In vitro susceptibility of *Trichomonas vaginalis* to AT-specific minor groove binding drugs

Porntip Chavalitshewinkoon-Petmitr1*, Muhamin Ramdja1, Somsri Kajorndechakiat1, Raymond K. Ralph2, William A. Denny3 and Prapon Wilairat4

1Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, Bangkok; 2Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, Thailand; 3School of Biological Sciences, The University of Auckland, Auckland; 4Auckland Cancer Society Research Centre, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand

Received 14 July 2002; returned 5 February 2003; revised 11 February 2003; accepted 6 May 2003

Trichomoniasis is one of the most common sexually transmitted diseases, with around 120 million world-wide suffering from *Trichomonas vaginalis*-induced vaginitis every year. Although trichomoniasis can be treated with metronidazole, the prevalence of metronidazole-resistant *T. vaginalis* seems to be increasing. Since the percentage of AT base pairs in *T. vaginalis* DNA (71%) is very much higher than in human cells, in this study a series of bisquaternary quinolinium salt compounds with high AT-binding specificity were tested for their antitrichomonal activities. Minimum inhibitory concentrations (MICs) were determined for these compounds against a local strain of *T. vaginalis* in culture. Among 14 bisquaternary quinolinium compounds tested, an N-ethyl derivative was the most effective drug against *T. vaginalis*, being nearly as potent (MIC = 0.16 µM) as metronidazole (MIC = 0.096 µM), and with low toxicity towards human cells. The nature of the substitution at the quinolinium quaternary centre appears to be important in terms of effectiveness of bisquaternary compounds against the parasite. In contrast, no clear relationships could be seen for substituents on the quinolinium ring; Me and Cl substituted analogues showed higher activity against trichomonads, whereas OMe, NHMe and NH₂ substituents decreased activity.

Keywords: *Trichomonas vaginalis*, AT-specific drugs, DNA minor groove

Introduction

*Trichomonas vaginalis* is one of the most common organisms causing vaginitis in women world-wide. For the most part, *T. vaginalis* is considered to present a classical venereal disease, despite its slight potential for non-venereal transmission. When the antitrichomonal metronidazole was first introduced in 1959, cure rates approximated 95%, but within 2 years of its introduction, the first case of metronidazole resistance was reported in Canada4 and nitroimidazole resistance has now been observed in most areas of the world. In the trichomonad, metronidazole is enzymically reduced to its cytotoxic metabolite by a single electron transfer from ferredoxin to the nitro group of metronidazole and metronidazole-resistant organisms have reduced levels of ferredoxin gene transcription and intracellular ferredoxin.5 Thus, there is a need for development of new antitrichomonal drugs.

DNA minor groove binding drugs (MGBs) are small compounds that bind specifically to the minor groove of B-DNA. MGBs can be divided into two classes based on their binding sequence preferences. One class of MGB (e.g. anthramycins) binds preferentially to GC sites by irreversible alkylation at the 2-amino group of guanines, but the majority of such compounds preferentially bind reversibly at AT-rich regions in the minor groove (AT-specific MGBs), without major distortion of the DNA structure.6 These drugs display a broad spectrum of antiviral, antibacterial, antitumour and antiprotozoal activity.7 DNA topoisomerase I may be the pharmacological target of AT-specific minor groove binding ligands.8

The AT-specific MGB class of bisquaternary quinolinium compounds (e.g. 4-[4-[4-[4(N-methylquinolinium)-amino]benzamido]anilino]-N-methylpyridinium, SN 6999; Figure 1) were developed initially as antileukaemic drugs.9 As the genome of *T. vaginalis* is heavily weighted in favour of AT base pairs (71%),10 we tested a series...
of compounds related to SN 6999 to determine whether they could exhibit antitrichomonal activity in culture.

Materials and methods

Parasite

A local strain of *T. vaginalis* employed in this study was obtained from a patient at Rajavithi Hospital, Bangkok, Thailand.

Axenic culture of *T. vaginalis*

Trypticase yeast extract maltose (TYM) medium was used for culturing *T. vaginalis*. A single colony of *T. vaginalis* was obtained aseptically from a patient at Rajavithi Hospital, Bangkok, Thailand.

Drug preparation

Bisquaternary quinolinium compounds were initially dissolved in DMSO at a concentration of 9 mM. Metronidazole was dissolved in sterile distilled water at a concentration of 1 mM. Working drug solutions were freshly prepared before use by diluting with TYM medium to the required concentrations.

Test of AT-specific minor groove binding drugs on *T. vaginalis*

Standard microtitre plates with 96 flat-bottomed wells were used in the test. Drugs of various concentrations were added to the wells in 200 µL aliquots. A 200 µL suspension containing 1 × 10^4 T. vaginalis was then placed into each well. All the drug and control studies were carried out in duplicate; a minimum of two independent experiments was conducted for each drug test. The plates were incubated at 37°C and were examined after 24 h with an inverted microscope (25× magnification) for motile trichomonads. The minimal inhibition concentration (MIC) is defined as the lowest drug concentration in which no motile organism was seen. DMSO at 2% (v/v) concentration had no effect on parasite motility.

