Clinical and Toxicodynamic Evidence that High-Dose Pyrazinamide Is Not More Hepatotoxic than the Low Doses Currently Used[▽]

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Antimicrobial pharmacokinetic-pharmacodynamic studies suggest that pyrazinamide doses higher than those currently recommended may be more efficacious. However, high pyrazinamide doses are believed to be hepatotoxic. Searches for clinical trials in MEDLINE, EMBASE, and the Cochrane Controlled Trial Register were made. Studies that employed pyrazinamide dose scheduling and pharmacokinetic analysis design were examined. Population pharmacokinetic modeling methods were utilized to identify parameters associated with toxicity. At an equivalent area under the concentration-time curve, the time that concentration persisted above some thresholds was associated with overall adverse events ($P = 0.032$), arthralgia ($P = 0.089$), and an elevated serum aspartate aminotransferase level at 3 months ($P = 0.067$). Next, a meta-analysis was utilized to compare rates of adverse events (i) between different pyrazinamide doses, (ii) between different dosing schedules, and (iii) between pyrazinamide-containing and non-pyrazinamide-containing antituberculosis regimens. The 29 studies selected were heterogeneous (Cochrane Q statistic P value of <0.001 ; I^2 of $>95\%$). For the once-a-day dosing schedule, arthralgia was dose dependent ($r^2 = 0.996$). However, arthralgia was less common with intermittent dosing, consistent with the time concentration persisted above the threshold. Arthralgia was generally clinically inconsequential. The frequencies of hepatotoxicity were 0.057 (95% confidence interval [CI], 0.021 to 0.141) for pyrazinamide monotherapy, 0.044 (CI, 0.033 to 0.059) for pyrazinamide-containing combination regimens, and 0.040 (CI, 0.023 to 0.040) for non-pyrazinamide-containing combination regimens. The frequencies of hepatotoxicity were 0.042 (CI, 0.026 to 0.067) for 30 mg/kg of body weight, 0.055 (CI, 0.031 to 0.094) at 40 mg/kg, and 0.098 (CI, 0.047 to 0.193) at 60 mg/kg of pyrazinamide. Thus, high-dose pyrazinamide did not significantly increase hepatotoxicity. This suggests that a considerable portion of hepatotoxicity rates may be idiosyncratic.

Pyrazinamide was responsible for shortening the duration of antituberculosis rifampin-containing regimens to the current 6-month standard for drug-susceptible tuberculosis (TB) (3, 18, 31). In addition, pyrazinamide is often part of the treatment for multidrug-resistant and extensively drug-resistant TB. Recent antimicrobial pharmacokinetic-pharmacodynamic studies suggested that doses higher than the dose of 15 to 30 mg/kg of body weight/day currently used would be more efficacious (19). In addition, pharmacokinetic studies have demonstrated increased pyrazinamide serum clearance and volume of distribution with weight (16, 53), so higher doses than those currently used are needed to achieve the same exposures in heavier patients than in leaner patients. However, the main limitation of high-dose pyrazinamide therapy has been a high rate of adverse reactions (8, 11). Frequently described adverse events (AE) are arthralgia and hepatotoxicity (3, 17). The latter is also common to many anti-TB drugs used in combination with pyrazinamide, so causality is not established. While guidelines for attributing AEs to drugs are now clear (12), most previous reports that detailed pyrazinamide AE (8, 11, 13) fell short of these standards. Unfortunately, such reports form the backbone of the idea that pyrazinamide causes most of the observed AEs in anti-TB regimens.

Toxicodynamics of antimicrobial drugs have of late started to benefit from a better understanding of pharmacokinetic variability and dose fractionation techniques that allow the identification of particular aspects of the concentration-time curve that best determine drug toxicity (35, 42). Using these techniques as well as a meta-analysis, we examined how often toxicity occurs with pyrazinamide monotherapy and with pyrazinamide combination therapy for active TB. Second, we wanted to identify the time to occurrence of nonidiosyncratic pyrazinamide AEs. Third, we wanted to determine the role of dose and dose schedule on the frequency of AEs.

MATERIALS AND METHODS

Definition of terms. The Council for the International Organization of Medical Sciences and the Food and Drug Administration definitions for drug AEs were used in our study (12, 50). AE refers to any unfavorable and unintended sign, symptom, disease, overdoses, and medication errors temporally associated with the use of a drug regardless of causality (12). For more recent studies, serum alanine aminotransferase (ALT) levels greater than three times the upper limit of normal with symptoms of hepatitis or greater than five times the upper limit of normal were defined as hepatotoxicity (3, 12). For older studies, standard laboratory values for the bromsulphthalein and/or the cephalin-cholesterol flocculation test used at the time were acceptable (39). Arthralgia was any joint pain reported during a study. Mortality refers to any deaths occurring during a study regardless of the cause.

Search strategy. Searches were performed in the MEDLINE, EMBASE, and the Cochrane Controlled Trial Register (Cochrane Library 2004, issue 2) databases. A search for title and abstract was made by using the following search Medical Subject Heading (MeSH) terms: “tuberculosis or *Mycobacterium tuberculosis*” and “antituberculosis therapy or antituberculosis drugs” and “drug toxicity.” Searches were limited to English-language literature published between January 1950 and December 2008. We supplemented the electronic data search

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FIG. 1. Literature search strategy and results of trials included in the present analysis.

with a manual search of all references listed from the studies obtained in the above-described search using the same standardized abstraction form. The references from these articles were used to supplement the search for clinical trials performed prior to 1963 since some of them were not adequately captured by electronic databases. The search strategy is depicted in Fig. 1.

