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Effect of extracorporeal shock wave lithotripsy on bacterial viability

Relationship to the treatment of struvite stones

G. Reid[1,2], M. A. S. Jewett[3], J. C. Nickel[4], R. J. C. McLean[5], and A. W. Bruce[1]

[1]Division of Urology Research Laboratories, Department of Surgery, [2]Department of Microbiology, Toronto General Hospital and University of Toronto, Toronto, and
[3]Department of Surgery (Urology), The Wellesley Hospital and University of Toronto, Toronto, and
[4]Department of Urology, [5]Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, Canada

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Summary. The aim of this study was to determine whether extracorporeal shock wave lithotripsy (ESWL) affected the viability of the infecting bacteria within a simulated struvite stone matrix. A strain, *Proteus mirabilis* 28cii, was prepared in three forms: (1) suspended in saline and urine, (2) artificially encapsulated by suspending in agar beads and (3) artificially encapsulated and mineralised by suspending in agar beads with calcium carbonate crystals. The preparations were placed in capped vials partially immersed in degassed water and held in the focal point of the Siemens Lithostar and given 1,000 shocks. Subsequent viability testing showed that bacteria suspended in urine were greatly affected by shock treatments (55% loss in viability), but incorporation into agar beads negated this effect (even if the cells were exposed to 2000 shocks). Mineralisation of the beads with calcium carbonate crystals caused a decrease in viability of 82% that was significantly different from controls. However, this still left 2.3×10^8 viable organisms (82% of 2.8×10^8), easily enough to form the focus for further infections. A series of control experiments carried out using an ultrasonic cell sonicator probe gave comparable results to those obtained with ESWL. These results demonstrate that ESWL treatment of infected stones must be accompanied by antimicrobial coverage.

Key words: Extracorporeal shock-wave lithotripsy – Bacteria – Stones

Urinary calculi represent a continuing concern for the clinician. Whilst the vast majority of calculi are comprised of calcium oxalate or calcium phosphate, approximately 10%–20% contain struvite ($MgNH_4PO_4 \cdot 6H_2O$) and carbonate apatite ($Ca_{10}(PO_4)_6 \cdot CO_3$). These are often referred to as "infection stones" due to their association with urinary infection [4]. A characteristic staghorn morphology occurs when the stones fill the renal pelvis and collecting system [4]. Left untreated, they can cause significant morbidity from renal damage and even mortality [17]. The infecting organisms produce an enzyme, urease, which hydrolyses urea in a reaction leading to crystallization, crystal aggregation and struvite stone deposition. The most common urease producers associated with stones are gram-negative enteric bacteria, particularly *Proteus* [7, 15]. Electron microscopy studies have shown that bacterial microcolonies are integrated into the crystal matrix of the calculi. These organisms are important in nidus formation, matrix production and crystal aggregation and growth [10]. The bacterial growth and biofilm structure within calculi and the crystalline lattice itself generate a remarkable resistance to antimicrobial agents [3, 9, 14]. Therefore, it is not surprising that urinary tract infection has been found to persist in 40% of patients with calculi, even after conventional antibiotic therapy [5, 7].

Extracorporeal shock-wave lithotripsy (ESWL) represents an exciting new "non-invasive" treatment for urinary calculi. However, its use in eradicating struvite calculi has, at best, been disappointing [11, 16] due to a high rate of recurrences. This present study was undertaken to assess the impact of ESWL on the viability of bacteria associated with the majority of infection stones.

