In Silico Children and the Glass Mouse Model: Clinical Trial Simulations To Identify and Individualize Optimal Isoniazid Doses in Children with Tuberculosis

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Received 4 June 2010/Returned for modification 29 August 2010/Accepted 14 November 2010

Children with tuberculosis present with high rates of disseminated disease and tuberculous (TB) meningitis due to poor cell-mediated immunity. Recommended isoniazid doses vary from 5 mg/kg/day to 15 mg/kg/day. Antimicrobial pharmacokinetic/pharmacodynamic studies have demonstrated that the ratio of the 0- to 24-h area under the concentration-time curve (AUC0–24) to the MIC best explains isoniazid microbial kill. The AUC0–24/MIC ratio associated with 80% of maximal kill (80% effective concentration [EC80]), considered the optimal effect, is 287.2. Given the pharmacokinetics of isoniazid encountered in children 10 years old or younger, with infants as a special group, and given the differences in penetration of isoniazid into phagocytic cells, epithelial lining fluid, and subarachnoid space during TB meningitis, we performed 10,000 patient Monte Carlo simulations to determine how well isoniazid doses of between 2.5 and 40 mg/kg/day would achieve or exceed the EC80. None of the doses examined achieved the EC80 in >90% of children. Doses of 5 mg/kg were universally inferior; doses of 10 to 15 mg/kg/day were adequate only under the very limited circumstances of children who were slow acetylators and had disease limited to pneumonia. Each of the three disease syndromes, acetylation phenotype, and being an infant required different doses to achieve adequate AUC0–24/MIC exposures in an acceptable proportion of children. We conclude that current recommended doses for children are likely suboptimal and that isoniazid doses in children are best individualized based on disease process, age, and acetylation status.

Standard guidelines for isoniazid dosing in children vary greatly. The World Health Organization (WHO) recommends a regimen of 5 mg/kg daily or 10 mg/kg three times per week in children with tuberculosis (TB) (49). In countries that follow the WHO, the doses administered in the field are actually 6 to 7 mg/kg/day, raising concerns about overdosing (7). Recently, another WHO panel proposed 10 to 15 mg/kg daily (48). The American Thoracic Society/Centers for Diseases Control (ATS/CDC) also recommends 10 to 15 mg/kg daily (2). These recommendations are driven by naive pooled pharmacokinetic data; there are poor population pharmacokinetic data for children. The recommended regimens by and large evolved from those for adults, which are themselves based on peculiarities of children. The recommended regimens by and large evolved from areas where HIV is endemic (9, 27, 34). Pulmonary disease, when it occurs, is cavitary in only 6% of cases (49). This means that in this age group, indices of isoniazid penetration into meninges, phagocytes, and epithelial lining fluid (ELF) are important determinants of outcome.

There have been several attempts to design new isoniazid doses for children. One attempt identified a daily isoniazid dose that would achieve a peak serum concentration (Cmax) of 3 to 5 mg/liter, which is considered optimal (38). Using such an approach, it was demonstrated that the optimal daily dose that would achieve such a Cmax in children is 8 to 12 mg/kg (29). This was an important step in designing optimal doses, because it gave a clearly defined drug concentration target for clinicians to aim for. In order to improve on this, three other factors vital in determining pathogen response to drug concentration need consideration. The first is the MIC for the isolate. It is a general principle of antimicrobial pharmacokinetics-pharmacodynamics (PK/PD) that the higher the MIC, the poorer the response (1, 4, 10, 32, 42, 43). Thus, the variability of MICs is an important determinant of response. Therefore, drug concentrations should be indexed to MIC. Second, the optimal target exposure should be prospectively derived and defined from a full exposure-effect curve of the PK/PD index linked to effect. For isoniazid, the PK/PD index associated with optimal kill is unequivocally the ratio of the 0- to 24-h area under the concentration-time curve (AUC0–24) to the MIC, based on results from four independent studies (3, 17, 21, 35). The isoniazid AUC/MIC exposure associated with maximal or near-maximal kill of Mycobacterium tuberculosis is the best target to aim for in therapy. A third important issue is that of drug penetration to reach the pathogen; to state the obvious, optimal drug exposures are relevant only if they are achieved at the site of infection. In children with TB these anatomic sites are the subarachnoid space, phagolysosomes inside phagocytes, and pulmonary lesions.

