

Biological Effects of Two Successive Shock Waves Focused on Liver Tissues and Melanoma Cells

J. BENEŠ¹, P. ŠUNKA², J. KRÁLOVÁ³, J. KAŠPAR⁴, P. POUČKOVÁ¹

¹First Faculty of Medicine, Charles University, Prague, ²Institute of Plasma Physics, Prague, ³Institute of Molecular Genetics, Prague, ⁴Faculty of Biomedical Engineering, Czech Technical University, Prague, Czech Republic

Running title:
Biological Effects of Shock Waves

Summary

A new generator of two successive shock waves focused to a common focal point has been developed. Cylindrical pressure waves created by multichannel electrical discharges on two cylindrical composite anodes are focused by a metallic parabolic reflector – cathode, and near the focus they are transformed to strong shock waves. Schlieren photos of the focal region have demonstrated that mutual interaction of the two waves results in generation of a large number of secondary short-wavelength shocks. Interaction of the focused shockwaves with liver tissues and cancer cell suspensions was investigated. Localized injury of rabbit liver induced by the shock waves was demonstrated by magnetic resonance imaging. Histological analysis of liver samples taken from the injured region revealed that the transition between the injured and the healthy tissues is sharp. Suspension of melanoma B16 cells was exposed and the number of the surviving cells rapidly decreased with increasing number of shocks and only 8 % of cells survived 350 shocks. Photographs of cells demonstrate that even small number of shocks results in perforation of cell membranes.

Keywords

Focused shock waves • Double shocks • Cavitation collapse • Secondary shocks • Liver injury

Introduction

Extracorporeal shock waves are microsecond acoustic pressure pulses with peak pressures of 400-1200 atmospheres (Delius and Adams 1999). Shock wave treatment was introduced by Chaussy *et al.* (1980) and it is the treatment of choice worldwide for urinary tract stones and a treatment alternative for most other types of stones in the human body, such as gallstones and pancreatic and salivary duct stones (Delius 1994). Since the cancer tissue does not differ acoustically from the health tissue, localized action of the shock waves in such acoustically homogeneous medium is generally attributed to cavitations produced by the rarefaction wave. Collapsing cavitations create strong secondary shock waves of tens of nanosecond in duration that damage cell membranes. Local thermal effects accompanying cavitation collapse and production of chemical radicals may also play some role in the cell killing. To enhance the effect of cavitations, gas bubbles or cavitation nucleation agents have been injected into the studied tissues and only in such cases an improved therapeutic effect has been observed (Yu et al. 2004). Interaction of focused shock waves with soft tissues has been studied as a possible method for an extracorporeal treatment of some kind of cancers (Muller and Song 2004).

We have developed a novel method for generation of focused shock waves where the primary pressure wave is formed by a multi-channel discharge on a composite anode immersed in water with an increased electrical conductivity. The cylindrical composite anode consists of a metallic cylindrical electrode (60 mm in diameter, 100 mm long) covered with a thin (0.2-0.3 mm thick) porous ceramic layer. At the applied voltage of 30 kV a large number of short discharge channels distributed homogeneously on the anode surface are initiated (Šunka 2001). Each discharge channel creates a semi-spherical pressure wave, and by superposition of all of the waves a cylindrical pressure wave propagating from the anode is formed. The cylindrical pressure wave is focused by a metallic parabolic reflector (cathode)

and near the focus it is transformed into a strong shock wave. In such arrangement we are able to produce either a single shock wave that overcomes the cavitation level near the focal point, or two successive shock waves focused to a common focal point. The first wave produces some acoustic inhomogeneity and cavitations on which the second, main, shock wave dissipates. The amplitude of the pressure wave reached 100 MPa. The rarefaction wave at an amplitude of 20 – 25 MPa produced cavitations (Šunka *et al.* 2004).

By varying the time delay between the two discharges we were able to study the interaction of the second wave with the inhomogeneity and cavitations created by the first wave. The aim of the experiments was to demonstrate the toxic effect and to localize the action of the shock waves propagating in an originally acoustically homogeneous medium. We exposed rabbit liver tissues for spatial demonstration of tissue necrosis. In a second experiment we exposed B16 melanoma cells *in vitro* and after exposure the cells were injected into mice.

