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ORIGINAL PAPER

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Evaluation of adrenomedullin levels in renal parenchyma subjected to extracorporeal shockwave lithotripsy

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Abstract Despite its safety and efficacy, the traumatic effects of high-energy shock waves (HESW) on renal morphology and function during long-term follow-up have yet to be elucidated. Although the main target of shock waves is the stone located in the kidney, the surrounding tissue and other organs are also subjected to trauma during this procedure. In contrast to renal blood flow evaluation after shock wave treatment, ischemic development, causing varying degrees of damage at the tissue level, has not been well evaluated. . The renoprotective peptide adrenomedullin (AM) is a potent vasorelaxing, natriuretic and cell growth modulating peptide, which is thought to act as an autocrine/paracrine regulator in renal glomeruli and tubules. In this experimental study, renal parenchymal AM levels were assessed in an attempt to evaluate the effect of HESW on the tissue levels of this peptide, which may be responsible for the regulation of ischemia induced by extracorporeal shock wave lithotripsy(ESWL), in a rabbit model. Thirty white New Zealand rabbits, each weighing 3–5 kg were used. The animals were divided into three main groups, and varying numbers of shock waves (1,000, 1,500, 2,000) were applied under fluoroscopic localization to the same kidney of all animals. Ketamine HCl anesthesia was administered (15–20 mg/kg) and all of the procedures were performed with a Multimed 2000 lithotriptor. Untreated contralateral kidneys were evaluated as controls. Following HESW application, the treated and untreated kidneys of each animal were removed through bilateral flank incisions under ketamine HCl anesthesia after 24 h and 7 days, respectively. Tissue AM levels were assessed with immunohistochemistry. During the early follow-up period (24 h), both treated and untreated kidneys showed a moderate to high degree of AM positivity. The number of tubules stained with AM increased as the number of shock waves increased and the expression of this protein became evident, possibly due to a higher degree of tissue damage. Additionally, a limited degree of AM positivity was noted in the contralateral kidneys although this was not as evident as the positivity seen in the treated kidneys. Assessment of tissue AM levels during late follow-up (7 days) in both kidneys demonstrated a moderate or limited degree of positivity in the treated kidneys. Limited or no positivity could be demonstrated in the contralateral kidneys at this time. Taking the certain traumatic effects of HESW, which causes transient ischemia during ESWL, into account, we conclude that the application of HESW results in a transient decrease in renal perfusion, causing ischemic injury in treated as well as in contralateral (untreated) kidneys. This ischemic event lasts for a short time and seemed to be dose- and time-dependent. Increased tissue levels of AM appear to be a potential defence against ESWL induced ischemia.

Keywords ESWL · Adrenomedullin · Renal injury

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Introduction

Despite its safety and efficacy, the short- and long-term adverse effects of high-energy shock waves (HESW) on renal morphology and function have yet to be elucidated [2, 21, 25]. Although the main target of shock waves is the stone located in the kidney, the surrounding tissue or

other organs are also subjected to trauma during this procedure [12, 13]. Studies dealing with the immediate vascular supply and total effective renal plasma flow to kidneys treated with HESW indicate a transient decrease in renal perfusion, returning to the normal range in a definite period of time [4, 5, 10]. In contrast to renal blood flow evaluation after shock wave treatment, ischemic development at the tissue level together with regulatory mechanisms in response to this kind of trauma within the kidney itself have not been well evaluated.

The renoprotective peptide adrenomedullin (AM) is 52 amino acids long and is produced in many tissues including adrenal medulla, lung, hearth and kidney [8]. It is a potent vasorelaxing, natriuretic and cell growth modulating peptide, which 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 it has a glomerular and tubular action in an autocrine/paracrine fashion. The reduced blood pressure induced by AM was almost completely abolished by NOS inhibition, suggesting that the hypotensive action of AM is mediated mainly via a NO dependent mechanism [7, 11, 15].

In this experimental study, renal parenchymal AM levels were assessed in an attempt to evaluate the protective effects of AM against the adverse reactions of transient ischemia induced by high energy shock wave application in a rabbit model.

Materials and methods

A total of 30 white New Zealand rabbits, each weighing 3–5 kg, were used. The animals were fed a normal diet and treated for the presence of urinary tract infection and parasitic infection which may alter the tissue AM levels.

