Urol Res (1993) 21:279–282

Urological Research

Determination of the energy-dependent extent of vascular damage caused by high-energy shock waves in an umbilical cord model

P. Steinbach[1], **F. Hofstaedter**[1], **H. Nicolai**[2], **W. Roessler**[2], **W. Wieland**[2]

[1] Institut für Pathologie der Universität Regensburg, Franz-Josef-Strauss-Allee 11, D-93042 Regensburg, Deutschland
[2] Abteilung für Urologie, Krankenhaus St. Josef, Landshuter Strasse 65, D-93053 Regensburg, Deutschland

Received: 15 September 1992 / Accepted: 5 March 1993

Summary. To determine the spatial extent of shock-wave-induced vascular damage human umbilical cords were exposed to electromagnetically generated, focused ultrasound waves of different energy densities. During treatment macroscopically visible hematoma and superficial holes appeared. Following exposure specimens were fixed and examined histologically. In addition to vessel wall necrosis and rupture, complete detachment of endothelial cells in defined regions was observed. A correlation of the extent of the damage with the energy density distribution revealed that a local energy density of 0.3 mJ/mm^2 is the lower threshold for the occurrence of severe vascular damage.

Key words: Energy threshold – Shock waves – Umbilical cord – Vascular damage

High-energy shock waves (HESW) are routinely used in medical treatment for the disintegration of renal, ureteral or gallbladder stones [10]. In the last few years HESW have been applied to tumor cells in vitro and tumors in vivo to study the possibility of tumor treatment with the help of HESW [2, 9, 11, 12]. In particular, combined treatment with biological response modifiers (e.g. tumor necrosis factor alpha, TNFα) seems to be a promising new therapy modality; complete tumor regression was obtained in some xenograft models [8]. The reason for this synergistic effect is not known, but vascular damage is probably involved [13]. Hoshi et al. [5] and Brendel et al. [1] showed that HESW as a monotherapy induces vascular damage which may promote tumor necrosis. Recently, Smits [13] showed that vascular effects are more pronounced if shock waves are combined with TNF. Therefore, detailed knowledge about HESW-induced damage to the vascular system is of major interest in order to achieve a better understanding of the interaction between HESW and tissue. This ultimately lead to the development of the most effective tumor therapy modalities. The same knowledge may also help to prevent side effects in conventional lithotripsy.

In the present study we analyzed HESW-induced vascular damage in an umbilical cord model that allows an exact determination of the spatial extent of damage and its correlation with the given physical shock wave parameters (i.e. energy density distribution). As it is possible to give an energetic threshold for the occurrence of severe vascular damage these results may well be of some importance concerning the safety of stone treatment.

Correspondence to: Dr. P. Steinbach

Materials and methods

Umbilical cords

Human umbilical cords were clamped immediately after birth with the native blood inside the vessels. Until required for the study they were refrigerated at 8°C in 0.9% saline for 2–8 h. Coagulation of the blood could not be prevented completely but when removing the clamps after treatment a large amount of liquid blood ran out of the vessels. Occasional cultivation of endothelial cells according to the method of Jaffe et al. [6] was taken as an indicator of cell viability.

The use of immediately clamped umbilical cords should prevent the introduction of artificial cavitation nuclei and therefore resemble the in vivo situation at least from this point of view. As this seems to be of some importance we preferred the model system just described despite some obvious disadvantages (poorer reproducibility as compared with in vitro systems employing cultivated endothelial cells, inability to prevent completely the coagulation of blood).

Shock wave treatment

Shock waves were generated by an electromagnetic shock wave source kindly provided by Siemens (Erlangen, Germany). The source and lens are identical to those in the commercially available Lithostar Plus. Briefly, at the focal position positive pressures range from 20 to 60 MPa, and negative pressures from 5 to 10 MPa. With increasing distance from this point the shock wave parameters

