Anti-staphylococcal activity of indolmycin, a potential topical agent for control of staphylococcal infections

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Received 26 April 2004; returned 26 May 2004; revised 4 June 2004; accepted 6 June 2004

Objectives: We sought to investigate the anti-staphylococcal activity of indolmycin, with particular emphasis on comparing its activity with fusidic acid and mupirocin.

Methods: Established procedures were used to examine the activity of indolmycin against a range of clinical isolates, including strains resistant to fusidic acid and mupirocin. Indolmycin-resistant mutants were recovered and characterized phenotypically and genotypically.

Results: Indolmycin was bacteriostatic and demonstrated good activity against MSSA (methicillin-susceptible Staphylococcus aureus), MRSA (methicillin-resistant S. aureus) and VISA (vancomycin-intermediate S. aureus), including strains resistant to mupirocin or fusidic acid. Spontaneous indolmycin-resistant mutants occurred at a lower frequency than those selected by mupirocin or fusidic acid and exhibited no cross-resistance with the comparative drugs. High-level resistance (indolmycin MIC 128 mg/L) that was associated with an H43N mutation in tryptophanyl-tRNA synthetase (TrpS), the target enzyme of indolmycin, resulted in loss of bacterial fitness. However, the locus responsible for low-level indolmycin resistance (indolmycin MICs 8–32 mg/L) was not identified.

Conclusions: Indolmycin is a potent anti-staphylococcal agent, which exhibits activity against mupirocin- and fusidic acid-resistant strains. Indolmycin might be a candidate for development as a topical agent in the treatment of staphylococcal infections and nasal carriage of MRSA.

Keywords: Staphylococcus aureus, antibiotics, dermatological infections, control of MRSA carriage

Introduction

Mupirocin and topical formulations of fusidic acid have important roles in the management of superficial infections caused by Staphylococcus aureus. Both agents have been used as topical therapies for atopic eczema and impetigo and mupirocin is also used for eradication of nasal carriage of methicillin-resistant S. aureus (MRSA). However, the rising prevalence of resistance to mupirocin and fusidic acid in S. aureus is challenging the benefit of these drugs. There is therefore a growing need to find alternative topical antimicrobial agents for the control of MRSA carriage and dermatological infections caused by S. aureus.

Indolmycin, which selectively inhibits bacterial tryptophanyl-tRNA synthetase (TrpS), was discovered in 1960. However, this antibiotic was not developed as a systemic agent because it lacks sufficient activity against the majority of commonly occurring pathogenic bacteria. Nevertheless, early work demonstrated that indolmycin has potent anti-staphylococcal activity which suggests that this antibiotic could be a candidate for a new topical agent effective against S. aureus. Since no recent work has been conducted with indolmycin, we decided to re-evaluate this antibiotic as a potential anti-staphylococcal agent, with particular emphasis on comparing its activity with fusidic acid and mupirocin.

In this study, we demonstrate that the anti-staphylococcal activity of indolmycin against clinical isolates of S. aureus is comparable to both fusidic acid and mupirocin. Furthermore, there is a lower potential for development of indolmycin resistance relative to the comparative agents and high-level resistance to indolmycin is accompanied by significant reductions in bacterial fitness.

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Materials and methods

Bacterial strains, growth media and antibiotics

*S. aureus* 8325-4 was used as a standard laboratory strain. Clinical *S. aureus* used for comparative susceptibility testing were isolates obtained from around the world and maintained in a culture collection belonging to the University of Leeds. Clinical isolates expressing resistance to either fusidic acid or mupirocin were also investigated. Strains were grown in Iso-Sensitest broth (ISB) or agar (ISA) (Oxoid, Basingstoke, UK). Indolmycin was a gift from Pfizer (Sandwich, Kent, UK).

Determination of susceptibility to antimicrobial agents

Minimum inhibitory concentrations (MICs) were determined by microdilution in ISB. Microdilution was carried out in 384-well microtitre plates with inocula of $10^6$ bacteria, and the MIC defined as the lowest concentration of antibiotic completely inhibiting bacterial growth after 18 h of incubation at 37°C.