Data entry. Data were abstracted from the spreadsheets, text, and tables and entered into Microsoft Excel spreadsheets. All the AEs defined above, time to AE, daily pyrazinamide dose, duration of therapy, cumulative dose, and gender were captured. The data were then exported to different programs for analysis.

Toxicodynamic analysis. For the purpose of determining pharmacokinetic parameters associated with toxicity, studies were chosen if they used a dose-scheduling (dose fractionation) design and measured serum pyrazinamide concentrations at multiple time points. In the East African pyrazinamide study, patients were treated with a dose of either pyrazinamide at 0.5 g three times a day ($n = 92$), 1.5 g daily ($n = 96$), or 3 g every other day ($n = 192$) for 6 days of each week, for a cumulative weekly oral dose of 9 g (15). The patients all received streptomycin intramuscularly. Patients were treated in the hospital for 6 months, with directly observed therapy. A proportion of these patients had serum and urine concentrations of pyrazinamide and pyrazinoic acid measured (16). We used the previously reported values of the “Nairobi crossover” groups in the study to identify the compartmental pharmacokinetics of pyrazinamide using ADAPT 5 software (2), and we used the identified parameters to calculate the 0- to 168-h area under the concentration-time curve (AUC_{0-168}) for each regimen. The peak maximum concentrations of drug in serum (C_{max}) and the percentage of time that the concentration persisted above 5 mg/liter, 10 mg/liter, or 20 mg/liter utilized were those observed for the original pharmacokinetic study (16). Comparisons of the proportion of patients with all AEs (excluding vestibular toxicity, which is due to streptomycin), elevated aspartate aminotransferase (AST) levels, and arthralgia were made between each of the three dosing regimens as well as between the two daily doses as a combined group versus the intermittent group (every other day) using Pearson’s chi-squared test.

Monotherapy studies. Monotherapy studies that used many different doses of pyrazinamide were examined for relationships between dose and AE (37, 43, 54). Because data for some of the patients were missing, we used only all AEs, daily dose, and cumulative dose for analyses of associations with AEs in a logistic regression model. The model included a constant, with 20 maximum iterations, and P values of 0.05 for entry and 0.10 for removal at each backward step.

Meta-analysis of controlled studies. Prospective controlled studies and observational studies with concurrent controls that reported the presence and absence

of AEs as an endpoint and reported the AEs in a standard manner met the inclusion criteria (12). Other inclusion criteria were (i) studies that clearly confirmed active TB by microscopy and/or culture; (ii) AEs reported as a study outcome with clear, standard, and clinically acceptable definitions; and (iii) the use of ethical and clinically acceptable methods for that time. Studies were excluded if they were retrospective or if they combined previously reported data already included in the search. Case reports, case series, as well as comparative studies with historical controls, case-control, and retrospective design were excluded because of the higher risk of bias associated with these designs. Since the observed frequencies of AEs associated with treatment regimens were the outcomes of interest, studies done with highly selected populations were excluded. These populations were defined as incarcerated or posttransplant patients or children or selected minorities as well as patients in phase I/phase II studies done as a proof of concept or to test new medicinal products.

Both authors reviewed selected abstracts and articles considered for inclusion. Discrepancies were resolved by consensus. The quality of each trial was graded by use of a validated score whose maximum possible score is 5 (25). Full description of evaluation methods, description of participants who withdrew from or dropped out of the trial, and randomization of participants were given 1 point for each item present. If randomization was concealed and the method of double-blind evaluation was appropriate, the study was assigned 1 additional point.

Individual-level data from controlled clinical trials that compared various established anti-TB treatment regimens were examined. Three categories of anti-TB regimens were examined: pyrazinamide monotherapy regimens (category A), combination regimens that included pyrazinamide (category B), or combination regimens that excluded pyrazinamide (category C). The outcomes of interest were (i) all AEs, (ii) hepatotoxicity, (iii) arthralgia, (iv) proportion of patients interrupting therapy, and (v) death.

Statistical analysis. The number of patients randomized to each treatment regimen was used as the denominator. Weighted mean percentages of patients with each AE were estimated by using either fixed- or random-effects models according to established methods (4). For both models, each study datum was weighted by the inverse of its variance. However, because random-effects models combine within-study data variance and between-study data variances, DerSimonian and Laird methods were used to obtain between-study data variance (4, 20). Heterogeneity across studies was compared by using the Cochrane chi-squared Q test and quantified by I^2 index methods. Sensitivity analysis was performed to assess the robustness of obtained estimates of hepatotoxicity frequencies when the quality of pooled studies was considered.

Software. Population pharmacokinetic analysis was performed by using ADAPT 5 software. GraphPad Prism (version 5) and Comprehensive Meta-Analysis software were used for statistical analysis and meta-analysis. All software was run on a personal computer.