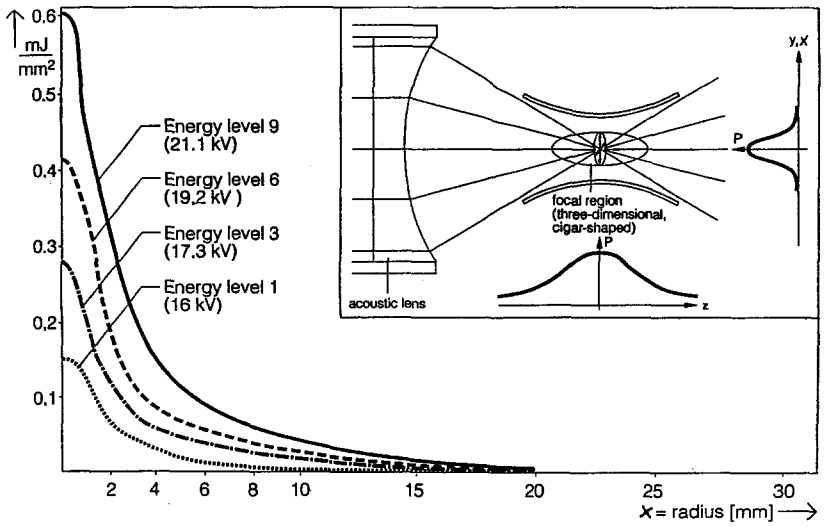

Fig. 1. Energy density distribution in the focal plane perpendicular to the acoustical axis (diagram provided by Siemens Company, Erlangen)

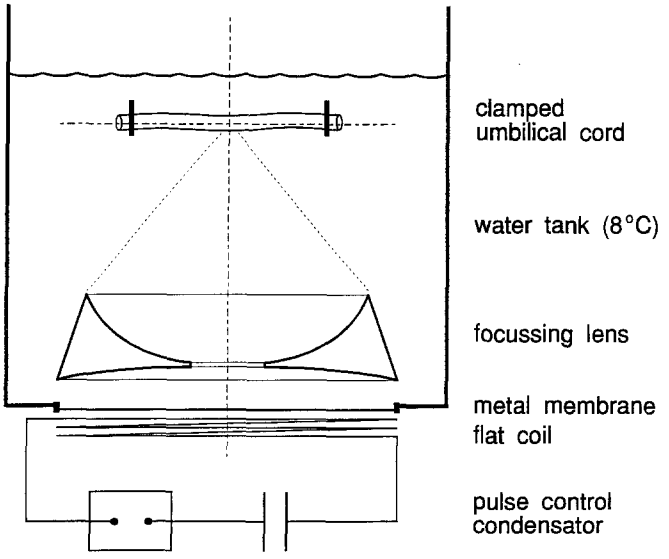

Fig. 2. Schematic view of the experimental set-up used to expose umbilical vessels to shock waves

change and the corresponding energy density decreases markedly (Fig. 1). For further details see Folberth et al. [3]. Shock wave source and lens are directly coupled to a $35 \times 35 \times 35\,cm^3$ water bath equipped with a thermostat. Exposure was performed in water at 8° C with the clamped umbilical cords perpendicular to the acoustical axis as shown in Fig. 2; focussing was performed on one of the three umbilical vessels by means of two crossing laser beams marking the focal point.

Two thousand pulses with focal energy densities of 0.2 (energy level 1), 0.4 (energy level 6) or $0.6\,mJ/mm^2$ (energy level 9) were administered. The pulse repetition frequency was 2 Hz. After treatment the position of the focus was marked with a piece of thread stitched carefully through the outer periphery of the umbilical cord.

Histologic sections

Specimens were fixed in 10% phosphate buffered formalin for at least 12 h and cut into 5 mm slices, the stitch being in the center of one slice (= focal slice); from each side of the focal slice three further slices were prepared. All slices were embedded in paraffin and 5 µm sections were stained with hematoxylin and eosin. The focal slice was routinely cut into 30–50 equally spaced sections. Where necessary, neighboring slices were treated identically. All sections were analyzed microscopically for defects of the exposed vessel to determine the spatial extent of damage.

Results

Macroscopically visible hematoma (Fig. 3a) always occurred after treatment with energy level 9, and in 6 of 12 cases after exposure to energy level 6. In addition to these subepithelial lesions, in many cases we found small (about 1 mm in diameter) superficial holes (Fig. 3b). These occurred either at the entrance or the exit site of the shock waves. No clear-cut correlation with the direction of the ultrasound beam could be detected. No macroscopic defects were visible after treatment with energy level 1.

