PHARMACOLOGY

Effects of Ultrasound, the Polyene Antibiotic Amphotericin B, and Their Combination on Biomembranes of Yeastlike *Candida* Fungi

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Treatment of candidiasis continues to present a difficult problem [1,2] because of the low efficacy of anticandidal agents and the development of resistance to them in the fungi. There are reports that pyodermas, *Proteus vulgaris* infections, and some other diseases respond well to treatment with drugs in combination with ultrasound [3,4], but the impact of such combinations on *Candida* infections has not been investigated. The purpose of the present study was to see how yeastlike *Candida* fungal cells respond to a combination of the antibiotic amphotericin B and ultrasound.

MATERIALS AND METHODS

The effect of amphotericin B (AB) and ultrasound (US) on cells of *Candida albicans* was assessed by measuring the chemiluminescence (CL) of these cells. The intensity of CL can give an indication of the degree to which lipid structures of cell membranes have been damaged [5-9]. In addition, the ultrastructure of *Candida albicans* cells was examined by electron microscopy.

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Suspensions of a 24-hour culture of *Candida* fungi (museum strain № 707) grown on a dense Sabouraud medium were used. A total of four se-

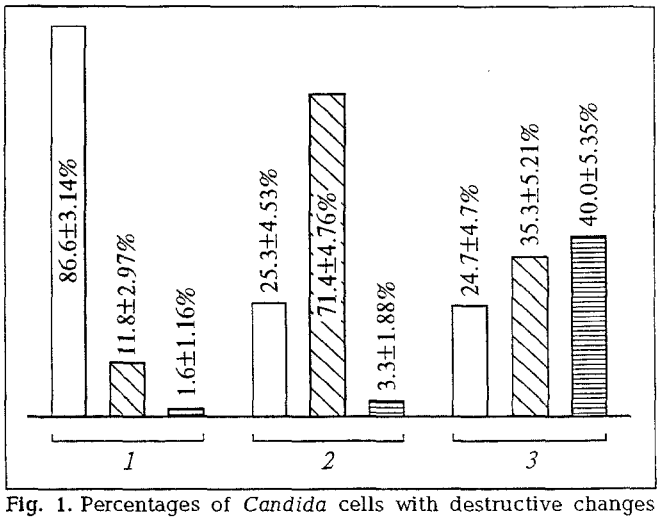

Fig. 1. Percentages of *Candida* cells with destructive changes of varying degree following exposure to ultrasound (US), the polyene antibiotic amphotericine B (AB), or their combination. *1*) cells exposed to US; *2*) cells exposed to AB (in a subbactericidal dose); *3*) cells exposed to US and AB (in the same dose). White bars: apparently unchanged cells; obliquely hatched bars: cells with moderate ultrastructural changes; vertically hatched bars: inviable cells with gross ultrastructural changes, primarily in biomembranes.

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ries of tests were run. In the 1st (control) series, fungal cells were exposed neither to US nor to AB; in the 2nd, they were exposed to US alone (1 W/cm², 2640 kHz, 20 min); in the 3rd, unsonicated fungal cells were incubated for 3 h with AB applied in a subbactericidal dose. In the 4th series, fungal cells were similarly exposed to both US and the antibiotic.

Chemiluminescence (CL) of fungal cell samples was recorded with specially designed equipment consisting of a sensitive photomultiplier detector, a high-voltage stabilizer, a direct-current amplifier, and a recording potentiometer. To a temperature-controlled cuvette, 5 ml of fungal suspension (5×10^9 reproductive bodies per ml) were added, followed by incubation for 2 min in a dark chamber with continuous agitation, after which 1 ml of a 0.02 mol/liter solution of the CL inducer ($FeSO_4\times7H_2O$) was added to the system, and the level of CL was recorded for 20-30 sec, followed by determination of the intensity (amplitude) of the fast CL burst (in relative units).

The ultrastructure of the fungal cells, unexposed or exposed to US and/or AB, was examined in a Geol JSM-1000S transmission electron microscope (Japan). The examination, the material for which was prepared as previously described [10], included both assessment of the degree to which fungal cell components (primarily biomembranes) were preserved and determination of the relative proportions of cells with various ultrastructural changes caused by the antibiotic, sonication, and their combination.