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Published ahead of print on 22 November 2010.

Published ahead of print on June 23, 2017 by guest
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Clinical trial simulations that utilize Monte Carlo methods have been performed for identification of optimal dose and susceptibility breakpoints in adults with TB (13–18, 35). These simulations are stochastic rather than deterministic and take into account both pharmacokinetic and MIC variability as well as drug penetration to the site of infection. The central step is the ability to achieve the PK/PD exposures associated with maximum microbial kill as prospectively identified in animal TB models or in the hollow-fiber PK/PD model of TB. The latter model has been called the “glass mouse.” Recently Laer et al. called for simulations to identify optimal doses for the pediatric patient, the “in silico child” (25). In this study we used Monte Carlo simulations to identify optimal doses for children with TB who are 10 years old or younger, with target isoniazid exposure derived from our isoniazid PK/PD studies in the “glass mouse.”

MATERIALS AND METHODS

Isoniazid PK/PD targets. Isoniazid PK/PD exposure effect curves derived in the hollow-fiber, guinea pig, and mouse models have demonstrated similar inhibitory sigmoid Emax model parameters (17, 21, 35). The hollow-fiber PK/PD model of TB has no immune system, making it a particularly good model for deriving exposures for disseminated TB in children under 10 years old, in whom CMI is expected to be poor, especially in the context of HIV coinfection (34). In that model the isoniazid AUCl0–24/MIC ratio that mediates 80% of maximal kill of M. tuberculosis (80% effective concentration [EC80]) (considered the optimal effect) was 287.2 (17).

Isoniazid population pharmacokinetics in children less than 10 years old. There are no published population pharmacokinetic data for isoniazid in children. However, a study by Rey and colleagues in which isoniazid peak concentration, systemic clearance (SCL), and volume of distribution (Vd) were identified in 33 children and listed by age and acetylation status (41) was used to construct median parameter estimates and variance for children 10 years old. Because Schaat et al. demonstrated that the elimination rate constant increases with decreasing age in children (45), we also explored the effect of age on SCL and Vd in the results of Rey et al. However, the absorption constant (Ke) could not be calculated based on any of the published data, and we set it at 4.2 ± 5.64 h−1 for fast acetylators and 6.6 ± 7.49 h−1 for slow acetylators, similar to the values for adults (39). The effect of weight as an independent variable could not be assessed.

Disease pattern and isoniazid penetration into infected sites. Because of the problem of system hysteresis, multiple simultaneous samples of cerebrospinal fluid (CSF) and plasma from children with TB meningitis are needed. Donald et al. studied 1,431 children age 0.3 to 8.6 years multiple times and reported means (± standard deviations [SD]) of simultaneous CSF and plasma concentrations at seven time points (8). We analyzed these concentrations in order to compare the shapes of the isoniazid concentration-time curves for plasma and CSF. Isoniazid CSF and plasma concentrations at seven time points were analyzed with the ADAPT II program (6), with plasma data examined using a one-compartment model with first-order absorption and CSF data examined using a one-compartment model and first-order absorption with a lag. From these, the ratio of isoniazid AUCl0–24 in CSF to that in plasma was then identified and utilized for subsequent simulations. On the other hand, there are no data on isoniazid epithelial lining fluid (ELF)-to-plasma ratio in children, but a ratio of 2 has been calculated for adults and was used in this study (5, 22). Intracellular M. tuberculosis were assumed to be within neutrophils and macrophages (11). Based on drug penetration at four or five sampling time points, the isoniazid macrophage:plasma concentration ratio was calculated as 0.74 (95% confidence interval [CI], 0.45 to 1.03), while the neutrophil:plasma ratio was 1.26 (95% CI, 0.91 to 1.60) (5, 19, 40). Thus, for intracellular bacilli, isoniazid AUCl0–24 exposures were assumed to be equal to those in the central compartment. The protein binding of isoniazid is only 1% to 10%, and therefore protein binding in ELF and CSF is expected to be negligible (23, 37).

