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Efficacy of the Novel Topical Antimicrobial Agent PXL150 in a Mouse Model of Surgical Site Infections

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Antimicrobial peptides have recently emerged as a promising new group to be evaluated in the therapeutic intervention of infectious diseases. This study evaluated the anti-infectious effect of the short, synthetic, broad-spectrum antimicrobial peptide PXL150 in a mouse model of staphylococcal surgical site infections. We found that administration of PXL150, formulated in an aqueous solution or in a hydroxypropyl cellulose gel, significantly reduced the bacterial counts in the wound compared with placebo treatment, warranting further investigations of the potential of this peptide as a novel local treatment of microbial infections.

The dramatic increase in bacterial resistance to conventional antibiotics in recent years emphasizes the importance of identifying novel, more potent antimicrobial therapies. One disease area where bacteria cause significant mortality and morbidity is surgical site infections (SSIs) affecting either the incision or deep tissues at the operation site (1). Despite advances in environment and surgical practice, SSIs are the third most frequently reported nosocomial infections (2). According to a surveillance study performed in the United States, SSIs account for 14 to 16% of all nosocomial infections among hospital inpatients and for 38% of nosocomial infections among surgical patients (1, 2). Similarly, European data suggest that the incidence of SSIs may be as high as 20%, depending on the surgical procedure (3).

The pathogens responsible for SSIs depend on the procedure. Most commonly isolated bacteria in SSIs are Gram-positive cocci: *Staphylococcus aureus* is the most frequently isolated organism, followed by coagulase-negative staphylococci and *Enterococcus* spp. Approximately one-third of the isolates are Gram-negative bacilli, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterobacter* species (4). The most significant change in the microbiology of SSIs over recent years has been the increased involvement of antibiotic-resistant bacteria, in particular, methicillin-resistant *S. aureus* (MRSA), as well as a progressive variation in causative pathogens. A dramatic increase in MRSA infections has been reported in different geographic locations in connection with a wide range of surgical procedures (5–8). According to different surveillance studies, as many as 50% of *S. aureus* isolates are resistant to methicillin (9–11). The Gram-negative bacilli isolated from patients with SSIs also demonstrate an increased resistance profile (12, 13). Polymicrobial infections in SSIs are increasingly frequently reported, involving both Gram-positive and Gram-negative organisms, especially *S. aureus* together with *P. aeruginosa* (9). On the basis of this evidence, new antimicrobial agents with a wide spectrum of activity, a low potential for resistance development, and activity against resistant and multiresistant strains are required for the treatment of SSIs.

Antimicrobial peptides (AMPs), a group of natural compounds constituting an integral part of the innate immune response, have attracted attention as a possible novel strategy for treatment of infections (reviewed in reference 14). AMPs present several advantages over conventional antibiotics, including a rapid, broad-spectrum microbicidal activity against a wide range of microorganisms (15), low levels of induced resistance (16), and concomitant immunomodulatory activities (17).

We recently described a novel short synthetic AMP, PXL150, that exhibits broad-spectrum microbicidal action against both Gram-positive and Gram-negative bacteria, including resistant strains such as MRSA (18). *S. aureus* and MRSA failed to develop resistance to PXL150 under continued selection pressure. In human cell lines, PXL150 downregulated the secretion of the proinflammatory markers tumor necrosis factor alpha (TNF-α) and plasminogen activator inhibitor type 1 (PAI-1), suggesting that the microbicidal effect of the peptide is accompanied by anti-inflammatory properties. PXL150 in an aqueous solution demonstrated a pronounced anti-infectious effect in an *in vivo* model of full-thickness excision wounds infected with MRSA in rats and in an *ex vivo* model of pig skin infected with *S. aureus* (18).

To evaluate the pharmaceutical potential of PXL150 as a novel local treatment for SSIs, the antimicrobial effect of this peptide was investigated in a murine SSI model. In this model, a silk suture contaminated with the most common pathogen in SSIs, *S. aureus*, was implanted into an incision wound on the back of mice and assessment of the infection was performed by counting viable bacteria in the tissue homogenate. The same model has been used previously to assess the effect of systemic and topical antimicrobial agents, and the results observed have been shown to correlate closely with efficacy in clinical trials with human subjects (19–21). For further details of the experimental model used, see the supplemental material.