Effects of indolmycin on culture turbidity and bacterial viability and synergic interactions with other antibiotics

Time–kill curves in ISB with indolmycin were carried out on 8325-4 as previously described. The susceptibility of strain 8325-4 to either fusidic acid or mupirocin combined with indolmycin was determined by a modified chequerboard technique in ISB using 384-well microtitre plates.

Selection and characterization of indolmycin-resistant mutants

Mutation frequencies to resistance, growth rates and relative competitive fitness were determined as previously described. PCR amplification of the entire *trpS* gene was carried out using oligonucleotide primers *trpS*-55 (5'-AAA CCT AAT TTT TCA GAT AAG TTT CTA CAC) and *trpS*-1244 (5'-CTT TTA ATC CTA GTA GAG GTA GAC GTT A) and DNA sequencing was carried out from these same primers.

Results

In vitro activity of indolmycin against clinical *S. aureus* isolates, including strains resistant to fusidic acid and mupirocin

Indolmycin was active against all organisms in the panel of 67 clinical strains tested, displaying MICs in the range 0.125–2 mg/L (Table 1). Specific clinical isolates of *S. aureus* known to express resistance to either mupirocin or fusidic acid were also susceptible to indolmycin (Table 1).

Effects of indolmycin on growth and survival of *S. aureus* 8325-4

The MIC of indolmycin for *S. aureus* 8325-4 was 0.25 mg/L. The effects of indolmycin on the growth and viability of this strain at multiples of the MIC ($\times 4 \times 32$) were evaluated over a 6 h period. Indolmycin exhibited a bacteriostatic action since there was no decline in the absorbance or viability of cultures (Figure 1).

Activity of indolmycin combined with fusidic acid or mupirocin

Indifferent responses were observed when indolmycin was combined with fusidic acid or mupirocin. Thus the results obtained when indolmycin was combined with each of the other antibiotics did not differ from inhibition observed in the presence of a single agent (data not shown).

Mutation frequency for resistance to indolmycin and comparison with fusidic acid and mupirocin

Indolmycin-resistant mutants of *S. aureus* 8325-4 selected on plates containing indolmycin at 1.0 mg/L ($\times 4$ the ISB microdilution MIC) arose at a frequency of $9.8 \pm 0.84 \times 10^{-9}$. In contrast, with similar selections, mutants resistant to fusidic acid arose

Table 1. Activity of indolmycin against clinical strains of *S. aureus* and in comparison with fusidic acid and mupirocin

<table>
<thead>
<tr>
<th>Organisms (no. of strains)</th>
<th>Antibiotic</th>
<th>MIC mg/L</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>range</td>
</tr>
<tr>
<td>MSSA (32)</td>
<td>fusidic acid</td>
<td>0.06–0.125</td>
</tr>
<tr>
<td></td>
<td>mupirocin</td>
<td>0.06–0.25</td>
</tr>
<tr>
<td></td>
<td>indolmycin</td>
<td>0.125–0.5</td>
</tr>
<tr>
<td>MRSA (28)</td>
<td>fusidic acid</td>
<td>0.06–2</td>
</tr>
<tr>
<td></td>
<td>mupirocin</td>
<td>0.06–&gt;512</td>
</tr>
<tr>
<td></td>
<td>indolmycin</td>
<td>0.125–2</td>
</tr>
<tr>
<td>VISA (7)</td>
<td>fusidic acid</td>
<td>0.06–0.125</td>
</tr>
<tr>
<td></td>
<td>mupirocin</td>
<td>0.06–1</td>
</tr>
<tr>
<td></td>
<td>indolmycin</td>
<td>0.125–2</td>
</tr>
<tr>
<td>FusR-SA (10)$^a$</td>
<td>fusidic acid</td>
<td>2–256</td>
</tr>
<tr>
<td></td>
<td>mupirocin</td>
<td>0.125–0.25</td>
</tr>
<tr>
<td></td>
<td>indolmycin</td>
<td>8–512</td>
</tr>
<tr>
<td>MupR-SA (10)$^b$</td>
<td>mupirocin</td>
<td>0.125–0.5</td>
</tr>
<tr>
<td></td>
<td>indolmycin</td>
<td>0.125–2</td>
</tr>
</tbody>
</table>

$^a$Fusidic acid resistance resulting from mutations in *fusA* ($n=7$), *fusB* ($n=1$) and uncharacterized mechanisms ($n=2$).

