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Experimental therapy on induced methicillin-resistant *Staphylococcus aureus* infection in canine model

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Abstract : A randomized experimental study was done to evaluate short course therapeutic efficacies of two types of mupirocin ointment (Bactroban Nasal and Bactroban) in the elimination of methicillin-resistant *Staphylococcus aureus* (MRSA) nasal colonization (16 dogs) and wound infection (3 dogs or 18 wound sites) in dogs. In each model, dogs being assigned to TR-1 treatment group was given ointment twice a day for two consecutive days, and those that assigned to TR-2 treatment group was given the same dose for three days. Neither TR-1 nor TR-2 regimen was effective to clear nasal carriage completely with a clearing rate of 62.5% and 87.5%, respectively. Whereas, for 2 days at least twice daily application of mupirocin for wound infection was quite enough to eliminate MRSA, with a clearing rate of 83.3~100% by 4 weeks follow-up. No apparent side effects were observed in each model, and in no case was it necessary to discontinue the treatment. Further controlled studies on the elimination of nasal colonization are required to establish cost-effective and efficient regimen on companion animals.

Key words : methicillin-resistant *Staphylococcus aureus*, canine colonization, wound infection, therapy.

Introduction

The multiple resistant strains of staphylococci have been emerging a new medical threat to animal and human health[1~3]. In particular, *Staphylococcus aureus* has proved to be an extremely versatile species, responding to the challenge of new antibiotics by acquiring resistance to many of them within a fairly short time. In human medicine, endemic and epidemic nosocomial infections caused by methicillin-resistant *S aureus* (MRSA) represent an increasing problem throughout the world[4~8].

Since mid-1980 several reports published on methicillin-resistant staphylococci infection of animals including equine[9~10], canine[11~12], feline[13], bovine[14~15] and others[16~20]. These infections were associated with mastitis, metritis, dermatitis, and postoperative wound infection. MRSA isolates have been shown to be fully virulent and can cause a wide range of

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spectrum of diseases that are difficult to control[21~24]. Furthermore, these isolates are usually resistant to multiple antibiotics, including beta-lactam, aminoglycosides, quinolones, macrolides, and lincosamides[25~26], and efficient colonizers of immunocompromized hosts. Therefore, accurate identification of MRSA strains or types, and their carriers within a hospital, are of special importance in coping with these outbreaks.

The aim of our report was to evaluate short course therapeutic efficacies and safety of intranasal preparation ('Bactroban Nasal') on the treatment of experimental canine nasal colonization and of topical skin preparations ('Bactroban') for wound infection model due to MRSA.

Materials and Methods

Antimicrobial agents : Two percent calcium mupirocin ('Bactroban Nasal', lot no. 670566-c) for intranasal use was purchased from Smith-Kline Beecham Pharmaceuticals (Philadelphia, USA). Bactroban ointment was kindly provided from Hanol Pharmaceutical Co., Korea.

Bacterial strain : *S aureus* field strain which was confirmed to have a oxacillin minimum inhibitory concentration (MIC) value of 〉128μg/ml and *mec* A, *fem* A, and *fem* B gene-positive was used for colonization. For control group, a strain of *S aureus* ATCC 29213 was challenged with the clinical isolate.

Preparations of bacterial inoculum : For nasal colonization model, the isolate was prepared by inoculating a single colony of the isolate into 5ml of Mueller-Hinton broth, incubating for 4~6h at 37℃, adjusting the culture to a density of no. 0.5 McFarland, and further diluting to give a final inoculum of 1.5×10^6CFU/ml. For wound infection model, the challenge inoculum size was adjusted to the concentration, ranging from 10^7 to 10^8CFU/ml in PBS.

Study design and medication :

Nasal colonization : Sixteen clinically healthy dogs were assigned to two groups; 2 day-q12h group (TR-1) and 3 day-q12h group (TR-2). All dogs in the study had not received any antimicrobial agents for at least 48h prior to inoculating bacterial suspensions, and clinically evaluated, including physical examination, complete blood counts (CBC), differential leukocyte counts, and serum chemistry. The 2% calcium mupirocin ointment was applied to both anterior nares twice daily - once in the morning and once in the evening - for 2 or 3 consecutive days (4 or 6 doses); this was followed by a short nasal massage for 1 min to insure its even distribution.

Wound infection : Three dogs (18 wound sites) were assigned to TR-1 and TR-2 treatment group, respectively. A 1cm length of the mupirocin ointment was either squeezed onto a sterile swab or fingertip, and it was applied to each wound site. (A 1cm length of calcium mupirocin ointment weighted 250mg and contained 5mg of mupirocin). For dogs being assigned to control groups in each model were not received any treatment.