RESULTS

Toxicodynamic analysis. Pharmacokinetic exposures reported in the East Africa study as well as those calculated using compartmental population pharmacokinetic analysis are shown in Table 1. For elevated aspartate aminotransferase (AST) levels, only a cutoff point of >50 Karmen units was reported. This is

TABLE 1. Pharmacokinetic parameters of patients in a dose-scheduling study design

Parameter ^a	Value for dosing regimen of:		
	500 mg 3 times a day	1,500 mg daily	3,000 mg alternate days
SCL (liters/h)	4.11	3.97	4.16
AUC_{0-168} (mg · h/liter)	2,189.2	2,267.00	2,162.94
C_{max} (mg/liter)	20.2	33.8	67.5
Time > 20 mg/liter (%)	9	24	23
Time > 10 mg/liter (%)	67	49	35
Time > 5 mg/liter (%)	92	74	48

^a SCL, systemic clearance; AUC_{0-168} , area under the concentration-time curve from 0 to 168 h; C_{max} , peak concentration; Time >, time above which the concentration persists.

equivalent to >54 international units/liter and was used to define hepatotoxicity for the purposes of the toxicodynamic analysis. No differences in overall AEs or arthralgia were found when each of the three dose schedules was compared to the other ($P = 0.1$). However, hepatotoxicity at 3 months occurred in 11/49 patients on three-times-a-day therapy, 19/51 patients with once-a-day therapy, and 18/90 patients with the every-other-day regimen ($P = 0.067$). When both daily therapy regimens were compared to the every-other-day regimen, all AEs (14/188 versus 5/190 patients; $P = 0.032$) and arthralgia (7/190 versus 2/192 patients) were more common with the daily therapy regimens ($P = 0.09$). Since the cumulative AUC was similar regardless of dose schedule, and the $C_{\max}$ was lowest with 0.5 g three times a day but highest with 3 g every other day (Table 1), the AUC and $C_{\max}$ were least associated with either all AEs or arthralgia. Since the percentage of time when the concentration exceeded 20 mg/liter was lowest with the 0.5-g three-times-a-day regimen, it too was inconsistent with the pattern of the rate of AEs. However, since the percentage of time when the concentration exceeded 5 mg/liter and, to a lesser extent, 10 mg/liter increased with more frequent dosing, these two parameters better explained the pattern of the higher rates of AE and arthralgia encountered more often with the daily therapy regimens.

Pyrazinamide monotherapy studies. Three separate pyrazinamide monotherapy studies were identified (Table 2) (37, 43, 54). Only the study by Yeager et al. (54) had results presented that were comprehensive enough, and these results were further analyzed. Severely ill TB patients received a mean pyrazinamide daily dose of 3.7 g (range, 1.2 to 7.6 g) for 3 to 75 days as salvage therapy. AEs were encountered for 22/43 patients, with the most frequent AEs being arthralgia (12/43 patients), palpitations (4/43), and pyrexia (4/43). The majority of AEs occurred after 30 days of therapy. Hepatotoxicity was reported for only 2 (5%) patients, both of whom had abnormal flocculation tests and jaundice after 52 and 87 days of treatment. In both cases, hepatotoxicity was resolved after the pyrazinamide therapy was stopped. Five patients died, two during therapy and the remainder after therapy. None of the deaths were judged to be drug related.

In multivariate logistic regression analysis, only the daily dose significantly predicted pyrazinamide-induced arthralgia, with a 2-fold increase in arthralgia with each 1-g increase in the daily dose ($r = 0.580$; $P = 0.014$). The relationship between daily dose and all AEs is shown in Fig. 2. When all adverse events were combined and plotted against the daily pyrazinamide dose, the inflection point for increased toxicity was at 4.5 g/day (data not shown).

Meta-analysis of controlled studies. Twenty-nine controlled clinical trials with over 13,000 patients from Africa, Asia, Europe, and the Americas met inclusion criteria. Characteristics of included patients and the studies that met the inclusion criteria are shown in Table 2. The median follow-up period ranged from 3 to 24 months but was statistically similar between the regimens tested. The majority of the trials (18/29 trials) had patients randomized to assigned treatment regimens. The agreement between the two reviewers was associated with κ values of 0.9 for the inclusion of trials and 0.9 for the rating of trials on considered methodological aspects (25). Tests for heterogeneity for all outcomes are shown in Fig. 3. A

pooled analysis of all studies showed substantial heterogeneity across studies (Cochrane Q statistic P value of <0.001 ; I^2 of $>95\%$), indicating that there is no certain frequency rate for the outcomes of interest around which all studies can be pooled (Fig. 3). Consequently, random-effects point estimates were used to infer statistical differences between groups. The funnel plot and classical fail-safe methods demonstrated a z value of 44.476, with a P value of <0.001 . Thus, no significant bias was observed.

Over 3,000 of 13,000 (22%) patients reported one or more AEs, and 211 (2%) patients died. Twenty-nine percent of patients who received pyrazinamide monotherapy reported an AE. Pyrazinamide monotherapy studies were monitored for longer periods than were studies of combination regimens. Among those patients who received combinations that included pyrazinamide, 19% reported AEs, while among those who received combination therapy with no pyrazinamide, 14% reported AEs (Fig. 3). The mean times to AEs were 1.48 months for monotherapy and 1.21 months for combinations ($P = 0.3017$ by log rank test). Arthralgia was the most frequent AE and was encountered in 16% of patients receiving pyrazinamide alone, compared to 2.5% of patients receiving combinations that included pyrazinamide and 0.4% of patients receiving combinations that did not have pyrazinamide ($P < 0.0001$) (Fig. 3). However, those studies were highly heterogeneous. Pooled frequency estimates for the other AEs were statistically similar whether pyrazinamide was added to the regimen or not (Fig. 3). Specifically, the frequency of hepatotoxicity and jaundice was less than 6% across all regimens tested and did not differ whether or not pyrazinamide was included in a regimen (Fig. 4).

