Chapter 5

Surgical irrigation with pooled human IgG to reduce post-operative spinal implant infection

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Abstract

A multiple site, non-lethal rabbit surgical model of spinal implant infection was used to assess the efficacy of a spinal wound lavage to reduce post-operative infection from methicillin resistant Staphylococcus aureus (MRSA). Multiple aqueous lavages of isotonic saline were compared to the same procedure using 1wt% pooled human immunoglobulin G (IgG) applied directly to the surgical implant sites. Visually observed clinically relevant signs of infection (e.g, swelling, erythema, pus) were supported by bacterial enumeration from multiple biopsied tissue and bone sites post-mortem, 7- and 28-days post-challenge. Clinical signs of infection were significantly reduced in IgG-lavaged infected spinal sites. Bacterial enumeration also exhibited statistically significant reductions in soft tissues, bone and on K-wire spinal implants using IgG lavage compared with saline. Complete healing of all surgical wounds was seen after 28 days, although isolated fibrosed abscesses were observed in autopsied sites treated with both IgG- and saline-lavages. Local use of IgG wound lavage is proposed as infection prophylaxis against antibiotic resistant implant-centered or surgical wound infection.

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New innovations in spinal instrumentation have brought major advantages for improving treatment of a large number of spinal pathologies. Stabilization of the spine permits more rapid mobilization of the patient, facilitates more reliable and effective correction of deformities and maintenance and reconstruction of the spine after decompressive surgery. Hardware failure and neurologic injury caused by the introduction of stabilizing equipment are relatively rare, but risk of postoperative infection clearly increases after implantation of spinal instrumentation. Depending on the patient population, procedure-specific incidences of infection after application of posterior instrumentation vary from 0.1 to 12%, with *Staphylococcus aureus* as the most common wound site pathogen.[1-10] These infections carry a high morbidity for the patient and are extremely costly due to requirements of prolonged hospital stay, surgical re-intervention and aggressive antibiotic therapy.[1,5,11,12] Unfortunately, many wound-site pathogens have become resistant to first-line antibiotics. According to the National Nosocomial Surveillance

Systems, methicillin resistance is present in 80% of all *S. aureus* isolates (MRSA).[13] Most recently, vancomycin resistant MRSA emergence has been reported.[14,15]

One routine surgical prophylaxis attempting to reduce this potentially devastating postoperative complication involves intra-operative wound irrigation with 0.85% saline together with routine systemic antibiotic infusion.[16] Various other anti-infecting irrigation solutions (e.g., containing antibiotics) investigated recently in an attempt to decrease infection rates have met with inconsistent results.[17-21] Topical application of antibiotics carries associated risk of promoting antibiotic resistance at the site of application as this strategy often results in sublethal antibiotic doses in the site after wound closure.[22]

One possible alternative for conferring protection against infection at wound sites while avoiding antibiotic resistance involves local potentiation of host immune responses using natural immune components. Manipulation of inflammatory, immunomodulatory and healing cascades in wound sites has not yet been explored in detail. Passive local immunotherapy, involving the direct application of pooled immunoglobulins (e.g., IgG, IgA, IgM), has been recognized for decades as a strategy to locally and rapidly bolster host immune response to confer anti-infective protection.[23-27] Application of a local bolus dose of IgG to sites of potential infection enhances opsonization of pathogens, facilitates neutralization, and promotes clearance by recruited invading neutrophils and macrophages.[25-27] Local, direct immunotherapy will also be effective against antibiotic resistant bacteria, since acquisition of pathogen antibiotic resistance does not change their susceptibility to opsonization and phagocytosis.[28] This strategy, however, has not been investigated as a topical wound lavage to prevent postoperative spinal implant infection.

