PHOTOLON ENHANCEMENT OF ULTRASOUND CYTOTOXICITY

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Objective: To evaluate the impact of Photolon on cytostatic and cytotoxic effects of therapeutic range ultrasound in C6 glioma cells. Methods: C6 glioma cells in suspension or monolayer cell culture were exposed to ultrasound (880 kHz, 0.2–0.7 W/cm²) in the presence or absence of Photolon at the concentration of 1 μg/ml in the culture medium, and then cell viability was evaluated. Results: Photolon increased the cytotoxic effect of ultrasound by 1.5–2.3-fold but had no effect on its cytostatic activity. Conclusion: Photolon produces a pronounced sonosensitizing effect on glioma C6 cells and is a promising drug for sonodynamic treatment of malignant tumors. Key Words: photolon, ultrasound, C6 glioma cells.

Ultrasound is widely used in medicine for diagnosis and treatment of many diseases. The use of ultrasound for therapeutic purposes is based on the specific nature of the interaction between ultrasound and biological tissues. Structural and functional changes in biological tissues exposed to ultrasound depend on the parameters of ultrasonic radiation: its frequency, intensity and duration of exposure. High-frequency ultrasound (1.0–31.5 MHz) is characterized by predominant thermal effect, whereas ultrasound with low frequency produces cavitation. Physiotherapeutic practice uses ultrasonic waves with 800 to 3000 kHz frequency and up to 3 W/cm² intensity.

Investigation of biological effects of ultrasonic waves with different frequency, intensity and duration of action has shown that ultrasound exerts antitumor activity. In experimental studies, ultrasound of therapeutic range was found to cause growth inhibition and death of human tumor cells: leukemia K562, HL-60, KG1a and Nalm-6 cells [1, 2], breast adenocarcinoma MCF-7 cells, ovarian carcinoma SC-OV-3 [3], epidermoid cancer [4], cervical carcinoma HeLa cells [5–7], colon carcinoma HT-29 cells, gingival carcinoma Ca9-22 cells [7], glioma C6 cells [8, 9], as well as implanted rat tumors: Zeidel hepatoma, M-1 sarcoma [4], RS-1 cholangiocellular carcinoma [10], C6 glioma [11]. High-intensity (3 W/cm² to 104 W/cm²) focused ultrasound with 1–10 MHz frequency, the absorption of which results in a focal temperature of 40–100 °C, is used in oncology for non-invasive surgery of deep-seated tumors, as well as for induction of local hyperthermia.

Moreover, exposure to ultrasound of a specific frequency range, intensity and duration was found to improve the efficacy of anticancer drugs both in vitro and in vivo without causing direct destruction of tumor cells, which is attributed to increased permeability of cell membranes for cytostatics [10, 12]. The potential gain in radiosensitivity of tumor cells due to ultrasound pretreatment was also demonstrated [13, 14].

A new trend, sonodynamic therapy of malignant tumors is being developed, which is based on tumor destruction as a result of synergetic enhancement of ultrasound effect by a sonosensitizer drug. The terms “sonodynamic therapy” and “sonosensitizer” were proposed by Umemura et al. (1990) by analogy with the terms of photodynamic therapy, as the classical photosensitizer hematoporphyrin had also been shown to possess sonosensitizing properties [15]. The mechanism of sonodynamic therapy seems to be associated with the damage of cell membranes. The advantage of sonodynamic therapy over photodynamic one is its ability to focus ultrasound in the area of deep-seated tumors.

In experimental models in vitro on tumor cells and in vivo on transplanted tumors in laboratory animals, the sonosensitizing properties of porphyrin photosensitizers and a number of other compounds were studied [12]. Such photosensitizers as hematoporphyrin, Photofrin, Photofrin II, mesoporphyrin, protoporphyrin, protoporphyrin IX, TPPS, ATX-70, ATX-S10, pheophorbide-a, AlPcS4, chlorine PADS31 [15], methylene blue [16], erythrosine, rhodamine [17], NPe6 [18], rose bengal [11], 5-ALA [19], chlorin e6 [20] have been shown to enhance ultrasound cytotoxic effect on tumor cells and implanted tumors. The results of the experimental studies suggest that sonodynamic therapy is a promising treatment modality for malignant tumors.

The purpose of this study was to investigate a sonodynamic activity of the original Belarusian photosensitizer Photolon.

