Thermal Eradication of Prosthetic Joint Associated Bacteria

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Abstract

While the number of joint replacement surgeries has increased in recent decades, little progress has been made in treating deep prosthetic joint infections (PJI) without resorting to surgical revision.[1] Non-surgical solutions to PJI will not only avoid costly revision surgeries, but may reduce the likelihood of infection relapse compared to current treatment options. We are developing a non-invasive thermal technique to destroy biofilm on the metal surfaces of prosthetic joints using alternating magnetic fields (AMF). One hypothesized benefit of using AMF therapy is that it could be used in conjunction with traditional antibiotics to produce a synergistic bactericidal effect. The purpose of this study is to characterize the thermal sensitivity of planktonic bacteria in the presence of antibiotics to aid in the development of appropriate parameters for AMF dosing.

Introduction

Induction heating via AMF is employed commercially and industrially as a safer alternative to conventional conduction heating methods. By passing alternating electric current along a metal coil, a magnetic field is generated and a secondary current flows through the center of the coil. When ferromagnetic material is placed in the path of induced current, internal resistance and rapidly changing direction of current results in lost energy, or heat. Unlike conduction, induction generates heat from within the metal object, sparing non-ferromagnetic material (e.g. living tissue); a characteristic which we hope may provide an unconventional opportunity to disrupt biofilm on metal surgical implants and allow greater exposure to conventional antibiotics in the surrounding joint fluid.[2]

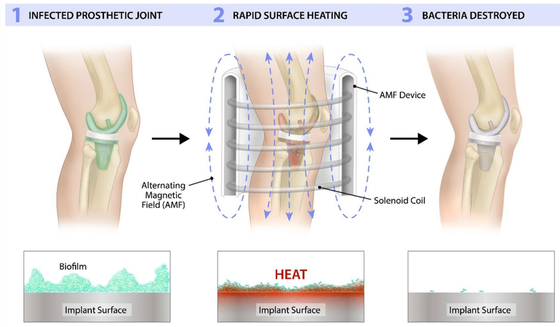

Figure 1 Biofilm on prosthetic joints is highly resistant to penetration by antibiotics, but by exploiting the ferromagnetic properties of the metal in prosthetic devices, the metal surfaces can be heated sufficiently to destroy bacteria and disrupt the biofilm.

A critical goal in designing an AMF device for use on surgical implants is to achieve sufficient bactericidal effect while sparing the surrounding host tissue. While AMF therapy itself will not directly damage living tissue, the heated metal surface of the targeted prosthetic joint poses a potential burn risk to surrounding tissue, and thus combination with antibiotics could be invaluable for lowering AMF dosing requirements to safer levels.

Methods

Planktonic cultures of *Pseudomonas aeruginosa* (1 x 10^9 CFU/mL PAO1) were aliquoted (100 uL) into 1.5 mL Eppendorf tubes prior to heat treatment. The experimental variables and controls were as follows: a control with no heat or antibiotic treatment, a "heat-only" control, three "antibiotic-only" controls and three "heat + antibiotic" experimental groups. For heating, samples were subjected to a single thermal shock treatment on a heating block calibrated to a target temperature. Tubes were then placed in a spinning incubator at 37°C, 110 rpm for 24 hours to simulate normal physiological conditions.

A sub-zero time point (-1 h) was used to represent those samples which were plated prior to receiving any antibiotic or heat treatment, and this time point therefore represents the absolute starting concentration of *P. aeruginosa*. Zero time points (0 h) mark the brief interval immediately following application of heat and ciprofloxacin, but prior to incubation.

Prior to this experiment, it was necessary to characterize the thermal sensitivity of *P. aeruginosa* at various temperatures. The resulting graph served as a guide for selecting temperature exposures during the current study (Figure 2).

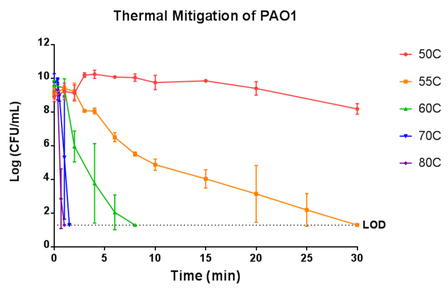

Figure 2 In order to characterize the thermal sensitivity of planktonic PAO1 at various temperatures and exposures, samples were plated immediately after heating without an incubation period for regrowth.

Results

A single 10-minute dose of 55°C thermal shock reduced bacterial concentrations by 1.47-log CFU/mL. However, incubation allowed surviving bacteria to quickly reach and surpass original starting concentrations. Ciprofloxacin (2 ug/mL) alone achieved a 3.78-log reduction within six hours and prevented significant regrowth during 24 hours of incubation. However, when bacteria were subjected to heat and antibiotic in combination, a 6.20-log reduction was achieved within 24 hours of incubation.

