amoebicidal effects against Acanthamoeba castellanii and Balamuthia mandrillaris (N. A. K., K. O. and G. J. G., unpublished data). Future work will include identification of the nature of the compound(s), such as the chemical structure and properties, as well as its mode of action, and whether this is a known or novel compound.

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Transparency declarations

None to declare.

References


Antifungal activity against Candida albicans of nikkomycin Z in combination with caspofungin, voriconazole or amphotericin B

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Keywords: antifungal combinations, in vitro activity

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Sir,

Until the last decade, antifungal therapy was based mostly on drugs acting on the fungal membrane, such as amphotericin B and azoles, and the rationale for the use of combination therapy remained questionable.1 Thus, the only drug combination of two antifungals with two modes of activity used clinically, primarily in cryptococcosis, was amphotericin B and 5-fluorocytosine.2 The introduction of echinocandins, which act on the fungal cell wall by inhibiting glucan synthesis, opened the approach to explore different drug combinations, such as echinocandin and polyenes, or echinocandins and azoles,3,4 for various mycoses.

Nikkomycin Z inhibits chitin synthesis, by acting as a competitive analogue of chitin synthase substrate UDP-N-acetylglucosamine.5 Since chitin is found in most fungal cell walls,
This study reports on the assessment of in vitro activity of nikkomycin Z in combination with either amphotericin B, voriconazole or caspofungin against Candida albicans. C. albicans CBS 562, a laboratory strain, and a clinical strain, 58919, were used throughout the study. For the susceptibility tests, the Candida strains were grown on Sabouraud agar for 24 h and suspended in PBS to a concentration of 0.5–2.5 × 10⁶ cells/mL using haemocytometer counts. This stock suspension was further diluted in yeast nitrogen base (YNB) to a final concentration of 0.5–2.5 × 10³ cells/mL.

Caspofungin, voriconazole, amphotericin B and nikkomycin Z stock solutions were prepared at concentrations of 5 and 1 mg/mL, respectively, and kept at −70 °C. At the initial stage of the study, the sensitivity of Candida was tested for each drug using a broth microdilution technique, determining minimal inhibitory concentration (MIC)—the lowest drug concentration inhibiting yeast growth) and minimal fungicidal concentration (MFC—the lowest drug concentration killing the yeasts). The MIC and MFC values of amphotericin B, caspofungin and voriconazole from three to four experiments (data not shown) were compatible with the data reported in the literature, with no significant differences between the test strains. The nikkomycin Z values were high for both C. albicans strains, with differences between the strains. Drug combinations were assessed by checkerboard assays using the broth microdilution methodology. The experiment was performed in 96-well microtitre plates using YNB as a medium. The results presented are MIC values and are those recorded after 48 h of incubation at 37 °C. Each plate included rows with combinations of drugs and control rows of the drugs alone. The results were used to determine the fractional inhibitory concentration (FIC; in mg/L) of the combination of nikkomycin Z and either caspofungin, voriconazole or amphotericin B. FICs were calculated for MIC endpoint measurements taken from the micro-well with the lowest concentration of the drug combination needed to achieve the respective endpoints. The FIC of a drug for an individual isolate was calculated as the MIC of the drug when used in combination with another drug divided by the MIC of the drug when used alone. The FIC index (FICI) value was calculated by adding the FIC of nikkomycin Z to that of each of the other drugs. FICI values were interpreted as follows: FICI ≤ 0.5, synergistic; 0.5 < FICI ≤ 4, no interaction; FICI > 4, antagonistic. Table 1 shows the susceptibility of the C. albicans strains for three different drug combinations: amphotericin B + nikkomycin Z, voriconazole + nikkomycin Z and caspofungin + nikkomycin Z. For each combination, three separate tests were carried out for each C. albicans strain. The assays showed that the MIC values of nikkomycin Z in combination with voriconazole or caspofungin were lower in comparison with those of the drug alone. The analysis of the FICI data indicates that the effect of the three combinations is similar for both C. albicans strains. In two of the combinations (caspofungin + nikkomycin Z and voriconazole + nikkomycin Z), the result is synergy, whereas in the third combination (amphotericin B + nikkomycin Z), there was no interaction.

This study explored the in vitro anti-Candida activity of drug combinations from four classes: the polyenes, second-generation triazoles, echinocandins and nikkomycins. While both the polyenes and triazoles act on the fungal cell membrane, although through different mechanisms, the echinocandins and nikkomycins affect the cell wall, interfering with the synthesis of glucan and chitin, respectively. Our data show that combining voriconazole or caspofungin with nikkomycin Z resulted in a synergistic effect. To the best of our knowledge, the specific drug combinations used in our study against C. albicans have not been described before. Whether combinations of antifungals with different modes of activity could enhance the therapeutic efficacy beyond that of monotherapy should be corroborated in animal models, leading eventually to use in humans.

