Pharmacodynamics of Voriconazole in Children: Further Steps along the Path to True Individualized Therapy

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Voriconazole is the agent of choice for the treatment of invasive aspergillosis in children at least 2 years of age. The galactomannan index is a routinely used diagnostic marker for invasive aspergillosis and can be useful for following the clinical response to antifungal treatment. The aim of this study was to develop a pharmacokinetic-pharmacodynamic (PK-PD) mathematical model that links the pharmacokinetics of voriconazole with the galactomannan readout in children. Twelve children receiving voriconazole for treatment of proven, probable, and possible invasive fungal infections were studied. A previously published population PK model was used as the Bayesian prior. The PK-PD model was used to estimate the average area under the concentration-time curve (AUC) in each patient and the resultant galactomannan-time profile. The relationship between the ratio of the AUC to the concentration of voriconazole that induced half maximal killing (AUC/EC50) and the terminal galactomannan level was determined. The voriconazole concentration-time and galactomannan-time profiles were both highly variable. Despite this variability, the fit of the PK-PD model was good, enabling both the pharmacokinetics and pharmacodynamics to be described in individual children. (AUC/EC50)/15.4 predicted terminal galactomannan (P = 0.003), and a ratio of >6 suggested a lower terminal galactomannan level (P = 0.07). The construction of linked PK-PD models is the first step in developing control software that enables not only individualized voriconazole dosages but also individualized concentration targets to achieve suppression of galactomannan levels in a timely and optimally precise manner. Controlling galactomannan levels is a first critical step to maximizing clinical response and survival.

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of the pharmacokinetic and pharmacodynamic data were necessarily sparse, we buttressed the pharmacokinetics by using richer data obtained from the early phases of drug development. Such an approach enabled us to ensure robust estimates of the pharmacokinetics, which would have otherwise been extremely difficult or resulted in biased parameter estimates. The development of a linked PK-PD model is a further step in the provision of true individualized therapy where a drug is administered to control a biomarker that is itself intricately linked to therapeutic responses and optimal clinical outcomes.

MATERIALS AND METHODS

Patients. All patients aged <18 years receiving voriconazole, with at least one voriconazole serum concentration and galactomannan level measured, within the 9-year period from January 2005 to March 2014 were eligible for inclusion in this study. The medical, pharmacy, and laboratory records at the University Medical Center Groningen were reviewed. Demographic, microbiological, and clinical data were collected using standardized case report forms. The voriconazole treatment regimen and serum concentrations of voriconazole were also collected. Information that could potentially influence the voriconazole serum concentrations was identified and reviewed for potential inclusion of these serum concentrations into the population PK-PD model. The EORTC/MSG criteria (12) were used to determine the probability of invasive fungal disease for each patient at the start of voriconazole therapy. The Medical Ethical review board of the University Medical Center Groningen (metc 2013-491) waived the requirement to obtain informed consent from individual patients.

TDM. All patients at the University Medical Center Groningen who were treated with voriconazole underwent therapeutic drug monitoring (TDM). The first sample was typically taken after 2 days, and results were reported the same day. The therapeutic trough concentration targets were >1 mg/liter and <6 mg/liter. Concentrations outside these values prompted a change in dosage. There was no algorithm for dosage adjustment, but typically the dose of voriconazole was increased or decreased by 30 to 50% and concentrations were remeasured after several days. Galactomannan was not used to make decisions about dosage adjustment.

Voriconazole assay. The voriconazole serum concentrations were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (16). All measurements were performed on a Thermo Fisher (San Jose, CA, USA) triple-quadrupole LC-MS/MS with a Finnigan Surveyor LC pump and a Finnigan Surveyor autosampler, which was set at a temperature of 20°C. The Finnigan TSQ Quantum Discovery mass selective detector was operating in electrospray positive-ionization mode and performed selected reaction monitoring. The ion source spray voltage was set at 3,500 V, the sheath and auxiliary gas pressures at 35 and 5 arbitrary units, respectively, and the capillary temperature at 350°C. Cyanomipramine was used as internal standard. Analyses were performed on a 50-mm by 2.1-mm C18 5-μm analytic column (Hypersil Aquastar; Interscience Breda, The Netherlands). The column temperature was set at 20°C. The mobile phase consisted of an aqueous buffer (containing ammonium acetate [10 g/liter] water, acetic acid [35 mg/liter] water, and trifluoroacetic acid [2 ml/liter]), water, and acetonitrile. Chromatographic separation was performed using a gradient with a flow of 0.3 ml/min and a run time of 3.6 min. Sample preparation was performed by protein precipitation and found to be suitable, resulting in linear calibration curves in the range of 0.1 to 10 mg/liter. The peak height ratios of voriconazole and the internal standard were used to calculate concentrations. This method was validated in accordance with the Guidance for Industry Bioanalytical Method Validation of the Food and Drug Administration. The validation showed an overall bias ranging from 0.1 to 2.3%, a within-run coefficient of variation (CV) ranging from 1.9 to 7.8%, and a between-run CV ranging from 0.9 to 3.1%.

