the Dunn's post-hoc test. A p value of less than 0.05 was considered statistically significant.

Results

Biochemical Analysis

Remarkably elevated TOS values were observed in rats undergoing SWL i.e. group 2 (Table 1). However, group 3 (SWL + NAC) had significantly low TOS values compared to group 2 (p=0.027). The median TOS value was 15.38 µmol H2O2 Eqiv./L and 16.27 H2O2 Eqiv./L in rats with SWL + saline (group 2) at short term (14 day) and long term (28 day), respectively. NAC administration (group 3) decreased these values to 8.58 H2O2 Eqiv./L at short term and 10.92 H2O2 Eqiv./L at long term (Table 1). Consequently, NAC administration improved the TOS almost two times in the third group. Similarly, the median TAS value was 0.96 µmol TroloxEqiv./L in the short term of group 2 (SWL-saline). This value was increased to 1.19 µmol TroloxEqiv./L by 14 day NAC injection (group 3). This difference was significant (p=0.006). As a result of these, OSI (TOS/TAS) was lower in the NAC group (p=0.013). This effect was particularly significant in the short-term subgroup. As much as two times higher OSI values were measured in rats that underwent SWL without NAC. While the median OSI was 7.21 arbitrary units at the 14 day with NAC (group 3), it was 16.72 arbitrary units in the SWL group without NAC (group 2) (Table 1). Even the control group had an OSI value of 10.555 arbitrary units that was higher but not significant compared to NAC group.

Histological Investigation

Tubular damage as an acute damage parameter was found to be prominent in rats receiving SWL. Control group had a median score of 0.5 for tubular damage. This value was increased up to 2 in the SWL + saline group (group 2) both in short- and long-term subgroups. This difference was significant (p=0.022). Although not significant, NAC application decreased this tubular damage score down to 1 and, 1.5 at short and long term, respectively. In figure 2, histological views of the tubular damage are shown with respect to groups. Similarly, interstitial inflammation was not observed at all in the control group. SWL caused a prominent increase in this score up to 2 both at 14 and 28 days in group 2. These changes in interstitial inflammation were statistically significant (p=0.002). Although not-significant, this score was decreased to 1 by NAC application in group 3 (Table 2). The rest of the acute damage parameters namely, dilatation-congestion

Table 1.The comparison of biochemical oxidative stress parameters between groups

	Group	n	Mean	Median	Standard deviation	min	max	p
TOS (μmol H_2O_2Eqiv./L)	I	6	11.126	11.12[ab]	1.5804	8.46	12.89	0.027
	2-short	5	15.254	15.38[b]	3.8641	11.52	20.70	
	3-short	5	8.976	8.58[a]	1.4356	7.64	11.00	
	2-long	4	21.595	16.27[b]	15.885	9.01	44.83	
	3-long	4	10.43	10.92[ab]	3.1326	6.25	13.63	
TAS (μmol TroloxEqiv./L)	I	6	1.0866	1.09[ab]	0.0826	1.00	1.23	0.006
	2-short	5	0.976	0.96[b]	0.0622	0.92	1.06	
	3-short	5	1.204	1.19[a]	0.0823	1.09	1.31	
	2-long	4	1.2675	1.165[ab]	0.2518	1.10	1.64	
	3-long	4	1.855	1.685[ab]	0.8456	1.09	2.96	
OSI (Arbitrary unit)	I	6	10.253	10.555[a]	1.3485	7.69	11.50	0.013
	2-short	5	15.634	16.72[a]	3.7261	11.42	19.53	
	3-short	5	7.508	7.2[b]	1.5439	6.11	10.09	
	2-long	4	18.105	11.85[ab]	15.350	7.97	40.75	
	3-long	4	7.1175	6.93[ab]	4.5532	2.11	12.5	

