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Effects of high-energy shock waves on the viable human kidney

Wolfgang Roessler[1], **Pia Steinbach**[2], **Heinz Nicolai**[1], **Ferdinand Hofstaedter**[2], **Wolf F. Wieland**[1]

[1] Abteilung für Urologie, Krankenhaus St. Josef, Landshuter Strasse 65, D-93053 Regensburg, Deutschland
[2] Institut für Pathologie der Universität, Universitätsstrasse, D-93042 Regensburg, Deutschland

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Summary. Between September 1990 and July 1991, we treated 17 patients with renal-cell carcinoma by radical nephrectomy and two patients with urothelial carcinoma of the kidney pelvis by ureteronephrectomy. Immediately after nephrectomy, perfusion of the kidneys with cold HTK solution was performed and the organs were kept in hypothermia of 8°C. The tumor-free parenchyma of the kidneys was treated 4 h later with shock waves of different energy levels in an experimental shock-wave system (Siemens Company, Erlangen). Light microscopy and examinations by scanning laser microscopy were performed after treatment. High-energy shock waves (HESW) produce significant changes in the tubulary and blood-vessel system of the viable human kidney, depending on the energy applied. Although our model is limited by hypothermia of the explanted kidneys, the effects of shock waves on the organs can be studied. Our model is suitable for testing the effects of different lithotriptors on the human kidney.

Key words: High-energy shock waves – Human kidney – Side effects

Extracorporeal shock-wave lithotripsy is a widely accepted method for treating calculi in the urinary collecting system. Many authors have suggested that the application of high-energy shock waves may cause tissue damage as a side effect of stone fragmentation. It is currently unknown whether the positive or negative component of the shock wave causes the calculus fragmentation, but the negative pressure seems to be responsible for the damaging effects on the surrounding tissues during therapy in the human kidney. Different authors have demonstrated various degrees of side effects after the application of high-energy shock waves (HESW) in animal models [1, 8, 10, 12–14]. Tissue damage and renal intraparenchymal hemorrhage seem to correlate with the energy density or the peak pressure in the focal area. To our knowledge, no author has yet demonstrated the acute histological effects of HESW on the human kidney after stone treatment. The purpose of our study was to determine the morphological effects of HESW on the viable human kidney.

Correspondence: W. Roessler

Patients and methods

From December 1990 to June 1991, we treated 17 patients with renal-cell carcinoma by radical nephrectomy and 2 patients with urothelial carcinoma of the kidney pelvis by ureteronephrectomy. Table 1 shows the age and sex distribution of the patients.

Immediately after nephrectomy (warm ischemic time, less than 1 min) the organs were perfused with 2000–3000 ml cold HTK solution. The standardized perfusion method has been described elsewhere [4, 7]. The composition of the solution is presented in Table 2. It is similar to the Euro-Collins solution and is used worldwide in liver, heart, and kidney preservation for organ transplantation. When the kidneys were bloodless and cold, they were stored for a maximum of 4 h in hypothermia of 8°C in polyvinyl bags filled with HTK solution.

Application of HESW was performed in an experimental electromagnetic shock-wave system (Siemens Company, Erlangen) by the delivery of 2000 shocks with energy-output levels of 15, 17, 19, and 21 kV at a frequency of 1 Hz. The corresponding shock-wave energy density is shown in Fig. 1. Pressure-field measurements were performed using a polyvinylidene difluoride (PVDF)-needle hydrophone (Imotec) connected with a 250-MHz oscilloscope. The temperature of the water was maintained at 8°C by a special pump system guided by a thermoregulator. The center of the focal area (4×40 mm) was determined by bidimensional laser-guided localiza-

