Original

José Antonio Lepe[1,2,3]
Emilio García-Cabrera[2,3]
María Victoria Gil-Navarro[4],
Javier Aznar[1,2,3]

Rifampin breakpoint for *Acinetobacter baumannii* based on pharmacokinetic-pharmacodynamic models with Monte Carlo simulation

[1] Infectious Disease, Clinical Microbiology and Preventive Medicine Clinical Unit. Hospital Universitario Virgen del Rocío. Sevilla. Spain.
[2] The Seville Biomedical Research Institute, Hospital Universitario Virgen del Rocío /CSIC/ Universidad de Sevilla.
[3] Spanish Network for Research in Infectious Disease (REIPI).
[4] Pharmacy Service. Hospital Universitario Virgen del Rocío. Sevilla. Spain.

ABSTRACT

Objective: The aim of this study is to develop a pharmacokinetic–pharmacodynamic (PK–PD) rifampin breakpoint for *Acinetobacter baumannii* based on Monte Carlo simulation and to compare it with the reference value establish by the French Society for Microbiology (SFM).

Methods: A 10,000 subject's Monte Carlo simulation for rifampin with intravenous dose of 10 mg/Kg/day and 20 mg/Kg/day was performed. The distribution of MIC was calculated using unique clinical isolates of *A. baumannii*. The PK–PD parameter calculated was $Cmax_{free}$/MIC.

Results: The isolates rifampin MIC_{50} and MIC_{90} were 2 and 32 mg/L respectively, ranging between 0.023-32 mg/L. According to interpretive criteria established by the SFM: 468 (75.8%) isolates were susceptible (MIC ≤ 4 mg/L) and 150 (24.2%) were non susceptible (MIC > 4 mg/L).

For 10 mg/Kg/day dose: the probability (%) of attaining $Cmax_{free}$/MIC ratio values = 8 by Monte Carlo simulation in the study population was 0.4%, the rifampin MIC cut off value obtained from an optimal treatment (target ≥ 90%), was 0.125 mg/L. The probability of obtaining a $Cmax_{free}$/MIC ratio equal to 10 was 0.2% and the MIC cut off value obtained <0.125 mg/L.

At doses of 20 mg/kg/day: the probability of obtaining a $Cmax_{free}$/MIC ratio equal to 8 was 0.8%, the rifampin MIC cut off value obtained was 0.25 mg/L. For a $Cmax_{free}$/MIC = 10, it was 0.6% and 0.125 mg/L, respectively. The percentage of susceptible isolates ranging 0% to 1%, depending on the dose and therapeutic target used.

Conclusion: the rifampin breakpoints obtained from our PK/PD Monte Carlo simulation differ from those established by SFM, although further clinical studies in patients are needed to confirm our findings and improve the use of this antibiotic.

Keywords: rifampin, Monte-Carlo, *Acinetobacter*, PK/PD.

Correspondencia:
Jose Antonio Lepe
Hospitales Universitarios Virgen del Rocío
Av Manuel Siurot s/n
41013 Sevilla. Spain
Telephone: 34-955013203
Email: jalepe@cica.es

Evaluación de un punto de corte farmacocinético-farmacodinámico para rifampicina en *Acinetobacter baumannii* mediante simulación de Monte-Carlo

RESUMEN

Introducción: El objetivo de este estudio es desarrollar un punto de corte farmacocinético (PK/PD) de rifampicina para *Acinetobacter baumannii* basado en modelos de simulación de Monte Carlo y compararlo con el valor de referencia establecido por la Sociedad Francesa de Microbiología (SFM).

Materiales y Métodos: Se ha realizado una simulación de Monte Carlo de 10.000 individuos que se administraba una dosis intravenosa de rifampicina a dos dosis 10 mg/kg/día y 20 mg/kg/día. La distribución de CMI se calculó utilizado aislados clínicos de *A. baumannii*. Los parámetros farmacocinéticos calculados fueron $Cmax_{libre}$/CMI.

