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● *Original Contribution*

ANTIBACTERIAL EFFECTS OF EXTRACORPOREAL SHOCK WAVES

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Abstract—Despite considerable knowledge about effects of extracorporeal shock-wave therapy (ESWT) on eukaryotic tissues, only little data are available concerning their effect on prokaryotic microorganisms. The objective of the present study was to determine the bactericidal activity as a function of energy flux density and shock-wave impulse number. Standardised suspensions of *Staphylococcus aureus* ATCC 25923 were exposed to different impulse numbers of shock waves with an energy flux density (ED) up to 0.96 mJ mm^{-2} (2 Hz). Subsequently, viable bacteria were quantified by culture and compared with an untreated control. After applying 4000 impulses, a significant bactericidal effect was observed with a threshold ED of 0.59 mJ mm^{-2} ($p < 0 \cdot 05$). A threshold impulse number of more than 1000 impulses was necessary to reduce bacterial growth ($p < 0 \cdot 05$). Further elevation of energy and impulse number exponentially increased bacterial killing. ESWT proved to exert significant antibacterial effect in an energy-dependent manner. Certain types of difficult-to-treat infections could offer new applications for ESWT. (E-mail: Gerdesmeyer@aol.com) © 2005 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Infection, Lithotripsy, Shock wave, ESWT, Bactericidal, Antibacterial, Energy flux density, Impulse number.**

INTRODUCTION

Since the introduction of extracorporeal shock-wave therapy (ESWT) for the treatment of nephrolithiasis by Chaussy et al. (1980), a multitude of new indications for ESWT have arisen. Nowadays, extracorporeal shock waves are not only applied for the treatment of kidney stones, but also for the fragmentation of gallstones, pancreas stones and salivary gland stones (Delhaye et al. 1992; Iro et al. 1992; Sauerbruch et al. 1986). Apart from physical disintegration of calculi as an approved standard therapy in humans, enthesiopathies like tennis elbow, plantar heel spur or calcified tendonitis of the shoulder and bone pathologies (pseudarthroses and delayed unions) represent classical indications for ESWT (Dahmen et al. 1993; Gerdesmeyer et al. 2003; Kaulesar Sukul et al. 1993; Rompe et al. 1996a, 1996b; Schleberger and Senge 1992).

However, despite considerable knowledge about effects of shock waves on eukaryotic soft tissues, only few data are available concerning their effect on prokaryotic microorganisms. In a first approach, we evaluated the direct effect of extracorporeal shock waves on staphylococci *in vitro*. These results indicated a highly significant bactericidal effect of extracorporeal shock waves on viable *Staphylococcus aureus* cells with a mean decrease by a factor of approximately 1.3×10^3 or 3.1 orders of magnitude (von Eiff et al. 2000). Moreover, a significant bactericidal effect of high energy shock waves was found for different gram-positive and gram-negative pathogens such as *Staphylococcus epidermidis*, *Enterococcus faecium* and *Pseudomonas aeruginosa* (Gollwitzer et al. 2004).

In this study, we evaluated the bactericidal activity as a function of energy flux density (ED) and shock-wave impulse number (IN) to define the optimal *in vivo* conditions. An appropriate animal infection model is now warranted to further evaluate the data defined in these experiments. Our results may provide the basis of novel treatment for certain types of bacterial infections.

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METHODS

Preparation of bacteria

Before shock-wave treatment, *S. aureus* ATCC 25923 was cultured to late logarithmic growth phase in trypticase soy broth at 37 °C for 18 h. Bacteria were then washed twice in phosphate-buffered saline (PBS), resuspended in normal saline and adjusted to 1×10^7 c.f.u. mL^{-1}. Suspensions were prepared in normal saline and soft Pasteur pipettes (Micro-Bio-Tec-Brand, Giessen, Germany) were filled with 3.0 mL of the suspension, sealed and proceeded to ESWT.

Shock-wave application

High-energy shock waves were applied with an "Alpha Compact Lithotrypter" (Dornier Med Tech®, Wessling, Germany) in a water bath. A custom-made sample holder was used for reproducible placement of the specimens in the shock-wave focus. Sirios red (Sigma-Aldrich, München, Germany) was applied as an indicator dye before the microbiologic investigations to examine sealed plastic pipettes for leakage. Therefore, vials were filled with standard concentrations of the dye ($n = 3$), sealed by fusion of the tip and proceeded to 4000 shock waves at an ED of 0.96 mJ mm^{-2}. Absorbance was determined in quadruplicate with a conventional photometer before and after shock-wave application of both the dye solution and the surrounding water bath.

The influence of both IN and ED was examined under standardised conditions and untreated bacterial suspensions served as a control ($n = 5$ for each group). At first, increasing IN up to 4000 impulses (with steps of 1000 impulses) was applied with a constant ED of 0.96 mJ mm^{-2}. Afterwards, different levels of ED with $0 \cdot 38$ mJ mm^{-2}, $0 \cdot 59$ mJ mm^{-2} and $0 \cdot 96$ mJ mm^{-2} were administered with a constant number of 4000 impulses. After shock-wave application, serial aliquots of each test and control sample were plated on Mueller–Hinton agar plates and viable counts were determined after incubation at 37°C for 48 h.

