ABSTRACT

INVESTIGATION OF THE PHARMACOKINETICS AND LOCAL AND SYSTEMIC MORBIDITY OF A GENTAMICIN IMPREGNATED COLLAGEN SPONGE IMPLANTED IN THE CANINE STIFLE

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Dr Noel Moens

This thesis is an investigation of a gentamicin impregnated collagen sponge (GICS) product implanted into an inflamed canine stifle joint. Project goals were to determine the duration for which drug concentrations remained above minimal inhibitory concentrations within the joint following sponge implantation; to determine whether there was systemic exposure to the drug following local implantation; to evaluate the impact of the sponge on local joint inflammation and lameness; and to evaluate whether sponge use resulted in any renal injury.

The study design was a randomized controlled experimental trial (2 x n=9) performed with research hounds. GICS were arthroscopically implanted at a dose of 6mg/kg. Pharmacokinetic parameters were modeled using statistical moment analyses. Joint inflammation was measured by synovial fluid cell counts and cytokine concentrations, lameness was measured by force plate asymmetry indices, and renal function was measured by glomerular filtration rate (GFR) study using technetium 99 plasma clearance. The prevalence of lesions associated with aminoglycoside nephrotoxicity was assessed by renal biopsy and electronmicroscopy.

Intra-articular gentamicin concentrations fell to sub-MIC for Staphylococcus sp. (4ug/ml) by 22.4hrs (95% CI=18.6-26.2) following sponge implantation. C\textsubscript{max synovial} was 2397ug/ml (95%
Plasma gentamicin concentrations achieved levels of $C_{\text{max, plasma}} = 8.0 \text{ug/ml} \ (95\% \ CI=6.1-10.0 \text{ug/ml})$ at 1.5hrs (95% CI=0.8-2.1) following GICS placement and fell below target trough of 2.0ug/ml by 5.6hrs (95% CI=4.7-6.5hrs) following GICS placement. GICS implantation caused joint inflammation ($p<0.01$), lameness ($p=0.04$), and decreased GFR ($p=0.04$). No dog developed clinical renal failure. No difference was observed in the prevalence of renal lesions on biopsy between treatment and control group ($p=0.49$).

Intra-articular gentamicin concentration following GICS placement at an IV-equivalent dose reached high levels and declined rapidly. The maximum plasma levels attained were approximately $1/3^{rd}$ of the recommended sub-toxic target for human patients following parenteral gentamicin administration. GICS implantation in the inflamed joint caused additional inflammation and joint dysfunction that is likely to be of clinical relevance. GICS implantation affected renal function at the dose assessed. Renal effects may be exacerbated in septic patients, and care should be taken with GICS dosing in clinical patients.
ACKNOWLEDGEMENTS

This thesis would not have been possible without the advice, assistance and support of many people. I would particularly like to thank Dr Tom Gibson who provided the original idea and groundwork for this project, and whose commitment, hard work, and endless good humor in the face of adversity was a constant source of inspiration. I would also like to thank Dr Noel Moens for his tact and always sound advice. I would also like to thank the other members of my advisory committee and research team- Dr Stephanie Nykamp, one of the most reliable people I know; Dr Ron Campbell for generously allowing me the use of his computer one Sunday; Dr Darren Woods for his great smile; Dr Rob Foster for his assistance with renal ultrastructure evaluation; Dr Bob Harris for his expertise with the electronmicroscopy; Amanda Hathaway AHT for her technical and organizational skills as well as huge capacity for humor and hard work; all the CAF staff; and last but not least the research hounds that provided us with such useful data.
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CHAPTER ONE

LITERATURE REVIEW: A REVIEW OF LOCAL ANTIBIOTIC IMPLANTS AND APPLICATIONS TO VETERINARY ORTHOPAEDIC SURGERY

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Introduction

Post-operative surgical site infections (SSI) remain an inherent risk of any surgical procedure. The Centre for Disease Control and Prevention (CDC) standard definition of a SSI is shown in Table 1 (1). The incidence rate of SSIs in clean orthopaedic procedures performed on human patients is reported to range from 0.3-1.3%, while the equivalent range in veterinary procedures is 2.6-10% (2). The reasons for the differences are not clear. While absolute prevention of SSIs is not achievable, there is a continued focus on SSI prevention in veterinary medicine.

Table 1 CDC definition of a surgical site infection (1)

<table>
<thead>
<tr>
<th>An infection occurring EITHER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within 30 days of a surgical procedure OR</td>
</tr>
<tr>
<td>Within 1 year of a surgical procedure if an implant is used</td>
</tr>
</tbody>
</table>

WITH AT LEAST ONE OF:

- Purulent drainage or abscess formation at the surgical site
- Organisms cultured from an aseptically obtained sample
- Characteristic clinical signs
- Clinician diagnosis
The epidemiology of infection following surgical incision can be conceptualized as a patient/pathogen/procedure triangle. Patient factors include those related to the local wound (e.g. depth of adipose tissue, oxygen tension) and systemic host defences against infection (e.g. diabetes, poor nutritional status) (3,4). Pathogen factors include bacterial load and behaviour. Procedural factors may be the most modifiable, and include those related to both perioperative management (exposure to hypothermia, transfusion therapy, antibiotic prophylaxis) and intraoperative management (aseptic technique, tissue handling, drain placement) (3-5). Successful infection control protocols address all of these factors. While antibiotic prophylaxis/treatment constitutes only one avenue of SSI prevention/ control, it is one of the most emphasized. Antibiotic prophylaxis is defined as the use of antibiotic therapy to prevent infection, while treatment occurs in the face of established infection.

Although systemic antibiotics are considered standard of care for both SSI prophylaxis and treatment, a number of factors may compromise efficacy. These include antibiotic penetration to provide adequate concentrations for sufficient time at the surgical site, acquisition of antibiotic resistance traits by the infective organism, administration compliance, and dose-limiting antibiotic toxicity profiles (6). In response to these issues, there has been increasing interest in products providing local antibiotic therapy. There are several purported advantages of local antibiotic use, both for treatment and prophylaxis. High local antibiotic concentrations can be achieved at the surgical site, improving penetration of biofilm and necrotic tissue and increasing bacterial kill for antibiotics with concentration dependent kill characteristics (7). Improved bacterial kill reduces the risk of bacterial mutation and acquisition of horizontally transmissible resistance traits in polymicrobial infections (6). Exposure to high antibiotic concentrations may achieve kill even for organisms classified as resistant according to standard
pharmacokinetic profiling (8). As long as systemic uptake from the site is minimised and there is no cytotoxicity, acceptable safety may be maintained despite very high local concentrations - a significant advantage for antibiotics of the aminoglycoside class. Finally, when antibiotic agents are implanted directly at the surgical site, administration compliance is assured. Local antibiotics may be used alone or in combination with systemic therapy. In common with all therapeutic antibiotic use, good practice mandates culture and susceptibility testing to assist antibiotic selection. Prophylactic use should be evidence-based rather than speculative, ideally following studies demonstrating measurable patient benefit.

The incidence of multi-drug resistant nosocomial infections in veterinary species appears to be rising (8,9). This is postulated to be a direct consequence of increased and in some cases inappropriate antibiotic use. Therapeutic options for local antibiotic therapy in the form of antibiotic impregnated cements, gels, and sponges as well as antibiotic or antibacterial coated implants and devices are becoming increasingly available, however high quality evidence supporting their use, particularly in veterinary species, is lacking. The purpose of this review is to summarize available information on the unique challenges of orthopaedic infections, and the advantages, disadvantages, and available evidence for clinical use of local antibiotics.

**Biology of implant-associated infections**

Many orthopaedic procedures involve the use of implants. For this reason, orthopaedic surgical site infections pose unique challenges. Bacteria adhering to the surface of implants change their behaviour, exhibiting both biofilm formation and facultative intracellular dormancy (10). Starting at the time of implant placement, a ‘race for the surface’ begins, as adhering bacterial contaminants compete with integrating host tissue for dominance of the implant surface
environment (10). One of the most studied SSI pathogens are the staphylococci, especially Staphylococcus aureus and S pseudintermedius, which account for >50% of orthopaedic infections in both dogs, and Staphylococcus aureus and other coagulase-negative staphylococcus species which account for >50% of prosthetic joint infections in humans (11,12). Once devices have been implanted, they acquire a film of extracellular matrix (ECM) proteins, consisting of fibrinogen, fibronectin, albumin and collagen. The ECM coating can then serve as a platform for host cell adhesion and fibroblast colonization. However, staphylococci also express receptors for the ECM. Biofilm formation is initiated in the first 1-2 hours post-implantation, as bacteria interact with the ECM. In the following hours, irreversible molecular bridging occurs between the bacteria and the implant surface. The bacteria then begin to secrete an exo-polysaccharide layer, and a multi-layered biofilm develops (13). Within the biofilm, they are protected from phagocytosis and antibiotics. In some cases, it has been found that killing bacteria in a biofilm requires roughly 1000 times the local antibiotic concentration required to kill bacteria in suspension (13). Biofilm density can increase with exposure to sub minimum inhibitory concentrations (MIC) of some antibiotics, indicating an adaptive response (14).

Within the Staphylococcus sp, small colony variant strains are recognized. These strains exhibit a slow metabolism and can occupy a facultative intra-cellular position within host cells. Both biofilm formation and intracellular dormancy render these bacteria relatively resistant to antibiotic therapy in the context of implant-associated infections (15).

While Staphylococcus sp. are a key pathogenic species in the biology of SSIs, a number of other bacteria are frequently implicated, including E.coli, enterobacter, pasteurella and pseudomonas (11, 16). Plasmid mediated transmission of multi-drug resistant traits are common among these species. Antibiotic treatment selects for resistance in both pathogenic and
commensal Enterobacteriaceae, and is considered the most important risk factor for acquiring extraintestinal infection with multi drug resistant strains (16). This emphasises the need for appropriate antibiotic use guided by culture and susceptibility results.

**Principles of local antibiotic use**

Any exposure of infective organisms to an antibiotic applies a selection pressure. This in turn predisposes to the emergence of drug resistance traits and the potential for therapeutic failure (6). For systemic antibiotic therapy, information on the typical tissue concentrations reached with standard antibiotic dosing is integrated with pharmacodynamic information (notably the minimum inhibitory concentration or MIC) to determine the minimum inhibitory concentration breakpoints (MIC<sub>BP</sub>) which are reported for each antibiotic drug by the Clinical and Laboratory Standards Institute (CLSI)(17). It should be emphasised that the CLSI has made recommendations for relatively few veterinary pathogens and that each recommendation is specific for a single host species, a particular dosage regimen, and often a single site of infection. The relationship between the measured MIC for the infective organism population and the reported MIC<sub>BP</sub> for that antibiotic determines whether the infection is reported as susceptible, intermediate, or resistant to that therapy in that patient. This system has been established both to guide individual therapy and ensure that across the patient population exposure to sub-therapeutic antibiotic concentrations is minimized. However, inherent in the system are a number of assumptions. The antibiotic in question is assumed to be dosed appropriately, with full owner and patient compliance, and fully penetrate to the infection site. The preferential concentration within certain organ systems shown by some antibiotics is not accounted for. The ability of the
infective organism to show *in vivo* ‘escape’ behaviour by biofilm formation or facultative intracellular dormancy is not accounted for (18). The cultured isolate is assumed to be representative of the infecting organism, despite the possibility of off target sampling and the time lag inherent in culture results. These pitfalls may account for some of the discrepancies between culture result predictions and therapeutic response.

While the MIC:MIC<sub>BP</sub> relationship is a good guide to effectiveness, additional recommendations have been made with respect to antibiotic tissue concentrations to minimize the incidence likelihood of emergent resistance. Following the mantra ‘dead bugs don’t mutate’, concentration dependent antibiotics (e.g. aminoglycosides, fluoroquinolones) should have a peak plasma drug concentration (PDC)/ MIC of >10-12 at the infection site (6). In contrast, the efficacy of time dependent antibiotics (e.g. β-lactams) is best predicted by the time that PDC > MIC; this should be 50-100% of the dosing interval (6), depending on the antibacterial agent and target pathogen. These targets may not always be achievable with standard antibiotic dosages or dosing intervals, and more research is needed to help guide the clinician attempting to meet these targets. In addition, the appropriate duration of antibiotic therapy is frequently poorly established. Recent studies investigating this issue have shown a trend toward identifying shorter courses to be of equivalent efficacy (19, 20, 21). As antibiotic exposure is well established as a risk factor for generating clonal expansion of antibiotic resistant endogenous microflora which may subsequently occupy a pathogenic niche (16), the ideal course duration can be defined as the minimum duration required to achieve clinical resolution in the majority of patients.

