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Article

The role of modeling and simulation to improve the treatment of fungal infections caused by *Cryptococcus*: A literature review

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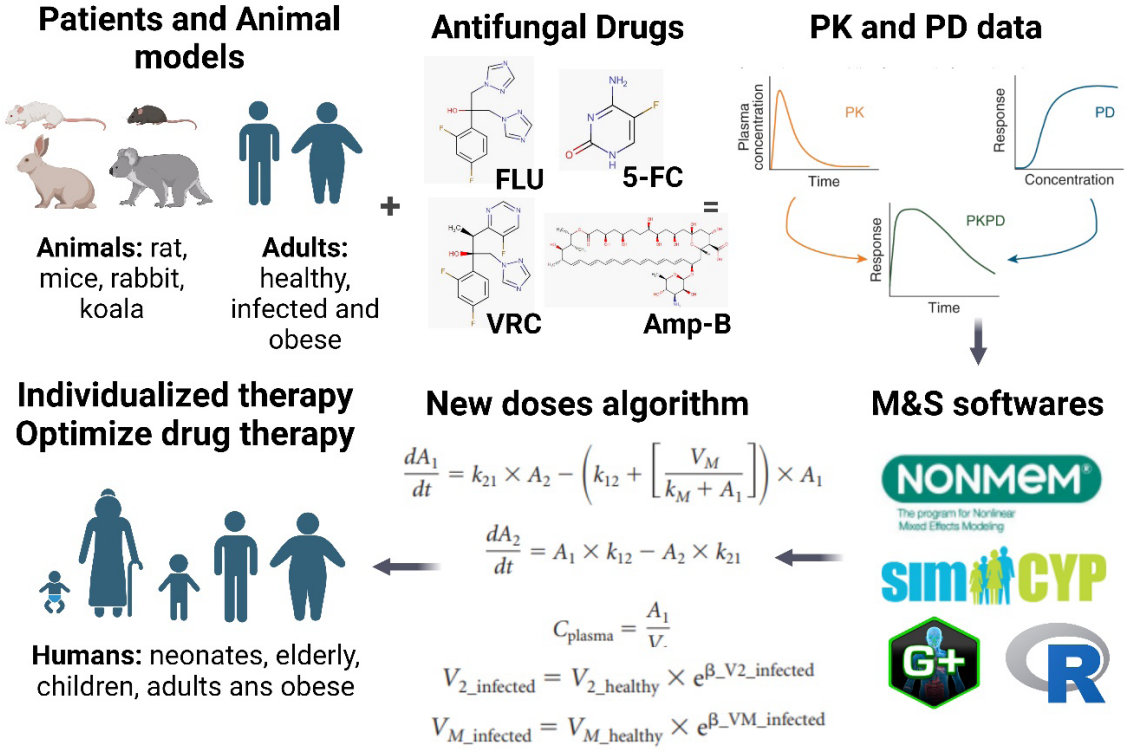
GRAPHICAL ABSTRACT



The treatment of fungal infections presents problems in relation to toxicity, pharmacokinetic properties, and undesirable side effects among other factors. An alternative to clarify some of these problems is the use of mathematical modeling and simulation of the pharmacokinetics and pharmacodynamics data of antifungals, in order to seek greater support in decision making regarding the treatment of *Cryptococcus* infection. Here, we describe the results of a literature review focusing on studies that used mathematical modeling and simulation of pharmacokinetic and pharmacodynamic data of antifungals used in the treatment of cryptococcosis. Through this review, it was possible to identify that most of the content

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presented refers to studies of modeling, which refer to two very important modeling approaches that provide subsidies for an adequate treatment. Studies that performed Monte Carlo simulations and evaluated the probability of reaching the target show that many treatments used are ineffective, and it is necessary to investigate new models that include more information about these difficult to treat infections. These mathematical tools are extremely important, because through the correlation of pharmacokinetics and pharmacodynamics data of an antifungal, it is possible to make an appropriate decision for the treatment of fungal infections caused by *Cryptococcus* spp.

Keywords: Antifungals. Cryptococcosis. Mathematical modeling. Pharmacodynamics. Pharmacokinetics.

INTRODUCTION

The frequency of fungal infections has been increasing in recent decades. Most of them are superficial and easy to treat. However, in recent years these infections have changed their outcome profile, being associated with invasive diseases that present themselves as causes of morbidities and mortality, caused by inadequate diagnoses, invasive surgeries, inefficacy of antifungals available on the market, and patients with HIV (Human Immunodeficiency Virus). And among the pathogens frequently isolated in these infections are *Candida* spp., *Cryptococcus* spp., and *Aspergillus* spp. (McCarty, Pappas *et al*., 2016; Armstrong-James *et al*., 2017; Li *et al*., 2018).

Cryptococcosis is a systemic fungal infection whose main organs affected are the central nervous system (CNS) and the lungs. It is caused by fungi of the genus *Cryptococcus* that affects animals and humans (Reuwsaat *et al*., 2018) and has an annual incidence of almost 220,000 individuals, of which 181,000 cases result in death (Fisher *et al*., 2021) and many patients require emergency department. *Cryptococcu*s is described as a complex fungus, as it can remain dormant in the host for decades before its reactivation, and in addition, it has several characteristics that allow its survival and adaptation in the host, making this infection difficult to diagnose and treat (Bahn *et al*., 2020).

The treatment of invasive fungal infections presents difficulties in clinical practice, both in relation to the limited number of antifungal agents available and to the difficulties of correct diagnosis. This can lead to the incorrect use of drugs, not showing therapeutic success and leading to more and more to causes of resistance, which further reduces the option of antifungals for treatment (Nett, Andes, 2016; Vieira, Nascimento, 2017). In addition, reports in the literature show that antifungals have issues with toxicity, low tolerance to high doses, narrow spectrum of activity, pharmacokinetic properties and undesirable side effects (Nett, Andes, 2016). Five main classes are currently available to treat fungal infections: azoles, polyenes, echinocandins, allylamines and pyrimidine analogs (Nett, Andes, 2016; Vieira, Nascimento, 2017; Flevari *et al*., 2013; Pappas *et al*., 2016).

With the increasing number of cases of infections, limited efficacy, and reports of resistance to antifungals it is necessary to optimize the dosage regimens, and that requires knowledge of the mechanisms involved in the effect of antifungals - pharmacodynamics (PD) and knowledge about the concentrations that are reached at the site of action - pharmacokinetics (PK). This PK/PD relationship, together with the use of mathematical modeling and simulation with the construction of models for data integration, assists in describing the concentration profiles of a drug in an organism over time and in defining the dose regimens necessary for therapeutic success, as well as minimizing side effects and the emergence of resistance (Mould, Upton, 2013; Kristoffersson *et al*., 2016; Rathi, Lee, Meibohm, 2016).

With the use of modeling and simulation (M&S) it is possible to use different mathematical methods, from compartmental, population or physiological models, which depend on the desired results. Several studies have shown that through modeling, it is possible to relate the most diverse aspects of the medication, the individual and the disease in order to seek the best clinical outcome and understand the sources of variability observed.

Developed countries already use M&S in drug-discovery and to improve clinical outcomes. In those countries, regulatory agencies such as the FDA (U.S Food&Drug Administration) and EMA (European Medicines Agency) provide documents to guide its use.

In Brazil, pharmacometrics (PmX) are still not used by regulatory sources to assist in decision-making and in hospitals to assist the treatment of these fungal infections, even though it presents reliable and accurate results, which can be used to optimize drug therapy. However, initiatives that seek to insert this tool in the hospital, regulatory, and pharmaceutical industries in order to improve drug therapy, development of new drugs, and precision medicine, are being observed.

This shows that it is necessary to train people to use these very important tools, and the regulatory agency Agência Nacional de Vigilância Sanitária (ANVISA) has also been trying to implement this approach to assist in decision-making regarding medication reports. Therefore, M&S of antifungals provide subsidies for adequate treatment, and thus, this work aimed brings together recent articles on the most varied mathematical strategies in the management of infections caused by *Cryptococcus* ssp. to assist in decision-making for its treatment.

MATERIAL AND METHODS

The integrative review was carried out by research in the PubMed database, using original papers published in English between the years 2010 and 2022, which addressed mathematical modeling on treatment of fungal infections. The combinations of the following keywords were used in the search: "fungal brain infection", "cryptococcus", "pharmacokinetics", "pharmacodynamics", "antifungals", "microdialysis", "tissue penetration", "Physiologically Based Pharmacokinetic".

A total of 397 articles were identified for a preliminary assessment of the databases, where we evaluated the abstracts and the full text, considering the inclusion and exclusion criteria that can be seen in Figure 1. The final selection comprised 41 articles, together with the articles that were included during the reading and preparation of the manuscript.

In the initial search strategy, 397 articles were identified, mapped and analyzed by VOSviewer Software® 1.6.15 according to: high frequency keyword counting, creation of a co-occurrence map and grouping of keywords in clusters. VOSviewer allowed the text mining functionality that was used to visualize conceptual networks, based on co-words of terms extracted from the articles, especially titles and abstracts.