Table 1. MICs of bisquaternary quinolinium compounds against *Trichomonas vaginalis*

<table>
<thead>
<tr>
<th>Drug number</th>
<th>R</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>MIC (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>Et</td>
<td>H</td>
<td>H</td>
<td>0.16</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>Me</td>
<td>NH₂</td>
<td>H</td>
<td>12.8</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>Me</td>
<td>H</td>
<td>5-NH₂</td>
<td>9.6</td>
</tr>
<tr>
<td>4</td>
<td>6-Me</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>0.64</td>
</tr>
<tr>
<td>5</td>
<td>6-OMe</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>9.6</td>
</tr>
<tr>
<td>6</td>
<td>6-NMe₂</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>6.4</td>
</tr>
<tr>
<td>7</td>
<td>6-NH₂</td>
<td>Me</td>
<td>H</td>
<td>NH₂</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>6-NH₂</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>128</td>
</tr>
<tr>
<td>9</td>
<td>6-Cl</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>0.64</td>
</tr>
<tr>
<td>10</td>
<td>7-Cl</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>0.96</td>
</tr>
<tr>
<td>11</td>
<td>7-NH₂</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>9.6</td>
</tr>
<tr>
<td>12</td>
<td>8-Me</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>6.4</td>
</tr>
<tr>
<td>13</td>
<td>8-OMe</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>0.64</td>
</tr>
<tr>
<td>14</td>
<td>8-Cl</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>12.8</td>
</tr>
</tbody>
</table>

Results and discussion

A local strain of *T. vaginalis* employed in this study had an MIC of 0.096 µM for metronidazole under aerobic conditions. In the study of a series of bisquaternary quinolinium compounds with substituents mainly in the quinolinium ring, together with limited additional compounds bearing amino substituents in other positions, the most effective (MIC = 0.16 µM) against *T. vaginalis* was the unsubstituted compound 1, but this was also the only compound with an ethyl substitution at R₁ (Table 1 and Figure 1). No compounds with other R₁ substitutions were available to determine the significance of this.

In the R₁ = methyl series, the effects of substitution in the quinolinium ring were also examined. Substitution of Me and Cl groups at C6 (4 and 9) gave analogues with higher activity against trichomonads (MIC = 0.64 µM in both cases) compared to other C-6 substituents (OMe, NMe₂, NH₂; compounds 5–8). A Cl substituent at the C7 position was best (10; MIC = 0.96 µM), compared with NH₂ (11; MIC = 9.6 µM). Finally, in the C-8 position, OMe was the best (13; MIC = 0.64 µM).

Structure–antileukaemic activity relationships of bisquaternary quinolinium compounds have been analysed previously. For this activity, there was an absolute requirement for a quaternary centre; the free bases where R₁ = H showed no antileukaemic effect, whereas the methyl bisquaternary salt (R₁ = Me) was highly inhibitory, and there was relatively little difference between R₁ = Me and R₁ = Et. Derivatives with electron donor substituents (CH₃, OCH₃, NMe₂, NH₂) in the quinoline ring provided the most active antileukaemic compounds. Both 6- and 7-aminquinoline variants were also highly active, and showed less chronic toxicity, with several 6-aminquinoline variants giving a high percentage of indefinite survivors in L1210 tests.

Against *T. vaginalis*, in the R₁ = Me series, NH₂ substitution at R₂ (compound 2) or 5-NH₂ at R₃ (compound 3) were less effective than the unsubstituted compound 1. The MICs for compounds with substituents (CH₃, OCH₃, NMe₂, NH₂) in the quinoline ring providing electron donation to the 4-NH position were also higher than for 1. Several 6- and 7-aminquinoline variants (compounds 7, 8 and 11), highly active as antileukaemic compounds, also showed low comparative efficacy against *T. vaginalis*. Again in contrast to the antileukaemic activity, compounds with substituents (6-Cl, 7-Cl, 8-OMe; compounds 9, 10, 13) providing electron withdrawal from the 4-NH position were more active than those with electron-donating groups (although still not as active as the unsubstituted compound 1). Amino group substitution on the anilino ring (R₃) in compounds 3 and 7 also decreased the activity against *T. vaginalis*.
The most active compound, 1, was almost as potent as metronidazole against wild-type *T. vaginalis*. This compound has also been shown to have relatively low toxicity to human cells (IC$_{50}$ Jurkat leukaemia cells > 20 µM; R.K. Ralph, unpublished data). Although the MGB compounds were not tested against metronidazole-resistant strains of *T. vaginalis*, the targets of the two drugs are different. Whether or not this renders cross-resistance unlikely must be tested in future studies. More work needs to be done to decide whether bis-quaternary quinolinium compounds can be successfully developed for use in the chemotherapy of trichomoniasis. In particular, the nature of the substitution at R$_1$ should be studied further.

Acknowledgements

We would like to thank Mr. Saiyud Incheng and Miss Niramol Thima, Department of Protozoology, Faculty of Tropical Medicine, Mahidol University for their technical assistance. This work was supported by a grant from Mahidol University. PW is a Senior Researcher of the Thailand Research Fund.

References