Galactomannan assay. The samples for the determination of the galactomannan index were measured using the Platelia Aspergillus enzyme immunoassay (EIA) kit (Bio-Rad Laboratories) as described by the manufacturer. A cutoff value for positivity in serum of >0.5 was used.

PK-PD modeling. The pharmacokinetic (i.e., serum voriconazole concentrations) and pharmacodynamic (i.e., galactomannan values) data from the 12 children were necessarily sparse, as they were collected as part of routine clinical care rather than as part of a prospective clinical trial. In addition, these sparse pharmacokinetic and pharmacodynamic data were not necessarily optimally informative. Fitting any pharmacokinetic model to a limited, sparse, and nonoptimally informative data set either is not possible or will lead to biased parameter estimates. We circumvented this problem using a two-step process. In the first step, each of the 12 new patients had their pharmacokinetics estimated using a previously described population PK model in which the PK model served as the Bayesian prior (11). In the second step, the Bayesian posterior estimates for each patient’s PK parameters were fixed, and the pharmacodynamic parameters were then estimated by fitting the pharmacodynamic component of the model to each patient’s galactomannan data. The population program Pmetrics was used for all modeling (17).

The structural pharmacokinetic mathematical model consisted of three differential equations that described the rate of change of the amount of voriconazole within each compartment. A fourth equation described the rate of change of galactomannan in the serum. These four inhomogeneous ordinary differential equations are as follows:

$$\frac{dX_1}{dt} = -K_g \cdot X_1$$  \hspace{1cm} (1)

$$\frac{dX_2}{dt} = K_g \cdot X_1 + \text{RateIV} - \frac{V_{\text{max}}}{K_{\text{m}} + V + X_2} \cdot X_2 - K_{\text{cp}} \cdot X_2 + K_{pc} \cdot X_4$$  \hspace{1cm} (2)

$$\frac{dX_3}{dt} = K_{cp} \cdot X_2 - K_{pc} \cdot X_1$$  \hspace{1cm} (3)

$$\frac{dX_4}{dt} = K_{\text{prod}} \cdot \left[ 1 - \left( \frac{x_4}{\text{POP}_{\text{max}}} \right) \right] \left[ 1 - \frac{X_4^H}{V} \right]$$

$$K_{\text{lim}} \cdot X_4$$  \hspace{1cm} (4a)

where $X_1$, $X_2$, and $X_4$ are the amounts (in milligrams) in the gut, central compartment, and peripheral compartment, respectively, and $dX_3/dt$ is the instantaneous rate of change in the amount of drug in compartment $n = 1, 2, 3, K_{pc}$ is the first-order rate constant of drug absorption after an oral bolus dose from the gut compartment (compartment 1) to the central serum compartment (compartment 2), RateIV is the rate of intravenous voriconazole infusion, $V_{\text{max}}$ is the maximum rate of the enzyme activity in metabolism of voriconazole (mg/h) and was allometrically scaled for body weight (kg) using the equation $V_{\text{max}} = V_{\text{max0}} \cdot \text{weight}^{0.77}$. $K_{pc}$ is the concentration of voriconazole in the central compartment at which voriconazole clearance is half-maximal, $V$ is the volume of the central compartment (liters) and was also allometrically scaled as $V = V_0 \cdot \text{weight}$, $K_{pc}$ and $K_{\text{lim}}$ are the first-order rate constants connecting the central compartment and peripheral compartment (compartment 3), $X_4$ is the concentration of galactomannan in the serum, $K_{\text{prod}}$ is the maximal rate of production of galactomannan in the central compartment, and $\text{POP}_{\text{max}}$ is the maximal achievable galactomannan value, $K_{\text{lim}}$ is the maximal rate of elimination of galactomannan from the central compartment, $H$ controls the steepness of the relationship between drug concentration and reduction in galactomannan production in the central compartment, and $E_{\text{conj}}$ is the concentration of voriconazole at which half-maximal reduction in galactomannan production is achieved. The oral bioavailability of voriconazole, $F$, was included because patients received voriconazole both orally and intravenously. In Pmetrics, $F$ is a multiplier on oral doses, and it is not included within the differential equations.
Equations 1, 2, and 3 describe the rates of change of voriconazole in the gut, central serum, and peripheral tissue kinetic compartments, respectively. Equation 4 describes the rate of change of galactomannan in the central serum kinetic compartment, and we divide this equation into two separate terms for clarity. First, equation 4a describes the production of galactomannan, which is limited by a maximum value and also by voriconazole concentrations in a sigmoidal function, such that when concentrations are infinite, production is zero; second, equation 4b describes the sum of all physiologic galactomannan elimination mechanisms. A baseline galactomannan value within compartment 4 on the day of the first voriconazole dose was also estimated within Pmetrics, with a possible range of 0.1 to 12, reflective of clinically observed extremes. The fit of the mathematical model to the data was assessed using visual inspection and linear regression of the observed versus predicted values both before and after the Bayesian step.