The aim of this study was to compare the effectiveness of routine surgical saline lavage with a lavage solution containing 1wt% pooled human IgG in reducing postoperative surgical wound infection in the absence of any systemic antibiotics. The approach utilized a recently reported spinal implant infection model in rabbits that has been shown to produce a reliable, localized spinal implant infection using MRSA.[29]

Materials and Methods

Animals. After approval of all protocols by the institutional animal care and use committee, twelve New Zealand white (NZW) female rabbits were obtained, weighing 2.5 – 3.0 kg each. Rabbits were allowed one week of routine care and feeding to acclimate prior to surgery.

Bacterial inoculum. Methicillin resistant *Staphylococcus aureus* (MRSA, ATCC 33593) was prepared in TSB (BBL® Tryptic Soy Broth USP, Becton Dickinson, Cockeysville, MD 21030, USA, Lot H8DFLS) and washed by centrifugation in isotonic saline just prior to surgery. Inoculum concentrations of $5x10^2$ - $1x10^4$ Colony Forming Units (CFU) per 100 µl were adjusted using a standard spectrophotometric assay and confirmed by direct bacterial enumeration on Tryptic Soy Agar (TSA, Difco - Becton Dickinson, Sparks MD 21152, USA, Lot 128882XA).

Immunoglobulin and saline lavage preparation. Pooled human immunoglobulin (Gamimune® N, 10%, Bayer Corporation, Pharmaceutical Division, Elkhart, IN 46515, USA, Lot 648R009B) was diluted in sterile glycine according to recommendations from the manufacturer to provide a 1% IgG solution (10mg IgG/ml). This solution (3ml) was stored in sterile syringes with 30G needles for use in the animal model. Control lavage of 0.85% saline was drawn into identical syringes with 30G needles. The use of saline or IgG as a lavage treatment was blinded and randomized, with two IgG- and one saline-lavaged surgical site per rabbit as described below.

In vitro IgG-MRSA titer determination. An anti-human IgG ELISA[23,24] was used to determine polyclonal human IgG titers against MRSA strain ATCC 33593. The ELISA was modified to detect gram positive MRSA by using 3% rabbit serum as a blocking agent. Titer numbers express the inverse log dilution of IgG concentration at 50% ELISA optical absorbance (450nm) from the inflection mid-point on each IgG-MRSA binding curve. A higher titer number reflects higher IgG binding to MRSA.

Surgical model. The non-lethal rabbit spinal implant surgical infection model has been described recently in detail.[29, this thesis] The following represents a brief description of the implantation and lavage procedure. Each rabbit was premedicated with butorphanol tartrate (0.1 mg/kg) and anaesthetized via intramuscular cocktail comprising ketamine HCl (44 mg/kg), xylazine (5 mg/kg) and acepromazine maleate (0.75 mg/kg) thirty minutes later. The positions of the desired vertebrae --thoracic 13 (*Th13*), lumbar 3 (*L3*) and lumbar 6 (*L6*) -- were marked on the back of the animal, and 0.5 - 1.0 ml of antibiotic-free Marcaine® 0.5% was injected subcutaneously at every site as local anaesthetic. After preparing the shaved animal with povidone-iodine, a 2.5 cm dorsal skin incision was made longitudinally in the midline on top of each desired vertebra, followed by an incision into the fascia to expose the spinous process. Using a small rongeur, the entire spinous process with surrounding musculature and ligaments, was excised from the base, creating a hollow self-contained defect, mimicking a partial laminectomy defect. The ligamentum flavum was not violated, nor was the dura exposed. At this time, 1ml of either IgG or saline (selected blind, randomized) was used to lavage the pocket and sponged out immediately using sterile gauze.

