In Vivo Activity of a Novel Polymeric Guanidine in Experimental Skin Infection with Methicillin-Resistant *Staphylococcus aureus*[▽]

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The in vivo efficacy of the novel polymeric guanidine AKACID Plus was evaluated in a guinea pig model of experimental skin infection with methicillin-resistant *Staphylococcus aureus* (MRSA). Topical application of AKACID Plus at concentrations of $\geq 0.5\%$ was as effective as mupirocin 2% cream in the treatment of superficial skin infection with MRSA.

The polymeric guanidine AKACID Plus, a 3:1 mixture of poly-(hexamethylen-guanidinium-chloride) (Chemical Abstracts Service registry no. 57028-96-3) and poly-[2-(2-ethoxy)-ethoxy-ethyl]-guanidinium-chloride] (Chemical Abstracts Service registry no. 374572-91-5), is a novel member of the cationic family of polymeric antimicrobials. Due to their own positively charged molecules, cationic antiseptics have a high binding affinity to the negatively charged cell walls and membranes of bacteria and fungi. A disruption of the target cells is brought about by the perturbation of these sites (4, 6, 12). AKACID Plus shows high water solubility and combines low acute toxicity (2) and antiproliferative effects (10) with a broad antimicrobial spectrum against bacteria, yeasts, and filamentous fungi (7, 9, 13). Nebulized AKACID Plus solution 0.5% was active in eradicating bacterial quality control strains and multiantibiotic-resistant clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* in a room disinfection assay (8). However, the in vivo activity of the novel polymeric guanidine has not been studied so far. The aim of the present study was to evaluate the in vivo activities of different concentrations of AKACID Plus cream formulation in a guinea pig model of experimental skin infection with methicillin-resistant *S. aureus* (MRSA) compared to the activities of mupirocin, a topical antistaphylococcal antibiotic.

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AKACID Plus as a 25% aqueous solution (Ch. 1007) was acquired from Polymers of Creativity Produktion GmbH, Vienna, Austria, and diluted in Ultraphil (cream vehicle) to the desired test concentrations of 0.01, 0.1, 0.25, 0.5, and 1%. These doses were chosen due to bactericidal activity in basic and extended quantitative suspension tests (9). A commercially available mupirocin 2% cream formulation (Bactroban; Smith-

Kline Beecham Pharmaceuticals, Crawley, United Kingdom) was used as the standard substance (positive control).

Experimental skin infection was carried out by using a mupirocin-sensitive clinical MRSA strain, 912, isolated from a patient with a wound infection in 2004 at the Department of Internal Medicine I, Division of Infectious Diseases and Tropical Diseases, Medical University of Vienna. Previously, MICs of 0.125 and 0.25 $\mu\text{g/ml}$ were determined for mupirocin and AKACID Plus, respectively (2). Female and male Him-Dunkin-Hartley guinea pigs (Institute of Laboratory Animal Science, Veterinary Medicine Vienna, Austria) weighing 350 to 400 g were used for the infection experiments after an acclimatization period of 10 days. The animals were housed in single cages. Food and water were given ad libitum. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of the Medical University of Vienna (GZNo. 66.009/0028-BrGT/2005). Prior to infection, guinea pigs were randomized to six different therapy groups (AKACID Plus 0.01% cream, AKACID Plus 0.1% cream, AKACID Plus 0.25% cream, AKACID Plus 0.5% cream, AKACID Plus 1% cream, and mupirocin 2% cream), a placebo group (Ultraphil alone), and an untreated control group. Sixty-eight guinea pigs were included in the study. Animals were anesthetized by intraperitoneal injection of xylazine (5 mg/kg of body weight)-ketamine (100 mg/kg of body weight). The backs of the animals were shaved with electric clippers, followed by wet shaves. To break down the skin integrity of the animals, a 4-cm² round iron stamp was dipped into liquid nitrogen and was applied to the dorsal right and left sides of the animals' backs for 5 s. After 5 min, each locus was inoculated with a fresh overnight bacterial suspension of MRSA 912 in tryptone soy broth (2×10^8 CFU/ml) in a volume of 0.1 ml. The liquid was gently rubbed into the skin with a sterile pipette tip until no more visible fluid was observed. Each animal had two infected areas. Placebo, test, and standard substances (0.2 g of cream per locus) were topically administered three times daily for a period of 7 days, starting 1 day after the initial infection, by which time erythematous lesions had already formed. The two lesions on each animal received either different topical treatment regimens on both sides or one topical treatment on one side and