On a microscopic level hematoma was associated with vessel wall necrosis (Fig. 4a) or even rupture of the wall (Fig. 4b). In addition to these gross effects it was obvious that endothelial cells were missing in distinct areas along the intima as demonstrated in Fig. 5. This was common to all sections where any definite indication of HESW-induced damage could be detected. Therefore, this was taken as the most characteristic lesion and was thus evaluated quantitatively with respect to its spatial dimension.

The line of missing endothelium around the perimeter of the vessel was strongly dependent on the applied focal intensity. Similarly, the expanse along the vessel, i.e. in longitudinal direction, was governed by the focal energy density (Table 1). It is interesting to compare the two different areas of damage (at energy levels 6 and 9) in longitudinal direction with the respective intensity distribution curves. At energy level 6 the damage extends up to the point $x_1 = 0.85$ mm; at energy level 9 the distance is $x_2 = 2.25$ mm, as shown in Table 1. Comparing the values of the energy density distribution of level 6 at x_1 and of level 9 at x_2 we find $0.3\,mJ/mm^2$ in both cases. Thus this value can

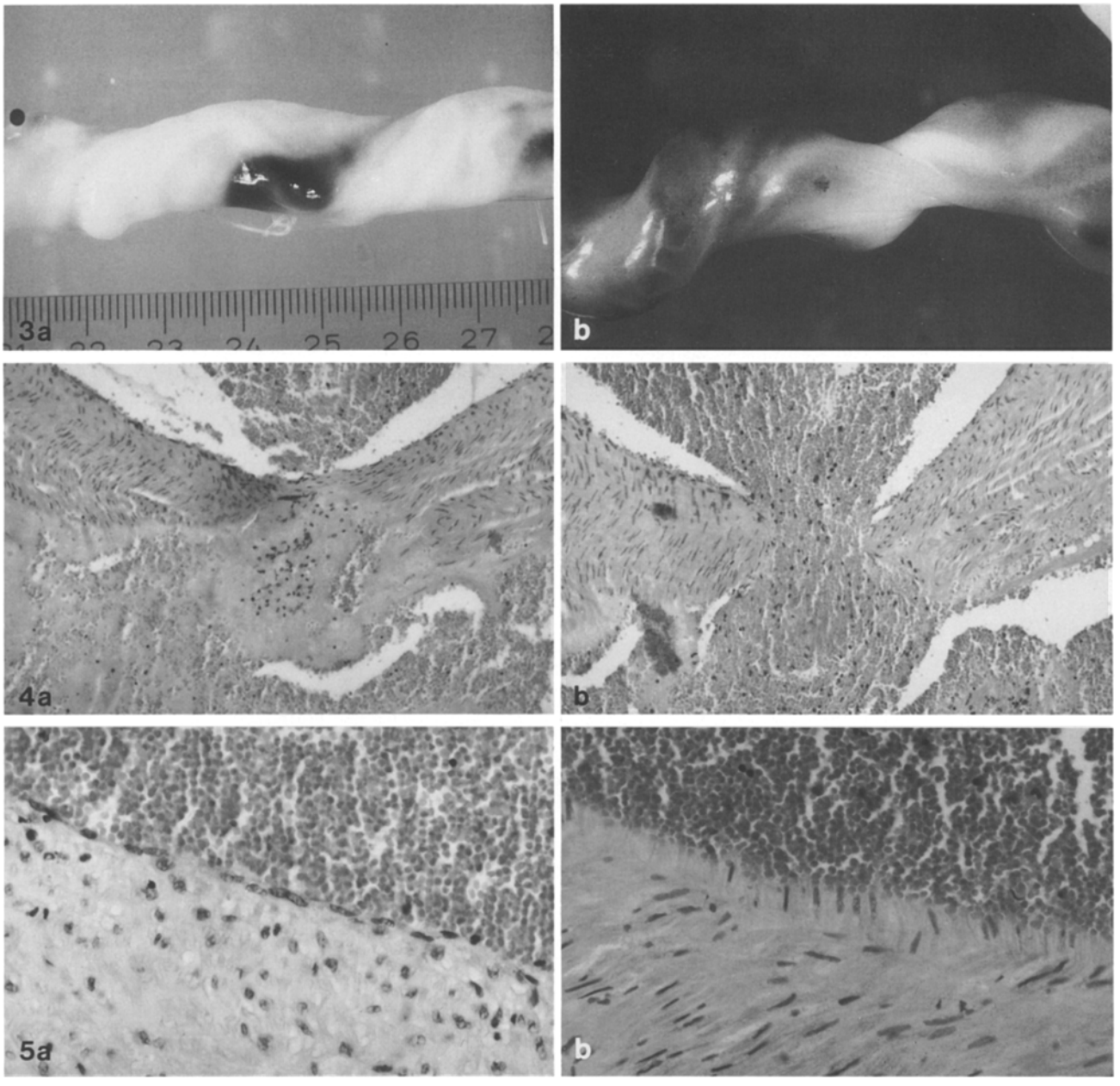

Fig. 3a, b. Macroscopically visible shock-wave-induced lesions: *a* hematoma; *b* superficial hole. For details see text

Fig. 4a, b. Shock-wave-induced defects of the vessel wall: *a* vessel wall necrosis; *b* rupture of the wall. (Objective magnification ×10)

Fig. 5a, b. Characteristic lesion after shock wave treatment: *a* intact endothelium (far outside the focal area); *b* intima without endothelium after treatment with a local energy density exceeding 0.3 mJ/mm^2. (Objective magnification ×40).

be termed the absolute energetic threshold, as it is the *local* intensity below which no HESW-induced damage occurred in our model system. This explains the absence of damage after treatment with energy level 1, where the maximum, i.e. focal, intensity is only 0.2 mJ/mm^2.

Discussion