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### References
Cluster of multidrug-resistant Neisseria gonorrhoeae with reduced susceptibility to the newer cephalosporins in Northern Greece

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Keywords: gonorrhoea, therapy, STD

Sir,

Therapeutic options for gonococcal infection are nowadays limited due to the spread of gonococcal resistant to a wide variety of antimicrobials. The dramatic increments in the rates of quinolone-resistant gonococci and the current shortage of spectinomycin leave the newer cephalosporins as the last safe choice among the drugs that are currently recommended as first-line treatment. Therefore, increasing reports of reduced in vitro activities of expanded-spectrum cephalosporins against Neisseria gonorrhoeae are of serious concern.

The aim of this report from the Greek National Reference Centre for Neisseria gonorrhoeae (NRCNG) is to be aware of the presence, in Northern Greece, of a cluster of multiresistant gonococci that exhibit decreased susceptibility to the newer cephalosporins.

From December 2006 through January 2008, a total of 195 gonococcal strains were submitted to the NRCNG, and all were characterized by serovar identification with the GC serotyping panel (BACTUS AE, Sweden) and tested for susceptibility to various antimicrobials using the Etest (AB Biodisk, Sweden). By analysing the results, a group of 17 isolates showed cefotaxime MICs 0.25–1 mg/L, as opposed to ≤0.002–0.125 mg/L for the remaining gonococci. By extending susceptibility testing to other cephalosporins, these isolates were found to exhibit raised MICs to cefixime and ceftriaxone also, while being highly resistant to cefuroxime and non-susceptible to cefotaxin, cefazidime and cefepime. Cefalotin and aztreonam MIC50 values were increased up to 8-fold when compared with the ATCC 49266 strain, which was tested in parallel. This group of cephalosporin decreased susceptibility (CEDS) isolates consisted of non-penicillinase-producing, fluoroquinolone-resistant and multidrug-resistant strains with low-level resistance to penicillin G, tetracycline, erythromycin and chloramphenicol (Table 1).

CEDS isolates were allocated to a distinct serovar, Bpyut, not exhibited by any other strain isolated during the study period. Additionally, all shared the same plasmid content, harbouring only the cryptic gonococcal plasmid, and showed highly similar PFGE-generated SpeI restriction patterns, 14 of them being identical. CEDS strains were also of the same origin, all submitted from the Skin and Venereal Disease Hospital located in Thessaloniki. They accounted for 63% of 27 viable gonococci that were received from this setting and represented 39% of all 43 gonorrhoea cases reported from Northern Greece during the 14 month period running from December 2006 through January 2008.

We conclude that CEDS gonococci were disseminated in Northern Greece by clonal spread of a single strain. Epidemiological data were obtained through a standard questionnaire answered by the patients, including demographic and sexual behaviour data, previous sexually transmitted disease history and an area of acquisition of infection. No relation between the CEDS strains and any particular group of the population was apparent. Data were available for all CEDS strains apart from the first one isolated in December 2006. The second strain was isolated in the same month from a Spanish–English heterosexual contact, while the remaining 15 isolates were obtained after February 2008 from Greek heterosexual men who affirmed contact with female partners in Thessaloniki.

Due to only slight reductions in susceptibility to cefotaxime, which is the cephalosporin regularly tested, CEDS strains passed unnoticed. As a consequence, patients were given the standard regimen used for gonorrhoea in the Venereal Hospital of Thessaloniki since 2002, which included cefotaxime sodium (single intramuscular dose of 1.5 g), plus tetracycline (100 mg twice daily for 8 days). Although therapeutic failures were not reported, the fact that the CEDS strains were subsequently found resistant to both agents does not guarantee this treatment’s outcome, and this could be a cause for the persistence of these strains.

The susceptibility profile of the CEDS cluster of gonococci resembles those of N. gonorrhoeae strains with reduced susceptibility to newer β-lactams reported previously from Japan1–3 and elsewhere.4,5 Cephalosporin susceptibility reductions have been associated with various types of mosaic penicillin-binding protein 2, occurring via homologous recombination between penA genes of N. gonorrhoeae and other Neisseria species.2,5

Most of the strains with decreased susceptibility to the newer cephalosporins reported so far, including CEDS in this...