Resultados: Los valores de CMI_{50} y CMI_{90} fueron 2 y 32 mg/L respectivamente, obteniéndose un rango de 0,023-32 mg/L. De acuerdo con el criterio establecido por la SFM 468 aislamientos (75,8%) eran sensibles (CMI ≤ 4 mg/L) y 150 (24,2%) resistentes (CMI > 4 mg/L).

Para una dosis de 10 mg/Kg/día: la probabilidad (%) de alcanzar un cociente $Cmax_{libre}$/CMI igual a 8 por simulación de Monte Carlo fue 0,4%, el valor de CMI de rifampicina por debajo del cual se podría inferir un escenario óptimo de tratamiento (objetivo ≥ 90%) fue ≤ 0,125 mg/L. La probabilidad de obtener un cociente $Cmax_{libre}$/CMI igual a 10 fue 0,2% y el punto de corte <0,125 mg/L.

A dosis de 20 mg/Kg/día: la probabilidad de obtener un cociente $Cmax_{libre}$/CMI igual a 8 fue 0,8% y el punto de corte 0,25 mg/L. Para $Cmax_{libre}$/CMI de 10, fue 0,6% y 0,125 mg/L respectivamente. En base a estos resultados, el porcentaje de sensibilidad osciló entre 0 a 1%, dependiendo de la dosis y del objetivo terapéutico evaluado.

Conclusión: los puntos de corte de rifampicina obtenidos en nuestra simulación de Monte Carlo difieren de los establecidos por la SFM, aunque estudios clínicos deberían corroborar estos resultados y mejorar el uso de este antibiótico.

Palabras clave: rifampicina, Monte-Carlo, *Acinetobacter*, PK/PD

INTRODUCTION

A high number of nosocomial infections are caused by *Acinetobacter baumannii,* and due to its extraordinary ability to develop resistance to all available antibiotics pose a challenge to the clinicians for an empiric antibiotic treatment. Nowadays, the resistance rates to carbapenems, the gold standard for empiric treatment, ranges in our country from 50 to 80%[1], and the therapeutic arsenal is limited to colistin, tigecycline, minocycline and rifampin[2].

Rifampin has demonstrated in vitro and in vivo bactericidal activities against multi-drug resistant (MDR) *A. baumannii*[3]. Experimental models show that rifampin is efficacious in the treatment of severe infections caused by imipenem-resistant *A. baumannii* strains[4]. Antibiotic combinations represent a therapeutic option in the treatment of MDR *A. baumannii* infections. In treatments involving antibiotics like rifampicin, combination therapy is used to avoid the appearance of antimicrobial resistance. In fact, in our hospital the combination of rifampin and colistin, is the unique available treatment due to the high rate of carbapenem resistant strains. However, rifampicin, has the problem of ease of acquisition of resistance due to changes in the RNA polymerase encoded by chromosomal mutations that occur rapidly in the presence of the drug and hence the need to be associate with other antibiotics.

The antibacterial effect of rifampin is concentration dependent. Moreover, the post-antibiotic effect of rifampin and the suppression of resistance is also concentration dependent. A previous work; have demonstrated that those effects were best correlated with the maximum concentration of drug Cmax/MIC ratio[5,6].

EUCAST and CLSI agencies do not establish breakpoints for rifampin in Gram negatives organisms. However, The French Society for Microbiology (SFM) is the unique that establishes a rifampin breakpoint for *A. baumannii* based on MIC distributions (susceptible MIC of ≤ 4 mg/L, intermediate 8-16 mg/L and resistant MIC of >16 mg/L)[7]. These breakpoints are used routinely in our clinical laboratory setting to guide clinical decision-making but without pharmacokinetic–pharmacodynamic (PK–PD) later confirmation.

The aim of this study is to develop a PK–PD rifampin breakpoint for *A. baumannii* based on Monte Carlo simulation and to contrast with French reference value.