Subsequently, a 0.85 mm diameter stainless steel threaded Kirschner wire (ASTM F138, donated by Smith&Nephew-Richards, Memphis, TN 38116, USA) was screwed through both vertebral transverse processes, crossing the laminectomy defect, and cut adjacent to the lateral wall of the left transverse process at the appropriate length. Again, 1ml of either IgG or saline (blinded) was used to lavage the site and sponged out using sterile gauze. In implant sites, bacterial inoculum in 100 µl saline was then applied from a sterile syringe needle (30G) onto both the K-wire implant and the tissue bed inside the defect pocket and left for exactly 60 seconds. Subsequently, the last blinded 1ml lavage of either IgG or saline was performed and sponged out. The wound was closed in multiple sutured layers using Vicryl® and silk (donated by Ethicon Inc., Somerville, NJ 08876, USA). [29]

On the same rabbit, the next two spinal sites were laminectomized and inoculated in random order, following the same protocol and using new sterile equipment. The

MRSA-infected sites were proven to be reliably locally infected, isolated, non-communicative and non-contiguous in earlier control experiments, even when local inoculations exceeded 10^5 CFU MSRA. [29] All three spinal surgical sites were therefore inoculated in this model and used to compare the 1wt% IgG lavage solution with the standard saline surgical lavage solution. Variability was minimized and surgical trauma was standardized by using the same surgeon (KP) to perform all operations. After the procedure, the closed wounds were left uncovered to prevent bandage irritation of the skin. The animals were housed individually in standard cages, provided with water and standard antibiotic-free rabbit chow and monitored daily with special regard to their wounds, temperature, signs of sepsis and body weight. [29]

In another series of rabbits, the same surgical procedure was performed as described above, but no K-wire was implanted. To establish consistent infections in this case without a biomaterial implant, spinal sites were inoculated with 500, 1,000, 5,000 or 10,000 CFU MRSA and lavaged following the same blinded protocol described above.

Evaluation. According to approved animal protocols, rabbits were sacrificed by intravenous injection of pentobarbital (10mg/kg) after either 7- or 28-days post-surgery. Prior to euthanasia, blood was drawn from the ear vein and cultured to determine systemic sepsis. Still blinded for the applied lavage fluid, the surgical sites were individually visually evaluated for clinical signs of infection: swelling, erythema, pus. Different areas were rated by the same surgeon that performed the procedures (- : no signs; + : moderate signs; ++ : severe clinical signs of infection). Under sterile conditions, biopsies of the skin (suture area), the fascia and muscle (suture area), the vertebral lamina, the soft tissue that surrounded the implant in the defect (fibrous tissue), the K-wire implants and both transverse processes were removed from all sites. Additionally, a piece of the right liver lobe (approximately 5 g) was removed to assess systemic presence of MRSA infection. Harvested tissue samples were immediately homogenized (Omni-International GLH homogenizer) and implants were sonicated (NEY Ultrasonik 100) for 30 minutes in saline to detach bacteria. Serial dilutions of each sample were plated on TSA and incubated for 24 hours at 37°C to enumerate

pathogens at every site. Randomly, different samples were also plated on TSA containing 5µg/ml nafcillin (Sigma Chemical, St Louis MO 63178, USA, Lot 66H0044) to determine if indeed MRSA was cultured in the tissues. Data analysis was performed using a statistical package component of Microsoft® Excel97 for Windows.

Results

No animal morbidity or mortality problems were encountered with either the anesthesia or surgical procedure. All animals demonstrated a small rise in temperature one day post-surgery that renormalized quickly. After completing the entire process of harvesting and enumerating bacteria in all the sites post-mortem, the identity of the surgical lavage fluids used in the different sites per rabbit was revealed.

Table 1 lists data acquired for the 12 animals in the 7-day short-term study for MRSA burdens at various sites following each lavage treatment and clinical signs of infection observed per site per animal. The difference in visually observed clinical signs of infection at 7 days between the different lavaged sites was calculated following a (subjective) numerical representation of (-)=0, (+)=1, (++)=2. A statistical T-test analysis between saline- (avg. value = 1.50) and IgG-lavaged (avg. value = 0.83) sites resulted in a p-value of 0.005 in favor of the IgG lavage treatment providing improved visual signs of wound healing.