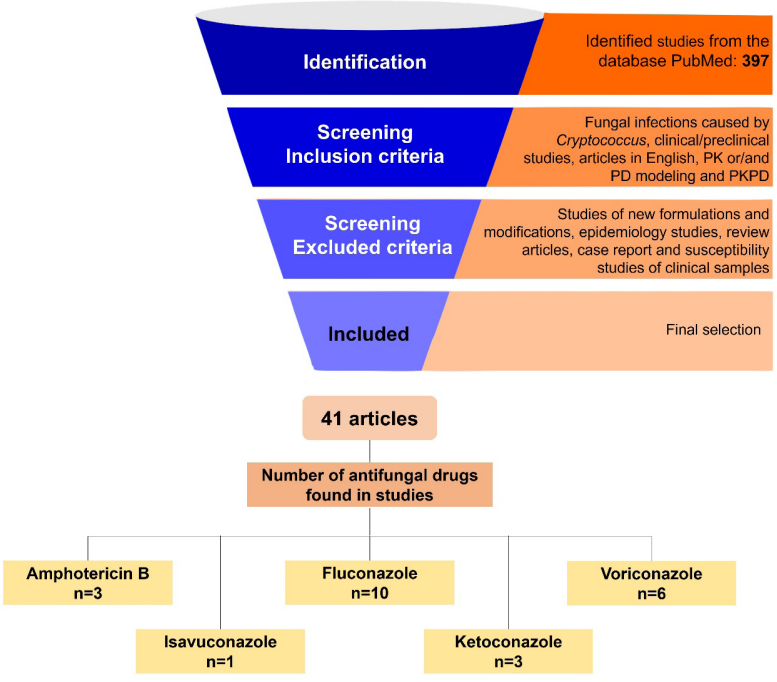

FIGURE 1 - Flowchart of literature searching and screening.

RESULTS AND DISCUSSION

VOSviewer allows each keyword to reflect a specific theme of the text. Once a keyword is related to a particular topic, the more frequently the keyword shows, the more important the topic will be. Figure 2 illustrates the most frequent keywords and the most associated keyword pairs. The interpretation of the results showed that four clusters were formed from 48 keywords identified algorithmically by the software.

Based on the high frequency keywords in the articles and their relevance score for other keywords, cluster 1 (red dots) consists of 20 keywords focused on the use of antifungal agents in animal models (relating cryptococcosis models, drug combinations, microbial sensitivity tests, etc.). Cluster 2 (green dots), composed of 14 keywords, presents biological models in humans, use of PK/PD and PBPK models and simulation of antifungal PK parameters. Cluster 3 (blue dots), composed of 7 keywords, indicates properties related to antifungals (solubility, permeability, drug liberation, etc.). Finally, cluster 4 (yellow dots), composed of 7 keywords, indicates factors related to the distribution of antifungals (in plasma, tissues, microdialysis technique, etc.).

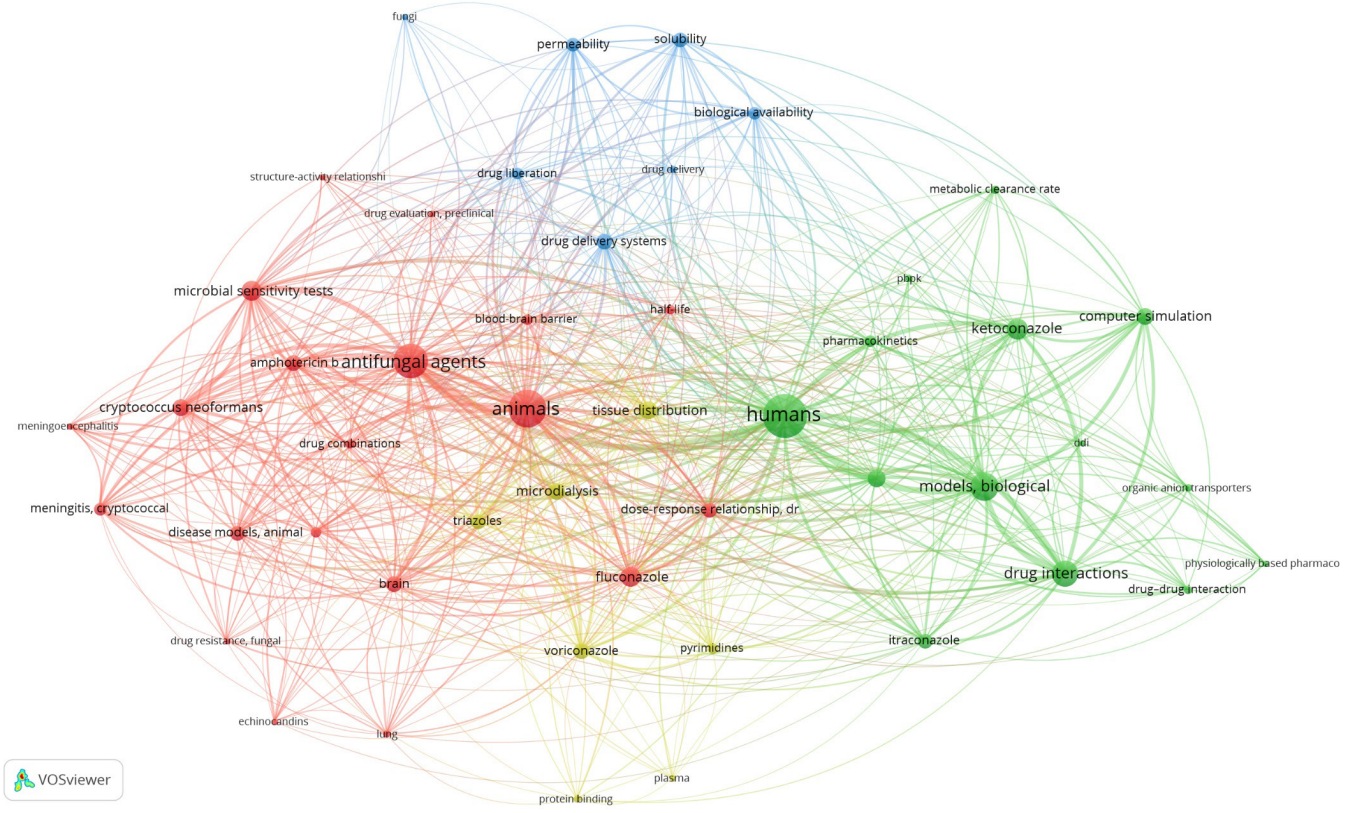

FIGURE 2 - Map of co-occurrence keywords related in papers focusing on pharmacokinetics, pharmacodynamics, antifungals, *Cryptococcus*, microdialysis, tissue penetration, Physiologically Based Pharmacokinetic, through pubmed database (between 2010 to 2022).

Mathematical Modeling

In the articles we found, the following approaches were used: pharmacokinetic (PK) evaluation, PK/PD models, population PK modeling (popPK) and physiologically dependent pharmacokinetic (PBPK) modeling, along with PK/PD indices and Noncompartmental PK Analysis (NCA). In this way, using PK, modeling and simulation, we can build mathematical models that allow us to describe or simulate

concentration profiles over time of a drug in an organism, from in vitro or in vivo data. This approach can include compartmental, population, physiological models, and comprises pharmacometrics, which is a science that quantifies the behavior of drugs, medications and diseases, seeking to answer the most diverse questions (Mould, Upton, 2013).

PK is a science that studies and contemplates the course of drugs in the biological organism through the processes of absorption, distribution, metabolism and excretion (ADME). In preclinical studies, PK evaluation is of great importance, because through this evaluation it is possible to obtain the PK parameters that derive from the plasma concentrations of the drug. One of the main objectives of this evaluation is to determine the dosage and number of doses necessary for the treatment to be effective for the disease that is to be treated (Fan, De Lannoy, 2014). In Table I we can see studies that evaluated the PK of two antifungals used in the treatment of cryptococcosis.

The PK/PD index are established through the relationship of a measure of potency of the drug in vitro, the minimal inhibitory concentration (MIC), with a measure of exposure of the organism to the drug, using the PK parameters area under the plasma concentration (AUC) versus time curve, peak plasma concentration (C_{max}) and time. So that those who use the AUC/MIC and C_{max}/MIC index have a concentration-dependent effect, and those who use %T>MIC have a time-dependent effect (Figure 3). Knowing which of the three PK/PD indexes describes antifungal activity provides the basis for determining the dose frequency at which a drug is most effective (Sy, Zhuang, Derendorf, 2016; Lepak, Andes, 2011). The PK/PD index were used in four antifungals studies with special situations caused by *Cryptococcus*, which will be reported in the text below and can be seen in Table I.