TOS: Total oxidant status; TAS: Total antioxidant status; OSI: Oxidative Stress index, Group 1: Control group, Group 2: SWL + saline, Group 3: SWL + NAC, Short:14 days, Long: 28 days SWL: Extracorporeal shock wave lithotripsy, NAC: N-acetylcysteine, [a, b, ab]: The letters (a, b, ab) in the upper right side of median values of the groups were used to show whether the differences between the groups were significant. If there is a statistically significant difference between the groups, they carry different letters (like a and b). If there is the letter (ab), it does not differ from the group with a, and the group with b letter, Min: Minimum, Max: Maximum

in the glomerular and vascular structures, and increase in inflammatory cells, were not different between the groups (Table 2). Similarly, no difference in chronic renal damage parameters, such as tubular atrophy and interstitial fibrosis, was noticed between the groups.

Discussion

SWL constitutes a milestone in the management of Urinary Stone disease (2). Particularly, the higher clinical success in children with renal stones signifies the clinical utilization of SWL. However, possible histological and functional alterations in renal tissue after SWL treatment has gained a tremendous concern, and several well-designed experimental trials have focused on this issue.

One of the possible mechanisms for SWL-related renal damage is increased oxidative stress due to renal ischemia-reperfusion injury (3,4,5). An experimental model on rabbits demonstrated a statistically significant decrease in tissue scavenger enzyme levels (i.e. formation of free oxygen radicals) after SWL in dose dependent manner (5). A similar trail on 69 rats clearly demonstrated a significant increase in malondialdehyde (MDA) levels and decrease in superoxide dismutase (SOD) activity as markers for oxidative stress after SWL (14). They further reported that total saponins of astragalus which is the main component of. A mongholicus can decrease shock wave-induced kidney injury not only by scavenging oxygen free radicals but also inhibiting the expression of p-selectin (14).

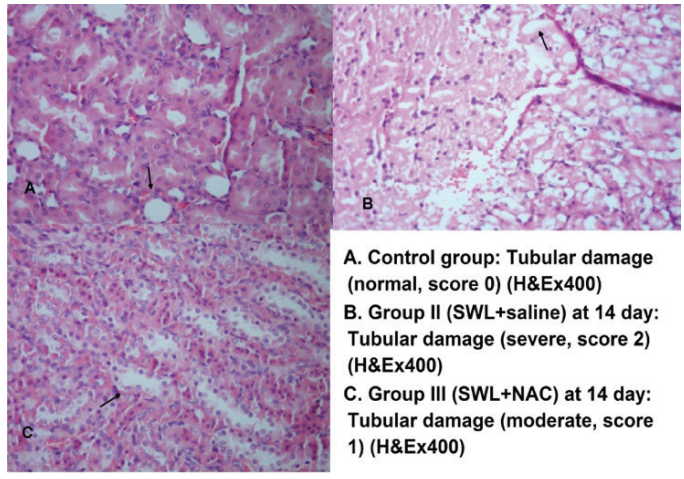

Figure 2. Tubular damage between the groups. A) Control group: tubular damage (normal, score 0) (h&te x400). B) Group 2 (swl + saline) at 14 day: tubular damage (severe, score 2) (h&te x400). C) Group 3 (swl + nac) at 14 day: tubular damage (moderate, score 1) (h&te x400)

The same authors performed a subsequent trial on rats; they confirmed that shock wave significantly increased the level of MDA and decreased SOD activity in both blood and renal homogenates (7). The study group receiving astragalosides, a novel antioxidant agent, could be protected from shock wave-induced renal oxidative injury. Furthermore, a clinical trial on 120 patients with renal SWL treatment proposed that oral antioxidant administration was associated with reduced mean serum concentration of MDA, higher levels of serum ascorbic acid and serum albumin, lower alpha-tocopherol/cholesterol ratio, lower urinary albumin and beta-2 microglobulin levels

