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The effect of isolated high-energy shock wave treatments on subsequent bacterial growth

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Summary. To determine wether high-energy shock waves possess bactericidal potential, ATCC strains of *Eschericha coli, Streptococcus faecalis, Pseudomonas aeruginosa* and *Staphylococcus aureus* were suspended in solution at concentrations approximating 10^6 bacteria per milliliter, placed in polypropylene cryovials, and immersed in the water bath of a Dornier HM3 lithotriptor. Each cryovial was then fluoroscopically guided to the epicenter of the f2 focal point and 2000 shocks at 20 kV applied. Suspensions were then serially diluted and colony counts obtained. The procedure was then repeated with 4000 shocks at 20 kV from the Dornier HM3 and 4000 shocks at intensity level 4 from a Wolff Piezolith 2200 shock wave lithotriptor. Comparison of shock-wave-treated and sham-treated bacterial suspensions revealed no significant difference in bacterial growth according to the colony count technique. We conclude that high-energy shock waves, whether generated by spark gap or piezoelectric array, do not possess significant bactericidal activity.

Key words: High-energy shock waves – Bactericidal effect

Previous clinical studies [8, 10] gave rise to reports of decreases in both persistent urinary tract infection and bacteriuria after high-energy shock wave lithotripsy of infection stones. Medical management of patients involved in these studies included perioperative intravenous antibiotics as well as lithotripsy. It was assumed by the authors of these reports that the bactericidal effect that occurred following high-energy shock-wave lithotripsy was secondary to stone pulverization, which had exposed the bacteria within the infection stone to concurrent antimicrobial treatment.

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A possible alternative effector of bactericidal activity during shock-wave treatment is direct bacterial cytoxicity generated by high-energy shock waves. High-energy shock waves have been shown to possess powerful physical forces, and previous investigators have demonstrated tumor cell cytoxicity in vitro and tumor growth suppression in vivo [12]. This study was designed to determine whether isolated high-energy shock waves generate bycterricidal activity when administered to suspensions of different bacterial pathogens.

Materials and methods

Bacterial suspensions

ATCC (American Type Culture Collections) strains (Difco Laboratories, Detroit Mich.) of *Pseudomonas aeruginosa* (ATCC #27853), *Streptococcus faecalis* (ATCC #29212), *Staphylococcus aureus* (ATCC #25923) and *Escherichia coli* (ATCC #25922) were cultured on trypticase soy agar with 5% sheep blood. Several of the resultant colonies were mixed with sterile normal saline to make bacterial suspensions. These suspensions were compared with spectrophotometrically derived absorbance/concentration curves (Fig. 1) and diluted as necessary with sterile normal saline in order to approximate the bacterial concentration to 10^6 bacteria per ml. Equal aliquots of each bacterial suspension were each transferred to 2.0 ml polypropylene cryovials (Vanguard International). Each tube was filled completely, and the cryovial caps were packed with paraffin wax in order to exclude air bubbles which could interfere with shock-wave transmission.

Shock-wave treatments

Cryovials containing aliquots of bacterial suspensions for high-energy shock-wave treatment were secured to the mock stone fixture attached to the patient gantry of a Dornier HM3 lithotriptor. The cryovial was fluoroscopically positioned at the epicenter of the f2 focal point. All contents of the cryovial fit within the dimensions of the f2 focal point (Fig. 2; shaded area denotes f2 focal point). Initial fluoroscopic visualization was assisted by placing a metal sleeve over the cryovial, which was manuevered to the epicenter of the f2 focal point using the patient-positioning hydraulic system. The position of the tip of the metal antenna attached to the mock stone fixture was

