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Kinetics of Isoniazid Transfer into Cerebrospinal Fluid in Patients with Tuberculous Meningitis

Sang-Goo Shin, M.D., Jae-Kyu Roh*, M.D., Nam-Soo Lee*, M.D., Jae-Gook Shin, M.D., In-Jin Jang, M.D., Chan-Woong Park, M.D., Ho Jin Myung*, M.D.

Clinical Pharmacology Unit, Department of Pharmacology and Department of Neurology, Seoul National University College of Medicine*

For the pharmacokinetic analysis of Isoniazid transfer into CSF, steady-state isoniazid concentrations of plasma and CSF were measured in eleven tuberculous meningitis patients confirmed with findings of CSF and neuroimazing.

Peak plasma levels (4.17-21.5 ug/mL) were achieved at 0.25 to 3hours after multiple isoniazid dose (600mg/day). Terminal half-life, total clearance (Cl/F) and volume of distribution (Vd/F) were 1.42±0.41 hr, 0.47±0.22 L/kg/hr and 0.93±0.48 L/kg, respectively.

Isoniazid concentrations in CSF collected intermittently were highest at 3 hr (Mean, 4.18 ug/mL) and were 0.54±0.21 ug/mL at 12 hrs after the last dose of isoniazid 10mg/kg/day. CSF/plasma partitioning of isoniazid and equlibration rate were estimated using modified pharmacokinetic/pharmacodynamic model. Disposition rate constant from CSF to plasma and CSF/plasma partitioning ratio of isoniazid were estimated to be 0.39 h^{-1} and 1.17, respectively.

Key Words: *Isoniazid, Acetylisoniazid, Pharmacokinetics, Pharmacokinetic-Pharmacodynamic model, CSF level,CSF/Plasma partitioning*

INTRODUCTION

Pharmacokinetic studies of antimicrobial agents should be ideally undertaken with a goal, not limited to determination of the characteristics of absorption and disposition process, which explain the process to deliver the drugs to the foci of infection for destruction of the infecting microorganisms. To accomplish this goal, It is necessary to obtain samples of some biological fluid for the quantification of drug and/or metabolites. In particular, it is most desirable to sample a biological fluid, that is in equilibrium with the site of action. Moreover, to estimate the details of time dependent changes of the drug in infected foci, attempt to analyse the data with appropriate pharmacokinetic model might be needed. At present, little attempts have been made to analyse the concentration time course in infected foci in terms of pharmacokinetics or their relation to drug concentration in plasma as a guidelines for optimum usage of antituberculous drugs for tuberculous meningitis.

Data concerning the concentration of the antituberculosis drug in cerebrospinal fluid (CSF) are sparse and scattered throughout the literature, though a recent survey reveals 8 of these 12 drugs have documented CSF penetration (Hodiness, 1985). In case of isoniazid (INH), only three reports have been published for the penetration into CSF, inspite of the wide use of the drug more than two decades. Moreover, different values from 20% to more than 90% have been recorded among three reports (Elmendorf et al., 1952;Barclay et al., 1953; Forgan-Smith et al.,1973). However, none of these reports give important information about the rate of penetration and the equilibrium partitioning of INH between plasma and CSF, and the effective duration of INH concentration

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Address for Correspondence: Sang-Goo Shin, M.D. Department of Pharmacology, Seoul National University College of Medicine, Seoul 110-460. Tel (02)7601-3391

in CSF.

In trying to obtain a detailed information for INH penetration into CSF and to estimate the time course of drug concentration in CSF, it is necessary to use a pharmacokinetic model to elucidate the link between plasma and CSF compartment. We have adapted the pharmacokinetic-pharmacodynamic model proposed by Holford and Sheiner (1981) with a minimum assumption to allow a simultaneous analysis of plasma and CSF isoniazid concentration-time data.