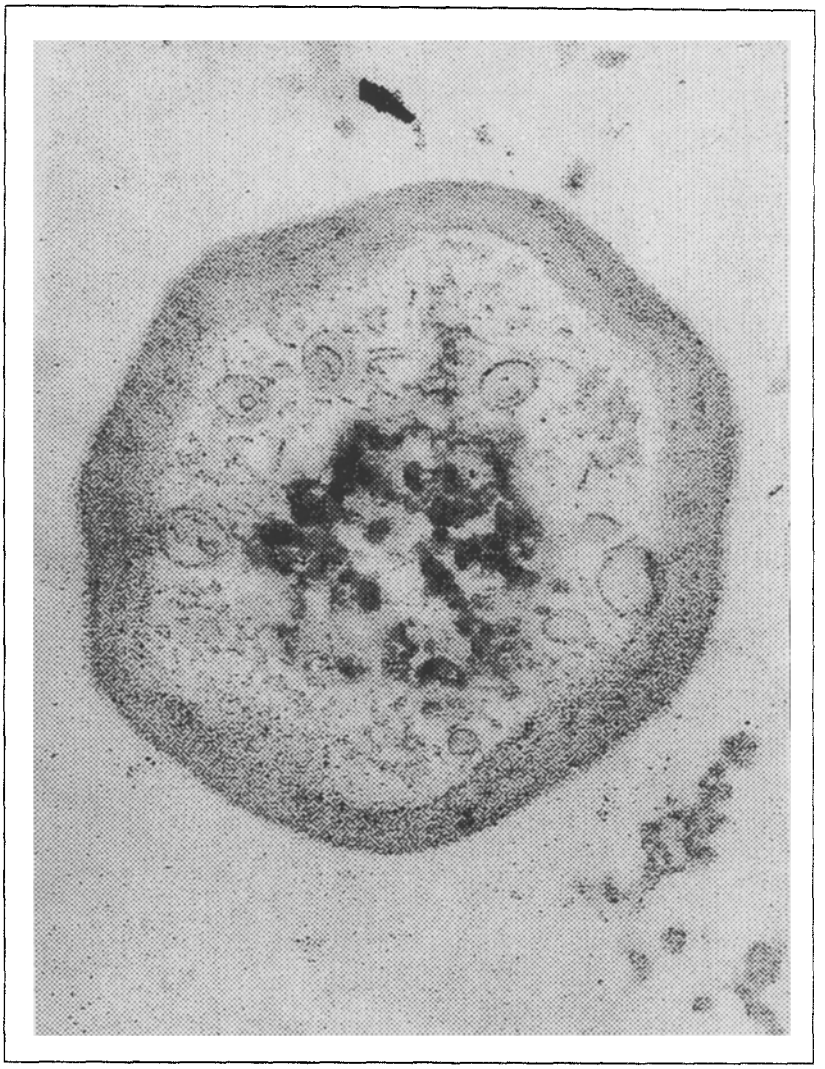

Fig. 2. A *Candida* cell exposed to ultrasound and then incubated with amphotericin B in a subbactericidal dose. The blastospore is deformed; the cytoplasm is contracted, torn away from the cell wall, and shows lipid degeneration. Membrane structures appear as fragments scattered in a haphazard manner. Organelles show signs of gross destruction. The cell wall is osmiophilic (the overall picture corresponds to the final stage of autolysis). ×14,000.

The results were processed statistically using the parametric Student t test to evaluate the significance of differences between the test series.

RESULTS

As shown in Table 1, sonication alone (2nd series) increased the fast burst of Fe^{2+}-induced CL by 23% relative to its control value (1st series); exposure to AB alone (3rd series) increased the fast burst amplitude by 60% and 37% relative to its values in the 1st and 2nd series, respectively; sonication in combination with antibiotic treatment (4th series) increased the amplitude by more than 2-fold over its values in the 1st and 2nd series and by more than a half over its value in the 3rd series. These differences were all statistically significant.

The electron microscopic examination of *Candida* cells exposed to US alone showed that membrane structures were all preserved in more than 80% of the cells (Fig. 1). After the incubation of unsonicated cells with AB, only about one-fourth of the cells had retained the normal shape and membrane structures; their cytoplasm showed low electron density in extensive areas. However, only about 3% of the blastospores were grossly deformed (Fig.1).

After the combined exposure to US and AB, electron micrographs revealed membranes with grossly impaired integrity and with signs of autolysis in its final stage (Fig. 2); inviable cells were

TABLE 1. Effects of Ultrasound (US), Amphotericin B (AB), and Their Combination on Fast Burst Amplitudes of Fe^{2+} – Induced Chemiluminescence (CL) by *Candida* Cells

Test series	Treatment	Amplitude of fast CL burst, relative units
1st	Control (unexposed fungal cell suspension)	11.5±0.6
2nd	US alone	14.2±0.1*
3rd	AB alone	18.4±0.2*,**
4th	US+AB	29.1±1.6*,**,***

Note. The values are means of 7–12 tests. Asterisks indicate significant differences (at $p<0.01$) from the 1st (control) (*), 2nd (**), and 3rd (***) test series.

present in greatly increased numbers, constituting 75% of all blastospores (Fig. 1); this is 25-fold more than the proportion of such cells when ultrasound was used alone and about 12-fold more than when the antibiotic was used alone. In this test series, as already noted, the amplitude of the fast CL burst was 2.5 times greater than in the control series.

The amplitude of the fast CL burst has been shown to provide an indication of the amount of peroxidation products, including lipid hydroperoxides, that has accumulated in the cells, which in turn reflects the rate of lipid peroxidation (LPO) processes [5-10]. It is therefore apparent that exposure of fungal cells to a combination of ultrasound and amphotericin B results in a more pronounced development of free-radical processes (by which the structural barriers of these cells are disrupted) than does their exposure to either of these two agents alone. The accumulation of peroxides causes the mitochondria to swell and promotes uncoupling and inhibition of oxidative phosphorylation, thereby throwing the cell into a vicious circle of impaired bioenergetics [5,6,7,11]. The peroxidation process is accompanied by deformation of the membrane lipoprotein complex (through the "detergent" action of free radicals and, especially, secondary LPO products), by increases in membrane permeability for protons and water, and by the appearance of "lesions" in the membranes; if the LPO progresses, rupture of mitochondrial and lysosomal membranes occurs [12], with consequent leakage of lysosomal hydrolases into the cytoplasm, resulting in cytolysis and death of the cell [8,9].

In conclusion, the results of this biophysical and morphological study, which correspond to our clinical data (a recovery rate of 87±6% achieved for patients with candidiasis), indicate that the structural damage to cell membranes of *Candida* fungi is considerably greater when these fungi are treated with the antifungal antibiotic amphotericin B after being sonicated than when they are treated with this antibiotic alone, and, therefore, that the antibiotic treatment of candidiasis should preferably be supplemented with exposure of the causative agent to ultrasound.

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