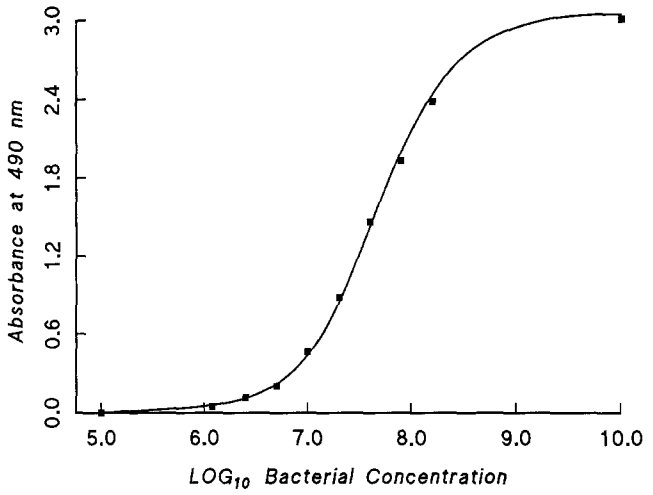

Fig. 1. Turbidimetric approximation of bacterial concentration – *E. coli*

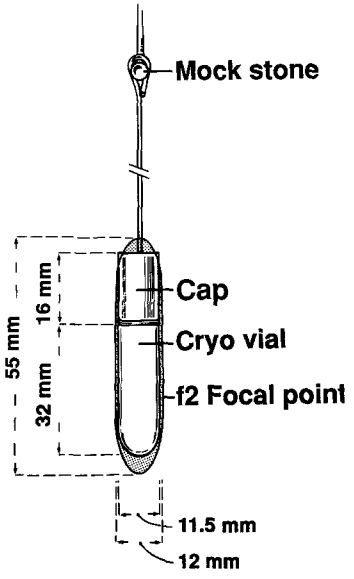

Fig. 2. Positioning of bacterial suspension in f2 focal point (*shaded area* represents dimensions of f2 focal point)

noted in both fluoroscopic monitors and the metal sleeve removed from the water bath. The positioning of the tip of the antenna was frequently reevaluated by fluoroscopy during shock wave administration, and after each 1000 shocks the metal sleeve was reinserted and placement of the cryovial in the epicenter of the f2 focal point was fluoroscopically reconfirmed.

A sham-treated control bacterial suspension was submerged in the water bath and secured at the periphery furthermost from the f2 focal point (approximate distance 2 m). Each aliquot of the bacterial suspensions in the shock-wave treatment group then received 2000 shock waves. Shock waves were administered at a rate of 100 per min at 20 kV. Electrodes were replaced after each 2100 shocks, and the first 100 shock waves were generated on an empty water bath to avoid the high degree of energy variability previously noted among the initial 100 shock waves [5]. The water bath was composed of degassed, deionized water maintained at a constant 37°C. All cryovials containing aliquots of the same bacterial suspension underwent shock-wave treatment on the same day.

The experiment was then repeated using different aliquots of the same bacterial strains, delivering 4000 shocks at 20 kV and 100 shocks per min to each bacterial suspension. Again, each set of four cryovials containing aliquots of the same bacterial suspensions were treated on the same day and sham-treated suspensions were exposed to identical environmental conditions.

Using the same technique, aliquots of bacterial suspensions of each of the four bacterial strains were exposed to 4000 shock waves generated by a Wolff Piezolith 2200 at intensity level 4 and a rate of 120/min. Cryovials were secured to a laboratory stand and localized to the focal point using ultrasonic guidance. Water bath temperatures were again maintained at 37°C. There was a sham-treated control for each aliquot for comparison.

Bacterial counts

Bacterial suspensions were serially diluted with sterile normal saline to 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} concentrations using a sterile technique. Two 100-ul aliquots each of 10^{-3}, 10^{-4} and 10^{-5} dilution were plated on trypticase soy agar with 5% sheep blood using the colony technique. Bacterial suspensions were vortexed to produce maximum uniformity and minimize clumping prior to each dilution and prior to plating for colony counting. Agar plates were incubated for 24 h at 37°C, then counted to establish total numbers of bacterial colonies.

Results

Initially, single aliquots of each bacterial suspension were treated with 2000 shocks from a Dornier HM3 lithotriptor. Dual colony counts were prepared from each bacterial suspension at serial dilutions to 10^{-3}, 10^{-4} and 10^{-5} concentration. Plates that showed signs of contamination were discarded. Plates with the highest countable concentrations of bacteria (100–1000) and the next serial dilution were used in subsequent computations of bacterial concentrations. Single aliquots of each bacterial suspension underwent sham treatment and the resultant bacterial cell count was computed in a similar manner. This initial study revealed no significant difference in bacterial cell count when aliquots of the same suspension treated with 2000 shocks and subjected to sham treatment were compared; therefore, four aliquots of each bacterial strain were treated with 4000 shock waves from the Dornier HM3 lithotriptor. Serial Dilution and colony count plating for computation of bacterial cell count were performed in a similar manner. A single sham-treated control suspension with dual colony count plating of each serial dilution was used to compute control bacterial cell counts. Comparison of suspensions treated with 4000 shock waves versus control suspensions revealed no significant difference among any of the four strains of bacteria tested (Fig. 3, Table 1).