Treatment of orthopaedic surgical infections, specifically osteomyelitis, poses some unique challenges. Following fracture and vascular impairment, the medullary cavity constitutes
a relatively closed compartment with a paucity of local phagocytic cells. The inflammatory cascade may potentiate additional vascular obstruction and tissue damage from free radical release. The combination of implants, surgical contamination, and impaired vascular supply with consequent impaired endogenous immunity and impaired penetration of systemically administered antibiotics sets the stage for nosocomial infection (22). In response to these challenges, local antibiotic therapy has found a niche in the management of osteomyelitis.

The biological reasons for treatment failure can be broadly categorised into three groups: 1) the drug fails to reach its target 2) the drug is not active against the target pathogen or 3) the target is altered (23). Penetration of drugs into sites of infection almost always depends on passive diffusion and is thus proportional to the driving concentration gradient (24). For systemically administered drugs, this mandates good vascular supply to the target site. The outer membrane of gram-negative bacteria is a semi-permeable barrier in which are embedded porin protein channels that restrict the entry into the cell of small polar molecules such as antibiotics (24). Porin channel absence or mutation may prevent antibiotic entry reducing drug concentration at the target site. β-lactam antibiotics depend on this mechanism of bacterial cell entry. For drugs requiring active transport across the cell membrane, a mutation closing down this transport mechanism can confer resistance. For example, gentamicin transport depends on energy generated by respiratory enzymes during oxidative phosphorylation (24). A mutation in the key enzyme or anaerobic conditions slows entry of gentamicin into the cell, resulting in resistance. Drugs may also be transported out of the cell by efflux pumps, and resistance to numerous drugs is mediated by this mechanism, for instance chloramphenicol, fluoroquinolones, and β-lactams (24, 25, 26, 27).
Drug inactivation is the second general mechanism of treatment failure. The contents of pus can bind antibiotics, reducing the active free drug fraction. Antibiotic modifying enzymes can be produced by the target bacteria, for example β-lactamas (24).

The third general mechanism of drug resistance is target alteration, for example a mutation in the binding domain of the target DNA gyrase enzyme in the case of fluoroquinolones (27).

It is of note that while the first and second mechanisms may be at least partially overcome by sufficient increases in drug concentration gradients, the third is not likely to be assisted by this approach.

While the therapeutic framework for systemic antibiotics is relatively well established, no such equivalent system applies for antibiotics administered locally into the surgical wound bed, or used to coat implants or other devices. The antibiotic concentrations achieved locally may be much higher than those that typically result from systemic administration, and thus standard susceptibility reporting criteria and MIC\textsubscript{BP} will not apply. The change in drug concentration with time or pharmacokinetics of local therapy is also very different to systemic therapy, with a profile typically characterized by the rapid onset of a single peak concentration followed by a variable elimination phase, rather than the pulsatile pattern of sequential dosing. Thus the dose delivered by local administration may be more sustained than that delivered by systemic administration. To complicate matters further, evidence-based information on the appropriate duration of antibiotic therapy is frequently lacking. There is also the potential for local antibiotic therapy to compromise the wound environment either by a direct cytotoxic effect or by introduction of a delivery vehicle which persists long after the antibiotics have dissipated; these issues are typically not addressed in \textit{in-vitro} studies (28). The delivery vehicle itself, particularly
if non-biodegradable, may act as an implant and subsequently become colonised as well as potentiating the emergence of resistance. There are reports of antibiotic loaded cement beads contributing to the emergence of gentamicin resistant staphylococci sp (29). Theoretical advantages and disadvantages of local therapy are shown in Table 2. The following information attempts to summarise available data on the characteristics of various local antibiotic therapies.

**Table 2 Advantages and disadvantages of local antibiotic therapy**

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>High local antibiotic concentrations achievable in the wound bed may eliminate bacteria not susceptible to systemic therapy and penetrate biofilm</td>
<td>Risk of direct host cytotoxicity from antibiotic or carrier</td>
</tr>
<tr>
<td>Focused delivery may maximize therapeutic benefit while minimizing systemic toxicity</td>
<td>Risk that delivery vehicle may have a negative effect on wound healing, act as a nidus for persistent infection, or require surgical removal</td>
</tr>
<tr>
<td>Reduced systemic exposure and consequent fecal output of antibiotics may reduce environmental antibiotic exposure and selection for resistant traits⁸</td>
<td>Risk of promotion of resistant traits, if the PK profile provides a period of sub-therapeutic antibiotic exposure</td>
</tr>
<tr>
<td>Antibiotic delivery not dependent on the presence of vascularized tissue</td>
<td>Limited available information on dosing, efficacy, and wound or species specific pharmacokinetic and pharmacodynamic profile</td>
</tr>
<tr>
<td></td>
<td>Concurrent systemic therapy may still be appropriate</td>
</tr>
</tbody>
</table>


**Antibiotic impregnated cement**

Various forms of bone cement, including polymerized polymethylmethacrylate (PMMA), calcium sulphate (CS), and hydroxyapatite (HA) are in use in veterinary orthopaedics,
predominantly for prosthesis implantation and management of SSI with antibiotic-impregnated cement beads. All of these cements may be impregnated with antibiotics for either infection prophylaxis or treatment.

Powdered PMMA mixed with liquid methylmethacrylate undergoes an exothermic reaction to form non-absorbable bone cement 5 to 10 minutes later. The antibiotic powder or liquid solution is mixed with the PMMA prior to the addition of methylmethacrylate. Therefore, the antibiotic used must be stable in the face of the heat generated during the polymerization reaction. The cement material can then be formed into non-absorbable beads or used for infection prophylaxis of cemented arthroplasties (30). Conversely, calcium sulphate (CS) (plaster of Paris) and hydroxyapatite cement (HA) undergo no exothermic reaction during setting, are bioabsorbable, use water for admixture rather than a chemical polymer, and have also been studied as beaded antibiotic release vehicles (19,20).

There are a number of in-vitro studies evaluating antibiotic elution from these materials. Unfortunately, inter-study comparison and extrapolation of in-vitro results to a clinical setting are hampered by lack of a standardised or validated model for the drug elution environment. Models typically use differing elution volumes and volume change intervals, and make no attempt to replicate the dynamic flow state of the in-vivo environment. The cement/antibiotic mix ratios investigated as well as the elution concentrations considered efficacious are also highly variable. All studies investigating cement mixed with more than one antibiotic found the elution times were substantially shorter than when the antibiotics were used as a single agent (31-33). Use of a liquid rather than powdered antibiotic was not necessarily associated with loss of efficacy (31-33). The rate of elution of gentamicin from PMMA was similar for both the powdered and liquid form, however amikacin eluted faster when powdered rather than liquid
form was used (31). Idiosyncrasies were observed- for instance metronidazole delayed cement setting by 12 hours, and meropenem lost all biological activity when autoclaved (34,35). Findings from 7 in-vitro and 2 in-vivo studies are summarized in Table 3. There have been no good quality randomized controlled trials in human or veterinary clinical patients investigating the therapeutic benefit of antibiotic impregnated beads to treat osteomyelitis when compared with systemic therapy alone or in combination. Existing studies are either underpowered or experienced difficulties with protocol lapses. An experimental study in dogs evaluated treatment of induced Staphylococcus aureus osteomyelitis of the tibia with PMMA bead implants.

Systemic gentamicin therapy for 4 weeks was compared with gentamicin-impregnated PMMA, using a 1:1 mix with a single 1cm x 1.5cm bead implanted in each dog at the site of infection. An improved rate of resolution was identified in the gentamicin impregnated PMMA group (89%) compared with the systemic therapy group (63%), p=0.049 (36). A randomised controlled trial evaluated systemic versus local therapy for 52 adult human patients undergoing debridement and reconstruction for infected non-unions. Four weeks of intravenous antibiotics were compared to local gentamicin PMMA beads together with two to 5 days of peri-operative systemic therapy. Resolution rates were 83% versus 89% (p=0.53) respectively (37). A multi-center randomized controlled trial compared systemic to local therapy using a similar methodology and also found no difference in resolution rates, however 75% of the patients in the local therapy group broke protocol and exceeded the 5 day limit to concurrent systemic therapy set by the investigators (38).

The effect of using antibiotic loaded cement for infection prophylaxis in hip and knee arthroplasties in human patients has been investigated. A recent systematic review reported an
absolute risk reduction of 8% and relative risk reduction of 81% when antibiotic loaded cement is used (p<0.001) (39).

Findings regarding the effect of antibiotic admixture on cement mechanical properties have been variable. The addition of cefazolin powder to PMMA powder at a 1:40 dry weight ratio was reported to have no effect on compressive strength, while gentamicin powder at a 1:400 ratio caused significant reductions in compressive strength (40). The use of gentamicin in liquid form has been reported to have a greater negative impact on mechanical characteristics of cement than use in powder form, with a reduction in elastic modulus, but no difference in ultimate load (7). A recent review on the influence of antibiotic admixture on mechanical characteristics of cement concluded that there is limited consensus on either the effect of the antibiotic or on the method used to blend the antibiotic with the cement, with conflicting results found across multiple studies. However, in general, the addition of antibiotic powder was found to cause a significant reduction in the fatigue life of the cement (41).

Antibiotic beads have also been investigated for infection prophylaxis in the context of open fracture management. A retrospective non-randomised study in 914 human patients identified an absolute risk reduction of 8.3% when comparing treatment with aminoglycoside-impregnated PMMA beads in conjunction with systemic therapy against systemic therapy alone (42).

Potential negatives surrounding the use of antibiotic impregnated cement include the risk of systemic toxicity and the generation of resistant organisms by prolonged exposure to sub-therapeutic concentrations of antibiotic. The available pharmacokinetic data for use of antibiotic impregnated cement in veterinary patients is very limited, and it would appear prudent to take concurrent parenteral dosing into account. However, there have so far been no veterinary reports
of adverse patient events for this treatment modality. Serum levels of gentamicin remained undetectable when mongrel dogs were treated with PMMA containing 100mg of gentamicin per dog (36). The generation of resistant organisms remains a concern. A cross-sectional study identified a 22% prevalence of bacterial colonisation of antibiotic loaded PMMA beads, with documented emergence of resistance (29). There is a case report of gentamicin impregnated beads removed from a human patient 5 years after placement, at which time low levels of eluting gentamicin were still detectable, and the bead surface had been colonized by a gentamicin resistant coagulase negative staphylococci Staphylococcus (CoNS) spp (43). Where possible, the timely removal of PMMA implants may reduce this risk.