In terms of dose and dose schedule, the 15 studies (1, 6, 7, 9, 10, 21–23, 26, 29, 36, 40, 41, 44, 48) examined for these factors were homogeneous (Cochrane Q statistic of 1.681; $P = 0.195$). The pooled frequency estimate for a pyrazinamide dose of ≥ 60 mg/kg was 0.098 (confidence interval [CI], 0.047 to 0.193), that for 40 mg/kg was 0.055 (CI, 0.031 to 0.094), and that for 30 mg/kg was 0.042 (CI, 0.026 to 0.067) (Fig. 5). Among patients on pyrazinamide-based combination therapy, hepatotoxicity was more frequent in patients treated with daily doses (frequency, 0.054 [CI, 0.034 to 0.086]) than in those treated with intermittent doses (frequency, 0.027 [CI, 0.016 to 0.047]) ($P = 0.058$), even though the cumulative weekly dose with intermittent therapy was 10.5 g versus 9 g for daily therapy. Thus, hepatotoxicity occurred with more frequent dosing despite a slightly lower cumulative dose, which means that it was associated with the percentage of time that the concentration exceeded a threshold but not total exposure (AUC) or peak concentration.

Sensitivity analysis. To test for alternative explanations for these data, pyrazinamide and rifampin doses were correlated against peak serum AST level data. No significant correlation between the pyrazinamide dose and peak serum AST levels was observed ($P = 0.5561$; $r = 0.0161$). The rifampin dose was also not correlated with liver function ($P = 0.8633$; $r = 0.0507$). Further analysis of only high-quality trials, i.e., a quality score greater than 3, did not significantly alter conclusions compared to those obtained when all trials were included in the analysis.

TABLE 2. Characteristics of selected controlled clinical trials

Study	Study author(s) (reference) ^b	Yr	Place	Sample size	Regimen ^a	Trial quality score	Trial participants
Pyrazinamide monotherapy (category A)							
S1	Yeager et al. (54)	1952	United States	43	Z ₇ 0.2 g/kg/day	1	Advanced TB; retreatment
S2	Schwartz and Moyer (43)	1954	United States	19	Z ₇ 3.0 g/day	1	Advanced TB; retreatment
S3	Phillips et al. (37)	1954	United States	11	3Z ₇ 3.0 g/day	1	Advanced TB; retreatment
Combinations regimens including pyrazinamide (category B)							
S4	Indian Council of Medical Research (49)	1997	India	438	2EHRZ ₇ /6EH ₇	3	New smear-positive TB
				374	2HRZ ₇ /4HR ₂		
S5	Hong Kong Tuberculosis Treatment Services/BMRC (24)	1976	Hong Kong	371	6SHZ ₃	3	New smear-positive TB
				185	6SHZ ₃		
				182	6SHZ ₃		
				41	6SHRZ ₇		
S6	Hong Kong Chest Service/BMRC (23)	1978	Hong Kong	354	6SHZ ₇	3	Smear negative
S7	Hong Kong Chest Service/BMRC/Chennai (21)	1989	Hong Kong/India	276	2SHRZ ₇ /4SHZ ₂	3	
				447	3SHRZ ₇		
				234	4SHRZ ₇		
				416	3SHRZ ₇		
				645	4SHRZ ₃		
				235	6SHRZ ₃		
S8	Potter and Chang (40)	1955	United States	60	6SZ ₇	1	Advanced TB; retreatment
S27	Phillips and Horton (36)	1956	United States	38	18HZ ₇	2	New smear-positive TB
S9	Mathews (29)	1959	United States	261	HZ ₇	2	New smear-positive TB
S10	Kamal et al. (26)	1976	India	31	6SHZ ₇ (Z = 30 mg/day)	2	
				50	HZ ₇ (Z = 60 mg/kg)		
S11	Allison (1)	1956	United States	63	HZ ₇ (Z = 0.5 g t.i.d.)	2	
S12	Ramakrishnan et al. (41)	1968	India	19	SHZ ₇ (Z = 70 mg/kg)	1	Advanced TB; retreatment
S13	Campagna et al. (7)	1953	United States	21	HZ ₇ (Z = 350 mg q.i.d.)	1	
S14	Hong Kong Chest Service/BMRC (22)	1981	Hong Kong	244	6HRSZ ₇	3	
				243	6HRSZ ₃		
				241	6HRZE ₃		
				239	6HRZE ₇		
S15	Cohn et al. (9)	1990	United States	125	6HRZ ₇	2	New smear-positive TB
S16	East African/BMRC Pyrazinamide Investigation (15)	1969	East Africa	96	6SZ (t.i.d.)	3	
				102	6SZ (o.d.)		
				104	6SZ ₃		
				94	6SZ ₃		
S17	Combs et al. (10)	1990	United States	617	2HRZ ₇ /4HR ₇	2	
S18	British Thoracic Association (6)	1981	United Kingdom	170	2HRSZ ₇ /4HR ₇	3	
				164	2HREZ ₇ /4HR ₇		
S19	Singapore Tuberculosis Services/BMRC (44)	1979	Singapore	397	2SHRZ ₇ /4HRZ ₇	3	Smear positive
S20	Tuberculosis Research Centre (48)	1986	India	297	3SHRZ ₇	3	New smear-positive TB
				307	3SHRZ ₇ /2SHZ ₂		
				304	3SHZ ₇ /2SHZ ₂		
S21	Okwera et al. (34)	1994	Uganda	101	2RHZ ₇ /7HR ₇	5	
S28	USPHS Tuberculosis Therapy Trial (51)	1959	United States	194	6HZ ₇	2	Advanced TB; retreatment
				122	3HZ ₇ /3HP ₇		
				197	6HZ ₇		
				115	3HZ ₇ /3HP		
S22	Velu et al. (52)	1961	India	36	12ZH ₇	1	Smear positive
S23	Small (45)	1959	United States	114	HZ ₇	1	Smear positive
S24	McDermott et al. (30)	1954	United States	55	HZ ₇ (Z = 50 mg/kg)	1	Advanced TB; retreatment
S25	Livings (28)	1956	United States	376	HZ ₇	1	
S26	Zierski and Bek (55)	1980	Poland	119	2HRSZ ₇ /4HR ₂	5	
Combinations regimens excluding pyrazinamide (category C)							
S5	Hong Kong Tuberculosis Treatment Services/BMRC (24)	1976	Hong Kong	137	SPH ₇	3	Smear positive
S6	Hong Kong Chest Service/BMRC (23)	1978	Hong Kong	224	SHR ₇	3	Smear positive

