

SHORT REPORT

The bioavailability of mupirocin in nasal secretions in vitro

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J Clin Pathol 2002;**55**:233–235

Aim: To determine the bioavailability of mupirocin in human nasal secretions and to assess whether the contents of nasal secretions interact appreciably with this antibiotic.

Methods: The comparative bioavailability of mupirocin and chlorhexidine in nasal secretions was determined by bioassay after one, four, and eight hours of incubation with pooled secretions from three subjects. The interaction of mupirocin with nasal secretions was characterised by matrix assisted laser desorption time of flight mass spectrometry (MALDI-TOF).

Results: MALDI-TOF analysis showed that mupirocin was not absorbed by the main fraction of pooled nasal secretions and should remain active. In bioassay, mupirocin retained 100% of its antistaphylococcal activity in nasal secretions, whereas chlorhexidine was significantly reduced from 100 mg/litre to 1.5 mg/litre and from 1000 mg/litre to 38.5 mg/litre, irrespective of incubation time.

Conclusions: The high bioavailability of mupirocin in nasal secretions results from the lack of appreciable molecular interactions.

There is renewed interest in developing intranasal antimicrobial agents to eliminate methicillin resistant and sensitive *Staphylococcus aureus*¹ and fungi.² High in vitro activity may not be the sole indicator of clinical efficacy because agents must be able to resist inactivation by nasal secretions. Mupirocin has been the mainstay for the elimination of nasal carriage of *Staphylococcus aureus*,³ including methicillin resistant strains (MRSA), during hospital outbreaks.⁴⁻⁶ Chlorhexidine, which also has good antistaphylococcal activity,⁷ is relatively ineffective intranasally,⁵ and topical vancomycin has little effect.¹

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As a simple in vitro model to identify potential intranasal efficacy, the activity of mupirocin and chlorhexidine in nasal secretions was compared, and potential complexes between nasal secretions and mupirocin examined by a revolutionary mass spectrometry method.⁸

METHODS

Nasal secretions were obtained from three healthy subjects (two men, one woman) after vigorous exercise for five to 10 minutes in air temperatures of $\leq 10^{\circ}\text{C}$: dripping and blown secretions were collected in 30 ml plastic universals.⁹ Mupirocin or chlorhexidine were added to 0.45 ml of pooled nasal secretions to achieve final concentrations of mupirocin or chlorhexidine of 50, 100, 250, 500, and 1000 mg/litre. After one, four, and eight

Table 1 Effect of nasal secretions on the activity of mupirocin or chlorhexidine at 37°C

	Average amount recovered from initial concentrations (mg/l) of				
	50	100	250	500	1000
Mupirocin	50	100	250	500	1000
Chlorhexidine	1.2	1.5	2.0	12.5	385

Aliquots from the incubation mix were bioassayed after one, four, and eight hours.

hours incubation at 37°C, active values of mupirocin or chlorhexidine in 200 μl aliquots removed from each sample at each time interval were determined in a well plate assay using DST agar (CM261; Oxoid, Basingstoke, UK) buffered by 0.2 M biphasic phosphate buffer to pH 7.2 for mupirocin, or Muller-Hinton agar (CM337; Unipath) for chlorhexidine. The Oxford staphylococcus NCTC 1675 was used as the indicator organism and the inoculum size was controlled by spectrometry.⁹ The interaction of mupirocin with a water solubilised fraction of pooled nasal secretions, dialysed against a cellulose ester membrane with a molecular weight cutoff point of 1000 Da (Sigma, Poole, Dorset, UK) to remove salts and minor fractions released during solubilisation, was examined by a matrix assisted laser desorption time of flight mass spectrometer (MALDI-TOF MS 4; Kratos, Manchester, UK), as described previously.⁹

RESULTS

MALDI-TOF analysis showed that mupirocin, which had a mass/charge peak of 592 (fig 1A), was not absorbed by the main fraction of pooled nasal secretions (fig 1B) and should still remain active (fig 1C). This was confirmed in bioassay when, unlike chlorhexidine, the activity of mupirocin remained unaltered when incubated with nasal secretions for up to eight hours at 37°C. Table 1 shows that the activity of chlorhexidine was significantly reduced from 1.5% to 38.5% of the initial concentration of 100 and 1000 mg/litre, respectively. Recoverable activities of mupirocin and chlorhexidine were unaffected by time.

DISCUSSION

In a molecular assessment, MALDI-TOF mass spectroscopy, which allows direct capture and characterisation of complex spectra,¹⁰ showed that the extract of nasal secretions did not significantly absorb mupirocin. This has not been described previously. Nasal secretions serve to eliminate substances introduced into the nose, and consist of mucopolysaccharides and mucoprotein¹¹ which, like mupirocin, possess an overall

Abbreviations: MALDI-TOF, matrix assisted laser desorption time of flight mass spectrometry; MRSA, methicillin resistant strain