Figure 1 graphically compares log values (CFU/g wet tissue) of MRSA enumeration from tissue biopsies of saline- and IgG-lavaged surgical sites after seven days. Differences between bacterial enumeration after the two different lavage treatments summed over all tissues is statistically significant (IgG log CFU = 6.56, saline log CFU = 6.94, t-test, p=0.02), indicating that IgG-lavage reduces MRSA growth in surgical sites. Statistics improve for IgG-treated sites when directly quantified biopsied MRSA colony numbers are compared, although the difference from saline controls is within the same log value (IgG = 3.65×10^6 *vs.* saline = 8.80×10^6, p-value 0.01, student's t-test).

Rabbit	Site	Lavage	Clinical Score
1	Th13	Saline	++
	L3	IgG	-
	L6	IgG	-
2	Th13	IgG	-
	L3	IgG	+
	L6	Saline	++
3	Th13	Saline	++
	L3	IgG	+
	L6	IgG	-
4	Th13	IgG	++
	L3	Saline	+
	L6	IgG	+
5	Th13	IgG	++
	L3	Saline	+
	L6	IgG	+
6	Th13	IgG	+
	L3	IgG	+
	L6	Saline	++
7	Th13	IgG	++
	L3	IgG	-
	L6	Saline	+
8	Th13	Saline	++
	L3	IgG	-
	L6	IgG	-
9	Th13	IgG	++
	L3	Saline	+
	L6	IgG	+
10	Th13	IgG	+
	L3	IgG	+
	L6	Saline	+
11	Th13	IgG	++
	L3	IgG	-
	L6	Saline	+
12	Th13	Saline	++
	L3	IgG	-
	L6	IgG	+

Table 1:

Directly observed clinical description of MRSA-inoculated spinal implant sites after days: 12 rabbits, 36 implant sites, lavage fluid used per site and clinically observed infection score after 7 days. No sign of infection (-), moderate infection (+) and severe infection (++).

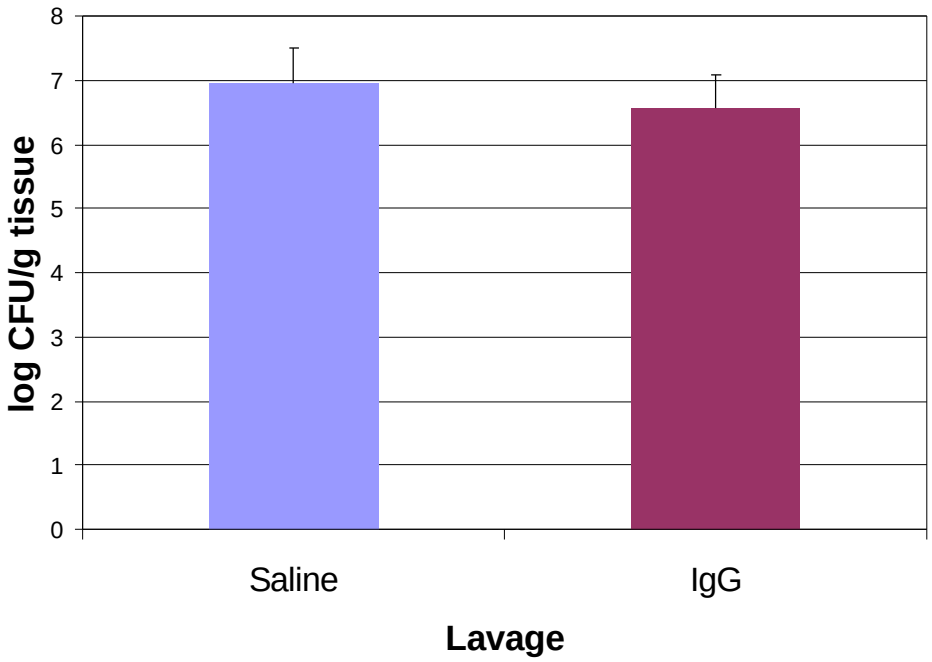

Figure 1:

Enumeration of methicillin resistant Staphylococcus aureus ATCC 33593 cultured and collectively summed from all tissue biopsies in the saline- versus IgG-lavaged spinal implant sites (mean CFU value per gram tissue) 7 days post-infection. P-value for the difference is 0.02 (student's t-test).