Sutures carrying an inoculum of *S. aureus* were able to cause marked infection in the wounds, which persisted during the whole 96-h period of observation. Consistent with previously published

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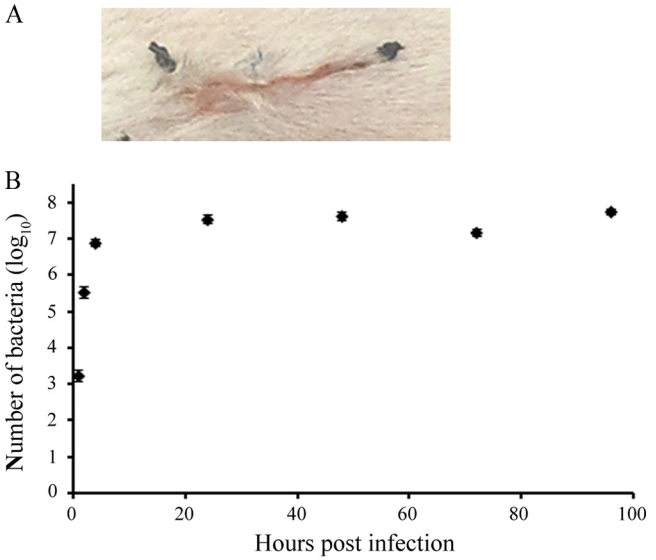

FIG 1 Infection establishment in a mouse SSI model. (A) A 1-cm-long, full-thickness incision wound was created on the dorsal side of the mouse. Approximately 1 cm of silk suture infected with *S. aureus* (approximately 5×10^3 cells/cm of suture) was placed into the wound and secured in the skin by knotting. One single nylon suture was attached over the middle of the incision. (B) Viable counts of bacteria per wound were analyzed in the tissue homogenate at 1, 2, 4, 24, 48, 72, and 96 h postinfection ($n = 5$ mice per time point).

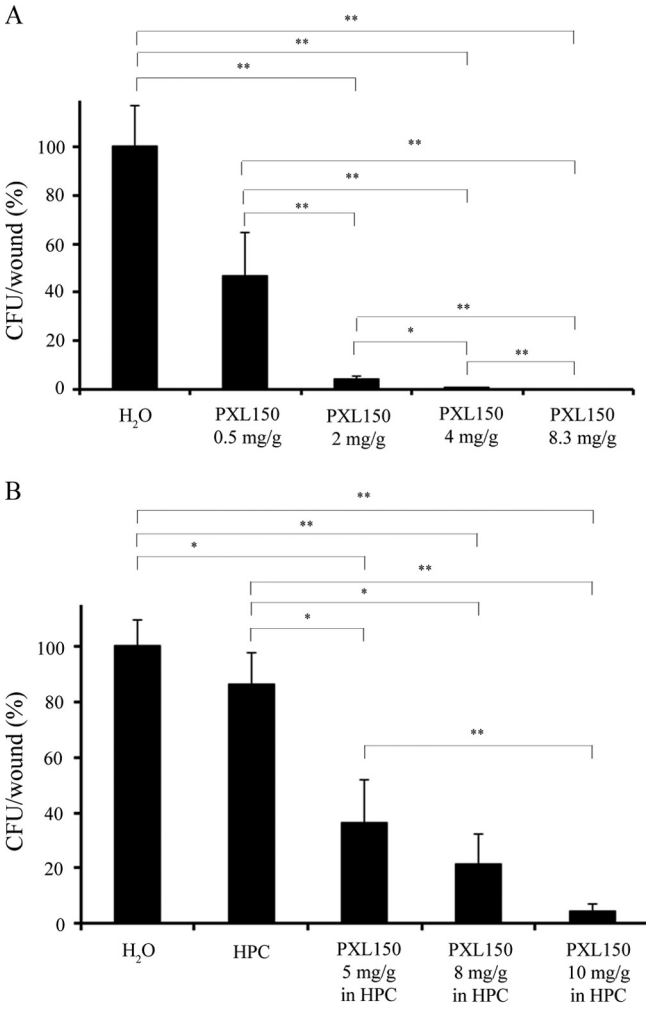

FIG 2 Antimicrobial dose-response effect of PXL150 administered in an aqueous solution (A) or in 1.5% HPC gel (B) in a mouse SSI model. Treatment was applied at 2 h postinfection, and analysis of bacterial survival in wounds was performed at 2 h posttreatment ($n = 5$ to 7 mice per group). *, $P < 0.05$; **, $P < 0.01$. HPC, hydroxypropyl cellulose.

results (20), the viable count of bacteria in the wounds increased exponentially during the first 4 h and reached a plateau of approximately $10^{7.5}$ CFU/wound at 24 h postinfection. Little variation between bacterial counts in individual wounds assessed over time was seen, indicating that a contaminated suture could cause a reproducible experimental infection (Fig. 1).