Materials and methods

Bacterial suspensions

A representative *Proteus mirabilis* urinary isolate, strain 28 cii, was used due to its urease activity and adherence to urinary tract cells [13]. The bacteria were stored at −70 °C and inoculated into brain heart infusion yeast extract broth (Difco, Detroit) for three subcultures before being incorporated into suspensions of (i) pooled, filter-sterilised human urine (from volunteers without a history of urinary stones), (ii) sterile saline; (iii) agar beads as previously described [12] and (iv) agar beads with calcium carbonate powder added to a concentration of 2.5%. In brief, agar beads were formed by adding 1 ml bacterial suspension to 1 ml 2% molten agar, vortexing, adding 3 ml peanut oil, vortexing on ice and washing away single organisms and excess oil by three centrifuge spins in phosphate-buffered saline. The agar beads varied in size, as described previously [12], each containing several log counts of viable organisms. The distribution, sizes and number of beads were similar for each sample, and this

Table 1. Percentage reduction in viable counts of *Proteus mirabilis* strain 28 cii after 1,000 extracorporeal shock-wave lithotripsy shocks and 3 × 30-s pulses with sonication. All the experimental values are included to demonstrate the material used for statistical analysis. The minimum requirement of duplicate experiments was upheld in all cases

	1,000 Shocks		Sonication	
	Viability (mean ± SD)	Percentage reduction	Viability (mean ± SD)	Percentage reduction
Bacteria in urine:				
Test	$1.525 \pm 0.125 \times 10^8$ (mean of 1.4 and 1.65)	55%[a]	$2.61 \pm 0.67 \times 10^8$ (mean of 3.2, 2.2, 4.3, 3.2, 2.1 and 1.6)	21%[b]
Control	$3.4 \pm 0.1 \times 10^8$ (mean of 3.5 and 3.3)		$3.3 \pm 0.4 \times 10^8$ (mean of 3.1, 3.1, 3.0, and 4.0)	
Bacteria in saline:				
Test	NT		$7.8 \pm 1.72 \times 10^8$ (mean of 5.8, 7.4, 8.4, 11.1, 6.3 and 7.9)	14%[b]
Control	NT		$9.05 \pm 0.94 \times 10^8$ (mean of 9.5, 9.2, 7.5, and 10.0)	
Bacteria in agar beads:				
Test	$3.15 \pm 1.5 \times 10^8$ (mean of 3.0 and 3.3)	0%[b]	$6.0 \pm 2.16 \times 10^7$ (mean of 5.0, 5.0, 9.0, 4.0, 9.0 and 4.0)	11%[b]
Control	$1.7 \pm 0.2 \times 10^8$ (mean of 1.5 and 1.9)		$6.75 \pm 1.48 \times 10^7$ (mean of 6.0, 7.0, 9.0, and 5.0)	
Bacteria in agar beads with 2.5% $CaCo_4$:				
Test	$5.0 \pm 1.0 \times 10^7$ (mean of 6.0 and 4.0)	82%[a]	$0.66 \pm 0.94 \times 10^7$ (mean of 0.5, 0.52, and 0.49)	67%[b]
Control	$2.8 \pm 1.5 \times 10^8$ (mean of 1.4, 1.6, 5.3 and 2.9)		2.0×10^7 (mean 2.0 and 2.0)	

NT not tested; [a] alpha at 0.5 statistical significance; [b] no statistical difference

does not explain any resultant variation in data. In addition, the use of untreated beads provided an effective control of the number of bacteria contained within the bead preparations, and the same suspensions formed the source of test and control samples. The washing procedure after bead formation ensured that few single bacterial cells were present. Bacterial viability was measured by disrupting each suspension through a glass pipette (this breaks up the beads and releases single cells for culture) prior to performing dilution plating on brain heart infusion yeast extract agar. Similar techniques are used in microbiology for the disruption of bacterial aggregates. Each colony forming unit represented single viable bacterial cells. The agar beads were used to simulate the outer matrix of certain kidney stones [1]. The calcium carbonate crystals were utilized to simulate the crystalline lattice of struvite stones [7]. These crystals were contained within the bacteria/agar beads.

Shock treatments

The bacterial suspensions were placed in 3 cm long, capped plastic vials with no air/fluid interface. The vials were partially immersed in degassed water (room temperature), coupled to a device and held in the focal point of the Siemens Lithostar before application of 1,000 shocks (19 kV). In certain instances 100 and 2,000 shocks were applied. Controls consisted of bacterial suspensions placed in the focal point of the Lithostar but not treated with shocks. The temperature of the degassed water did not rise substantially and did not reach a level that could have killed bacteria.