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FIG. 1. Culture results for infective skin lesions with MRSA 912 following different therapy regimens with either AKACID Plus (A), mupirocin (MUP), or Ultraphil alone (placebo) compared to results for an untreated control group. The actual bacterial counts per biopsy specimen ($\log_{10}$ CFU/biopsy specimen) are represented by circles for all therapy groups. The median value of the data for each group is shown as a horizontal bar. Sixteen lesions of 16 different guinea pigs per experimental group were investigated.

placebo treatment on the other side or one topical treatment on one side and no treatment on the other side. In accordance with the study protocol, 16 lesions (1 on each of the dorsal right sides of eight guinea pigs and 1 on each of the dorsal left sides of eight other guinea pigs) per therapy group, placebo group, and control group were treated. To evaluate the possible risk of contamination between animals in different cages, two single guinea pigs were shaved and irritated by the ice-cold iron stamp but were not infected. To test the risk of contamination between lesions on same animals, two additional animals received an uninfected lesion on one side and an infected lesion on the other side which was either treated with the placebo or received no treatment.

On day 8 postinfection, 18 to 20 h after the last topical application, the guinea pigs were humanely killed by using an intraperitoneal anesthesia overdose. A part of each infected skin lesion (approximately 5 mm²) was excised, weighed (17.3 mg $\pm$ 5.1 mg), and homogenized in 1 ml of sterile sodium tryptone solution supplemented with neutralizing substances, i.e., 3% saponin, 3% polysorbate 80, 0.1% histidine, and 0.1% cysteine, as described previously (9). The homogenate was diluted by serial 10-fold dilution, and the diluent was spread on tryptone soy agar supplemented with neutralizing substances to enumerate viable staphylococci. After incubation at 37°C for 48 h, the number of bacterial cells was calculated. Bacterial colonies grown on tryptone soy agar were plated on chromogenic oxacillin resistance screen agar (Oxoid, Hampshire, United Kingdom) (1) to confirm the presence of MRSA.

Actual values of the $\log_{10}$ -transformed data ($\log_{10}$ CFU/biopsy specimen) and corresponding median values are presented in Fig. 1. The log dose-effect curve for AKACID Plus is provided in Fig. 2. For statistical analysis, the $\log_{10}$ -transformed data for the six therapy groups, the placebo group, and the control group (no treatment) were tested for normality using the Kolmogorov-Smirnov test and a probability-probability plot. However, no normality was found in any therapy group. Therefore, the nonparametric Kruskal-Wallis test was used to detect a statistical difference between the different

therapy regimens. To assess the dose-dependent activity of AKACID Plus, pairwise treatment comparisons by the Mann-Whitney U test adjusted for multiplicity by the Bonferroni-Holm method were made for AKACID Plus 1% treatment versus AKACID Plus 0.25% treatment, AKACID Plus 0.5% treatment versus AKACID Plus 0.1% treatment, and AKACID Plus 0.1% treatment versus AKACID Plus 0.01% treatment. All testing was performed at an overall 5% significance level, meaning that *P* values of less than 0.05 were considered statistically significant differences. The SPSS statistical software system (version 12.0 for Windows; SPSS Inc., Chicago, IL) was used for calculations.

One day after the initial infection, each animal showed one skin lesion on the dorsal right and one on the dorsal left side. The lesions were characterized by strictly bordered redness and swelling which resulted from the irritation of the skin surface by the ice-cold iron stamp. On days 5 to 8 postinfection, the erythematous areas with erosions were covered by crusts and scabs. All animals included in the experimental study sur-

FIG. 2. Log dose-effect curve for AKACID Plus. Percentages of maximum effects of different AKACID Plus concentrations (0.01 to 1%) are defined by the following formula: percentage of negative biopsy cultures $\div$ number of all biopsy samples per therapy group ($n = 16$).