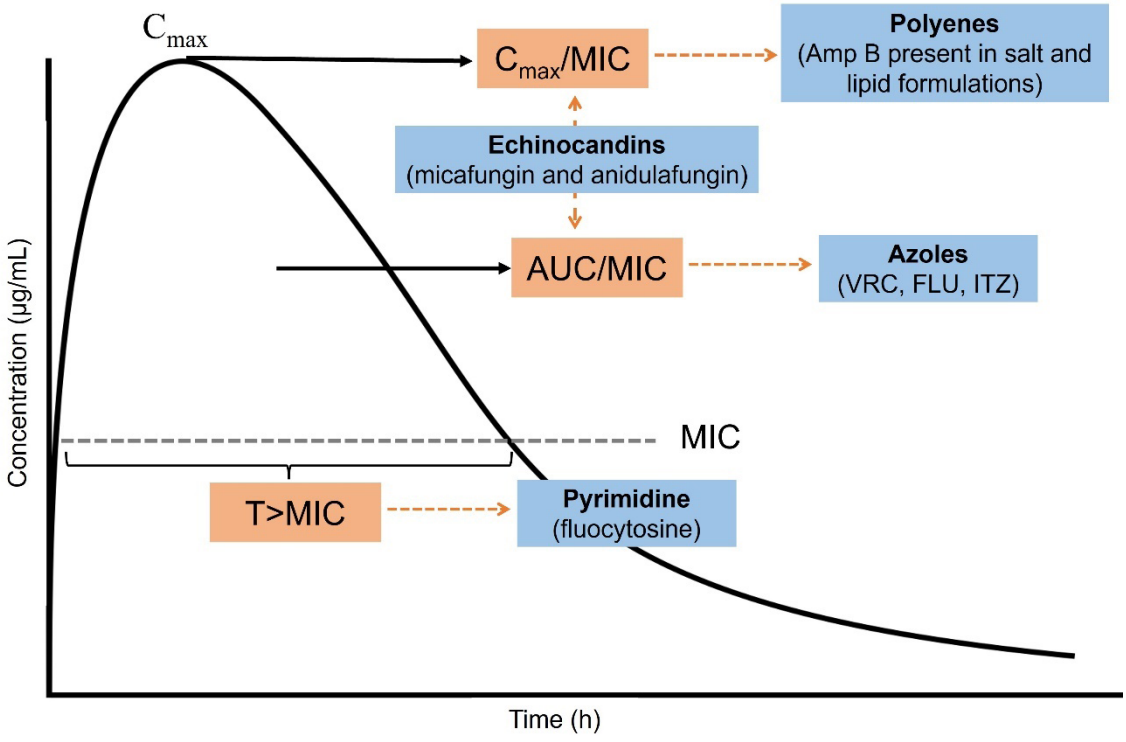

FIGURE 3 - PK/PD indices used for the classes of antifungals.

NCA is a model-independent method where no assumptions are made about drug behavior, that is, we do not assume body compartments. They are faster and less costly to execute, as they rely almost exclusively on algebraic equations to estimate PK parameters. However, they provide little information about variabilities that can arise from one individual to another. The NCA is used as a reference for comparison with the PK parameters calculated by the compartmental analysis, in order to verify the results (Lepak, Andes, 2011). Through this analysis, we can obtain parameters that help us understand the PKs of antifungals. Table II presents the summary of the results referring to this analysis.

Currently, pharmacokinetic/pharmacodynamic (PK/PD) modeling is an approach that supports making important decisions, including outlining the ideal dosage regimen (Schmidt *et al*., 2008). PDs models relate concentrations at the effect site to the pharmacological response, and PKs models allow predicting the concentration of drugs in different tissues of the human body as a function of time (Schmidt *et al*., 2008; Hope, Drusano, 2009). This approach was used in clinical and pre-clinical studies, shown in Table III.

PopPK has the capacity to expand traditional structural models, through the addition of statistical and error models capable of representing the magnitude of variability of model parameters between individuals (Joerger, 2012) identifying covariates is important in explaining interindividual variability, and thus increasing the predictive value of the model. Nonlinear mixed-effects modeling with stepwise covariate modeling is frequently used to build structural covariate models, and the most commonly used software-NONMEM-provides estimations for the fixed-effect parameters (e.g., drug clearance). Thus, PopPK can be applied in PK studies carried out in a population of individuals who receive the same drug in an identical dose and dosage regimen in order to understand the relationships between the specific characteristics of the subjects and the changes in PK parameters and, thus identify sources of variability across the population that can be divided into several factors such as demographic data, environmental factors, genetic phenotype, etc., (Joerger, 2012; Sime, Roberts, Roberts, 2015) identifying covariates is important in explaining interindividual variability, and thus increasing the predictive value of the model. Nonlinear mixed-effects modeling with stepwise covariate modeling is frequently used to build structural covariate models, and the most commonly used software-NONMEM-provides estimations for the fixed-effect parameters (e.g., drug clearance). Relating these covariates to the PKs parameters of antifungals can explain the variability of the parameters and facilitate the understanding of the diseases and the adjustment of the dose. Some studies carried out with this approach can be seen in Table IV.

Monte Carlo simulations are being used to predict doses of antimicrobials that effectively eradicate microorganisms, incorporating some of the important variables that influence this result. Knowledge of the factors that are fundamental for the calculation of probabilities in Monte Carlo simulations allows us both to anticipate when failures may occur and offer new recommendations for successful therapy, since the PK/PD indices are often used as targets in the antimicrobial dose selection process. The PK/PD indices used in the evaluation of antifungals are AUC/MIC, C_{max}/MIC and Time>MIC (Nielsen, Cars, Friberg, 2011).

The Time>MIC value or percentage of the time at which the plasma levels are above the MIC, C_{max}/MIC expresses at the rate between the maximum concentration the MIC and finally at AUC/MIC, expressed at the ratio between the area sob at curve and the MIC. The indices are expressly based on specific numerical values for each type of infection, taking into account the MIC values. In Monte Carlo simulations, the variability between patients in PK parameters is considered, as well as in PD (in terms of MIC), and the probability of reaching the goal (PTA) is determined based on these stochastic simulations of the model (Nielsen, Cars, Friberg, 2011). This tool has been used to predict the effect of various antifungals in special situations against yeasts in clinical and preclinical studies, which will be reported in the text below and in the Table V.

And finally, PBPK models are bottom-up models that integrate physiological information from the organism with physical-chemical properties from the drug that allows a priori simulation of pharmacokinetic profiles. In the clinical and preclinical setting, this

strategy can be used for drug-drug interaction (DDI) studies, translational studies, and for understanding the influence of pathophysiology on pharmacokinetic and pharmacodynamic processes, which is the case of fungal infections (Kuepfer *et al*., 2016) as can be seen in Table VI.

TABLE I - Main information's of PK evaluation and PK/PD index of antifungals using in treatment of cryptococcosis

Ref.	Patients/animal model characteristics	Drug and Dosage regimen	Yeast and MIC	PK/PD index	Outcomes
Wu *et al*., 2014	Murine with cryptococcal meningitis	3 mg/kg/d i.v. of Amp-B, 10 mg/kg i.v. of VERA and ITZ	*C. neoformans* (clinical isolate)	n.a.	This study demonstrated that Amp-B is probably a substrate for Pg-P and that they can increase Amp-B uptake through BBB, leading to a reduction in fungal load on the brain.
Sudan *et al*., 2013	Nonimmunosuppressed murine model of cryptococcal meningitis	125 and 250 mg/kg p.o. once a day for 9 days of FLU	*C. neoformans* (4 clinical isolates) 3; 3.33; 3.67 and 7.33 µg/mL	AUC/MIC = 389	The MIC was important to understand the exposure-response relationships, because the AUC/MIC ratio of 389 was the index mor efficient for FLU.
Santos *et al*., 2016	Murine model of cryptococcosis	75 mg/kg p.o. daily of FLU	*C. gattii* L27/01= 16µg/mL and *C. gattii* $L27/01_F$ = 128 µg/mL	AUC/MIC	The maximum concentration of FLU in the brain was lower than the MIC for both strains.
Alhadab *et al*., 2019	HIV-infected Ugandan patients with cryptococcal meningitis	100, 200, 300, 400 mg/day of SER; 0.7 – 1.0 mg/kg per day and 800 mg/day of FLU	*Cryptococcus* clinical isolates Mean 4 µg/mL (SER)	No PK/PD index correlated	They observed that the concentrations of unbound SER do not reach MIC concentrations. None of the selected PK/PD index correlated with the decrease in the $\log_{10}$ CFU/mL.
Hope *et al*., 2019	Murine model of cryptococcal meningitis	125 and 250 mg/kg p.o once a day of FLU	*C. neoformans* (ATCC 208821) 4 µg/mL	*f*AUC/MIC = 231,4	The emergence of resistance was observed in vivo with a *f*AUC/MIC of 231.4.

Ref. – references; Amp-B - Amphotericin B; VERA – Verapamil; ITZ – Itraconazole; BBB – Blood-brain barrier; Pg-P - glycoprotein-P FLU -Fluconazole; SER – Sertraline; AUC/MIC - area under the plasma concentration/minimal inhibitory concentration; PK/PD - Pharmacokinetic/pharmacodynamic; n.a. - not applicable.