MATERIAL AND METHODS

Determination of rifampin MIC in *A. baumanii* clinical isolates. A total 618 unique and non-duplicate *A. baumannii* (24. 8% imipenem susceptible and 99, 5% colistin susceptible) isolates obtained from abscesses and wounds [175, (28.3%)], respiratory specimens [299, (48.4%)], sterile fluids (including CSF) [34, (5.5%)], blood [37, (6%)], and urine [73, (11.8%)] from individual patients attended in the period 2007-2010 were

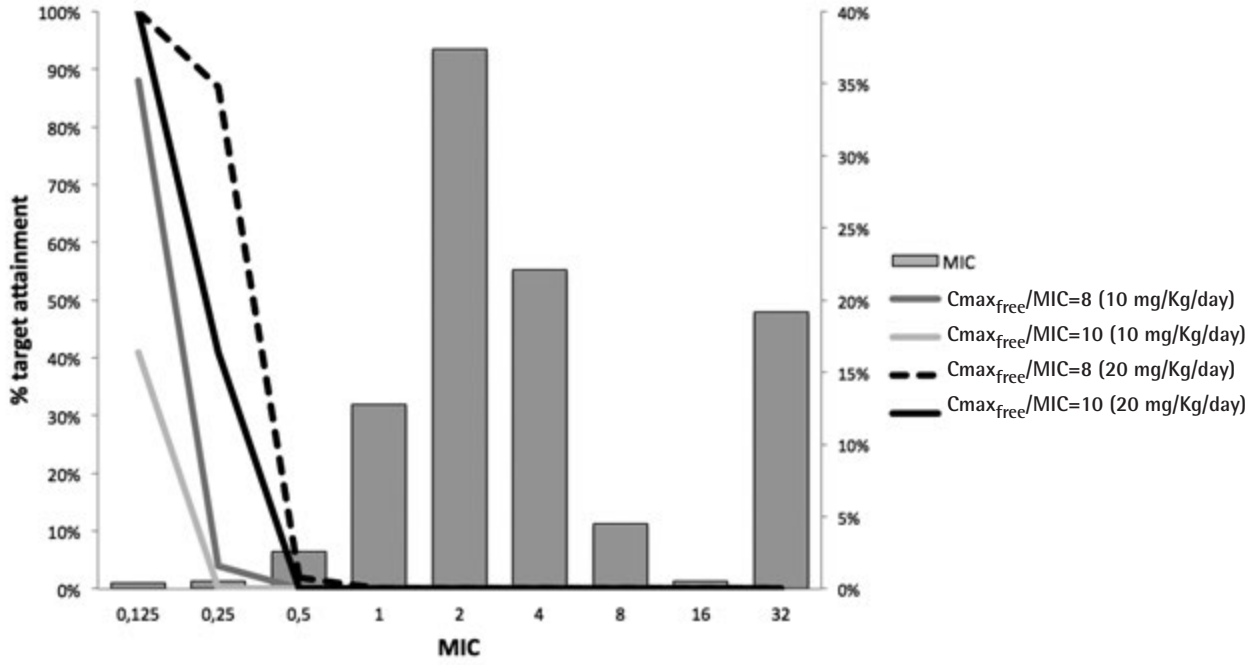

Figure 1 MIC values distribution of *Acinetobacter baumannii* isolates against rifampin and MIC values vs % PK/PD target attainment.

