ACTION OF LOW FREQUENCY ULTRASOUND ON THE PERITONEUM, PERITONEAL EXUDATE CELLS, AND THE COURSE OF EXPERIMENTAL PERITONITIS

V. S. Paukov, P. N. Morozov, O. Ya. Kaufman, N. G. Ermakova, and E. G. Ambrozevich

UDC 616.831.002-085.837.3-036.8-07:616.381-091.8+616.381-008.853]-092.9

KEY WORDS: ultrasound, experimental peritonitis, peritoneum, exudate.

Low-frequency ultrasound (LFUS) has been used with good results in the treatment of patients with peritonitis [1, 5]. The exposure dose used in such cases (6-8 sec/cm of peritoneum) is based on the absence of gross changes in the serous membrane [1, 2]. However, the present writers have shown clinically that with a duration of exposure to ultrasound as specified above, individual polymorphonuclear neutrophils (PNN) in the substance of the peritoneum and in the exudate are destroyed [4] and desquamation of mesotheliocytes takes place. At the same time it has been shown that LFUS can clear the peritoneal microvessels from thrombi and stasis and can activate emigration of PNN. Essentially no other information is available on the mechanism of the therapeutic action of LFUS *in vivo*. By analogy with high-frequency ultrasound it can be tentatively suggested that LFUS increases the powers of absorption of the peritoneum. LFUS, acting *in vivo*, can perhaps kill microorganisms present in the exudate and peritoneum, for such an effect has been found during long-term irradiation of a microbial suspension with ultrasound *in vitro* [2].

For the reasons given above, in the present investigation mechanisms of action of LFUS were analyzed in the intact organism and during the treatment of peritonitis, and experimental evidence was obtained on which to base an optimal duration of irradiation of the peritoneum and peritoneal exudate with ultrasound.

EXPERIMENTAL METHOD

Experiments were carried out on 50 noninbred male albino rats weighing 260-300 g and on 190 guinea pigs of both sexes weighing 250-420 g. Ether anesthesia was used during any painful procedures. The peritoneum and exudate were irradiated with ultrasound at a frequency of 26.5 kHz and with an amplitude of 30 μ. Altogether five series of experiments were carried out. In the first two the duration of exposure of LFUS was studied. For this purpose the action of LFUS was studied on the peritoneum of intact animals (20 guinea pigs and 30 rats — series I) with exposures of between 20 and 120 sec. The phagocytic power of PNN and their chemotaxis also were studied in a modified Boyden's chamber [7] after different exposures of peritoneal exudate *in vivo* to ultrasound (series II). Exudate containing PNN was obtained by intraperitoneal injection of a 0.5% solution of amylodextrin 4 h before irradiation with ultrasound [3].

In the remaining three series of experiments, the mechanism of action of LFUS in peritonitis were analyzed. In the experiments of series III, to determine the direct bactericidal action of LFUS a suspension of *Staphylococcus aureus* (strain Zhaev) or a 3% fecal suspension was injected into the peritoneal cavity and seedings of standard volumes of suspension ($1 \cdot 10^{-5}$ ml) were taken before and after the action of LFUS. In the experiments of series IV the effect of LFUS on the ability of the peritoneum to absorb antibacterial preparations was determined. For this purpose, after exposure of guinea pigs to LFUS, tritium-labeled isoniazid (specific activity 168 mCi/mmole) was injected intraperitoneally in a dose of 100 mCi/100 g body weight into three groups of animals (Fig. 1a). Blood was taken from the femoral veins. Radioactivity of the samples was determined in Unisolv II dioxane scintillator (from Koch Light, England) in a Wallac 1210-Ultrobeta liquid scintillation counter LKB, Sweden). The peritoneum was irradiated with LFUS through a solution of black ink for the same purpose.

Department of Pathological Anatomy, No. 2 Faculty of Internal Medicine, I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Sciences of the USSR A. I. Strukov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 98, No. 8, pp. 168-172, August, 1984. Original article submitted June 3, 1983.

