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Ultrasonic Enhancement of Antibiotic Action on Gram-Negative Bacteria

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The effect of gentamicin upon planktonic cultures of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* was measured with and without application of 67-kHz ultrasonic stimulation. The ultrasound was applied at levels that had no inhibitory or bactericidal activity against the bacteria. Measurements of the MIC and bactericidal activity of gentamicin against planktonic cultures of *P. aeruginosa* and *E. coli* demonstrated that simultaneous application of 67-kHz ultrasound enhanced the effectiveness of the antibiotic. A synergistic effect was observed and bacterial viability was reduced several orders of magnitude when gentamicin concentrations and ultrasonic levels which by themselves did not reduce viability were combined. As the age of the culture increased, the bacteria became more resistant to the effect of the antibiotic alone. Application of ultrasound appeared to reverse this resistance. The ultrasonic treatment-enhanced activity was evident with cultures of *P. aeruginosa* and *E. coli* but was not observed with cultures of gram-positive *S. epidermidis* and *S. aureus*. These results may have application in the treatment of bacterial biofilm infections on implant devices, which infections are usually more resistant to antibiotic therapy.

Bacterial biofilms usually colonize chronic transdermal medical devices such as catheters, dialysis tubing, drug delivery tubing, and electrical (e.g., pacemaker) leads. Bacterial biofilms are also known to colonize totally implanted devices such as artificial vascular grafts, heart valves, left ventricular assistance devices, internal catheters, prosthetic joints (hips, knees, etc.), and other polymeric or metallic implants. Bacterial biofilm formation on transdermal devices is usually attributed to bacteria migrating from the skin along the surface of the device into the body. In totally internal devices, the infection is thought to result from nonsterile surgical procedures or hematogenously disseminated bacteria that adhere to and subsequently colonize the device. In each of these cases, the infection can often be controlled by antibiotic therapy but it is extremely difficult, if not impossible, to eradicate the infection completely (5, 10, 14). Previous studies have shown that the concentrations of antibiotics needed to eradicate an in vitro bacterial biofilm on a polymeric or metal substrate completely is 50 to 5,000 times higher than that needed to kill planktonic (suspended) bacteria (10, 14). There are several current hypotheses about the possible mechanisms producing this resistance. Most hypotheses attribute the resistance to the exopolysaccharide and other exopolymers exuded by the bacteria in the formation of the biofilm (1, 5, 7, 9, 13). The exopolysaccharide is believed to establish a diffusional (or transport) barrier that protects the cells (1). Other researchers have suggested that the exopolysaccharide binds the antibiotic before it can reach the bacteria (12). Still other hypotheses have proposed that the bacteria in mature biofilms are metabolically dormant and thus cannot take up and/or metabolize the antibiotics (1, 2, 4, 6, 13). All of these scenarios concur that the biofilm environment somehow protects the bacteria such that not all of the bacteria in the biofilm are killed by the antibiotic. Thus, the infection reappears when the antibiotic therapy is stopped, and the device usually must be removed to eliminate the infection.

If a bacterial biofilm infection could be completely eradicated from an infected device, one could avoid chronic antibiotic therapy or subsequent surgeries for removal or replacement. In addition, this may allow the use of devices that are currently restricted by the problems of infection, such as polymeric prostheses in the urinary tract. We are studying the synergistic use of ultrasound and antibiotics to kill the bacteria sequestered in a biofilm. This report presents results of the first step in that study, which is investigation of the in vitro response of planktonic bacteria to combinations of ultrasound and an antibiotic.

MATERIALS AND METHODS

Organisms. Cultures of *Pseudomonas aeruginosa* (GNRNF-P_SA-1), *Escherichia coli* (ATCC 10798), *Staphylococcus epidermidis* (ATCC 14460), and *Staphylococcus aureus* (ATCC 12260) were maintained on sheep blood agar plates. Twenty-four hours before an experiment, an inoculum was transferred from a colony on the agar plate to 10 ml of tryptic soy broth (TSB) without glucose (Difco, Detroit, Mich.) and grown overnight at 37°C. After 24 h, 0.01 ml of the culture was transferred to 9.99 ml of sterile TSB and grown at 37°C in 50-ml Erlenmeyer flasks on a rotary shaker. Growth curves showed that the bacteria were in the exponential phase of growth between 2 and 10 h. The numbers of bacteria in the suspensions were measured and bacterial viability was verified by plating serial dilutions onto nutrient agar and incubating the agar plates for 24 h.