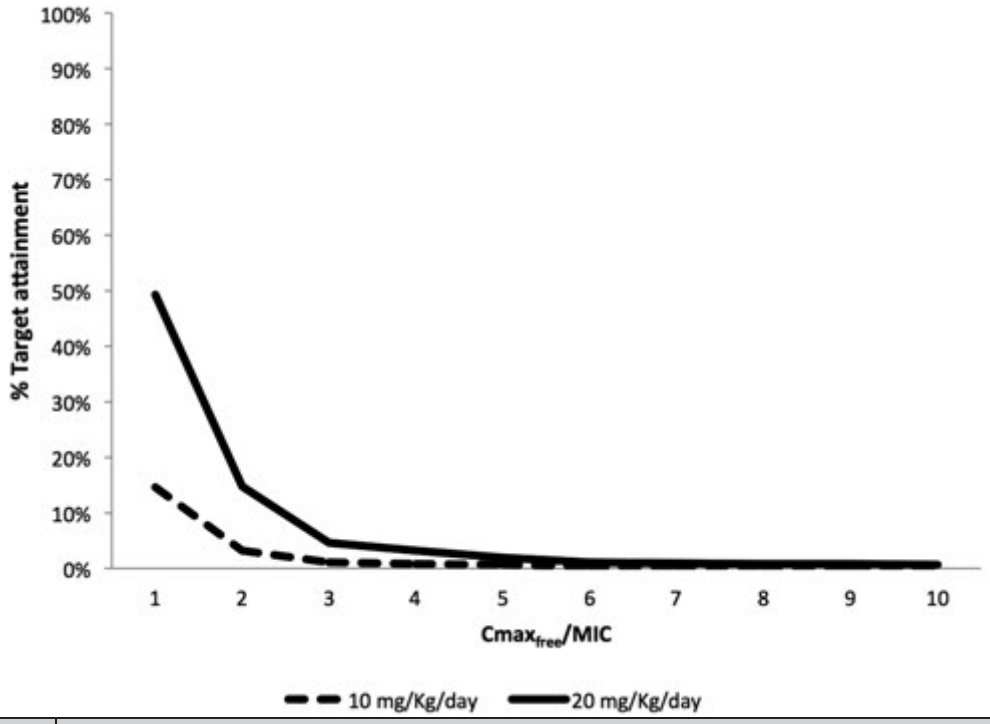

Figure 2 | **Probability (%) of attaining Cmax$_{free}$/MIC ratio values by Monte Carlo simulation.**

studied. MIC to rifampin was determined by the Epsilon-test® (AB biodisk, Biomerieux, France) according to manufacturer's instructions with *S. aureus* ATCC 29213 used for quality control purposes. The modal MIC was reported as MIC_{50} and MIC_{90}, the percent susceptibility was calculated according to interpretive criteria established by the French Society for Microbiology[7].

Monte Carlo simulation. To calculate rifampin breakpoint, Microsoft Excel was used to perform a 10.000 subjects Monte Carlo simulation for the intravenous rifampin dose of 10 mg/Kg/day and 20 mg/Kg/day (patient weight 70 Kg) using the following PK-PD equation:

Where Cmax *free*: was the maximum concentration achieved in the serum (mg/L), dose: the dose of antibiotic (mg), Bioavailability: the fraction unbound to protein and *Vss*: the antimicrobial volume of distribution on steady state (L/Kg). Pharmacokinetic parameters included in the model were obtained from mean value and CI of the previous published data of Houin et al[8]. The pharmacodynamic parameters included in the model were obtained from the rifampin MIC study of *A. baumanii* isolates from our hospital. The model permitted variation in protein binding. All the PK-PD parameters are assumed to be log-normally distributed in the population, and MICs were accepted at single values from 0.125 to 32 mg/L.

A Cmax$_{free}$/MIC of 10 was assumed as the target attainment[9]. Additionally, a ratio of Cmax$_{free}$/MIC of 8 (likely effectiveness) was also evaluated[9]. The PK/PD susceptible breakpoint was defined as the MIC at which the probability of target attainment (PTA) was 90%[10].

RESULTS

The isolates rifampin MIC_{50} and MIC_{90} were 2 and 32 mg/L respectively, ranging between 0,023-32 mg/L. The MIC distribution is shown in figure 1, we highlight that two different populations of *A. baumannii* with different susceptibility of rifampin has been found, most of the isolates [496, (80.3%)] with MIC ≤ 8 mg/L and the remaining [122, (19.7%)] with MIC > 8 mg/L. According to interpretive criteria established by the SFM: 468 (75.8%) isolates were susceptible (MIC ≤ 4 mg/L) and 150 (24.2%) were non susceptible (MIC > 4 mg/L).

For 10 mg/kg/day (figure 1 and 2): the probability (%) of attaining Cmax$_{free}$/MIC ratio values = 8 by Monte Carlo simulation in the study population was 0.4%, the rifampin MIC cut off value obtained from an optimal treatment (target ≥ 90%), was 0.125 mg/L. The probability of obtaining a Cmax$_{free}$/MIC ratio equal to 10 was 0.2% and the MIC cut off value obtained < 0.125 mg/L.

