

Sandra Gil-Alonso (1), Nerea Jauregizar (1), Ignacio Ortega (2), Elena Eraso (3) and Guillermo Quindós (3)

(1) Department of Pharmacology, Faculty of Medicine, University of the Basque Country (UPV/EHU), Spain. UFI11/25 “Microbios y Salud” (2) Research Development and Innovation Department, Faes Farma S.A. Leioa. Spain (3) Department of Immunology, Microbiology and Parasitology, Faculty of Medicine, University of the Basque Country (UPV/EHU), Spain. UFI11/25 “Microbios y Salud”

sandra.gil@ehu.es

BACKGROUND AND OBJECTIVES

The use of echinocandins (anidulafungin (AF), caspofungin (CF) and micafungin (MF)), a new class of antifungal agents, to treat serious *Candida* infection is increasing.

In vitro time-kill curves are attractive tools for studying the pharmacodynamics of antimicrobial agents as they provide detailed information of antimicrobial efficacy as a function of both time and concentration.

Once these experiments have been performed, a mathematical model can be simultaneously fitted to the data and the respective pharmacodynamic (PD) parameters can be calculated.

The aims of this study were (i) to establish a general mathematical model that is appropriate for characterizing the in vitro PDs of echinocandins determined in static time-kill curve experiments and (ii) to apply this model in order to compare the in vitro PD features of the three antifungal agents against different *Candida* species.

MATERIALS AND METHODS

Study design

Nine American Type Culture Collections (ATCC) reference strains of *Candida albicans*, *Candida dubliniensis*, *Candida africana*, *Candida glabrata*, *Candida bracarensis* and *Candida nivariensis* were studied.

AF (Pfizer, Spain), CF (MSD, Spain) and MF (Astellas Pharma) were the investigated antifungal agents. MICs were determined using the CLSI-approved microdilution method (1).

Time-kill procedures

Time-kill curves were determined starting from 1x10⁵ to 5x10⁵ CFU/ml inoculum and 200 µl RPMI 1640 medium. Echinocandins concentrations tested were 0.06, 0.125 and 0.5 µg/ml. Aliquots of 6-10 µl were removed and serially diluted in PBS. Volumes of 5, 10, 50 or 100 µl were spread onto SDA plates and incubated at 37°C for 24 to 48 h to determine the numbers of CFU per milliliter at 0, 2, 4, 6, 24 and 48 h.

Data analysis

Different models were tried fitting the log-transformed data of the static time-kill curve experiments of the three drugs simultaneously. A first order conditional estimation method algorithm was used, as implemented in NONMEM VII (ICON Development). The interindividual variability of the model parameters was estimated by using exponential error models. The residual variability was estimated by using additive model. The “Drug” was introduced in the model as a covariate in order to evaluate the difference in PD parameters related to the three drugs.

Evaluation of the model performance included the analysis of standard diagnostic plots, objective function value and the precision of the parameter estimates, as well as the visual inspection of the data for the quality of fit.

RESULTS

1- An adapted sigmoidal E_{max} model provided the best fit for the echinocandins time-kill data against *Candida* isolates:

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

In this final model, dN/dt is the change in the number of *Candida* as a function of time, K_g (h⁻¹) is the candidal growth rate constant in the absence of drug, E_{max} is the maximum killing rate constant (i.e. maximum effect), EC₅₀ (µg/mL) is the drug concentration necessary to produce 50% of maximum effect, C (µg/mL) is the concentration of antifungal drug at any time (t) and N (colony forming units CFU/mL) is the number of viable *Candida*. Since drug concentration did not change during the time-kill experiment, C was constant for the entire fitted time period.

This model also took into account: 1) The maximum number of *Candida* (N_{max}), which potentially corrected for the limitations of the in vitro system; 2) Isolates had not reached yet the logarithmic growth phase at time zero, i.e. delay in growth: (1-e^{αt}); 3) Delay of the onset of killing: (1-e^{βt}); 4) A Hill factor (h) modified the steepness of the slopes and smoothed the curves. This factor was fixed for CF and MF.

This model was capable of describing the static time-kill curves (Figure 1). The corresponding model parameter estimates are listed in table 1. In the final model, N_{max} and K_g were estimated from the growth control data and were assumed to be constant.

2- In time-kill experiments, PD effects can be described by at least three parameters: K_g, E_{max} and EC₅₀. Even no differences in EC₅₀ values were detected among the three drugs against *C.glabrata* NCPF 3203, the EC₅₀ values demonstrate that the three echinocandins are effective at easily achievable serum levels against *C.glabrata* and also against the other studied species and strains (data not shown). Differences in the rates of maximal killing (E_{max}) were detected, being micafungin the drug with the highest E_{max} against *C.glabrata*.