In summary, there is some evidence, although cross-species, for the use of antibiotic-impregnated cement in joint arthroplasty, although this may come at the expense of shortened fatigue life. There is also good experimental data demonstrating the efficacy of antibiotic-impregnated cement beads in the treatment of osteomyelitis.
Table 3  Summary of in-vivo and in-vitro studies investigating antibiotic elution from cement beads

<table>
<thead>
<tr>
<th>Antibiotic and current CLSI MIC&lt;sub&gt;BP&lt;/sub&gt; for Staph. sp</th>
<th>Study author</th>
<th>Drug/ cement mix evaluated (dry weight ratio)</th>
<th>Cement type</th>
<th>Elution concentration considered efficacious (C&lt;sub&gt;effic&lt;/sub&gt;)</th>
<th>Time&gt; C&lt;sub&gt;effic&lt;/sub&gt; (days)</th>
<th>Maximum elution concentration documented (ug/ml)</th>
<th>Time&gt; MIC&lt;sub&gt;BP&lt;/sub&gt; (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefazolin 8ug/ml</td>
<td>Phillips&lt;sup&gt;21&lt;/sup&gt;</td>
<td>1:6</td>
<td>PMMA</td>
<td>4 x MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>&gt;30</td>
<td>~530</td>
<td>&gt;30</td>
</tr>
<tr>
<td></td>
<td>Adams&lt;sup&gt;a,70&lt;/sup&gt;</td>
<td>1:9</td>
<td>PMMA</td>
<td>N/R</td>
<td>N/a</td>
<td>88</td>
<td>&gt;28</td>
</tr>
<tr>
<td></td>
<td>Udomkusonsrij&lt;sup&gt;71&lt;/sup&gt;</td>
<td>1:10</td>
<td>PMMA</td>
<td>0.125 ug/ml</td>
<td>&gt;14</td>
<td>~1200</td>
<td>&gt;14</td>
</tr>
<tr>
<td></td>
<td>Udomkusonsrij&lt;sup&gt;71&lt;/sup&gt;</td>
<td>1:10</td>
<td>CS</td>
<td>0.125 ug/ml</td>
<td>&gt;14</td>
<td>~1300</td>
<td>&gt;14</td>
</tr>
<tr>
<td></td>
<td>Weisman&lt;sup&gt;26&lt;/sup&gt;</td>
<td>1:40</td>
<td>PMMA</td>
<td>N/a</td>
<td>7-13</td>
<td>N/a</td>
<td>N/a</td>
</tr>
<tr>
<td>Ceftriaxone 2ug/ml</td>
<td>Ethell&lt;sup&gt;19&lt;/sup&gt;</td>
<td>1:10</td>
<td>PMMA</td>
<td>2 ug/ml</td>
<td>N/a</td>
<td>~350</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Ethell&lt;sup&gt;19&lt;/sup&gt;</td>
<td>1:10</td>
<td>PMMA</td>
<td>2 ug/ml</td>
<td>7</td>
<td>7</td>
<td>&lt;9</td>
</tr>
<tr>
<td>Ticarcillin 64ug/ml</td>
<td>Adams&lt;sup&gt;a,70&lt;/sup&gt;</td>
<td>1:33</td>
<td>PMMA</td>
<td>N/a</td>
<td>N/a</td>
<td>6100</td>
<td>N/a</td>
</tr>
<tr>
<td>Vancomycin 2ug/ml</td>
<td>Atilla&lt;sup&gt;20&lt;/sup&gt;</td>
<td>1:30</td>
<td>CS</td>
<td>4 ug/ml</td>
<td>84</td>
<td>1776</td>
<td>&gt;84</td>
</tr>
<tr>
<td></td>
<td>Adams&lt;sup&gt;a,70&lt;/sup&gt;</td>
<td>1:10</td>
<td>PMMA</td>
<td>N/a</td>
<td>N/a</td>
<td>48.1</td>
<td>&gt;28</td>
</tr>
<tr>
<td>Meropenem 4ug/ml</td>
<td>Baez&lt;sup&gt;23&lt;/sup&gt;</td>
<td>1:5</td>
<td>PMMA</td>
<td>4 ug/ml</td>
<td>15-18</td>
<td>~1100</td>
<td>15-18</td>
</tr>
<tr>
<td>Gentamicin 4ug/ml</td>
<td>Ramos&lt;sup&gt;22&lt;/sup&gt;</td>
<td>1:20</td>
<td>PMMA</td>
<td>N/a</td>
<td>&gt;21</td>
<td>N/a</td>
<td>N/a</td>
</tr>
<tr>
<td></td>
<td>Ethell&lt;sup&gt;19&lt;/sup&gt;</td>
<td>1:20</td>
<td>PMMA</td>
<td>~0.5 ug/ml</td>
<td>&gt;30</td>
<td>~50ug/ml</td>
<td>N/a</td>
</tr>
<tr>
<td></td>
<td>Ethell&lt;sup&gt;19&lt;/sup&gt;</td>
<td>1:20</td>
<td>HA</td>
<td>~0.5 ug/ml</td>
<td>&gt;30</td>
<td>~2000ug/ml</td>
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<tr>
<td></td>
<td>Anagnostakos&lt;sup&gt;,72&lt;/sup&gt;</td>
<td>1:80</td>
<td>PMMA</td>
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<td>&gt;30</td>
<td>116ug/ml</td>
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<tr>
<td></td>
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<td>1:400</td>
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<td>6-12</td>
<td>N/a</td>
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<tr>
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<td>Phillips&lt;sup&gt;21&lt;/sup&gt;</td>
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<td>PMMA</td>
<td>8 x MIC&lt;sub&gt;90&lt;/sub&gt;</td>
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<td>MIC</td>
<td>&gt;30</td>
<td>~200</td>
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<td>N/a</td>
<td>N/a</td>
<td>1517</td>
<td>&gt;28</td>
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<sup>a</sup> in-vivo study, 5 x 9mm diameter beads implanted in medullary cavity in dogs and tissue/bone concentrations measured at 28d
Gentamicin impregnated collagen sponge

Gentamicin impregnated collagen sponge (GICS) is a local antibiotic delivery product finding increasing application worldwide for prophylaxis and treatment of surgical infections. The product consists of bovine or equine collagen impregnated with gentamicin and lyophilized to provide uniform drug distribution. This facilitates accurate patient dosing by unit sponge area. Applications in human patients include the treatment of implant-associated infections (44, 45), soft tissue infections (46-48) and infection prophylaxis in oncologic, orthopaedic and cardiac surgery, including the management of open fractures at the time of open reduction/ internal fixation (49-51). Reported veterinary usages are similar, with a focus on the management of intra-articular infections (52-54). Randomised controlled trials investigating human patient populations have reported prophylactic GICS to reduce infection rates following median sternotomy (55), however conversely the sponge was reported to increase infection rates when used prophylactically during colo-rectal surgery (56).

The reported advantages of the GICS include the delivery of very high local antibiotic concentrations combined with rapid biodegradability (Innocoll product information). A study investigating antibiotic release following GICS implantation in equine tarso-crural joints at 0.26mg/kg identified median peak intra-synovial concentrations of 169ug/ml, although gentamicin concentrations fell to sub-MIC (4ug/ml) by 48 hours post implantation (57). A similar study investigating pharmacokinetics following GICS implantation in canine stifles at a dose of 6mg/kg identified mean peak intra-synovial concentrations of 2397 ug/ml with a decline to sub-
MIC concentrations by 23 hours post implantation. Plasma levels reached approximately 33% of those anticipated after IV gentamicin administration (58). While the standard mg/kg dosing used for systemic therapy does not extrapolate well to the local environment in terms of efficacy, reporting dosing in this manner may be of assistance when considering potential systemic toxicity concerns, The PK studies performed to date support the manufacturer’s claims for delivery of high local antibiotic concentrations. Studies investigating the biodegradation of GICS following subcutaneous and intra-muscular implantation identified a marked inflammatory response persisting to at least 5 days following implantation (59, 60). A study investigating intra-articular inflammation following GICS implantation identified elevated cytokines and cellular inflammatory response persisting to at least 4 weeks post implantation (61). Thus delayed biodegradation may be an issue, and persistence of a collagen nidus in the face of sub-therapeutic local antibiotic concentrations may explain the increased infection risk found in some studies.

Toxic serum gentamicin levels and compromised renal function have been reported in association with intra-articular sponge use in human patients at gentamicin doses ranging from 7-9mg/kg (62). A study investigating renal function in normal dogs following subcutaneous implantation of GICS found normal serum creatinine for at least 7 days post-operatively; the gentamicin dose used was not stated and it is therefore not possible to know the clinical significance of this finding (63). A study investigating sensitive renal markers following intra-articular implantation of the GICS at 6mg/kg in dogs found a reduction in glomerular filtration rate in the treatment group (61).
In summary, cross-species data suggests that while there may be a role for GICS in antibiotic prophylaxis for clean surgeries where the consequences of infection may be devastating (55), there is also the potential for GICS to worsen infection rates when used in the face of bacterial contamination (56). Canine data evaluating intra-articular GICS suggests a rapid decline in eluted gentamicin to sub-MIC levels, with persistence of collagen-associated inflammation for several weeks (58, 61). The persistence of the collagen sponge may be the cause of the deleterious effects on wound healing seen in some circumstances (56). There is currently no high quality data on the efficacy or safety of GICS for the treatment of established infections.

**Antibiotic impregnated gel**

An injectable, antibiotic impregnated dextran polymer hydrogel has recently become available. The gel consists of two ingredients that form a gel within two minutes of mixing, and is suitable for injection or topical application. The residence time of the gel *in vivo* is 4 to 5 weeks, with degradation via hydrolysis. The gel is reported to be fully biodegradable and non-immunogenic. Available antibiotics include amikacin, vancomycin, or amikacin/clindamycin (R-gel product information, Royer Animal Health). A recent in-vitro study suggested release of high local concentrations of antibiotic within the first 24 hours (C\textsubscript{max}:MIC>300 for amikacin and clindamycin) with concentrations sustained above the MIC for 10 days (64). To date, no case series or treatment trials have undergone peer-reviewed publication.
Antibiotic impregnated demineralised bone matrix

Demineralised bone matrix impregnated with tobramycin and gentamicin has been investigated in vitro, with a view to clinical application in the management of infected non-unions. Potential advantages include osteoinduction and osteoconduction together with no requirement for a second procedure to remove the implants. In vitro, continued osteoblastic activity was identified, and antibiotic levels were maintained above MIC for 13 days (65, 66). Reduction in positive cultures in an experimental model was demonstrated when compared with standard demineralized bone matrix alone, however effect on bone healing in vivo was not evaluated, and no comparison was made between antibiotic impregnated demineralized bone matrix and standard bone matrix in combination with systemic antibiotic therapy (66).

Antibacterial properties and coatings for surgical implants

Veterinary metal orthopaedic implants are typically composed of 316L stainless steel. This is a 2.8% molybdenum, 15% nickel, 18% chromium and 64.2% iron alloy (67). Alternative materials include titanium alloys, and various absorbable materials such as polycaprolactone and polylactide (68, 69). Stainless steel implants have been associated with significantly greater infection rates than titanium implants (70, 71, 72), although the topic is controversial. A postulated reason for this is that soft tissue adheres firmly to titanium implant surfaces, while a fibrous capsule containing a fluid filled void is formed around steel implants. The consequent dead space is more susceptible to bacterial colonization, and less accessible to host defence mechanisms (15). An
Experimental study investigating Staphylococcus aureus biofilm formation found biofilm to form more readily on steel versus titanium implant surfaces (73).

Various metal implant coatings, including hydrophobic materials, antibiotics, nitric oxide releasing compounds and silver, have been assessed in both in-vitro and in-vivo studies for potential to decrease implant colonization. The hydrophobic material polycation N,N- dodecyl,methyl-PEI (PEI = polyethylenimine) was shown to prevent implant colonization in a sheep osteomyelitis model (74). Various antibiotic-loaded, biodegradable polymers have shown efficacy as implant coatings (75,13) however there is a theoretical risk of inducing resistant bacterial strains if the antibiotic release profile shows prolonged periods of sub-MIC antibiotic concentrations. Experimental studies in rats have demonstrated efficacy of a gentamicin-coated tibial nail for improving bone healing when used in the face of bacterial contamination (76), and a follow-up case series reported minimal complications when gentamicin-coated nails were used for management of open fractures in human patients (77). Gentamicin coated polyurethane sleeves fitted to external skeletal fixator pins have been investigated as a method of prevention of pin tract infections (78). Nitric oxide releasing coatings have been applied to external skeletal fixator pins and shown to reduce bacterial colony counts in a rat model (79). A method of covalently linking antibiotics to a titanium implant surface has been developed, offering the theoretical advantages of no additional delivery vehicle together with long-term activity and reduced tissue toxicity. No clinical trials have been performed to date (80).
Silver is bactericidal, disrupting the function of bacterial cell membranes and metabolic proteins (81). Silver-resistant bacterial strains are reported (82). In vitro studies evaluating silver nanoparticle coatings have shown promise (83, 84) however the technology has yet to be fully assessed in appropriate in-vivo models or clinical trials. A randomised controlled trial investigating silver-coated external fixator pins compared with standard stainless steel pins in 24 human patients identified a 10% reduction in positive culture rates. This difference did not reach statistical significance due to the small number of patients enrolled in the study and associated lack of power. Silver coated pin implantation resulted in a significant increase in silver serum level, resulting in termination of this study (85). A subsequent randomised controlled trial evaluated the effect of applying a silver coating to a titanium-vanadium megaprosthesis used for limb salvage in 51 human patients following major oncologic resection. Infection rates were 5.9% in the silver coating group versus 17.6% in the uncoated group (p=0.062), however the groups lacked homogeneity, with operating times on average 30 minutes shorter in the silver coating group (p=0.03). A single patient in the treatment was reported as suffering from silver toxicity (86).

