# Short Communication

# Changes in Microtubules and Microfilaments Due to a Combined Effect of Ultrasound and Cytostatics in HeLa Cells

( ultrasound bioeffect / cellular changes / cell proliferation / cytoskeleton / cytostatic effect )

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**Abstract. Treatment of HeLa cells with low intensity ultrasound and two cytostatic drugs, cycloplatin and methotrexate, resulted in a partial disassembly of microtubules and microfilaments. This disassembly was due to depolymerization and subsequent erroneous repolymeration of essential cytoskeletal proteins, resulting in formation of unusual arrangements, mainly small, granule-like structures. The combined action of ultrasound and cytostatics had a synergistic effect dependent on both the concentration of the drug and the time of sonication. The demonstrated changes in the cytoskeleton are considered to be non-specific to ultrasound treatment, reflecting only an altered vital state of the treated cells.**

Isolated cells or cell cultures provide excellent experimental models for investigation of effects of different physical and chemical stimuli. This influence can be studied directly, without participation of numerous various internal factors operating in the whole organism. It has been demonstrated repeatedly that ultrasound is able to damage animal and/or plant cells according to its intensity level (Dvořák and Hrazdira, 1966; Hrazdira and Havelková, 1977; Adler et al., 1988; Azadiv et al., 1996). Relatively little attention has been paid to the influence of ultrasound on the fibrous system of cytoplasmic proteins known as the cytoskeleton (Adler et al., 1993). The specific determination of these cytoplasmic components by the immunofluorescence technique has extended the range of cell investigation, enabling examination of the important role that the cytoskeletal system plays in intracellular communication and cell locomotion. The immunofluorescence method may be of value in studying the effect of cytostatic drugs in interaction with some physical or chemical factors that may influence the primary cytotoxicity of cytostatic drugs.

The aim of this study was to investigate the sensitivity and changes in cytoskeleton components after exposure of HeLa cells to a simultaneous effect of ultrasound at low therapeutic intensities and selected cytostatic drugs.

## Material and Methods

### *Cell line*

The HeLa cell line, grown in Dulbecco's minimal essential medium (DMEM) (PAA Laboratories GmbH, Linz, Austria), supplemented with 10% bovine foetal serum (PAA), 2 mM glutamine (PAA), 100 U $ml^{-1}$ penicillin and 100 μg $ml^{-1}$ streptomycin (Bio-Wittaker Inc., Walkersville, MD) at 37°C under anaerobic conditions was used. Cell monolayers attached to cover slips were treated by the following experimental procedures:

1. treatment with ultrasound at low therapeutic intensities
2. incubation with two different cytostatic drugs
3. combined action of both factors

### *Visualization of the cytoskeleton*

Cytoskeletal components were visualized by indirect immunofluorescence. The cell monolayers were rinsed with phosphate buffer, before adding 0.1% Triton X-100, to wash out non-cytoskeletal proteins. The cells were subsequently fixed by means of 3% paraformaldehyde. After fixation, microtubules were determined by adding primary antibody TU-01 (Institute of Molecular Genetics, Prague, Czech Republic) diluted 1 : 500 by phosphate-buffered saline (PBS) and secondary antibody SwAM FITC (Institute for Sera and Vaccines, Prague, Czech Republic) for 45 min each, and TRIC phalloidin (Sigma-Aldrich, Ltd., Prague, Czech Republic) diluted 1 : 100 by PBS was used to detect

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Abbreviations: FITC – fluorescein isothiocyanate, PBS – phosphate-buffered saline, PVDF – polyvinylidenfluoride, SwAM – conjugated swine anti-mouse globulin, TU-01 – tubulin monoclonal antibody.

microfilaments. Between each two treatments, the cells were washed with PBS. The samples were viewed in a fluorescence microscope (Leitz Laborlux S, Wetzlar, Germany), and the main attention was paid to alterations in microtubules and microfilaments.

Changes in microtubules and microfilaments of all the cytoskeletal components were evaluated independently by two professional observers.

### *Ultrasound treatment*

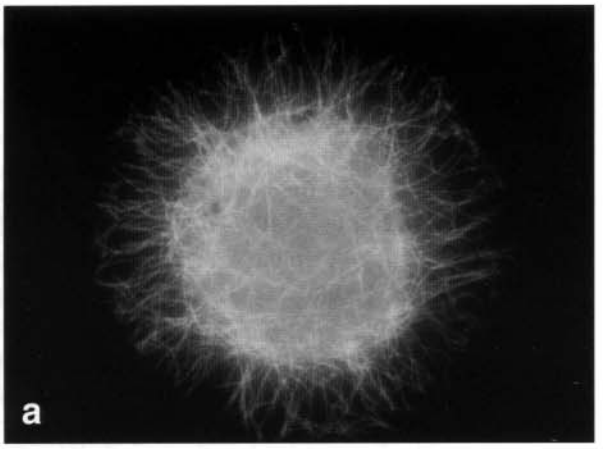
A Beautyline BTL-07 therapeutic generator (Beautyline Ltd., Prague, Czech Republic), operating at a frequency of 1 MHz, was used as the source of continuous ultrasound. Glass cover slips with cell monolayers were sonicated in a special chamber under standard conditions, minimizing the production of standing waves, described elsewhere (Mornstein and Grec, 1996). The ultrasound intensity was 500 mW/cm$^2$, the time of treatment was either 5 or 10 min. The ultrasound intensity was controlled by means of a calibrated PVDF hydrophone, type MH28-6 (Force Institute Copenhagen, Denmark). The heating effect was controlled by means of a two-probe differential thermometer, made in our laboratory.