S27	Phillips et al. (37)	1954	United States	261	SHRE/SHRE ₂	1	
S9	Mathews (29)	1959	United States	55	18HP ₇	1	
S11	Allison (1)	1956	United States	237	HP ₇	1	
S14	Hong Kong Chest Service/BMRC (22)	1981	United States	51	HP ₇	3	
S17	Combs et al. (10)	1990	Hong Kong	238	6HRSE ₃	2	
S18	British Thoracic Association (6)	1981	United States	445	HR ₇	3	
S29	BMRC Co-Operative Study (5)	1973	United Kingdom	177	2HRE/7HR ₇	3	
				122	3SHF/9HP ₇	3	
				116	3SHR/9HE ₇	5	New smear-positive TB
				112	3SHR/9HR ₇		
				116	3SHP/9S2H ₇		
				103	2HRE/4HRE (daily)		
				105	2HRE/4H2R ₂		
				102	2HRE/4HRE		
					(once a week)		
				101	2HRE/4H2R2E ₂	5	
S21	Okwera et al. (34)	1994	Uganda	90	2STH/10TH ₇	2	
S28	USPHS Tuberculosis Therapy Trial (51)	1959	United States	152	6HP ₇		
S26	Zierski and Bek (55)	1980	Poland				

^a Z, pyrazinamide; H, isoniazid; S, streptomycin; E, ethambutol; P, para-aminosalicylic acid; R, rifampin; T, thiacetazone; t.i.d, three times daily; o.d., once daily. The pyrazinamide dose/kg used in the study is indicated in parentheses.
^b BMRC, British Medical Research Council.

FIG. 2. Relationship between proportions of patients receiving pyrazinamide monotherapy who developed adverse events and cumulative pyrazinamide dose. Dose-dependent toxicity was driven by arthralgia. For the two patients who had hepatotoxicity, one received 2.7 g/day and the other received 3.8 g/day of pyrazinamide.

DISCUSSION

For all the AEs, the presence or absence of pyrazinamide did not result in a significantly higher frequency of side effects. However, the studies were not homogeneous. The most frequent AE associated with pyrazinamide was arthralgia, which occurred late (after 1 month) and was nonsevere (Fig. 2). Second, the data did not demonstrate a relationship between pyrazinamide dose and hepatotoxicity. In this pooled analysis, hepatotoxicity was infrequent and also occurred late, after 30 days. These data question prior attestations that the addition of pyrazinamide to a regimen, or increasing pyrazinamide doses, significantly increases anti-TB-drug-related adverse events.

Polyarthralgia occurred more frequently in pyrazinamide-based regimens, consistent with data from previous reports (46, 47, 55). Pyrazinamide is hydrolyzed by microsomal deaminase to pyrazinoic acid (POA) and then hydroxylated to 5-hydroxypyrazinoic acid by xanthine oxidase for excretion by the

NB A=Pyrazinamide Monotherapy; B=Combinations including Pyrazinamide; C=Combinations excluding Pyrazinamide. Cochrane Q-test $p < 0.001$ for overall adverse event, interruption of therapy, hepatotoxicity, jaundice, arthralgia and death, respectively.

FIG. 3. Frequency of adverse events reported across tuberculosis treatment regimens.

kidneys. Pyrazinamide-induced arthralgia is caused by hyperuricemia due to the competitive inhibition of xanthine oxidase by POA. Serum pyrazinamide concentrations correlate closely with the POA concentration in serum and urine at a POA/pyrazinamide ratio of 1/3 (16). Our pharmacokinetic analysis results established that the percentage of time for the concentration to exceed 10 mg/liter, but most strongly exceed 5 mg/liter, best explained arthralgia. The meta-analysis revealed that the same, intermittent dosing reduced the frequency of arthralgia compared to the frequency of arthralgia for daily therapy for roughly equivalent cumulative doses, so the time above a certain threshold concentration was also the parameter driving

arthralgia. The relationship between dose and arthralgia in monotherapy studies also reflects the same finding, since without a dose-scheduling design, all three parameters (time above threshold or AUC and/or peak concentration) will increase proportionately with the dose. We propose that xanthine oxidase gets saturated at relatively low POA concentrations so that the longer the concentration persists above the threshold, the longer the maximal inhibition of xanthine oxidase, which leads to prolonged urate deposition in joints (16). Once therapy stops, the concentrations fall and urate deposition stops, and the patients feel better.