**Conclusion**

In the face of increasing incidence of multi-drug resistant implant infections, local antibiotic modalities are receiving increased attention for both infection prophylaxis and treatment. Local antibiotic therapy, achieving very high antibiotic concentrations at the site of the implant, may represent an avenue for treatment of infections including those
by biofilm-forming bacterial pathogens. Randomised controlled trials in human patients have demonstrated an infection risk reduction when antibiotic impregnated cement is used for infection prophylaxis in implanted joint prostheses (39), and when a gentamicin impregnated collagen sponge is used for infection prophylaxis in midline sternotomy (55). The other modalities discussed have for the most part yet to be evaluated in randomized controlled trials in veterinary or human patients. In general, the in-vivo concentration-time profile and appropriate dosing regimen for local antibiotic modalities have yet to be elucidated. Toxicity is possible locally and systemically, and attention to the dose and characteristics of the dosage form applied are warranted (62). There is currently an emphasis on in-vitro studies in the veterinary literature on this topic, which do not necessarily assist robust clinical decision making. A central web-based registry of orthopaedic surgical site infections providing a running database documenting clinical isolate, context, treatment and outcome might go some way towards alleviating the veterinary-specific information void on this important topic.

In summary, for therapy of established orthopaedic surgical site infections, the principles governing responsible antibiotic use should be adhered to. Antibiotic selection should be targeted by culture and susceptibility results, and narrow spectrum agents should be used wherever possible and appropriate. The use of local antibiotics fulfilling these criteria in addition to standard systemic therapy may be judicious if there are reasonable clinical concerns regarding vascularity at the surgical site which can not be addressed by surgical debridement, or if there is an implant burden which can not be removed until healing has further progressed. In these instances, either antibiotic
impregnated cement or collagen have undergone the greatest clinical research. If PMMA beads are used, removal of the beads together with the implants once the infection has resolved and bone healing progressed is recommended.

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CHAPTER TWO: INTRODUCTION

Motivation for research and research objectives

Gentamicin impregnated collagen sponge (GICS) is a widely available surgical implant used both for treatment and prophylaxis of surgical site infections. The product consists of type I bovine collagen impregnated with gentamicin. The purported advantages of GICS include its biodegradability and biocompatibility, and ability to achieve high, sustained, local concentrations of gentamicin with minimal systemic exposure. The commercial product has found increasing application in surgery on humans for prophylaxis of surgical site infections at median sternotomy, as well as treatment of infected total hip replacements (1,2). However, a recent randomised controlled trial identified no benefit and a possible association with increased infection rates with prophylactic sponge use following colorectal surgery (3). Commercial product information suggests both rapid and prolonged release, with no risk of systemic toxicity and complete absorption within one to seven weeks. The incidence of implant-associated methicillin resistant staphylococcus pseudintermedius (MRSP) infections in veterinary orthopaedic surgery is increasing (4). It has been our observation that many of these infections occur following tibial plateau levelling osteotomy, with a concurrent septic arthropathy of the stifle. In addition, a significant fraction of these infections are gentamicin susceptible, with a potential role for clinical use of GICS. Unfortunately, much of the available information on GICS in the context of management of implant associated orthopaedic infections in anecdotal in nature, with little specific data on gentamicin pharmacokinetics and no randomised controlled trials. A single case series suggested an association
between renal injury and GICS use for management of infected total hip replacements (5). The goals of this research were to evaluate the local and systemic pharmacokinetics of gentamicin release from GICS following implantation in the stifle, and to assess for any harm caused by the sponge. Areas of potential morbidity included a local inflammatory effect together with renal injury if systemic exposure was greater than anticipated.

Anticipated challenges

A randomised placebo controlled blinded clinical trial, while technically feasible, was not felt to be ethically appropriate in the face of potential morbidities. Instead, an experimental trial using research hounds was elected. Financial limitations limited animal numbers, which in turn mandated appropriate outcome of interest selections to achieve adequate power. The research was designed as a laboratory randomised controlled trial. The treatment arm provided observational data for assessment of pharmacokinetics, while comparison of the treatment and control groups allowed assessment of the causal relationships between GICS and any identified morbidities. One anticipated challenge was the effect of joint inflammation on local pharmacokinetics. The synovial membrane is highly vascular, and in normal circumstances acts as a dialysis membrane allowing rapid transfer of small molecules between the systemic circulation and intra-articular environment (6). An active inflammatory response, such as that commonly found in patients with a septic arthropathy, could be anticipated to alter drug pharmacokinetics following local delivery in comparison to a normal, uninflamed joint. Options included using a canine model with an induced septic arthropathy, or an induced chemical (urate)
synovitis. The urate synovitis model was elected due to increased predictability, although at the acknowledged expense of reduced clinical parallel. Animal numbers and the use of clinically realistic GICS dosages mandated the use of relatively sensitive markers to allow any differences in outcome measure to reach statistical significance—thus renal injury was assessed by the use of GFR measurement and electron microscopy of renal ultrastructure.

Chapter Three presents the observational data evaluating local and systemic pharmacokinetics of gentamicin release from GICS following intra-articular implantation. Chapter Four presents the data from the randomised controlled trial evaluating the effect of GICS on joint inflammatory response, lameness, and renal changes.

References


CHAPTER THREE

INTRA-ARTICULAR PHARMACOKINETICS OF A GENTAMICIN IMPREGNATED COLLAGEN SPONGE- HIGH PEAK CONCENTRATION, SHORT DURATION

Accepted for publication in Veterinary Surgery

Abstract

Objective: To investigate local and systemic pharmacokinetics of gentamicin following intra-articular implantation of a gentamicin impregnated collagen sponge (GICS) in the inflamed canine joint.

Study Design: Descriptive repeated measures experimental study.

Animals: 9 dogs

Methods: Stifle joint inflammation was generated by urate injection. Twenty-four hours later a GICS was arthroscopically implanted at gentamicin dose=6mg/kg. Synovial fluid and plasma gentamicin concentrations were measured for 14 days post implantation, and pharmacokinetic parameters modeled using statistical moment analyses.

Results: Intra-articular gentamicin concentrations fell to sub-MIC for Staphylococcus sp. (4ug/ml) by 22.4hrs (95% CI=18.6-26.2) following sponge implantation. C_{\text{max synovial}} was 2397ug/ml (95% CI=1161-3634 ug/ml) at 1.2 hrs (95% CI=0.5-1.8hrs). Plasma gentamicin concentrations achieved levels of C_{\text{max plasma}} =8.0ug/ml (95% CI=6.1-10.0 ug/ml) at 1.5hrs (95% CI=0.8-2.1) following GICS.
placement and fell below target trough of 2.0ug/ml by 5.6hrs (95% CI=4.7-6.5hrs) following GICS placement.

Conclusions: Intra-articular gentamicin concentration following GICS placement at an IV-equivalent dose reached high levels and declined rapidly. The maximum plasma levels attained were approximately 1/3rd of the recommended sub-toxic target for human patients following parenteral gentamicin administration.

Clinical relevance: Intra-articular gentamicin concentrations reached are theoretically sufficient to kill even multi-drug resistant staphylococci. It is unclear whether the collagen sponge formulation offers any advantage over intra-articular injection of the parenteral formulation, given the rapid decay profile. GICS should be used with caution or avoided in patients with renal insufficiency. Concurrent parenteral aminoglycoside doses should be avoided or adjusted.

5 keywords: gentamicin, collagen, dogs, intra-articular, pharmacokinetic
Introduction

Gentamicin impregnated collagen sponge (GICS) is a local antibiotic delivery product finding application for prophylaxis and treatment of surgical infections. The product consists of bovine or equine collagen impregnated with gentamicin and lyophilized to provide uniform drug distribution. This facilitates dosing by unit sponge area. Applications in human patients include the treatment of implant-associated infections, soft tissue infections, and infection prophylaxis in oncologic, orthopedic and cardiac surgery. Reported veterinary usages are similar, with a focus on the management of intra-articular infections.

Septic arthropathies are a source of morbidity in both human and veterinary patient populations. The commonest infective organisms are the staphylococcal species, accounting for over 50% of cases, and these infections are frequently hospital-acquired. Biofilm formation, the increasing prevalence of genes conferring multi-drug resistance, and the ability of staphylococci to undergo facultative intracellular dormancy all pose challenges to effective bacterial killing in septic arthropathies. Intra-articular local antibiotic therapy can be employed either as an adjunct or as a replacement for systemic therapy, and theoretically offers several advantages. These include the provision of very high local drug concentrations, with minimal systemic exposure and the potential for reduced financial cost of treatment. High local drug concentrations maximize bacterial kill at the septic focus for organisms expressing biologically defensive or antibiotic resistant characteristics. Minimal systemic exposure
reduces or eliminates toxicity. Reduced requirement for parenteral drug administration shortens hospital stay and reduces financial costs.

Gentamicin impregnated collagen sponges may provide an effective local antibiotic delivery system for both prophylaxis and treatment of surgical infections, including septic arthritis. In general, gentamicin exhibits good activity against Staphylococcal sp., coliforms, Enterobacter sp., Enterococci sp., and Pseudomonas sp. displaying both concentration dependent killing and post antibiotic effect. Gentamicin typically retains activity even in the face of multi-drug resistant strains. Current CLSI breakpoints for dogs classify staphylococci as susceptible, intermediate, and resistant if growth is first inhibited at gentamicin concentrations of <=4ug/ml, 4-8ug/ml, and >=16ug/ml respectively. Organisms characterized as gentamicin-resistant by survival at an MIC breakpoint of 4ug/ml may still be killed if exposed to sufficiently high gentamicin concentrations. An in-vitro study investigating 103 methicillin resistant Staphylococcus pseudintermedius isolates from dogs in 8 countries found that of 72 isolates characterized as gentamicin resistant at standard breakpoints, all were killed by a gentamicin concentration of 256ug/ml. While this would not be a physiologically viable concentration with systemic administration, it might be achievable with local therapy.

Gentamicin has the potential to cause concentration dependent renal and ototoxicity. Toxic effects are potentiated by sepsis and contraction of circulating volume. Gentamicin is highly water and poorly lipid soluble, resulting in a steep plasma: tissue gradient following parenteral use. The use of gentamicin in a local delivery
format may minimize toxicity while maximizing tissue concentrations where most required. In contrast to PMMA beads, the gentamicin collagen implant does not require surgical removal, and is biodegraded in-situ by endogenous collagenases. However, the potential for systemic exposure to gentamicin following local delivery remains, and has not been well characterized to date. Data from elderly human patients following GICS implantation to treat infected total hip arthroplasty identified toxic serum gentamicin levels between 1 and 10 days after surgery, together with decreased creatinine clearance.

The purpose of this study was to elucidate the pharmacokinetic profile of elution of gentamicin from a GICS following implantation into the inflamed joint in the dog. The sponge was dosed at 6mg/kg. Gentamicin concentrations in synovial fluid and plasma were measured for 14 days. Specific goals were to determine the intra-articular $C_{\text{max}}$ as well as the time for which intra-articular drug concentrations remained above MIC for relevant pathogens, and to assess systemic exposure to gentamicin following intra-articular administration.

**Materials and methods**

**Ethical approval**

This study was carried out in accordance with U.S. National Institutes of Health “Guide for the Care and Use of Laboratory Animals” and the Animal Welfare Acts (US
Study design

This was a prospective interventional repeated measures study performed under experimental conditions.

