ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2002, p. 2358–2364 Vol. 46, No. 8
0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.8.2358–2364.2002
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Effects of Gender, AIDS, and Acetylator Status on Intrapulmonary Concentrations of Isoniazid

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Received 21 May 2001/Returned for modification 23 November 2001/Accepted 17 April 2002

The objective of the present study was to evaluate the effects of gender, AIDS, and acetylator status on the steady-state concentrations of orally administered isoniazid in plasma and lungs. Isoniazid was administered at 300 mg once daily for 5 days to 80 adult volunteers. Subjects were assigned to eight blocks according to gender, presence or absence of AIDS, and acetylator status. Blood was obtained prior to administration of the first dose, 1 h after administration of the last dose, and at the completion of bronchoscopy and bronchoalveolar lavage (BAL), which was performed 4 h after administration of the last dose. The metabolism of caffeine was used to determine acetylator status. Standardized bronchoscopy was performed without systemic sedation. The volume of epithelial lining fluid (ELF) recovered was calculated by the urea dilution method. Isoniazid concentrations in plasma, BAL fluid, and alveolar cells (ACs) were measured by high-performance liquid chromatography. AIDS status or gender had no significant effect on the concentrations of isoniazid in plasma at 1 or 4 h. Concentrations in plasma at 4 h and concentrations in ELF were greater in slow acetylators than fast acetylators. The concentration in plasma (1.85 ± 1.60 μg/ml [mean ± standard deviation; $n = 80$]) at 1 h following administration of the last dose was not significantly different from that in ELF (2.25 ± 3.50 μg/ml) or ACs (2.61 ± 5.01 μg/ml). For the entire study group, concentrations in plasma at 1 h were less than 1.0, 2.0, and 3.0 μg/ml for 34.7, 60, and 82.7% of the subjects, respectively; concentrations in ELF were less than 1.0, 2.0, and 3.0 μg/ml in 30 (37.5%), 53 (66.0%), and 58 (72.5%) of the subjects, respectively; and concentrations in ACs were less than 1.0, 2.0, and 3.0 μg/ml in 43 (53.8%), 59 (73.8%), and 65 (81.3%) of the subjects, respectively. The concentrations of orally administered isoniazid in plasma were not affected by gender or the presence of AIDS. The concentrations in plasma at 4 h and the concentrations in ELF, but not the concentrations in ACs, were significantly greater in slow acetylators than fast acetylators. Concentrations in plasma and lungs were low compared to recommended therapeutic concentrations in plasma and published MICs of isoniazid for *Mycobacterium tuberculosis*. The optimal concentrations of isoniazid in ACs and ELF are unknown, but these data suggest that the drug enters these compartments by passive diffusion and achieves concentrations similar to those found in plasma.

Isoniazid is an essential drug in the treatment of tuberculosis. For humans without tuberculosis who received a single 250-mg oral dose of isoniazid, elimination half-lives have been reported to be 1.2 and 3.3 h in fast and slow acetylators, respectively, and peak concentrations in plasma (at 1 h postdosing) have been reported to be 2.44 and 3.64 μg/ml, respectively (25). Several investigators in the United States have reported that the absorption of antimycobacterial agents is impaired in patients with AIDS (18, 27, 29; S. E. Berning, G. A. Huitt, M. D. Iseman, and C. A. Peloquin, Letter, N. Engl. J. Med. **327:**1817-1818, 1992; C. A. Peloquin, A. A. MacPhee, and S. E. Berning, Letter, N. Engl. J Med **329:**1122-1123, 1993). However, this effect was not demonstrated in Kenyan patients, in whom concentrations in plasma were not different among individuals with or without AIDS or with or without diarrhea (9). Low concentrations of isoniazid and rifampin in serum have also been reported in non-HIV-infected patients with tuberculosis who received directly observed therapy. Serum isoniazid and rifampin concentrations were below the suggested therapeutic range in 68 and 64% of the patients, respectively (22). The subjects with AIDS who were selected for the present study were stable, did not have diarrhea, and had no signs or symptoms of acute illness.

In clinical practice, an oral dose of 300 mg of isoniazid per day is recommended regardless of gender. The 300-mg once-daily dose was therefore administered to both men and women in this study to determine the drug concentrations that would be expected in practice. Since women, as a group, are significantly lighter than men and therefore might have greater drug concentrations in plasma, epithelial lining fluid (ELF), and alveolar cells (ACs), we included gender in the stratification.

In subjects receiving isoniazid, free isoniazid, acetylated isoniazid, and isonicotinic acid appear in the urine in various amounts depending on the acetylator phenotype of the subject. Acetylator status can be ascertained by measuring the metabolism of caffeine (19, 32). Knowledge of acetylator status is important because it affects the pharmacology of isoniazid (25). Therefore, it was included in the stratification of our subjects.

Isoniazid is active against tubercle bacilli that are growing within cultured human macrophages (16). Drug concentrations

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within cultured human macrophages obtained by bronchoalveolar lavage (BAL) approximate the concentrations in the extracellular fluid, suggesting that drug enters the cells by passive diffusion (20). Whether isoniazid penetrates into alveolar macrophages or ELF in vivo in humans has not previously been reported.

