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Review

Synergistic bactericidal effects and mechanisms of low intensity ultrasound and antibiotics against bacteria: A review

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ABSTRACT

Low intensity ultrasonic therapy is always an important research area of ultrasonic medicine. This review concentrates on low intensity ultrasound enhancing bactericidal action of antibiotics against bacteria in vitro and in vivo, including planktonic bacteria, bacterial biofilms, *Chlamydia*, and bacteria in implants. These literatures show that low intensity ultrasound alone is not effective in killing bacteria, while the combination of low intensity ultrasound and antibiotics is promising. Low intensity ultrasound facilitating antibiotic treatment is still in its infancy, and still requires a great deal of research in order to develop the technology on medical treatment scale.

1. Introduction

Antibiotics are among the most frequently prescribed medicine that cure diseases from minor discomforts to life-threatening infections. They kill or injure bacteria by interfering with biosynthesis of DNA, proteins, or cell wall components. Unfortunately, bacteria exhibit more and more significant antibiotic resistance, for many antibiotic failures in the clinic. In Europe, over 25,000 people die from infectious disease with the selected multidrug-resistant bacteria every year [1].

Antibiotics resistance mechanism of bacteria includes: bacteria produce hydrolytic enzymes to break down antibiotics or antagonize antibacterial activity; the antibiotic targets are changed as result of bacteria structure or gene mutation; bacteria reduce their cell membrane permeability to prevent antibiotics from getting into intracellular targets or form drug efflux pumps to pump antibiotics out of cells [2–4]. Bacterial biofilm is formed with lots of aggregating bacteria colonies. Its antibiotics resistance is 500–5000 times greater than that of planktonic bacteria [5]. There are two main reasons. On one hand, compact extracellular matrix covering the bacterial biofilm prevents aminoglycoside drugs from entering into biofilms. On the other hand, due to the lack of oxygen and nutrition, the bacteria inside the bacterial biofilm is not susceptible to antibiotics that is effective to aerobic bacteria and bacteria under growth and proliferation [6–9]. The antibiotics resistant mechanism of *Chlamydia* mainly includes: it is resistant to tetracyclines, quinolones, macrolides and rifampicin due to gene transmission and mutation [10] and persistent *Chlamydial* forms are more resistant to doxycycline than acute forms because of the decreased antibiotic uptake by host cells [11].

Currently, noninvasive and proven effective physical methods with antibiotics that can affect bacterial adhesion, growth and even kill bacteria include magnet field, electrical field and low intensity ultrasound [12–16]. Among them, low intensity ultrasound is a very promising method for enhancement of antibiotic actions on bacteria because of its beam directivity, noninvasiveness of treating deep tissue targets and capability for drug delivery. There have been a series of literatures published on the combination of low intensity ultrasound and antibiotics is effective on the antibiotics resistant mechanism of bacteria and kills more bacteria than antibiotics used alone. The following concerns the synergistic effects and mechanisms of low intensity ultrasound and antibiotics.

2. Bactericidal action against planktonic bacteria

2.1. Synergistic bactericidal effects

It was first observed by Pitt et al. in 1994 that the combination of ultrasound operating at 67 kHz and 0.3 W/cm^2 and 12 μg/ml gentamicin showed 10^5 CFU/ml greater in killing *Pseudomonas aeruginosa* in 24 h culture than 12 μg/ml gentamicin does alone, and 10^7 CFU/ml greater in killing *Escherichia coli*, and the ultrasound alone did not kill bacteria [15]. Rediske et al. noted that

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compared with application alone of three kinds of antibiotics that are aminoglycosides antibiotics (gentamicin, streptomycin, and kanamycin), tetracycline antibiotics (tetracycline), and penicillin antibiotics (ampicillin) on each of Gram-negative organisms (*Enterobacter aerogenes, Serratia marcescens*, and *Salmonella derby*) and Gram-positive organisms (*Streptococcus mitis* and *Staphylococcus epidermidis*), combination of antibiotics and ultrasound operating at 70 kHz and 3 W/cm^2 decreases viable counts by 2–4 orders of magnitude ore more, and the ultrasound alone did not kill bacteria [17]. Ayan et al. observed that compared with application alone of five kinds of antibiotics that are penicillin (penicillin, and oxacillin), glycopeptides (teicoplanin, and vancomycine), macrolides (erythromycin), lincomycin (clindamycine), fluoroquinolones (levofloxacine, and ciprofloxacin), the combination of antibiotics and ultrasonic operating at 1.5 MHz and 30–161 mW/cm^2 decreases viable counts by 3.66 $\log_{10}$ CFU/ml to 5 $\log_{10}$ CFU/ml ($P < 0.001$) [18]. Liu et al. discovered that compared with antibiotics alone, the combination of fluoroquinolones (levofloxacine and ciprofloxacin) and ultrasound operating and 40 kHz and 1 W/cm^2 enhanced inhibitory ratio on *E. coli* by 20% [19].