Animals

Nine research-specific purpose bred dogs (Marshall BioResources, North Rose, NY) of the hound type were studied (5 male, 4 spayed female), with a mean weight of 22.8kg (range 19.5-27.6kg) and age of 2.1yrs (range 1.1-4yrs). Dogs were conditioned to routine handling prior to study use. Baseline physical and orthopedic examinations, complete blood counts, urinalysis, and biochemistry profiles identified no abnormalities.

Acute synovitis model

Dogs were sedated using 0.05mg/kg hydromorphone and 2ug/kg dexmedetomidine IV (Dexdomitor; Pfizer Animal Health, Madison, NJ) to facilitate intra-articular injection and placement of a jugular catheter (16G, 20cm; MILA International, Erlanger, KY). For each dog, a stifle joint was randomly selected using computer randomisation and injected with 10mg sodium-urate (Sigma-Aldrich Canada Ltd, Oakville, ON) as sterile urate suspension according to previously reported techniques for predictable generation of an
Aspiration of synovial fluid was performed prior to urate injection to confirm intra-articular location. Aspirated fluid was submitted for baseline evaluation of cell counts and cytology. Standard aseptic technique was used for all interventional procedures. Tramadol 2mg/kg PO Q 8 hrs was administered to maintain post-procedural comfort, and continued for the following 3 days.

**Stifle arthroscopy**

Twenty-four hours later, acute synovitis was confirmed in each dog by the presence of lameness, palpable joint effusion, and documentation of synovitis on arthroscopic examination. Dogs were pre-medicated with acepromazine 0.03mg/kg IV and hydromorphone 0.05mg/kg IV. General anesthesia was induced using propofol 10mg/ml to effect (mean administered dose 3.2mg/kg), and maintained with isoflurane carried by 100% oxygen following endotracheal intubation. Arthroscopy of the previously injected stifle was performed by a board certified surgeon (NM) using a standardized technique, as follows.

A 22g needle was introduced into the stifle joint and synovial fluid aspirated. Following aspiration 6mls sterile saline were instilled to distend the joint. Synovial fluid was submitted for post-urate total nucleated cell counts and cytology. Using a blunt-tip trochar a distolateral arthroscopy portal was established and advanced in a proximomedial direction. An egress port was established in the proximal medial parapatellar region. A 2.4mm arthroscope (Karl Storz, Canada Ltd, Mississauga, ON) was introduced and the joint explored. The joint was assessed for pre-existing pathology.
and a modified Outerbridge score\textsuperscript{32} assigned. Synovial inflammation was assessed and a visual analogue score assigned (Figure 1). A modified multi-fenestrated intra-articular sampling catheter (catheter volume 0.5mls, MILA International, Erlanger, KY) was placed in the lateral parapatellar pouch using the Seldinger over-wire technique and position confirmed with arthroscopic visualization (Figure 2). Following confirmation of correct location the catheter was secured using both skin sutures at the hub and a single deep suture anchoring the catheter hub to the fascia lata (3 metric Surgipro; Covidien, Norwalk, CT). The scope was then removed leaving the scope portal in situ, positioned at the intercondyloid fossa.

Figure 1 Synovial membrane during arthroscopy of the stifle joint 24 hours following induction of an acute chemical synovitis using injection of sodium urate. Note the increased vascularity and vascular engorgement.
Figure 2 Intra-articular placement of a sampling catheter into the lateral para-patellar pouch under arthroscopic guidance using the Seldinger technique.

Figure 3 Gentamicin impregnated collagen sponge in the stifle joint immediately following intra-articular placement via an arthroscopy portal.
Placement of gentamicin impregnated collagen sponge

The sponge consisted of sterile renatured Type I bovine collagen impregnated with gentamicin. The commercial sponge product contained 2.8mg of collagen and 2.0mg of gentamicin sulphate per cm\(^2\), and was available in units of 10 x 10x0.5cm (Collatamp G, Theramed Corp, Mississauga, ON). The sponge was dosed to bodyweight at 6mg/kg of gentamicin, and the appropriate dosage for each dog was administered by cutting the sponge to the correct area according to the manufacturer’s instructions. The sponge dose was then divided into small segments, rolled, and inserted through the scope portal. The sponge remained dry until intra-articular placement. Following sponge delivery, the scope was re-inserted in the scope portal and intra-articular placement of the sponge confirmed (Figure 3).

Sampling for pharmacokinetic data
Samples of blood and synovial fluid were collected using a standard 3 syringe technique in the case of blood samples from the jugular catheter, and a two syringe technique (no flush) for the intra-articular catheter. Synovial fluid samples were collected from the intra-articular catheter until 48 hrs following GICS placement, and thereafter by arthrocentesis under sedation. Plasma and synovial fluid samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 48, 72, 96, 168 and 336 hours post GICS placement. Any discrepancy in actual vs intended sampling time was noted. Blood samples were collected into heparinized blood collection tubes and centrifuged at 2000 x G for 3 minutes to obtain heparinized plasma. Synovial fluid and heparinized plasma samples were immediately frozen at -70°C and held until analysis.

**Measurement of gentamicin concentrations in plasma and synovial fluid**

Gentamicin concentrations were measured using a fluorescence polarization immunoassay (Abbott TDx Analyzer, Abbott Diagnostics, Toronto, ON). The limit of quantification (LOQ) for the assay was 0.27ug/ml. The assay manufacturer reports a cross-reactivity of <1% for hydromorphone and tramadol. The target (range) concentrations for low (L), medium (M) and high (H) quality control samples (QC) were 1.0 (0.85-1.15), 4.0 (3.60-4.40) and 8.0 (7.20-8.80) ug/ml, respectively. The nominal concentrations for the gentamicin calibrators were 0, 0.5, 1.5, 3.0, 6.0 and 10.0 ug/ml. Prior to assessment of experimental samples, the assay was validated for canine plasma and synovial fluid as follows. Blank canine plasma and synovial fluid samples were fortified with gentamicin to duplicate the LQC, MQC and HQC ranges. The synovial
fluid blank was pre-treated with hyaluronidase (type 1-S, Sigma Aldrich, St Louis, Missouri) at a concentration of 1ug/uL. Three replicate samples were assessed at each concentration. These were then analyzed and assessed against manufacturer-provided human serum QC samples. Acceptance criteria were defined as an assay coefficient of variation of <6% at all QC levels. All canine samples assessed (n=18) fell within the acceptance criteria.

Samples with concentrations >10ug/ml required dilution prior to analysis. Dilution was performed with a diluent provided by the manufacturer (XSYSTEMS dilution buffer, Abbott Laboratories Diagnostics Division, Abbott Diagnostics, Toronto, ON). The dilution factor (DF) was calculated as (sample volume + dilution reagent volume)/ (sample volume). Synovial fluid samples were pre-treated with hyaluronidase at 1ug/ml prior to analysis. Final sample concentration was defined as (DF x reported concentration).

**Sampling for validation of catheter vs arthrocentesis methodology**

Concurrent sampling by arthrocentesis and catheter aspiration for validation of technique equivalency was performed at 24 and 48 hours post GICS placement.

**Animal care and husbandry**

Dogs were housed in individual runs for the duration of the study. They were fed commercial dry dog food (Adult maintenance; Purina) and provided with water ad lib.
Exercise was restricted for the first 48 hours of the study, and thereafter they were walked outside on leash several times daily. Bandages and Elizabethan collars were used to prevent removal of jugular and intra-articular catheters. All interventional procedures (catheter placement and removal, sponge placement, arthrocentesis) were performed under sedation or general anesthesia. All dogs were assessed for discomfort by a veterinarian at least every 6 hours for the first 48 hours of the study, and thereafter at least every 12 hours, and adjunctive analgesia provided as needed (hydromorphone 0.05mg/kg IV or IM).³³

**Pharmacokinetic and statistical data analysis**

Data was assessed for normality using the Shapiro Wilk test, and means +/-SD or 95%CI reported where a normal distribution applied, and median (IQR) where not normal. The equivalence of synovial fluid sampling methodologies was assessed using Bland-Altman plots and calculation of Bland-Altman’s limits of agreement.³⁴ Paired samples with a normal distribution and constant variance were compared using a paired t-test. Statistical analyses were performed using Stata (StataCorp, College Station, TX, USA). Pharmacokinetic data for synovial fluid and plasma were explored graphically using scatter plots of concentration vs time and log(concentration) vs time for each dog. Pharmacokinetic analysis was performed using a commercial software program (WinNonlin® Version 5.2.1; Pharsight Corporation, Mountain View, CA, USA) using a standard two stage approach. The pharmacokinetic parameters were calculated for both matrices (plasma and synovial fluid) using non-compartmental analyses for extravascular
These parameters included time above a gentamicin concentration of 2 and 4ug/ml for both plasma and synovial fluid; terminal half life (T₁/₂) and the slope of the terminal elimination phase (λ₂), maximum drug concentration (Cₘₐₓ), time to reach peak levels (Tₘₐₓ) and area under the concentration-time curve (AUC). The terminal half life was calculated as ln(2)/λ₂. The slope of the terminal elimination phase of gentamicin in each matrix was calculated using the last 6 points on the curve for synovial fluid, and the last 5 points on the curve for plasma. The linear up, log down trapezoidal method was used to calculate areas under the curve. Parameters were calculated for all 9 dogs, and reported as means and 95% CI.

Results

Documentation of lack of pre-existing pathology and development of acute synovitis

No joint showed any evidence of pre-existing pathology on arthroscopic examination. All modified Outerbridge scores were 0, and cruciate ligaments all appeared normal. All dogs demonstrated mild weight-bearing lameness and stifle effusion in the treated stifle following urate administration. Synovial inflammation was graded on arthroscopy at mean +/-SD of 79 +/-7 where a 0 score was equivalent to worst possible, and 100 score was normal. Total nucleated cell counts increased significantly from 2.5
+/− 0.7 x10⁹/L at baseline to 77.0 +/− 17.6 x10⁹/L (p<0.01) twenty-four hours following urate administration, with a shift to a neutrophilic cell population.

**Validation of intra-articular sampling catheter**

There was good agreement between gentamicin concentrations in synovial fluid whether collected by sampling catheter or arthrocentesis. Over the range assessed (0.52-544ug/ml) the mean difference was 1.0 ug/ml +/− 2.3ug/ml, with 95% limits of agreement= -3.6 – 5.5ug/ml. Synovial fluid production was in general profuse, and no problems with blood contamination were experienced during sample collection.

**Missing data**

Results were not available for 8/153 synovial fluid samples, and 2/153 plasma samples. Lack of availability was typically due to human error, transient catheter sampling difficulties, or insufficient sample volume. Plasma data from one animal at one time point was excluded due to suspected incorrect labeling.

**Pharmacokinetics of gentamicin concentration in synovial fluid.**

Mean +/− SD of the gentamicin concentration in synovial fluid for n=9 dogs over the time 0-336 hours is shown in Figure 4. The gentamicin concentration remained above the lower LOQ for the assay for 8 out of 9 dogs at 336 hours. The highest concentrations of gentamicin in synovial fluid were reached at 1.2 hrs (95% CI=0.5-1.8hrs) after sponge
implantation, with an average $C_{\text{max \ synovial fluid}}$ of 2397ug/ml (95% CI=1161-3634 ug/ml) and a range of 405ug/ml to 4615ug/ml. There was substantial variability between the different dogs in the gentamicin concentrations reached at all time-points, with a mean coefficient of variation of 79.7% (range 29-107%). The fall in intra-articular gentamicin concentration over time was characterized by a rapid initial decline, with a prolonged lag phase during which gentamicin concentrations were sub-MIC but had not yet declined to zero. A non-compartmental model was used to estimate the time from sponge implantation to gentamicin concentration in synovial fluid falling below the MIC recommended by the CLSI for susceptible staphylococci (4ug/ml). This occurred at an average of 22.4hrs (95% CI=18.6-26.2) following sponge implantation. Gentamicin concentrations were on average 0.41ug/ml (95% CI=0.29-0.54ug/ml, n=8) at 14 days after GICS placement.