On the other hand, hepatotoxicity is the most dreaded side effect attributed to anti-TB drugs (3, 8, 13, 14, 17, 31). Contrary to data from previous reports (8, 13, 14, 17, 40, 51), our meta-analysis demonstrated no significant relationship between pyrazinamide dose and hepatotoxicity. As shown in Fig. 4, hepatotoxicity was infrequent and occurred in fewer than 6% of patients across all anti-TB regimens and was rarely encountered prior to a 30-day duration of therapy. In four previous studies (7, 41, 43, 52), pyrazinamide doses ranging from 43 mg/kg to >60 mg/kg were associated with sporadic hepatotoxicity that did not significantly differ in frequency compared to that for patients who had received lower pyrazinamide doses. On the other hand, higher rates of hepatotoxicity with the 25-mg/kg dose than with the 40-mg/kg daily dose were demonstrated in a U.S. Public Health Service (USPHS) study from 1959 (51). However, in that USPHS study, cephalin cholesterol flocculation tests and bromsulfalein retention tests were used to evaluate liver function. These tests have since

FIG. 6. Time to developing hepatotoxicity from the 1959 USPHS study.

been abandoned because of poor clinical predictive value, so the misclassification bias would have been high (39). In addition, pyrazinamide was given for prolonged periods compared to the current practice of 2 months. Furthermore, our reanalysis of the USPHS study data (51) using their own liver function tests demonstrated no significant difference in proportions of patients developing hepatotoxicity at 2 months between 25-mg/kg and 40-mg/kg daily doses (Fig. 6). Our current findings suggest that, indeed, doses of up to 60 mg/kg for patients with a mean weight of 86 kg, currently encountered in the United States (33), would probably not lead to higher rates of hepatotoxicity.

The mechanisms underlying drug-induced liver injury are not clearly understood (27, 38). While we should not generalize from the two hepatotoxic events in patients treated with pyrazinamide monotherapy, it is nevertheless intriguing that both patients had liver biopsy specimens that showed marked eosinophilia (54). Other researchers have also demonstrated features more consistent with immunoallergic liver damage (11, 32). Given the lack of dose-dependent hepatotoxicity in our analysis, we speculate that non-dose-dependent mechanisms such as immune-based damage, among others, may explain a large proportion of pyrazinamide-induced liver damage. This suggests that a portion of pyrazinamide hepatotoxicity rates may perhaps be from idiosyncratic events.

There are several limitations to our analysis. First, the selection was not exhaustive because only studies published in English were selected. Second, the identified studies were heterogeneous; however, meta-analysis is best suited for the generation of a correct hypothesis and not a proof. Third, we did not examine studies of hepatotoxicity encountered in studies of prophylactic treatment of latent TB. That analysis will be performed separately for that group of patients. In addition, only data from East Africa were available for population pharmacokinetic analyses. Despite these limitations, however, arthralgia was unequivocally induced by pyrazinamide but was clinically inconsequential. No relationship between pyrazinamide dose and hepatotoxicity was demonstrated. While Fig. 5 may appear to show that higher doses were associated with greater proportions of toxicity, this is driven mainly by the large variance in patients who received >60 mg/kg. These patients were sicker patients with more advanced TB, as reflected in Table 2. Given the important role of pyrazinamide in current anti-TB

therapy and the potential to further optimize efficacy by increasing the dose, our data suggest that higher doses should be examined for both efficacy and safety. Nevertheless, caution is still needed in the conduct of such studies.