Treatment with shock waves generated by the Wolff Piezolith was performed in a similar manner; however, only single aliquots of each bacterial strain were subjected to shock-wave treatment. Comparison of bacterial concentrations among Piezolith shock wave treated suspensions and controls also revealed no statistically significant difference among any of the four strains of bacteria tested (Fig. 4, Table 2).

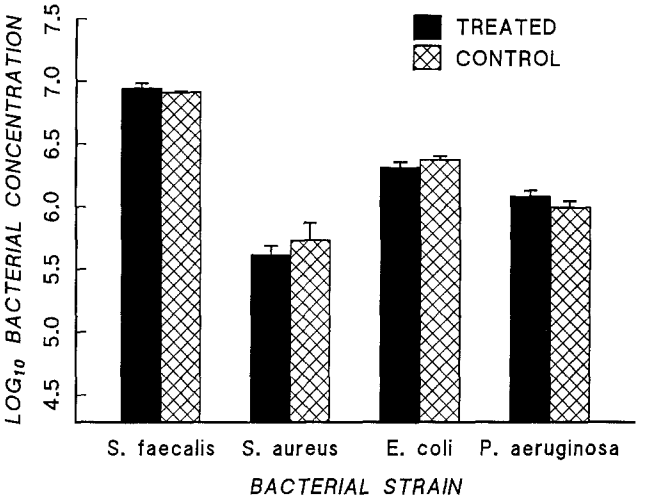

Fig. 3. Comparison of bacterial concentration between Dornier treated (4000 shocks at 20 kV) and control bacterial suspensions (*error bars* represent standard error of the mean)

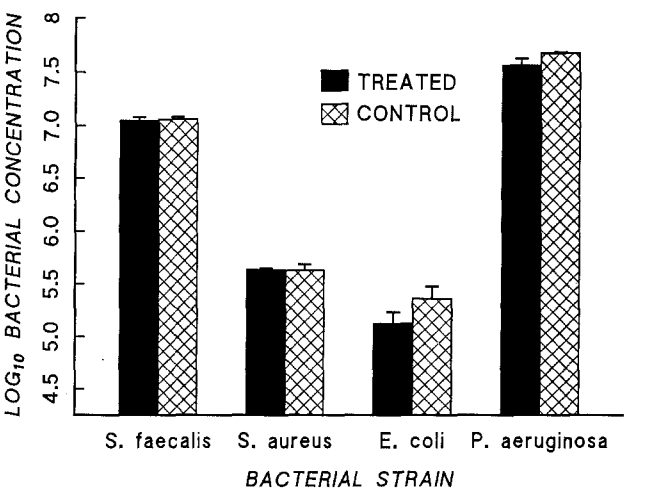

Fig. 4. Comparison of bacterial cocentration between Piezolith-treated (4000 shocks, intensity level 4) and control bacterial suspensions (*erro bars* represent standard error of the mean)

Table 1. Comparison of treated (4000 shocks from a Dornier HM3 lithotriptor) vs control bacterial cell counts

	n^a	$\bar{x}$	SEM
Streptococcus faecalis			
Treatment	8	8.87×10^6	0.77×10^6
Control	2	8.16×10^6	0.11×10^6
Staphylococcus aureus			
Treatment	22	4.12×10^5	0.72×10^5
Control	6	5.37×10^5	2.00×10^5
Escherichia coli			
Treatment	16	2.05×10^6	0.21×10^6
Control	4	2.34×10^6	0.17×10^6
Pseudomona aeruginosa			
Treatment	15	1.21×10^6	0.13×10^6
Control	3	0.98×10^6	0.13×10^6

[a] Number of colony count agar plates; varies significantly between groups owing to contamination, bacterial clumping or presence of innumerable bacterial colonies