Depending on the number of ESWL applied, the animals were divided into three main groups. Following injection of an intravenous contrast agent through an ear vein, a varying number of shock waves (1,000, 1,500 or 2,000) were applied to the right kidney of all animals under fluoroscopic localization. The kV value was kept constant during all procedures (14 kV). Ketamine HCl anesthesia was administered (15–20 mg/kg) and all of the procedures were performed with a Stonelith V5 lithotriptor which used a spark gap system as energy source with a focal point of 23×10 mm and a focal pressure of 1,200 bar. Untreated left kidneys were evaluated as controls.

Following HESW application, the treated and untreated kidneys of each animal were removed through bilateral flank incisions under ketamine HCl anesthesia after 24 h or 7 days. The kidneys were washed with saline solution and cut into small pieces. Following the addition of 2 volumes of saline solution, each tissue piece was homogenized (Braun Melsungen homogenizer, 1 min at 7,000 rpm and 1 min at 1,000 rpm). The sections of the kidneys were deparaffinized in xylene and rehydrated in graded ethanol. The endogenous peroxidase was blocked by incubation in 0.3% H_2O_2 solution in methanol for 30 min. The sections were boiled in 10 mM sodium citrate (pH 6.0) for 10 min in a microwave oven. They were then incubated for 20 min at room temperature with the primary antibody (Peninsula Laboratories, San Carlos, Calif.), diluted at 1:500. Each antibody kit contained 50 μl of lyophilized rabbit antiserum to AM 1-52 (human). It was reconstituted with 50 μl distilled water for the equivalent of undiluted antiserum and then with additional buffer for diluted antiserum. The reaction was visualized using a LSAB 2 kit and DAB chromogen (Dako, Denmark). Sections were then slightly counterstained with hematoxylin. Staining without primary antibody served as a negative control, whereas formalin-fixed, paraffin-embedded sections of an adrenal medulla of the rabbits were used as a positive control. Cytoplasmic immunoreactivity was counted as positive. Following the evaluation of ten different high power fields, the proportion of positive cells was recorded as: – (negative), + (less than 5%), + + (5–50%), and + + + (more than 50%).

All procedures were performed at +4°C. To eliminate personal interpretation errors, all examination and calculation procedures were performed three times by the same pathologist.

Results

Evaluation of the tissue AM levels in treated as well as untreated (contralateral) kidneys showed that during the early follow-up period (24 h) both treated and untreated kidneys had an evident degree of positivity. The number of cells in both the glomeruli and tubules staining positive for AM increased as the number of shock waves increased (Fig. 1) and the degree of AM positivity was closely related to the number of shock waves applied (Table 1). This was possibly due to a higher degree of tissue damage. Another interesting finding was the widespread involvement of distal tubular and collecting duct cells in terms of AM positivity. On the other hand, a certain degree of AM positivity was also present in the contralateral kidneys, although this was not as evident as the changes in the treated kidneys (Fig. 2).

The assessment of tissue AM levels after 7 days in both kidneys demonstrated a moderate or limited degree of positivity in the treated kidneys. Limited or no positivity could be found in the contralateral kidneys at this time (Fig. 3).

Discussion

Despite its non-invasive and practical nature, increasing experience with ESWL has shown that HESW applica-

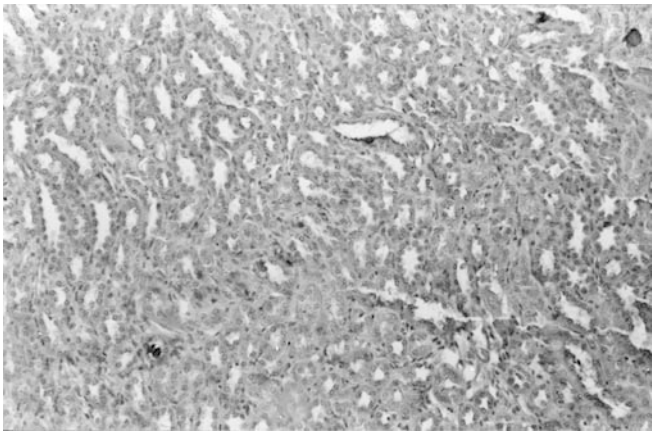

Fig. 1 AM positivity is evident in a specimen obtained from a treated kidney during early follow-up (24 h). (1,500 SW, ×100)

Table 1 Evaluation of the AM positivity (recorded as the percentage of positively stained cells) in tissue sections obtained from treated and untreated kidneys during early(24 h) and late (1 week) follow-up periods. Five kidneys were evaluated in each groups. – Negative, + < 5%, ++ 5–50%, +++ > 50%