Selective bacterial enumeration from the implanted K-wires alone, calculated per gram implant, lavaged with either IgG or saline, is presented in Figure 2. Differences are close to significance (IgG log CFU = 3.86, saline log CFU = 4.25, t-test, p=0.07), with the mean MRSA count post-harvest being reduced in the IgG-lavaged sites seven days post-surgery. Statistics do not improve when enumerated cultured CFU values are compared (IgG = 7.25×10^3 *vs.* saline = 1.76×10^4, p-value 0.07, student's t-test).

Significantly, eleven implants of the 24 IgG-lavaged sites had no countable bacteria (46%), while only three of the 12 saline-lavaged sites (25%) presented no culturable bacterial colonies. This correlated with the lack of clinical signs of infections described in Table 1. If non-colonized implants in these two groups are excluded from the comparison, MRSA enumeration for 13 IgG-lavaged implants and 9 saline-lavaged implants are 2.19×10^4 and 2.34×10^4, respectively (no significant difference).

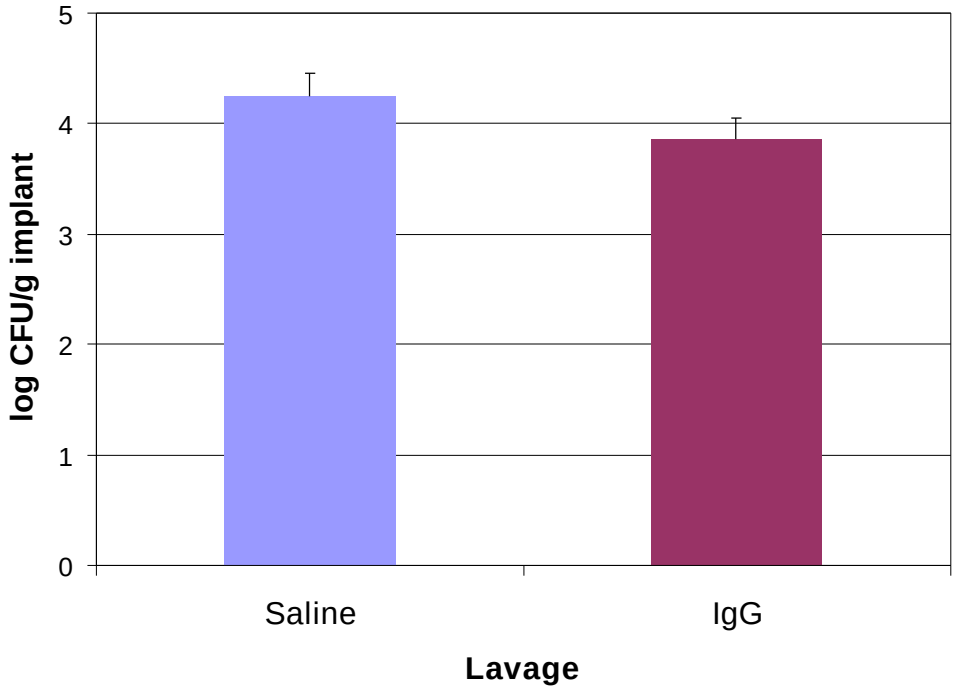

Figure 2:

Enumeration of methicillin resistant Staphylococcus aureus ATCC 33593 detached and cultured from the stainless steel K-wire spinal implants from the saline- versus IgG-lavaged spinal implant sites after 30 minute sonications (mean CFU value per gram implant) 7 days post-infection. P-value for the difference is 0.07 (student's t-test).

Table 2 presents the data from four rabbits in the 28-day long-term study, including the MRSA CFU values enumerated after plating both the tissue biopsies and harvested implants on TSA. In this long-term study, the center surgical site was left unchallenged with MRSA (sham site) to prevent any direct cross-over contamination from the inoculated head (*Th13)* and tail *(L3)* sites over the 28-day time period.