The primary aim of the present study was the determination of the spatial extent of damage to intact human vessels and its correlation with physical shock wave parameters. For the first time (to the knowledge of the authors) an exact area of shock-wave-induced vascular damage with respect to the applied energy density distribution could be determined. Based on these data an absolute energetic threshold (i.e. the value of the *local* energy density) for the occurrence of shock-wave-induced damage could be defined. Energy density is a physical quantity that can be defined independently of a specific shock wave source. Thus, these results should be applicable to other apparatus with different focal areas if the physical parameters of the pulses (e.g., rise time, peak height, negative pressure) are comparable. This restric-

Table 1. Frequency and extent of HESW-induced vascular damage after exposure to 2000 shock waves of various focal energy densities

Focal intensity (mJ/mm^2)	No. of umbilical cords showing vascular damage	Extent of endothelial lesions in the longitudinal direction (mm)	Endothelial lesions around the perimeter of the vessel (% of perimeter)
0.2 (level 1)	0/10	–	–
0.4 (level 6)	7/13	1.7 ± 0.8 ($n = 5$)	24 ± 15
0.6 (level 9)	9/9	4.5 ± 1.3 ($n = 8$)	60 ± 30

All values are the mean ± SD; they are significantly different at the $P < 0.05$ level (*t*-test);
n, Number of umbilical cords used for quantitative evaluation

tion has to be made unless it is known whether or not these parameters affect tissue damage. As far as the destruction of concrements is concerned it has been shown by Köhler et al. [7] that peak height and pressure rise time have no influence on the fragmentation efficacy, but corresponding data do not exist for tissue.

Knowledge of the spatial dimension of a specific HESW-induced lesion may have an impact on the future treatment modalities of a larger tumor. If its size exceeds the range of the effective shock wave energies a scanning system should be applied. The results of the present study should be taken as a basis for extending these type of studies to tumors in vivo. In particular, in the latter case a distinction will need to be made between primary shock wave effects (that would be comparable to the effects presented here) and secondary effects (due to the reaction of the tumor-bearing organism to the primary damage).