We (10, 11, 13, 14) and others (1–6) have developed techniques for the measurement in vivo of the concentration of drugs in pulmonary ELF and ACs. The purpose of this study was to compare the steady-state concentrations of isoniazid in the plasma, ELF, and ACs of healthy volunteers and men and women with AIDS and to assess the effect of acetylator status on the concentrations of the drug in these compartments.

MATERIALS AND METHODS

Study design and subjects. The investigation was prospective and nonblinded. After giving informed consent, subjects underwent a medical history; physical examination; purified protein derivative skin test; and baseline laboratory testing, including complete blood count, platelet count, and tests for blood urea nitrogen, serum creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and total bilirubin levels. Healthy subjects were required to be 18 years of age or older and within 10% of the acceptable weight for height according to the Metropolitan Life height and weight tables (23). If the subject was a woman, the subject was required to be nonlactating and not pregnant. Subjects were excluded from the study if they had a history of asthma requiring daily therapy, tuberculosis or a positive skin test result (induration of greater than 5 mm [subjects with AIDS] or greater than 10 mm [healthy volunteers] at 48 to 72 h), intolerance to isoniazid or lidocaine, or clinically significant organ dysfunction; if they were required to take on a regular basis medications other than self-prescribed vitamins, birth control pills, or thyroid replacement therapy; or if they had abnormal serum creatinine levels or values by other laboratory screening tests that were outside the normal range (greater than twice the normal values for subjects with AIDS). Patients with AIDS were required to meet the revised criteria of the Centers for Disease Control and Prevention for the diagnosis of AIDS (8) and to have the following: (i) less than four soft stools per day without hematochezia, (ii) no abdominal pain or cramping, (iii) no nausea or vomiting, and (iv) a negative chest X ray within 2 weeks of enrollment. If a chest X ray had not been done, it was performed as part of the study. Thirty-six of the 40 subjects with AIDS were receiving one or more drugs as part of their medical care. When the preenrollment evaluation was completed, 80 subjects were assigned to one of the following groups: 10 healthy men who were fast acetylators, 10 healthy women who were fast acetylators, 10 men with AIDS who were fast acetylators, 11 women with AIDS who were fast acetylators, 10 healthy men who were slow acetylators, 10 healthy women who were slow acetylators, 10 men with AIDS who were slow acetylators, and 9 women with AIDS who were slow acetylators. This research has complied with all relevant federal guidelines and institutional policies.

Isoniazid was obtained from a commercial source (Barr Laboratories, Inc., Pomona, N.Y.). A 300-mg tablet was administered orally once daily for a total of 5 days. The first dose of study medication was administered under direct supervision on day 1 in the General Clinical Research Center at the University of California, San Francisco. Subjects were observed for 30 min afterward for adverse effects. The second, third, and fourth doses were taken at home on the respective days according to verbal and written instructions and as documented in writing by the subjects. The fifth (last) dose was taken on the fifth study day under direct supervision. All doses were taken at approximately 9:30 a.m.

Bronchoscopy and BAL. Standardized bronchoscopy and BAL (10–12, 14, 15) were performed 4 h after the administration of the fifth (last) dose. The subjects' blood pressure, pulse, respiratory rate, and temperature were recorded prior to and at the completion of the procedure. Throughout the procedure the subjects were monitored by fingertip oximetry.

Subjects were prepared with a 4% topical lidocaine gargle followed by a 4% topical lidocaine spray. Pledgets soaked with 4% topical lidocaine were then applied to each side of the posterior pharynx, followed by the application of topical 1% lidocaine more distally. No systemic sedation was used. A fiberoptic bronchoscope (FB-19H; Pentax) was inserted in the right middle lobe. The instrument was in place for approximately 3 to 5 min.

A total of four 50-ml aliquots of normal saline were instilled into the right middle lobe, and each was immediately aspirated into a trap. The specimens were kept on ice until they were frozen. The first aspirate was discarded. The second, third, and fourth aspirates were pooled. The volume of the pooled BAL fluid was measured and recorded. Measured aliquots of the pooled BAL fluid were sent to the clinical laboratory at the University of California, San Francisco, for cell count and differential.

Specimen handling. Blood collected in tubes containing sodium heparin was immediately centrifuged. The plasma was then separated and stored frozen at −80°C until it was assayed.

A measured volume of BAL fluid was immediately centrifuged at 400 × *g* for 5 min. The supernatant was decanted, and both cells and the supernatant were frozen at −80°C until they were assayed. Immediately prior to assay, the cell pellets were resuspended in 5% acetonitrile, sonicated for 2 min on a Dismembrator (model 550; Fisher Scientific, Pittsburgh, Pa.), centrifuged at 3,000 × *g* for 15 min, and decanted. The supernatant was then evaporated to dryness and reconstituted in 5% acetonitrile.