At doses of 20 mg/kg/day (figure 1 and 2): the probability of obtaining a Cmax$_{free}$/MIC ratio equal to 8 was 0.8%, the ri-

fampin MIC cut off value obtained was 0.25 mg/L. For a $Cmax_{free}$/MIC = 10, it was 0.6% and 0.125 mg/L, respectively.

The percentage of susceptible isolates ranging 0 to 1%, depending on the dose and therapeutic target used (figure 1).

DISCUSSION

This work shows that in the population studied to achieve a rifampin $Cmax_{free}$/MIC ≥ 4 or 10 and AUC_{0-24h}/MIC = 30 are not always attained with doses of 10 and 20 mg/kg/day, especially at the level of MIC_{50} and MIC_{90} level of our *A. baumannii* range MIC.

Several studies have been conducted to evaluate the rifampin bactericidal and sterilizing efficacies but it is difficult to identify the PK-PD parameter that best describes the rifampin's efficacy[6]. Rifampin exhibited an exposure-dependent killing kinetics, as the ratio AUC_{0-24h}/MIC the best parameter that correlated with a reduction of bacterial count[5] and Cmax/MIC if we considering the post-antibiotic effect[6]. Other authors argue that for concentration-dependent drugs such as fluoroquinolones, aminoglycosides and rifampin a high ratio of maximum concentration to MIC ($Cmax_{free}$/MIC ratio above 8 to 10) is a better predictor of a successful treatment outcome[9]. Based on the foregoing, the simulation was carried out using $Cmax_{free}$/MIC.

A clinical MIC breakpoint, derived from pharmacological indices, can be used to divide the pathogens into the categories of clinically susceptible or clinically resistant[11]. Our findings, based on $Cmax_{free}$/MIC = 8 or 10 for which was available a known target[6,9,10], suggest that lower breakpoints (0.125-0.25 mg/L) than the SFM breakpoint should be used. Therefore, based on the rifampin simulation, one would expect a high probability of sub-optimal rifampin $Cmax_{free}$/MIC ratio, for patients infected with organisms with rifampin MICs ≥ 0.125 mg/L and being treated with standard doses. Using higher doses such as 20 mg/kg/day, previously employed on clinical studies in combination therapy, this percentage would increase minimally, leaving cover a wide range (99-100%) at MICs ≥ 0.25 mg/L of our *A. baumannii* distribution.

The results could conflict with previous studies; Montero et al showed that imipenem and rifampin, colistin and rifampin, tobramycin and rifampin were effective against *A. baumannii* in a mouse pneumonia model[3], although in a small clinical study[12], a rifampin plus imipenem regimen for carbapenem-resistant *Acinetobacter* infections, was not associated with clinical benefits, moreover 7 out of 10 patients developed a high level of resistance to rifampicin during treatment. In another study[4], rifampicin in monotherapy was effective against *A. baumannii* in experimental model of pneumonia, although rifampin resistance were development during the experiments. In general, one might infer that the emergence of rifampicin resistance during treatment in these studies is consistent with the results of our simulations.

In brief, these results suggest that protein binding may be a key parameter in the pharmacodynamics of rifampin[13]. Therefore, protein binding may explain the suboptimal clinical efficacy of current dose of rifampin.

Therefore, the results of our simulations allow us to ensure that microorganisms included in the sensible category according to the guidelines of the SFM can be considered as not susceptible decreasing the *A. baumannii* population capable of being treated with this antibiotic. Despite this, our study could be affected by some limitations, while PK/PD simulation can assist to establish more adjusted breakpoint, we do not forget that are based on number assumption. Moreover, in our study all pharmacokinetic parameter pertain to values measured in serum but it is well known that rifampin is widely distributed throughout the body. It is present in effective concentrations in many organs and body fluids, including cerebrospinal fluid[14]. Other limitation is that intracellular neither 25-O-desacetyl metabolite activities have been considered, neither the variability of concentration due to the interaction by co-administered antibiotic.

In conclusion, the rifampin breakpoints obtained from our PK/PD Monte Carlo simulation differ from those established by SFM, although further clinical studies in patients are needed to confirm our findings and improve the use of this antibiotic.

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