$^b$Mupirocin resistance resulting from mutations in *ileS* ($n=1$), *mupA* ($n=5$), and uncharacterized mechanisms ($n=4$).
Anti-staphylococcal activity of indolmycin

Figure. 1. Effect of indolmycin on growth (a) and survival (b) of S. aureus 8325-4. Indolmycin (×4–×32 MIC) was added to an early logarithmic phase culture in ISB at time zero and samples taken at the times indicated for determination of culture absorbance at 600 nm (a) and viable bacteria (b). The remainder of the culture served as a drug-free control.

with a frequency of 7.6 ± 1.3 × 10^{-7} and those resistant to mupirocin with a frequency of 7.2 ± 0.93 × 10^{-8}.

Indolmycin-resistant mutants did not exhibit cross-resistance to fusidic acid or mupirocin

Twenty-one indolmycin-resistant mutants were selected following plating of strain 8325-4 on indolmycin-selective ISA. Indolmycin MICs for 20 strains ranged from 8 to 32 mg/L, whereas a single strain exhibited high-level resistance (MIC 128 mg/L). Additional mutants exhibiting high-level resistance to indolmycin were selected by plating cultures onto ISA containing indolmycin at 32 mg/L. None of the indolmycin-resistant mutants displayed cross-resistance to fusidic acid or mupirocin (data not shown).

Genetic basis of indolmycin resistance

Mutations at His-43 within the TrpS enzyme, confer indolmycin resistance in Bacillus stearothermophilus.\(^6\) Whether such mutations occur in S. aureus is unknown. The trpS genes of eight low-level indolmycin-resistant mutants (indolmycin MICs 8–32 mg/L) were sequenced and compared with trpS from strain 8325-4 (indolmycin MIC 0.25 mg/L). No differences were detected. In contrast, high-level indolmycin-resistant mutants (indolmycin MIC 128 mg/L) possessed a single nucleotide substitution resulting in an amino acid change of H43N in TrpS.

Low-level resistance to indolmycin in S. aureus has been reported to result from alterations in the affinity of tryptophan and tyrosine transport systems that are used for uptake of the antibiotic.\(^5\) The genes encoding the tryptophan and tyrosine transporters have not been characterized in S. aureus. However, candidate transporter genes were identified in the S. aureus Mu50 genome on the basis of protein homology with such transporters from Escherichia coli and Bacillus subtilis. Candidate genes SAV2838 and SAV2316 were PCR amplified and sequenced. However, no differences in the nucleotide sequences of these genes were detected when compared to 8325-4.

Fitness of indolmycin-resistant mutants

Antibiotic resistance in bacteria is often accompanied by reductions in bacterial fitness.\(^7\) The fitness of indolmycin-resistant S. aureus mutants was therefore examined. Low-level indolmycin resistance was associated with only a minor fitness cost (doubling times of 39.4 ± 0.2 min to 45.1 ± 2.7 min compared to 38.8 ± 0.8 min for the parental strain 8325-4 and a reduction of 7% in relative competitive fitness). In contrast, high-level indolmycin-resistant mutants carrying the H43N mutation were unfit, demonstrating significant increases in their doubling times (range 81.7 ± 5.0 min to 87.6 ± 4.1 min) and a 40–44% decrease in relative competitive fitness.

Discussion

Fusidic acid and mupirocin are widely used as topical antibiotics for the treatment of superficial staphylococcal infections and mupirocin also plays an important role in eradicating MRSA carriage.\(^1\) Unfortunately, resistance to both agents is emerging in S. aureus,\(^2,3\) which is likely to limit the effectiveness of these antibiotics as topical preparations. Consequently, alternative agents are required. Ideally, such agents should not be used both systematically and topically because development of resistance through topical use may then threaten efficacy of the antibiotic when administered systemically for serious staphylococcal infections.\(^2\) Data presented in this paper suggest that indolmycin, a narrow-spectrum antibiotic discovered more than 40 years ago,\(^4\) which has not been developed for systemic use, could be a candidate for a new topical agent to control staphylococcal infections.