Table 2. The comparison of histological alterations between groups

	Group	n	Mean	Median	Standard deviation	min	max	p
Tubular damage	I	6	0.5	0.50[a]	0.548	0	1	**0.022**
	1-short	5	1.6	2.00[b]	0.548	1	2	
	3-short	5	1.2	1.00[ab]	0.837	0	2	
	2-long	4	2.0	2.00[b]	0.0	2	2	
	3-long	4	1.5	1.50[ab]	0.577	1	2	
Interstitial inflammation	I	6	0.0	0.00[a]	0.00	0	0	**0.002**
	2-short	5	2.0	2.0[b]	0.707	1	3	
	3-short	5	1.0	1.0[ab]	1.00	0	2	
	2-long	4	2.0	2.00[b]	0.00	2	2	
	3-long	4	1.0	1.00[b]	0.00	1	1	
Congestion	I	6	0.5	0.50	0.548	0	1	0.291
	2-short	5	1.2	1.00	0.447	1	2	
	3-short	5	0.6	1.00	0.548	0	1	
	2-long	4	0.75	1.00	0.50	0	1	
	3-long	4	0.75	1.00	0.50	0	1	
Increase in inflammatory cells	I	6	0.00	0.00	0.00	0	0	0.287
	2-short	5	1.20	1.50	0.20	0	2	
	3-short	5	1.00	1.50	0.50	0	2	
	2-long	4	0.75	1.00	0.541	0	1	
	3-long	4	0.75	1.00	0.541	0	1	
Fibrosis	I	6	0.00	0.0	0.0	0	0	0.299
	2-short	5	2.00	2.00	0.50	1	3	
	3-short	5	1.00	1.50	0.50	1	2	
	2-long	4	2.00	2.00	0.0	2	2	
	3-long	4	1.00	1.00	1.00	1	1	
Tubular atrophy	I	6	0.00	0.00	0.00	0	0	**1.00**
	2-short	5	0.00	0.00	0.00	0	0	
	3-short	5	0.00	0.00	0.00	0	0	
	2-long	4	0.00	0.00	0.0	0	0	
	3-long	4	0.00	0.00	0.00	0	0	

Group 1: Control group, Group 2: SWL + saline, Group 3: SWL + NAC, Short: 14 days, Long: 28 days SWL: Extracorporeal shock wave lithotripsy, NAC: N-acetylcysteine
[a, b, ab]: The letters (a, b, ab) in the upper right side of median values of the groups were used to Show whether the differences between the groups were significant. If there is a statistically significant difference between the groups, they carry different letters (like a and b). If there is the letter (ab), it does not differ from the group with a, and the group with b letter

(15). They concluded that SWL generates free radicals through ischemic/reperfusion injury mechanism, and oral administration of antioxidant may protect these patients from short-term renal injury caused by SWL. A similar study by Ozguner et al. (6) showed that shock wave exposure caused a significant rise in MDA, urine N-acetyl-beta-glucosaminidase (NAG) activity, uric acid and white cell counts as markers of oxidative stress. On the other hand, a novel free radical scavenger, caffeic acid phenethyl ester, reduced the rise in MDA, NAG, uric acid and white cell counts providing a protection against SWL-induced free radical damage. In another study on 12 rats, it was demonstrated that SWL caused oxidative stress and impaired the antioxidant and trace element levels in the kidneys of rats (16). They reported higher MDA levels, and reduced glutathione levels, lower SOD, and glutathione peroxidase activities in SWL group. A clinical trial reported elevated plasma and urinary nitric oxide levels after SWL (3). Plasma and urinary MDA levels also showed statistically significant elevation after SWL. Another clinical trial proposed that SWL caused a severe alteration in activities of glucose-6-phosphate dehydrogenase, SOD and catalase in the erythrocyte haemolysate (17). They concluded that erythrocyte lipid peroxidation might be induced and antioxidative defense