TABLE II - Main information's of NCA of antifungals

Ref.	Patients/ animal model characteristics	Drug and Dosage regimen	Pharmacokinetic parameters (median)						Outcomes
			λ	Vss	CL	$AU_{C0-}\infty$	F	ppb	
Black *et al*., 2014	Clinically normal koalas	10 mg/kg p.o and i.v. of FLU	4.69 h p.o. 2.25 h i.v.	0.92 L/ kg i.v.	0.31 L/h/ kg i.v.	22.87 µg/mL*h p.o. 34.54 µg/mL*h i.v.	53%	39.5%	This study demonstrated striking differences in t½, CL, Vss, F and PPB for koalas compared to other mammals.
Wang *et al*., 2017	Male Sprague–Dawley rats	10 and 20 mg/ kg i.v. of FLU	n.a.	n.a.	n.a.	**AUC_{0-300} plasma** 2619 ±135 (dose 10 mg/kg); 4587 ± 207 (dose 20mg/kg) without probenecid 2975 ± 113 (dose 10 mg/kg); 5156 ± 229 (dose 20mg/kg) with probenecid **AUC_{0-300} ECF** 1587 ±110 (dose 10 mg/kg); 2853 ± 207 (dose 20mg/kg) without probenecid 2176 ± 114 (dose 10 mg/kg); 4113 ± 201 (dose 20mg/kg) with probenecid	n.a.	11.0%	The combined use of probenecid increased the penetration of FLU into the BBB, which would increase the concentration of FLU in a brain lesion without increasing the dose of FLU.
Alves *et al*., 2018	Model of cryptococcal meningoencephalitis in rats	20 mg/kg i.v. of FLU	Healthy Plasma 0.11 h Brain 0.19 h Infected Plasma 0.15 h Brain 0.16 h	Healthy Plasma 0.336 L Infected Plasma 0.255 L	Healthy Plasma 0.031 L/h Infected Plasma 0.034 L/h	Healthy Plasma 220.88 µg*h/mL Brain 135.50 µg*h/mL Infected Plasma 183.20 µg*h/mL Brain 166.68 µg*h/mL	93%	90%	In infected animals the infection caused an influence on the distribution of FLU to the brain.
Kirbs *et al*., 2019	Healthy male volunteers	i.v. infusions day 1 (6 mg/ kg over 2 h) and day 2 (4 mg/kg over 1.3 h). Day 3 and 4, 200 mg every 12 h of tablets of VRC	**Plasma** s.d. (0-12h) 0.09; m.d. (48-60h) 0.05; m.d. (72-84h) 0.06 **ISF** s.d. (0-12h) 0.09; m.d. (48-60h) 0.03; m.d. (72-84h) 0.04	n.a.	**Plasma** m.d. (72-84h) 16.3 L/h **ISF** m.d. (72-84h) 14.8 L/h	**Plasma** s.d. (0-12h) 7.30 mg*h/L; m.d. (48-60h) 14.7 mg*h/L; m.d. (72-84h) 12.3 mg*h/L **ISF** s.d. (0-12h) 5.39 mg*h/L; m.d. (48-60h) 14.7 mg*h/L; m.d. (72-84h) 13.5 mg*h/L;	96%	47.1 %	In this study, a high interindividual variability was observed in the concentration-time profiles of VRC, although doses have been adapted to body weight in the first i.v. administrations.

Ref. – references; NCA- Noncompartmental Pharmacokinetic Analysis; PPB - Plasma protein binding; FLU -Fluconazole; VRC - Voriconazole; BBB - Blood-brain barrier; ECF - Brain extracellular fluid; λ - elimination rate constant; t½ - elimination half-life; CL- clearance; Vss- steady-state volume of distribution; F- oral bioavailability, AUC-area under the plasma concentration; ISF - Interstitial fluid; s.d. - Single dose; m.d. - Multiple dose; n.a. - not applicable.

TABLE III - Main information's of PK/PD modeling of antifungals using in treatment of cryptococcal meningitis in animal's models

Ref.	Patients/animal model characteristics	Yeast and MIC	Drug and Dosage regimen	Sample	Assessment of activity and MIC methods	Model	Outcomes
Sudan *et al.*, 2013	Nonimmunosuppressed murine model of cryptococcal meningitis	*C. neoformans* 3; 3.33; 3.67 and 7.33 µg/mL	125 and 250 mg/kg once a day for 9 days of FLU	Plasma and cerebrum	CLSI method	5-compartment mathematical model consisting by 5 ordinary differential equations and the relationship between the AUC/MIC ratio versus decline in fungal density was modeled using the sigmoid E_{max} model	The mathematical model was adjusted to the PK/PD data and showed that FLU may be an inferior drug for induction therapy because many patients are unable to achieve the pharmacodynamic goal, being advised to use the highest possible dose.
O´Connor *et al.*, 2013	Murine model of cryptococcal meningoencephalitis	*C. neoformans var. grubii* (ATCC 208821) 1 µg/mL for LAmp-B and 0.125 µg/mL for flucytosine	LAmp-B 3, 10 and 20 mg/kg i.v. daily and flucytosine 20, 50 and 100 mg/kg/d i.v.	Plasma and cerebrum	CLSI M27-A3 method	A Greco model implemented within ADAPT 5 was used to modeled the effect of the combination of LAmp-B and flucytosine	This study demonstrated that both LAmp-B and flucytosine produced a dose-dependent reduction in fungal load. And the effect of the combination of agents on the brain was additive.
Santos *et al.*, 2016	Murine model of cryptococcosis	*C. gattii* L27/01= 16µg/mL and *C. gattii* $L27/01_F$ = 128 µg/mL	75 mg/kg daily and of FLU 0.2 mg/kg daily of Amp-B	Serum, lungs, and brain	CLSI M27-A3 method	Modified E_{max}-model and sigmoid E_{max} model	The maximum concentration of FLU in the brain was lower than the MIC for strains. The treatment of mice infected with the resistant strain was ineffective even with the use of high doses of FLU or when testing Amp-B, confirming cross-resistance between these drugs.
Lestner *et al.*, 2017	Murine and rabbit models of cryptococcal meningoencephalitis	*C. neoformans var. grubii* (ATCC 208821)	LAmp-B i.v.	Plasma and cerebrum	EUCAST and CLSI methods	Population model used PK and PD murine data with the program Pmetrics was performed using four differential equations	The rabbits study suggests that more than a single dose is needed to treat cryptococcal meningitis, whereas the study in mice may underestimate the total exposure of the drug that is necessary for fungicidal activity in humans, as they had antifungal effects beyond the time in which that it was possible to detect the drug.
Hope *et al.*, 2019	Murine model of meningoencephalitis	*C. neoformans* (ATCC 208821) 4 µg/mL	125 and 250 mg/kg once a day of FLU	Plasma, cerebrum and CSF	CLSI microdilution method	3-compartment PK model linked to two equations that describe the time course of the densities of susceptible and resistant subpopulations	The PK/PD model described that FLU at clinically relevant exposures does not cause fungi eradication in the central nervous system and is ineffective in preventing the rapid onset of resistance.

TABLE III - Main information's of PK/PD modeling of antifungals using in treatment of cryptococcal meningitis in animal's models

Ref.	Patients/animal model characteristics	Yeast and MIC	Drug and Dosage regimen	Sample	Assessment of activity and MIC methods	Model	Outcomes
Kovanda *et al.*, 2019	Rabbit model of cryptococcal meningoencephalitis	*C. neoformans* (ATCC 208821) 0.008 µg/mL for ISA and 1 µg/mL for FLU	ISA orally and FLU i.v.	Plasma, brain and CSF	CLSI M27-A3 macrodilution method	Population PK/PD mathematical model was performed using nonparametric estimation with Pmetrics software using five differential equations	Similar significant reductions in fungal load on the brain and CSF were observed in rabbits treated with ISA sulfate and FLU. Showing that the treatment of cryptococcal meningoencephalitis with the two drugs are similar.

Ref. – references; FLU – Fluconazole; ISA – Isavuconazole; Amp-B - Amphotericin B; PK/PD – pharmacokinetic/pharmacodynamic; CLSI - Clinical & Laboratory Standards Institute; AUC/MIC - area under the plasma concentration/minimal inhibitory concentration; LAmp-B- Liposomal amphotericin B; CSF- cerebrospinal fluid; EUCAST- European Committee on Antimicrobial Susceptibility Testing.

TABLE IV - Main information's of popPK models of antifungals using in treatment of cryptococcosis

Ref.	Patients/ animal model characteristics	Yeast and MIC	Drug and Dosage regimen	Sample	Model	Covariates	PK parameters	Outcomes
Alobaid *et al.*, 2016	Critically ill nonobese, obese and morbidly obese patients	n.a.	n.a	Plasma	Two-compartment popPK model	CL_{CR} for FLU clearance and BMI for FLU Vc	CL = 0.95 L/h Vc = 15.10 L KCP = 5.41 h^{-1} KPC = 2.92 $^{h-1}$	In this study, which included patients with severe obesity and morbid obesity, it was shown that the clearance of FLU was correlated with measured CL_{CR}, and Vc was better correlated with BMI.
Alves *et al.*, 2017	Model of cryptococcal meningoencephalitis in rats	*C. neoformans* (ATCC 28957) 0,03 - 0,5 µg/mL	5 mg/kg i.v. of VRC	Plasma and µd	Two compartments and MM elimination describes the VRC concentration-time profile in plasma and tissue	Infection was included in V_2 and V_M	V_{1pop} = 0.241 L $V_{2\ healthy}$ = 0.352 L $V_{2\ infected}$ = 0.216 L $B_{V2\ infected}$ = -0.487 $K_{12\ pop}$ = 8.530 h^{-1} $K_{21\ pop}$ = 6.130 h^{-1} $V_{M\ healthy}$ = 0.324 µg/mL $V_{M\ infected}$ = 0.186 µg/mL $B_{VM\ infected}$ = -0.557 k_M = 0.540 µg/mL	In this study, the brain penetration rate showed an increase in exposure in infected animals when compared to healthy animals. The levels of VRC that reached the infected tissues were higher than the MIC, which indicates a potential treatment for cryptococcal meningitis.