	Treated kidney								Untreated kidney							
	24 h				1 week				24 h				1 week			
	–	+	++	+++	–	+	++	+++	–	+	++	+++	–	+	++	+++
1,000	1	3	1	0	3	2	0	0	4	1	0	0	5	0	0	0
1,500	1	1	2	2	2	2	1	0	2	2	1	0	3	1	1	0
2,000	0	0	2	3	1	3	1	1	1	2	2	0	2	3	0	0

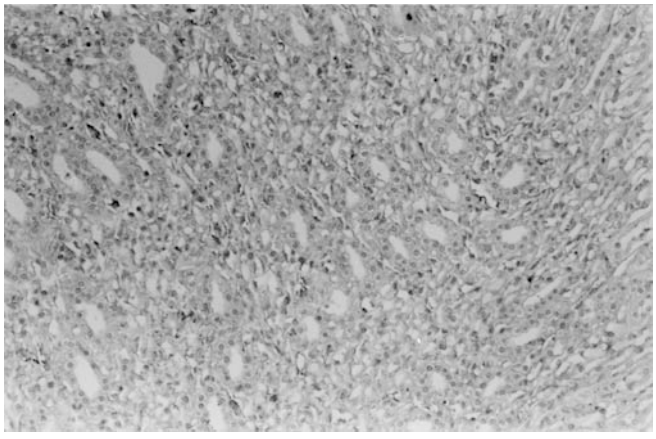

Fig. 2 A moderate degree of AM positivity in a contralateral kidney specimen obtained during early follow-up (24 h). (1,500 SW, ×100)

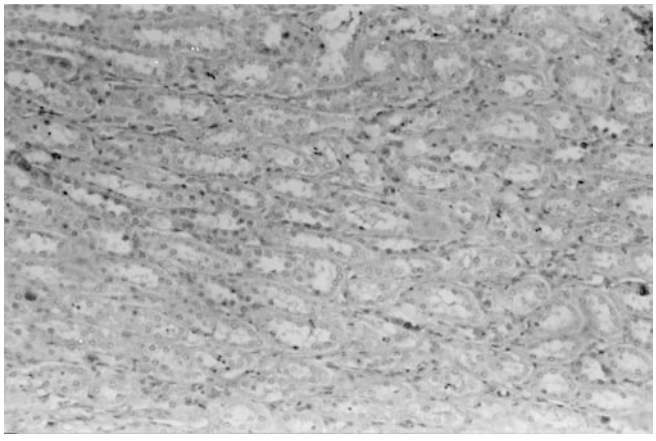

Fig. 3 A limited degree of AM positivity in a treated kidney specimen during long-term follow-up (1 week). (1,000 SW, ×100)

tion can cause some morphological and functional alterations to tubular as well as glomerular structures [2, 5, 6, 9, 18, 19, 21, 23, 25]. Certain protective agents have been used in an attempt to limit these alterations, with varying success rates [1, 20, 22, 26].

Although studies dealing with the immediate vascular supply and the total effective renal plasma flow of kidneys treated with ESWL indicated a transient decrease in renal blood flow [4, 5, 10], ischemia at the tissue level has not been thoroughly evaluated. Among the possible etiological factors, having been found to be responsible for tissue damage during ischemic events in some organs [3, 18, 24], oxygen free radical formation could occur and cause some alterations at the tissue level. Transient ischemia and subsequent reperfusion disrupt tightly controlled oxygen metabolism and subsequently give rise to oxygen free radical formation [17]. Renal ischemia-reperfusion injury in the ESWL treated kidneys, as well as other conditions, is characterized by a decreased renal blood flow and glomerular filtration rate (GFR), decreased glomerular capillary ultrafiltration, tubular dysfunction due to obstruction from cellular debris and edema formation.

Another interesting finding related to the ischemic changes after ESWL is that in contrast to the traumatic effects of ESWL application in treated kidneys, post-ESWL GFR and plasma renal flow were also found to be reduced in the contralateral, control kidneys. Some authors have claimed that apart from the activation of renal vasoconstrictor nerves by the shock waves, the local or systemic release of vasoregulatory (vasoconstrictor/vasodilator) substances such as endothelin, nitric oxide and AM may also play an important role [25]. Additionally, it has been shown that many mediators are involved in the pathophysiology of post-ischemic renal dysfunction, among which reactive oxygen metabolites and vasoconstrictive substances (endothelin, angiotensin II, tromboxane, prostaglandins) clearly play a very important role [3, 14].

