Research article/Raziskovalni prispevek

IN VITRO ACTIVITY AND EMERGENCE OF RESISTANCE TO MUPIROCIN IN STAPHYLOCOCCUS AUREUS

IN VITRO AKTIVNOST IN RAZVOJ ODPORNOSTI PROTI MUPIROCINU PRI BAKTERIJI STAPHYLOCOCCUS AUREUS

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Ključne besede: *mupirocin; Staphylococcus aureus; odpornost*

Abstract – Background. *Nasal carriage of Staphylococcus aureus is an important risk factor for later infection with the same strain. Topical mupirocin is used for S. aureus nasal decolonization. However, due to increasing mupirocin misuse resistance may develop rapidly. We determined the in vitro activity of mupirocin and compared the emergence of resistance among 30 methicillin-susceptible S. aureus (MSSA) isolates and 30 methicillin-resistant S. aureus (MRSA) isolates.*

Methods. *Minimal inhibitory concentrations (MICs) were determined according to the National Committee for Clinical Laboratory Standards (NCCLS). Emergence of resistance studies were performed by incubating the isolates with increasing concentrations of mupirocin (0.125 to 16 µg/ml) over 8 days in Mueller-Hinton broth (MHB). Isolates were screened for resistance using Mueller-Hinton agar plates with 4 µg/ml of mupirocin.*

Results. *MICs were determined for all recovered isolates. Before mupirocin exposure, MSSA and MRSA MIC_{90} (range) in µg/ml were 0.25 (0.06–4) and 0.25 (0.06–0.5). After one day of mupirocin exposure, all recovered isolates demonstrated decreased susceptibility to mupirocin (MSSA MIC_{90} 64 µg/ml and MRSA MIC_{90} 32 µg/ml). On subsequent days, no further significant changes in the mupirocin MIC_{90} was detected.*

Conclusions. *The in vitro study suggests that mupirocin resistance emerges easily and early after exposure to low antimicrobial concentrations. Therefore, mupirocin should be used prudently and should always be combined with other decolonization interventions such as gargling and skin wash with chlohexidine.*

Izvleček – Izhodišča. *Nosilstvo Staphylococcus aureus v nosu predstavlja pomemben dejavnik tveganja za kasnejšo okužbo z istim sevom. Topični mupirocin se uspešno uporablja za dekolonizacijo nosu v primeru nosilstva bakterije S. aureus. Vse večja uporaba in predvsem zloraba mupirocina je lahko povezana z razvojem odpornosti bakterije S. aureus na ta antibiotik. V raziskavi smo določili in vitro aktivnost mupirocina in primerjali razvoj odpornosti med 30 izolati za meticilin občutljivega S. aureus (MSSA) in 30 izolati proti meticilinu odpornega S. aureus (MRSA).*

Metode. *Minimalne inhibitorne koncentracije (MIK) smo določili po priporočilih National Committee for Clinical Laboratory Standards (NCCLS). Sposobnost razvoja odpornosti stafilokokov smo določili z inkubacijo izolatov v gojišču Mueller-Hinton z naraščajočo koncentracijo mupirocina (0,125 do 16 µg/ml) v času 8 dni. Na mupirocin odporne izolate S. aureus smo osamili s pomočjo presejalnega gojišča Mueller-Hinton z vsebnostjo mupirocina v koncentraciji 4 µg/ml.*

Rezultati. *MIK smo določili za vse izolate, ki so porasli na presejalnem gojišču. Pred izpostavljenostjo mupirocinu je MIK_{90} (razpon) v µg/ml za MSSA in MRSA znašal 0,25 (0,06–4) in 0,25 (0,06–0,5). Po enem dnevu izpostavljenosti mupirocinu so vsi izolati kazali zmanjšano občutljivost za mupirocin (MSSA MIK_{90} 64 µg/ml in MRSA MIK_{90} 32 µg/ml). Pri nadaljnji inkubaciji z naraščajočo koncentracijo mupirocina v naslednjih dneh nismo več opazili značilne spremembe MIK_{90} mupirocina.*

Zaključki. *Naša raziskava in vitro nakazuje, da se lahko odpornost proti mupirocinu razvije zlahka in zgodaj po izpostavljenosti nizkim koncentracijam tega antibiotika. Zato je nujno, da mupirocin uporabljamo preudarno in vedno v povezavi z drugimi dekolonizacijskimi ukrepi, kot sta grgranje in umivanje kože s kloheksidinom.*

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Introduction

Nasal carriage has been recognized as an important risk factor for *S. aureus* infection in patients with concomitant human immunodeficiency virus infection, with intravascular devices, undergoing surgical procedures, on hemodialysis or continuous ambulatory peritoneal dialysis (CAPD) and in liver transplant recipients (1–4). Decolonization of nasal carriers with mupirocin has shown to reduce the incidence of *S. aureus* infections in surgical patients and in those on hemodialysis and CAPD (2).