Table 1. Age and sex distribution of 19 patients

Sex	*n*	Age (years)
M	13	Range, 61–83 Mean, 69.2
F	6	Range, 54–81 Mean, 64.5

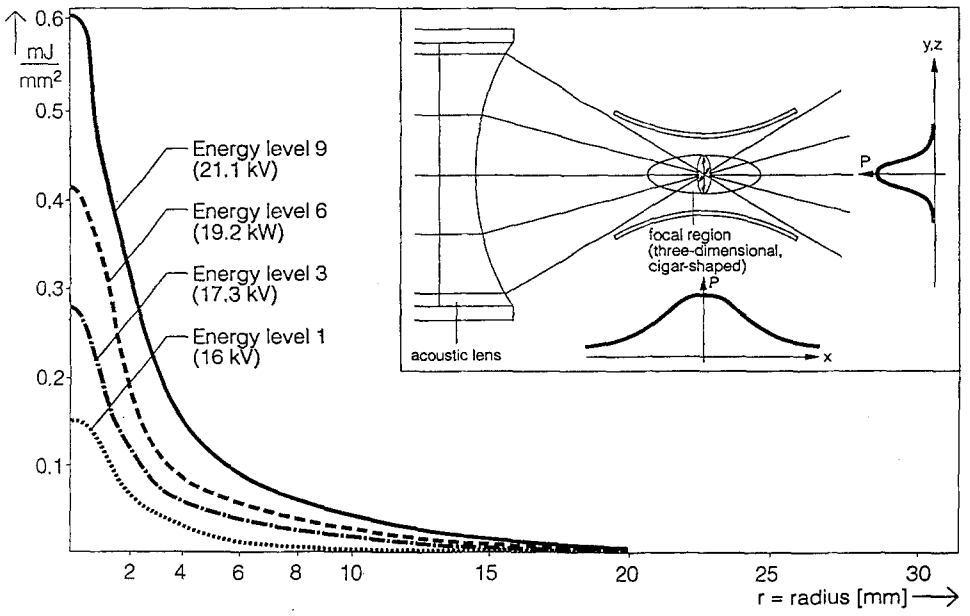


Fig. 1. Energy-output levels and corresponding high-pressure field levels (data given by Siemens)

Table 2. Composition of the HTK solution

a-Ketoglutarate	HTK solution of Bretschneider
Na^+	15 mmol/l
K^+	10 mmol/l
Mg^{2+}	4 mmol/l
Cl^-	50 mmol/l
Tryptophan	2 mmol/l
Ketoglutarate	1 mmol/l
Histidine/histidine-HCl	180/18 mmol/l
Mannitol	30 mmol/l
Osmolarity	310 mosmol/l
pH	7.3 (8°C)

tion. The polyvinyl bags filled with HTK solution and the kidneys to be treated affected the shock-wave parameters or pressure fields in no way as determined before the start of our HESW experiments.

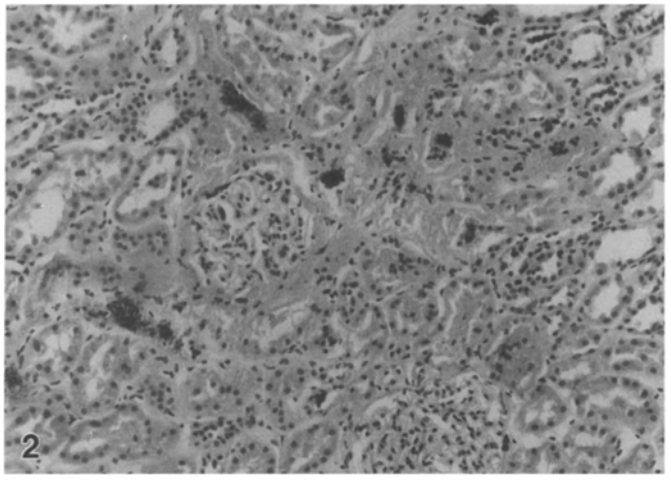
After treatment, histological examinations of the kidney using regions from the focal zone and controls were performed. Specimens from the focal area and controls were immediately fixed in 10% buffered formalin and marked by ink. Thus, the direction of the HESW source and the focal area could be exactly defined under light microscopy. A minimum of four specimens were investigated in each case (serial sections).

