International Journal of Antimicrobial Agents 47 (2016) 178–183

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

In vitro pharmacodynamic modelling of anidulafungin against *Candida* spp.

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ARTICLE INFO

Article history:
Received 6 May 2015
Accepted 16 December 2015

Keywords:
Pharmacodynamic
In vitro
Modelling
Anidulafungin
Candida

ABSTRACT

The aim of this study was to fit anidulafungin in vitro static time–kill data from nine strains of *Candida* with a pharmacodynamic (PD) model in order to describe the antifungal activity of this drug against *Candida* spp. Time–kill data from strains of *Candida albicans*, *Candida glabrata* and *Candida parapsilosis* clades were best fit using an adapted sigmoidal E_{max} model and resulted in a set of PD parameters (E_{max}, EC_{50} and Hill factor) for each fungal strain. The data were analysed with NONMEM 7. Anidulafungin was effective in a species- and concentration-dependent manner against the strains of *C. glabrata* and *C. parapsilosis* clades as observed with the EC_{50} estimates. Maximum killing rate constant (E_{max}) values were higher against *C. glabrata* and *C. parapsilosis* complex strains. In conclusion, we demonstrated that the activity of anidulafungin against *Candida* can be accurately described using an adapted sigmoidal E_{max} model.

1. Introduction

Invasive candidiasis remains a significant cause of global morbidity and mortality, especially among patients with underlying immunosuppression. *Candida albicans* remains the predominant cause of candidaemia and invasive candidiasis, accounting for 50% of all cases. However, the incidence of infections due to non-*albicans Candida* spp., such as *Candida parapsilosis* and *Candida glabrata*, is increasing [1]. These species are closely related to two phenotypically similar cryptic species. *Candida dubliniensis* and *Candida africana* are within the *C. albicans* clade; *Candida bracarensis* and *Candida nivariensis* are two species closely related to *C. glabrata*, and *Candida orthopsilosis* and *Candida metapsilosis* are newly recognised members of the *C. parapsilosis* complex of species.

The use of anidulafungin, a new class of antifungal agent, to treat serious *Candida* infections is increasing. Anidulafungin inhibits 1,3-β-D-glucan synthase, an enzyme that is necessary for synthesis of an essential component of the cell wall of several fungi. There are reports of species with decreased susceptibility to anidulafungin, such as isolates of *C. parapsilosis* and *C. glabrata* [2–4].

The parameter most commonly used to quantify the antifungal activity of a drug is the minimum inhibitory concentration (MIC). Although the MIC is a well-established in vitro pharmacodynamic (PD) parameter routinely determined in microbiology, this parameter has several disadvantages. For instance, the MIC does not provide information on the rate of fungal kill, and since MIC determination depends on the number of micro-organisms at a single time point, many different combinations of growth and kill rates can result in the same MIC.

Antifungal activity is a dynamic process, whereas the MIC is only a threshold value, a one-point measurement with poor precision determined in two-fold dilution steps. An alternative PD approach, namely microbial time–kill curves, has been proposed to offer detailed information about the antimicrobial efficacy as a function of both time and drug concentration [5,6].

Although time–kill curves can be studied using animal models of infection, in vitro models offer significant advantages in cost,

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http://dx.doi.org/10.1016/j.ijantimicag.2015.12.011
0924-8579/

convenience and time, as well as allowing direct investigation of the drug–microbe interaction in a controlled and reproducible manner [7]. Once the specific time–kill experiments have been performed, the results can be accurately described using PD mathematical models and the respective PD parameters can be calculated.

The aims of this study were: (i) to establish a general mathematical model that is appropriate for describing the in vitro pharmacodynamics of anidulafungin in static time–kill curve experiments, and to obtain model parameters such as the concentration producing 50% of the maximal effect (EC_{50}) and the maximal effect (E_{max}); and (ii) to apply this model in order to compare the in vitro PD features of anidulafungin against different *Candida* strains.

2. Materials and methods

2.1. Fungal strains

Nine reference strains were included in this study, including *C. albicans* NCPF 3153, *C. dubliniensis* NCPF 3949, *C. africana* ATCC 2669, *C. glabrata* ATCC 90030, *C. nivariensis* CECT 11998, *C. bracarensis* NCYC 3133, *C. parapsilosis* ATCC 22019 (quality control strain), *C. metapsilosis* ATCC 96143 and *C. orthopsilosis* ATCC 96139.

2.2. Antifungal agents

Anidulafungin (Pfizer SLU, Madrid, Spain) was dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution of 5120 mg/L. The dilutions were prepared in RPMI 1640 medium with L-glutamine and without $NaHCO_2$ buffered to pH 7 with 0.165 M morpholinepropanesulfonic acid (MOPS) (Sigma-Aldrich, Madrid, Spain). Stock solutions were stored at −80 °C until use.