0007-4888/84/9808-1049$08.50

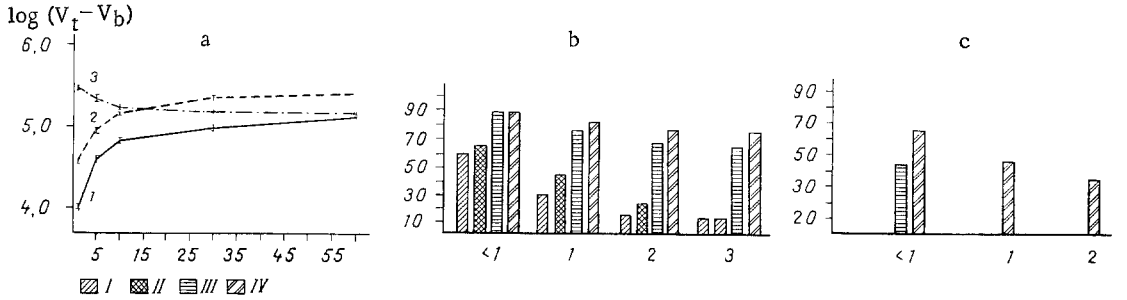

Fig. 1. Effectiveness of LFUS in experimental peritonitis: a) absorption of [^{3}H]isoniazid from peritoneal cavity of guinea pigs; 1) control; 2) peritonitis; 3) peritonitis + LFUS. Abscissa, time after injection of preparation (in min); ordinate, log ($V_t - V_b$) where V_t and V_b denote the counting rate for the test and background (in cpm) respectively. Immediately after irradiation the intensity of absorption by the peritoneum was one order of magnitude higher than the absorptive power in control guinea pigs; b) survival of guinea pigs after intraperitoneal injection of 0.8 ml/100 g of a 33% fecal suspension (LD_{100}) and different methods of treatment: I) peritonitis; II) peritonitis + LFUS; III) peritonitis + kanamycin; IV) peritonitis + LFUS + kanamycin; c) survival rate of guinea pigs after injection of fecal suspension in a dose of $3\times LD_{100}$ (2.4 ml/100 g body weight): I) peritonitis + kanamycin; II) peritonitis + LFUS + kanamycin. Abscissa, time (in days): ordinate, number of surviving guinea pigs (in %). Survival rate in control was 0.

In the experiments of series V the action of LFUS on survival of guinea pigs with experimental fecal peritonitis was studied [3, 6]. In this series six groups of animals, each containing 15-25 guinea pigs, were used (Fig. 1b, c). Irradiation and the other therapeutic measures were carried out on guinea pigs with peritonitis lasting 2 h, after which survival of the animals during the next 3 days was noted. In all series, samples of peritoneum were taken for light and electron-microscopy.

EXPERIMENTAL RESULTS

After irradiation of the peritoneum of intact animals for 10-20 sec no changes were found in its structure. The use of therapeutic doses of LFUS (duration of exposure 60 sec) led to focal desquamation of single mesotheliocytes (Fig. 2a), small hemorrhages, and focal changes in muscle fibers of striated muscles adjacent to the peritoneum (Fig. 2a, b). These changes consisted of focal translucency of the matrix and disturbance of the integrity of the mitochondrial cristae (Fig. 2c), marked dilatation of tubules of the T system (Fig. 2d), and focal coagulation necrosis of the sarcoplasm of the myons (Fig. 2b), and they reached their maximum 2 h after irradiation. The plasmalemma of the muscle fibers was unchanged. During the next 2 days these fibers regenerated and the integrity of the mesothelial layer was restored.

After irradiation of the exudate for 10-20 sec most PNN were preserved and capable of performing intensive phagocytosis of bacteria (up to 10-15 cells per PNN; Fig. 2e). Irradiation of the exudate for 40 sec led to degenerative changes in individual PNN, consisting of homogenization of the nuclei, translucency of the cytoplasm, and release of granules through tears in the cell membrane. After irradiation for 60 sec, these leukocytes numbered about 30-40%; PNN with their plasmalemma intact, but with zones of translucency of the cytoplasm were the majority. These PNN were able to phagocytose single cocci.

The action of LFUS on mobility of PNN was studied from two aspects: 1) its effect on chemotactic activity of the exudate; 2) effect on the ability of the cells themselves to move in a particular direction. When irradiated (5 min) and unirradiated exudate containing remnants of stimulating factor (amylodextrin) were used as the chemical attractant, both fluids exhibited high but equal activity. If washed peritoneal exudate leukocytes were used as chemical attractant, their activity before irradiation with ultrasound was two to three times higher than after destruction by sonication. This result is in agreement with data in the literature, according to which PNN produce a chemotactic factor [8]. The results obtained by analysis of mobility of irradiated PNN showed that their chemotaxis can vary widely depending on the chemical attractant used. Nevertheless, it can be concluded that with a short