**Exposure-response relationships.** The relationship between a traditional pharmacodynamic measure of drug exposure, such as the ratio of the area under the concentration-time curve (AUC) to the MIC, and a therapeutic response is often not possible to determine for invasive aspergillosis because the invading pathogen is usually not recovered. Therefore, we used a new concept in these analyses. The AUC/EC50 is the ratio of the voriconazole daily AUC to the EC50, which is the posterior Bayesian estimate of the (in vitro) concentration of voriconazole required to induce half-maximal reduction in galactomannan levels in each individual patient. Thus, the EC50 is analogous to the more traditional in vitro estimate of drug potency, which is the MIC, but instead reflects an in vivo estimate of potency that can be derived from the change in galactomannan and voriconazole drug concentrations. The average daily AUC was calculated by estimating the total fitted AUC for each patient and dividing by the number of 24-hour treatment intervals. The average AUC circumvents the problem of defining which AUC is important for treatment effect (e.g., the AUC following the first dose or after a week of dosing). The relationship between the AUC/EC50 ratio and the final galactomannan level or survival was explored.

**RESULTS**

**Demographics.** The demographic data for the study population are summarized in Table 1. Fifty percent of patients had either acute myelogenous leukemia (AML) or acute lymphoblastic leukemia (ALL). The total mortality rate of the patient population was distressingly high: 10 (83.3%) of the 12 children died. For 4 of the 12 patients, *Aspergillus* spp. were recovered, and three of these four patients died from invasive aspergillosis. In the remaining patients, a diagnosis of probable invasive aspergillosis was established using galactomannan.

**TDM data for voriconazole and galactomannan.** There were a total of 261 and 33 measurements available for voriconazole concentrations and galactomannan levels from the 12 children, respectively. The concentration-time profiles for these respective readouts are shown in Fig. 1.

**Population PK-PD model.** The fit of the population PK-PD model to the data was acceptable despite the extreme pharmacokinetic and pharmacodynamic variability that is evident in Fig. 1. The Bayesian posterior estimates for the pharmacokinetics and pharmacodynamics are shown in Fig. 2 (left and right panels, respectively). The pharmacodynamics (i.e., galactomannan) were not well described using either the mean or median values for the parameters from the population model (data not shown). There simply was not a single set of parameter values that could be identified that was adequate to describe the time course of galactomannan in every patient. In contrast, however, the time course of galactomannan in each individual patient was readily described with a high degree of precision using the Bayesian posterior estimates for each patient. The heterogeneity of the different trajectories of galactomannan in individual patients is evident in Fig. 1 and 3. Of note, the initial condition (i.e., the galactomannan level at the commencement of treatment) was strikingly different between patients, potentially reflecting differences in underlying fungal burden. Furthermore, the time course of galactomannan in response to voriconazole therapy was also highly variable and ranged from a prompt decrease through to persistent antigenemia with no apparent therapeutic response.