2.3. Determination of minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs)

The MIC, defined as the minimum concentration producing ≥50% growth reduction, was determined following Clinical and Laboratory Standards Institute (CLSI) guidelines [8,9]. The MFC, defined as the lowest drug concentration that killed ≥99.9% of the final inoculum, was determined as described by Cantón et al. [10]. All MICs and MFCs were measured in RPMI 1640 medium buffered to pH 7.0 with 0.165 M MOPS and the results were read after 24 h of incubation.

2.4. Time–kill procedures

Before time–kill curve studies were performed, the antifungal carryover effect was determined as previously described by Cantón et al. [11]. Time–kill studies were performed as previously described [12–15]. Time–kill studies were carried out on microtitre plates for the computer-controlled microbiological incubator Bio-Screen C MBR (LabSystems, Vantaa, Finland) in RPMI (final volume 200 μL) using an inoculum size of $1–5 \times 10^5$ CFU/mL. Anidulafungin concentrations assayed ranged from 0.015 mg/L to 32 mg/L. These concentrations were selected based on the MIC determined for each species complex. When these concentrations did not lead to the maximum effect, additional higher concentrations were tested to achieve this target. Once the E_{max} was attained, the concentration range was considered acceptable for parameter estimation. Plates were incubated for 48 h at 36 ± 1 °C (30 ± 1 °C for *C. africana*) without agitation. At predetermined time points (0, 2, 4, 6, 24 and 48 h), 10 μL (0–6 h) or 6 μL (24–48 h) was removed from both the control well (without drug) and each test solution well and was serially diluted in phosphate-buffered saline to determine the number of CFU/mL. Technical variability for the ranges of volumes used was coefficient of variation (CV) = 0.5–0.8%. Volumes of 5, 10, 50 or 100 μL (depending on the dilution and concentration of the drug) were plated onto Sabouraud dextrose agar and were incubated at 36 ± 1 °C (30 ± 1 °C for *C. africana*) for 24–48 h. When the colony counts were expected to be <200 CFU/mL, samples of 5 μL were taken directly from the test solution and were plated. The lower limit of accurate and reproducibly detectable colony counts was 200 CFU/mL. Time–kill curve studies were conducted in duplicate and on two different days.

2.5. Mathematical modelling of time–kill data

Time–kill curve analysis and mathematical modelling of the time–kill curve data were performed using a non-linear mixed-effects approach as appropriate with NONMEM 7 (ICON Development Solutions, Ellicott City, MD).

A previously described adapted E_{max} model [16] was tried to fit to the log-transformed data of the static time–kill curve experiments of anidulafungin. This model accounts for delays in *Candida* growth and onset of killing as well as the maximum number of *Candida:*

$$\frac{dN}{dt} = \left[K_g\left(1-\frac{N}{N_{max}}\right)\cdot(1-e^{-\alpha t}) - \left(\frac{E_{max}\cdot C^h}{EC_{50}^h + C^h}\right)\cdot(1-e^{-\beta t})\right]\cdot N$$

In this model, dN/dt is the change in the number of *Candida* as a function of time; K_g (h^{-1}) is the cell growth rate constant in the absence of drug; E_{max} (h^{-1}) is a model estimated parameter that accounts for the maximum killing rate constant, it is not the maximum observed effect; EC_{50} (mg/L) is the drug concentration necessary to produce 50% of the maximum effect; C (mg/L) is the concentration of antifungal drug at any time (t); and N is the number of viable *Candida* (CFU/mL).

This model also took into account the following factors: (i) in in vitro systems, available space and nutrients are limited. The factor that accounts for the resulting saturation of growth is the maximum number of fungi (N_{max}); (ii) isolates have not yet reached the logarithmic growth phase at time zero, i.e. delay in growth: $(1-e^{-\alpha t})$; (iii) delay in the onset of killing: $(1-e^{-\beta t})$; and (iv) a Hill factor or sigmoidicity factor (h) modified the steepness of the slopes and smoothed the curves with a concentration increase.

Since anidulafungin concentrations did not change during the time–kill experiments, C was constant for the entire fitted time period. For each fungal strain, the initial estimates of K_g, N_{max} and α were determined using the data for the growth rate in the absence of anidulafungin (control data). Thereafter, K_g, N_{max} and α were fixed in each model at their determined values in the initial fit, whereas the drug parameters E_{max}, EC_{50}, β and h were fitted simultaneously to the experimental data. The correlation between growth rate and E_{max} was analysed by non-parametric correlation test of Spearman.

A first-order conditional estimation method algorithm was used, as implemented in the non-linear mixed-effects modelling software NONMEM 7. Interindividual variability of the model parameters was not included in the final model because the fungal inocula were obtained from a pure culture so that all of the experimental fungal cultures were assumed to be genetically identical. The residual variability was estimated by using an additive model. Evaluation of the model performance included analysis of standard diagnostic plots, objective function value and the precision of the parameter estimates, as well as visual inspection of the data for the quality of fit.