MATERIALS AND METHODS

Patients and study design

Eleven patients with tuberculous meningitis (proved by the presence of the organism in CSF or suspected by initial cerebrospinal fluid differential cell count, protein, glucose and neuroimaging techniques) participated in this pharmacokinetic study while they are under antituberculosis therapy. Clinical status was assessed by findings from complete history and physical examination, chest X-ray, electrocardiogram, complete blood cell count, serum chemistries and creatinine clearance. Patient were excluded in the study if they have any serious disease other than tuberculosis and hepatic, or renal insufficiency. Informed consent was obtained from each patient before the study.

The steady-state pharmacokinetic study of INH was carried out in 9 male and 2 female patients (age,48±16.5 years; weight, 60.6±10.8 kg). Patients received fixed dose triple antituberculous drugs (INH 600 mg/day, pyrazinamide 1500 mg/day, refampin 600 mg/day) more than four days before starting pharmacokinetic study. The combination of three drugs was continued during entire study period. Concomittant medications with those having known drug interaction with isoniazid were avoided. Isoniazid was administered orally as tablets in the morning after an overnight fast; the ingestion of food was allowed two hours after the dose was taken.

Blood samples were withdrawn from an indwelling heparin-locked (100 unit/mL) intravenous catheter, prior to the morning dose, and then 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12 and 24 hours after dosing. Plasma was obtained by centrifugation (500 g, 4°C, 15 min), deproteinized by addition of 10% trichloroacetic acid and stored at -80°C until analyzed. Cerebrospinal fluid collection were made just before the morning dose, and 1, 2, 3, 4, 5, 6, 8 and 12 hours after dosing. Cerebrospinal fluid were collected 3 to 4 times intermittantly in same patient within two weeks next to blood sampling. Individual CSF sampling times were allocated randomly. Cerebrospinal fluid were deproteinized and stored just as plasma samples.

Assay of Isoniazid and Acetylisoniazid in body fluids

Plasma and cerebrospinal fluid samples were analyzed for INH and acetylisoniazid (AcINH) within 2 days of collection by the spectrofluorometric methods of Olson et al. (1977).

The INH in the aliquots that are to be used for determination of AcINH was converted to an azide, which does not react with salicylaldehyde. This was done by adding 0.1 mL of the $NaNO_2$ solution to the 0.4 mL aliquots of TCA treated supernatant and allowing the mixture to stand for 10 min, then 0.1 mL of ammonium sulfamate solution was add to destory excess nitrate. After 5 min, to hydrolyze the AcINH to INH, 0.1 mL of 6 mol/L HCL was added and heated at 80°C for 1 hr, the tubes allowed to cool at room temperature for 10 min and nutralized by adding 0.1 mL of 6 mol/L NaOH.

The following procedures were used for measuring both INH and AcINH. From the hydrazone by adding salicylaldehyde solution (0.5 mL/tube) to the diluted biological fluid of total volume of 0.8 mL, and adjust the pH to 4.0±0.1 with 0.5 mol/L HCL or 0.5 mol/L NaOH. After 15min, 1 mL of the bisulfite solution was added. After adding reducing agent (0.1 mL of 10% the ascorbic acid solution), the pH of the mixture was adjusted to 5.7±0.05 and the tubes were heated at 50°C for 10min. After the tubes have again reached room temperature, 1.5 ml of isobutanol was added. The tubes were placed in an ice bath for 10 min, then separate the phases by centrifugation at 500 xg for 10 min and place the tubes in an ice bath until the fluorescence of the organic layer is determined at 386 (excitation) and 462 (emission) nm. The coefficients of variation at the concentrations of 1 and 10 ug/mL of plasma were below 3% for INH and 6% for AcINH, respectively. The lowest limit of senisitivity in our hands was 0.04 ug/mL for INH and 0.1 ug/mL for AcINH.

