# Application of NMR-Tomography and Histological Analysis to Study Cavitation Processes Induced by Ultrasonic Shock Waves in Biological Objects

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Acoustic shock wave stimulation induces cavitation and changes in the tumor tissue, which can be detected by NMR-tomography and pathohistologic examination.

**Key Words:** *acoustic shock waves; cavitation; tumor; NMR-tomography*

The apparatuses generating the focused acoustic shock waves (ASW) are widely used in medicine. They were also applied in the treatment of tumors, in particular in combination with cytostatic agents [3,7]. Localization of the effect of ASW within the limits of focal area requires strict control and visualization of the results of acoustic stimulation. An orienting parameter for such control is the phase shift of the signal caused by temperature rise (to 90°C) in the focus of a radiator [5]. However, ASW induce short-term and insignificant local changes in temperature, because energy dissipation in the shock waves is negligible, while the secondary processes generated by the waves (primarily, cavitation) decay during milliseconds. In this case, it is more informative to record the changes in tissue structure caused by acoustic stimulation. NMR-tomography provides unique possibilities of precise contrast visualization of soft tissues. It can also be used to aim the focuser and to control changes produced in tissues by acoustic stimulation.

Our aim was to study *in vivo* the results of ASW stimulation of tumors using NMR-tomography combined with histological control.

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## MATERIALS AND METHODS

The study was carried out on mice intramuscularly inoculated with Krebs-2 tumor 1 week before the experiment. The mice were narcotized with ether sulfate and subjected to 10 ASW pulses (Fig. 1) in such a way that the focal area was inside the tumor. After stimulation the mice were placed in the receiver coil of a microtomographic transducer of an MSL-300 NMR spectrometer with a diameter of 25 mm. NMR-tomographic recording was performed by the methods of spin and gradient echo with the following parameters: 30×30 mm visual field, 1 mm section width, 0.1×0.1 mm plane resolution. The repetition period of the pulse series was 100 msec and 3 sec for gradient and spin echo, respectively. Recordings were performed 15, 45, and 60 min after ASW stimulation. Due to the difficulties of mouse positioning in the transducer with 1-mm accuracy, the site in Fig. 2, *a* is somewhat shifted relatively to the site in Fig. 2, *b-d*, although the non-stimulated tumor core is quite homogeneous and produces signals of high intensity.

After recordings the mice were sacrificed, the tumor was analyzed by pathohistologic methods. The tissue was fixed and embedded in paraffin and 5-7-μ histological sections were stained with hematoxylin and azure-eosin. The histological data were compared with the results of NMR-tomography. Histological examination was performed for non-radiated control

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specimens and for experimental specimens taken 1 h and 1 week after exposure.

## RESULTS

Characteristic darkening corresponding to radiator focal zone (circle in Fig. 2) is clearly visualized by gradient echo method. Then the light areas appear in the focal zone due to capillary rhexis and microhematoma. Since the object visualized by gradient echo tomogram is characterized by a high density and the short $T_1$=300±50 msec and $T_2$=80±30 msec (measured by multiecho and inversion-reconstruction methods [2]), the light areas can correspond to fluid with large relaxation time, which explains saturation and attenuation of the signal. They can be also underlain by appearance of local heterogeneity and/or a decrease in the amount of substance in this area. To test the hypothesis on the appearance of free fluid, we recorded spin echo tomograms with echo period of 80 msec and the train repetition time 3 sec. However, the signal was practically absent in the $T_2$-weighted spin echo in NMR-tomograms, which attests to the absence of the free liquid in the focal area. Therefore, darkening in the focal area is presumably related to the local heterogeneities produced by structural changes in the specimen.

The histological section obtained after stimulation demonstrated wide necrotic areas with hemorrhagic sites. The tumor cells were mostly destroyed, being at various stages of dystrophic and necrobiotic processes. Round-cell inflammatory infiltration was moderately expressed. There were cavities at the boundary of the preserved tumor tissue and necrotic area presumably produced by cavitation bubbles generated by acoustic stimulation. The role of cavitation in biological tissues is disputable [1,4,6]. Most often cavitation bubbles were observed at the boundary of tissues that have different density. The ASW-treated specimens had cavities with a diameter of tens microns (Fig. 3, *a, b*). avitation in the focal area generates expanding bubbles. However, their growth is arrested by high viscosity and large energy dissipation in the medium. Then intercellular fluid fills the bubbles. There are data on similar effects produced by powerful ultrasonic radiation focused in biological tissues [4,6], where such formations were called as "pseudobubbles", because they were only traces left by real gaseous bubbles.