Methods

The arrangement of the present experiment is schematically shown in Fig.1. A cylindrical composite anode consists of two insulated parts (A1- \varnothing 60 \times 70 mm, A2- \varnothing 77 \times 20 mm) energized from separate pulse power supplies that can be switched on with a different time delay. If they are switched on simultaneously, the shock wave from the larger diameter anode A2 reaches the focus 5 μ s before the wave from the smaller diameter anode A1. The pulse power supplies consist of low inductive capacitors charged up to 30 kV and two triggered spark gaps. The generator can produce either a single shock wave or two successive waves focused on a common focal point. The amplitude of the shocks at the focus can be varied by the surface areas of the anodes and by parameters of the discharge circuits. The generator was placed in a tank where the conductive liquid was separated from the experimental volume containing distilled water by an acoustically transparent membrane.

Schlieren photography was used for visualization of the pressure field in the focal region. Temporal courses of the focused shock waves were measured by polyvinylidene fluoride (PVDF) shock gauges.

B 16 melanoma syngenic cells were grown in a tissue culture. In each ampoule, 10^7 tumor cells were situated in 1 ml of PBS. Individual ampoules were exposed to the effects of acoustic shock waves. Immediately after the exposure, a sample of exposed cells was taken from each ampoule and transferred into a tryptophan blue solution for the determination of percent survival of the cells.

Injury of soft tissues of experimental animals induced by the shock waves was identified *in vivo* by the magnetic resonance imaging. The rabbits were anesthetized, depilated on their abdomen and exposed to 1200 double shocks with a fixed time delay between the waves of 5 μ s. The focus of the shock waves was targeted into the rabbit's liver. On the next day and 6 days after the exposure the rabbits were examined by magnetic resonance imaging. Thereafter, they were sacrificed and histological analysis of the injured samples of the liver was performed. The settings of MR images were found empirically, looking for a compromise between MR image quality and acquisition time. Despite the narcosis, the rabbit body movement causes several artifacts during the long imaging sequence. Special body fixation was applied for best results. The picture is taken in transverse plane; field of view (FOV) is 20 \times 20 cm.

Results

The first stage of the study of shock wave effects was targeted at studying the effects on B16 melanoma cells. We found (Fig. 1.B,C) that the shock waves damage tumor cells depending on the number of shocks the tumor cells were exposed to *in vitro*. If the baseline number of cells was 6 million, then the numbers of surviving cells were of 4 million after 90 shocks, 3.5 million after 190 shocks, less than 2 million after 270 shocks, and about half a million after 350 shocks. Higher numbers of administered shocks induced a nearly almost

complete destruction of all the tumor cells. The cell suspension exposed to 600 shock waves was subcutaneously transplanted to C57B16 mice. In comparison with the intact controls, the latency period in the experimental group was extended by 13 days and the tumor growth was delayed. The differences were statistically significant.

Interaction of the focused shock waves with soft animal tissues was studied *in vivo* on laboratory rabbits. An example of the MRI scan of the rabbit is shown in Fig. 2. The MRI machine was SISCO 85 with the field strength of 2 T. Classical Spin Echo method, the most suitable for measuring tissue properties, was used. Scans were taken at 2-mm steps. Injury of the liver tissue and partially also the front wall of the stomach are apparent (Fig. 2a). The scans revealed that the injury extends 15 mm, roughly according to the breath excursions. The destroyed tissue is clearly visible as a bright round area. The impact is presumably followed by edema (Fig. 2b on the left). After two weeks, the edema disappeared; the impact is obvious as a small bright point (Fig. 2b on the right). Histology of the injured liver tissue was done 6 days after the exposure. The injured necrotic tissue is sharply separated from the surrounding healthy tissue. The large number of leukocytes accumulated at the boundary of the injured tissue displays the immunity reaction to the injury.

Our results demonstrate the mechanical toxicity of two bound couple shocks but there is still insufficient knowledge concerning possible dissemination of tumor cells into the body.

Conclusions

A generator of two successive shock waves focused on a common focal point was developed. Interaction of the second wave with the inhomogeneity created in water by the first wave results in strong attenuation of the second wave and in generation of a large number of secondary short wavelength shocks. Local injury of the rabbit liver induced by the shock waves was demonstrated by magnetic resonance imaging. A very sharp boundary between the injured and health tissues was shown by their histological analysis.