Antibiotic. Gentamicin sulfate (lot no. G3632; Sigma) was used without further purification.

Ultrasonication. These experiments employed a Sonicor

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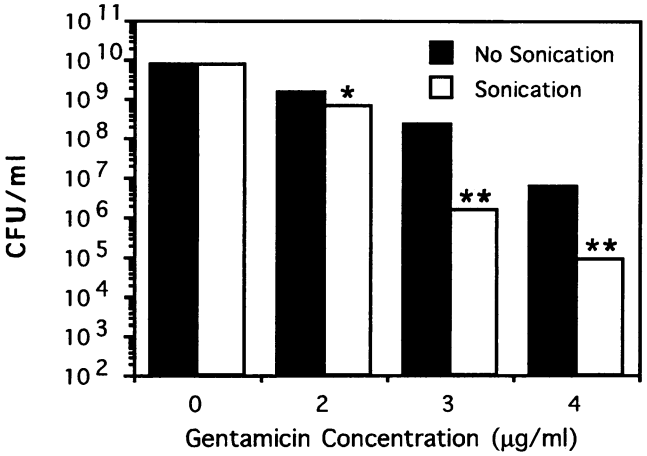

FIG. 1. Concentration of *P. aeruginosa* in 14-h cultures without and with 67-kHz ultrasonication. The data are averages of five replicated experiments. The single asterisk indicates statistical significance at the 0.05 level, and the two asterisks indicate significance at the 0.025 level.

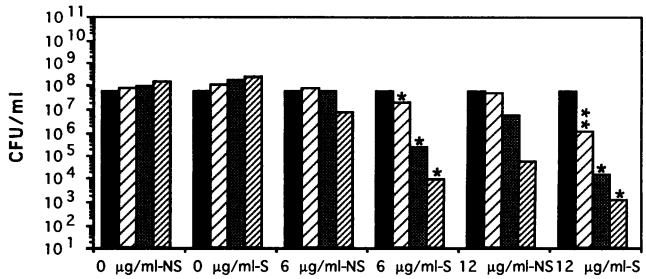

FIG. 2. Concentration of viable *P. aeruginosa* in a 3-h culture subsequently exposed to gentamicin and/or ultrasonication. The *x* axis gives exposure to gentamicin and/or ultrasonication, where NS indicates no sonication and S indicates 67-kHz sonication. The bar shading represents subsequent exposure times as follows: black, 0 h; light hatching, 0.5 h; grey, 1 h; heavy hatching, 2 h. The single asterisk indicates statistical significance at the 0.05 level, and the two asterisks indicate significance at the 0.025 level.

SC-100 sonicating bath (Sonicor Instrument Co., Copiaque, N.Y.) operating at 67 kHz and a power density of 0.3 W/cm^2 as specified by the manufacturer. (We did not measure the frequency or power density ourselves.) This ultrasonic power was generated by two lead zirconate crystals connected to the underside surface of a stainless steel bath. The bath was filled with water to a volume of 1,500 ml and maintained at a constant temperature by recirculating the water through a temperature control unit (Cole-Parmer Refrigeration Circulator no. 1268-14). The ultrasonicating bath temperature was monitored and controlled on the basis of the temperature of the bacterial suspensions inside the test tubes.

Measurements of MIC. The MIC of gentamicin was measured by preparing a series of glass test tubes containing TSB and gentamicin at concentrations of 0, 1, 2, 3, 4, and 5 μg/ml for *P. aeruginosa* and *S. aureus*; 0, 2, 3, 4, 6, 8, 10, and 12 μg/ml for *E. coli*; and 0, 0.1, 0.2, 0.3, 0.4, and 0.5 μg/ml for *S. epidermidis*. Each tube was inoculated with exponential-growth-phase organisms to a concentration of 10^5 bacteria per ml and incubated at 37°C for 14 h. After 14 h of incubation, the turbidities of the cultures were assessed and the concentrations of the cultures were determined by plate counting. The MIC was defined as the lowest concentration of antibiotic in the test tube that showed no turbidity and had viable counts of less than 10^7 CFU/ml. The MICs for *P. aeruginosa*, *E. coli*, *S. epidemidis*, and *S. aureus* were 4, 6, 0.3, and 3 μg/ml, respectively. In similar experiments, we measured the effect of temperature on the MIC for *P. aeruginosa*.