Establishment of nasal colonization and wound infection :

Nasal colonization : After dogs were positioned recumbent for easy administrations of bacterial suspension an aliquot of 1ml of prepared inocula was injected slowly into both left and right nostrils using a syringe without needle. This procedure was repeated for three times with 30 min interval for the sake of complete colonization of the bacteria.

Wound infection : For superficial wounding six circular areas, each $3.14cm^2$, 6 areas on each side of the dorsal midline, were used on each of the 6 dogs. On the injection, dogs were sedated intravenously with 0.3ml of ketamine per kg. The dorsal area of the dog was shaved with electric small animal clippers, and scraped with a no. 24 scalpel blade until blood was appeared. Barrels of 20ml syringe were cut approximately 2cm from the flanged end. The designated test sites were demarcated by attaching the syringe barrels to the skin surface with cyanoacrylate adhesive (Super Glue, UNI 102, Japan). The flanged end was in contact with the skin and the adhesive provided an air- and water-tight seal. The 100μl of the each prepared culture suspension was applied to the wounded skin, using micropipette, and were spread evenly with sterile spatulas. At the following day, bacterial cultivations were done to examine colonization on each site. After confirmation of colonization, barrels were removed, and then wound sites were occluded with dressings.

Clinical and microbiological evaluation of dogs : The primary end point for efficacy was defined as the bacteriologic elimination of MRSA strain by 4 weeks after therapy, which was assessed by cultivations of samples obtained from the anterior nares and wound sites. The post-treatment nasal swab was taken 48h after the last application of treatment in order to avoid the carry-over effect of the antibiotic onto culture media. A final clinical evaluation of the nasal mucosa was performed at the 8-week follow-up. For wound infection model, the post-treatment swab was taken from 24h to 28 day after the last application of treatment. Therapy was declared a failure if nasal carriage was not eliminated. Therapy was considered successful if *S aureus* was completely eradicated from the anterior nares, with no evidence of relapse at each evaluation period. A safety profile which included CBC, creatinine, serum levels of urea nitrogen (BUN), bilirubin, electrolytes, and tests of liver function (alanine and aspartate aminotransferase, and alkaline phosphatase) was performed at the first and second day of posttherapy. Cultures of the nares were obtained by inserting the premoistened cotton-tipped swabs into the nostrils for at least 1cm and drawing them outward in a firmly rotating motion against the walls of each nostril. The swabs were inoculated directly onto the mannitol salt agar. The plates were incubated at 37℃ in aerobic condition and were examined at 24 and 48h. For pure culture, all mannitol-positive colonies were subcultured onto blood agar plates and were then identified by standard procedures. All isolates obtained during therapy and those that failed to be cleared of MRSA, if possible, were tested by the Bauer-Kirby disk diffusion method for susceptibility to 1μg of oxacillin.

Statistical analysis : The clearing rate between the two therapeutic regimens was evaluated by a two-sample *t*-test. A P-value of ≤ 0.05 was considered statistically significant. All analyses were done with PC-SAS (release 6.04 ; SAS Institute, Cary, NC, USA)[27].

Results

Clinical response : For nasal carriage model, all of the 8 dogs experimentally infected with MRSA clinically responded, regardless of the therapeutic regimen used. No significant adverse events were associated with therapy on clinical examination or in laboratory profiles. Percentage of nasal eradication with two mupirocin therapeutic regimens are shown in Table 1 and were as follows : success was achieved in 7 (87.5%) and 8 (100%) at the first week, 5 (62.5%) and 7 (87.5%) at the second week, 5 (62.5%) and 7 (87.5%) at the fourth week, and 5 (62.5%) and 5 (62.5%) at the eighth week. For wound infection model treated with TR-2 regimen, all sites were culture negative until the end of observation period, whereas for those that were treated with TR-1 regimen, 83.3% of wound sites were cleared of bacteria (Table 2). Follow-up studies disclosed similar percentage of eradication between groups.