Mupirocin resistance in *S. aureus* was first reported in 1987, just two years after mupirocin was introduced into clinical practice. Mupirocin resistance in staphylococci has been classified as low-level (MIC 8-256 µg/ml) and high-level (MIC > 256 µg/ml) (5). The widespread use of mupirocin has been accompanied by the emergence of both types of mupirocin resistance in *S. aureus.* Miller *et al.* reported an increase in mupirocin resistance, predominantly high-level resistance, among methicillin-resistant *S. aureus* (MRSA) from 2.7% in 1990 to 65% in 1993 (6). In contrast, Watanabe *et al.* reported emergence of low-level mupirocin resistance in MRSA shortly after introduction of mupirocin in Japan (7). High-level resistant strains are not eradicated with mupirocin and the clinical significance of low-level mupirocin resistant strains is controversial (8). However, Harbarth *et al.* reported a statistically significant association of low-level mupirocin resistance in MRSA with failure of nasal decolonization. Alternatives to mupirocin are needed for *S. aureus* nasal decolonization (9).

We examined the *in vitro* activity of mupirocin and compared the emergence of resistance to mupirocin in *S. aureus*.

Materials and methods

Collection of clinical isolates

Thirty clinical isolates each of MRSA and methicillin-susceptible *S. aureus* (MSSA) from the Mayo Clinic (Rochester, MN) and the Cleveland Clinic (Cleveland, OH) collected between January 1985 and December 2002 and stored at –70 °C before studied. Five isolates (8%) were from patients with endocarditis and 16 isolates (27%) were from patients with prosthetic joint infection. The source was not documented for the remaining 39 isolates (65%). One isolate per patient was studied. The isolates were typed using *Sma*I pulsed-field gel electrophoresis (PFGE) and the band patterns were scored and interpreted according to published criteria (10).

Mupirocin

Mupirocin lithium salt powder was purchased from USP (Rockville, MD).

Determination of MIC values

MIC values were determined by broth microdilution according to NCCLS (National Committee for Clinical Laboratory Standards) guidelines with cation-adjusted Mueller-Hinton broth (MHB) using an inoculum of 10^5 colony-forming units/ml (10). For the emergence of resistance studies, mupirocin was tested in serial twofold dilutions ranging from 0.03 to 32 µg/ml for day 0, and 0.250 to 256 µg/ml for day 1 to 8. Differences in MIC values of one or two twofold dilution were not considered significant (in accordance with NCCLS guidelines). *S. aureus* ATCC 29213 was used as a control strain.

Selecting for resistant isolates

S. aureus isolates were exposed to increasing concentrations of mupirocin in 10 ml of MHB containing the following concentrations of mupirocin: 0.125 µg/ml (day 1), 0.25 µg/ml (day 2), 0.5 µg/ml (day 3), 1 µg/ml (day 4), 2 µg/ml (day 5), 4 µg/ml (day 6), 8 µg/ml (day 7) and 16 µg/ml (day 8). Following overnight incubation, the broth culture was centrifuged for 10 minutes at 900 g and the pellet divided into two aliquots. One aliquot was resuspended in 10 ml of fresh MHB containing twice the mupirocin concentration of the previous day; the other aliquot was screened for resistance on a Mueller-Hinton agar plate containing 4 µg/ml of mupirocin. The plate was incubated in room air at 37 °C for 48 hours. The MIC value of five to ten colonies recovered from screening agar was determined at each day of serial passage. This procedure was repeated for 8 days.

Results

MIC values of mupirocin are shown in Table 1. MIC values for the studied agent were similar for MSSA and MRSA isolates. After 1 day of exposure, the mean mupirocin MIC_{90} values had increased by eight and seven twofold dilutions for MSSA and MRSA, respectively. On subsequent days no further significant changes in the mupirocin MIC_{90} values were detected (Figure 1).