TABLE IV - Main information's of popPK models of antifungals using in treatment of cryptococcosis

Ref.	Patients/ animal model characteristics	Yeast and MIC	Drug and Dosage regimen	Sample	Model	Covariates	PK parameters	Outcomes
Alves *et al.*, 2018	Model of cryptococcal meningoencephalitis in rats	*C. neoformans* (ATCC 28957) 4 µg/mL	20 mg/kg i.v. of FLU	Plasma and µd	Two compartments describe the FLU concentration-time profile in plasma and tissue	Infection was included in k_{21}, V_1 and $_V2$	$V_{1\ pop}$ = 0.176 L $B_{V1\ WT}$ = 1.080 $V_{2\ pop}$ = 0.059 L $B_{V2\ WT}$ = 0.951 $K_{21\ pop}$ = 6.260 h^{-1} $B_{k21\ CAT}$ = -0.635 $K_{12\ pop}$ = 1.612 h^{-1} $K_{10\ pop}$ = 0.156 $^{h-1}$	This study showed a brain penetration rate and increase in exposure in infected animals when compared to healthy animals. This study shows also, that when used in monotherapy, FLU is limited to treat a cryptococcal meningoencephalitis.
Stott *et al.*, 2018	Adults with cryptococcal meningitis	*C. neoformans* 8 µg/mL	800 mg q24h orally of FLU and 1mg/kg q24h of Amp-B deoxycholate	Plasma and CSF	Four-compartment PK model	Patient's weight was associated with the estimated volume of distribution	K_a = 8.78 h^{-1} SCL/F = 0.72 L/h V_c/F = 18.07 L K_{cp} = 12.20 h^{-1} K_{pc} = 18.10 h^{-1} K_{cs} = 35.43 h^{-1} K_{sc} = 28.63 h^{-1} V_{cns}/F = 32.07 L	The popPK model suggests that current FLU regimens are unsuitable for induction therapy for cryptococcal meningitis. What can be attributed to the notion that FLU is a fungistatic agent.
Alhadab *et al.*, 2019	HIV-infected Ugandan patients with cryptococcal meningitis	*Cryptococcus* clinical isolates Mean 4 µg/mL	100, 200, 300, 400 mg/ day of SER; 0.7 – 1.0 mg/ kg per day and 800 mg/ day of FLU	Plasma and CSF	One-compartment PK model with first-order absorption and elimination an exploratory sigmoidal E_{max} model	The use of antiretrovirals was included as a covariate in CL/F	CL/F = 52.7 L/h V/F = 1780 L ART CL = 0.948 K_a = 0.101 h^{-1}	SER oral clearance was 1.95 times higher in patients receiving ART, which represents 49% less exposure to the drug. SER increased the exposure of FLU in the brain which increased the fungal clearance of CSF.

Ref. – references; popPK- Population pharmacokinetic; VRC - Voriconazole; FLU- Fluconazole; µd – microdialysate; MM- Michaelis-Menten; V_1 - volume of the central compartment; V_2- Volume of distribution in the peripheral compartment; V_M- maximum rate of metabolism; β_V_2- exponential scaling factor for V_2; β_VM - exponential scaling factor for VM; Vc- volume of distribution of the central compartment; CL- clearance; KCP- rate constant for fluconazole distribution from the central to peripheral compartment; KPC- rate constant for fluconazole distribution from the peripheral to central compartment;k_{10}, k_{12}, k_{21} - first-order distribution rate constants; VM - maximum enzymatic velocity; kM - MM constant; Ka - absorption rate constant from the gut to the central compartment; SC -, clearance; F- bioavailability; Kpc, Kcp, Ksc and Kcs- first-order rate constants; Vcns - volume of distribution in central nervous system compartment SER – Sertraline; CSF -cerebrospinal fluid; CL_{CR}- creatinine clearance; BMI- body mass index; ART – antiretroviral; n.a. - not applicable.

TABLE V - Main information's of Monte Carlo simulations of antifungals

Ref.	Patients/ animal model characteristics	Drug and Dosage regimen	Model	PK/PD target	Outcomes
Alhadab *et al.*, 2019	Critically ill nonobese, obese, and morbidly obese patients	200 mg/d of FLU, weight-based loading dose of 12 mg/kg and maintenance dose of 6 mg/kg	Two-compartment popPK model	*f*AUC/MIC of 100	In this study it was demonstrated that a FLU dose of 200 mg daily was insufficient to achieve *f*AUC/MIC of 100 for pathogens with MICs of >2 mg/liter in patients with BMI of >30 kg/m^2 and loading dose of 12 mg/kg and maintenance dose of 6 mg/kg/day achieved pharmacodynamic targets for higher MICs.
Stott *et al.*, 2018	Adults with cryptococcal meningitis	400 mg q24h, 800 mg q24h, 1,200 mg q24h, and 2,000 mg q24h of FLU	Four-compartment PK model	AUC/MIC of ≥389.3	This study provides the PD rationale for the long-recognized fact that FLU monotherapy is an inadequate induction regimen for cryptococcal meningitis
Alves *et al.*, 2018	Model of cryptococcal meningoencephalitis in rats	125 mg/kg dose for rats and 400–2000 mg for humans of FLU	Two-compartmental popPK model	*f*AUC/MIC >389	This study demonstrated which FLU is limited use in monotherapy to the treatment of cryptococcosis in rats and humans to value of MIC >8 μg/mL.

Ref. – references; popPK- Population pharmacokinetic; FLU- Fluconazole; BMI- body mass index; *f*AUC/MIC - unbound drug fraction/MIC ratio; MIC – minimal inhibitory concentration.

TABLE VI - Main information's of PBPK models of antifungals

Ref.	Software	Drug and ADM	Study type	Population	Dose Regimen	Main Results	Acceptance Criteria	Conclusion
Li *et al.*, 2019	PK-SIM	VRC; Oral	TDI	Healthy Volunters	m.d.	VRC causes TDI of CYP3A4; 200 mg BID is sufcient for CYP2C19 intermediate metabolizers to reach the tentative therapeutic range while 400 mg BID more suitable for rapid metabolizers.	Geometric mean error (fold 0.5 - 2.0)	The study support TDI of CYP3A4 by VRC as an important characteristic of this drug's PK. The PBPK model developed here could support individual dose adjustment of VRC according to genetic polymorphisms of CYP2C19, and these results are fundamental for the management of DDI's risk.

TABLE VI - Main information's of PBPK models of antifungals

Ref.	Software	Drug and ADM	Study type	Population	Dose Regimen	Main Results	Acceptance Criteria	Conclusion
Cristofoletti *et al.*, 2016	SIMCYP	FLU; Oral	Intraspecie (from adults to chidren)	Healthy volunters	s.d.	The absortion of FLU in fasted condition within the terapeutic range is dose indepent. The most absortion area is jejuno and the reduce of this surface area in children does not affect the absortion of FLU. Its administration in children is not solubility limited.	n.a.	The study corroborates to the development of models to predict diferences in absortion between children and adults after oral drug administration.
Hens *et al.*, 2018	SIMCYP	POSA; Oral	Absortion model	Healthy Volunters	s.d.	Acid suspension overpredicted the simulated concentrations over the observed concentration in early times. The amount of dissolved POSA in the stomach is crucial for the amount of POSA that will be absorbed. The fast gastric emptying rate (0.175 h) remained unchanged during these simulations, the effect of SITT on systemic drug exposure was more visible after the absorption phase.	5% percentile and 95% percentile	The study evaluated the impact of these GI variables on the systemic outcome of a 40 mg acidic (pH 1.6; 70% predissolved) and a 40-mg neutral (pH 7.1; 2.3% predissolved) suspension. It was clearly observed that gastric/duodenal pH and gastric emptying are the most sensitive parameters that may cause variability in systemic outcome of the drug, for both suspensions.
Zhao *et al.*, 2018	SIMCYP	FLU; i.v	Interspecie	Neonates	s.d.	From juvenile mice to neonates, a correction factor of maximum lifespan potential should be used for extrapolation, while a "renal factor" taking into account renal maturation was required for successful bridging based on adult and *in vitro-in silico* data. Simulations results demonstrated that the predicted drug exposure based on bridging approach was comparable to the observed value in neonates. The prediction errors were -2.2%, +10.1% and -4.6% for juvenile mice, adults and *in vitro-in silico* data, respectively.	Prediction error (PE)	The results approach to predict the PK of FLU in neonates from juvenile mice, adults and *in vitro-in silico* data. Our results firstly support the feasibility of PK bridging approach in neonates.