Isoniazid assay. Isoniazid concentrations in plasma, BAL fluid, and ACs were measured by high-pressure liquid chromatography (HPLC), as we have reported previously (17). Analyses were performed with a Waters (Milford, Mass.) autosampler and an ESA (Chelmsford, Mass.) electrochemical detector set at +0.6 V. The isoniazid in plasma and alveolar cells was extracted with a chloroform-butanol mixture and was then back-extracted into dilute acid.

The mobile phase consisted of 70% 50 mM KH_2PO_4 and 30% acetonitrile-isopropanol (4:1; vol/vol) flowing at 0.8 ml/min. It was pumped through a 5-μm Phenomenex CN (Torrance, Calif.) reversed-phase column (250 by 4.6 mm) at ambient temperature. The retention times for isoniazid and the internal standard (diphenylcarbazide) were 4.7 and 8.3 min, respectively. The BAL fluid was injected directly onto a Polymer Labs Inc. (Amherst, Mass.) polystyrene divinylbenzene column (4.6 by 250 mm). A mobile phase consisting of 5% acetonitrile and 15 mM ammonium hydroxide flowing at 0.5 ml/min was used. The isoniazid retention time was 14.6 min. No internal standard was used. Peak areas were monitored, and the calibration curves were created with EZ Chrom software (Scientific Software, San Ramon, Calif.). The sensitivities of the assay with plasma, BAL fluid, and ACs were 100, 10, and 25 ng/ml, respectively. Weekly determinations of isoniazid concentrations in spiked plasma and phosphate-buffered saline stored at −80°C revealed no degradation of the drug. The precisions of the assay, defined by the coefficient of variation (CV), with plasma, BAL fluid, and ACs, were 6.7, 15.4, and 8.5%, respectively. The accuracies, defined by the relative error, with plasma, BAL fluid, and ACs were 2.4, 7.7, and 28%, respectively.

Determination of acetylator status. (i) Procedures.

Caffeine was used to determine acetylator status (19). A detailed history was taken. In addition, a physical examination was performed to assess factors that might influence drug metabolism, including smoking habits; intake of caffeine, food, and alcohol; ethnicity; drug therapy; and the presence of other diseases. Subjects were instructed to refrain from ingesting all methylxanthine-containing foods or beverages for 24 h. On the following morning, subjects received 200 mg of caffeine orally. Urine was collected between 4 and 8 h after administration of the dose. Urine samples were stored at −80°C until analysis.

(ii) To assess whether a subject was a slow or a fast acetylator, concentrations of 5-acetylamino-6-amino-3-methyluracil (AAMU), 1-methylxanthine (1X), and 1-uric acid (1U) were measured. The 5-acetylamino-6-formylamine-3-methyluracil (AFMU) in each sample was converted to AAMU with dilute sodium hydroxide; and the AAMU, 1X, and 1U concentrations were measured. The molar ratio of AAMU/[AAMU + 1X + 1U] in the urine collected at 4 h was used to classify slow and fast acetylators. Subjects with molar ratios <0.35 were classified as slow acetylators, and those with molar ratios >0.35 were classified as fast acetylators (19, 31).

Pharmacologic probe. (i) Method for xanthines and urates.

Urinary caffeine and its xanthine and urate metabolites were measured by the HPLC procedure of Muir et al. (24), with the following modification. A solid-phase cartridge technique instead of a liquid-liquid extraction was used to prepare samples for HPLC. Samples were applied to Certify cartridges (Varian Associates) with ethyl acetate and hexane washes. After the xanthines and urates were eluted with 15% methanol, HPLC was performed with a 3-μm reversed-phase Microsorb column and a 15% methanol mobile phase at a flow rate of 0.3 ml/min. A Hewlett-Packard 1090 HPLC instrument diode array detector and chem-station were used. The xanthines (including 1,8-methylxanthine as the internal standard) and urates were monitored at 270 and 290 nm, respectively. The chromatography was complete in 15 min. The precision of the assay, defined by the CV, was 3.8% for 1X, 1.1% for 1,7-dimethylxanthine, and 4.8% for 1-methylurate.

(ii) Method for AAMU. AAMU, the major metabolite of caffeine, was assayed by a separate HPLC procedure described by Tang et al. (31), with minor mod-

TABLE 1. Recovery of cells from BAL fluid in 80 subjects by gender, AIDS status, and acetylator phenotype