Comparison of bacterial enumeration demonstrated that only the superficial fascia immediately beneath the skin consistently contained countable bacterial colonies, with no evidence for deep MRSA wound infection. The fascia was nearly always associated in these sites with fibrosed, isolated self-contained abcesses containing viable MRSA. Specifically, bacterial enumeration from IgG-lavaged sites ($1.12x10^6$ CFU/gram tissue, 89% of biopsies exhibit no bacteria) and saline-lavaged sites ($5.40x10^4$ CFU/gram tissue, 85% of biopsies exhibit no bacteria) show no differences at 28 days. Importantly,

no bacteria were detected on any stainless steel K-wire at the 28-day point: the host consistently cleared the MRSA biomaterial-centered infection regardless of the type of treatment at this point, indicating the non-lethal, localized nature of the acute infection.

Rabbit	Site	Lavage	Clinical Score	Mean CFU/g
1	Th13	IgG	-	5.09E+06
	L3	sham	-	0
	L6	IgG	+	1.54E+06
2	Th13	IgG	-	0
	L3	sham	-	0
	L6	IgG	-	0
3	Th13	IgG	-	5.00E+04
	L3	sham	-	0
	L6	Saline	+	1.08E+05
4	Th13	IgG	+	5.17E+04
	L3	sham	-	0
	L6	Saline	-	0

Table 2:

Directly observed clinical description of MRSA-inoculated spinal implant sites after 28 days: 4 rabbits, 12 sites, lavage fluid used per site, clinically observed infection score and mean bacterial enumeration from the fascia immediately beneath the skin after 28 days. No sign of infection (-), moderate infection (+) and severe infection (++).

Consistent with previous studies on biomaterial-centered infections,[30-33] surgical sites lacking any K-wire implant required substantially greater MRSA challenge (10^4 CFU MRSA) in order to establish consistent infection in the different spinal sites. Doses of 500, 1,000 and 5,000 CFU MRSA did not produce consistent infections.

This contrasts the results from implanted K-wire sites, where 500 CFU MRSA proved sufficient for reliable infection, even for the bone. Table 3 presents the data for bacterial enumeration after 7 days from four rabbits in this no implant group challenged with 10^4 CFU MRSA. Bacterial enumeration from IgG-lavaged sites was lower (3.06×10^6 CFU/gram tissue, 40% of biopsies contained no bacteria) than that from saline-lavaged sites (7.93×10^6 CFU/gram tissue, 15% of biopsies contained no bacteria) indicating IgG's ability to reduce bacterial burden over the 7-day period ($p = 0.02$, student's t-test) in the absence of implanted biomaterials.

Rabbit	Site	Lavage	Clinical Score	Mean CFU/g
1	Th13	IgG	++	5.33E+06
	L3	Saline	+	1.30E+07
	L6	IgG	+	8.60E+06
2	Th13	Saline	++	5.71E+06
	L3	IgG	++	1.45E+06
	L6	IgG	+	3.46E+06
3	Th13	IgG	++	2.23E+06
	L3	Saline	++	1.99E+06
	L6	IgG	++	1.59E+06
4	Th13	Saline	++	1.11E+07
	L3	IgG	-	4.68E+05
	L6	IgG	-	1.32E+06

Table 3:
Directly observed clinical description of MRSA-inoculated spinal implant sites after 7 days: 4 rabbits, 12 sites, lavage fluid used per site, clinically observed infection score and mean bacterial enumeration from all samples biopsied from different sites after 7 days. No sign of infection (-), moderate infection (+) and severe infection (++).