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REFERENCES

- Allison, S. T. 1956. Pyrazinamide-isoniazid in low dosage in treatment of pulmonary tuberculosis. *Am. Rev. Tuberc.* **75**:400–405.
- Biomedical Simulations Resource. 2009. ADAPT 5 user's guide: pharmacokinetic/pharmacodynamic systems analysis software. Biomedical Simulations Resource, Los Angeles, CA.
- Blumberg, H. M., W. J. Burman, R. E. Chaisson, C. L. Daley, S. C. Etkind, L. N. Friedman, P. Fujiwara, M. Grzemska, P. C. Hopewell, M. D. Iseman, R. M. Jasmer, V. Koppaka, R. I. Menzies, R. J. O'Brien, R. R. Reyes, L. B. Reichman, P. M. Simone, J. R. Starke, and A. A. Vernon. 2003. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. *Am. J. Respir. Crit. Care Med.* **167**:603–662.
- Borenstein, M., L. V. Hedges, J. P. T. Higgins, and H. R. Rothstein. 2009. Introduction to meta-analysis. John Wiley & Sons, Ltd., West Sussex, United Kingdom.
- British Medical Research Council Co-Operative Study. 1973. Co-operative controlled trial of a standard regimen of streptomycin, PAS and isoniazid and three alternative regimens of chemotherapy in Britain. *Tubercle* **54**:99–129.
- British Thoracic Association. 1981. A controlled trial of six months chemotherapy in pulmonary tuberculosis. *Br. J. Dis. Chest* **75**:141–153.
- Campagna, M., A. Calix, and G. Hauser. 1954. Observations on the combined use of pyrazinamide (Aldinamide) and isoniazid in the treatment of pulmonary tuberculosis; a clinical study. *Am. Rev. Tuberc.* **69**:334–350.
- Centers for Disease Control and Prevention. 2003. Update: adverse event data and revised American Thoracic Society/CDC recommendations against the use of rifampin and pyrazinamide for treatment of latent tuberculosis infection—United States, 2003. *MMWR Morb. Mortal. Wkly. Rep.* **52**:735–739.
- Cohn, D. L., B. J. Catlin, K. L. Peterson, F. N. Judson, and J. A. Sbarbaro. 1990. A 62-dose, 6-month therapy for pulmonary and extrapulmonary tuberculosis. A twice-weekly, directly observed, and cost-effective regimen. *Ann. Intern. Med.* **112**:407–415.
- Combs, D. L., R. J. O'Brien, and L. J. Geiter. 1990. USPHS tuberculosis short-course chemotherapy trial 21: effectiveness, toxicity, and acceptability. The report of final results. *Ann. Intern. Med.* **112**:397–406.
- Corbella, X., M. Vellido, C. Cabellos, P. Fernandez-Viladrich, and G. Rufi. 1995. Hypersensitivity hepatitis due to pyrazinamide. *Scand. J. Infect. Dis.* **27**:93–94.
- Council for International Organizations of Medical Sciences/World Health Organization. 2006. The development safety update report (DSUR): harmonizing the format and content for periodic safety reporting during clinical trials. World Health Organization, Geneva, Switzerland.
- Danan, G., D. Pessayre, D. Larrey, and J. P. Benhamou. 1981. Pyrazinamide fulminant hepatitis: an old hepatotoxin strikes again. *Lancet* **ii**:1056–1057.
- Durand, F., J. Bernuau, D. Pessayre, D. Samuel, J. Belachie, C. Degott, H. Bismuth, J. Belghiti, S. Erlinger, and B. Rueff. 1995. Deleterious influence of pyrazinamide on the outcome of patients with fulminant or subfulminant liver failure during antituberculous treatment including isoniazid. *Hepatology* **21**:929–932.
- East African/British Medical Research Council Pyrazinamide Investigation. 1969. A controlled comparison of four regimens of streptomycin plus pyrazinamide in the treatment of pulmonary tuberculosis. *Tubercle* **50**:81–112.
- Ellard, G. A. 1969. Absorption, metabolism and excretion of pyrazinamide in man. *Tubercle* **50**:144–158.
- Girling, D. J. 1978. The hepatic toxicity of antituberculosis regimens containing isoniazid, rifampicin and pyrazinamide. *Tubercle* **59**:13–32.
- Girling, D. J. 1984. The role of pyrazinamide in primary chemotherapy for pulmonary tuberculosis. *Tubercle* **65**:1–4.
- Gumbo, T., C. S. Dona, C. Meek, and R. Leff. 2009. Pharmacokinetics-pharmacodynamics of pyrazinamide in a novel in vitro model of tuberculosis for sterilizing effect: a paradigm for faster assessment of new antituberculosis drugs. *Antimicrob. Agents Chemother.* **53**:3197–3204.
- Higgins, J. P., S. G. Thompson, J. J. Deeks, and D. G. Altman. 2003. Measuring inconsistency in meta-analyses. *BMJ* **327**:557–560.
- Hong Kong Chest Service/Tuberculosis Research Centre, Madras/British Medical Research Council. 1989. A controlled trial of 3-month, 4-month, and 6-month regimen of chemotherapy for sputum-smear-negative pulmonary tuberculosis. Results at 5 years. *Am. Rev. Respir. Dis.* **139**:871–876.