Group (no. of subjects)[a]	ELF vol (ml)	No. of cells/liter	Percent				
			PMNs[b,c]	Lymphocytes[c]	Macrophages[c]	Eosinophils	Degenerated or other cells
Men with AID							
FA (10)	1.10 ± 0.41	$1.78 \times 10^8 \pm 1.97 \times 10^8$[d]	0.7 ± 1.1	14.9 ± 14.2	81.5 ± 15.7	0.9 ± 2.5	1.7 ± 5.4
SA (10)	0.99 ± 0.54	$2.05 \times 10^8 \pm 1.80 \times 10^8$	1.3 ± 1.9	9.8 ± 8.4	84.6 ± 15.2	1.3 ± 2.7	3.0 ± 9.5
Healthy men							
FA (10)	0.81 ± 0.34	$1.81 \times 10^8 \pm 2.70 \times 10^8$[d]	1.8 ± 3.0	19.6 ± 17.1	75.3 ± 18.3	0.7 ± 1.6	2.6 ± 5.6
SA (10)	0.93 ± 0.41	$1.40 \times 10^8 \pm 1.01 \times 10^8$[d]	2.9 ± 4.1	10.4 ± 10.1	82.6 ± 14.4	2.0 ± 4.6	2.0 ± 6.3
Women with AIDS							
FA (11)	1.20 ± 0.60	$3.20 \times 10^8 \pm 2.0 \times 10^8$	3.1 ± 4.5	6.4 ± 5.3	88.0 ± 8.7	0.5 ± 1.2	2.1 ± 4.3
SA (9)	1.88 ± 0.10[e]	$4.78 \times 10^8 \pm 2.6 \times 10^8$[d]	3.4 ± 5.2	25.6 ± 18.9	68.2 ± 20.7	2.3 ± 4.8	0.3 ± 1.0
Healthy women							
FA (10)	0.76 ± 0.31[e]	$8.92 \times 10^7 \pm 2.02 \times 10^7$[d]	1.2 ± 1.3	14.4 ± 11.6	84.0 ± 11.8	0.1 ± 0.3	0.3 ± 0.9
SA (10)	0.58 ± 0.23[e]	$1.61 \times 10^8 \pm 1.36 \times 10^8$[d]	1.4 ± 1.7	19.8 ± 17.6	75.4 ± 17.2	0.9 ± 2.2	2.5 ± 7.9

[a] FA, fast acetylator phenotype; SA, slow acetylator phenotype.
[b] PMNs, polymorphonuclear leukocytes.
[c] No significant differences among the groups.
[d] The numbers of cells from women with AIDS who were slow acetylators was significantly greater than the numbers of cells from healthy women who were slow acetylators ($P = 0.042$), healthy men who were fast acetylators ($P = 0.006$), healthy men who were slow acetylators ($P = 0.025$), and men with AIDS who were fast acetylators ($P = 0.028$).
[e] The volumes of ELF recovered from women with AIDS who were slow acetylators were significantly greater than the volumes recovered from healthy women who were fast or slow acetylators ($P > 0.05$); no other differences in ELF volumes between groups were significant ($P < 0.05$).

ifications, as follows: urine was incubated for 20 min with 25 mM NaOH at 40°C to convert AFMU quantitatively to AAMU. After neutralization with HCl, 0.5-ml aliquots of samples were applied to Bond Elut cartridges (Varian Associates). The analyte was then eluted with bicarbonate-buffer (pH 7.4) and applied to a TSK-20 size-exclusion HPLC column. The AAMU emerged in 15 min by use of a 0.1% acetic acid mobile phase and a flow rate of 0.8 ml/min. Monitoring was performed with a Waters 481 variable detector. The CV for AAMU was 8.4%.

These techniques resulted in sharp peaks and separation for the urinary xanthines and urates, AAMU, and the internal standard.

Quantitation of volume of ELF and concentrations of antibiotics in ELF and ACs. The amount of ELF recovered was calculated by the urea dilution method (28). The concentration of urea in serum was analyzed by the clinical laboratory at the University of California, San Francisco, by a coupled urease-glutamate dehydrogenase enzymatic method (30) modified by Boehringer Mannheim Corporation (Indianapolis, Ind.). Measurements were made at a fixed time interval that permitted automated analysis with a BM 747 analyzer (Boehringer Mannheim). The urea concentration in supernatant of the BAL fluid was measured by a modified enzymatic assay (BUN kit UV-66; Sigma, St. Louis, Mo.), as reported previously (5, 10, 11, 14, 15, 28). The assay is linear ($R^2 = 0.99$) for concentrations of urea in BAL fluid from 0.047 to 0.750 mg/dl. The accuracies of the assay with low and high concentrations were 7.0 and −0.04%, respectively. Controls were included with every run, and if the result for the control was not within 10% of the known value, the assays for generation of the standard curve and with controls and specimens were repeated.

The volume of ELF in BAL fluid was derived from the following relationship: $V_{ELF} = V_{BAL} \times (Urea_{BAL}/Urea_{SER})$, where V_{ELF} is the volume of ELF sampled by BAL, V_{BAL} is the volume of BAL fluid aspirated, $Urea_{BAL}$ is the concentration of urea in BAL fluid, and $Urea_{SER}$ is the concentration of urea in serum.

The concentration of antibiotic in the ELF was derived from the following relationship: $ABX_{BAL} \times (V_{BAL}/V_{ELF})$, where ABX_{BAL} is the measured concentration of antibiotic in BAL fluid.

The volume of ACs collected in the pellet suspension was determined from the cell count obtained with BAL fluid. Cells were counted in a hemocytometer, which has a lower detection limit of 1.0×10^6/liter. The calculated number of cells in 1.0 ml of the pellet suspension was determined to be equal to the number of cells per liter of BAL fluid/100. It has been noted, however, that centrifugation causes an average loss of 21% of the cells, so that the actual number of cells recovered may be lower than the number counted and the actual antibiotic concentration may be proportionately greater than the concentration calculated (33). Differential cell counting was performed after the specimen was spun in a cytocentrifuge. The volume of ACs in the pellet suspension was determined by using a mean macrophage cell volume of 2.42 μl/10^6 cells (2).