Shock waves damage B16 melanoma tumor cells, the damage being dependent on the number of shocks, the cells are exposed to *in vitro*. If the exposed tumor cells are transplanted to inbred C57B16 mice, the latency period of the tumor growth is prolonged and the tumor growth is also slowed down compared with the controls.

Acknowledgement

This work was supported by the Grant Agency of the Czech Republic under contract No. 202/05/0685 and MSM 0021620808

References

- CHAUSSY C, BRENDDEL W, SCHMIEDT E.: Extracorporeally induced destruction of kidney stones by shock waves. *Lancet*, **ii**: 1265–1268, 1980.
- DELIUS M: Medical applications and bioeffects of extracorporeal shock waves. *Shock Waves* **4**: 55–72, 1994.
- DELIUS M, ADAMS G: Shock wave permeabilization with ribosome inactivating proteins: A new approach to tumor therapy. *Cancer Res* **59**: 5227–5232, 1999.
- MILLER DL, SONG JM: Lithotripter shock waves with cavitation nucleation agents produce tumor growth reduction and gene transfer in vivo. *Ultrasound Med Biol* **28**: 1343–1348, 2002.
- ŠUNKA P: Pulse electrical discharges in water and their applications. *Physics of Plasmas*, **8**: 2587–2594, 2001.
- ŠUNKA P, BABICKÝ V, ČLUPEK M, BENEŠ J, POUČKOVÁ P: Localized damage of tissues induced by focused shock waves, *IEEE Trans Plasma Sci* **32**: 1609–1613, 2004.
- YU TH, WANG GY, HU K: A microbubble agent improves the therapeutic efficiency of high intensity focused ultrasound: a rabbit kidney study, *Urol Res* **32**: 14–19, 2004.

Author for correspondence:

Jiří Beneš, First Medical Faculty, Charles University, U Nemocnice 2, Prague 2, Czech Republic; e-mail: benesji@volny.cz.

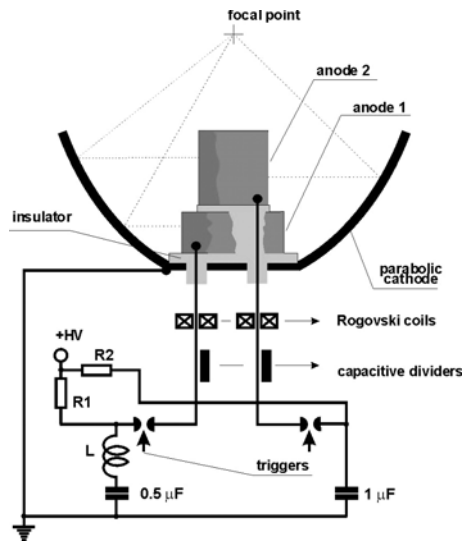


Fig. 1A. Schematic arrangement of the experiment.