Figure 4 Synovial fluid gentamicin concentration vs time curve following intra-articular implantation of GICS at a dose of 6mg/kg in 9 dogs with experimentally induced synovitis. Data are expressed as mean +/- SD.
Figure 5 Plasma gentamicin concentration vs time curve following intra-articular implantation of GICS at a dose of 6mg/kg in 9 dogs with experimentally induced synovitis. Data are expressed as mean +/- SD.
Pharmacokinetics of gentamicin concentration in plasma.

Mean +/- SD of the gentamicin concentration in plasma for n=9 dogs over the time 0-336 hours is shown in Figure 5. The highest concentrations of gentamicin in plasma were reached at 1.5hrs (95% CI=0.8-2.1) following GICS implantation, with average $C_{\text{max, plasma}}=8.0\,\text{ug/ml}$ (95% CI=6.1-10.0 ug/ml). There was again substantial inter-dog variability, with the coefficient of variation at each time-point ranging from 19-99%. A non-compartmental model for extravascular administration was used to estimate the times until plasma gentamicin concentration fell below 4ug/ml (Staphylococcal sp. MIC) and 2ug/ml (target trough). These times were estimated at 3.3hrs (95% CI=2.1-4.4hrs) and 5.6hrs (95% CI=4.7-6.5hrs). Plasma levels were undetectable (<0.27ug/ml) in all dogs by 24 hours after GICS placement.
Table 4. Pharmacokinetic parameters for gentamicin concentrations in plasma and synovial fluid following intra-articular implantation of a GICS at 6mg/kg in 9 dogs with experimentally induced synovitis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma Mean (95% CI)</th>
<th>Synovial fluid Mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum concentration $C_{\text{max}}$ (ug/ml)</td>
<td>8.0 (6.1-10.0)</td>
<td>2397.2 (1160.9-3633.6)</td>
</tr>
<tr>
<td>Coefficient of variation for $C_{\text{max}}$ (%)</td>
<td>32.1</td>
<td>67.1</td>
</tr>
<tr>
<td>Time to maximum concentration (hrs) $T_{\text{max}}$</td>
<td>1.5 (0.8-2.1)</td>
<td>1.2 (0.5-1.8)</td>
</tr>
<tr>
<td>Time above 4ug/ml (hrs)</td>
<td>3.3 (2.1-4.4)</td>
<td>22.4 (18.6-26.2)</td>
</tr>
<tr>
<td>Time above 2ug/ml (hrs)</td>
<td>5.6 (4.7-6.5)</td>
<td>30.8 (24.8-36.8)</td>
</tr>
<tr>
<td>Terminal phase half life $T_{1/2}$ (hrs)</td>
<td>2.9 (1.6-4.1)</td>
<td>200.2 (117.2-283.2)</td>
</tr>
<tr>
<td>Rate constant for terminal slope $\lambda_z$ (1/hr)</td>
<td>0.30 (0.20-0.40)</td>
<td>0.0056 (0.0006-0.0106)</td>
</tr>
<tr>
<td>AUC (hr.ug/ml)</td>
<td>35.1 (28.4-41.8)</td>
<td>6064.8 (2717.8-9411.9)</td>
</tr>
<tr>
<td>% AUC extrapolated</td>
<td>5.5 (3.0-7.9)</td>
<td>3.2 (0.3-6.1)</td>
</tr>
</tbody>
</table>

Discussion

In this study we performed a pharmacokinetic investigation under experimental conditions of a commercially available gentamicin impregnated collagen sponge (Collatamp G, Theramed Corp) placed in an inflamed joint. Key results included identification of very high but rapidly declining intra-articular drug concentrations, a relatively prolonged period of sub-MIC concentrations, systemic exposure to gentamicin, and high inter-individual variability in release profiles.

Previous studies evaluating the pharmacokinetics of gentamicin released from this product in surgical wounds report that local gentamicin concentrations can be anticipated to remain above MIC for 7 to 10 days.\textsuperscript{20,36} We found the elution of gentamicin from the
sponge to be more rapid in an intra-articular location, with decline below MIC by 22.4hrs (95% CI=18.6-26.2) after placement. In line with previous reports, we found that the maximum intra-articular concentrations reached are very high. The recommended minimum \( C_{max} \) for a concentration dependent drug is 12 x MIC.\(^{37}\) The local \( C_{max} \) following intra-articular GICS implantation averaged approximately 50x this concentration at 2397ug/ml. However, there was large inter-individual variation in the maximum local drug concentrations achieved, with the \( C_{max} \) ranging from 405ug/ml to 4615ug/ml. This may suggest lack of consistency in dosing, wide variation in the volume of the dosing compartment, or lack of uniformity in release pharmacokinetics. The corresponding wide variation in the range of maximum plasma levels achieved (4.5 to 13.1 ug/ml) indicates an inconsistent and therefore potentially unsafe profile for dogs with pre-existing renal insufficiency, although it should be noted that in no dog did plasma levels reach those typically recorded after parenteral gentamicin administration at the same dose (54.4 +/- 18.1ug/ml).\(^{38}\)

Contrary to previous reports, systemic uptake was substantial. The maximum plasma concentration occurred approximately 18 minutes after peak intra-articular concentrations were reached, and average peak plasma concentrations following intra-articular GICS administration reached 30-50% of peak plasma concentrations typically targeted for human patients following IV gentamicin administration (16-24ug/ml).\(^{29}\) Systemic uptake of gentamicin may have been increased in this study compared with previous reports due to both the location of sponge placement (intra-articular vs subcutaneous) and the presence of established inflammation at the time of placement. In
view of the systemic uptake identified, we would recommend some caution in using the
GICS in any dog with actual or potential renal insufficiency, particularly in the presence
of circulating volume contraction or sepsis. Concurrent systemic aminoglycoside therapy
should be avoided or the dose appropriately adjusted over the initial 24 hours period
following GICS placement.

The rapid initial decline in intra-articular gentamicin concentrations likely
reflected re-distribution of the gentamicin released from the sponge from the intra-
articular space into the vascular space. The rate of decrease of gentamicin following
GICS implantation into a normal joint has been previously reported for horses. In the
fast phase of decay, the elimination rates were significantly more rapid (22.4 +/- 5.0 vs
33.3 +/- 13.3hrs to sub 4ug/ml, p=0.04) in the inflamed joints reported here than in the
normal joints reported previously. Cross-species comparisons must be treated with
cautions, however this difference may suggest that the redistribution of gentamicin from
the intra-articular to the vascular space is accelerated in the presence of inflammation.
This phenomenon has been previously documented following intra-articular
administration of parenteral products in horses, and has several implications. Firstly,
given that the level of synovial inflammation generated by the urate model was relatively
mild compared with that seen by the authors in clinical cases with septic arthropathies,
the estimates of intra-articular persistence of gentamicin and of the maximum plasma
concentration reached may be optimistic. A higher magnitude of inflammatory response
may result in faster dissipation of antibiotic from the joint and higher maximum plasma
concentrations. Secondly, it emphasizes the need for consideration of an appropriate
model when assessing pharmacokinetic parameters in a manner transferable to the clinical setting. It is certainly possible that GICS implanted into body cavities in the presence of pre-existing peritonitis or pleuritis show very different pharmacokinetic characteristics to those implanted into normal cavities in animal models, and the same may apply to surgical wounds.

Gentamicin is a concentration dependent antibiotic. Thus bacterial kill is more closely related to the peak concentrations reached than the time for which they are maintained. The rapid decay to sub-MIC levels identified here may not negatively impact on the efficacy of the sponge for preventing local infections in a clinical setting, and in fact the rapid decay in drug concentration in plasma may be advantageous in minimizing patient toxicity. When the sponge is used prophylactically and placed immediately prior to skin closure, maximum local concentrations are likely to coincide chronologically with the maximal load of bacterial contaminants. Efficacy is supported by the almost universally positive results reported in clinical trials investigating prophylactic use for reducing the incidence of surgical site infections.\textsuperscript{7,8,41} In addition, in view of the increasing incidence of multi-drug resistant methicillin resistant Staphylococcus pseudintermedius infections in veterinary medicine, it should be noted that gentamicin is one of the few remaining parenteral and non-glycopeptide antibiotics with a reasonable in-vitro activity against MRSP if delivered at sufficiently high concentrations.\textsuperscript{27} In a recent study investigating MRSP resistance patterns in dogs across Europe and North America, all 103 MRSP isolates assessed showed susceptibility if exposed to concentrations higher than 256ug/ml, which was well below the estimated mean
maximum concentration documented in this study.\textsuperscript{27} However, the rapid decay profile does suggest that the sponge may offer little advantage over an intra-articular injection, as previously suggested.\textsuperscript{39} Finally, the relatively prolonged period of sub-MIC intra-articular concentrations may pose a risk factor for acquired resistance in the bacteria not killed by the initial pulse.\textsuperscript{42}

Studies investigating the collagen component of the GICS implant reported necrosis and an inflammatory response associated with the collagen implant, together with the persistence of observable collagen in surgical wounds from 15 to 28 days post-operatively.\textsuperscript{36, 43} When used in the context of established infection, there may be the greater potential for the collagen component to become colonized and act as an additional wound burden once bacteria remaining viable after the initial gentamicin pulse re-establish.

The methodology selected in this study deserves some discussion. Urate was selected over endotoxin to induce inflammation primarily for welfare reasons. Urate has been previously reported in multiple studies to offer a consistent local inflammatory response, while endotoxin carries the risk of inducing systemic illness.\textsuperscript{31, 44} An intra-articular catheter was placed to facilitate joint sampling for both welfare and convenience purposes. The sampling frequency required over the 24 hours following GICS placement made serial sedation for arthrocentesis impractical. Finally the GICS sponge dose was selected as equivalent to the IV dose typically used clinically in both dogs and humans.

Limitations of this study included lack of a truly parallel clinical model. These dogs did not have established septic arthropathies at the time of sponge implantation, and
there is the possibility that sponge pharmacokinetic parameters may be different in this context. In addition, direct comparison with a group receiving an intra-articular injection of a parenteral gentamicin formulation at an identical dose would have been informative, as we suspect PK parameters would be similar and injectable gentamicin is considerably cheaper. Finally, we made no attempt to assess the clinical efficacy of the gentamicin sponge over either parenteral therapy alone or intra-articular injection – clinical trials are needed, particularly to assess the efficacy of the sponge in managing established infection.

In conclusion, intra-articular gentamicin reaches extremely high concentrations 1.2 hrs following GICS placement at a dose of 6mg/kg, with concentrations theoretically sufficient to kill even multidrug resistant organisms. Intra-articular concentrations decay rapidly in the presence of inflammation, reaching sub-MIC levels for staphylococci by 22.4 +/- 5.0 hours after placement, and remain sub-MIC but non-zero for at least 14 days. It is unknown whether the GICS offers any pharmacokinetic advantage over intra-articular injection for treatment of joint infections. There is substantial systemic uptake of the drug, and concurrent systemic administration should be adjusted accordingly. GICS use should be considered only with caution in patients with documented or potential renal insufficiency, particularly in the presence of concurrent endotoxemia. Parenteral doses of aminoglycosides scheduled within the same 24 hour period as GICS implantation should be adjusted or omitted.
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CHAPTER FOUR

INTRA-ARTICULAR IMPLANTATION OF A GENTAMICIN IMPREGNATED COLLAGEN SPONGE: ARTICULAR AND RENAL MORBIDITY IN A CANINE MODEL.

Abstract

Background: Nosocomial septic arthropathy can occur following intra-articular surgery. The infection is frequently multi-drug resistant and may be refractory to systemic therapy. Increased use of gentamicin impregnated collagen sponge (GICS) for management and prevention of surgical site infections (SSI) is reported, and GICS may have a role in facilitating rapid control of intra-articular infections. The purpose of this study was to investigate the safety of intra-articular use of GICS using a canine model.