Table 1: Final model parameters of anidulafungin, micafungin and caspofungin against *C.glabrata* (NCPF 3203)

Parameter	Estimate (RSE,%)
Structural model	
K _g (h ⁻¹)	0.83 (25.5)
EC ₅₀ (µg/mL)	0.11 (5.5)
N _{max} (CFU/mL)	7.72 (0.5)
α	0.11 (37.4)
β	0.06 (43.8)
Covariate model	
E _{max} (h ⁻¹)	
Anidulafungin	1.92 (42.7)
Caspofungin	0.41 (21.7)
Micafungin	2.53 (34.9)
h (fixed)	
Anidulafungin	3.82 (17.8)
Caspofungin(fixed)	10 (---)
Micafungin(fixed)	10 (---)
Interindividual variability (%)	
ω(β)	74.09 (53)
Residual variability (%)	
	36.19 (31)

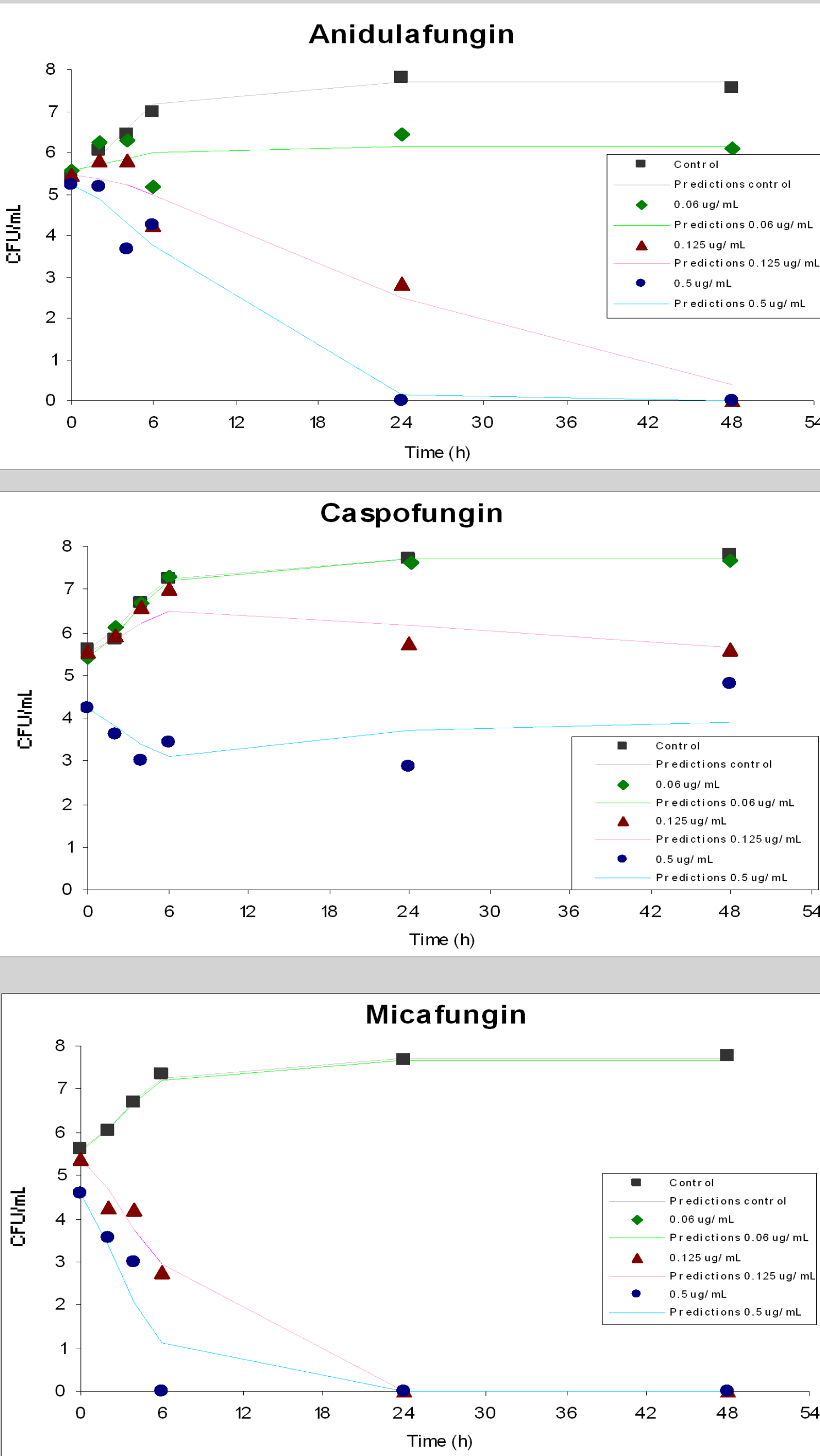


Figure 1: Fitted time-kill curves derived using the mathematical model for anidulafungin, micafungin and caspofungin against *C.glabrata*. Lines represent individual predictions based on the developed model