Pharmacokinetic Analysis of Drug plasma concentration-time curves

Plasma concentrations of INH and AcINH were analyzed for terminal half-life ($t_{1/2}$), maximum concentration (Cmax), and the time to reach maximum concentration (Tmax) by standard techniques. The area under the plasma concentration-time curve (AUC) from time 0 to 24 hours at steady-state was calculated from the linear/log trapezoidal summation. Total oral clearance (CL/F) was calculated from the following

equation.

$$CL/F = Dose/AUC \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Volume of distribution (Vd/F), absorption rate constant (ka) and elimination rate constant (ke) of isoniazid were estimated by fitting the drug concentration-time course in plasma to one compartmental model (Fig. 1) using

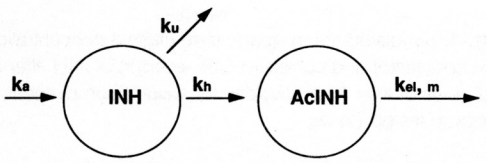


Fig. 1. Single compartmental pharmacokinetic disposition model for INH and AcINH. Ka is the first-order absorption rate constant, kh, ku and kel, m are acetylation rate constant, rate constant for renal excretion and other unknown pathway, and elimination rate constant for acetylisoniazid, respectively.

PCNONLIN non-linear regression computer program (Metzler, 1986).

Pharmacokinetic Model for Isoniazid CSF transfer

The pharmacokinetic model in this study is based on the assumption that the amount of distributed INH from plasma to CSF is negligible relative to the total amount of the drug in remaining body compartment. Besides, passive INH distribution from plasma to CSF and from CSF to body compartment were assumed. According to the above assumptions, the cerebrospinal fluid space is assigned to so called "effector compartment" connected to the central compartment by first-order rate constants,kie and keo,respectively (Fig. 2). Then, concentration-time course of effector compartment (C_{CSF}) after single oral dose can be expressed by following equation, including the CSF/plasma ratio at equilibrium (ki) and disposition rate constant of INH from CSF (Keo) to vas culas space.

$$Ccsf\ (t) = \frac{Ki \cdot F \cdot Dose \cdot Keo}{Vd} \left(\frac{e^{-ket}}{(ka-ke)\ (keo-ke)} + \frac{e^{-kat}}{(ke-ka)\ (keo-ka)} + \frac{e^{-kect}}{(ke-keo)\ (ka-keo)} \right) \cdots (2)$$

This allows a simultaneous analysis of plasma and CSF isoniazid concentration-time courses.

The pharmacokinetic parameters (ki,keo) for INH transfer into CSF were calculated by naive-pooled simultaneous fitting of plasma and CSF concentration-time data. In the fitting procedure the residuals were weighted with the inverse of the observed values, because the error of the assay is a more or less constant percentage of the concentration.

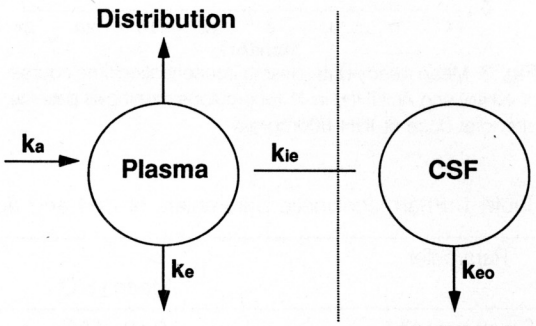


Fig. 2. Modified pharmacokinetic/pharmacodynamic model. describing isoniazid CSF transfer kinetics, ka is the first-oder absorption rate constant, ke and keo are elimination rate constant of isoniazid from body and disposition rate constant from CSF, respectively.