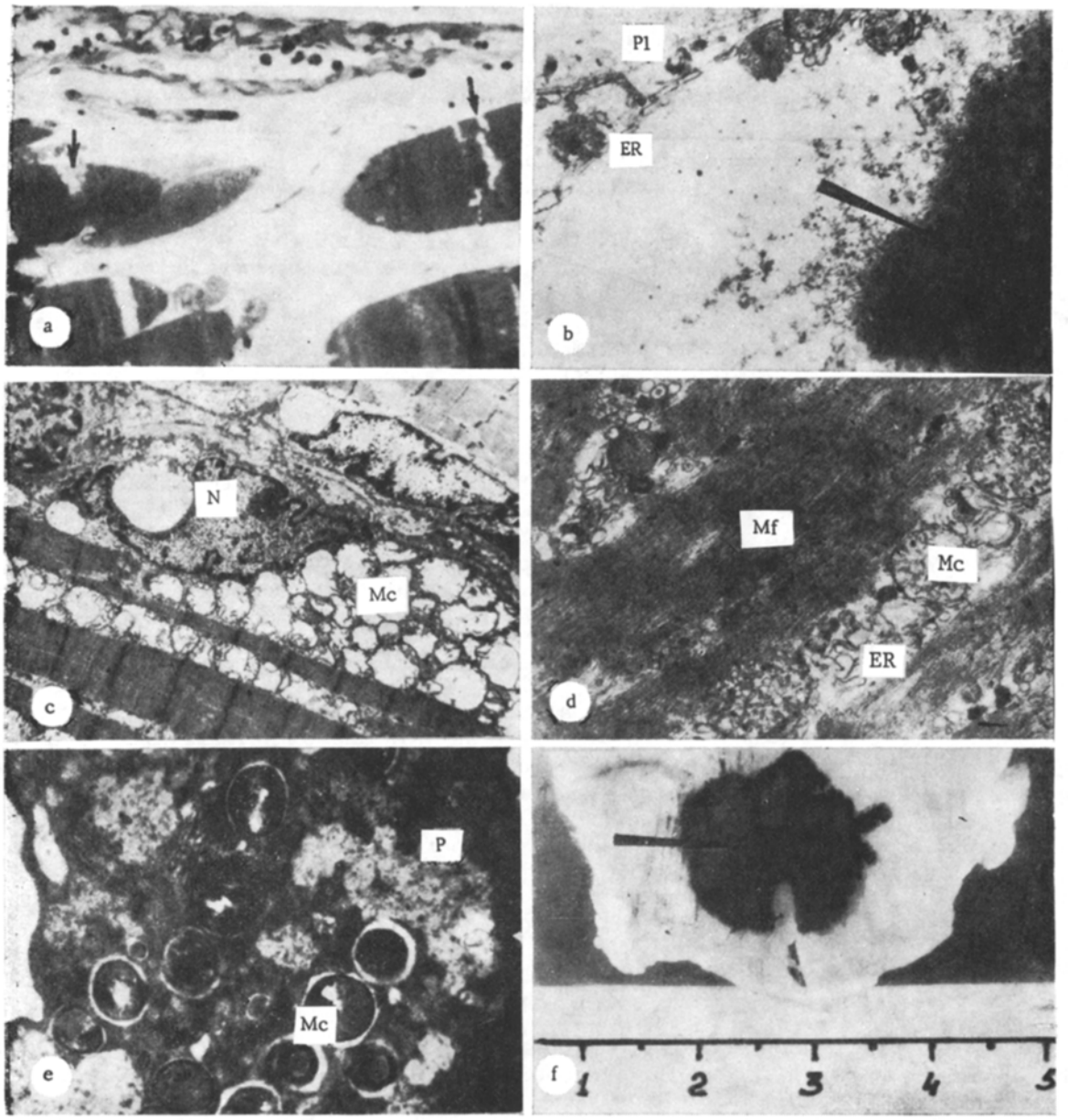

Fig. 2. Structural changes in peritoneum and adjacent striated muscles after exposure to LFUS: a) histological preparation of rat peritoneum with adjacent muscles after irradiaton with LFUS with exposure of 6-8 sec/cm^2: desquamated mesotheliocytes, focal lesions, visible in underlying muscles (arrow). Semithin section, azure II and methylene blue, 150 ×; b) fragment of same peritoneum examined under electron microscope. Arrows indicate focus of coagulation necrosis; plasmalemma (Pl) intact, single mitochondria and tubules of endoplasmic reticulum (ER) visible beneath the plasmalemma. Two hours after LFUs, 10,000×; c) ultrastructure of Myons of guinea pigs located subperitoneally, after exposure to LFUS for 6-7 sec/cm^2. Cristae of mitochondria (Mc) destroyed. 5000 ×; d) dilatation of tubules of smooth endoplasmic reticulum (ER) and disturbance of regular arrangement of sarcomeres in myons of subperitoneal striated muscles. Mf) Myofilaments, 10,000 ×; e) intensive phagocytosis of staphylococci by polymorphs (P) after irradiation with LFUS for 20 sec *in vivo*. 12,000 ×; f) penetration of colloidal solution of black ink into peritoneum at site of action of LFUS. Native preparation of parietal peritoneum, 1.7 ×.

exposure, LFUS had marked chemotactic mobility, whereas lengthening of the exposure to LFUS reduced this mobility through destruction of the cells.