TABLE VI - Main information's of PBPK models of antifungals

Ref.	Software	Drug and ADM	Study type	Population	Dose Regimen	Main Results	Acceptance Criteria	Conclusion
Watt *et al*., 2018	PK-SIM	FLU; oral	Intraspecie (from adults to children)	Healthy voluters and patients with ECMO	s.d.	The pediatric PBPK model only captured 74% of the observed data for critically ill infants, but this was expected because the model was based on healthy infants. Critically ill children have more variability in exposure due to disease. By accounting for the developmental changes in body size and physiology as children age, the current ECMO PBPK model was able to predict the optimal loading dose for each age group. However, the optimal regimen may be too complicated for clinical implementation.	Fold error 0.7-1.3	The study described in this study should provide an approach to dose determination in this difficult to study population that provides needed flexibility to account for different and evolving extracorporeal circuit components and reduces the number of children enrolled in clinical trials.
Frechen *et al*., 2013	NONMEN	VRC; i.v and oral	DDI	Healthy Volunters	m.d.	Identification of an apparent sustained inhibitory effect by VRC due to a proposed quasi accumulation at the enzyme site, a significantly reduced inhibitory potency of intravenous VRC for oral substrates.	90% of CI's	The proposed model focused on *in vivo* clinical trials is a powerful tool for maximizing knowledge obtained from clinical DDI studies and serves as a complementary instrument in addition to mechanistically test the classical NCA and specifically in vitro-based (physiological-based) simulation models the magnitude of DDIs.
Hens *et al*., 2017	SIMCYP	POSA; oral	ADAM	Healthy Volunters	s.d.	For the first time, this research demonstrated predictive in silico simulations of GI dissolution, supersaturation, and precipitation for a weakly simple compound, told in part by modeling of in vitro dissolution studies and validated with clinical measurements in both GI fluids and plasma.	n.a.	This research illustrates the predictive capacity of PBPK modeling in evaluating the effect of formulation pH on systemic exposure for two suspensions of a dose of POSA.

TABLE VI - Main information's of PBPK models of antifungals

Ref.	Software	Drug and ADM	Study type	Population	Dose Regimen	Main Results	Acceptance Criteria	Conclusion
Liu *et al*., 2016	SIMCYP	KETO; oral	ADAM/ IVIVE	Clinical trials	s.d.	Established KTZ PBPK model successfully identified the effect of supersaturation and precipitation on the systemic plasma concentration profiles of KETO.	n.a.	These findings show that IVIVE can be used in biopharmaceutical experiments to understand and gain trust in the accuracy of the input parameters and mechanistic models used for in vivo mechanistic oral absorption simulations, thus enhancing the prediction efficiency of PBPK models.
Ruff *et al*., 2016	STELLA	KETO; oral	ADAM/ IVIVE	Clinical trials	s.d.	Oral solution plasma profile simulations with *in-vitro* transition experimental results were conducted using the Nizoral® tablets predissolved. In the case of an oral solution, no disintegration must be assumed and gastric emptying is the most critical stage in bringing the dissolved substance to the absorption site.	ANOVA	The proposed standard transfer models therefore provided the most precise simulation of the observed profile and support the hypothesis of making the in vitro sample more physiologically important when coupled with PBPK.
Silva *et al.,* 2018	GASTROPLUS	KETO; oral	ADAM	Patients with Hypochlorhydria	s.d.	The developed model accurately predicted the impact of increased pH and beverage co-administration on drug dissolution and absorption, confirming that complete gastric dissolution is required.	Two-fold error	Reliable models of PBPK can be used to forecast future pharmacokinetic pitfall, opening up additional approaches for researching clinical practice dosing strategies.
Johnson *et al*., 2018	SIMCYP	KETO; oral	ADAM	Pediatrics	s.d.	The tmax of KETO was estimated to be around 1h in both neonates and adults, but the former had a higher fa rating.	**	While some performance testing was carried out, the components of the model obviously need to be extended as new research on GI tract ontogeny is available and evaluated against further in vivo proof of real drug absorption age-related results.

TABLE VI - Main information's of PBPK models of antifungals

Ref.	Software	Drug and ADM	Study type	Population	Dose Regimen	Main Results	Acceptance Criteria	Conclusion
Qi *et al*., 2017	SIMCYP	VRC; oral	DDI	Healthy Volunters	s.d.	The findings showed that the plasma concentration–time profiles of VRC and PPIs simulated by the PBPK model were compatible with the observed profiles. Furthermore, the DDI simulations indicated that VRC PK values improved to varying degrees when paired with different PPIs.	AFE < 2.0	However, due to the medicinal concentration spectrum, VRC dose modification is inappropriate regardless of which PPI was co-administered.
Zane, Thakker, 2014	SIMCYP	VRC; i.v.	Intraspecie (from adults to children)	Healthy children and adults	s.d.	The estimation of oral bioavailability increased significantly after integrating intestinal first-pass metabolism into the model, indicating that VRC is subject to intestinal first-pass metabolism in children but not in adults.	20% prediction error of the observed values	The PBPK method employed in this research provides a mechanistic explanation for the discrepancies in bioavailability between adults and infants. This will be the first instance of unequal first-pass metabolism in children and adults, if verified.

Ref. – references; VRC - Voriconazole; FLU- Fluconazole; ADM – Administration; s.d. - Single dose; m.d. - Multiple dose; pbpk - physiologically based kinetic; TDI - time-dependent inhibition; POSA – Posaconazole; KETO – ketoconazole; SITT - small intestinal transit time; GI – gastrointestinal; ECMO - extracorporeal membrane oxygenation; ADAM - Advanced Dissolution Absorption and Metabolism; NCA- Noncompartmental Pharmacokinetic Analysis; PPI - proton pump inhibitors; DDI - drug–drug interaction; *T*max - time to maximum plasma concentration; IVIV E - Integrated in Vitro in Vivo Extrapolation; n.a.- not applicable.

Antifungal Drugs

The drugs indicated for the treatment of infection caused by *Cryptococcu*s according to the Consensus on Cryptococcosis are AmB, AmB lipid formulations (lipid and liposomal complex), FLZ, voriconazole (VRC), itraconazole and 5-FC in various combinations (Kon, 2008). Figure 4 shows the stages of meningoencephalitis treatment along with the indicated drugs.

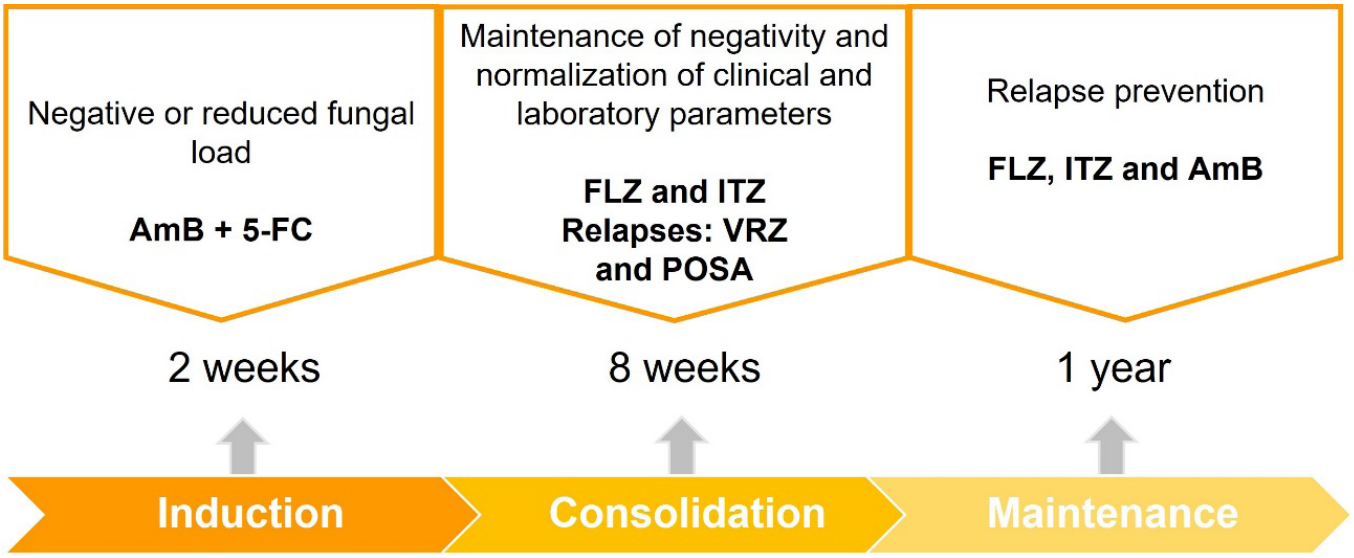

FIGURE 4 - Graphic representation of the treatment of cryptococcal meningitis as recommended by WHO (2018).

Amphotericin B

AmB is the antifungal drug of choice recommended for treatment of CNS cryptococcosis (Lepak, Andes, 2011), including an intensive period of induction therapy (O'Connor *et al*., 2013). All AmB formulations available for use must be administered by intravenous infusion because their enteral absorption is negligible. It is 95-99% bound to plasma proteins. Due to toxicity problems, lipid encapsulation was used to improve the tolerability of AmB. And so, three formulations with different chemical composition, particle size and shapes were developed: liposomal AmB (LAmB), colloidal AmB dispersion (colloidal amphotericin) and the lipid complex of AmB. However, LAmB is the only widely available lipid formulation and presents non-linear PK (Bellmann, Smuszkiewicz, 2017).