On all 5µg/ml nafcillin TSA plates selective for MRSA culture, bacterial colony presentation and quantification was no different from that observed on normal TSA plates, meaning that only MRSA was isolated from the infected sites and that no exogenous bacteria contaminated the surgical wounds during surgery. All twenty rabbits from the three experimental groups had no countable bacteria on TSA in 5ml whole blood or 5g liver tissue cultures, signifying that systemic spread of bacteria from the separate localized spinal infections does not occur in this model. [29]

Discussion

Use of immunoglobulins against infections has a substantial history, particularly when administered systemically.[25,26,34] Local application of immunoglobulins to various non-surgical sites against specific pathogens has also been reported.[23-26] Use of broad spectrum pooled polyclonal IgG confers advantages of wide-ranging specificity and relatively ready commercial availability, while monoclonals tailored against specific virulence factors provide opportunities to improve titers and antimicrobial potency.

In this work, efficacy of a 1wt% IgG wound lavage in reducing bacterial burden in a rabbit spinal implant infection model[29] was compared to standard saline surgical lavage fluid. Rabbits received multiple non-contiguous spinal K-wire implantations challenged with 500 CFU methicillin resistant *Stahpylococcus aureus* (MRSA in 100 µl saline carrier). Control procedures lacking K-wire implantations required substantial higher inocula doses (10^4 CFU) to establish MRSA infection. MRSA was chosen as a pathogen because of its frequency in postoperative spine infection and the difficulty currently encountered in successfully treating clinical, biomaterial-centered, osteomyelitic, postoperative spinal implant infections. Results indicate that IgG-lavage significantly reduces bacterial burden in spinal wound site tissues and bone adjacent to implants, as well as clinical indicators of infection in these sites. Prophylactic protection that inhibits MRSA tissue and bone colonization is therefore important in preventing MRSA infection in a surgical wound.

A pilot *ex vivo* contamination trial, applying MRSA inocula followed by the aqueous lavage procedure to freshly harvested rabbit gluteal muscle, demonstrated that either blinded lavage fluid used in this comparison (saline or 1wt% IgG) consistently removed ~65% (SD=9%) of the MRSA inoculum from challenged wound sites (results not shown), leaving ~175-200 CFU MRSA inoculum to induce infection in tissue and bone surrounding K-wire implants.

In clinical settings, local levels of endogenous host immunoglobulin typically drop in wound sites following extensive surgical procedures, compromising the host's ability to efficiently opsonize adventitious, contaminating planktonic bacteria to prevent tissue adherence and biofilm modes of pathogen growth. The initial 6 hours post-surgery, or "decisive period", are critical in this process, with the introduced pathogens still quiescent in the so-called lag-phase, or metabolically inactive state.[35] This is the optimal window of opportunity for the host immune system to neutralize invading microorganisms, assisted by routinely applied systemic prophylactic antibiotics. Complex spinal surgical procedures are at high risk for postoperative infection, reflected by incidences reported up to 12% [1-9] and increasingly virulent wound site pathogens are

becoming antibiotic resistant.[14,15,36] Additionally, the host is often immunocompromised or malnourished (often after both anterior and posterior spine surgery),[37] increasing the risk of bacterial colonization, osteomyelitis and wound site biofilm formation that results in devastating postoperative wound infection. Local application of IgG supplements wound site immune defenses to potentially enhance pathogen killing and is indifferent to antibiotic resistant mechanisms in this susceptible phase.

Doughty reviewed topical antiseptics for use in wounds and could only identify Dakin's solution as a useful efficacious antiseptic lavage without cytotoxicity.[38] A study on the efficacy of various irrigation solutions for removing an important slime-producing *Staphylococcus* from stainless steel cortical bone screws showed that standard antibiotic-containing solutions for intra-operative irrigation had no significant effect on bacterial removal when compared to saline alone.[19,22] Others have reported beneficial effects from topical antibiotic application over regular saline, either as a lavage or introduced by different carriers into wound sites.[17,20,39]