Table 1. Success rate(%) at each evaluation period after mupirocin treatment for all dogs with nasal colonization of MRSA strain

Regimen	Days after the last treatment				
	2	7	14	28	56
2d, q12h(TR-1)	8(100)	7(87.5)	5(62.5)	5(62.5)	5(62.5)
3d, q12h(TR-2)	8(100)	8(100)	7(87.5)	7(87.5)	5(62.5)
Control	0	0	0	0	0

Table 2. Success rate(%) at each evaluation period after mupirocin treatment for all dogs with wound infection caused by MRSA strain

Regimen	Days after the last treatment				
	1	2	7	14	28
2d, q12h(TR-1)	18(100)	18(100)	18(100)	16(66.6)	17(83.3)
3d, q12h(TR-2)	18(100)	18(100)	18(100)	18(100)	18(100)
Control	0	0	0	2(16.7)	2(16.7)

Bacteriological response and safety profile : None of the strains isolated from the dogs with relapse were resistant to mupirocin. Also, no dogs developed measurements of CBC, BUN, creatinine, and liver enzyme higher than twice normal during the study period.

Carriage-free period : For nasal carriage model, a total

of 3 dogs who were treated with TR-1 or TR-2 regimen were persistently colonized by 8 weeks of observation period. By comparison of two regimens, TR-2 tended to protract the carriage-free period, but not statistically significant ($p > 0.05$). For wound infection model, both regimens showed highly effective for elimination of MRSA carriage, with a clearing rate of 100% by 1 week. During the entire observation period, all wound sites that were treated with TR-1 regimen were cleared of bacterial colonization except one at 28 day of observation while no culture positive sites were observed those applied with TR-2 regimen.

Discussion

The therapy of infections caused by MRSA or the eradication of colonization is particularly difficult because of its characteristics of multiple resistance and limited choice of therapeutic options. The eradication of MRSA colonization has been attempted by using several approaches, including whole-body bathing with specified agents, as well as therapy with selected systemic and topical antimicrobial agents[28]. Long-term eradication (defined as greater than equal to 6 months) eradication of MRSA has generally not been achieved with any of these methods[29~32]. The development and early investigations with new fluoroquinolones, particularly ciprofloxacin, demonstrated their *in vitro* activity against both MRSA and MSSA[33~35]. While some resistance developed in MRSA when patients were treated with ciprofloxacin as a single agent, the early results reported with the fluoroquinolones suggested that combination therapy may prove to be even more efficacious[33].

Mupirocin (pseudomonic acid A) is an antibiotic produced by the subfermentation of *Pseudomonas fluorescens* and has FDA-approved. It has a unique chemical structure unrelated to any other antibiotic class. The mechanism of action is consisting of inhibition of bacterial RNA and protein synthesis. Calcium mupirocin ointment was developed for intranasal administration in a white soft-paraffin base to eliminate adverse effect such as irritation of nasal mucosa. Mupirocin is rapidly metabolized to inactive monic acid and the plasma half-life of mupirocin is less than 30 min, thus it is suitable only for topical use. It is not recommended for use against gastrointestinal carriage of *S aureus* because of gastric acidity. In the current study, therapeutic efficacies of 2 or 3 days of short course treatment regimen were compared using the model of nasal colonization and wound infection. Two parameters are important when assessing the efficacy of any regimen for clearing nasal carriage of *S aureus* : the number of days therapy to achieve clearance and the post-treatment interval to recolonization. With this in mind, a little modified regimen that having fewer applications per day and reducing the number of days of treatment was designed to reduce treatment cost and to increase dog's compliance to the therapy. TR-1 regimen in nasal colonization model does not seem to effective for elimination of MRSA carriage, compared with TR-2 regimen. During the entire observation period of 8 weeks in nasal model using TR-2 regimen, one dog relapsed at the 2nd week of post-therapy and another 3 dogs relapsed by the end of observation. During the 8 weeks of follow-up the overall relapsing rate of 37.5% was relatively higher than the results from human beings[36]. Bulanda *et al*[37] reported the effects of mupirocin treatment (5 days-q12h) on nasal carriage of *S aureus*, with 42% of relapsing rate during a 1-year period of follow-up. This result indicates that TR-2 regimen was not effective to eliminate the bacteria from nasal colonization. For wound infection model, of the 18 sites treated with TR-1 regimen, all but one dog were cleared of MRSA within the entire study period; 18 remained clear until 1 weeks later and two became recolonized 2 weeks after the course. Of the 18 sites treated with TR-2 regimen, all were negative for follow-up 4 weeks after the course. Although MRSA in animals so far has been reported in the geographically limited countries, the use of mupirocin may be of value in MRSA infection.

Legends for figures

Fig 1. Inflammatory skin lesions with multi-focal small pustules caused by MRSA (a) on day 2 after bacterial challenge and (b) scar formation on day 7 after initiation of mupirocin treatment.

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