### *Application of cytostatics*

Two cytostatic drugs, cycloplatin (cis-diamine-1-cyclobutandicarboxylate platinum complex) and methotrexate (4-amine-N-10 methylpteroylglutamic acid, Lachema Ltd., Brno, Czech Republic), were chosen for the experiments. Both drugs inhibit DNA synthesis, but each by a different molecular mechanism. The effect of cycloplatin is produced by inhibition of DNA replication while methotrexate causes competitive inhibition of folate reductase, a necessary component of DNA synthesis. Both cycloplatin and methotrexate, diluted by sterile PBS, were used at a concentration of 1 mg/ml. The cells were incubated with the cytostatics for either 30 or 60 min. At the concentrations used, the two cytostatic drugs produce a similar degree of cytoskeleton alteration.

### *Combined action*

For the study of combined action of drugs and ultrasound, the cell monolayers on cover slips were first



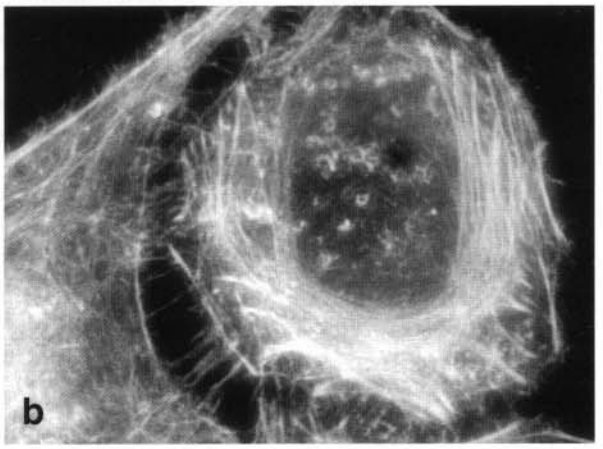
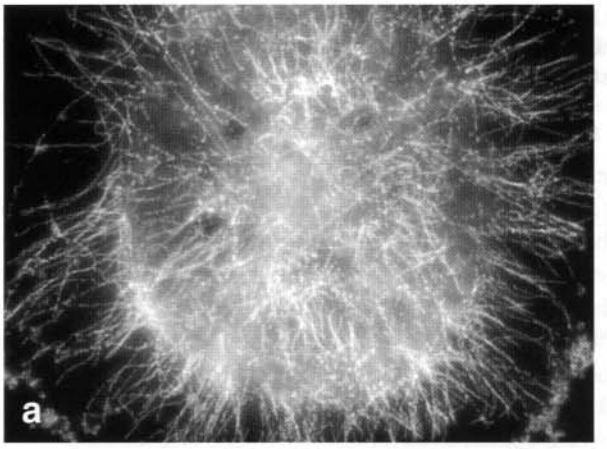




*Fig. 1.* Untreated HeLa cells. Primary magnification 400x. a) microtubules, b) microfilaments



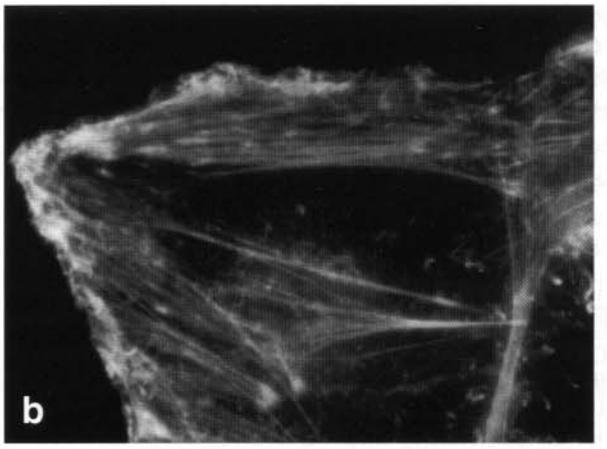




*Fig. 2.* HeLa cells treated only with ultrasound. Exposure: 500 mW/cm$^2$, 5 min, primary magnification 400x. a) microtubules, b) microfilaments