Monte Carlo simulations. Clinical trial simulations were performed in silico for 10,000 children who each received a dose of isoniazid for which we determined the probability of achieving or exceeding the AUCl0–24/MIC ratio of 287.2 in either (i) phagocytic cells, (ii) CSF, or (iii) ELF, by use of the Monte Carlo method (30, 31). The experimental doses examined were 2.5, 5, 7.5, 10, 15, 20, and 40 mg/kg/day. The optimal dose was the minimum dose that achieved the EC80 in ≥90% of children. Isoniazid pharmacokinetic parameter estimates and their variances (i.e., domain of inputs), based on our calculations from the data of Rey et al. (41), were entered as prior data in subroutine PRIOR of the ADAPT II program (6). After input of a random-number generator, the program output is a distribution of 10,000 SCLs. It is known that 88% of variability in the isoniazid SCL is due to polymorphisms of the arylamine N-acetyltransferase 2 (nat-2) enzymes encoded by NAT-2*4 alleles (24). Therefore, simulations were performed separately for fast and slow acetylators. Both normal and log-normal distributions were examined, and the final distribution was chosen on the basis of fidelity of recapitulating the original pharmacokinetic data. For a drug such as isoniazid, whose absorption is rapid and whose bioavailability approaches 100%, the AUCl0–24 is easily computed from the dose for each SCL value. At each MIC, AUCl0–24/MIC values that are ≥EC80 are identified and the target attainment probability (TAP) is summarized, based on proportions of isolates at each MIC in the distribution. For the purposes of this study, we utilized the MIC distribution for 1,217 clinical M. tuberculosis isolates from Japan, the largest known published set (50). However, it should be noted that for each locale, the final TAP depends on the MIC distribution unique to that place. As an example, we explored the TAP in fast-acetylator children in a hypothetical locale where bacilli are more susceptible to isoniazid by shifting the Japanese MIC distribution two tube dilutions lower.

Software and hardware. The ADAPT II program was developed by D’Argenio and Schumitzky by use of NIH funding and is therefore free software that can be downloaded from anywhere (6). Simulations utilizing this software were performed on a PC. For better graphing, some of the ADAPT II results were imported into GraphPad Prism 5 on the same PC.

RESULTS

Pharmacokinetics. The full pharmacokinetic parameters identified in the children are shown in Table 1. We found no overall correlation between age and either V or SCL when age was computed as a continuous variable (Fig. 1). However, when age was computed as a categorical variable for infants, who were defined as children 1 year old or younger versus those 1 to 10 years old, slow-acetylator infants had an SCL different from those of others (Table 1). In regard to CSF pharmacokinetics, Fig. 2 is based on our population pharmacokinetic modeling and depicts concentrations observed in the 27 children with TB meningitis who were treated with 20 mg/kg isoniazid, reported by Donald et al. (8), superimposed on the 24-h concentration-time profile based on our population pharmacokinetic modeling with their data. These data, however, are based on the report which had averaged concentrations for slow- and fast-acetylator children, so that only parameter estimates could be calculated. However, these data enabled us to calculate the isoniazid CSF/plasma AUCl0–24 ratio, which was 0.80. All these pharmacokinetic data are presented in Table 1 and were used in subsequent Monte Carlo simulations.
Monte Carlo simulations for children who are fast acetylators. The probabilities of each of the several doses achieving or exceeding EC80 in fast acetylators are shown in Fig. 3. Whether considering the treatment of disseminated TB with no meningitis (Fig. 3A), TB meningitis (Fig. 3B), or even pneumonia (Fig. 3C), doses of 2.5 to 10 mg/kg performed poorly within an MIC range for “susceptible” isolates. Indeed, for TB meningitis even doses of 15 to 20 mg/kg performed poorly in this group of children. An important finding, shown in Fig. 3A to C, is that different isoniazid doses perform differently according to site of infection, and therefore by disease syndrome, so that doses should be individualized by the type of TB that a child has.