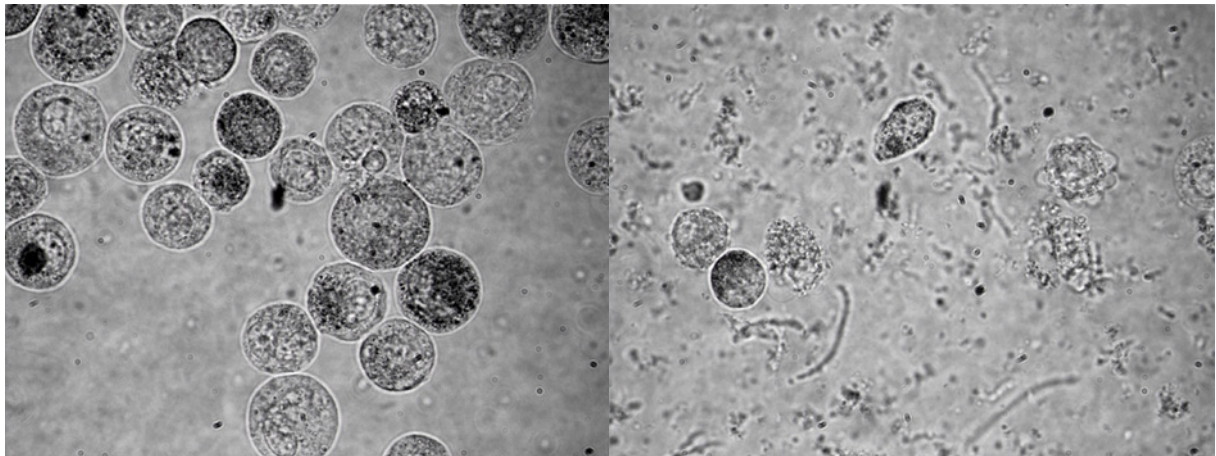


Fig. 1B. Left: control, non-exposed cells; right: B16 melanoma cells exposed to 270 shocks, after which the tumor cells are almost completely destroyed.

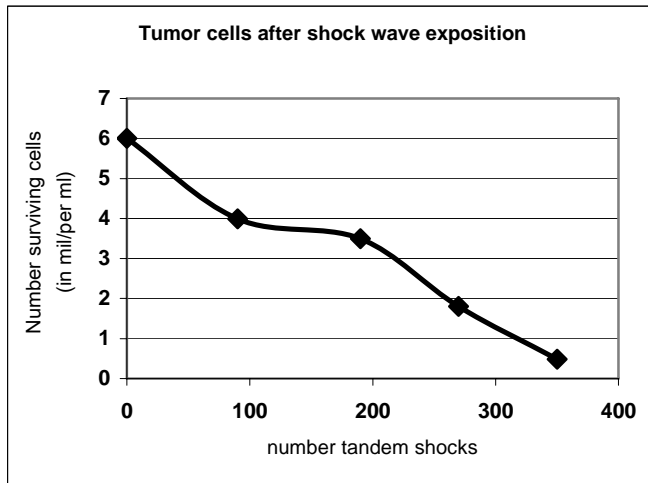


Fig. 1C. The figure shows numbers of surviving tumor cells after their exposure to 90, 190, 270 and 350 shocks.

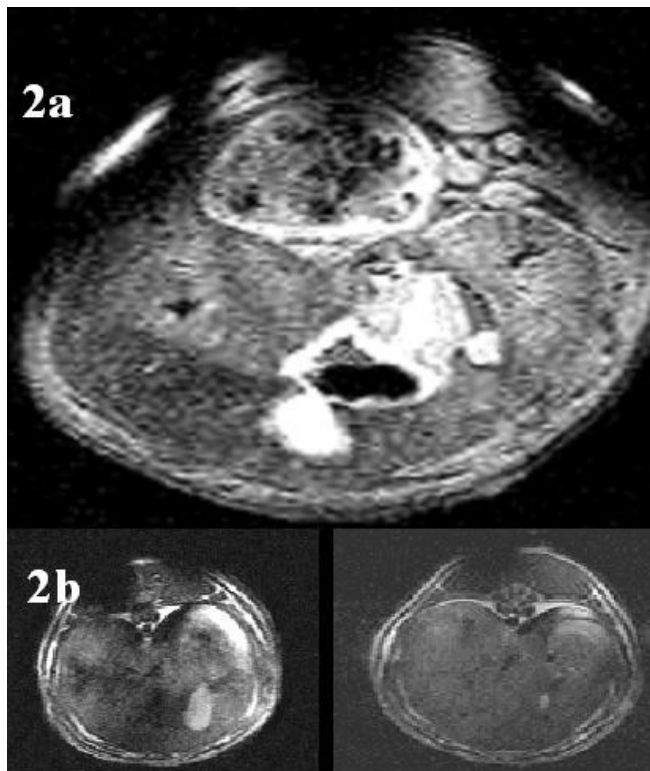


Fig. 2.a. *Ex vivo* images of rabbit liver after shock-wave impact. Nonstandard image contrast is observed due to cooled rabbit body. FOV = 20cm, TR = 0.7 s, TE = 0.013 s. Tomography magnetic field 2 T **b.** In vivo MRI scan of rabbit liver after shock-wave impact. Left: snapshot immediately after the impact; right: two weeks later. FOV = 20 cm, TR = 0.7 s, TE = 0.013 s. Tomography magnetic field 2 T.