AmB was investigated as a substrate of P-glycoprotein (Pg-P) in BBB (blood brain barrier), in a model of cryptococcal meningitis, in non-infected CD-1 mice and infected with a clinical isolate of *C. neoformans*, after a dose of 3 mg/kg/ d of AmB i.v for 4 days. And 24 h after the last dose of AmB, the animals were divided into 3 groups that received or did not receive a single i.v. dose of 10 mg/kg of verapamil (VERA) or itraconazole (ITZ). In this study, both groups that received AmB + VERA had higher concentrations of AmB in the brain than those treated with AmB alone, while in the animals treated with AmB + ITZ, the concentration of AmB in the brain was similar to that of the group that only received AmB. Both groups, at all treatments, showed low plasma concentrations. This study demonstrates that AmB is probably a substrate for Pg-P and that this combination can increase AmB uptake through BBB, leading to a reduction in fungal load in the brain (Wu *et al*., 2014).

In the study by O'Connor *et al*., (2013) a model of cryptococcal meningoencephalitis caused by *C. neoformans* was used to define the PK and PD of Liposomal AmB (LAmB) and 5-FC alone and in combination. A Greco model implemented in ADAPT 5 was used to model the effect of the combination of drugs. Both monotherapy with LAmB and 5-FC showed rapid penetration into the brain and that time profiles of concentration of both drugs were similar in plasma and in the brain. Using the Greco model, drug combination was additive, and this study indicates that a regimen of LAmB 3 mg/kg/d with 50 mg/kg/d 5-FC would be associated with an almost maximum antifungal activity which could be less toxic than other dosages of agents used to treat cryptococcal meningoencephalitis (O'Connor *et al*., 2013).

Lestner *et al*., (2017) used two models of cryptococcal meningitis (mice and rabbit) to evaluate 3-day regimens

with LAmB for induction therapy. They developed a PK/PD model to describe the observed data in mice, with the program Pmetrics. The main challenge was to model the "deposit" type effect that AmB had on the brains of mice they had antifungal effects beyond the time in which that it was possible to detect the drug concentrations in the brain (Lestner *et al*., 2017) with near-maximal efficacy achieved with LAmB at 10 to 20 mg/kg/day. The terminal elimination half-life in the brain was 133 h. The pharmacodynamics of a single dose of 20 mg/kg was the same as that of 20 mg/kg/day administered for 2 weeks. Changes in quantitative counts were reflected by histopathological changes in the brain. Three doses of LAmB at 5 mg/kg/day in rabbits were required to achieve fungicidal activity in cerebrospinal fluid (cumulative area under the concentration-Time curve, 2,500 mg h/liter).

Fluconazole

FLZ is a synthetic triazole antifungal used in the treatment of cryptococcal meningoencephalitis and there are countries where it is the only available drug for treating these infections (Sudan *et al*., 2013; Wang *et al*., 2017). FLZ is recommended as the main therapeutic agent for the maintenance phase of cryptococcal meningoencephalitis, it has a safety profile, low toxicity, long half-life, physicochemical characteristics that carry on good tissue distribution, is orally bioavailable, has excellent CNS penetration, and has a linear PK (Wang *et al*., 2017; Santos *et al*., 2016; Alves *et al*., 2018; Hope *et al*., 2019).

The pharmacokinetics of FLZ were evaluated in healthy koalas after administration of a single dose of 10 mg/kg p.o. and i.v. NCA showed a shorter elimination half-life (t½) of 2.25 h after i.v. and 4.69 hours after p.o., administration and oral bioavailability was variable and low (0.53). This study demonstrated striking differences in t½, CL, Vss, F and ppb for koalas compared to other mammals. This makes it difficult to predict PK parameters for koalas based on allometric scaling (Black *et al*., 2014).

A study, in addition to evaluating the PK of FLZ in the plasma, evaluated it in the cerebral extracellular fluid (ECL) and cerebrospinal fluid (CSF), using the microdialysis technique that allows the determination of free concentrations at the site of interest (Hammarlund-Udenaes, 2017), to compare the distribution of FLZ in the rat brain with and without co-administration of probenecid. Probenecid significantly increased FLZ AUC_{0-300} in the brain ECF. However, the increases in the AUC_{0-300} values of the plasma and CSF with probenecid were not significant. Furthermore, probenecid significantly increased the penetration of FLZ into the brain (Wang *et al*., 2017).

Sudan *et al*., (2013) estimated the PK/PD index of FLZ for cryptococcal meningoencephalitis using a mice infected with clinical isolates of *C. neoformans* after dosing regimens of 125 and 250 mg/kg once daily for 9 days. A PK/PD model was used to evaluate these doses using plasma and cerebrum concentration data, and to perform extrapolations to humans. The AUC/MIC ratio = 389 was the most predictive index for the efficacy of FLZ, and only 66.7% of patients who received 1,200 mg/kg reach or exceed the stipulated AUC/MIC ratio. The study demonstrates that FLZ when used in monotherapy may be an inferior drug for therapy because many patients are unable to achieve the index, being advised to use the highest possible dose (Sudan *et al*., 2013).

With a similar result, Hope *et al.,* (2019) also used a PK/PD model to evaluate two doses of FLZ in a murine model of cryptococcal meningitis using data from plasma, cerebrum and CSF. The 3-compartment PK/PD model was used to perform extrapolations to humans. This model described that FLZ at clinically relevant exposures does not cause fungi eradication in the CNS and is ineffective in preventing the rapid onset of resistance (Hope *et al*., 2019).

Santos *et al*., (2016) developed a murine model of cryptococcosis caused by two strains of *C. gattii*, one resistant and the other susceptible, and administered a dose of 75 mg/kg of FLZ daily. In this study, it was observed that the AUC/MIC index was associated with the result of anti-cryptococcal therapy. The maximum concentration of FLZ in the brain was lower than the MIC for both strains. However, the treatment of mice infected with the resistant strain was ineffective even with the use of high doses. In addition, they evaluated the correlation between PK/PD modeling and antifungal resistance using a modified E_{max}-model and a sigmoid

E_{max}-model. They observed that the treatment of mice infected with the resistant strain was ineffective even with the use of high doses of FLZ (Santos *et al*., 2016).

Another study that also evaluated the PK of FLZ was carried out by Alves *et al*., (2018) using the microdialysis technique. In this study, FLZ PK was evaluated in healthy and *C. neoformans*-infected mice in a model of cryptococcal meningoencephalitis, after a dose of 20 mg/kg iv. Statistical differences were observed in the tissue penetration factor (*f*T) values $fT_{healthy} = 0.69$ versus $fT_{infected} = 1.04$. A two-compartment popPK model was used to describe time profiles of FLZ concentration in plasma and brain. The covariate infection was associated with parameters K_{21}, V_1 and V_2. This study demonstrated that the infection was able to alter the distribution of FLZ in the brain of infected animals. Furthermore, this study shows that when used in monotherapy, FLZ is of limited use in monotherapy to the treatment of cryptococcosis in rats and humans to a value of MIC >8 µg/mL (Alves *et al*., 2018).

Stott *et al*., (2018) came to a similar conclusion, after investigating the impact of a series of clinically relevant covariates on the penetration of FLZ into the CNS in adults with cryptococcal meningitis, after oral doses of 800 mg every 24 h in combination with AmB deoxycholate at a dose of 1 mg/day. kg every 24 hours. A four-compartment PK model described the parameters. The covariable patient weight was associated with the estimated volume of distribution. Monte Carlo simulation was used to assess the implications of PK variability in terms of reaching a target AUC/MIC = 389.3 after doses of 400 mg, 800 mg, 1200 mg and 2000 mg (all q24h). The recommended dosage of FLZ for cryptococcal meningitis induction therapy fails to attain the target in respect to the MIC distribution for *C. neoformans*. This study suggests that current FLZ regimens are inadequate for induction therapy for cryptococcal meningitis (Stott *et al*., 2018) and Monte Carlo simulations were performed for a range of fluconazole dosages. A meta-analysis of trials reporting outcomes of CM patients treated with fluconazole monotherapy was performed. Adjusted for bioavailability, the PK parameter means (standard deviation).

The study by Alhadab *et al*., (2019) investigated the role of sertraline as an adjunct in the treatment of HIV-associated cryptococcal meningitis in Ugandan patients receiving AmB, antiretrovirals (ART) and FLZ. A one-compartment PK model with first order absorption and elimination by exploratory sigmoidal E_{max}-model was used. The use of antiretrovirals was included as a covariate in CL/F. SER increased the exposure of FLZ in the brain which increased the fungal clearance of CSF (Alhadab *et al*., 2019).

A clinical study described popPK of FLZ in a cohort of critically ill nonobese, obese, and morbidly obese patients. In this study, popPK modeling of the plasma concentrations of 21 patients was performed and a two-compartment popPK model described these data points. The covariate CL_{CR} were included for clearance and body mass index for central compartment volume of distribution. This study demonstrated that a FLZ dose of 200 mg daily was insufficient to achieve an area under the concentration-time curve for the free, unbound drug fraction/MIC ratio (*f*AUC/MIC) of 100 for pathogens with MICs of >2 mg/L in patients with BMI of >30 kg/m^2 (Alobaid *et al*., 2016) obese, and morbidly obese patients. Critically ill patients prescribed fluconazole were recruited into three body mass index (BMI).