Among the possible responsible peptides, AM, as a renoprotective peptide with potent vasorelaxing, natriuretic and cell growth modulating properties, is thought to act as an autocrine/paracrine regulator in renal glomeruli and tubules. AM was found immunohistochemically to be localized in the glomeruli, cortical distal tubules and in medullary collecting cells, suggesting that it has a glomerular and tubular action in an autocrine and paracrine fashion. AM administration causes a potent natriuretic and vasodilatory effect in experimental models [7, 8, 11, 15]. The reduced blood pressure induced by AM, was almost completely abolished by NOS inhibition, suggesting that the hypotensive action of AM is mediated mainly via a NO dependent mechanism. The secretion and synthesis of AM in endothelial cells are also increased by oxidative stress, which is one

of the major metabolic abnormalities in vascular walls during hypertension, atherosclerosis and diabetic complications [14, 15, 16].

AM exerts potent antiproliferative effects on various cell types, together with potent vasodilatory and natriuretic actions. 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 AM binding and action are actually increased in these injured kidneys.

ESWL induced trauma in renal parenchyma is associated with a transient ischemia during which some morphologic as well functional alterations in the renal tubules were be observed. In this experimental study, we aimed to determine whether ESWL induced renal trauma has any effect on renal parenchymal levels of AM and to investigate whether this peptide can be used as a marker to detect shock-wave induced impairment of renal hemodynamics. There have been no published reports on the effect of ESWL on the tissue levels of AM. Hence, AM positivity in the kidney tissue may indicate the potential of the kidney to act against the functional as well as morphological changes induced by possible ischemia. Itmay be regarded as a protective mechanism against trauma induced vasoconstriction.

In the present study, the determination of the concentration of AM levels in the parenchymal tissue of treated and untreated kidneys revealed evident positivity in both kidneys during early follow-up. The degree of positivity in both tubular and glomerular cells was more evident in the treated kidneys, and this positivity was found to increase with the number of ESWL applied. An interesting finding was the AM positivity detected in untreated (contralateral) kidneys. This observation in turn led us to consider a possible limited degree of ischemia formation after ESWL application in these kidneys.

In light of our findings and the results reported in the literature, we conclude that the application of HESW results in a transient decrease in renal perfusion, causing ischemic insult in treated as well as in contralateral (untreated) kidneys. This ischemic event lasts for a short time and seems to be dose-and time-dependent. Increased tissue levels of AM appear to be a potential defence against ESWL induced ischemia. We believe that further studies with a larger series of subjects and including other potentially involved peptides are needed to provide more reliable results on the possible protective effects of these peptides against the ischemia induced alterations in renal parenchyma undergoing ESWL.