Bacterial quantification results from this non-lethal rabbit spinal surgical model demonstrate a high number of viable MRSA present in nearly all challenged sites, regardless of the lavage treatment. The efficacy of this infection model to consistently produce such high bacterial burden is attributed to the surgical creation of a laminectomy dead-space defect that fills with blood after closure, providing an ideal medium for applied MRSA pathogens to multiply. Additional K-wire implants provide continuous biomaterial-based seeding centers for proliferating MRSA colonies to infect surrounding tissues. Biomaterial implantation is well-documented to both increase infection risk and serve as a preferred colonization site.[30-33,40-42] In this worst-case dead-space infected implant scenario, the IgG-lavaged sites show significantly improved clinical signs of infection incidence and magnitude compared to the saline controls. Data for animals without K-wire implantations show that increased amounts of MRSA are necessary to cause an infection, and that subsequent IgG-lavage treatment significantly lowered the bacterial burden post-mortem. Data from the long-term 28-day study show that the animals successfully overcome localized infections, regardless of lavage-treatment.

We caution against direct extrapolation of these results to clinical situations due to limitations intrinsic to this model. First, these outbred New Zealand white rabbits appear to be extremely susceptible to inoculation with MRSA ATCC 33593 when compared to other *in vivo* models in which higher 10^5 - 10^7 CFU MRSA burdens establish a consistent infection.[31,43] These rabbits fail to develop effective phagocytic responses to MRSA even in the presence of high titer sera.[44] Additionally, this susceptibility seems to be limited to MRSA: the same spinal model employing high inoculum doses (>10^7 CFU) of either *Staphylococcus epidermidis* (strain RP-12) or *Enterococcus faecium* (strain UMMC) do not produce any sign of local spinal infection after 7 days (unpublished data). The issue of whether such an apparently vulnerable animal-pathogen pairing accurately assesses new anti-infective therapies remains unanswered. Additionally, no comparisons of efficacy have yet been made with existing, reported antibiotic lavages.[17,19,38] Amounts of IgG remaining inside the surgical defects over time post-lavage and its half-life are also unknown. Lastly, effectiveness of immunopotentiating agents in poorly vascularized musculoskeletal intravertebral wound sites may be hindered by slow wound site infiltration of phagocytic cells necessary to facilitate bacterial clearance.

Pooled human immunoglobulin did not exhibit overwhelming efficacy alone as a topical wound lavage prophylaxis in this rabbit model to fight postoperative biomaterial-centered surgical wound infection. This is consistent with previous *in vitro* tests of commercial human preparations against MRSA in human blood preparations that failed to demonstrate bactericidal activity.[44] ELISA-based titer data for this specific human pooled polyclonal preparation used here show substantial IgG binding activity against this MRSA strain (titer = 3912). Opsonization of MRSA in wound sites would be predicted. Opsonophagocytic data for MRSA bacterial clearance *in vitro* using freshly harvested human blood neutrophils (data not shown) indicates that presence of this pooled IgG does not significantly enhance MRSA killing efficacy. Nevertheless, significant reductions in colonization frequency, and wound site tissue and implant-adherent bacteria were noted for local IgG treatment. Additionally, no systemic bacteria were detected either 7- or 28-days post-infection.

Because clinical surgical intervention will continue to routinely employ prophylactic systemic antibiotics, use of IgG alone does not represent a relevant anti-infective measure, but serves as a useful benchmark to continue toward more clinically relevant assessment. Combination therapy of systemic antibiotics and local IgG is complimentary. Recently, this combination strategy in other animal infection models shows both additive and synergistic[45] efficacy against several different virulent pathogens. Such an approach represents a logical, more relevant extension of the current strategy, and provides the benefit of protection against antibiotic resistant infection. Other implant models and application methods (e.g., IgG delivered from biodegradable wound-filling gel or fibrin sealant) could also improve bactericidal efficacy by providing extended delivery of immunoglobulin directly to the wound bed as phagocytes infiltration, inflammatory responses, and healing cascades proceed. These strategies will be subject to comparison with other standard topical anti-infective methods in fighting antibiotic resistant infection.

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