The results reported here demonstrate that indolmycin exhibits good activity against numerous clinical strains of S. aureus, including methicillin-susceptible S. aureus (MSSA), MRSA and vancomycin-intermediate S. aureus (VISA). Furthermore, the anti-staphylococcal activity of indolmycin was comparable to the established topical agents fusidic acid and mupirocin. Moreover, a collection of S. aureus clinical isolates, which possessed common resistance mechanisms to either fusidic acid or mupirocin, were also fully susceptible to indolmycin. These results are consistent with the separate target sites for each inhibitor and the absence of structural similarity between the antibiotics.

As a structural analogue of L-tryptophan, indolmycin competitively inhibits bacterial TrpS\(^5\) and therefore belongs to the class of antibacterials, including mupirocin, that prevents the aminocacylation of tRNA.\(^7\) The accumulation of uncharged tRNA in turn arrests protein synthesis and induces the stringent response pathway, causing cessation of bacterial growth.\(^9\)
Inhibition by indolmycin is therefore predicted to produce a bacteriostatic response, consistent with the observations reported here for \textit{S. aureus} 8325-4. In contrast, indolmycin exhibits concentration-dependent killing of \textit{Helicobacter pylori} suggesting that it may have additional modes of action in this organism.

The potential for emergence of resistance to indolmycin in \textit{S. aureus} is obviously an important consideration with regard to its possible application as a topical antimicrobial agent. Although the future emergence of a transferable indolmycin-resistance determinant cannot be ruled out, genomic mutation currently appears to be the only mechanism for development of indolmycin resistance in \textit{S. aureus}. Indeed, we have demonstrated that high-level resistance to indolmycin is associated with a point mutation (H43N) in TrpS. Furthermore, although we were unable to identify the mutation, or mutations, responsible for low-level indolmycin resistance, these have previously been ascribed to point mutations in the structural genes for aromatic amino acid transporters. Although there appear to be several routes by which mutants resistant to indolmycin can arise, it is reassuring to note that the frequency of development of resistance to indolmycin is lower than the development of spontaneous resistance to fusidic acid or mupirocin.

Furthermore, high-level resistance to indolmycin in 8325-4 was accompanied by significant reductions in bacterial fitness. These unfit mutants all carried the amino acid substitution H43N within the TrpS enzyme. The histidine residue at position 43 is directly involved in binding tryptophan as well as stabilization of the Trp-adenylate intermediate. It is therefore likely that the loss of this key histidine residue accounts for the significant reduction in bacterial fitness associated with this genotype. If this mutation also arose \textit{in vivo} then organisms expressing high-level resistance to indolmycin might be counter-selected in the absence of indolmycin-selection pressure, i.e. their carriage might cease upon removal of the topical medication.

Since inhibitors of aminocycl-tRNA synthetases are specific for their corresponding enzyme, cross-resistance between these agents and other drug-classes at the level of the drug target are not expected. However, mutations within genes encoding membrane transporters might confer cross-resistance to structurally unrelated drugs. Nevertheless, low-level indolmycin-resistant mutants (presumed permease mutants) did not exhibit cross-resistance to fusidic acid or mupirocin, suggesting that neither fusidic acid nor mupirocin utilize indolmycin-specific transport systems.

In conclusion, the bacteriostatic mode of action of indolmycin is comparable to the anti-staphylococcal activities of both fusidic acid and mupirocin and the agent has excellent \textit{in vitro} activity against fusidic acid- and mupirocin-resistant organisms. We therefore suggest that further studies be conducted with indolmycin to determine whether it might be developed as a topical agent for the treatment of staphylococcal infections and control of MRSA carriage.

Acknowledgements

We thank Drs S. Bradley and C. Kauffman of the Department of Veterans Affairs Medical Center, University of Michigan Medical School, Ann Arbor, MI, USA for providing mupirocin-resistant clinical isolates of \textit{S. aureus}. J.G.H. acknowledges receipt of a PhD scholarship from the Association of Commonwealth Universities, UK.

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