The most characteristic primary lesion occurring in our model system was the complete detachment of endothelial cells in an area exposed to local energy densities higher than $0.3\,mJ/mm^2$. In an in vivo system this defect would cause thrombosis, probably resulting in occlusion of the vessel. In a tumor vessel hypoxia and ultimately death of tumor cells may result. This is in accordance with an increased sensitivity of strongly vascularized tumors to HESW [13]. The highly synergistic effect of shock waves and TNF may be explained by enhanced damage to the tumor vascularization. The cause might be the direct cytotoxic effect of TNF to endothelial cells [13]. However, to gain a better understanding of these phenomena an analysis of endothelial damage at a cellular level would be of major interest. Such experiments are currently in progress in our laboratory.

Little is known about the physical mechanisms underlying tissue and vessel damage. Direct, cavitational or streaming effects may be considered. Direct effects are mechanical forces due to the particle velocity and displacement during the propagation of a longitudinal wave. They will be particularly effective at a boundary layer between materials of different acoustic impedances [14]. In the present case this would mainly be the layer of endothelial cells between the blood (impedance = $16.6 \times 10^5\,kg/m^2s$) and the vessel wall (impendance = $15.6 \times 10^5\,kg/m^2$) [4], which is in accordance with our experimental results. Cavitational effects occurring within the liquid (i.e., blood) might damage the endothelial cells and the vessel wall as a result of the emission of secondary shock waves, high-speed jet streams or formation of radicals. However, the loss of endothelial cells might well be explained by mechanical effects alone. HESW-induced streaming arises due to the propagation of a pressure wave in a viscous medium (in this case blood). As a result of friction, damaging forces might act on the endothelial cells leading to their detachment.

Any of the abovementioned effects alone or in any combination could be the cause of HESW-induced vascular damage. Further experiments are required to achieve a better understanding of the interaction between shock waves and tissue.

Acknowledgements. The authors would like to thank Sigrid Appel and Beate Rothgangel for excellent technical assistance. We acknowledge the support of this work by Siemens Company, Erlangen, Germany.

References

1. Brendel W, Delius M, GoetzA (1987) Effects of shock waves on the microvasculature. Progress in applied microcirculation, vol 12. Karger, Basel, p 41
2. Dongen JW van, Steenbrugge GJ van, Romijn JC, Schröder FH (1989) The cytocidal effect of high energy shock waves on human prostatic tumour cell lines. Eur J Cancer Clin Oncol 25:1173
3. Folberth W, Köhler G, Rohwedder A, Matura E (1992) Pressure distribution and energy flow in the focal region of two different electromagnetic shock wave sources. J Stone Dis 4:1
4. Goss SA, Johnston RL, Dunn F (1974) Comprehensive compilation of empirical ultrasonic properties of mammalian tissues. J Acoust Soc Am 64:1640
5. Hoshi S, Orikasa S, Kuwahara M, Suzuki K, Yoshikawa K, Saitoh S, Ohyama C, Satoh M, Kawamura S, Nose M (1991) High energy underwater shock wave treatment on implanted urinary bladder cancer in rabbits. J Urol 146:439
6. Jaffe EA, Nachman RL, Becker CG, Minick CR (1973) Culture of human endothelial cells derived from umbilical veins: identification by morphologic and immunologic criteria. J Clin Invest 52:2745
7. Köhler G, Schätzle U, Granz B (1991) Fundamentals for the development of a lithotripter of the new generation. Eur Urol 20:327
8. Oosterhof GON (1990) High energy shock waves in urological tumors. Thesis, Nijmegen
9. Randazzo RF, Chaussy CG, Fuchs GJ, Bhuta SM, Lovrekovich H, deKernion JB (1988) The in vitro and in vivo effect of extracorporeal shock waves on malignant cells. Urol Res 16:419
10. Reichenberger H (1988) Lithotripter systems. Proc IEEE 76:1236
11. Russo P, Stephenson RA, Mies C, Huryk R, Heston WDW, Melamed MR, Fair WR (1986) High energy shock waves suppress tumor growth in vitro and in vivo. J Urol 135:626
12. Russo P, Mies C,Huryk R, Heston WDW, Fair WR (1987) Histopathologic and ultrastructural correlates of tumor growth suppression by high energy shock waves. J Urol 137:338
13. Smits GAHJ (1992) High energy shock wave induced biological effects in different tumor models. Thesis, Nijmegen
14. Steinbach P (1992) Cellular effects of ultrasound shock waves (in German). Thesis, Regensburg