Time-temperature kill curves had previously been generated to assess bacterial response to thermal shock alone, but until the current experiment, regrowth had not been assessed.

In the absence of antibiotic, regrowth at 37°C rapidly restored bacterial concentrations to pre-treatment levels. Therefore, combination therapy produced a superior bactericidal effect when compared to antibiotic or heat shock alone.

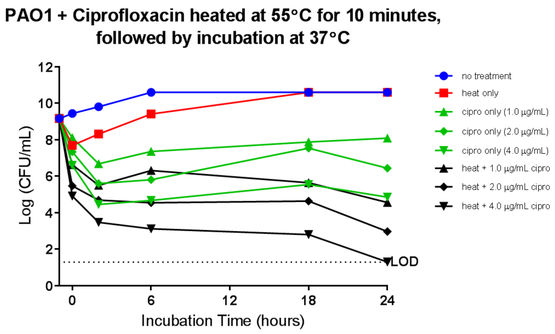

Figure 3 Initially, the reduction in planktonic concentrations of *P. aeruginosa* by a single thermal shock was comparable to the bacteriostatic effect of ciprofloxacin, however, the reduction was transient as bacteria were still capable of rapid replication in the absence of additional inhibitory factors. In combination, ciprofloxacin provided a means of halting regrowth following heat shock.

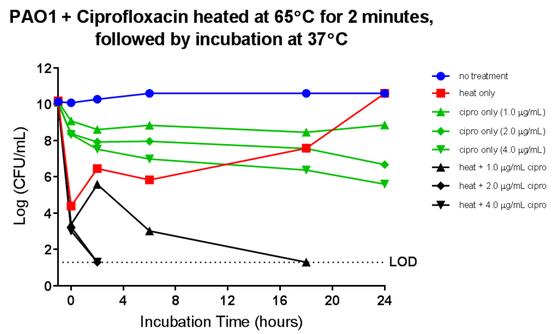

Figure 4 In this experiment, the temperature was increased but the heat exposure time was reduced. The results suggest that high heat alone may have a longer lasting inhibitory growth effect than that observed at lower temperatures.

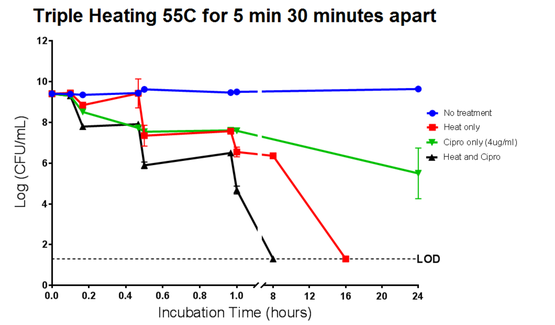

Figure 5 Three nonconsecutive 5-minute 55°C thermal shocks spaced 30 minutes apart. By decreasing heat exposure time, but increasing the number of thermal shocks, time to limit of detection was reduced from 24 to eight hours.

When multiple doses of heat were applied in conjunction with ciprofloxacin, a step-down effect over time was observed (Figure 5).

In multiple heat dosing, the log reduction achieved by each successive heat shock was enhanced by the simultaneous inhibitory activity of ciprofloxacin, but it is worth noting that unlike single dose heat experiments, multiple doses of heat are capable of reaching LOD even in the absence of antibiotics.

Conclusions

- The bactericidal effects of thermal shock are enhanced and prolonged by the addition of ciprofloxacin, and show an advantage over either treatment being used alone.
- Whether ciprofloxacin was added before or after thermal shock did not affect results.
- Unexpectedly, colonies from heated samples were smaller than those of non-heated samples. Longer incubation periods were often required to accurately count these colonies (i.e. 22 hours rather than 18 hours for other samples). This delayed growth was also observed graphically at higher temperatures (Figure 4) and multiple heat dosing, and may represent periods of structural repair and replacement of denatured proteins which persists beyond the initial thermal insult[3] and thus contributes a bacteriostatic benefit even hours after treatment.
- Multiple spaced heat dosing provides a practical means of lowering the minimum inhibitory temperature exposure while still achieving steep logarithmic reductions in bacterial load.

Future Goals

- Determine whether the beneficial combination of heat and ciprofloxacin is synergistic or merely additive.
- Apply combination therapy to biofilm using AMF in order to determine how dosing requirements differ between planktonic bacteria and biofilm.
- Since desired therapeutic temperatures for metal implants will cause heat to dissipate into surrounding joint fluid, it is important to assess how bacterial thermal sensitivity may change after repeated heat exposures to anticipate and possibly avoid the development of thermotolerance.
- The delayed bacterial growth observed in heat-treated samples could be explored in order to predict which antibiotic mechanisms might best exploit the heat stress response.

References

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3. Richter K, Haslbeck M, Buchner J. The heat shock response: life on the verge of death. *Molecular Cell*. 2010;40(2):253-266.