To measure the effect of ultrasound on the MIC, parallel experiments were performed in which identical sets of cultures were placed in the ultrasonicating bath and in an incubator in a separate room well removed from the noise and vibrations of the ultrasonicating bath.

Measurement of bactericidal activity. The bactericidal activity of gentamicin was measured by inoculating TSB in a shaker flask with the organisms and growing the suspensions for 3, 6, 8, 10, 12, 24, or 36 h at 37°C. At each of the time intervals indicated, 0.9 ml of the suspension was placed into each of several test tubes and 0.1 ml of gentamicin in a physiologic saline solution (PSS) was added to produce concentrations of 3, 6, and 12 μg/ml for *P. aeruginosa*, *E. coli*, and *S. aureus* and 0.3, 0.6, and 1.2 μg/ml for *S. epidermidis*. These tubes were incubated at 37°C without shaking for 0, 0.5, 1, 2, 3, 6, 12, and 24 h, after which times the contents of the tubes were vortexed and samples were removed and plate counted to determine viable concentrations.

To measure the effect of ultrasound on bactericidal activity, parallel and identical sets of cultures were prepared at the time gentamicin was introduced. One set of test tubes was placed in the ultrasonicating bath, and the other set was placed in an incubator in a separate room.

In some experiments with *P. aeruginosa* only, the culture was grown for 3 h in TSB without gentamicin prior to initiation of ultrasonication. During this initial 3 h, some cultures were ultrasonicated while others were not to determine whether sequential exposure to ultrasound and gentamicin has the same effect as simultaneous exposure.

Other experiments. To elucidate possible mechanisms leading to the synergistic effect of ultrasound and gentamicin, several other similar experiments were performed. To examine whether ultrasound could change (break down) the TSB nutrient, we sonicated TSB for 24 h before using it as a growth medium and then measured growth rates of *P. aeruginosa* in shaken cultures. The oxygen concentrations in ultrasonicated and nontreated samples of PSS, TSB, and a culture in TSB were measured with an oxygen electrode (97-08; Orion Research, Cambridge, Mass.).

To examine changes in *P. aeruginosa* cellular function caused by ultrasonic exposure, cell suspensions were subjected to ultrasonication for up to 24 h. The resulting cultures were examined on a hanging-drop slide, and the percentage of cells having normal or abnormal motility was estimated.

RESULTS

MIC. Figure 1 shows the results of the MIC experiments with gentamicin and *P. aeruginosa* in the presence and absence of 67-kHz ultrasound. These data show that the MIC decreased from 4 to 3 μg/ml in the presence of ultrasound. At higher gentamicin concentrations, ultrasonication decreased the viable cell counts by 2 or 3 orders of magnitude below that of the nonsonicated culture. This observation was consistent through eight replications of this experiment.

To verify that the observed decreased MIC of gentamicin was not caused by any slight temperature changes due to ultrasonic heating, the MIC of gentamicin (in the absence of ultrasound) was measured at 35, 37, and 39°C. Results showed

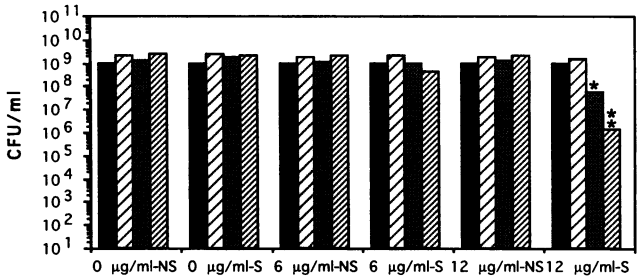

FIG. 3. Concentration of viable *P. aeruginosa* in a 6-h culture subsequently exposed to gentamicin and/or ultrasonication. The *x* axis gives exposure to gentamicin and/or ultrasonication, where NS indicates no sonication and S indicates 67-kHz sonication. The bar shading represents subsequent exposure times as follows: black, 0 h; light hatching, 0.5 h; grey, 1 h; heavy hatching, 2 h. The single asterisk indicates statistical significance at the 0.05 level, and the two asterisks indicate significance at the 0.025 level.

