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ORIGINAL PAPER

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Effects of shock wave lithotripsy on plasma and urinary levels of nitrite and adrenomedullin

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Abstract Purpose: In this prospective clinical study, we aimed to determine whether shockwave lithotripsy (SWL) has any specific effect on plasma as well as urinary nitrite, a stabile metabolite of nitric oxide (NO) and adrenomedullin (AM) concentrations, and to investigate whether these variables can be used as a marker for detecting shockwave-induced impairment of renal tubular and glomerular cells. Material and Methods: A total of 20 patients with renal pelvic or caliceal stones ≤ 2 cm undergoing anesthesia-free SWL without auxiliary measures and a control group of ten patients without any urological symptoms were included in this study. The plasma and urinary concentrations of nitrite and AM were measured before, 24 h, and 7 days after SWL. Nitrite levels were measured by Griess reaction. Reverse-phase high-performance liquid chromatography (HPLC) was used to determine AM levels. Results: Application of high-energy shock waves (HESW) in our study caused a statistically significant increase in plasma levels of both NO and AM, which reflected an organized response of the kidney to this type of trauma in an attempt to maintain normal renal hemodynamics. Mean plasma nitrite concentration before SWL application was 29.9 ± 7.6 μmol/l and this value was found to be 39.02 ± 8.45 μmol/l at 24-h follow-up. Comparative evaluation of the plasma concentrations of AM revealed a significant increase at the 24-h examination: 20.51 ± 3.0 pmol/ml and 32.54 ± 4.3 pmol/l, respectively. On the other hand, comparative evaluation of urinary levels of both nitrite and AM levels before as well as 24 h after SWL application revealed a statistically significant increase related to markers. Conclusion: This first clinical study on plasma-urinary nitrite and AM levels in patients undergoing the SWL procedure indicated that plasma and urine levels of both peptides were increased. Our findings in turn suggested that SWL application to kidneycan stimulate the NO-cGMP signalling pathway to increase NO production in the kidney. Our findings also indicated that the increased levels of NO and AM secretion during renal parenchymal ischemia may be protective enough for renal pathological alterations resulting from SWL-induced renal trauma. We suggest that this increase may be a compensatory response to SWL induced injury.

Keywords SWL · Renal injury · Adrenomedullin · Nitric oxide

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Introduction

Despite its proved safety and efficacy in a number of studies, it has been reported recently that high-energy shock waves may cause adverse effects on the function and morphology of the visceral organs. Although the main target of high-energy shock waves(HESW) is the stone located in the kidney, the surrounding tissue or other organs are also subjected to trauma during this procedure [1]. Increased excretion of small proteins (β2- and α1-microglobulin) and an enzyme(N-acetyl-β-glucosaminidase) and a decrease in Tamm-Horsfall protein excretion have been reported after SWL as signs of proximal and tubular impairment, respectively [2].

NO, a highly reactive substance, is enzymatically generated by nitric oxide synthase (NOS). The major source of NO that causes vasodilatation is endothelial

NOS. The physiological role of endothelial-derived NO in regulating vasodilatation via vascular smooth muscle cells has been well elucidated. In this way, NO antagonizes the vasoconstrictive effect of angiotensin II on the afferent arteriole and helps regulate renal blood flow, glomerular filtration rate and sodium homeostasis. In addition to these effects, nitric oxide has also been found to influence many aspects of function, including autoregulation of blood flow, renin secretion, glomerular mesangial and epithelial cell activity and tubular functions [3].

AM, which is a potent vasorelaxing, natriuretic, cytoprotective and cell growth-modulating peptide, is thought to act as an autocrine/paracrine regulator in renal glomeruli and tubules. AM was found immunohistochemically to be localized in glomeruli, cortical distal tubules and in medullary collecting cells, suggesting that AM exerts glomerular and tubular actions in an autocrine and paracrine fashion. The reduced blood pressure induced by AM was almost completely abolished by NOS inhibition, suggesting that the hypotensive action of AM mediated mainly via a NO-dependent mechanism [4, 5].