mechanism may be transiently impaired by SWL. A prospective, randomized, double-blind, placebo-controlled clinical trial on 100 patients with SWL treatment showed a clinical efficacy of coenzyme Q10 (CoQ10) as an oral powerful antioxidant with vasoactive properties in preventing renal injury (18). CoQ10 showed improvement in vasoactive hormone parameters, Vascular Resistance index on Doppler ultrasound and interleukin levels suggesting a protection in renal function, and also vasoactive and inflammation parameter values. In an experimental pig model of SWL, acute oxidative stress and inflammatory responses were localized to the renal medulla within the focal zone of the lithotripter (19). Melatonin as a potent endogenous free radical scavenger was tested on rabbits after SWL (20). The mean levels of MDA, uric acid and white cell counts as markers of oxidative stress were significantly lower in the melatonin-treated group suggesting a protective effect of melatonin. Another experimental rabbit model showed that antioxidant defense potential of the SWL-treated tissues was reduced, and MDA levels were increased (21). Administration of vitamin E plus C combination ameliorated antioxidant defense potential in part, prevented increases in MDA levels in the SWL-treated tissues. All these experimental SWL models and some clinical studies propose that SWL produces significant oxidative stress. Current trial also confirms that SWL application caused a remarkable decline in TAS levels and a significant rise in TOS levels. Consequently, a notable raise in OSI was observed in rats undergoing SWL. In this study, OSI values were reported by measuring TAS and TOS in blood samples of SWL-treated rats as the first time in the literature for detecting SWL-related oxidative stress. We also used a novel colorimetric and fully automated method for measuring total antioxidant response against potent free radical reactions (10,11).

Histological alterations due to SWL treatment were also reported by several well-designed experimental trials. Chronic morphological changes, such as tubular injury and interstitial fibrosis, were observed in rats after SWL (12). The authors also suggested that nuclear factor κB (NFκB) was important in the progression of SWL-induced long-term renal damage and also shock wave exposure up-regulated the expression of transforming growth factor-β1. According to an electron microscopic investigation on rabbit model, some significant subcellular changes, such as endothelial injury, damage to glomerular basal membrane, etc., were observed in the SWL-treated renal tissue (22). Acute morphological changes, such as glomerular bleeding, tubular dilatation, atrophy and partial necrosis, have been reported in rat kidneys after SWL (22). Investigation by scanning electron microscopy revealed a tubular loss of microvilli and cilia. It was postulated that long-term lesions were due to the venous rupture occurred during SWL, especially in thin arcuatae veins. This resulted in interstitial hematoma. Later on, hematomas progressed to interstitial fibrosis. Finally, blood supply in these areas was reduced and secondary changes, such as glomerular-tubular atrophy and sclerosis, followed. Similarly in a rat SWL model, gross interstitial hemorrhage, subcapsular hematomas, and hemorrhages into the renal pelvis were detected by using vascular casting procedures (23). All these histological investigations showed that SWL causes some significant morphological alterations in experimental models. In this study, tubular damage and interstitial inflammation, acute damage parameters, were also found to be prominent in rats receiving SWL. However, no finding of chronic renal damage, such as tubular atrophy and interstitial fibrosis, was noticed after SWL. This observation may be due to the fact that chronic changes require a longer period to develop. We can conclude that SWL produces significant oxidative stress which in turn results in acute renal damage such as tubular damage and interstitial inflammation.

NAC, a potent antioxidant, was tested to prevent oxidative damage in renal tissues. In a rat model, carbon dioxide pneumoperitoneum was used to induce oxidative stress (24). It was reported that administration of NAC provided a complete protection against decline in glomerular filtration rate following pneumoperitoneum. In a randomized, double-blind, controlled clinical trial on chronic hemodialysis patients, administration of NAC suggested to reduce oxidative stress without major side-effects (25). In another experimental study, it was shown that NAC could protect rat kidney against aspartame-induced oxidative stress (26). Similarly, it has been concluded that NAC showed effective restoration of oxidative stress biomarkers including MDA, SOD, and glutathione peroxidase (27). In this experimental model, rats that received intraperitoneal NAC at a dose of 300 mg/kg/day for 14 or 28 days showed a significant improvement of TOS as much as two times than the rats without NAC. Similarly, median TAS value was remarkably increased by NAC injection. Consequently, OSI was lower in the NAC group. Almost two times higher OSI values were measured in rats that underwent SWL without NAC. Therefore, this study showed that NAC has a protective role against oxidative stress associated with SWL. Moreover, tubular damage and interstitial inflammation as acute damage parameters were found to be less prominent in rats receiving NAC. The current trial suggests that NAC administration during SWL can prevent renal oxidative stress, and then, some subsequent morphological alterations may also be improved by NAC.