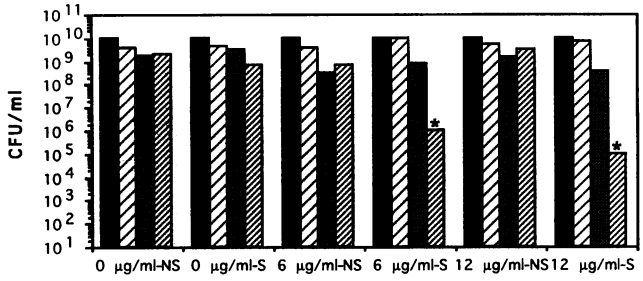

FIG. 5. Concentration of viable *P. aeruginosa* in a 24-h culture subsequently exposed to gentamicin and/or ultrasonication. The *x* axis gives exposure to gentamicin and/or ultrasonication, where NS indicates no sonication and S indicates 67-kHz sonication. The bar shading represents subsequent exposure times as follows: black, 0 h; light hatching, 3 h; grey, 12 h; heavy hatching, 24 h. The asterisk indicates statistical significance at the 0.05 level.

that there was no significant effect ($P > 0.05$) upon the MIC of gentamicin in this range of temperatures (data not shown).

The MIC of gentamicin for *E. coli* was reduced from 6 to 3 μg/ml by application of ultrasound. Similar experiments with the *S. epidermidis* and *S. aureus* strains showed no change in the gentamicin MIC in the presence of ultrasound.

Bactericidal activity. Figures 2 through 4 show the bactericidal activities of gentamicin against *P. aeruginosa* after 3, 6, and 12 h of growth in a shaker flask. In these figures, the *x* axis represents the particular combination of antibiotic and/or ultrasound and the *y* axis is the concentration of CFU. Each bar represents the time of exposure to the antibiotic and/or ultrasound, as explained in the legends. The data in Fig. 2 came from a 3-h-old culture, and the bacteria were rapidly growing. There was a slight increase in the bacterial population when no gentamicin was added. The similar increase in the second set of bars shows that in the presence of ultrasound the culture also continued to grow. This level of ultrasound by itself had no inhibitory effect. The third and fourth sets of bars show the concentrations of viable bacteria at 6 μg/ml with and without ultrasound. The antibiotic by itself had a slight effect, decreasing the viability by an order of magnitude after 2 h of exposure, but the combination of antibiotic and ultrasound decreased the viability by nearly 4 orders of magnitude. At 12 μg/ml, this synergistic killing effect was observed after only 30 min of antibiotic exposure.

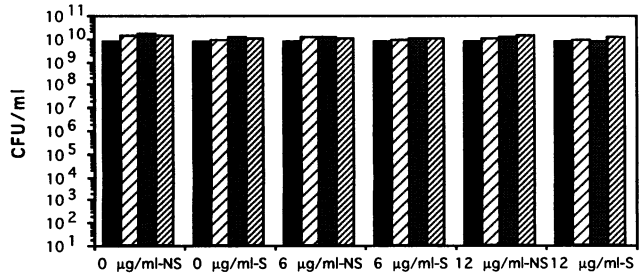

FIG. 4. Concentration of viable *P. aeruginosa* in a 12-h culture subsequently exposed to gentamicin and/or ultrasonication. The *x* axis gives exposure to gentamicin and/or ultrasonication, where NS indicates no sonication and S indicates 67-kHz sonication. The bar shading represents subsequent exposure times as follows: black, 0 h; light hatching, 0.5 h; grey, 1 h; heavy hatching, 2 h.

Figure 3 shows similar data for bacteria that were cultured for 6 h before application of gentamicin or ultrasonication. At this point, the bacteria were nearing the end of the exponential growth phase. The lack of change in the first, third, and fifth sets of bars indicates that without ultrasonication, the cultures were not affected by 12 μg of gentamicin per ml. The synergistic effect of ultrasound was evident at 1 and 2 h of exposure. At 2 h of exposure to 12 μg/ml, the synergistic effect resulted in killing of 99.9% of the bacteria whereas there was no killing by the antibiotic alone.