The aim of this prospective clinical study was to determine whether SWL has any effect on plasma as well as urinary NO and AM concentrations and to investigate whether these peptides can be used as a marker to detect shockwave-induced renal injury.

Patients and methods

In this prospective study, a total of 20 patients(12 women and 8 men) aged 24–48 (average 40.1) years with unilateral renal pelvic or caliceal stones ≤ 2 cm in diameter undergoing anesthesia-free SWL were included. None of the patients required an additional intervention either before or after SWL for any complication. In addition, a control group of ten patients (five men, five women), with an average age of 42.4 years (range, 26–55 years) with no urological symptoms were chosen among the hospital staff and included in this study program. No adjuvant surgical interventions (i.e. open or endourological procedures) was performed in these patients. In addition to a detailed history and physical examination, a complete blood count, urinalysis with cultures, routine biochemistry and renal ultrasound scan were done before treatment. In patients who had urinary tract infection, the urine was sterilized before SWL. Patients fulfilling the following criteria were included in this study: creatinine < 133 mol/l, no auxiliary measures before or after lithotripsy, no application of contrast medium during SWL, and no anesthesia, as these measures could influence renal tubular functions.

All patients were treated with the Stonelith V5 lithotriptor (equipped with a spark gap system with a 12.1-mm focal diameter) with varying kV values (16–24 kV). The patients received 770–2,500 shocks, with an average value of 1,687. During SWL sessions, the arterial blood pressure of all patients were regularly assessed and recorded. No significant change in these values was noted in any patient undergoing SWL.

To assess the value of plasma as well as urinary NO and AM as markers for shockwave-induced renal injury, blood samples and fresh morning urine specimens were collected before, 24 h, and 7 days after SWL.

Collection and preparation of plasma and urine samples for assay procedures

Blood samples were drawn into tubes with heparin and urine samples into tubes containing sodium tetraboric acid (0.5 g/l).

Plasma and urinary total nitrite levels

Total nitrite was quantitated by means of the Griess reaction after incubation of plasma or urine samples with *Escherichia coli* reductase to convert NO-3 to NO-2 [6]. One thousand microliters of the Griess reagent(1% sulfanilamide, 0.1% naphthalene diamine hydrochloride, and 2.5% phosphoric acid) (Sigma chemical Co., St. Louis MO) was then added to 1,000 µl of plasma or urine specimens. Absorbance was read at 545 nm after a 30-min incubation period. Standard curves were prepared with known concentrations (1–100 µmol/l) of sodium nitrite.

Plasma and urinary AM level

After extraction and purification, plasma and urine samples were applied to supelcosil C18 columns (Cecil 100HPLC). Loaded material was eluted 60% acetonitrile in 0.1% trifluoroacetic acid [7]. Rat adrenomedullin/1–50 (Phoenix Pharmaceuticals, Inc.) was used as the standard to determine plasma and urine adrenomedullin levels.

The urinary nitrite and AM levels were corrected using the urinary creatinine levels to avoid the influence of the concentration of the urine itself.

Statistical analysis

Results are given as mean $\pm$ SD and analyzed statistically by using the Wilcoxon Signed Rank test and Mann-Whitney U test. A p value less than 0.05 was considered as significant. Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 10.0)

Results

Comparative evaluation of the plasma levels of both NO and AM has shown a statistically significant elevation 24 h after HESW application ($p < 0.001$). The mean plasma nitrite concentrations before and 24 h after SWL application were 29.9 ± 7.6 µmol/l and 39.02 ± 8.45 µmol/l, respectively ($p < 0.001$). At the 7th-day evaluation, however, values tended to return to baseline values: 31.32 ± 7.95, $p > 0.05$

With respect to the changes in plasma AM levels, while a significant increase ($p < 0.001$) was observed during the examination at 24 h (20.51 ± 3.0 pmol/ml to 32.54 ± 4.3 pmol/l) (Table 1) the values were found to be within the limits observed before SWL (24.32 ± 3.2, $p > 0.05$), (Table 2). Thus, all these findings demonstrate a significant increase in plasma concentrations of both NO and AM at 24 h of examination after SWL application in the treated kidneys.