PBPK models can also indicate the first-in-human dose. A PBPK model proposed by Zhao *et al*., (2018) bridging method provided reliable estimates of FLZ PK parameters in neonates and demonstrated the viability of this approach to support the first-dose-in-neonates (Zhao *et al*., 2018). An ECMO PBPK model was used to derive FLZ dosing in children on ECMO across the pediatric age continuum, and simulations using the model fairly characterized observed PK data in infants on ECMO (Stott *et al*., 2018)and Monte Carlo simulations were performed for a range of fluconazole dosages. A meta-analysis of trials reporting outcomes of CM patients treated with fluconazole monotherapy was performed. Adjusted for bioavailability, the PK parameter means (standard deviation).

Voriconazole

VRZ is a second-generation triazole used in the treatment of invasive fungal infections exhibiting fungistatic activity against resistant strains of

Cryptococcus and has been recognized as a novel therapeutic option for the treatment of cryptococcal meningitis (Alves *et al*., 2017; Kirbs *et al*., 2019) it is crucial to know a given drug's free fraction that reaches the biophase. In the present study, we applied microdialysis (μD). VRZ is a moderately lipophilic compound, exhibits highly variable non-linear PK with large interindividual and intraindividual variability and is metabolized in the liver, primarily through CYP2C19 and, to a lesser extent, through CYP3A4 and CYP2C9 (Li *et al*., 2020).

Alves *et al*., (2017) evaluated the free levels achieved by VRZ in the brain of healthy and infected mice with *C. neoformans* in a model of cryptococcal meningoencephalitis using the microdialysis technique, after a dose of 5 mg/kg iv. A two-compartment popPK model and Michaelis-Menten (MM) elimination was used to describe the time concentration profiles of VRZ in plasma and brain. The rate of penetration into the brain showed an increase in exposure in animals infected with a $fT_{healthy} = 0.85$ versus $fT_{infected} = 1.86$. As a covariate, in this study the infection was included in V_2 and maximum metabolic rate (V_m). Another observation was that the drug levels that reached the infected tissues were higher than the MIC, which indicates a potential treatment for cryptococcal meningitis (Alves *et al*., 2017).

Another study also used the microdialysis technique to investigate VRZ in plasma and in the fluid of the interstitial space of the antifungal subcutaneous adipose tissue in healthy male volunteers after a sequence of dosages that began with iv infusions adapted to the total body weight every 12 h on day 1 (6 mg/kg over 2 h) and on day 2 (4 mg/kg kg over 1.3 h). On days 3 and 4, the dosage was changed to 200 mg tablets every 12 h. High interindividual variability was observed in the concentration-time profiles of VRZ, although doses have been adapted to body weight in the first i.v. administrations. Due to nonlinear PK, exposure to the target site of VRZ in healthy volunteers was considered highly comparable to plasma exposure, particularly after multiple doses (Kirbs *et al*., 2019).

The PK of antifungals can be modified by interaction with another compound. Li *et al*., proposed a PBPK model for VRZ. Knowing that VRZ is metabolized by CYP2C9 and CYP3A4, authors investigated the metabolism of VRZ to understand dose- and time-dependent alterations in the PK. The results show that VRZ causes time-dependent inhibition of CYP3A4. Simulations also showed that the standard oral maintenance dose of VRZ 200 mg twice daily would be sufficient for CYP2C19 intermediate metabolizers to reach the therapeutic range, while 400 mg twice daily might be more suitable for rapid metabolizers and normal metabolizers. When the model was integrated with independently developed CYP3A4 substrate models (midazolam (MID) and alfentanil), the observed AUC change of substrates by VRZ was within the 90% confidence interval of the predicted AUC change. The PBPK model developed could support individual dose adjustment of VRZ according to genetic polymorphisms of CYP2C19, and DDI risk management (Li *et al*., 2020).

To also look at DDI from VRZ and CYP3A4 substrates, Frechen *et al*., (2013) developed a PBPK model for VRZ and MID. The proposed semi physiological model approach generated a mechanistic explanation of the complex DDI occurring at major CYP3A expression sites and may thus serve as a valuable tool for maximizing knowledge obtained from clinical DDI studies (Frechen *et al*., 2013).

As we can see, VRZ has some interesting points in its PK. Not only for interactions with CYP3A4 substrates but also for proton pump inhibitors (PPI). According to Qi *et al*., (2017) various PPIs can affect the VRZ's PK. The findings show that the plasma concentration–time profiles of VRZ and PPIs simulated by the PBPK model indicated that VRZ's PK values improved to varying degrees when paired with different PPIs (Qi *et al*., 2017).

The metabolization of VRZ is the main cause of its interindividual variability,due to the knowledge that the VRZ's PK is different between adults and children. Zane, Thakker (2014) developed a model to predict PK parameters of VRZ in adult and pediatric populations. The pediatric oral model predicted oral bioavailability was twice greater than the observed levels. The estimation of oral bioavailability increased significantly after integrating intestinal first-pass metabolism into the model, indicating that VRZ is subject to intestinal first-pass metabolism in children but not in adults. The interest on changes of antifungal drugs in a pediatric population is growing (Zane, Thakker, 2014).

Isavuconazole

Isavuconazole (ISZ) is a new broad-spectrum antifungal agent and is the active moiety of the water-soluble prodrug isavuconazonium sulfate. ISZ presents values of MICs against *Cryptococcus* spp. from 0.008 mg/L to 0.5 mg/L. ISZ is a sensitive substrate and moderate inhibitor of cytochrome P450 (CYP) 3A4 in humans. It undergoes rapid hydrolysis by esterases in the gut and blood to release the poorly soluble but highly permeable active drug and presents an oral bioavailability of 98% (Kovanda *et al*., 2019; 2016).

The evaluation of the exposure and the effect of the dosage of isavuconazonium sulfate (prodrug of ISZ) in rabbits was carried out by Kovanda *et al*., (2019) using mathematical modeling. The PK/PD model described a similar significant reduction in fungal load on the brain and CSF were observed in rabbits treated with isavuconazonium sulfate and FLZ, showing that the treatment of cryptococcal meningoencephalitis with the two drugs are similar (Kovanda *et al*., 2019).

Ketoconazole

Ketoconazole (KTZ) is a chiral imidazole piperazine compound, which is a weak dibasic compound. KTZ is an antifungal agent with a broad-spectrum activity against various fungal infections. It is used to treat systemic opportunistic fungal infections that commonly occur in immunocompromised patients. Its absorption after oral administration is variable, as its solubility is pH-dependent, reaching the highest plasma concentrations at low gastric pH. KTZ is readily absorbed after conversion to the water-soluble salt by gastric acid, due to its high lipophilicity (Silva *et al*., 2018).

Johnson *et al*., (2018) investigated the absorption of KTZ in different ages of childhood. The t_{max} was estimated to be around 1h in both neonates and adults, but the former had a higher fa rating, indicating that more studies showing age-related differences in oral antifungal absorption (Johnson *et al*., 2018).

The changes in absorption in these drugs can be caused by many reasons. Silva *et al*., (2018) shows how hypochlorhydria – when hydrochloric acid is reduced on the stomach- can affect the absorption of KTZ. The findings shows that the disorder can cause incomplete drug's dissolution, which directly impacts the therapy's success (Silva *et al*., 2018). This finding is corroborated by Cristofoletti, Patel, Dressman (2016). Different situations were proposed by Pathak *et al*., (2017) and Hens *et al*., (2017; 2018) to identify the behavior in KTZ's and POSA's absorption, respectively (Hens *et al*., 2017; 2018; Pathak *et al*., 2017). The PBPK models successfully identified the effect of supersaturation and precipitation on the systemic plasma concentration. *In vitro* assays were carried out by Ruff *et al*., (2017) to imitate the supersaturation and precipitation behavior of KTZ. The findings were integrated in a PBPK model. In accordance with KTZ's high permeability, the simulated profiles were heavily affected by supersaturation, while precipitation was not expected to occur in vivo (Ruff *et al*., 2017).

CONCLUSION

This literature review showed that the use of pharmacometrics, which is a science applied to drug development, and which seeks to quantify the pharmacological effect through use of mathematical modeling and simulations of profiles of drug concentration versus time (PK) and pharmacological effect (PD), is an important tool for the integration of knowledge, in addition to allowing decision-making related to preclinical, translational and clinical studies of drugs. Our data resulting from this research allowed the identification of recent articles on this modeling and simulation approach, involving studies with PK/PD, popPK/PD and PBPK models, in addition to Monte Carlo simulations, PK evaluation and NCA. And as it was possible to observe, these mathematical tools are extremely important, because through the correlation of the PK and PD data of an antifungal it is possible to obtain more accurate and reliable results to carry out the appropriate decision making for the treatment of fungal infections caused by *Cryptococcus* spp, in addition to predicting other clinical scenarios, pathologies and optimizing specific dosages. In addition, several studies show that many of the treatments used are not effective,

and it is necessary to investigate new models that include more information about these difficult-to-treat infections.

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