Figure 4 shows the data for bacteria after 12 h of culture, when the bacteria were in the stationary phase with very little growth. No bactericidal effect was observed after 2 h of exposure to gentamicin-ultrasound treatment. Neither was any such effect observed on 24- and 36-h cultures exposed for 2 h. However, if the exposure was extended to 24 h, a bactericidal effect was observed, as exemplified in Fig. 5, which shows data from a 24-h culture of *P. aeruginosa*. Ultrasound alone did not kill cells, and gentamicin alone had only a slight bactericidal effect, but the combination of both stresses for 24 h decreased viable counts by 5 orders of magnitude. These data suggest that the bacteria were more recalcitrant to antibiotics and ultrasound when they were in the stationary growth phase, but they became susceptible given enough treatment time.

To determine whether sequential exposure to ultrasound and gentamicin had the same effect as simultaneous exposure, fresh cultures were exposed to ultrasound for 3 h before exposure to gentamicin. Table 1 shows the viability results for cultures subsequently subjected to 2 h of gentamicin exposure.

TABLE 1. Viable cell counts following sequential exposure of *P. aeruginosa* to ultrasound and gentamicin

Application of ultrasound[a] during:		Viable cell count after exposure to gentamicin concn (μg/ml) of:		
First 3 h of growth (no gentamicin)[b]	Subsequent 2-h exposure to gentamicin	0	6	12
−	−	3.7×10^8	9.3×10^6	1.4×10^5
+	−	3.6×10^8	3.8×10^7	3.2×10^5
−	+	5.8×10^8	2.4×10^5	7.1×10^3
+	+	2.8×10^8	9.0×10^3	4.7×10^3

[a] −, no ultrasound; +, ultrasound.

[b] Concentrations at the end of the 3-h growth period averaged 1.7×10^8 CFU/ml.

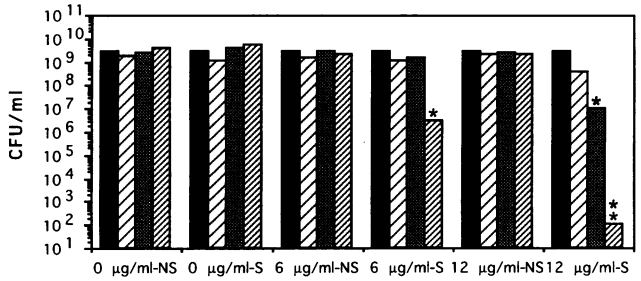

FIG. 6. Concentration of viable *E. coli* in a 6-h culture subsequently exposed to gentamicin and/or ultrasonication. The *x* axis gives exposure to gentamicin and/or ultrasonication, where NS indicates no sonication and S indicates 67-kHz sonication. The bar shading represents subsequent exposure times as follows: black, 0 h; light hatching, 2 h; grey, 6 h; heavy hatching, 24 h. The single asterisk indicates statistical significance at the 0.05 level, and the two asterisks indicate significance at the 0.025 level.

The data show that without gentamicin exposure, ultrasound alone had no significant impact on the cell population. When exposed to both the 6- and 12-μg/ml solutions, viable counts were decreased by orders of magnitude when ultrasound was applied. Comparison of the first two lines, or of the third and fourth lines, reveals that the presence or absence of ultrasound during initial growth (without gentamicin) had no significant impact on final cell viability.

Data from similar experiments with *E. coli* showed similar results. For example, Fig. 6 shows the viability of bacteria from a 6-h culture subsequently exposed to gentamicin and ultrasound for up to 24 h. Neither gentamicin alone nor ultrasound alone had any bactericidal effect, whereas the combination of the two decreased viability by 7 orders of magnitude. Not shown are results for 3-, 12-, and 24-h cultures of *E. coli*, all of which demonstrated the synergistic effect. As with *P. aeruginosa*, the older *E. coli* cultures were less susceptible than the younger cultures, but even the viability of the 24-h culture was decreased by 6 orders of magnitude after 24 h of exposure to ultrasound and 12 μg of gentamicin per ml.

In similar experiments with *S. epidermidis* and *S. aureus*, application of ultrasound appeared to have no statistically significant effect on viability.