**Relationship between AUC/EC50 ratio and terminal galactomannan level or survival.** The relationship between the AUC/EC50 ratio and the terminal galactomannan level is shown in Fig. 4. Using a simple nonlinear relationship, terminal galactomannan was strongly predicted by (AUC/EC50)/15.4 ($P = 0.003$). As a possible breakpoint, patients with an AUC/EC50 ratio $>6$ tended to have a more consistently lower terminal galactomannan level ($P = 0.07$). In contrast, the AUC/EC50 ratio was not associated with survival. The mean in those who died was 6.1, and that in those who survived was 7.6 ($P = 0.76$).

**DISCUSSION**

Much has been written about the use of therapeutic drug monitoring as an indispensable adjunct to the use of voriconazole for the treatment of invasive aspergillosis and other invasive fungal diseases (9). There is a strong and growing evidence base to support such an assertion. Patients with serum concentrations of $<1$ mg/liter appear to have poorer clinical outcomes and higher morality than patients with concentrations of $>1$ mg/liter (18). Similarly, patients with trough concentrations of $>5$ to 6 mg/liter
have a higher probability of having hepatotoxicity and confusion (18, 19). The case for routine TDM is further enhanced by the extreme pharmacokinetic variability that is characteristic of voriconazole and clearly evident in this study. The question raised by these analyses is whether TDM and dosage adjustment to achieve desired serum drug concentrations constitute the ultimate solution for using voriconazole and whether they constitute “true individualized therapy.”

The current strategy for TDM of voriconazole (or any other antimicrobial) is quite inconsistent with respect to individualization. The case for quantifying and controlling individual pharmacokinetic variability through dose modification has been made time and again by many people (including us). We have gone as far as use the information stored within population pharmacokinetic models to construct software that can be used for voriconazole dosage individualization in adults and children (10, 11). Importantly, the use of such software demands that the clinician define a drug concentration target that is deemed to have a high probability of therapeutic success and a low probability of toxicity. All the therapeutic targets that are used and cited in various guidelines are derived from large populations of patients, which are in effect “average” values. Such an approach is counter to all notions of individualized therapy and in fact is “one-size-fits-all” target selection. A significant advance that is enabled by the use of biomarkers such as galactomannan is the prospect of achieving true individualized target concentrations based on measured pharmacodynamics. Some patients will need more drug exposure, while others will need less. A different way of expressing this idea is that both the pharmacokinetics and pharmacodynamics are different from patient to patient but need to be optimized for an individual. A priori the trajectory of the voriconazole concentration-time profile or the galactomannan in an individual patient is unknowable. Variability in both pharmacokinetics and pharmacodynamics contributes to both good and poor clinical outcomes, and the achievement of optimal clinical outcomes requires control of both.

Figure 3 is particularly illustrative of the many challenges facing clinicians who are treating children with invasive fungal diseases. First, the pharmacokinetics of voriconazole are highly variable, as previously described by us and many others. Second, and perhaps more important, is the observation that the pharmacodynamics are also highly variable. There is no way of predicting which path (galactomannan trajectory) an individual patient will follow once voriconazole is started. Results from phase II and III clinical studies (20, 21) suggest that on average a satisfactory clinical response will be obtained when a fixed dosing strategy is used, but that does not provide any guarantee that the patient has been dosed such that the likelihood of a response is above average. Galactomannan provides a real-time indication of the patient’s individual response to voriconazole and whether a therapeutic response is being achieved or not. It is possible to react to changes in galactomannan directly rather than just to the voriconazole concentration. Consider the differences in galactomannan responses between patient 177 and patient 180 in Fig. 3. Both patients achieve comparable voriconazole serum concentrations in the first days of therapy, but their pharmacodynamics are com-

FIG 1 Voriconazole concentration-time profiles (A) and galactomannan-time profiles (B) for 12 pediatric patients who had concomitantly collected galactomannan and serum voriconazole concentration data. The samplings of voriconazole and galactomannan were not linked; hence, voriconazole serum concentration data were available after galactomannan sampling had stopped.