2.2. Bactericidal mechanisms

Williams et al. found that under the synergistic application of antibiotics and 70 kHz ultrasound with various intensities from 10 mW/cm^2 to 4.5 W/cm^2, the higher intensity, the lower bacteria activity. It suggests that stable cavitation of ultrasound contributed to the alteration in bacterial cell membrane, so antibiotics can penetrate into bacteria easily [20]. Using a macromolecule labeled 16-doxylstearic acid probe and spin-labeled gentamicin, Rapoport et al. observed that 80 kHz and 0.8–2.4 W/cm^2 ultrasound enhanced the permeability of cell membranes of *P. aeruginosa* and *E. coli* towards macromolecular compounds [21,22]. Rediske et al. and Runyan et al. further discovered that ultrasound increased the permeability of cell membranes of *P. aeruginosa* towards either large hydrophobic compounds or large hydrophilic compounds, indicating that sonoporation created holes in the bacterial cell membrane large enough for the rapid passage of a great deal of antibiotics instead of altering the characteristics of bacterial cell membrane [23,24]. Ayan et al. noted that partial destruction or disintegration of the cell walls was detected in some bacteria using the electron micrographs, as shown in Fig. 1. And they found that 1.5 MHz and 30–161 mW/cm^2 ultrasound had no effect on the antibiotic susceptibility of bacteria and did not cause any genetic difference detectable by AP-PCR process [18]. However, Hernot et al. suggested that the local heat in the region of oscillating microbubbles undergoing stable cavitation of ultrasound probably promoted biochemical reactions within cells which might facilitate antibiotics binding to their targets [25].

3. Bactericidal action against bacterial biofilms

3.1. Synergistic bactericidal effects on bacterial biofilms

Qian et al. found that the viable concentration of *P. aeruginosa* biofilms in 24 h cultures was decreased to 10^2 CFU/mm^2 after treatment with 12 μg/ml gentamicin and 500 kHz and 10 mW/cm^2 ultrasound, which is 1 log greater than gentamicin alone that decreased the viability to 10^3 CFU/mm^2 [26]. The biofilm viability of *P. aeruginosa* biofilms was reduced to as low as about 10 CFU/mm^2 after combined treatment with 70 kHz and 10 mW/cm^2 ultrasound and gentamicin [27]. But ultrasound alone did not reduce the viability of *P. aeruginosa* biofilms. No structural disintegration or complete removal of *P. aeruginosa* biofilms was detected using laser confocal scanning microscope [26]. A study from Johnson et al. showed that complete killing of *E. coli* biofilms in 14 h cultures was realized at exposure to ultrasound and gentamicin [28].

Rediske et al. implanted the *E. coli* biofilms grown on polyethylene disks subcutaneously on the backs bilaterally to the vertebral columns of New Zealand White female rabbits. Treatment with 28.48 kHz and 300 mW/cm^2 ultrasound and gentamicin reduced the viable counts of *E. coli* biofilms to as low as 0.89 $\log_{10}$ CFU/mm^2, which was far below a viable counts of 4.11 $\log_{10}$ CFU/mm^2 of biofilms treated with antibiotic alone. But, tissue damage to the skin was noted [29]. Herein, Rediske et al. performed similar experiments in vivo rabbit model using pulsed ultrasound with the same peak intensity. They found that the pulsed ultrasound showed equal reduction in viability at a lower total power intensity compared with continuous ultrasound in vivo experiment. More importantly, the skin showed no tissue damage [30]. For *S. epidermidis* infection that is often observed in orthopedic implants [31], Carmen et al. implanted *S. epidermidis* biofilms grown on polyethylene disks for 24 h subcutaneously on the backs bilaterally of rabbits. The rabbits were then treated with ultrasonic irradiation at 28.48 kHz and 300 mW/cm^2 (I_p) pulsed in a 1:3 duty cycle and vancomycin intravenously at the same time. After exposure to ultrasound for 48 h and vancomycin treatment, the viability of bacteria was reduced from 10^7 CFU/mm^2 to 10 CFU/mm^2 which was 2 logs much lower than viable counts of 10^3 CFU/mm^2 only treated with vancomycin [32]. They further discovered that *E. coli* biofilms implanted subcutaneously on the back of rabbits were almost eradicated after exposure to 28.5 kHz and 500 mW/cm^2 ultrasound pulsed in a 1:3 duty cycle for 48 h and gentamicin treatment [33].

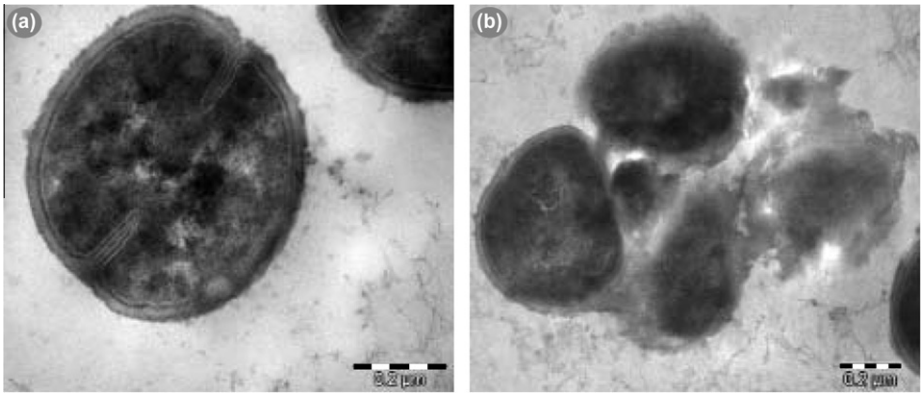

Fig. 1. (a) From the control group. A dividing bacterium with normal morphologies (×120,000). (b) From the test group. Some bacteria represent complete cell disruption in addition to the wall destruction (×75,000). Adapted from Ayan et al. [18].