Miscellaneous experiments. In parallel growth experiments using freshly autoclaved TSB and TSB which had been ultrasonicated for 24 h before sterilization, we found no significant difference in the growth rates of *P. aeruginosa* cultures. In normal TSB and ultrasonicated TSB, the average generation times were 26 and 27 min, respectively, over 3 h of growth and 53 and 55 min, respectively, over 10 h of growth.

The O_2 concentrations in PSS, TSB, and a growing culture before and after 24 h of ultrasonication are given in Table 2. There was no significant difference ($P > 0.05$) due to ultrasonication.

TABLE 2. Oxygen concentrations in PPM at 37°C

Solution	Mean O_2 concn ± SD (no. of measurements)	
	No sonication	Ultrasonication for 24 h
PSS	6.75 ± 0.09 (6)	6.65 ± 0.18 (6)
TSB	5.96 ± 0.30 (6)	6.29 ± 0.20 (3)
Growing culture	2.82 ± 0.25 (3)	2.78 ± 0.26 (3)

TABLE 3. Motility of cells subjected to ultrasound

Time (h)	No. of cells		% Motility	
	Ultrasonicated	Not ultrasonicated	Ultrasonicated	Not ultrasonicated
Expt 1				
0	2.7×10^8	3.1×10^8	95	95
1	3.0×10^8	3.0×10^8	90	95
2	5.6×10^8	5.6×10^8	80	95
3	7.8×10^8	4.0×10^8	70	95
Expt 2				
0	3.9×10^6	3.7×10^6	95	95
3	9.4×10^7	3.2×10^7	80	95
6	6.2×10^8	1.4×10^8	30	95
9	1.3×10^9	6.8×10^8	50	95
18	3.9×10^9	3.0×10^8	40[a]	65[b]
24	6.8×10^9	3.7×10^9	40[a]	70[b]

[a] Forward motility was not as quick as in younger or nonultrasonicated cells.
[b] There was much more general movement in the hanging drop from the nonultrasonicated sample than in that from the ultrasonicated sample.

Table 3 presents the motility of *P. aeruginosa* following exposure to ultrasound for various times. Two representative experiments are presented, each having a different initial concentration and duration. The percentages represent the portions of cells which exhibited a quick darting motion, typical of flagellated *P. aeruginosa*. Most of the balance of the cells exhibited a quivering or shaking motion without any net forward movement (clearly different from Brownian motion), and a small percentage of cells showed no motion. These data indicate that in nonultrasonicated cells, the quick darting motion continues for up to 24 h, although after some time the percentage of cells exhibiting this motion is reduced. At 18 and 24 h of growth, most of the nonultrasonicated cells still displayed the quick darting movement, whereas in the ultrasonicated cells, the cells with a net forward motion moved at a reduced speed. In all cultures exposed to ultrasonication for 9 h or more, a large fraction of the cells showed no motility. It is noteworthy that such flagellum-impaired cells were still viable and showed no population decrease upon plating.

DISCUSSION

The results of both the MIC experiments and the measurements of bactericidal activity show that addition of ultrasonic stimulation to gentamicin exposure enhances the effectiveness of the antibiotic in killing *P. aeruginosa* and *E. coli*. The statistical significance of the data was established by using a one-tailed *t* test to examine the null hypothesis that the bacterial populations were the same with and without application of ultrasound. In Fig. 1 to 6, the asterisks indicate samples in which there was a significant decrease in viability compared with that of a nonsonicated sample under identical growth conditions and gentamicin concentrations. There were no significant decreases in viability when ultrasound was applied without gentamicin.

The combination of 26.5-kHz ultrasound and gentamicin has been used clinically in the former Soviet Union to reduce bacterial colonization in surgical wounds (11). The reported procedure reduced the incidence of purulent-septic complications, but the mechanism of action was not addressed. Other researchers in the former Soviet Union have reported that the ultrastructure of fungi of the genus *Candida* was changed by the synergistic action of nystatin and 2.6-MHz ultrasound (15).