The concentration of antibiotic in alveolar cells was calculated from the following relationship: ABX_{PELLET}/V_{AC}, where ABX_{PELLET} is the antibiotic concentration in the 1-ml cell suspension, and V_{AC} is the volume of alveolar cells in the 1-ml cell suspension.

Statistical analysis. Database management was performed with a Sun 10 Sparcstation (Sun Microsystems, Milpitas, Calif.). Prophet software (version 5.0; Division of Research Resources, National Institutes of Health, Bethesda, Md., and Bolt, Beranek and Newman, Cambridge, Mass.) was used to compute descriptive statistics, and a different version of the software (Prophet, version 6.0, 1999; Abtech Corporation, Charlottesville, Va.) was used to perform linear regression, analysis of variance, and comparisons of means. Undetectable drug concentrations were recorded as zero and were included in calculations of means and standard deviations. Analysis of variance was used to assess the effects of gender and AIDS status on the subjects' physical characteristics, clinical laboratory values, drug dosage, drug concentrations, AC recovery, ELF recovery, and ratios of the concentration in ACs/concentration in plasma and concentration in ELF/concentration in plasma. The two-sample equal-variances *t* test (two-sided) was used to compare the weight-adjusted dosages in men and women, AC and ELF recovery, and drug concentrations in plasma, ACs, and ELF between women with AIDS who were smokers and women with AIDS who were nonsmokers. The equality of variances of the smoking and nonsmoking groups was calculated by the *F* test (Levene's test). The two-sample Mann-Whitney rank sum test (two-sided) was used to compare the ages and serum creatinine levels of subjects with and without AIDS and the CD4 counts in men and women with AIDS. The Shapiro-Wilk test was used to evaluate the normality of the distributions of the data sets prior to comparison. A *P* value <0.05 was regarded as significant.

RESULTS

Eighty subjects were enrolled in the study. The block assignments resulted in eight groups of subjects stratified by gender, AIDS status, and acetylator status (Tables 1 and 2). Because a fixed daily dose of isoniazid (300 mg) was used, the weight-corrected dose (mean ± standard deviation [SD]) was 19.1% greater for the 40 women (4.77 ± 0.88 mg/kg) than for the 40 men (4.00 ± 0.65 mg/kg) ($P = 0.0001$). The 40 subjects with AIDS were approximately 5.4 years older than the subjects without AIDS (38.3 ± 5 versus 32.9 ± 8 years) ($P = 0.0002$).

TABLE 2. Isoniazid concentrations in 80 subjects by gender, AIDS status, and acetylator phenotype[a]

Group	Isoniazid concn (μg/ml) in:			
	Plasma (1 h)[b]	Plasma (4 h)[c]	ELF (4 h)[d]	ACs (4 h)[e]
Men with AIDS				
FA	1.87 ± 1.96	0.47 ± 0.38	0.82 ± 0.79	3.08 ± 4.55
SA	1.61 ± 1.17	1.08 ± 0.81	2.15 ± 1.90	0.95 ± 1.70
Healthy men				
FA	1.18 ± 0.88	0.17 ± 0.21	1.00 ± 1.55	1.10 ± 2.73
SA	1.82 ± 1.09	0.87 ± 0.81	3.28 ± 4.68	3.83 ± 8.94
Women with AIDS				
FA	1.12 ± 1.0	0.71 ± 0.8	1.78 ± 1.7	1.05 ± 1.6
SA	2.00 ± 1.0	1.43 ± 1.0	1.96 ± 1.7	1.06 ± 2.0
Healthy women				
FA	2.29 ± 2.05	0.59 ± 0.69	1.21 ± 1.72	7.92 ± 6.72
SA	3.02 ± 2.52	1.03 ± 0.73	5.86 ± 6.92	1.93 ± 3.68

[a] All data are given as the mean ± 1 SD. FA, fast acetylator phenotype; SA, slow acetylator phenotype.
[b] The differences among gender ($P = 0.45$), acetylator status ($P = 0.25$), and AIDS status ($P = 0.10$) were not significant; there were no significant interactions among the three groups ($P > 0.05$).
[c] The differences between gender ($P = 0.07$) and AIDS status ($P = 0.1$) were not significant; the effect of acetylator status was significant ($P = 0.002$); there were no significant interactions among the three groups ($P > 0.05$).
[d] The differences between gender ($P = 0.16$) and AIDS status ($P = 0.22$) were not significant; the effect of acetylator status was significant ($P = 0.02$); there were no significant interactions among the three groups ($P > 0.05$).
[e] The differences among gender ($P = 0.45$), acetylator status ($P = 0.25$), and AIDS status ($P = 0.10$) alone were not significant; there was a significant interaction among the three groups ($P = 0.02$) (see text).