The limitations of the study include that pre-SWL oxidative stress measurements would be appropriate, however, obtaining blood samples from rats requires anesthesia. Second point, contrast material used to visualize the kidney may affect renal functions. Thirdly, larger study groups would be more powerful, however, the number of rats were restricted in accordance with the "3R (reduction, refinement, replacement) rule", also taking

into account similar studies in the literature and practices of the animal ethical committee.

Conclusion

Current study confirmed that SWL causes oxidative stress as expressed by remarkably increased plasma OSI in blood samples of SWL-treated rats as the first time in the literature. SWL also produced acute renal damage as tubular damage and interstitial inflammation. NAC was found to be effective in decreasing SWL-induced oxidative stress and also it was assumed that it may protect against certain histological alterations to some extent. This experimental model provides an important background for subsequent clinical trials on protective role of NAC in patients receiving SWL.

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Ethics

Ethics Committee Approval: All experiments in this study were performed in accordance with the Universal Declaration of Animal Rights (Paris, 1978) and were approved by the Local Animal Ethics Committee (10-01/03).

Informed Consent: Local Animal Ethics Committee approval was obtained and compliance with the international animal rights declaration.

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Authorship Contributions

Concept: D.B., K.Ç., Design: D.B., K.Ç., Y.Ş., Data Collection or Processing: D.B., Y.Ş., Analysis or Interpretation: D.B., K.Ç., H.E., Literature Search: D.B., K.Ç., Y.Ş., A.Y., Writing: D.B., A.Y., E.B.