In addition, replicate bacterial suspension were subjected to 3 × 30-s pulses of an ultrasonic cell sonicator probe (125 V, 1.5 amps), with 30-s intervals in an ice bath (used to prevent heat damage to the cells). This was undertaken as a control experiment, as sonic forces can remove the extracellular components of cells without affecting viability or can completely break open and kill bacteria. The force used here was intermediate, not aimed at complete destruction of the cells but at exposing them to sonic levels that might mimic lithotripsy shock wave effects.

Following treatment, all samples were examined microscopically and tested for viability by passing through a glass pipette and then by dilution plating on brain heart infusion yeast extract agar. Each experiment was performed twice, and bacteria were plated out in triplicate, resulting in six readings from which mean and standard deviation results were calculated.

Statistics

The logarithms of each bacterial plate count were taken and analysed using computerised *t*-testing. Statistical significance calculations were carried out between test and control values for each treatment.

Results

Both ESWL and sonication treatments caused a visible disruption of the agar bead suspensions, as seen under light microscopy. The results on bacterial viability are presented in Table 1. It was evident that the ESWL treatment greatly reduced the viability of *P. mirabilis* in urine (55%). However, incorporation into agar beads

negated this effect, and no loss of viability was found even after 2000 shocks ($1.9 \pm 0.2 \times 10^8$). The sonication results were similar, with bacterial killing being less in agar bead preparations.

The largest effect on *Proteus* viability was found with agar beads incorporating $CaCo_4$. Both ESWL and sonication treatment reduced bacterial viability (82% and 67%, respectively). An additional specimen was tested with 100 ESWL shocks, and a 55% reduction was noted, confirming the effect.

The reduction in viability was statistically significant where indicated in Table 1.

Discussion

The present results demonstrate that ESWL shock waves can somewhat affect the viability of uropathogenic bacteria. In relation to infection stones, two correlations exist. Firstly, the growth of bacteria as an encapsulated biofilm (modelled by the non-mineralized agar beads) is similar to that found in the interstices and at the periphery of struvite stones [7]. The limited success of ESWL treatment in reducing bacterial viability in these artificial agar beads, compared with planktonic cells, suggests that this biofilm in some way dampens the effect of the shock waves. Comparison of the results shows that this dampening was highly significant ($P < 0.001$). This is potentially an important microbiological finding. However, from a practical urological perspective, the agar bead structure was disrupted by the shock waves, suggesting that an accompanying antimicrobial therapy could have eradicated some or all of these organisms. This hypothesis is supported by the findings of Michaels et al. [8] which showed that effective combined ESWL and antimicrobial treatment can reduce the recurrence of bacterial infection.

Secondly, the simulation of a mineralized stone (agar beads with $CaCo_4$) resulted in a large percentage reduction in bacterial viability (82%). A similar effect was seen using sonication (67% reduction). From this, it is clear that the presence of calcium carbonate crystals intensified the shock-wave effects and increased bacterial kill. This would imply that ESWL treatment in vivo may not only disrupt infection stones but could also perhaps partly reduce bacterial numbers.

While ESWL treatment did result in a reduction of bacterial viability, a sizeable fraction still survived (18% or more, in this study). This means that even with 82% mortality, approximately 2.3×10^8 organisms still retained viability, a survival rate that is likely to be quite significant within the kidney tissues. Renewed growth of these survivors within calculi and in urine [2] with their accompanying urease activity, glycocalyx and crystal production [6] could lead to stone recurrences. This may in part explain the high recurrence rate of large struvite calculi following ESWL treatment [11]. As Michaels et al. [8] and others have stressed, it is most important that antimicrobial coverage be provided before and after ESWL to ensure that the patients are stone-free. Disrupted bacterial biofilms can be treated effectively with antimicrobial agents [3, 9], and such a protocol is likely to reduce complications caused by organisms such as *P. mirabilis*.

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Dr. Gregor Reid
SLB-328
University of Western Ontario
London, Ontario
Canada N6A 5B8