The CD4 counts (mean ± SD) for the 20 men and 20 women with AIDS were 125 ± 81 and 234 ± 118 cells/liter, respectively ($P = 0.002$). Serum creatinine levels for all subjects were within normal limits; however, as expected, the values were significantly greater for men (0.98 ± 0.14 mg/dl) than for women (0.86 ± 0.12 mg/dl) ($P < 0.0002$). Ten of the 20 female subjects with AIDS (5 fast acetylators and 5 slow acetylators) were cigarette smokers; the remainder of the subjects were nonsmokers.

All 80 subjects recruited for the study underwent and successfully completed the bronchoscopy and BAL. There were no major adverse events, and all of the subjects returned to their normal duties. Following bronchoscopy, 2 subjects (2.5%) experienced transient chest discomfort, 2 subjects (2.5%) experienced chills, and 12 subjects (15%) had an elevated temperature that subsided spontaneously. Transitory lightheadedness or dizziness probably secondary to lidocaine administration was common and occurred in 21 (26.3%) of the subjects.

Cell recovery from BAL fluid. The number (mean ± SD) of ACs recovered from BAL fluid in the eight groups ranged from $8.92 \times 10^7 \pm 2.02 \times 10^7$ to $4.78 \times 10^8 \pm 2.6 \times 10^8$ cells/liter (Table 1). As expected, the majority of these cells in each of the eight groups were in the monocyte/macrophage class. The proportions of monocytes/macrophages, polymorphonuclear leukocytes, and lymphocytes in BAL fluid were not different among the subjects in the eight groups. Women with AIDS who were slow acetylators had a greater total number of cells in their BAL fluid than healthy men and healthy women or men with AIDS who were fast acetylators. This observation was largely due to the effect of smoking on cell recovery. Cell recovery was greater for smoking women with AIDS ($5.35 \times 10^8 \pm 2.4 \times 10^8$ cells/liter) than nonsmoking women with AIDS ($2.47 \times 10^8 \pm 1.2 \times 10^8$ cells/liter) ($P = 0.004$). The proportions of monocytes/macrophages, polymorphonuclear leukocytes, and lymphocytes in BAL fluid were not different when the proportions in smoking women with AIDS were compared to those in nonsmoking women with AIDS ($P > 0.05$ for all cell types).

ELF recovery from BAL fluid. The volume (mean ± SD) of ELF in the eight groups ranged from 0.58 ± 0.23 to 1.88 ± 0.1 ml (Table 1). Significantly greater volumes of ELF were recovered from women with AIDS who were slow acetylators than from healthy women who were fast or slow acetylators. No other differences in the volumes of ELF recovered were significant. Five of the 9 women with AIDS who were slow acetylators, none of the 10 healthy women who were slow acetylators, and none of the 10 healthy women who were fast acetylators were smokers. None of the men were smokers. These observations are consistent with previous reports that the volume of ELF recovered is increased for current smokers (7).

Plasma isoniazid concentrations. Gender and AIDS status had no effect on the concentrations of isoniazid in plasma at 1 or 4 h (Table 2). The concentrations in plasma at 1 h (mean ± SD) were unaffected by acetylator status (2.1 ± 1.6 μg/ml in the slow acetylator group versus 1.6 ± 1.6 μg/ml in the fast acetylator group) ($P = 0.24$).

The concentrations in plasma at 4 h were greater in the slow acetylators than in the fast acetylators (1.1 ± 0.8 versus 0.5 ± 0.6 μg/ml) ($P = 0.0003$). Despite this difference, no single concentration adequately separated the fast and slow acetylator groups. The concentrations in plasma at 1 and 4 h were fitted to several empirical models [ratio of concentration at 1 h to concentration at 4 h, concentration at 1 h − concentration at 4 h, (concentration at 1 h − concentration at 4 h)/concentration at 4 h, comparison of the slopes of the linear curve connecting the two points, and others]. None of these models

TABLE 3. Subject characteristics and isoniazid concentrations in five Subjects with undetectable concentrations in plasma 1 h following dose administration[a]

Subject no.[a]	AIDS status	Gender	Concn (μg/ml) in:			
			Plasma at 1 h	Plasma at 4 h	ELF	AC
1	Negative	Male	0	0	0	0
2	Negative	Male	0	0	0.02	0
3	Positive	Male	0	0.7	0	1.1
4	Negative	Female	0	0	0	0.5
5	Positive	Female	0	2.5	0.10	0

[a] All subjects were fast acetylators, and none of the subjects was a smoker.

allowed separation of the fast and slow acetylator groups on the basis of the concentrations in plasma at 1 and 4 h.