Methods: Randomized controlled experimental trial, n=18. Stifle joint inflammation was generated by urate injection. Twenty-four hours later dogs were randomly assigned to arthroscopic implantation of GICS at gentamicin dose=6mg/kg (n=9) or sham operation (n=9). Primary endpoints were joint inflammation measured by synovial fluid cell counts and cytokine concentrations, lameness measured by force plate asymmetry indices, and renal function measured by glomerular filtration rate (GFR) study using technetium 99 plasma clearance. The prevalence of lesions associated with aminoglycoside nephrotoxicity was assessed by renal biopsy and electronmicroscopy.

Results: GICS implantation caused joint inflammation (p<0.01), lameness (p=0.04), and decreased GFR (p=0.04). No dog developed clinical renal failure. No difference
was observed in the prevalence of renal lesions on biopsy between treatment and control group (p=0.49).

**Conclusions:** GICS implantation in the inflamed joint causes additional inflammation and joint dysfunction that is likely to be of clinical relevance. GICS implantation affects renal function at the dose assessed. Renal effects may be exacerbated in septic patients, and care should be taken with GICS dosing.

**Clinical relevance:** The risk/benefit ratio of GICS therapy and the GICS dose implanted should be considered on a patient by patient basis.

**Introduction**

The gentamicin impregnated collagen sponge (GICS) was developed to prevent and treat surgical site infections by providing high gentamicin concentrations locally, avoiding the high systemic concentrations associated with nephrotoxicity.\(^1\) The GICS is currently approved for use in over 53 countries, and in the last 20 years more than 2 million sponges manufactured by Innocoll Technologies (Gallowstown, Ireland) have been used to treat clinical patients. The sponge has found numerous applications, including the management of septic arthropathies and infected arthroplasties.\(^2,3\) The sponge has also been used successfully as a prophylactic measure to reduce the incidence of surgical site infections following clean and clean-contaminated surgical procedures.\(^1,4,5\) The reported advantages of the GICS include the delivery of very high local antibiotic concentrations combined with rapid biodegradeability (Innocoll product information). An increasing role for infection prophylaxis in orthopedic surgery using GICS has been suggested.\(^6\) However, studies investigating the biodegradation of GICS following
subcutaneous and intra-muscular implantation identified a marked inflammatory response persisting to at least 5 days following implantation. A case series evaluating patients with an infected hip total arthroplasty managed with GICS reported an association with toxic serum gentamicin levels and reduced renal clearance.

The purpose of this study was to investigate whether intra-articular implantation of the GICS during arthroscopy in an inflamed joint causes (1) an additional and clinically relevant intra-articular inflammatory response compared with the arthroscopic procedure alone and (2) compromised renal function and/or resulted in renal lesions when implanted at standard systemic doses. An inflamed joint model was selected to reflect the likely context of use of the GICS in the clinical setting. The study was conducted in dogs in a randomized controlled laboratory environment. Inflammatory response and functional effect was assessed through sequential monitoring of synovial fluid cell counts, cytokine levels, and through force plate gait analysis. Renal function and morphology was evaluated with technetium 99 plasma clearance and renal biopsy with transmission electronmicroscopy.

Materials and Methods:

Ethical approval

This study was carried out in accordance with U.S. National Institutes of Health “Guide for the Care and Use of Laboratory Animals” and the Animal Welfare Acts (US
The study design was approved by the University of Guelph Animal Care Committee.

**Study design**

This was a randomized controlled experimental study.

**Animals**

Eighteen research-specific purpose bred dogs (Marshall BioResources, North Rose, NY) of the hound type were studied (10 male, 8 spayed female), with a mean weight of 22.5kg (range 18.9-27.6kg) and age of 2.0 yrs (range 1.1-4.2 yrs). Dogs were conditioned to routine handling prior to study use. Baseline physical and orthopedic examinations, complete blood counts, urinalysis, and biochemistry profiles identified no abnormalities. Dogs were randomized to the treatment (n=9) and control (n=9) groups using computer randomization.

**Acute synovitis model**

Dogs were sedated using 0.05mg/kg hydromorphone and 2ug/kg dexmedetomidine IV (Dexdomitor; Pfizer Animal Health, Madison, NJ) to facilitate intra-articular injection and placement of a jugular catheter (16G, 20cm; MILA International, Erlanger, KY). For each dog, a stifle joint was randomly selected using computer randomisation and injected with 10mg sodium-urate (Sigma-Aldrich Canada Ltd, Oakville, ON) as sterile urate suspension according to previously reported techniques for predictable generation of an
acute chemical synovitis. Aspiration of synovial fluid was performed prior to urate injection to confirm intra-articular location and absence of pre-existing pathology. Baseline evaluation of cell counts, cytokine concentrations and cytology was performed on aspirated fluid. Standard aseptic technique was used for all interventional procedures. Tramadol 2mg/kg PO Q 8 hrs was administered to maintain post-procedural comfort, and continued for the following 3 days.

**Stifle arthroscopy**

Twenty-four hours later, acute synovitis was confirmed in all dogs by the presence of lameness, palpable joint effusion, and documentation of synovitis on arthroscopic examination. Dogs were pre-medicated with acepromazine 0.03mg/kg IV and hydromorphone 0.05mg/kg IV. General anesthesia was induced using propofol 10mg/ml to effect (mean administered dose 3.2mg/kg), and maintained with isoflurane carried by 100% oxygen following endotracheal intubation. Arthroscopy of the previously injected stifle was performed by a board certified surgeon (NM) using a standardized technique as follows.

A 22g needle was introduced into the stifle joint and synovial fluid aspirated. Synovial fluid was submitted for post-urate total nucleated cell counts and cytology. Following aspiration 6mls sterile saline were instilled to distend the joint. Using a blunt-tip trochar a distolateral arthroscopy portal was established and advanced in a proximomedial direction. An egress port was established in the proximal medial parapatellar region. A 2.4mm arthroscope (Karl Storz, Canada Ltd, Mississauga, ON)
was introduced and the joint explored. The cartilage was assessed for pre-existing pathology and a modified Outerbridge score assigned. Synovial inflammation was assessed and a visual analogue score assigned. A modified multi-fenestrated intra-articular sampling catheter (catheter volume 0.5mls, MILA International, Erlanger, KY) was placed in the lateral parapatellar pouch using the Seldinger over-wire technique and position confirmed with arthroscopic visualization. The scope was then removed leaving the scope portal in situ, positioned at the intercondyloid fossa.

Placement of gentamicin impregnated collagen sponge

The sponge consisted of sterile renatured Type I bovine collagen impregnated with gentamicin. The commercial sponge product contained 2.8mg of collagen and 2.0mg of gentamicin sulphate per cm², and was available in units of 10 x 10x0.5cm (Collatamp G, Theramed Corp, Mississauga, ON). For the treatment group, the sponge was dosed to bodyweight at 6mg/kg of gentamicin, and the appropriate dosage for each dog was administered by cutting the sponge to the correct area according to the manufacturer’s instructions. The sponge dose was then divided into small segments, rolled, and inserted through the scope portal. The sponge remained dry until intra-articular placement. Following sponge delivery, the scope was re-inserted in the scope portal and intra-articular placement of the sponge confirmed. The control group received stifle arthroscopy only.
Assessment of joint inflammation

Synovial fluid samples were cytologically examined for all dogs at baseline, 2 days, 7 days, 15 days and 35 days following sponge placement. Samples were analysed within 12 hours of collection. Cytology and cell counts were assessed by a board certified veterinary pathologist (DW). Cytokines interleukin 6 (IL 6), interleukin 8 (IL 8), monocyte chemotactic protein (MCP) and keratinocyte chemoattractant (KC-like) were measured using a validated immunoassay (Millipore Bioplex Canine Cytokine Magnetic Bead Panel) with the recommended manufacturer’s protocol.

Force plate gait analysis

Force platform gait analysis was performed by completion of 5 valid trials at the trot (velocity 2.0-2.5 m/sec, acceleration +/- 0.5m/sec sec). Trials were videocaptured to facilitate contemporaneous limb identification. Trials were considered valid when all four feet were captured on adjacent forceplates on the same trial with no gait abnormalities. All dogs were handled during trials by a single investigator (TG), and trial data was assessed in a blinded manner. Trial data was normalized to bodyweight and analysed using established asymmetry indices. Force plate assessment was performed at baseline, 7 days and 14 days following arthroscopy.

GFR materials and methods

Glomerular filtration rate was assessed 6 days following sponge placement via plasma clearance of technetium99 diethylene triamine pentaacetic acid ($^{99m}$Tc-DTPA) using established techniques.
Renal biopsy materials and methods

Renal biopsies were obtained 7 days following sponge placement. Biopsies of the caudal pole of the left kidney were obtained with a 16 G E-Z Core biopsy needle using ultrasound guidance (GE Logic 5 with a microconvex curvilinear variable frequency probe set at 8 MHz). Renal biopsies were prepared for transmission electronmicroscopy using standard techniques. Images were recorded at 100kV on the LEO 912AB (Zeiss, Germany) using the Olympus/SIS (Germany/Japan) Cantega CCD camera with the Olympus/SIS iTEM software. Samples were assessed and semi-quantitatively scored for the presence of ultrastructure lesions consistent with aminoglycoside toxicity specifically vacuolation and cytosome/ mitochondrial swelling, by a boarded veterinary pathologist (RF) blinded to treatment group allocation.

Animal care and husbandry

Dogs were housed in individual runs for the duration of the study. They were fed commercial dry dog food (Adult maintenance; Purina) and provided with water ad lib. Exercise was restricted for the first 48 hours of the study, and thereafter they were walked outside on leash several times daily. Bandages and Elizabethan collars were used to prevent removal of jugular and intra-articular catheters. All interventional procedures (catheter placement and removal, sponge placement, arthrocentesis) were performed under sedation or general anesthesia. All dogs were assessed for discomfort by a veterinarian at least every 6 hours for the first 48 hours of the study, and thereafter at
least every 12 hours, and adjunctive analgesia provided as needed (hydromorphone 0.05mg/kg IV or IM).

**Statistical data analysis**

Data was assessed for normality using the Shapiro Wilk test, and means +/-SD or 95%CI reported where a normal distribution applied, and median (IQR) where not normal. Samples with a normal distribution and constant variance were compared using a t-test or paired t-test for paired samples. Samples with non-parametric data were compared using the Wilcoxon signed rank test. Statistical analyses were performed using Stata (StataCorp, College Station, TX, USA).

**Results:**

**Intra-articular inflammatory response**

Synovial fluid analysis at baseline revealed no abnormalities in any dog and no difference between the treatment and control groups. Baseline evaluation of cartilage Outerbridge scores and synovitis at arthroscopy identified no difference between the treatment and control groups. Following GICS implantation, synovial fluid cell counts remained elevated compared with baseline values until at least 35 days following sponge implantation (p=0.03) in the treatment group (Figure 1). In the control group, synovial fluid cell counts were no different from baseline by 14 days following urate injection/arthroscopy (p=0.78). Synovial fluid cytology was characterized by a neutrophilia in both
groups. Cytokines IL6, IL8, MCP and KC-like were elevated in the treatment group compared with the control group over all time points assessed (p<0.05). Mean values and CIs are shown in Table 1.

Figure 1 Changes in synovial fluid total nucleated cell counts by treatment group

Table 1. Cytokine concentrations by treatment group

<table>
<thead>
<tr>
<th>Cytokine concentration (pg/ml)</th>
<th>Treatment grp synovial fluid</th>
<th>Control grp synovial fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>95% CI (robust)</td>
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<tr>
<td>IL6</td>
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<td>IL8</td>
<td>198.1</td>
<td>92.9-303.4</td>
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<td>MCP</td>
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<td>68.0-156.1</td>
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<td>KC-like</td>
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</tbody>
</table>
Forceplate evaluation of gait found no difference between the treatment and control groups at baseline. At 7 days following surgery, the asymmetry indices for peak vertical force, z impulse and z maximum rise were found to be increased in the treatment group compared with the control group (p=0.04) as shown in Figure 2. No difference between the treatment and control groups was identifiable by 14 days following surgery (p=0.42).