References

1. Akbay C, Sayın C, Sarıca K, Soygür T, Sabuncuoğlu B (1998) Effect of verapamil on rabbit renal tissue after shock wave lithotripsy: an ultrastructural approach. Electron Microsc 4: 493
2. Baltacı S, Özer G, Soygür T, Yaman Ö, Sarıca K, Müftüoğlu YZ, Göğüş O (1996) Effects of extracorporeal shock wave lithotripsy (ESWL) on urinary epidermal growth (EGF) levels. J Endourol 10: 519
3. Bauer V, Bauer F(1996) Reactive oxygen species as mediators of tissue protection and injury. Gen Physiol Biophys 18: 7
4. Bedük Y, Erden İ, Sarıca K, Aytaç S, Karalezli G, Göğüş O (1994) Evaluation of renal morphology and function by CFDS immediately after ESWL. J Endourol 7: 457
5. Bomanji J, Boddy S, Britton KE, Nimmon CC, Whitfield HN (1987) Radionuclide evaluation pre-and post extracorporeal shock wave lithotripsy for renal calculi. J Nucl Med 28: 1284
6. Frick J, Sarıca K, Köhle R, Kunit, G (1991) Long-term follow-up after extracorporeal shock wave lithotripsy in children. Eur Urol 19: 225
7. Hirata Y, Hayakawa H, Suzuki Y, Suzuki E, Ikenouchi H, Kohmoto O, Kimura K, Kitamura K, Eto T, Kangawa K, Matsuo H, Omata M (1995) Mechanisms of adrenomedullin-induced vasodilatation in the rat kidney. Hypertension 25: 790
8. Jougasaki M, Wei CM, Aarhus LL, Heublein DM, Sandberg SM, Burnett JC (1995) Renal localization and actions of adrenomedullin: a natriuretic peptide. Am J Physiol 268: F675
9. Karalezli G, Göğüş O, Bedük Y, Köküuslu C, Sarıca K, Köksal O (1993) Histopathologic effects of ESWL on rabbit kidney. Urol Res 21: 67
10. Kaude JV, Williams CM, Miliner MR, Scott KN, Finlayson B (1985) Renal morphology and function immediately after ESWL. Am J Roentgenol 145: 305
11. Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, Eto T (1995) Adrenomedullin: a novel hypotensive peptide isolated from human pheochromacytoma. Biochem Biophys Res Commun 192: 553
12. Krysiewicz S (1992) Complications of renal extracorporeal shock wave lithotripsy reviewed. Urol Radiol 13: 139
13. Küpeli B, Sarıca K, Koşar A, Alçığır G, Budak M, Özdiler E (1995) Histopathologic effects of high energy shock waves on rat ovary. In: Tiselius HG (ed) Renal stones: aspects on their formation, removal and prevention. Proceedings of the 6th European Symposium on Urolithiasis. Stockholm, Sweden, p 242
14. Mashiach E, Sela S, Winaver J, Shasha SM, Kristal B (1998) Renal ischemia-reperfusion injury: contribution of nitric oxide and renal blood flow. Nephron 80: 458
15. Mukoyama M, Sugavara A, Nagae T, Mori K, Murabe H, Itoh H, Tanaka I, Nakao K (2001) Role of adrenomedullin and its receptor system in renal pathophysiology. Peptides 22: 1925
16. Nagata D, Hirata Y, Suzuki E, Kakoki M, Hayaakawa H, Goto A, Ishimitsu T, Minamino N, Ono Y, Kangawa K, Matsuo H, Omata M (1999) Hypoxia induced adrenomedullin production in the kidney. Kidney Int 55: 1259
17. Sarıca K, Koşar A, Yaman O, Bedük Y, Durak I, Gögüş O, Kavukcu M (1996) Evaluation of ischemia after ESWL: detection of free oxygen radical scavenger enzymes in renal parenchyma subjected to high-energy shock waves. Urol Int 57 :221
18. Sarıca K, Soygür T, Yaman Ö., Özer G, Sayın N, Akbay C, Küpeli S, Yaman LS (1996) Stone recurrence after shock wave lithotripsy: evaluation of possible enhanced crystal deposition in traumatized tissue in rabbit model. J Endourol 10: 513
19. Sarıca K, Süzer O, Yaman O, Küpeli B, Baltaci S, Bilaloglu E, Tasman S (1996) Leucine aminopeptidase enzymuria: quantification of renal tubular damage following extracorporeal shock wave lithotripsy. Int Urol Nephrol 28: 621
20. Sarıca K, Özer G, Soygür T, Yaman Ö, Özer E, Ustün H, Yaman LS, Gogus O (1997) Preservation of shock wave-induced renal histologic changes by dermatan sulphate. Urology 49: 145
21. Sarıca K, Türkölmez K, Koşar A, Alçığır G, Özdiler E, Göğüş O (1998) Evaluation of basal membrane antibody (immunoglobulin G) formation after high energy shock wave application in rats. J Endourol 12: 505

22. Sarıca K, Bakır K, Yağcı F, Topçu O, Akbay C, Sayın N, Korkmaz C (1999) Limitation of possible enhanced crystal deposition by Verapamil in renal parenchyma after shock wave application in rabbit model. J Endourol 13: 343
23. Strohmaier WL, Lahme S, Weidenbach PM, Bichler KH (1999) Reduction of high-energy shock-wave-induced renal tubular injury by selenium. Urol Res 27: 382
24. Thamilselvan S, Byer KJ, Hackett RL, Khan SR (2000) Free radical scavengers, catalase and superoxide dismutase provide protection from oxalate-associated injury to LLC-PK1 and MDCK cells. J Urol 64: 224
25. Willis LR, Evan AP, Connors BA, Blomgren P, Fineberg NS, Lingeman JE (1999) Relationship between kidney size, renal injury, and renal impairment induced by shock wave lithotripsy. J Am Soc Nephrol 10: 1753
26. Yaman Ö, Sarıca K, Özer G, Soygür T, Kutsal O, Yaman LS, Göğüş O (1996) Protective effect of Verepamil on renal histology following shock wave application in rabbit model. J Endourol.10: 329