ANTIMICROBIAL ACTIVITIES

Bactericidal effect of extracorporeal shock waves on *Staphylococcus aureus*

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Despite considerable knowledge about the effects of shock waves on eukaryotic soft tissues, no data are available concerning their effect on prokaryotic micro-organisms. In-vitro studies on the bactericidal effect of extracorporeal shock waves on *Staphylococci* were performed with energy levels that are standard for the disintegration of calculi. Suspensions containing $10^8$–$10^9$ cfu of *Staphylococcus aureus*/ml were sealed in plastic tubes and exposed to shock waves, resulting in a mean decrease of $\log_{10}$. Whereas impulse rates of $\geq 350$ resulted in a decrease of cfu/ml equalling the detection limit, lower numbers of impulses did not result in an appreciable bactericidal effect. The bactericidal effect of extracorporeal shock waves might provide the basis for the development of novel therapeutic strategies for bacterial infections.

Introduction

The use of shock waves for the treatment of urolithiasis by extracorporeal lithotripsy (ESWL) makes non-surgical intervention possible [1, 2]. In 1986, the clinical application of ESWL to human biliary duct stones was initially described by Sauerbruch et al. [3], and subsequent studies have documented its effectiveness for the disintegration of stones in other organs, such as the pancreas and salivary glands [4–6]. The positive effect of shock waves on bone healing and calcification has been observed, and shock wave treatment is now performed on pseudarthroses (non-unions) after bone fractures and tendinopathies [7]. Overall, this therapy is very well tolerated.

Shock waves are short mechanical impulses, which are characterised by an extremely fast increase of pressure and a high pressure maximum. They can be generated by an underwater spark which is produced when a high voltage arc between two electrodes. The following explosion-like vapourisation of the surrounding water results in a shock wave, spreading spherically in all directions. When a half ellipsoid reflector with the electrode in the focal point F1 is used, the generated shock waves are, according to the wave theory, reflected and focused in the focal point F2. For treatment purposes, urinary calculi, or in the present study the tubes, have to be brought into this focus F2. In lithotripsy, shock waves with an increasing time of 20–600 ns, a maximum pressure of 400–1000 bar and a mean pressure time of 0.5–1 ms are used [1, 6, 7].

As a result of the high pressure and the steep increase and decrease of the pressure slope, bleeding and haematoma can occur in adjacent organs or soft tissues [4, 7]. Micro-organisms from localised sites of infection may gain access to the bloodstream through damaged vessels, resulting in systemic infection. Therefore, shock wave treatment is not employed during acute infection of the urinary system or the bone in the case of pseudarthrosis. However, the exposure of bacteria to extracorporeal shock waves may feasibly result in physical damage to bacteria, as well as resulting in cell death. Future studies will be needed to define the value and safety of this technology for the treatment of certain difficult-to-treat infections, e.g., osteomyelitis and artificial valve endocarditis. As an initial approach, to determine its effect on bacterial viability, the present study evaluated the in-vitro effect of extracorporeal shock waves on micro-organisms [2, 7].

Received 21 Oct. 1999; revised version received 9 Dec. 1999; accepted 22 Dec. 1999. Corresponding author: Dr C. von Eiff (e-mail: eiffc@uni-muenster.de).

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Materials and methods

Shock waves were generated with a Dornier-Lithotripter XL 1, which was specifically developed for small specimens. Experiments were performed with Staphylococcus aureus ATCC 29213. S. aureus was chosen as the test organism because it is one of the most resistant organisms to killing by sonication [8]. Bacteria from overnight cultures grown in trypticase soy broth were serially diluted in phosphate-buffered saline to obtain final concentrations of c. 1 × 10^5 cfu/ml. The number of cfu was determined in duplicate by plating 10- and 50-μl samples on trypticase soy agar (TSA). Soft PVC tubes with an external diameter of 6 mm and a wall thickness of 1 mm (B. Braun Melsungen AG, Melsungen, Germany) were filled with 200 μl of the bacterial suspension, then sealed with a metal clip. Samples were tested for any leakage before, during and after experiments by exposing control tubes filled with an indicator dye to the shock waves and careful microscopic examination of the tubes and seals before and after shock wave treatment.

Four independent series of experiments were performed. The first three series of experiments (experiments 1–3) tested 33 tubes and were designed to evaluate the bactericidal effect of extracorporeal shock waves on staphylococci. A fourth experiment was designed to test the effect of different numbers of shock waves on bacterial survival. In the first three series of experiments, 21 PVC tubes containing a bacterial suspension were exposed to 1000 shock waves of 20 kV energy in de-gassed and de-calcified water (20°C) with an impulse rate of 120/min. In control experiments, 12 tubes were filled with 200 μl of the bacterial suspension (c. 10^5 cfu/ml), but were not exposed to shock waves. To evaluate the effect of different numbers of shock waves, in a fourth series of experiments, the PVC tubes were exposed to 10, 50, 100, 200, 350, 650 and 800 shock waves. After the experiments, 10- and 50-μl samples were recovered from tubes, including the control tubes, and viable counts were determined on TSA.

Statistical analysis was performed with the Mann-Whitney U test for analysis of individual test results [9].