Monte Carlo simulations for slow-acetylator children between 1 and 10 years old. Among children who were slow acetylators, isoniazid doses of 2.5 to 5 mg/kg performed poorly whether for disseminated TB, TB meningitis, or pneumonia (Fig. 4) caused by susceptible M. tuberculosis isolates. For TB meningitis, doses of up to 7.5 mg/kg were insufficient among a wide range of MICs (Fig. 4B).

Monte Carlo simulations for infants who are slow acetylators. Slow-acetylator infants had the lowest SCL, which means that relatively high AUCs could be achieved in this group (Fig. 5). Despite that advantage, doses of 5 to 7.5 mg/kg/day (Fig. 5B) were inadequate in infants with TB meningitis. Doses of up to 5 mg/kg were also inadequate in infants with disseminated disease (Fig. 5A), which means that 5 mg/kg would be useful only at low MICs and then only in infants with pulmonary TB not complicated by dissemination.

TAP. For each group of children, the overall performance of each dose, the target attainment probability (TAP), was calcu-
lated based on MICs from Japan (50); the TAPs are shown in Fig. 6. No dose of between 2.5 and 40 mg/kg/day achieved an EC80 in ≥90% of children. Doses of 5 mg/kg performed poorly across the board. Among fast acetylators, the performance of all doses, including WHO- and ATS/CDC-recommended doses, was extremely poor (Fig. 6A). Indeed, no dose achieved the EC80 in ≥90% of the children (Fig. 6A to C). Since the poor performance is driven not just by pharmacokinetics (and variability) but also by the MIC distribution, we examined a scenario for fast acetylators in which the MIC distribution curve reported by Yamane et al. (50) was shifted by two dilutions to the left, so that the median would shift from 0.03 mg/liter to 0.0078 mg/liter (i.e., making it really more susceptible). As shown in Fig. 6D, the TAP was somewhat better; however, 2.5 to 5 mg/kg still performed poorly, and 10 mg/kg was still inadequate for meningitis and disseminated TB.

**DISCUSSION**

In the treatment of latent TB, isoniazid is often administered as a single agent. Even though isoniazid is dosed in combina-
tion with rifampin and pyrazinamide for the treatment of active TB, it is nevertheless important to optimize the dose of this critical drug. Indeed, in diseases such as TB meningitis, where rifampin penetration into CSF/plasma is only 0.05 to 0.1 for a drug with 80 to 98% protein binding so that effective CSF exposures are only 1/1,000 to 2/100 of those in serum (26, 44, 47) and the efficacy of pyrazinamide is questionable given that the CSF pH is >7.0 and the drug is effective only at pHs below that (28, 51), the isoniazid effect is expected to play the predominant role in the combination. Clearly, from an efficacy standpoint the doses recommended by the WHO and the ATS/CDC are too low to achieve optimal exposures in many circumstances.

A second important point is the obvious need to individualize isoniazid doses in children. Despite the egalitarian desire for “standardized” therapy, in reality there is no “average” or “standard” child. They are as varied from each other as planets are from one another. In fact, variability is the point of evolution. Pharmacokinetic variability is one such manifestation, likely arising due to differential exposure of xenobiotic enzymes to plant chemicals in human ancestors (12). It is useful to assume that among the 2.2 billion children currently alive on earth, there will be 2.2 billion different concentration-time curves. Therefore, it is not a surprise that isoniazid pharmacokinetic parameters have a wide distribution, which means that a fixed dose will produce different AUC\(_{0-24}\) values in different children. Of course, in localities where fast acetylation is the overwhelmingly predominant phenotype, such as in countries of East Asia, the first doses administered would be those for fast acetylators, and the doses would be altered a few days later after establishment of the slow-acetylator phenotype by any one of several methods. The second factor to enter the decision on individualization of the isoniazid dose is the disease process, which is readily available from history and physical examination of the child. As an example, the small proportion of children in whom TB is unequivocally limited to a pneumonic process would get lower doses, while those with disseminated TB and TB meningitis would get higher doses, based on Fig. 6. The third important factor in individualizing the dose is the age of the child, so that there would also be a different dose for slow-acetylator infants.