FIG 2 Observed versus predicted values after the Bayesian step for voriconazole serum concentrations (left panel) and for galactomannan (right panel). The solid lines are the linear regressions of the observed-predicted concentrations.
is remarkable that the PK-PD mathematical model fits any of the
that could be used as a Bayesian prior. Despite some limitations, it
ting was difficult and required a preexisting population PK model
sparse and were not collected at optimally informative times. Fit-
trajectory (despite having voriconazole concentrations ordinarily
patient 180 achieved a sustained response in their galactomannan
mann antigenemia, and the patient ultimately died. In contrast,
ever, the population value may not have been appropriate for that
agent, or a second antifungal agent added. Instead, the dosage was
should have the dose increased, the drug changed to an alternative
Patient 177 appears not to be responding to voriconazole and
pletely different for reasons that may not be immediately obvious.
Patient 177 appears not to be responding to voriconazole and
should have the dose increased, the drug changed to an alternative
agent, or a second antifungal agent added. Instead, the dosage was
reduced, probably because the upper TDM target was exceeded
(again, this value is derived from a population of patients). How-
ever, the population value may not have been appropriate for that
patient. This suboptimal regimen resulted in sustained galacto-
mannan antigenemia, and the patient ultimately died. In contrast,
patient 180 achieved a sustained response in their galactomannan
trajectory (despite having voriconazole concentrations ordinarily
considered to be associated with a higher probability of toxicity)
and ultimately survived.

We do not claim that this is an ideal data set. The data are
sparse and were not collected at optimally informative times. Fitt-
ing was difficult and required a preexisting population PK model
that could be used as a Bayesian prior. Despite some limitations, it
is remarkable that the PK-PD mathematical model fits any of the
data, given that they were collected in routine clinical settings.

Importantly, however, there is an acceptable fit of the model to the
data only after the Bayesian step. In this regard, fitting models to
galactomannan data is similar to fitting mathematical models to
drug resistance data, where population fits are often notoriously
bad. The reason for this is obvious following a brief inspection of
the raw data in Fig. 1B. The galactomannan data are nonmono-
tonic. Some profiles rise unexpectedly, while others fall. Such het-
rogenicity in response makes it nearly impossible to derive a single
set of parameter values that account for all the data in a reasonably
unbiased yet satisfactorily precise manner. We could have per-
formed Monte Carlo simulation on the Bayesian posterior esti-
mates to explore the impact of both pharmacokinetic and phar-
codynamic variability on the therapeutic outcome, but we
ultimately decided that this would have produced unreliable
results given the paucity of data (some patients have only one or two
observations). However, this could easily be done in the future
with larger and more comprehensive data sets.

The AUC/EC_{50} ratio is a pharmacodynamic index that may
be helpful in future studies of invasive aspergillosis. While the

FIG 3 Serum voriconazole concentration-time profiles (solid black lines) and serum galactomannan-time profiles (gray lines) from the 12 children with concomitant PK and PD data. The raw data for voriconazole (black circles) and galactomannan (gray circles) are shown. In each case, the model fit is from the Bayesian posterior estimate. Only patients 159 and 180 survived.
EC_{50} requires at least one measured voriconazole level and galactomannan level in a patient and requires some PK-PD modeling expertise, it captures and quantifies much of the pharmacodynamic variability that is evident in this study. Thus, the AUC/EC_{50} ratio provides an understanding of the therapeutic response in terms of drug exposure (AUC) as well as the pharmacodynamics. A high estimate for EC_{50} may be caused by factors such as a high fungal burden, the presence of antifungal resistance mechanisms, a delay in the initiation of antifungal therapy, infection within sanctuary sites, and profound immunosuppression. In this way, we view it as potentially superior to the in vitro MIC, which does not account for the clinical therapeutic environment within a patient. The AUC/EC_{50} ratio is a fully individualized in vivo estimate of drug potency, and it significantly predicted terminal galactomannan levels, even in this small study. It did not predict survival, but the majority of this cohort died from a range of causes, including the underlying disease. Furthermore, terminal galactomannan is likely a more objective reflection of in vivo voriconazole efficacy than survival, which is multifactorial, especially in these kinds of patients with complex underlying medical problems.

The next steps are clear. Larger, richer data sets that contain optically informative sampling for both voriconazole and galactomannan will enable the construction of more robust pharmacokinetic-pharmacodynamic mathematical models. These models will form the basis of dual-output stochastic controllers where a clinician has the option to individualize dosing to control the serum drug concentrations, the circulating biomarker, or both. Such an advance represents a further critical step toward the provision of true individualized therapy, which is surely the ultimate goal of all clinicians treating any patient with a life-threatening invasive fungal infection. Such an approach is one key advance for better care of immunocompromised patients who usually have multiple comorbidities. Moreover, as circulating biomarkers are developed for other diseases, this approach can be applied to a wider range of infections.

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REFERENCES