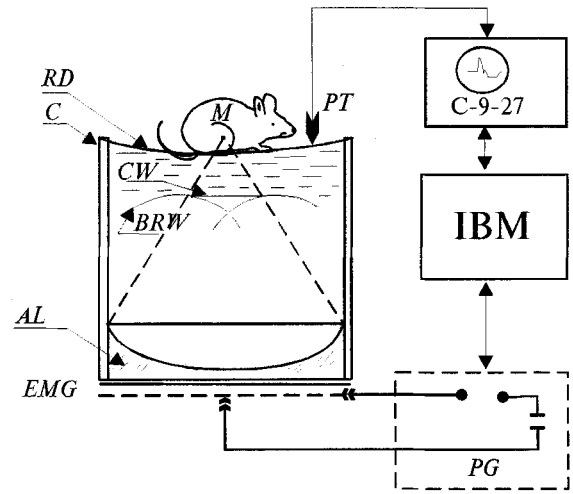


**Fig. 1.** Experimental setup. *M*: test mouse, *C*: cuvette with water for testing media and objects, *RD*: rubber diaphragm, *EMG*: electromagnetic generator producing acoustic shock waves, *AL*: acoustic lens, *CW*: compression wave, *BRW*: boundary rarefaction waves, *PG*: power generator, *PT*: pressure transducer.

At smaller magnification the specimens demonstrated relative homogeneity of tumor tissue before stimulation (Fig. 3, *a*). It was characterized by development of polymorphocellular sarcomatous tumor that infiltrated the muscle tissue. The tumor cells had the signs of polymorphism with giant abnormal nuclei and the nuclei with pronounced hyperchromatism. There was lysis of muscle tissue and destruction of the bone laminae.

Histological analysis of the tumor tissue from the ASW focal area, as well as their intravital tomograms demonstrated the development of cavitation and destruction processes induced in biological media and solutions by weak ASW. Thus, it is possible to record

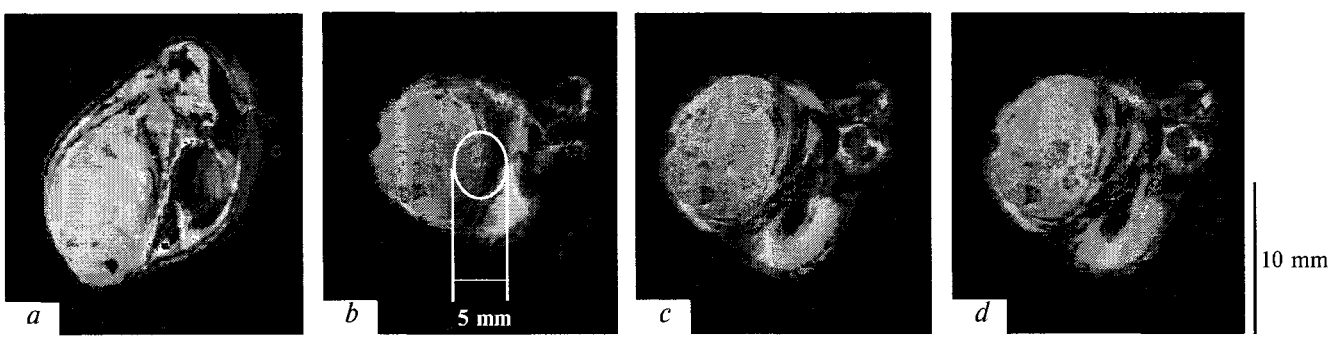


**Fig. 2.** NMR-tomography (gradient echo) in a mouse with inoculated Krebs-2 tumor before (*a*) and 15 (*b*), 45 (*c*), and 60 min (*d*) after 10 shock wave pulses; *b*) damaged area is encircled.