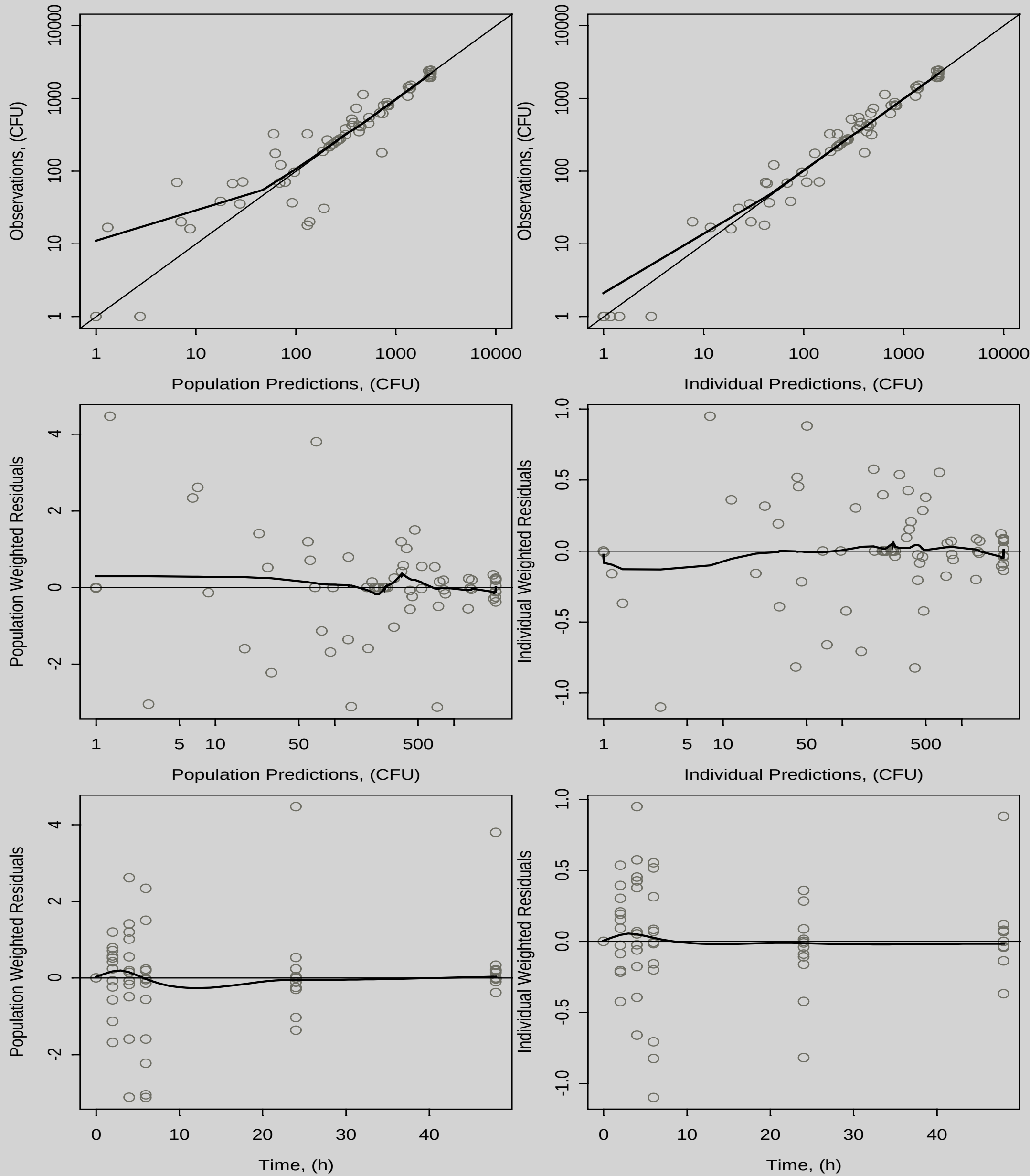


Figure 2: Observed versus population model predicted, observed versus individual model predicted with line of identity and linear regression line. Weighted residuals versus population predictions, weighted residuals versus individual predictions and weighted residuals versus time.