22. Hong Kong Chest Service/British Medical Research Council. 1981. Controlled trial of four thrice-weekly regimens and daily regimen all given for 6 months for pulmonary tuberculosis. *Lancet* **i**:171–174.
23. Hong Kong Chest Service/British Medical Research Council. 1978. Controlled trial of 6-month and 8-month regimens in the treatment of pulmonary tuberculosis: first report. *Am. Rev. Respir. Dis.* **118**:219–227.
24. Hong Kong Tuberculosis Treatment Services/British Medical Research Council. 1976. Adverse reactions to short-course regimens containing streptomycin, isoniazid, pyrazinamide and rifampicin in Hong Kong. *Tubercle* **57**:81–95.
25. Jadad, A. R., R. A. Moore, D. Carroll, C. Jenkinson, D. J. Reynolds, D. J. Gavaghan, and H. J. McQuay. 1996. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin. Trials* **17**:1–12.
26. Kamal, N., P. K. Mukerjee, K. Kishore, S. Kumar, and B. K. Khanna. 1976. Liver functions during pyrazinamide therapy. *Ind. J. Tuberc.* **23**:14–18.
27. Lee, W. M. 1993. Review article: drug-induced hepatotoxicity. *Ailment. Pharmacol. Ther.* **7**:477–485.
28. Livings, D. G. 1956. Pyrazinamide and isoniazid: summarized data from VA-Army-Navy cooperative study, p. 101. *Trans. 15th Conf. Chemother. Tuberc.*
29. Mathews, J. H. 1960. Pyrazinamide and isoniazid used in the treatment of pulmonary tuberculosis. *Am. Rev. Respir. Dis.* **81**:348–351.
30. McDermott, W., L. Ormond, K. Muschenheim, K. Deuschle, R. M. McCune, Jr., and R. Tompsett. 1954. Pyrazinamide-isoniazid in tuberculosis. *Am. Rev. Tuberc.* **69**:319–333.
31. Mitchison, D. A. 2000. Role of individual drugs in the chemotherapy of tuberculosis. *Int. J. Tuberc. Lung Dis.* **4**:796–806.
32. Morrissey, J. F., and R. C. Rubin. 1958. The detection of pyrazinamide-induced liver damage by serum enzyme determinations. *Am. Rev. Tuberc.* **80**:855–865.
33. Ogden, C. L., C. D. Fryar, M. D. Carroll, and K. M. Flegal. 2004. Mean body weight, height, and body mass index, United States 1960–2002. Advance data from vital and health statistics; no. 347. National Center for Health Statistics, Hyattsville, MD.
34. Okwera, A., C. Whalen, F. Byekwaso, M. Vjecha, J. Johnson, R. Huebner, R. Mugerwa, and J. Ellner. 1994. Randomised trial of thiacetazone and rifampicin-containing regimens for pulmonary tuberculosis in HIV-infected Ugandans. The Makerere University-Case Western University Research Collaboration. *Lancet* **344**:1323–1328.
35. Oleson, F. B., Jr., C. L. Berman, J. B. Kirkpatrick, K. S. Regan, J. J. Lai, and F. P. Tally. 2000. Once-daily dosing in dogs optimizes daptomycin safety. *Antimicrob. Agents Chemother.* **44**:2948–2953.
36. Phillips, S., and G. E. Horton. 1956. Pyrazinamide-isoniazid: comparison with isoniazid-para-aminosalicylic acid in active pulmonary tuberculosis with the choice of regimens determined by chance. *Am. Rev. Tuberc.* **73**:704–715.
37. Phillips, S., J. C. Larkin, Jr., W. L. Litzenger, G. E. Horton, and J. S. Haimsohn. 1954. Observations on pyrazinamide (Aldinamide) in pulmonary tuberculosis. *Am. Rev. Tuberc.* **69**:443–450.
38. Pohl, L. R. 1990. Drug-induced allergic hepatitis. *Semin. Liver Dis.* **10**:305–315.
39. Pohle, F. J., and J. K. Stewart. 1941. The cephalin-cholesterol flocculation test as an aid in the diagnosis of hepatic disorders. *J. Clin. Invest.* **20**:241–247.
40. Potter, B. P., and S. F. Chang. 1955. Experience with pyrazinamide. *Dis. Chest* **27**:44–50.
41. Ramakrishnan, C. V., B. Janardhanam, D. V. Krishnamurthy, H. Stott, S. Subbammal, and S. P. Tripathy. 1968. Toxicity of pyrazinamide, administered once weekly in high dosage, in tuberculous patients. *Bull. World Health Organ.* **39**:775–779.
42. Rybak, M. J., B. J. Abate, S. L. Kang, M. J. Ruffing, S. A. Lerner, and G. L. Drusano. 1999. Prospective evaluation of the effect of an aminoglycoside dosing regimen on rates of observed nephrotoxicity and ototoxicity. *Antimicrob. Agents Chemother.* **43**:1549–1555.
43. Schwartz, W. S., and R. E. Moyer. 1954. The chemotherapy of pulmonary tuberculosis with pyrazinamide used alone and in combination with streptomycin, para-aminosalicylic acid, or isoniazid. *Am. Rev. Tuberc.* **70**:413–422.
44. Singapore Tuberculosis Services/British Medical Research Council. 1979. Clinical trial of six-month and four-month regimens of chemotherapy in the treatment of pulmonary tuberculosis. *Am. Rev. Respir. Dis.* **119**:579–585.
45. Small, M. J. 1959. Treatment of pulmonary tuberculosis with isoniazid and pyrazinamide: experience in 114 cases. *Dis. Chest* **36**:265–279.
46. Solangi, G. A., B. F. Zuberi, S. Shaikh, and W. M. Shaikh. 2004. Pyrazinamide induced hyperuricemia in patients taking anti-tuberculous therapy. *J. Coll. Physicians Surg. Pak.* **14**:136–138.
47. Taki, H., K. Ogawa, T. Murakami, and T. Nikai. 2008. Epidemiological survey of hyperuricemia as an adverse reaction to antituberculous therapy with pyrazinamide. *Kekkaku* **83**:497–501. (In Japanese.)
48. Tuberculosis Research Centre. 1986. A controlled clinical trial of 3- and 5-month regimens in the treatment of sputum-positive pulmonary tuberculosis in South India. *Am. Rev. Respir. Dis.* **134**:27–33.
49. Tuberculosis Research Centre. 1997. A controlled clinical trial of oral short-course regimens in the treatment of sputum-positive pulmonary tuberculosis. *Int. J. Tuberc. Lung Dis.* **1**:509–517.
50. U.S. Department of Health and Human Services and U.S. Food and Drug Administration. 1 October 2009, accession date. CFR—Code of Federal Regulations title 21. U.S. Food and Drug Administration, Washington, DC. <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=314.80>.
51. U.S. Public Health Service Tuberculosis Therapy Trial. 1959. Hepatic toxicity of pyrazinamide used with isoniazid in tuberculosis patients. *Amer. Rev. Tuberc.* **80**:371.
52. Velu, S., R. H. Andrews, J. H. Angel, S. Devadatta, W. Fox, P. G. Jacob, C. N. Nair, and C. V. Ramakrishnan. 1961. Streptomycin plus pyrazinamide in the treatment of patients excreting isoniazid-resistant tubercle bacilli, following previous chemotherapy. *Tubercle* **42**:136–147.
53. Wilkins, J. J., G. Langdon, H. McIlleron, G. C. Pillai, P. J. Smith, and U. S. Simonsson. 2006. Variability in the population pharmacokinetics of pyrazinamide in South African tuberculosis patients. *Eur. J. Clin. Pharmacol.* **62**:727–735.
54. Yeager, R. L., W. G. Munroe, and F. I. Dessau. 1952. Pyrazinamide (aldinamide) in the treatment of pulmonary tuberculosis. *Am. Rev. Tuberc.* **65**:523–546.
55. Zierski, M., and E. Bek. 1980. Side-effects of drug regimen used in short-course chemotherapy for pulmonary tuberculosis. A controlled clinical study. *Tubercle* **61**:41–49.