Although it is true that ultrasonic treatment at sufficiently

high levels is capable of killing bacteria (8, 16), our data indicate that 67-kHz ultrasound by itself at these power levels did not decrease bacterial viability. In fact, cultures experiencing ultrasonication without gentamicin always grew slightly faster than the nonsonicated control. The increased growth rate usually resulted in an ultrasonicated population twice as large as control groups after 3 h of growth. We do not think that this increase is due to increased oxygen concentrations in the ultrasonicated cultures, because the oxygen concentrations in PSS, TSB, and growing cultures were not significantly changed by ultrasonication. In the literature, bacterial killing by ultrasound is usually attributed to cavitation in or on the bacteria or to generation of peroxides which subsequently kill the bacteria (8, 16). The levels of ultrasound used in this study were low and are apparently sublethal and subinhibitory for up to 24 h of exposure.

The motility observations, however, indicate that the cells were under some stress. The loss of the forward darting manner of motility suggests that ultrasonication impairs the flagellar function. The data suggest that the increased growth observed cannot be attributed to a higher oxygen concentration or modification of the TSB nutrient, but it may be possible that ultrasonicated cells are under some stress and show stress-induced growth.

Of particular interest is the effect of culture age upon susceptibility to the combination of ultrasound and the antibiotic. The data consistently show that as the culture becomes older, it becomes more recalcitrant to antibiotic activity. Figure 2 shows that the viability of a young (3-h) culture is reduced by gentamicin exposure at 1.5 or 3 times the MIC. However, *P. aeruginosa* and *E. coli* cultures that are 6 h old or older are not affected by 12 μg of gentamicin per ml in the absence of ultrasound. Growth curves show that both of these species are entering the stationary growth phase at about 6 h, and thus their metabolic processes are changing and their growth rate is slowing. These observations are consistent with previous reports (2, 4, 6) which show that growth rate is a primary modulator of antibiotic effectiveness. However, application of ultrasound eventually appears to restore bacterial susceptibility to gentamicin, and the duration of this refractory period (the time before susceptibility is restored) increases as the age of the culture increases.

The repeatability of the observed synergistic killing by ultrasound and gentamicin ensures that this phenomenon is real. However, the mechanisms by which it occurs remain elusive. Several possible hypotheses have been eliminated by the experiments described above. The measurements of O_2 concentrations in PSS, TSB, and bacterial cultures suggest that synergism is not due to a higher metabolic level caused by a higher oxygen concentration. Nor can it be attributed to changes in the TSB nutrient caused by ultrasonic treatment-mediated breakdown of nutrients.

The sequential-exposure experiments showed that ultrasound followed by gentamicin has no effect. Therefore, it seems that ultrasound alone does not disrupt the outer membrane of *P. aeruginosa* to allow easier access of gentamicin in a subsequent exposure. Or, if the membrane is disrupted, it is repaired sufficiently fast (within 1 min) that there is no enhanced killing over that done by gentamicin against nonstressed cells.

Of the four bacterial species studied to date, the two gram-negative species were affected by ultrasonic stimulation at 67 kHz, while gram-positive *S. epidermidis* and *S. aureus* were not affected. Owing to the limited number of species examined, we cannot determine whether or not this is a general phenomenon. Gentamicin by itself is effective against all four species.

The observation that ultrasonic stimulation can restore susceptibility to an antibiotic has significance with respect to the eradication of biofilms. Bacteria sequestered in biofilms appear to be much more resistant to antibiotic therapy than do planktonic cells. Explanatory hypotheses assume that the bacterial cells at the base of the biofilm are metabolically less active than those near the top of the biofilm (1, 13). The bacteria near the top have access to oxygen and nutrients; in addition, their waste products can easily diffuse away. Cells at the base of the biofilm may have limited supplies of oxygen and essential nutrients, and the metabolic waste products sequestered near the bacteria would be more concentrated. In a nutrient- or energy-deficient state, bacteria such as *P. aeruginosa* that actively transport gentamicin across the cytoplasmic membrane in energy-dependent processes (3) would be more resistant because of both lower internal antibiotic concentrations and reduced protein synthesis and other metabolic activities. The scenario implied by this hypothesis is that the cells at the base of a mature biofilm are sufficiently dormant that they are not affected by antibiotic therapy. However, they become active if the upper layers of the biofilm are killed and subsequently removed, thus making increased levels of nutrients available to the underlying bacteria. The significance of our present findings is that dormant bacteria in a biofilm may become susceptible to antibiotics through ultrasonic treatment. Evaluation of such a possibility and elucidation of the mechanisms by which ultrasound enhances antibiotic activity remain for future studies.

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