vived during the observation period. In all animals that were shaved and irritated by the ice-cold iron stamp but not infected, the initial reddish lesions disappeared within 5 days. Figure 1 shows the culture results for the infective skin lesions following different therapy regimens with either AKACID Plus, mupirocin, or Ultraphil alone (placebo) compared to the results for an untreated control group for 7 days. Untreated lesions harbored a median value of $5.3 \log_{10}$ CFU/biopsy specimen, with a range of 4.41 to $6.08 \log_{10}$ CFU/biopsy specimen. The placebo (Ultraphil alone) did not influence the bacterial persistence because all biopsy specimens yielded MRSA in the range of 4.13 to $6.42 \log_{10}$ CFU/biopsy specimen. A significant difference ($P < 0.001$) in the numbers of CFU/biopsy specimen was found between different groups (the untreated control and placebo groups) by the Kruskal-Wallis test. After the application of AKACID Plus 0.01% cream, all biopsy specimens were positive for MRSA and viable bacterial counts ranged from 3.48 to $5.48 \log_{10}$ CFU/biopsy specimen. AKACID Plus 0.1% cream could eliminate MRSA in 3 of 16 different biopsy specimens, with a median value of $3.33 \log_{10}$ CFU/biopsy specimen. AKACID Plus 1% cream achieved the highest bacterial activity specimen, with a median value of 0.00 and maximum value of $2.60 \log_{10}$ CFU/biopsy specimen, followed by AKACID Plus 0.5%, with a median value of 0.00 and a maximum value of $4.22 \log_{10}$ CFU/biopsy specimen. AKACID Plus 0.25% killed bacterial cells in 6 of the 16 different biopsy specimens. Treatment with mupirocin 2% cream resulted in a comparable range of bacterial counts (0.00 to $4.56 \log_{10}$ CFU/biopsy specimen). Significant differences in effectiveness were found following treatment with AKACID Plus at concentrations of 1 versus 0.25% ($P = 0.003$), 0.5 versus 0.1% ($P = 0.04$), and 0.1 versus 0.01% ($P = 0.008$). The log dose-log effect curve for AKACID Plus is shown in Fig. 2. No staphylococci were detectable in any of the six uninfected lesions.

In the present guinea pig model of skin infection with MRSA, we demonstrated dose-dependent activity of the novel polymeric guanidine AKACID Plus. AKACID Plus 0.5% cream was found to be as effective as the reference substance, mupirocin 2% cream, for eradicating *S. aureus*. Mupirocin was chosen as the positive control due to topical application in humans and high in vivo activity in *S. aureus* skin models. Gisby and Bryant demonstrated comparable efficacies of local mupirocin therapy and oral antibiotic therapy in a mouse surgical wound model (5). In patients with localized superficial skin infections or in patients colonized by MRSA, topical antibiotics offer a simple, cheap, and sufficient treatment option without systemic side effects. However, for deep-seated skin infections, orally or intravenously applied antibiotics have to be used. Perl et al. showed that prophylactic intranasal application of mupirocin to suppress or eradicate *S. aureus* in patients with postoperative nasal colonization can reduce nosocomial *S. aureus* infections (11). Recently, methicillin-resistant isolates of *S. aureus* and *Staphylococcus epidermidis* have developed increasing resistance to mupirocin (3). Similarly, we evaluated 32-fold increases of the MIC₉₀ of mupirocin against MRSA compared to methicillin-sensitive *S. aureus*, whereas similar MICs for antibiotic-sensitive and multiantibiotic-resistant

strains have been determined for AKACID Plus (2). Walker et al. demonstrated the persistence of mupirocin-resistant MRSA strains after treatment with 2% mupirocin ointment for 5 days, whereas mupirocin remained effective against mupirocin-sensitive MRSA (14). The results of our preclinical study of guinea pigs suggest that AKACID Plus 0.5% cream is a potent substance and might be a possible alternative to mupirocin 2% cream in the topical treatment of skin infections with MRSA. Additional investigations concerning safety, the optimal duration of therapy and the optimal cream or solution vehicle and assessment of the emergence of resistance to AKACID Plus during therapy will be necessary to clarify the significance of AKACID Plus in the treatment of *S. aureus* skin infections in the future.

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