Another important aspect arising from our study concerns the need for good population pharmacokinetic data. While we made use of line-listed SCL and \(V\) values published by others in the past, perhaps the greatest gap in knowledge that militates against optimal dosing for children is the lack of compartmental and population pharmacokinetics derived from a large and diverse enough population of children. This is indeed the major limitation of our current paper. Studies should examine children at different ages as well as with differences in nutritional status, presence of AIDS, concomitant antiretroviral therapy, and SNPs of genes encoding xenobiotic metabolism.

**FIG. 6.** Relationship between dose and target attainment probability. The results are for 10,000 children who are fast acetylators (A), slow acetylators and between 1 and 10 years old (B), slow acetylator infants (C), or fast acetylators with *M. tuberculosis* highly susceptible to isoniazid (D).
enzymes as covariates of population pharmacokinetic parameters. In this context, our current studies represent merely a first step. In regard to isoniazid CSF parameters and drug penetration, the only other study to sample the CSF multiple times that we could find was with adults; nevertheless, the elimination constants and CSF/plasma penetration ratios in that study were similar to our calculations (46). However, clearly both studies were small, and larger studies are needed. With better population pharmacokinetic parameter estimates from such studies, even more precise optimal doses will be identified and individualization of doses further improved beyond our current attempts.

Finally, while microbiological diagnosis is often difficult in children, there is nevertheless a great need to isolate enough strains of *M. tuberculosis* from children in many different countries so that true MIC distributions can be established. Given that evolution is also a pressure on *M. tuberculosis*, there is expected to be a genetically determined wide variability in MIC distribution from region to region. The current practice of categorizing isolates only as either “resistant” or “susceptible” based on epidemiological cutoff methods is problematic; the breakpoints themselves are questionable based on PK/PD grounds (15). While the dichotomous interpretation should be continued, it would be more informative if the actual MICs were also known.

There are other limitations to our proposals. It is likely that with increased dosing there will be increased toxicity. It is currently unclear which isoniazid pharmacokinetic parameter best predicts the development of toxicity. The increased isoniazid hepatotoxicity in adult TB patients who carry the NAT2*6A* haplotype (SNPs C282T and G590A), which is associated with slow acetylation, and the protection by the NAT2*4 haplotype, which is associated with fast acetylation (20), suggest that either the AUC0–24 or the time that concentration persists above a threshold is a predictor of toxicity. With studies to better understand the isoniazid toxicodynamics, regimens that optimize efficacy while reducing toxicity in the same way as suggested for pyrazinamide can be designed (35). A second important limitation may be encountered with individualized dosing in resource-limited countries, especially the determination of acetylation status. A solution may be a 3-h plasma sample after a few doses or performance of one of the surrogate urine tests for acetylation phenotype sent out as a routine test to a central laboratory or hospital in the country. Even if such results take a few weeks to come back, they could nevertheless be used to adjust the dose downward if the child is a slow acetylator. Faster and inexpensive colorimetric point-of-care tests could also be a focus for further research. Nevertheless, while resource limitations may limit the capacity of some pediatricians to individualize doses, the rational approach would be to attempt this as far as is practicable, since this is likely to lead to better patient outcomes (33). Finally, we did not attempt to design doses that could suppress resistance. Those would be higher than doses for optimal microbial kill and likely beyond the range that is clinically useful.

In summary, we examined several doses of isoniazid in children ≤10 years old in Monte Carlo simulations. Currently recommended doses are not expected to achieve optimal exposures in children, and higher doses are needed. In addition, doses should be individualized by disease process, age, and acetylation status. The precision of identifying optimal doses would be improved by further studies that establish population pharmacokinetics of anti-TB drugs in children as well as the drug MIC variability in clinical isolates of *M. tuberculosis* from pediatric patients.

REFERENCES