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Referances

1. Matlaga BR, Assimos DG. Changing indications of open stone surgery. Urology 2002;59:490-493.
2. Türk C, Petřík A, Sarica K, Seitz C, Skolarikos A, Straub M, Knoll T. EAU Guidelines on Interventional Treatment for Urolithiasis. Eur Urol 2016;69:475-482.
3. Aksoy H, Aksoy Y, Turhan H, Keleş S, Ziypak T, Ozbey I. The effect of shock wave lithotripsy on nitric oxide and malondialdehyde levels in plasma and urine samples. Cell Biochem Funct 2007;25:533-536.
4. Park JK, Cui Y, Kim MK, Kim YG, Kim SH, Kim SZ, Cho KW. Effects of extracorporeal shock wave lithotripsy on plasma levels of nitric oxide and cyclic nucleotides in human subjects. J Urol 2002;168:38-42.
5. Sarica K, Koşar A, Yaman Ö, Bedük Y, Durak I, Kavukçu M. Evaluation of ischemia after ESWL: detection of free oxygen radical scavenger enzymes in renal parenchyma subjected to high-energy shock waves. Urol Int 1996;57:221-223.
6. Ozguner F, Armagan A, Koyu A, Calıskan S, Koylu H. A novel antioxidant agent caffeic acid phenethyl ester prevents shock wave-induced renal tubular oxidative stress. Urol Res 2005;33:239-243.
7. Li X, He D, Zhang L, Cheng X, Sheng B, Luo Y. A novel antioxidant agent, astragalosides, prevents shock wave-induced renal oxidative injury in rabbits. Urol Res 2006;34:277-282.
8. Zafarullah M, Li WQ, Sylvester J, Ahmad M. Molecular mechanisms of N-acetylcysteine actions. Cell Mol Life Sci 2003;60:6-20.
9. De Stefani S, Micali S, De Carne C, Sighinolfi MC, Di Pietro C, Marzona L, Volpi N, Bianchi G. Shockwave lithotripsy and protective role of inosine: early and late evaluation in an experimental model. J Endourol 2008;22:1059-1063.
10. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38:1103-1111.
11. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004;37:112-119.
12. Li X, Xue Y, He D, Chen X, Zhang L. Shock wave induces chronic renal lesion through activation of the nuclear factor kappa B signaling pathway. World J Urol 2010;28:657-662.
13. Akgüllü Ç, Saruhan T, Eryilmaz U, Boyacıoğlu , Onbaşılı OA, Meteoğlu I, Kum C. The first histopathological evidence of trimetazidine for the prevention of contrast-induced nephropathy. Ren Fail 2014;36:575-580.
14. Li X, He DL, Cheng XF, Zhang LL, Yu LH, Li JJ. Effects of components isolated from Astragalus mongholicus on expression of p-selectin in shock wave induced kidney injury in rabbit model. Zhongguo Zhong Yao Za Zhi 2005;30:1606-1609.
15. Al-Awadi KA, Kehinde EO, Loutfi I, Mojiminiyi OA, Al-Hunayan A, Abdul-Halim H, Al-Sarraf A, Memon A, Abraham MP. Treatment of renal calculi by lithotripsy: minimizing short-term shock wave induced renal damage by using antioxidants. Urol Res 2008;36:51-60.
16. Gecit İ, Kavak S, Meral I, Pirinçci N, Güneş M, Demir H, Cengiz N, Ceylan K. Effects of shock waves on oxidative stress, antioxidant enzyme and element levels in kidney of rats. Biol Trace Elem Res 2011;144:1069-1076.
17. Aksoy Y, Malkoc I, Atmaca AF, Aksoy H, Altinkaynak K, Akcay F. The effects of extracorporeal shock wave lithotripsy on antioxidant enzymes in erythrocytes. Cell Biochem Funct. 2006;24:467-469.
18. Carrasco J, Anglada FJ, Campos JP, Muntané J, Requena MJ, Padillo J. The protective role of coenzyme Q10 in renal injury associated with extracorporeal shockwave lithotripsy: a randomised, placebo-controlled clinical trial Br J Urol int 2014;113:942-950.
19. Clark DL, Connors BA, Evan AP, Willis LR, Handa RK, Gao S. Localization of renal oxidative stress and inflammatory response after lithotripsy. Br J Urol int 2009;103:1562-1568.
20. Serel TA, Ozguner F, Soyupek S. Prevention of shock wave-induced renal oxidative stress by melatonin: an experimental study. Urol Res 2004;32:69-71.
21. Biri H, Ozturk HS, Buyukkocak S, Kacmaz M, Cimen MY, Unal D, Birey M, Bozkirli I, Durak I.. Antioxidant defense potential of rabbit renal tissues after ESWL: protective effects of antioxidant vitamins. Nephron 1998;79:181-185.

22. Recker F, Rubben H, Bex A, Constantinides C. Morphological changes following ESWL in the rat kidney. Urol Res 1989;17:229-233.

23. Weber C, Moran M, Braun E, Drach G. Injury of rat renal vessels following extracorporeal shock wave treatment. J Urol 1992;147:476-481.

24. Seguro AC, de Figueiredo LFP, Shimizu MHM. N-acetylcysteine (NAC) protects against acute kidney injury (AKI) following prolonged pneumoperitoneum in the rat. J Surg Res 2012;175:312-315.

25. Shahbazian H, Shayanpour S, Ghorbani A. Evaluation of administration of oral N-acetylcysteine to reduce oxidative stress in chronic hemodialysis patients: A double-blind, randomized, controlled clinical trial. Saudi J Kidney Dis Transpl 2016;27:88-93.

26. Finamor I, Pavanato MA, Pês T, Ourique G, Saccol E, Schiefelbein S, Llesuy S, Partata W. N-acetylcysteine protects the rat kidney against aspartame-induced oxidative stress. Free Radic Biol Med 2014;75:30.

27. Ribeiro G, Roehrs M, Bairros A, Moro A, Charão M, Araújo F, Valentini J, Arbo M, Brucker N, Moresco R, Leal M, Morsch V, Garcia SC. N-acetylcysteine on oxidative damage in diabetic rats. Drug Chem Toxicol 2011;34:467-474.