Absence of any direct bactericidal effect of LFUS was demonstrated in the next experiments with exposure of 60 sec *in vivo*: before irradiation of the staphylococcal suspension,

on average $(4.19 \pm 0.73) \cdot 10^7$ CFU (colony-forming units) were seeded, compared with $(4.26 \pm 0.82) \cdot 10^7$ after irradiation. Similar results were obtained in experiments with the fecal suspension.

When the peritoneum was irradiated through a solution of ink, the ink particles penetrated rapidly into the thickness of the peritoneum (Fig. 2f) to a depth of 2-3 mm, and were distributed in myons of the striated muscles and in the connective-tissue basis of the peritoneum. Similar results were obtained with isoniazid. In animals with peritonitis treated with LFUS (Fig. 1a) the quantity of the antibacterial preparation absorbed into the blood stream 5 min after the end of irradiation was about eight times higher than its concentration in the untreated and control animals. Considering that LFUS sharply increases the absorptive power of the peritoneum and penetration of fluid with dissolved or suspended substances in it into its thickness, it can be tentatively suggested that the combined use of LFUS and an antibiotic solution for the treatment of peritonitis will give better results than their use separately. This problem was studied in guinea pigs with fecal peritonitis (Fig. 1b, c). Its histological characteristics were described previously [3]. Irrigation of the peritoneal cavity of guinea pigs 2 h after injection of a fecal suspension with kanamycin solution leads to survival of 64% of animals 3 days after treatment (Fig. 1b). After ultrasonic irradiation of the peritoneal cavity of guinea pigs with peritonitis through kanamycin solution 76% of the animals survived the same period (Fig. 1b). Meanwhile, irradiation of the peritoneal cavity of guinea pigs with fecal peritonitis through Hanks' solution with LFUS was ineffective (Fig. 1c). After intraperitoneal injection of a fecal suspension into guinea pigs in a volume equal to $3 \times LD_{100}$, when irrigation of the peritoneal cavity with kanamycin solution was therapeutically ineffective, a combination of peritoneal irrigation with kanamycin solution and irradiation with LFUS led to survival of 34% of the animals (Fig. 1c).

The investigation thus showed that various structural and functional changes arise in the peritoneum and peritoneal exudate both of intact animals and of animals with fecal peritonitis under the influence of LFUS. Structural changes affect mainly the striated muscles, mesotheliocytes, and PNN. LFUS stimulates penetration of solutions (including those of antibiotics) into the thickness of the peritoneum and increases its absorptive power. Consequently, a combination of LFUS with irrigation of the peritoneal cavity with antibiotics gives a distinct therapeutic effect, whereas each component separately is ineffective.

LITERATURE CITED

1. V. V. Bagdasarov, "The use of energy of a low-frequency ultrasonic field in the combined treatment of patients with peritonitis," Author's Abstract of Candidate's Dissertation, Moscow (1982).
2. G. A. Bondarev, "The use of low-frequency ultrasound in the combined treatment of peritonitis under experimental and clinical conditions," Author's Abstract of Candidate's Dissertation, Moscow (1981).
3. O. Ya. Kaufman, A. S. Rostovshchikov, A. K. Kranchev, et al., Byull. Eksp. Biol. Med., No. 12, 100 (1982).
4. V. S. Paukov, V. I. Petrov, O. Ya. Kaufman, et al., Arkh. Patol., No. 2, 14 (1982).
5. V. I. Petrov, V. I. Loshchilov, V. V. Bagdasarov, et al., in: Abstracts of Proceedings of the 15th Scientific and Practical Conference to Commemorate the 110th Anniversary of the Birth of V. I. Lenin [in Russian], Ul'yanovsk (1980), pp. 197-198.
6. K. S. Simonyan, Peritonitis [in Russian], Moscow (1971).
7. S. Boyden, J. Exp. Med., 115, 452 (1982).
8. J. Palmblad, L. L. Mamsten, A. U. Uden, et al., Blood, 58, 658 (1981).