Unfixed specimens were used for scanning laser microscopy. The nuclear structure has been analyzed after staining with 2-[4-dimethylamino)styryl]-1-methylpyridinium iodide (DASPMI; Molecular Probes, Eugene, Ore., USA), a mitochondrial-specific dye [3]. In addition, nucleoli and nuclear membranes are brightly stained in human cells of different origin (Roessler et a., unpublished data). DASPMI was dissolved at 100 µg/ml in RPMI 1640 cell-culture medium (Seromed, Berlin) containing 10% fetal calf serum. Tissue slices (thickness, about 1 mm) were incubated for 1 h at 37°C in the dye solution, washed in phosphate-buffered saline (pH 7.2), and placed on slides. Subsequent analysis was carried out by a confocal scanning laser microscope (CSLM; Leica Lasertechnik, Heidelberg). Fluorescence was excited by an argon-ion laser (emission wavelength, 488 nm); the radiation emitted was collected at a wavelength of 515 nm. A 63×(NA 1.4) oil-immersion objective was used. To obtain comparable images, analysis was carried out with constant instrument settings except for the photomultiplier voltage (PMV). The PMV and focal plane were adjusted to obtain an optimal image of nuclear membranes within one section. Images of different focal planes except the uppermost plane (to avoid artefacts due to cutting) were recorded for subsequent morphological analysis.

Results

Histological changes in the normal human kidney parenchyma were mainly found in the tubular system and the mid-sized venous vessels. Within the focal area the tubular epithelial cells were focally destroyed. The nuclei were concentrated on specific points of the de-epithelized tubuli. The main target among these lesions was the convoluted tubuli. No change was seen in the glomeruli (Fig. 2). As mentioned above, the second target of HESW was the mid-sized vessels, especially arcuate veins (Fig. 3). Endothelial cells were broken apart from the intima and were sometimes found in dense clusters of aggregated cells. Both the tubular and the vascular lesions showed a patchy pattern. The area in which these lesions were found measured approximately 6×2×2 mm in diameter. The severity of the lesions was dependent on the energy used, but the size of the lesions was similar under all experimental conditions tested. The severity of the histological changes is shown in Table 3. We rated the changes as mild (+), moderate (++), or excessive (+++). As can be seen from the table, increasing energy-output levels increased the severity of histological events. These data conform well with the clinical experience in stone treatment by extracorporeal shock-wave lithotripsy.

Using scanning laser microscopy for cell-structure examinations in untreated specimens, we found distinct structures, probably tubuli, containing a number of clearly visible nuclei with a nuclear membrane and nucleoli (Fig. 4). In contrast, after HESW treatment the



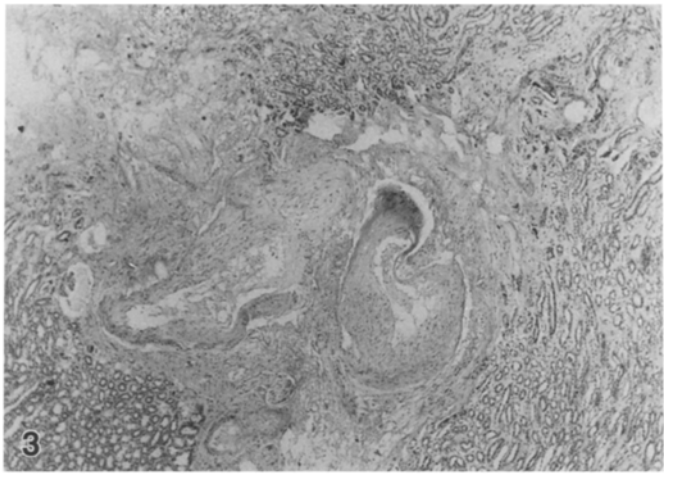


Fig. 2. Damage to the tubular epithelium with nuclear condensation (2000 shocks, 21 kV, H&E, ×100)