Table 2. Comparison of treated (4000 shocks from a Wolff Piezolith 2200 lithotriptor) vs control bacterial cell counts

	n^a	$\bar{x}$	SEM
Streptococcus faecalis			
Treatment	2	1.12×10^7	0.08×10^7
Control	2	1.14×10^7	0.06×10^7
Staphylococcus aureus			
Treatment	4	4.40×10^5	0.11×10^5
Control	4	4.28×10^5	0.60×10^5
Escherichia coli			
Treatment	4	1.35×10^5	0.37×10^5
Control	4	2.30×10^5	0.71×10^5
Pseudomonas aeruginosa			
Treatment	2	3.69×10^7	0.58×10^7
Control	2	4.76×10^7	0.04×10^7

[a] Number of colony count agar plates; varies between between groups owing to presence of innumerable bacterial colonies

Statistical analysis

Treatment versus control bacterial cell counts were compared using a two-tailed, unpaired *t*-test which demonstrated no statistically significant difference in bacterial cell count among any of the bacterial strains tested ($P \geq 0.2$ for each comparison). Minimal variability was present among the results.

Discussion

High-Energy shock waves generate powerful physical forces including free radical production [9], development of high barometric pressures [2, 5] and acoustic caviation [3]. Any or all of these factors may be responsible for the previously noted in vitro tumor cytotoxicity and in vivo tumor growth suppression. Similarly, previous clinical studies noted a decrease in bacteriuria and resolution of persistent urinary tract infections after treatment of infection stones with high-energy shock waves. Stoller [13] developed an ingenious technique to determine whether isolated high-energy shock waves affect the microbial flora of infection stones by comparing the bacterial population of portions of these stones treated with high-energy shock wave lithotripsy to the bacterial population of portions of the same stones crushed between sterile clamps. These data revealed no sterilization of any of the infection stones; however, the high degree of variability among experimental results made it impossible

to determine whether any significant number of bacteria were killed. In addition, this study did not identify the bacteria obtained during culture for colony counts. Therefore, many questions about possible bactericidal effects of high energy shock waves remained unanswered. Do high-energy shock waves kill bacteria? If so, is there a large enough kill to be clinically significant? Are high-energy shock waves bactericidal to all, some, or none of the different strains of bacteria? Are different types of shock wave more effective as bactericidal agents?

This study was designed to isolate the effects of high-energy shock wave treatment on bacterial cells and determine whether bactericidal activity exists. The limits of clinical feasibility were tested during this investigation using high numbers of shock waves directed against a broad range of standard bacterial pathogens. *E. coli* and *S. faecalis* were tested as examples of gram-negative and gram-positive bacteria that are relatively easily killed by conventional antibacterial measures, whereas *P. aeruginosa* and *S. aureus* were tested as examples of more robust and resistant bacteria.

Strict adherence to previously published methodology [7] was practiced in an effort to minimize the chance of false-negative results. Cryovials composed of polypropylene were utilized owing to an acoustic impedance which approaches that of water [7, 12]. Although no studies to date have documented either the presence or the absence of an acoustic interface between polypropylene and water, many scientific studies have been published in which polypropylene cryovials were used to contain tumor cells subsequently subjected to high-energy shock-wave treatement [1, 4, 6, 7, 11, 12, 14, 15]. Several of these studies have indicated that the acoustic impedance of polypropylene approximates that of the soft tissues encountered during clinical use [6, 12]. A 2.0-ml cryovial was chosen because, as seen in Fig. 2, it fits completely but snugly within the dimensions of the f2 focal point, allowing maximal shock-wave effect with uniform distribution. The cap of each tube was packed with paraffin wax and each tube was carefully checked for air bubbles to avoid air-fluid interfaces. The position of the cryovial at the epicenter of the f2 focal point was frequently confirmed during high-energy shock-wave lithotripsy.

The results of this series of experiments show no significant bactericidal effect of high-energy shock waves generated by either a spark go or a piezoelectric array technique. Similarly, there was no significant bactericidal activity exhibited by high-energy shock waves when a broad range of bacteria with a wide spectrum of susceptibility to standard bactericidal techniques was tested. The data contain a low level of variability and care was taken to avoid any air-fluid interface or other pitfalls common during laboratory testing of high-energy shock waves. We have shown that high-energy shock waves do not kill a significant amount of any of the four species of bacteria tested and conclude that isolatd high-energy shock waves do not possess significant bactericidal activity.

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