Figure 2 Asymmetry indices by treatment group

Renal impact

Glomerular filtration rate was lower in the treatment group compared with the control group at 6 days post sponge placement (p=0.04) with a mean GFR of 2.9 vs 3.6 ml/min/kg. No dog developed clinical renal failure.

Electronmicroscopy of renal biopsies identified mild lesions in both groups. No difference was identified in lesion severity or frequency between the treatment and control groups.
Discussion:

This study determined that GICS implantation during arthroscopy of an inflamed joint causes an additional inflammatory response and joint pain manifest as lameness when compared with arthroscopy alone, and that this inflammatory response persists until at least 35 days post-operatively. Implantation of GICS at the dose studied (6mg/kg; standard canine IV dose) causes a decrease in GFR, however in the healthy dogs evaluated this decrease was not associated with clinical renal failure or observable lesions on renal biopsy.

The pro-inflammatory effect of GICS has been documented in other studies. Local tissue necrosis has been observed following sponge implantation, postulated to be due to fleece acidity.\(^{(7,8)}\) The duration of persistence of the collagen component of the sponge has not been well investigated, and likely shows significant variation with implantation environment. One study documented persistence of collagen at 15 days following intra-muscular implantation, and did not re-evaluate further.\(^{(8)}\) Another study noted that antibiotic impregnated collagen implants used for prevention of SSI in fact increased SSI incidence, but that this effect was not evident until at least three weeks post-operatively, and postulated that this was due to the persistence of the collagen sponge in the surgical wound.\(^{(15)}\) In the current study, a local pro-inflammatory effect as evidenced by a persistent neutrophilic synovitis could be identified until at least 35 days post-operatively. Persistence of the sponge in the joint or surgical wound to a time point well beyond the anticipated decline of any incorporated antibiotic is likely to be of clinical concern both
due to the inflammatory effect on the local wound environment but also due to the potential for bacterial colonization of the sponge with subsequent nidus formation and ongoing clinical infection.

Intra-dermal immunization of mice with a mixture of type II collagen and Freund’s adjuvant is the most commonly studied autoimmune animal model of rheumatoid arthritis. Intra-articular implantation of type I collagen and the subsequent inflammatory response has not been well investigated, however the induction of systemic immune mediated arthritis would appear to be unlikely in the absence of adjuvant.

The methods employed in this study merit some discussion. The inflammatory effect of the sponge was evaluated in the context of a pre-existing urate synovitis to mimic the most likely clinical context of sponge use, that being the inflammatory environment of a septic arthritis. It was postulated that to assess the inflammatory effect of the sponge in the context of a normal joint might risk confusing statistical significance with clinical significance.

Limitations of this study include lack of long term follow-up beyond 35 days post sponge implantation, and small group sizes. The study was underpowered to detect a difference in ultrastructural renal lesions with approximately 20% power to detect a 50% difference at the prevalence observed. Thus caution must be applied to interpretation of this result. Finally, although the local inflammatory effect of GICS appears conclusive, we were unable to determine whether this was due to the collagen or gentamicin component of the sponge.
In conclusion, GICS implantation into the joint causes persistent inflammation manifest as a neutrophilic synovitis with elevated cytokine production together with transient lameness. GICS implantation also caused a reduction in GFR, although there was no clinical evidence of renal failure in these healthy experimental animals at the dose assessed. It is our recommendation that the potential risk/benefit ration of GICS should be carefully considered on a patient by patient basis prior to use, and that sponge use be avoided in patients either in or at risk for renal injury.

Bibliography:


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CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

Fulfillment of objectives

We fulfilled the primary objective of this research, which was to determine the pharmacokinetic profile of gentamicin release from a gentamicin impregnated collagen sponge placed in an intra-articular location. In addition we performed an evaluation of the effect of sponge placement on joint inflammation, limb use, and renal function. We determined that intra-articular gentamicin reaches extremely high concentrations 1.2 hrs following GICS placement at a dose of 6mg/kg, with concentrations theoretically sufficient to kill even multidrug resistant organisms. Intra-articular concentrations decay rapidly in the presence of inflammation, reaching sub-MIC levels for staphylococci by 22.4 +/- 5.0 hours after placement, and remain sub-MIC but non-zero for at least 14 days. It is unknown whether the GICS offers any pharmacokinetic advantage over intra-articular injection for treatment of joint infections. There is substantial systemic uptake of the drug, and concurrent systemic administration should be adjusted accordingly. GICS implantation into the joint causes persistent inflammation manifest as a neutrophilic synovitis with elevated cytokine production together with transient lameness. Joint inflammation persisted to at least 35 days post-operatively. GICS implantation also caused a reduction in GFR, although there was no clinical evidence of renal failure in these healthy experimental animals at the dose assessed. In our opinion, GICS use should be considered only with caution in patients with documented or potential renal insufficiency, particularly in the presence of concurrent endotoxemia. Parenteral doses of aminoglycosides scheduled within the same 24 hour period as GICS implantation should
be adjusted or omitted. It is our recommendation that the potential risk/ benefit ration of GICS should be carefully considered on a patient by patient basis prior to use, and that sponge use be avoided in patients either in or at risk for renal injury.

Study limitations

Limitations of this study included lack of a truly parallel clinical model. These dogs did not have established septic arthropathies at the time of sponge implantation, and there is the possibility that sponge pharmacokinetic parameters may be different in this context. In addition, direct comparison with a group receiving an intra-articular injection of a parenteral gentamicin formulation at an identical dose would have been informative, as we suspect PK parameters would be similar. An additional limitation was lack of long term follow-up beyond 35 days post sponge implantation, and small group sizes. The study was underpowered to detect a difference in ultrastructural renal lesions with approximately 20% power to detect a 50% difference at the prevalence observed. Thus caution must be applied to interpretation of this result. Finally, although the local inflammatory effect of GICS appears conclusive, we were unable to determine whether this was due to the collagen or gentamicin component of the sponge.

Future directions

This study focused on observation of pharmacokinetic parameters and morbidity assessment. We made no attempt to assess the therapeutic efficacy of GICS for achieving infection control in the context of a septic arthropathy. Given the rapid peak and decay profile of gentamicin release, and the persistent inflammatory response which we suspect
to be associated with the collagen carrier, it is possible that the GICS is inferior in risk/benefit ratio compared with a simple intra-articular infusion of parenteral gentamicin.

Conclusion

Over the course of this study we determined that intra-articular gentamicin concentrations reach high peak levels with a rapid decay profile following GICS implantation, and that GICS implantation at 6mg/kg causes a local inflammatory response that persists for several weeks, together with a reduction in GFR. This information has substantial clinical relevance- GICS may not be the most appropriate method of achieving infection control in the context of intra-articular SSIs, and should be used only with caution in patients at risk for renal injury or with pre-existing renal insufficiency.
APPENDIXES

APPENDIX I
Electronmicroscopy of renal ultrastructure

APPENDIX II
Methodology for gentamicin assay

The platform used for this assay is the Abbott TDx Analyzer. This system uses fluorescence polarization immunoassay (FPIA) technology. The TDx software calculates a best-fit curve equation that is used to generate a calibration curve. This curve is stored in the memory and concentrations of drug in unknown samples are calculated from this curve using polarization values generated for each sample is the assay. One level of quality control is performed once during each 8-hour shift using the Gentamicin Reagent Pack. The assay was also successfully validated for this research study. The TDx Gentamicin Assay has a limit of detection of <0.27 μg/mL and the maximum readout of 10.0 μg/mL. Any results below or above these limits are reported as out of range and dilutions were performed with high concentration samples in order to obtain a value within the range of the assay. The assay uses a minimum of 50 μl plasma or serum. In order to properly validate the assay for this study, control (blank) canine plasma and synovial fluid was fortified (spiked) with nominal concentrations of gantamicin. A pure analytical reference standard of gentamicin sulfate was obtained from the United States Pharmacopeia (www.USP.org) and used for the validation. Three levels of validation concentrations were used (low, medium, high) as well as a blank. Three replicates were analyzed for each concentration. Acceptance criterion was a value within 15% of the true
concentration. The validation study met the acceptance criterion at all three concentrations and for each matrix (plasma and synovial fluid). The incurred study samples used approximately 100 µl per test unit with no pretreatment or dilutions. However, due to the viscous state of the synovial fluid, samples were treated with hyaluronidase prior to assaying (100 µl synovial sample + 10 µL Hyaluronidase). Samples that read high and out of the range of the assay were diluted with phosphate buffered saline (PBS) at either a 500x or 100x dilution factor depending on time interval.

APPENDIX III
Glomerular filtration rate and renal biopsy materials and methods
Glomerular filtration rate was assessed via plasma clearance of technetium99 diethylene triamine pentaacetic acid ($^{99m}$Tc-DTPA) using established techniques. An average dose of 66.4 uCi (range 50.7 – 71.6 uCi) or 2.95 uCi/kg (range 2.23 – 3.64 uCi/kg) $^{99m}$Tc-DTPA was administered via a peripheral catheter. The activity in the dose syringe was measured before and after injection and subtracted to determine the delivered dose to the patient. At the time of injection a standard was made by placing 50 uCi of $^{99m}$Tc-DTPA in 1 L of sterile water. The activity in the syringe was measured pre and post injection into the standard to determine the exact dose delivered. The standard was used to convert the dose in uCi to activity in counts. Blood samples were obtained from a jugular catheter with the following procedure: 2 ml blood withdrawn and held, 4 ml drawn and placed in an EDTA tube, original 2 ml blood returned to patient, 2 ml heparinized saline flush. Samples were obtained at 15 min post injection and every 30 min post injection until 240 min. The exact time of sampling was recorded. The blood sample was
separated by centrifuging the samples for 5 minutes and 1 ml of plasma was placed in a sterile tube for counting. All samples were counted for a total of 60 seconds at the same time at the end of the day with a NaI well counter. A 1 ml sample of the standard and background counts were measured at the start, middle, and end of sample counting. These values were used to determine the average activity in the standard and background during the counting period (which took approximately 15 minutes). All values were entered into an excel spreadsheet to calculate GFR (ml/min/kg) using a single compartment model linear regression.

**Renal biopsy materials and methods**

In all but one dog 2-3 biopsies of the caudal pole of the left kidney were obtained with a 16 G E-Z Core biopsy needle using ultrasound guidance (GE Logic 5 with a microconvex curvilinear variable frequency probe set at 8 MHz). In one dog the left kidney was not identified so the caudal pole of the right kidney was biopsied.

**APPENDIX IV**

Sedation/ anesthesia and analgesia protocols

**Sedation for jugular catheter placement / arthrocentesis/ cystocentesis/ urate injection**

- inject 5ug/kg medetomidine and 0.05mg/kg hydromorphone IV, additional propofol sedation PRN as needed in 1 ml increments

  Post urate injection- tramadol 2mg/kg Q8hrs PO and hydromorphone 0.025-0.05mg/kg IV PRN
Anesthesia for arthroscopy

- pre-medication: acepromazine 0.03mg/kg IV and hydromorphone 0.05mg/kg IV
- induction: propofol to effect and endotracheal intubation
- maintenance: iso-flurane and fentanyl CRI at 5ug/kg/hr
- recovery: repeat of pre-medication drugs PRN
- post-op: tramadol 2mg/kg Q 8Hrs with hydromorphone 0.025-0.05mg/kg PRN
- intra-operative fluids PLA: 5ml/kg/hr, if MAP<70mmHg or systolic <100mmHg 10ml/kg fluid bolus

Sedation for manipulation/ removal of intra-articular sampling catheter if needed

- medetomidine 2ug/kg IV with butorphanol 0.2mg/kg IV

General anesthesia for tru-cut u/s guided renal biopsy

- pre-medication acepromazine 0.03mg/kg IV and hydromorphone 0.05mg/kg IV
- induction: propofol to effect and endotracheal intubation
- maintenance- isoflurane
- recovery- acepromazine 0.02mg/kg IV PRN
- intra-operative fluids PLA: 5ml/kg/hr, if MAP<70mmHg or systolic <100mmHg 10ml/kg fluid bolus
- if clinically significant renal haemorrhage (ass assessed by hemodynamic status variables and u/s evidence) then whole blood/ surgical intervention +/- dopamine