Fig. 3. Damaged blood vessels within the focus of HESW treatment (2000 shocks, 21 kV, H&E, ×30)

Table 3. Intensity of histological changes after the delivery of 2000 shock waves

kV	15	17	19	21	
Pressure (MPa)	16	32	50	65	
Shock-wave energy density (mJ/mm^2)	0.013	0.25	0.4	0.6	
+	3	4	2	0	
++	0	7	5	2	
+++	0	0	1	12	
Specimens (*n*)	3	11	8	14	36

Histological changes: +, mild; ++, moderate; +++, excessive

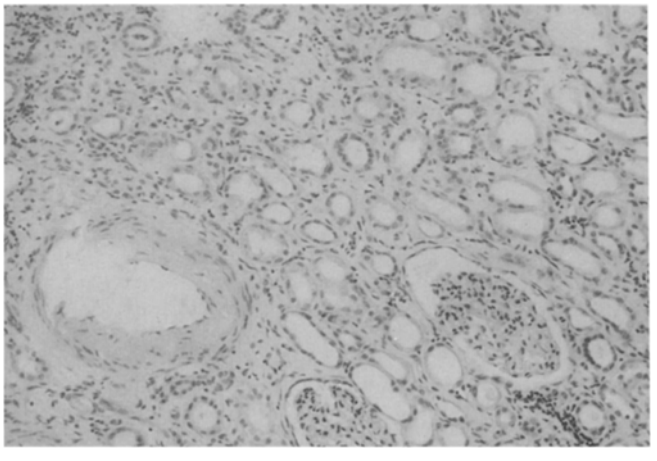
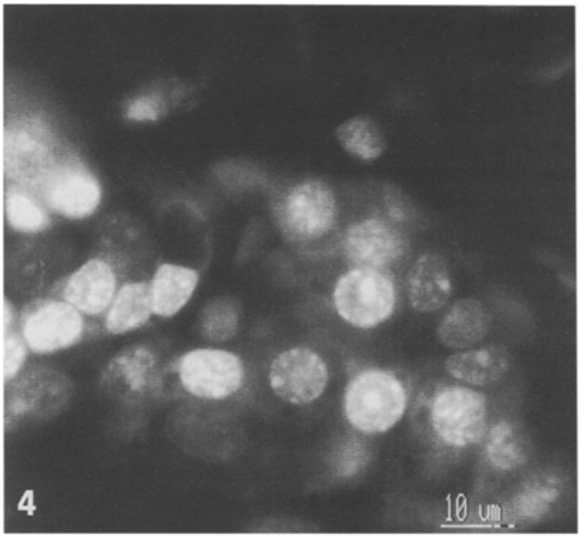


Fig. 6. Untreated control (H&E ×100)



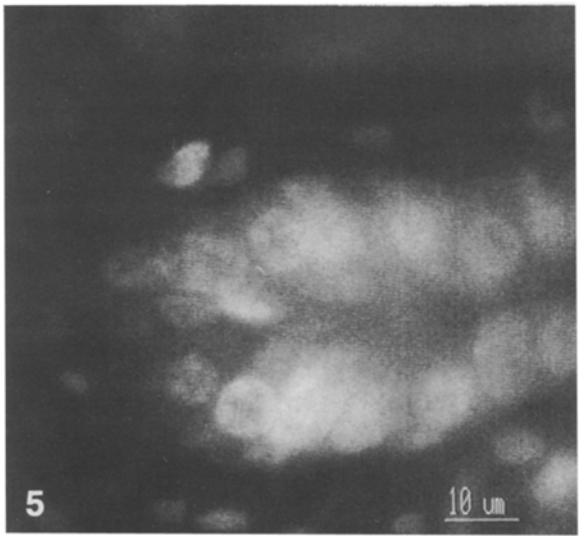


Fig. 4. Scanning laser microscopy: untreated specimen

Fig. 5. Scanning laser microscopy: 2000 shocks with an energy-output level of 19 kV

same structures were seen but nuclear staining was blurred, and no intranuclear structure was distinguishable (Fig. 5). In an untreated control (Fig. 6), no effect of perfusion or storage was detected, and the organs showed no morphological change.