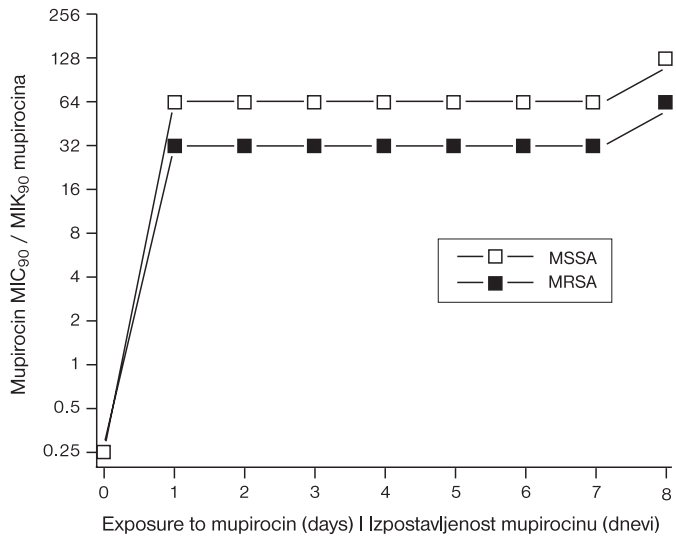

Figure 1. *Increase of MIC_{90} values of mupirocin for methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) S. aureus during 8 days of exposure to mupirocin.*

Sl. 1. *Naraščajoče vrednosti MIK_{90} mupirocina za meticilin občutljivi (MSSA) in proti meticilinu odporni (MRSA) S. aureus med izpostavljenostjo mupirocinu v obdobju 8 dni.*

Table 1. *MICs of mupirocin for methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) S. aureus. Results are summarized as MIC_{90} (range) in µg/ml.*

Razpr. 1. *MIK mupirocina za meticilin občutljivi (MSSA) in proti meticilinu odporni (MRSA) S. aureus. Rezultati so prikazani kot MIK_{90} (razpon) v µg/ml.*

Duration of exposure Trajanje izpostavljenosti	MSSA	MRSA
Day / dan 0	0.25 (0.06–4)	0.25 (0.06–0.5)
Day / dan 1	64 (32–64)	32 (8–32)
Day / dan 2	64 (8–64)	32 (8–32)
Day / dan 3	64 (4–64)	32 (16–64)
Day / dan 4	64 (8–128)	32 (16–64)
Day / dan 5	64 (16–64)	32 (16–32)
Day / dan 6	64 (32–64)	32 (8–64)
Day / dan 7	64 (32–128)	32 (16–64)
Day / dan 8	128 (32–128)	64 (32–128)

PFGE identified 10 unique MSSA and 7unique MRSA patterns (Table 2). Among the MSSA isolates, no single PFGE type predominated. Among the MRSA isolates, most [17 (57%)] belonged to a single PFGE group (PFGE type A).

Table 2. *Distribution of pulsed-field gel electrophoresis (PFGE) types among 30 methicillin-susceptible (MSSA) and 30 methicillin-resistant (MRSA) S. aureus isolates.*

Razpr. 2. *Razporeditev tipov gelske elektroforeze v pulznem polju (PFGE) med 30 izolati za meticilin občutljivega (MSSA) in 30 izolati proti meticilinu odpornega (MRSA) S. aureus.*

PFGE type Tip PFGE	No. (%) of MSSA isolates Štev. (%) izolatov MSSA	PFGE type Tip PFGE	No. (%) of MRSA isolates Štev. (%) izolatov MRSA
H	6 (20%)	A	17 (57%)
I	3 (10%)	B	3 (10%)
J	5 (17%)	C	2 (7%)
K	1 (3%)	D	1 (3%)
L	1 (3%)	E	5 (17%)
M	2 (7%)	F	1 (3%)
N	1 (3%)	G	1 (3%)
O	1 (3%)		
P	5 (17%)		
Q	5 (17%)		

Discussion

This study shows that mupirocin demonstrates *in vitro* activity against *S. aureus.* MIC values for mupirocin before antimicrobial exposure were similar to those reported previously (12). We were able to select resistant mutants following serial passage in increasing concentrations of mupirocin. In contrast to previous reports where at least 6 days of serial passage were needed to observe a significant increase in MIC values (13, 14), our isolates demonstrated decreased susceptibility to mupirocin after just one day of exposure at a concentration of 0.125 µg/ml. Discrepancies may be due to differences in methods, including inoculum size, use of agar versus broth culture and concentration of mupirocin studied. The ease with which we were able to select for mupirocin resistant isolates *in vitro* raises concerns that this may easily occur in patients. It has been suggested that the low concentration of mupirocin in the pharynx of patients after intranasal mupirocin application may facilitate emergence of mupirocin resistance (15).