Calculations and statistical methods

All data obtained by determining the viable counts were compared for statistical significance with $p < 0.05$ considered to be significant (Mann–Whitney test).

RESULTS

Influence of shock waves on permeability of soft Pasteur pipettes

Absorbance of the indicator dye before shock-wave application averaged 0.5835 ± 0.0064, and 0.5787 ± 0.0067 after 4000 impulses at the highest energy level ($p > 0.05$). Mean values for the surrounding water bath did not change significantly either, with 0.0364 ± 0.018 before ESWT and 0.0362 ± 0.019 after the treatment ($p > 0.05$). Consequently, leakage and dilution of the microbiologic test samples by the surrounding water bath could be excluded.

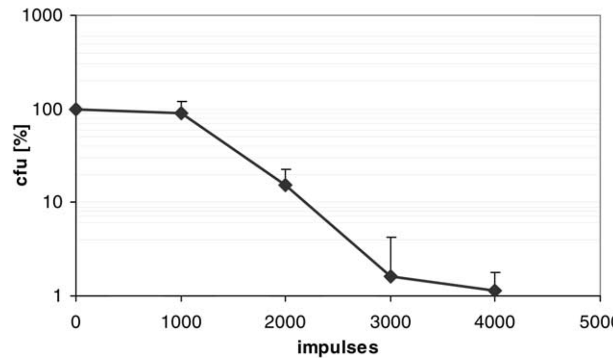

Fig. 1. Logarithmic scale of bacterial growth in relation to impulse number applied at high energy level (ED = 0.96 mJ mm^{-2}).

Influence of impulse numbers on antibacterial effectiveness

The relation of the applied impulse number to the antibacterial effectiveness of the ESWT is shown in Fig. 1. By applying up to 1000 impulses at an energy flux density of 0.96 mJ mm^{-2}, no significant reduction of bacterial viability was observed ($p > 0.05$). After exposure of at least 2000 impulses, bacterial growth was significantly reduced and directly related to the applied number of shock waves, if shock waves were applied at the same energy flux density level of 0.96 mJ mm^{-2} ($p < 0.01$). A further increased effect was found with increasing number of shock waves up to 4000 shocks. The reduction of bacterial viability increased significantly by application of a higher number of shock waves ($p < 0.01$).

Influence of shock-wave energy on antibacterial effectiveness

Bacterial growth after application of 4000 impulses with different energy levels is displayed in Fig. 2. Impulses with an level of energy flux density of 0.38 mJ mm^{-2} did not show any significant influence on *in vitro* growth of *S. aureus* ($p > 0.05$). With this experimental setting, a threshold energy flux density of 0.59 mJ mm^{-2} was necessary to exhibit significant antibacterial activity ($p < 0.05$). Similarly to the increased effect, correlated to the number of shock waves, shock waves with a higher energy flux density level also improved bacterial killing significantly up to two logarithmic levels for 0.96 mJ mm^{-2} ($p < 0.01$).

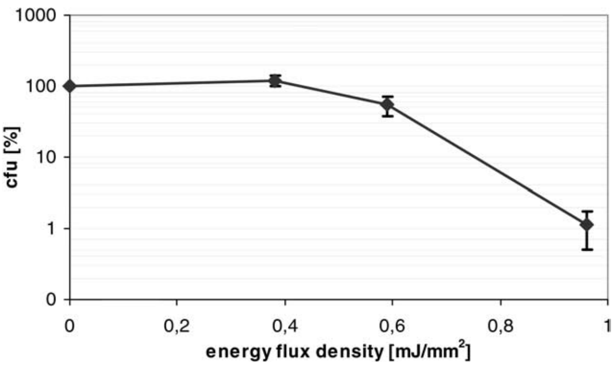

Fig. 2. Logarithmic scale of bacterial growth in relation to applied energy (ED); 4000 shock waves were applied at each energy level.

The antibacterial effectiveness of the ESWT seems to be related to the applied acoustic energy and the number of applied shock waves.

DISCUSSION

Since 1980, extracorporeal shock waves have been increasingly used in urologic and orthopaedic disorders, with the exception of acute infection because pathogens from localised sites of infection may gain access to the bloodstream through damaged vessels resulting in severe systemic infection. Therefore local infections are still considered to be a contraindication for shock-wave therapy of orthopaedic disorders. However, the exposure of bacteria to shock waves may feasibly result in physical damage to bacteria, as well as resulting in cell death.

Antibacterial effectiveness of ESWT has been previously reported for clinically relevant bacteria (von Eiff et al. 2000). Constant treatment parameters of 4000 impulses and 0.96 mJ mm^{-2} had been applied in our study and significant reduction of bacterial growth was achieved. Kerfoot et al. (1992) studied the effect of shock waves on bacterial growth in relatively rigid cryogenic vials and therefore failed to demonstrate any antibacterial effect, demonstrating the influence of the experimental setup on *in vitro* results.