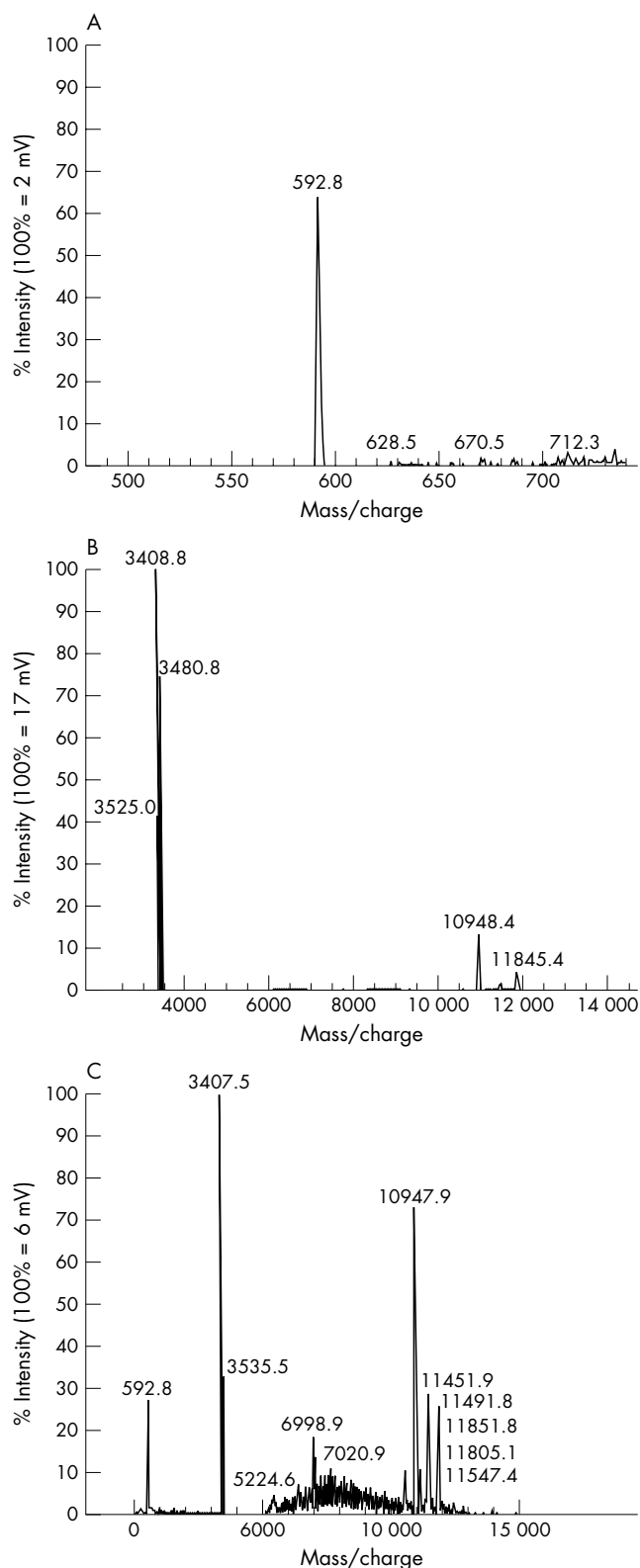


Figure 1 Matrix assisted laser desorption time of flight mass spectrometry analysis of the interaction of mupirocin with a solubilised fraction of nasal secretions. (A) Mass spectrum of mupirocin; (B) mass spectrum of a solubilised fraction of nasal secretions; (C) interaction of mupirocin with nasal secretions.

negative charge. It is probable that negative charges on the major components of nasal secretions repel mupirocin, preventing any appreciable interactions.

“Nasal secretions serve to eliminate substances introduced into the nose, and consist of mucopolysaccharides and mucoprotein”

Take home messages

- Nasal secretions, consisting of mucopolysaccharides and mucoprotein, eliminate substances introduced into the nose
- MALDI-TOF mass spectroscopy showed that the extract of nasal secretions did not significantly absorb mupirocin, explaining the clinical efficacy of this antibiotic for the treatment of nasal infections
- In contrast, chlorhexidine was shown to be inactivated by nasal secretions, explaining its lack of clinical efficacy for the treatment of nasal infections

This is the opposite to chlorhexidine, which has highly positively charged quaino groups¹² and is known to be inactivated by saliva which, like nasal secretions, contains negatively charged molecules.¹³ Indeed, bioassay confirmed that chlorhexidine is unlikely to have valuable intranasal activity because only the equivalent of 1.5 mg/litre remained from the initial 100 mg/litre and 385 from 1000 mg/litre. This is important because minimum inhibitory concentrations of chlorhexidine for several strains of MRSA have been reported to be from 2 to 8 mg/litre.⁷ By extrapolating our results it can be calculated that 0.26 ml of a 1% cream would be needed in each nostril to achieve a dose of 10 mg, compared with the “match head” sized amount required for mupirocin (~40–50 µl).³ Thus, the unreliability of chlorhexidine used intranasally to control MRSA carriage might result from the use of an insufficient amount.

Bioavailability in, and interaction with, nasal secretions could be a useful model for the development of newer analogues of mupirocin or similar agents, such as SB 205952.¹⁴ Indeed, using MALDI-TOF together with bioassay and the evaluation of killing kinetics predicted the effectiveness of 5% povidone-iodine against *S aureus* in a controlled trial (RLR Hill and JJ Wade. Presented at the 40th interscience congress on antimicrobial agents and chemotherapy, Toronto, 17–20 September 2000).

ACKNOWLEDGEMENTS

Thanks to A Jackson for assistance with mass spectrometry and the South London Public Health Laboratory and Joint Microbiology Research Unit for facilities at King's Denmark Hill Campus.

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Accepted for publication 6 July 2001

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