RESULTS

Following multiple oral administration of 600 mg INH per day the INH concentration-time curves in plasma showed a biexponential course suggesting that one compartmental body model would be appropriate for describing the kinetics (Fig. 3). The plasma Cmax of INH (4.17-21.5 ug/mL;average 9.46 ug/mL) was achieved in 0.25 to 3 hours after the dose of INH. The terminal half-life of INH ranged from 0.84 to 1.95 hours in all the subjects examined. The plasma levels of AcINH reached a maximum value at 1.0 to 3.0 hours after INH dose, and the peak levels ranged from 1.51 to 8.84 ug/mL with average value of 5.51 ug/mL. The elimination of AcINH from plasma was slower (average $t_{1/2}$ 4.54 hr; range, 2.64-7.89 hr) than that of INH. After chronic administration of INH, there was no accumulation of either INH or AcINH.

When phenotyped according to the criteria of Scott et al. (1969) or Reidenberg et al. (1980) for discriminating rapid from slow acetylators, all the subjects examined were classified as rapid acetylators. We used the criteria of terminal half-life for descrimicration of INH phenotypes, because Israili et al. (1987) recently reported increased plasma concentration ratio of AcINH/INH

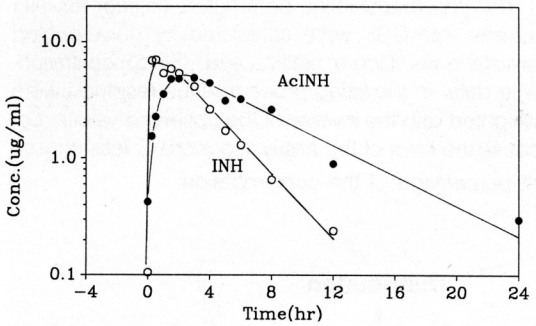


Fig. 3. Mean steady-state plasma concentration-time course of INH(o) and AcINH(•) in 11 tuberculous meningitis patients after oral dose of INH 600mg/day.

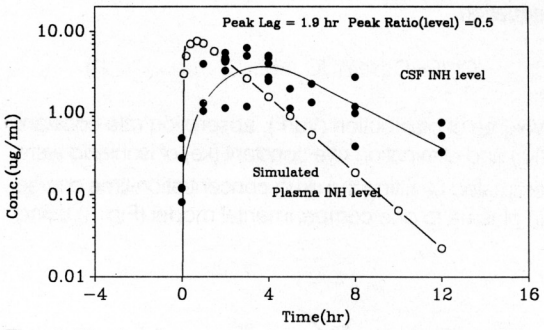


Fig. 4. Simulated mean steady-state plasma concentration-time courses(o) and corrected CSF levels(•) of INH after an oral dose of INH 10 mg/kg/day by assumption of linear kinetics in tested doses.

Table 1. Pharmacokinetic parameters of INH and AcINH in 11 patients with tuberculous meningitis

Parameter	Isoniazid	
	Mean±S.D.	Range
Cmax (ug/ml)	9.46±5.50	(4.17-21.5)
Tmax (hr)	1.35±0.88	(0.25-3.0)
AUC (ug/ml hr)	22.66±11.45	(12.10-48.33)
ka (hr^{-1})	3.36±3.29	(0.45-10.38)
Vd/F (L/kg)	0.93±0.48	(0.48-1.92)
CL/F (L/kg/hr)	0.47±0.22	(0.20-0.91)
$t^1/_2$ (hr)	1.42±0.41	(0.84-1.95)
	Acetylisoniazid	
Cmax (ug/ml)	5.51+2.22	(1.51-8.84)
Tmax (hr)	2.0+0.75	(1.0-3.0)
AUC (ug/ml hr)	47.10+23.82	(27.10-101.69)
$t^1/_2$ (hr)	4.54+2.0	(2.64-7.89)

ka: absorption rate constant
Cmax: peak concentration
CL/F: total clearance
Tmax: time to peak
Vd/F: volume of distribution
$t^1/_2$: terminal half-life

(R) at six hours after dose in two subjects among 14 subjects examined after concomittant administration of rifampin. However, rifampin did not show any significant effect on most of the pharmacokinetic parameters of INH.