The mechanism of mupirocin resistance has been well described. Mupirocin acts by competitively inhibiting bacterial isoleucyl-tRNA synthetase (IleRS), thereby blocking protein synthesis (16). The level of resistance correlates with the mechanism of resistance. Low-level resistance (MIC 8-256 µg/ml), as selected in our study, is due to chromosomal mutations in the endogenous IleRS (5). High-level resistance (MIC > 256 µg/ml) is a result of acquisition of *mupA,* a gene which codes for an exogenous IleRS which is not inhibited by mupirocin (17). It is suggested that the mupirocin resistance may be associated with decolonization failure. As the frequency of the chromosomal mutation responsible for the low-level resistance appears to be high and the gene for the high-level resistance is easily transferred, increased prevalence of mupirocin resistance is expected if this mupirocin will be used inappropriately.

Different PFGE patterns demonstrate the diversity of clones of the studied isolates and all showed emergence of resistance. This finding supports the hypothesis that mupirocin resistance can easily emerge in different strains of *S. aureus.*

In conclusion, our *in vitro* study demonstrated that mupirocin has a good activity against *S. aureus.* However, *in vitro* resistance to mupirocin was easily selected by serial passage and low-level mupirocin resistance developed already after one day of antimicrobial exposure. During the next seven days of antimicrobial exposure no further increase in resistance appeared. After 8 days of antimicrobial exposure we were unable to develop high-level resistance, probably because of different resistance mechanism, an acquisition of *mupA* gene. Misuse of mupirocin should be avoided.

References

1. Chang FY, Singh N, Gayowski T, Drenning SD, Wagener MM, Marino IR. *Staphylococcus aureus* nasal colonization and association with infections in liver transplant recipients. Transplantation 1998; 65: 1169-72.
2. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus:* epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 1997; 10: 505-20.
3. Von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. N Engl J Med 2001; 344: 11-6.
4. Weinke T, Schiller R, Fehrenbach FJ, Pohle HD. Association between *Staphylococcus aureus* nasopharyngeal colonization and septicemia in patients infected with the human immunodeficiency virus. Eur J Clin Microbiol Infect Dis 1992; 11: 985-9.
5. Cookson BD. The emergence of mupirocin resistance: a challenge to infection control and antibiotic prescribing practice. J Antimicrob Chemother 1998; 41: 11-8.
6. Miller MA, Dascal A, Portnoy J, Mendelson J. Development of mupirocin resistance among methicillin-resistant *Staphylococcus aureus* after widespread use of nasal mupirocin ointment. Infect Control Hosp Epidemiol 1996; 17: 811-3.
7. Watanabe H, Masaki H, Asoh N et al. Emergence and spread of low-level mupirocin resistance in methicillin-resistant Staphylococcus aureus isolated from a community hospital in Japan. J Hosp Infect 2002; 47: 294–300.
8. Annigeri R, Conly J, Vas S et al. Emergence of mupirocin-resistant *Staphylococcus aureus* in chronic peritoneal dialysis patients using mupirocin prophylaxis to prevent exit-site infection. Perit Dial Int 2001; 21: 554-9.
9. Harbarth S, Dharan S, Liassine N, Herrault P, Auckenthaler R, Pittet D. Randomized, placebo-controlled, double-blind trial to evaluate the efficacy of mupirocin for eradicating carriage of methicillin-resistant *Staphylococcus aureus.* Antimicrob Agents Chemother 1999; 43: 1412-6.
10. Tenover FC, Arbeit RD, Goering RV et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; 33: 2233-9.
11. NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, 6th ed, vol Approved standard M7-A6, Wayne, PA 2003.
12. Finlay JE, Miller LA, Poupard JA. Interpretive criteria for testing susceptibility of staphylococci to mupirocin. Antimicrob Agents Chemother 1997; 41: 1137-9.
13. Capobianco JO, Doran CC, Goldman RC. Mechanism of mupirocin transport into sensitive and resistant bacteria. Antimicrob Agents Chemother 1989; 33: 156–63.
14. Casewell MW, Hill RL. In-vitro activity of mupirocin (»pseudomonic acid«) against clinical isolates of Staphylococcus aureus. J Antimicrob Chemother 1985; 15: 523–31.
15. Watanabe H, Masaki H, Asoh N et al. Low concentrations of mupirocin in the pharynx intranasal application may contribute to mupirocin resistance in methicillin-resistant Staphylococcus aureus. J Clin Microbiol 2001; 39: 3775–7.
16. Yanagisawa T, Lee JT, Wu HC, Kawakami M. Relationship of protein structure of isoleucyl-tRNA synthetase with pseudomonic acid resistance of Escherichia coli. J Biol Chem 1994; 269: 24304–9.
17. Hodgson JE, Curnock SP, Dyke KG, Morris R, Sylvester DR, Gross MS. Molecular characterization of the gene encoding high-level mupirocin reisitance in Staphylococcus aureus J2870. Antimicrob Agents Chemother 1994; 38: 1205–8.