CD4 counts in the subjects with AIDS ($n = 40$) were not correlated with the concentrations of isoniazid in plasma at 1 h ($R = -0.03$; $P = 0.9$) or 4 h ($R = -0.07$; $P = 0.65$). As expected, the mean ± SD and median plasma isoniazid concentrations in all 80 subjects were significantly greater at 1 h (1.9 ± 1.6 and 1.45 μg/ml, respectively) than at 4 h (0.8 ± 0.8 μg/ml and 0.5 μg/ml, respectively) following administration of the last dose ($P < 0$ 0.05). The weights of all subjects ($n = 80$) were not correlated with the plasma drug concentrations at 1 h ($R = -0.17$; $P = 0.13$) or 4 h ($R = -0.14$; $P = 0.22$). Although the plasma isoniazid concentrations were greater in women than men at 1 h (2.1 ± 1.8 versus 1.6 ± 1.3 μg/ml) and at 4 h (0.9 ± 0.8 versus 0.6 ± 0.7 μg/ml), the differences were not statistically significant ($P > 0.05$ for both time periods). When nonsmoking women with AIDS were compared to smoking women with AIDS, the plasma drug concentrations (mean ± SD) at 1 h (1.3 ± 1.0 versus 1.8 ± 1.2 μg/ml) and at 4 h (1.1 ± 1.0 versus 1.0 ± 1.0 μg/ml) were not significantly different ($P >$ 0.05 for both time periods).

Five subjects had undetectable concentrations of isoniazid in the plasma specimen obtained at 1 h (Table 3). All five of the subjects were nonsmokers and fast acetylators, three of the subjects were men, and two of the subjects had AIDS. Of these five subjects, three also had undetectable concentrations in plasma at 4 h, three had undetectable concentrations in ELF, and three had undetectable concentrations in ACs. In one of the five subjects, isoniazid was undetectable in all three compartments. This subject was a healthy male volunteer who was a fast acetylator. The range of plasma isoniazid concentrations at 1 h in those subjects with measurable concentrations ($n =$ 75) was 0.2 to 7.1 μg/ml, with a mean ± SD of 2.0 ± 1.6 μg/ml.

Concentrations of isoniazid in ELF. There was a significant effect of acetylator status ($P = 0.005$) but no effect of gender or AIDS status ($P > 0.05$) on isoniazid concentrations in ELF (Table 2). For all 80 subjects the concentrations in ELF were 2.2 ± 4.5 and 1.2 ± 1.5 μg/ml in slow and fast acetylators, respectively ($P < 0.05$), and concentrations in ELF were not correlated with the volume of ELF that was recovered ($R =$ −0.3). The ratios of the ELF drug concentration to the plasma drug concentration at 1 h were 3.2 ± 8.1 and 1.2 ± 1.9 for slow and fast acetylators, respectively ($P = 0.08$). The ELF isoniazid concentrations (mean ± SD) in smoking (2.0 ± 1.9 μg/ml) and nonsmoking (1.8 ± 1.4 μg/ml) women with AIDS were not significantly different ($P > 0.05$).

The concentrations of isoniazid in ELF were undetectable in 25 subjects. AIDS status, gender, and acetylator status were not associated with undetectable concentrations of isoniazid in ELF ($P > 0.05$). Twelve of the 25 subjects had AIDS (48%), 11(44%) were women, and 17 were fast acetylators (68%). The concentrations of isoniazid in plasma at 1 h ($R = 0.15$; $P >$ 0.05) or 4 h ($R = 0.21$; $P > 0.05$) were not correlated with the concentrations of isoniazid in ELF.

Concentrations of isoniazid in ACs. For the entire group, the mean ± SD concentration of isoniazid in ACs was 2.5 ± 5.0 μg/ml and the ratio of the concentration in ACs to the concentration in plasma at 1 h was 2.1 ± 5.2. AC drug concentrations were not significantly different when the concentrations in smoking and nonsmoking women with AIDS were compared ($P > 0.05$). AC drug concentrations were significantly greater in healthy women who were fast acetylators than in healthy men who were fast acetylators and men with AIDS who were slow acetylators. The concentrations of isoniazid in ACs were undetectable in 31 subjects. Nineteen had AIDS (61%), 12 were women (39%), 16 were fast acetylators (52%), and 3 were smokers (10%). None of these factors were significantly associated with an undetectable isoniazid concentration in ACs ($P > 0.05$ for all comparisons). The concentrations of isoniazid in plasma at 1 h ($R = 0.15$; $P > 0.05$) or 4 h ($R =$ −0.15; $P > 0.05$) were not correlated with isoniazid concentrations in ACs.

DISCUSSION

The MICs of isoniazid for *M. tuberculosis* strains have been reported to be in the range of 0.025 to 0.05 μg (21). The minimum bactericidal concentration (MBC) is approximately the same as the MIC; therefore, the MBC/MIC ratio is approximately 1. A universal standard for susceptibility testing has not been accepted. The isoniazid susceptibility breakpoint recommended by the NCCLS is 0.5 μg/ml by use of the proportion method (24a). The following interpretations of radiometrically determined MICs of isoniazid for *M. tuberculosis* have also been recommended: ≤0.1, 0.5, 2.5, and >2.5 μg/ml for susceptible, moderately susceptible, resistant, and very resistant, respectively (21). For clinical purposes, a concentration in serum of 3 to 5 μg/ml following the administration of an oral dose of 300 to 450 mg has been recommended as a target range for the treatment of tuberculosis (27).