First, the anti-infectious effect of aqueous solutions of PXL150 was evaluated in this model. On the basis of our previous studies suggesting that PXL150 causes rapid membrane depolarization and killing of target bacteria (18), viable counts in the wounds were assessed at 2 h posttreatment. A single treatment with PXL150 at a concentration of 2, 4, or 8.3 mg/g produced bacterial counts in wounds significantly lower than those obtained with placebo (treatment with only water corresponded to 5.8 ± 0.8 $\log_{10}$ CFU/wound). There were statistically significant differences between all of the concentrations of the peptide tested, demonstrating a clear dose-response effect of PXL150. Concentrations of 4 and 8.3 mg/g PXL150 killed >99% of the bacteria, compared with placebo (Fig. 2A). The mean number of CFU of *S. aureus* recovered from the wounds treated with the highest concentration of the peptide (8.3 mg/g) was more than 3 $\log_{10}$ lower than that recovered from the placebo-treated wounds. Moreover, PXL150 at the highest concentration eradicated *S. aureus* from four out of the five treated wounds (<30 CFU estimated from the original suspension). In this experiment, no antibiotic control was included, which is considered a limitation of this work. However, it has previously been shown that retapamulin and mupirocin ointments reduced the numbers of *S. aureus* bacteria in this model by 3 to 4 $\log_{10}$ CFU/wound following application two or three times per day for 5 to 7 days when the initial infection was comparable to that in this study (approximately 6 $\log_{10}$ CFU/wound) (21).

As the next step, the anti-infectious effect of PXL150 at a concentration of 5, 8, or 10 mg/g formulated in 1.5% hydroxypropyl cellulose (HPC) gel was evaluated in this model. HPC is a nonionic, water-soluble polymer that is widely used as a thickening agent in pharmaceutical and cosmetic formulations in topical products (22). Previously it has been reported that placebo creams and ointments frequently affect the infection in this experimental model, leading to an increase in bacterial counts. This effect has been explained by the placebo formulation increasing the moisture content of the wound by preventing water loss or by the vehicle supporting the growth of pathogens *per se* (20). The vehicle used in this study (HPC gel) showed no effect by itself on bacterial counts, compared with the administration of water only (6.6 ± 0.1 $\log_{10}$ compared with 6.7 ± 0.1 $\log_{10}$, respectively). PXL150 in HPC gel exhibited a dose-dependent anti-infectious effect with statistically significant microbicidal activity observed at all of the concentrations of PXL150 tested, compared with treatment with water or HPC only (Fig. 2B). The highest concentration of PXL150 in HPC (10 mg/g) killed, on average, >95% of the bacteria, compared with water, while in three out of seven ani-

mals, bacterial counts were reduced by more than 99%. These results indicate that the microbicidal effect of PXL150 is preserved in the gel formulation. However, the lowest concentration of PXL150 that killed more than 95% of the bacteria was markedly higher when the peptide was formulated in HPC (10 mg/g) than when it was formulated in an aqueous solution (2 mg/g). The reason for this difference remains elusive; however, it is likely that the interaction of the cationic peptide with the HPC gel results in a slow release of PXL150, which results in an actual burst concentration of the peptide in the wound lower than that achieved with the administration of an aqueous solution of PXL150.

In all experiments, the treatment was applied at 2 h postinfection. This time point was selected on the basis of previous studies using a mouse surgical wound infection model (19–21), combined with our experiments on infection kinetics in the wound, suggesting that at 2 h postinfection, the exponential phase of bacterial growth with a high viable count of approximately $10^{5.5}$ CFU/wound was reached (Fig. 1B). However, this time interval is too short to allow assessment of the effect against bacterial biofilm, which is considered a limitation of this work.

In summary, our data demonstrate the potential benefit of using the peptide PXL150 for local antibacterial treatment of SSIs. In a mouse model of the management of experimental surgical wound infections, which is viewed as a valuable tool for predicting the effect of topical antibiotics in humans (19–21), PXL150 was shown to be efficient in reducing bacterial counts of the most common pathogen in SSIs, *S. aureus*. These results, in combination with our previous studies showing that PXL150 has a broad antimicrobial spectrum, an advantageous safety profile, and a low potential for resistance development (18), support the progression of PXL150 as a novel topical treatment of microbial infections.

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