On the other hand again, comparative evaluation of urinary levels of both variables before and 24 h after SWL application did demonstrate a statistically significant increase, like the plasma levels ($p < 0.001$ and $p < 0.001$). These values have tended to return to baseline values at the 1-week follow-up. The results are summarized in Tables 1 and 2. Although the plasma and urinary levels of both variables rose similarly with the increasing number of SWs (at the higher number of SW

Table 1 Evaluation of the plasma and urinary nitrite levels in patients undergoing SWL

	Study group			Control group
	Before SWL	24-h	7-day	
Plasma nitrite (μmol/l)	29.90 ± 7.60	39.02 ± 8.45	31.32 ± 7.95	27.90 ± 3.60
P value	< 0.001[a](before SWL, 24 h) > 0.05[a](before SWL, 7 day) > 0.05[b](control group, before SWL, 7.day)			
		< 0.05[b](control group, 24 h)		
Urinary nitrite (μmol/mg creatine)	0.18 ± 0.09	0.40 ± 0.28	0.22 ± 0.14	0.23 ± 0.29
P value	< 0.001[a](before SWL, 24 h) > 0.05[a](before SWL, 7 day) > 0.05[b](control group, before SWL, 7 day)			
		< 0.05[b](control group, 24 h)		

[a]Wilcoxon Signed Rank test.
[b]Mann-Whitney U test.

Table 2 Evaluation of the plasma and urinary AM levels in patients undergoing SWL

	Study group			Control group
	Before SWL	24-h	7-day	
Plasma adrenomedullin (pmol/l)	20.51 ± 3.0	32.54 ± 4.3	24.32 ± 3.2	23.90 ± 2.60
P value	< 0.001[a](before SWL, 24 h) > 0.05[a](before SWL, 7 day) > 0.05[b](control group, before SWL, 7.day)			
		< 0.05[b](control group, 24 h)		
Urinary adrenomedullin (pmol/mg creatine)	14.48 ± 8.0	30.17 ± 11.8	17.02 ± 9.6	15.02 ± 5.6
P value	< 0.001[a](before SWL, 24 h) > 0.05[a](before SWL, 7 day) > 0.05[b](control group, before SWL, 7.day)			
		< 0.05[b](control group, 24 h)		

[a]Wilcoxon Signed Rank test.
[b]Mann-Whitney U test.

applications, a clear increase was noted), this rise was not compared for the two variables.

Finally, we were not able to demonstrate a significant alteration in both plasma and urinary levels of these markers in control group patients before and after SWL application. A comparison between the results of the study group with the data obtained in control group patients has also been given at Tables 1 and 2.

Discussion

In addition to certain histomorphological changes demonstrated by animal model studies [8, 9], investigations dealing with the immediate vascular supply and total effective renal plasma flow (ERPF) to kidneys treated with HESWs indicated a transient decrease in renal perfusion, which might be related to shockwave-induced trauma in the treated kidneys [10, 11, 12, 13, 14]. While prolonged parenchymal transit time following HESW application was regarded as obstruction and ischemic formation due to vascular pathologies [10, 12, 13, 15], concerning the pathophysiology of postischemic renal dysfunction, reactive oxygen species (ROS) and vasoconstrictive substances (endothelin, renin, angiotensin II, thromboxane) were found to have important roles [16, 17].

Clinical and experimental studies have demonstrated that oxygen free radicals, that is toxic partial reduction production of oxygen, have a vital role in the development of renal parenchymal damage during ischemia [18, 19]. In one of our previous studies, decrease of antioxidant enzymes at the tissue level after SW application indicated parenchymal ischemia and free radical formation, which might be responsible for histological changes [14]. Use of some definite free radical scavenger agents against free radical-induced injury have also supported the vital role of ROS formation [20, 21]. In their original study, Cohen et al. [1] concluded that HESW lithotripsy is associated with increased lipid peroxidation products, which may cause further cellular damage. They also postulated that lipid peroxidation induced by SWL may be one of the several mechanisms that lead to other potential bioeffects

[10]. In another study, the antioxidant capacity of the treated kidney tissue was found to be reduced after SWL, indicating the presence of oxidant stress [11].

