conducting a dose–response. This is reinforced by the report by Schrage et al., who required a concentration range of 1–5 mg/mL IVIg to demonstrate the inhibition of 12 different superantigens. Moreover, these authors found that most commercial IVIg preparations had even better neutralizing activity than the preparation used in our study, reaffirming the point regarding the source of IVIg.

The main purpose of our study was to evaluate IVIg action on inflammation and outcome in S. pyogenes infection, rather than to re-evaluate the in vitro activity of IVIg against purified superantigens. For this reason, the superantigen-inhibiting properties of IVIg were assessed as a preliminary step only. Culture supernatants were used rather than purified superantigens, as these preparations had even better neutralizing activity than the preparations used in our study, reaffirming the point regarding the source of IVIg.

The key questions arising from our study include, first, whether the modulation of inflammation and bacterial clearance provided by IVIg in S. pyogenes-infected HLA-DQ8 mice has anything to do with the ability of IVIg to inhibit superantigen-induced mitogenesis? IVIg has several additional potentially protective actions, and studies are on-going to evaluate these. Secondly, it remains unclear if IVIg can confer additional benefit to ‘gold standard’ therapy in human streptococcal toxic shock, as, in HLA-DQ8 mice, this was not evident.

Transparency declarations

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References


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Antimicrobial effectiveness of ketoconazole against metronidazole-resistant Helicobacter pylori isolates from Iranian dyspeptic patients

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Keywords: antimycotics, oral yeasts, dual activity

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S. Massarrat2

Sir,

The rate of Helicobacter pylori infection in Iran reaches up to 85% and a considerable proportion of infected individuals develop gastric diseases.1 Furthermore, the eradication rate of H. pylori infection is low, mainly due to a considerable number (37%) of metronidazole-resistant strains.2 Accordingly, investigators have been trying to substitute this antibiotic. Since fatty acids, namely cholesterol glucosides, have been found in the cell membrane of Helicobacter species, investigators have speculated that imidazole antimycotics such as ketoconazole might interfere with the biosynthesis of these fatty acids from cholesterol.1 The dual activity of ketoconazole against both H. pylori and fungi might be valuable because oral yeasts have been proposed as potent reservoirs as well as protective vehicles for transmission of H. pylori to the human gastrointestinal tract.3 Thus design of chemothapeutic regimens containing ketoconazole will benefit patients by eradicating H. pylori as well as reducing the number of yeast microflora harbouring H. pylori.

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In this study the antibacterial activity of ketoconazole against metronidazole-resistant and -susceptible strains of *H. pylori* was assessed by agar dilution method (ADM) and disc diffusion method (DDM).

Fifty *H. pylori* isolates were obtained (2004–2005) from biopsy cultures of 50 patients who were referred to endoscopy units at the Digestive Diseases Research Centre of Tehran University of Medical Sciences, Tehran, Iran. Patients were grouped as those with oesophageal reflux (18), gastritis (17), ulcer (13) and gastric cancer (2). Biopsies were cultured on selective brucella agar (Merck) containing blood under microaerobic conditions. Bacterial isolates were identified as *H. pylori* on the basis of Gram’s stain, showing Gram-negative spiral forms, and positive urease, oxidase and catalase tests. The resistance of 50 *H. pylori* strains to metronidazole (Sigma) was assessed by ADM according to CLSI (formerly NCCLS) guidelines. Metronidazole in ethanol was added to Mueller–Hinton agar (Merck) plates containing blood, to reach final dilutions of 32, 16, 8, 4 and 2 mg/L. Aliquots (5 μL) of bacterial suspensions with turbidities equivalent to that of a no. 2 McFarland standard were spot-inoculated on the surface of agar. Plates were examined for growth or inhibition after 3 days of appropriate incubation. The MIC of metronidazole was determined as >8 mg/L. The antibacterial effectiveness of ketoconazole (Sigma) against 50 *H. pylori* isolates was assessed, using ADM (15 isolates) and DDM (35 isolates). In ADM, ketoconazole in DMSO was added to Mueller–Hinton blood agar, with final dilutions of 32, 16, 8, 4 and 2 mg/L. Among fifteen isolates, five were resistant to metronidazole. Aliquots (5 μL) of bacterial suspensions with turbidities equivalent to that of a no. 2 McFarland standard were spot-inoculated on the surface of agar plates. Plates were examined after 3 days of microaerobic incubation and MICs were determined. Similar bacterial suspensions of *Escherichia coli* were spot-inoculated on Mueller–Hinton agar plates with serial dilutions of ketoconazole (128–4 mg/L). In DDM, serial dilutions of ketoconazole (32, 16, 8, 4 and 2 mg/L) were prepared in DMSO. Among 35 recruited isolates, eleven were metronidazole-resistant. Aliquots (100 μL) of bacterial suspensions were surface-inoculated on Mueller–Hinton blood agar. Each ketoconazole dilution (10 μL) was introduced into paper discs on the surface of the agar. After microaerobic incubation, growth inhibition zones were measured. Strains with inhibition zone diameters (IZDs) for 35 *H. pylori* strains obtained with different dilutions of ketoconazole were determined. Susceptibility of bacterial isolates was determined according to IZDs at 8 mg/L. Nineteen isolates (54.1%) were susceptible and 16 isolates (45.9%) were highly susceptible to ketoconazole. Among 11 metronidazole-resistant strains, 7 (63.6%) were susceptible and 4 (36.4%) were highly susceptible to ketoconazole. *H. pylori* isolates from patients with gastritis, gastric ulcer or cancer were similarly susceptible to ketoconazole.

All the 50 isolates were inhibited by ketoconazole, although *E. coli* was highly resistant. The MIC of ketoconazole was determined as 8 mg/L in both ADM and DDM. Similarly, inhibitory concentrations of the antymycotic miconazole (MIC 2–32 mg/L) against *H. pylori* have been reported.

Ketoconazole can be considered as a substitute for metronidazole. Regarding the safety of ketoconazole, it is proposed that the dose required to inhibit mammalian cells is much higher than that required for fungi or bacteria. Since the intracellular existence of *H. pylori* in yeast plays an important role in the persistence of *H. pylori* in the human oral cavity, administration of ketoconazole not only leads to eradication of *H. pylori*, but might also reduce the chance of recurrence of bacterial infection by affecting colonization of yeasts in the gastrointestinal tract.

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**Transparency declarations**

None to declare.

**References**


**Table 1. Means of inhibition zone diameters for 35 *H. pylori* strains obtained with different dilutions of ketoconazole**

<table>
<thead>
<tr>
<th>Ketoconazole (mg/L)</th>
<th>Mean diameter (mm)</th>
<th>95% CI of the difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>32.257</td>
<td>30.81–33.71</td>
</tr>
<tr>
<td>16</td>
<td>27.000</td>
<td>25.74–28.26</td>
</tr>
<tr>
<td>8</td>
<td>22.029</td>
<td>20.81–23.25</td>
</tr>
<tr>
<td>4</td>
<td>16.343</td>
<td>15.32–17.36</td>
</tr>
<tr>
<td>2</td>
<td>1.486</td>
<td>–0.04–3.01</td>
</tr>
</tbody>
</table>

95% CI.95% confidence interval.