The study was conducted on C6 rat glioma cell line (Russian Collection of Cell Cultures, Cytology Institute of Russian Academy of Sciences, St. Petersburg). The cells were cultured in DMEM culture medium supplemented with 10% fetal calf serum. Exponentially growing cells as a monolayer or suspension culture were used for the experiments. To suspend the cells, the monolayer was detached from the culture flasks with 0.02% versen.

The drug Photolon (RUE Belmedpreparaty, Belarus) was diluted with saline just before the use. The cells were sonicated with ultrasound of 0.2, 0.4 or 0.7 W/cm² intensity at room temperature for...
60 s, using UST-1.040 apparatus for ultrasonic therapy (880 kHz, EMA Plant, Russia) in the continuous ultrasonic generation mode. The emitter was placed directly into the culture medium.

Photolon (1 μg/ml) was added to the culture medium 5 min before sonication of the cell suspension or 2 h before sonication of the cell monolayer (for maximum accumulation of Photolon in the cells).

The cytotoxic effect of ultrasound on C6 glioma cells in the suspension was assessed by trypan blue dye exclusion test 5 min after the exposure.

To evaluate the cytostatic effect of ultrasound, the monolayer culture growth of C6 glioma cells after the treatment was studied. 24 h after sonication, the monolayer was detached from the culture flasks with 0.02% versen, and viable (trypan blue unstained) cells were counted in a hemocytometer. The increase in the cell number compared to the control value was calculated from: $(N_t - N_o)/(N_c - N_o)$ if $N_t$ was greater than or equal to $N_o$, or $(N_t - N_o)/N_o$, if $N_t$ was less than $N_o$ ($N_c$ — cell number in the control culture; $N_t$ — cell number in the treated culture; $N_o$ — initial cell number before the treatment).

The values of $II_{50}$, ultrasound intensity required for a 50% inhibition of cell proliferation, and $LI_{50}$, ultrasound intensity required for a 50% cell death, were calculated using regression analysis of the data obtained. The enhancement ratio (ER) for inhibition of cell proliferation or cell killing was calculated as $II_{50}$ or $LI_{50}$ for ultrasound without Photolon divided by $II_{50}$ or $LI_{50}$ for ultrasound with Photolon.

The values obtained were processed using standard statistical methods of Origin 7.0.

To assess the cytotoxic effect of ultrasound, C6 glioma cell suspension (785 × 10³ cells/ml) was sonicated at 0.2, 0.4 or 0.7 W/cm² ultrasound intensity. Then the number of viable cells was calculated by trypan blue dye exclusion test.

Fig. 1, a, presents the number of viable C6 cells in suspension after the exposure to ultrasound alone or in combination with Photolon. The results suggest that sonication leads to a decrease in the number of viable cells. The cell death increased with ultrasound intensity; $LI_{50}$, ultrasound intensity required for a 50% cell death, was 0.51 W/cm².

Photolon addition to the cell suspension resulted in a significant enhancement of ultrasound cytotoxicity at 0.4 and 0.7 W/cm² intensity, with $LI_{50}$ decreasing to 0.33 W/cm². The differences in cell number after the exposure to ultrasound alone and ultrasound with Photolon were statistically significant ($p < 0.01$). Thus, the gain in ultrasound cytotoxicity in the presence of Photolon was 1.5.

It should be noted that Photolon alone caused a decrease in the number of viable cells only by 16% compared to the control. The temperature of the culture medium during cell treatment with ultrasound did not rise.

The effect of varying intensity ultrasound on the growth of the monolayer culture of C6 glioma cells was assessed by the number of viable cells 24 h after the exposure.

The data presented in Fig. 1, b, suggest that sonication leads to growth inhibition of C6 glioma cells, the number of viable cells reducing with increased ultrasound intensity. Regression analysis of the data revealed that $II_{50}$, the ultrasound intensity needed for a 50% inhibition of cell proliferation, was 0.27 W/cm², and $LI_{50}$, the ultrasound intensity required for a 50% cell death, was 1.32 W/cm².
and polyvinylpyrrolidone, is selectively accumulated in tumor tissues, displays strong photodynamic activity and is registered as an agent for photodynamic therapy of malignant neoplasms. Photolon is able to penetrate the blood-brain barrier, and it may be used for intraoperative photodynamic therapy in patients with primary and metastatic brain tumors [21].


REFERENCES