NO, a highly effective vasodilatory peptide, is enzymatically generated by endothelial NOS. Endothelial-derived NO regulates vasodilatation via vascular smooth muscle cells and antagonizes the vasoconstrictive effect of angiotensin II on the afferent arteriole, which helps to regulate renal blood flow, glomerular filtration rate and sodium homeostasis [22]. NO has also been found to influence certain renal functions, including autoregulation of blood flow, renin secretion, glomerular mesangial and epithelial cell activity and tubular functions. Regarding the potential role of NO in renal hemodynamics, contradictory results have been published in the literature. While some authors have expressed concern about its harmful effects, it has been clearly shown that in renal ischemic conditions, the production of NO is increased. Endothelial release of NO causes a local relaxation in vessel walls and as a result of renal blood flow improvement, ischemic tissue damage could be well limited. Thus, NO may also serve as a potent antioxidant due to this specific vasodilatory effect [3, 23, 24].

Ischemic kidneys increase NO production during reperfusion and it remains at elevated levels at least until the 7^{th} day [22]. During renal ischemia, in addition to endothelial damage and subsequent NOS depletion, inadequate reabsorption of the NO generated may result in the loss of NO necessary for maintenance of both glomerular and tubular functions. Based on the observations in the literature and the results of our study, increased urinary levels of NO is best explained by the injurious effects of ischemia on tubular function, which result in decreased tubular reabsorption of NO metabolites or increased renal synthesis against injury [22]. However, the cellular source of the increased urinary nitrite excretion observed in these patients cannot be deduced from this study. It may originate from vascular endothelial cells through the endothelial or calcium-dependent cellular isoforms of NOS [25] or it may be due to enhanced NO production by glomerular mesangial or renal tubular epithelial cells that contain calmodulin and calcium-independent-inducible NOS [26, 27].

On the other hand, another renoprotective peptide, AM is a potent vasorelaxing, natriuretic and cell growth-modulating peptide that is thought to act as an autocrine/paracrine regulator in renal glomeruli and tubules [4, 23]. This peptide was found immunohistochemically to be localized in glomeruli, cortical distal tubules and in medullary collection [28]. The reduced blood pressure induced by AM was almost completely abolished by NOS inhibition, suggesting that the hypotensive action of AM mediated mainly via a NO-dependent mechanism. Secretion and synthesis of AM in endothelial cells are also increased by oxidative stress, which is one of the major metabolic abnormalities on vascular walls in hypertension, atherosclerosis and diabetic complications [5, 29]. Furthermore, overexpression of AM ameliorated the tubular and glomerular damage as well as renal fibrosis in rat models of chronic renal injury. Therefore it is crucial to clarify whether the tissue levels of AM, with its specific actions, are actually increased in these injured kidneys [5, 28].

Evaluation of our data demonstrated that application of HESW caused a statistically significant increase in plasma levels of both NO and AM, which reflected a protective response of the kidney against this type of trauma in an attempt to maintain normal renal hemodynamics. These findings again suggested that SW application to kidney may stimulate the NO-cGMP signaling pathway to increase NO production in the kidney. There was an increase in the urinary levels of both markers and the difference has been found to be significant. Our findings also indicated the increase of NO and AM secretion during renal parenchymal ischemia may be protective enough for renal pathological alterations resulting from SW-induced renal trauma. On the other hand, membrane disorders and free radical formation are involved in shockwave-induced cellular damage and might cause an increase in NO and AM excretion.

In summary, it is clear that both NO and AM have a definite role in normal renal homeostasis, while there is a continuing debate as to their role under pathophysiological conditions. However, considering that both peptides may be helpful in maintaining renal function through their documented vasodilatory and hypotensive actions, we can say that evaluation of NO and AM levels in plasma may be helpful in predicting renal response against SW-induced morphological and functional injury. However, we believe that further studies including the tissue levels of both peptides will certainly be needed.

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