The present experiments demonstrated that a minimum energy threshold of 0.59 mJ mm^{-2} has to be applied before antibacterial effects arise. A further increase of ED far beyond the threshold level exponentially improved bacterial killing, emphasising the enormous influence of the shock-wave energy on bacterial survival. With energies above the threshold level, antibacterial effectiveness could be further improved by increasing the number of impulses. In contrast to a previous study demonstrating a minimum of 350 impulses to be necessary for significant staphylococcal killing (von Eiff et al. 2000), in this study, a threshold had to be overcome, being more than 1000 impulses. An exponential bactericidal effect was only observed with higher impulse numbers. These differences can be explained by different experimental setups and by using different sample volumes, with increasing the "probability-of-hit" between shock waves and suspended bacteria in smaller sample volumes, applied by von Eiff et al. (2000). Furthermore, recently improved shock-wave measurement techniques allow exact definitions of shock wave and devising parameters. Thus, due to the enormous influence of experimental setups, threshold levels of energy and impulse counts on bacterial killing cannot be generalised and are only valid for each specific study (Gerdesmeyer et al. 2002; Wess et al. 1997). The influence of energy on bacterial viability and growth has not been studied so far and the present study is the first to define a threshold ED for bacterial killing. Thus, by applying the corresponding technical parameters, subsequent investigations exposing bacteria to shock waves can be compared.

Different mechanisms of shock-wave tissue interaction have been proposed. Shock waves induce cavitation phenomena and shear forces at acoustic interfaces (Gerdesmeyer et al. 2002; Wess et al. 1997). Cavitation bubbles occur after high energy shock waves pass liquid structures. These bubbles collapse within a very short time and high-speed microjet streams with up to 800 m s^{-1} are generated and induce local lesions in surrounding tissue (Delacretaz et al. 1995; Gerdesmeyer et al. 2002). High local tensile and shear forces were also generated by shock waves, because of the extremely short rise time of high pressure within a defined small region, called the focus (Crum 1988; Delius et al. 1998). Large differences in impedance induce large transformations from acoustic energy to mechanical energy (Delacretaz et al. 1995; Delius et al. 1995; Gerdesmeyer et al. 2002), which directly affects surrounding tissue by mechanical stress. Shear forces and cavitation work synergistically, rather than independently (Zhu et al. 2002). These results have been confirmed by cell studies by analysing mechanical effects (e.g., cell lysis after shock-wave application) (Lokhandwalla et al. 2001).

The main targets of shock waves seem to be membrane systems, leading to an increased permeability of membranes and cell walls, comparable with hydrostatic pressure treatment (Diehl et al. 2003; Perrier-Cornet et al.1999). Shigehisa et al. (1991) reported a permeabilisation of bacterial cell walls by high hydrostatic pressure treatment followed by an increased discharge of cytoplasmatic RNA and membrane leakage, after high hydrostatic pressure treatment leading to increased intracellular staining was shown by Benito et al. (1999).

Thin cell layers of bacteria and intracellular structures get severely injured and leakage occurs, explaining the direct killing effects of shock waves (Lokhandwalla et al. 2001). Detectable local thermal or chemical effects have also been discussed, but no evidence of clinical relevance could be found (Delacretaz et al. 1995; Delius et al. 1995; Gerdesmeyer et al. 2002).

Additional biologic effects may add synergetic antibacterial properties. Several *in vivo* studies have found a direct vasculogenic effect, and extracorporeal shock waves were described to generate an increased perfusion (Wang et al. 2002, 2003). Mechanical and biologic effects could probably be used as additional treatment options in the infected condition.

Although a large number of studies examining effects of ESWT on a multitude of diseases exist, the potential therapeutic role for this form of energy to kill bacteria in humans has never before been evaluated. In fact, use of shock-wave treatment is considered to be a risk if bacterial infection is suspected. However, our findings suggest that ESWT should be evaluated and tested for use particularly in difficult-to-cure infections. For infections such as osteomyelitis or artificial valve endocarditis, development of novel therapeutic strategies is urgently warranted. Schaden (2000) applied high-energy extracorporeal shock waves on infected nonunions. Neither ESWT-related side effects nor systemic spreading of bacteria with secondary infections were observed. Furthermore, no difference in success rate was found between septic and aseptic nonunions after high-energy ESWT. Similar energy levels to those that we used in this study were also described in clinical trials where no clinical side effects were reported, except local petechial bleeding and haematoma (Ikeda et al. 1999). Our study showed significant bactericidal effects of extracorporeal shock waves. Therefore, infections should no longer be generally classified as a contraindication for shock-wave treatment. Further studies have to investigate whether the antibacterial effect of high-energy shock waves could be further improved and the presented results should be verified *in vivo* before clinical trials are designed. ESWT might be an important option in treatment of difficult-to-treat infections but results need to be confirmed in prospective clinical trials.

CONCLUSIONS / SUMMARY

High-energy shock waves were found to have direct antibacterial activity. This significant effect may have clinical relevance by reduction of bacterial growth up to several logarithmic levels. The antibacterial effect is dependent on energy and impulse number, and threshold levels have to be surpassed.

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