Discussion

Many side effects of extracorporeal shock-wave lithotripsy (ESWL) have been described by other authors using a variety of animal models and shock-wave sources [1, 2, 5, 8, 10, 12–14]. The degree of renal trauma was dependent on the shock-wave numbers and the generator voltage. In our in vivo/in vitro model of the viable human kidney, we found dose-dependent destruction of the renal blood-vessel and tubulary system. The glomerular system seemed to be unaffected by shock waves. We suggest that the hematuria occurring after the application of HESW is induced by the described defects (loss of endothelium) of the blood vessels in the focal area. These microscopic findings have also been described by Ryan et al. [14], who found capsular hematomas and hemorrhages around focally necrotic arcuate and interlobar veins in a rabbit model after HESW exposure. Begun et al. [1] demonstrated shock-wave-induced renal injury in a porcine model. The predominant injury pattern was interstitial and perivascular fibrosis (resulting from vascular defects). Hematoma and interstitial hemorrhage after shock-wave exposure has also been described by Newman et al. [13] and Karlsen et al. [10]. These data correlate well with the microscopic changes observed in our in vitro model.

Our model also explains many other known side effects of ESWL on the human kidney, such as a decreased glomerular filtration rate [9], an increase in urinary protein excretion [6, 15], and an increase in levels of serum lactic dehydrogenase [9]. These findings indicate a proximal tubular dysfunction as a result of the treatment and reflect the general effects on the tissues traversed by the shock waves. Karlsen et al. [9] estimated the acute changes in renal function after extracorporeal stone lithotripsy as being moderate, with the tubular functional changes being more pronounced than the glomerular changes. Those observations concur well with our findings in the tubular and glomerular system in our model.

A possible relationship between ESWL and the development of hypertension in a small percentage of patients has been postulated [11, 16]. In our opinion, this may theoretically occur because of the described changes in the renal blood-vessel system. Further investigations concerning this issue have to be performed, and this matter is being evaluated in a multicenter study under the auspices of the Food and Drug Administration. Our examinations by scanning laser microscopy revealed another, new issue. Besides the described histological changes, we found lesions in nuclear membranes and nucleoli of the renal tissue around the focal area as described above. These lesions were not visible by light microscopy and we cannot yet interpret them. Maybe there is a "biological focal zone" with special changes in cell structure beside or around the known "physical focal zone" of high energy density. Further studies and examinations concerning this issue will be performed by our group.

We conclude that our model is useful in explaining the side effects of shock waves, and our findings conform well to previously reported clinical results. Even if one assumes that the target (stone) remains in the focal zone during the entire treatment and that the pressure behind the stone is reduced to a minimum, the effects of the shock waves on the renal tissue surrounding the target will likely be similar to those reported herein. Our in vitro model of the human kidney has to be limited by special "artificial circumstances": in contrast to our model, the kidneys "in vivo" are surrounded by fat and muscles, but shock waves are known to pass those tissues nearly unchanged with minimal absorption, and the focal zone is the area of highest energy density. No change occurs in the organs due to operative intervention, clamping of veins and arteries, or perfusion with HTK solution, as we could prove in our controls. Our described technique of organ explantation is used in kidney transplantation worldwide. Moreover, we performed our studies under hypothermic conditions. Many investigators believe that much of the acute renal injury caused by ESWL may be due to the warm ischemia caused by intrarenal edema and hemorrhage. These conditions are reduced by hypothermia in our described study design. However, the total amount of renal injury might be underestimated in our model.

We suggest that our model adds much to the current understanding of the acute effects of shock-wave lithotripsy. We think that it is (1) suitable for testing the effects of different lithotriptors on the human kidney and (2) suitable for studying the side effects of stone therapy by HESW.

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