The values presented above are of importance in relationship to the findings from this study. In most of our subjects the plasma isoniazid concentrations at 1 and 4 h following oral drug administration observed by a witness were below those proposed for the treatment of patients with tuberculosis. For the entire study group, 12.5, 34.7, 60, and 82.7% of the subjects had peak concentrations plasma at 1 h of less than 0.5, 1.0, 2.0, and 3.0 μg/ml, respectively. These observations would agree with those of Kimerling et al. (22), who found inadequate concentrations of isoniazid in the sera of 68% of subjects who received directly observed therapy with isoniazid. Those investigators, however, used a twice-weekly dosing regimen and a target concentration in serum of 9 μg/ml, and thus, their results are not fully comparable to ours. Moreover, it is of concern that the peak concentrations in plasma at 1 h achieved in 10 (12.5%) subjects (5 with AIDS and 5 healthy volunteers) in this

study would be at or below the recommended critical concentration. The clinical significance of this is unknown. Five, 25, and 31 of the 80 subjects had isoniazid concentrations that were undetectable in plasma, ELF, and ACs, respectively. Since administration of the first and fifth doses was directly observed and administration of the second, third, and fourth doses was documented in writing by the subjects, lack of compliance is not a likely explanation for these results. We believe that the data suggest poor and unpredictable gastrointestinal absorption of isoniazid. The optimum dose of isoniazid has not been determined; and the relationship among the MICs, drug concentrations, and outcomes requires further investigation.

Peak plasma drug concentrations were not affected by AIDS status, as we defined in this study, or acetylator status and were not greater in women, despite the greater weight-adjusted dose received by the women. Our failure to find reduced concentrations in plasma in these AIDS patients, as has been reported by others (18, 27; Berning et al., letter; Peloquin et al., letter), may be due to differences in the clinical status of the AIDS patients, the presence of gastrointestinal tract diseases, or differences in other physiological factors between the groups. Plasma drug concentrations were not correlated with body weight for men, women, or the group as a whole. Plasma drug concentrations at 1 h were significantly greater than the concentrations at 4 h, consistent with previous reports that have described the kinetics of isoniazid in plasma (25, 26). As expected, plasma drug concentrations at 4 h following administration of the last dose were significantly greater in slow acetylators than in fast acetylators. The level of protein binding of isoniazid is low (<10%) and is not of clinical or kinetic importance. The level of protein binding has not been included in reports of the pharmacokinetics of isoniazid (25, 26).

The concentrations of isoniazid in ACs were not affected by gender, AIDS status, or acetylator status. Our data indicate that isoniazid appears to diffuse passively into ACs, as has been reported previously for an in vitro model (20). For the group as a whole, intracellular drug concentrations were not significantly different from the peak concentrations in plasma. However, as was true of the concentrations in plasma, the intracellular concentrations in many of the subjects were low relative to the reported MICs of isoniazid for *M. tuberculosis*. In our study, the ACs of more than a third of the subjects had undetectable concentrations of isoniazid and the intracellular concentrations were less 1 μg/ml for more than half of the subjects. The optimum intracellular concentration of isoniazid in vivo is unknown and requires further study. Since *M. tuberculosis* causes intracellular infections, it is likely, but unproven, that some critical intracellular concentration is desirable for a favorable outcome.

The concentration of isoniazid in ELF was not significantly different from the concentrations in plasma at 1 h and the concentrations in ACs, suggesting that isoniazid, unlike pyrazinamide, diffuses passively into ELF (14). The clinical significance of antibiotic concentrations in ELF is unknown for tuberculosis and other bacterial and fungal pneumonias.

Outcome studies that relate MIC data, drug concentrations in serum, ACs, and ELF, rates of cure, failure, and relapse, and the rates of response of *M. tuberculosis* organisms have not been performed. In general, high inhibitory or killing ratios are viewed as favorable in the treatment of infectious diseases, and such ratios are probably important for the treatment of tuberculosis. This study suggests that in some patients who are treated for tuberculosis, plasma and intrapulmonary (ELF and AC) isoniazid concentrations are at or below the concentrations necessary to inhibit (MIC) or kill (MBC) the organism. This may provide, in part, a pharmacokinetic explanation for the rapid emergence of isoniazid-resistant organisms when isoniazid alone is used to treat tuberculosis.

We were also unable to demonstrate a difference in plasma, AC, or ELF drug concentrations in smoking versus nonsmoking women with AIDS. However, the sample sizes were small (10 smokers and 10 nonsmokers), and study of a larger group of subjects might detect such differences. The study was not designed to detect interactions among the many other drugs taken by our AIDS patients and isoniazid. However, when the concentrations in the various compartments of subjects with AIDS were compared to those in the healthy subjects, no differences in plasma, ELF, or AC isoniazid concentrations were detected, suggesting that if interactions were present the effect on isoniazid kinetics was minimal.

ACKNOWLEDGMENTS

This work was carried out with funds provided by NIH grant AI36054 and NIH grant MO1RR00079 (General Clinical Research Center) at the University of California, San Francisco.

We acknowledge the assistance of Charles L. Daley, Tom Delahunty, and Farzaneh Raoufi for performing the assays and Eve Benton for manuscript preparation.

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