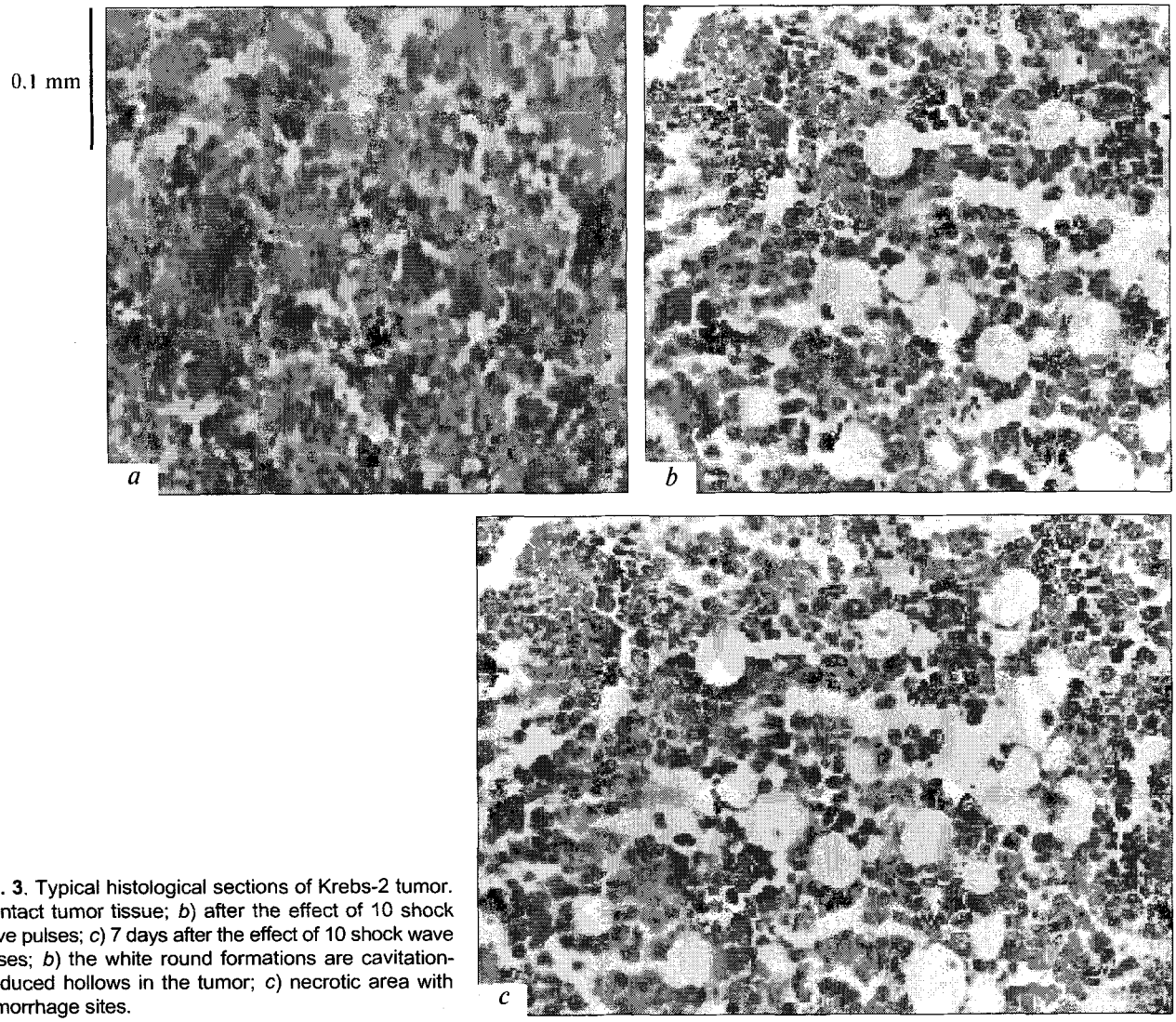


**Fig. 3.** Typical histological sections of Krebs-2 tumor. *a*) intact tumor tissue; *b*) after the effect of 10 shock wave pulses; *c*) 7 days after the effect of 10 shock wave pulses; *b*) the white round formations are cavitation-produced hollows in the tumor; *c*) necrotic area with hemorrhage sites.

cavitation-produced changes related to the formation of microcavities and accompanied by physiological alterations in the damaged tissue. ASW may be considered as a therapeutic mean in oncology diseases.

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## REFERENCES

1. E. Bell, *J. Cell. Comp. Physiol.*, **50**, 83-103 (1957).
2. P. T. Callaghan, *Principles of NMR Microscopy*, Oxford (1991).
3. A. H. Chung, K. Hynynen, V. Colucci, *et al.*, *Magn. Reson., Med.*, **36**, 745-752 (1996).
4. O. Hug and R. Pape, *Strahlentherapie*, **94**, 79-99 (1954).
5. K. Hynynen, C. A. Damianou, V. Colucci, *et al.*, *J. Magn. Reson. Imag.*, **5**, No. 3, 259-266 (1995).
6. J. F. Lehmann and J. Herrick, *Arch. Phys. Med.*, No. 3, 86-98 (1953).
7. *Physical Principles of Medical Ultrasonics*, Eds. C. R. Hill *et al.*, New York (1986).