Results

The results of the first three series of experiments testing the effect of extracorporeal shock waves on 21 tubes are shown in Table 1. All experiments were performed under identical conditions, with 1000 shock wave impulses. Whereas the viable counts in 12 control tubes (untreated; four tubes in each experiment) decreased by factors of 3.2, 4.4 and 3.7, respectively, the viable counts in tubes treated with extracorporeal shock waves decreased by factors of 2.5 × 10^5 (n = 5), 0.8 × 10^5 (n = 9) and 1.0 × 10^5 (n = 7).

These results indicate a mean decrease by a factor of 1.27 × 10^5 or 3.1 log_{10} orders of magnitude and were found to be significant (p < 0.01 for each experiment) by the Mann-Whitney U test. In 3 of 5, 5 of 9 and 6 of 7 determinations in independent experiments (experiments 1, 2 and 3, respectively), the viable counts were found to be below the detection limit; in these instances, the lower detection limit (20 cfu/ml) has been used for data analysis.

After demonstrating a bactericidal effect of extracorporeal shock waves on S. aureus with shock wave energy levels equivalent to those employed in treatment of human patients (as determined by maximal pressure, energy and type of electrode), the bactericidal activity was evaluated as a function of shock wave impulse numbers (Fig. 1). As shown, impulse numbers <200 had no appreciable effect on bacterial viability, whereas impulse rates ≥350 resulted in the full effect as seen in experiments 1–3 with viable counts below or close to the detection limit.

Discussion

This is believed to be the first report of the in-vitro bactericidal effect of extracorporeal shock waves on S. aureus in experiments with shock wave energy comparable to that used in patients for ESWL of renal and bile duct calculi [2–4]. Application of ultrasonic energy is a widely used method for disintegration, particularly of gram-negative bacteria and mycobacteria for laboratory purposes, e.g., for extraction of proteins.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Viable counts (cfu/ml) before experiment</th>
<th>Number of tubes</th>
<th>SW exposure</th>
<th>Median (sqd) viable counts (cfu/ml) after experiment</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.8 × 10^5</td>
<td>4</td>
<td>No</td>
<td>5.8 × 10^6 (2.7 × 10^6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2</td>
<td>9.0 × 10^4</td>
<td>5</td>
<td>Yes</td>
<td>2.1 × 10^5 (1.3 × 10^5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3</td>
<td>8.0 × 10^5</td>
<td>9</td>
<td>Yes</td>
<td>2.2 × 10^5 (7.8 × 10^5)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

The exposure conditions were: 1000 shock waves, 20 kV, 120/min (ranges of conditions commonly used clinically on stones, etc.: 500–3800 shock waves, 14–28 kV, 50–150/min). Results of individual experiments were analysed by the Mann-Whitney U test. SW, shock wave; sqd, interquartile distance.
EXTRACORPOREAL SHOCK WAVES AND S. AUREUS

Fig. 1. Viable counts of S. aureus ATCC 29213 exposed to the indicated numbers of shock waves. The means and SEM of five independent experiments are shown. Area under detection limit of 20 cfu/ml.

or nucleic acids or cleaning of laboratory equipment [10–12]. However, the energy levels and energy application methods used for these purposes differ widely from the methods used for therapy of human disease, i.e., application of ultrasonic energy from an energy-conducting metal tip immersed into the bacterial suspension. In the past, a quantitative sonication method has been developed to remove organisms from different types of vascular catheters in vitro for diagnostic purposes. Sherertz et al. used a quantitative sonication method with low intensity which does not lyse bacteria (55 000 Hz, 125 W, 1 min. Ultrasonic Industries, Plainview, NY, USA), to culture vascular catheters submitted to a clinical microbiology laboratory [13]. Staphylococci, including S. aureus, were among the most common organisms isolated. The sonication method allowed quantification of the number of cfu removed from a catheter and it was considered that this technique has considerable potential for use in clinical microbiology laboratories to aid in the diagnosis of vascular catheter infections and for clinical investigations into the pathogenesis of these infections [13].

Although there is a large body of literature describing indications and effects of extracorporeal shock waves on diseases as varied as urinary calculi, osseous non-unions and malignant tumours [1, 2, 4–7, 14, 15], the potential therapeutic role for this form of energy to kill bacteria in man has never been evaluated, and shockwave treatment is considered to be a risk if acute bacterial infection is suspected.

Certain types of bacterial infections – such as chronic osteomyelitis [16] or foreign body infections [17] – are difficult to treat, despite the availability of potent antimicrobial agents. The development of novel therapeutic strategies is warranted for these infections. The results of the present study suggest that extracorporeal shock wave treatment could be developed and tested for use in difficult-to-cure infections.

The bactericidal effect was obtained with shock wave impulse rates and energy levels equal to and safe for use in man [1, 6, 7]. Although the experimental set-up of the in-vitro system with bacterial cells sealed into PVC tubing is not completely equivalent to the physiological conditions of micro-organisms embedded in tissues, it has similarities to foreign body infections. A similar bactericidal effect might be achievable by application of shock wave energy to sites of localised infection by generating shock waves physically distant from the focus of application and conduction of energy through aqueous media (such as tissue).

In conclusion, this study demonstrated a bactericidal effect of extracorporeal shock waves on S. aureus. This might provide the basis for a novel treatment for certain types of bacterial infections in an appropriate animal model. In further experiments, the effect of extracorporeal shock waves on other micro-organisms, e.g., gram-negative bacteria, including the possibility of endotoxin release, should be tested.

This study was supported by a grant from the German Minister of Education and Research (grant no. 01KJ9750/9).

References