The pharmacokinetic data derived are summarized in Table 1. Pharmacokinetic variables as Vd and Cl should be viewed as approximated values since we administered INH orally not intravenously. There may be an appreciable first-pass effect of isoniazid (Weber and Hein, 1979) and this degree of first pass metabolism may invalidate Vd and Cl values when oral abosprtion of the drug is even assumed to be complete (Des Prez and Bonns, 1961; Weber and Hein, 1979) The Vd values of INH obtained from the oral dosing in this study varied between 0.48 and 1.92 L/kg in all the subjects. The mean values were slightly greater than the ranges of those reported previously from the intravenous dosing (Jenne, 1960; Ellard and Gammon 1976; Advenier et al., 1980).

CSF levels of INH observed were converted the corresponding levels for an oral dose of INH 10 mg/kg by assumption of lincar kinctics in tested dose (Fig. 4). In the cerebrospinal fluid the maximum concentration were achieved at 3 hrs (4.18±2.68 ug/mL) after the dose of INH. The average peak ratio of CSF/plasma was 0.5. CSF levels around 2 to 5 hrs after INH dose were identical to the corresponding plas-

ma levels. After then, CSF levels remained higher than plasma levels. The average CSF level at 12 hrs after the dose was 0.54±0.21 ug/mL, when the plasma levels are near detection limits of assay sensitivity. The disposition of INH from CSF was slower than that from plasma.

For the evaluation of penetration characteristics of INH into CSF, modified pharmacokinetic-dynamic model was applied assuming amount of drugs in CSF does not affect the time course of drug in the body. The pharmacokinetic characteristics drived from fitting simultaneously plasma and CSF levels to the model by naive pooled method were summarized in table 2. There was a favorable correlation between calculated and observed CSF concentrations in whole portion of the curve in CSF levels (r=0.77). The disposition rate constant from CSF, determinant of equilibrium rate between plasma and CSF (Holford and Sheiner, 1981), was 0.394 hr^{-1}, which is smaller than absorption (3.36 hr^{-1}) and elimination (0.53 hr^{-1}) rate constants of INH. Then, the terminal slope of isoniazid concentration-time course in CSF would be identical to the disposition rate constant. The CSF/plasma partitioning ratio at equilibrum was estimated to be 1.17. The lag of time to peak in CSF calculated by the model parameters was 1.9 hr after peak time in plasma.

Table 2. Cerebrospinal fluid penetration characteristics of INH

Parameter	Value
Disposition Rate Constant (keo)	0.394 hr^{-1}
Partition Coefficient (ki)	1.17
Peak Lag Time	1.90 hr
Peak Level Ratio (CSF/Plasma)	0.50
Terminal Half-life in CSF	1.76 hr

Terminal half-life of INH in CSF was calculated by 0.693/keo

DISCUSSION

A consquence of the experiments of Stüeben (1957), it became evident that the ratio of drug concentration between the vascular and tissue site changes over time because of distribution delay of the drug into extravascular site and shows anticlockwise histeresis over time (Fig. 5). Therefore, it is impossible to establish a disposition characteristics into specific tissue site by calculation of tissue/plasma ratio of drug concentration with scarce simultaneous measument of drug levels in plasma and target tissue. The disposition characteristics of a drug in vivo can only be evalualed by serial determination of a drug concentreation in tissue site as well as plasma concentration over time. More over, suitable pharmacokinetic models should be proposed, which would enable to link vascular compartment and tissue compartment. However, no pharmacokinetic models are yet available to analyse simultaneously two or more concentration time curves in different compartments. Several approaches were attempted to overcome these difficulties, but most of them depended on non-physiological assumptions (Cronberg, 1978; Mattie, 1978; Mattie et al., 1987). One exception seems to be the pharmacokinetic-pharmacodynamic model, where the concentration-time course in extravasular sampling site is assigned to so called "effector compartment". This allows a simultaneous analysis of at least the drug loss from the extravascular site to characterize the time course of drug level in extravascular site (Ganzinger et al., 1986).

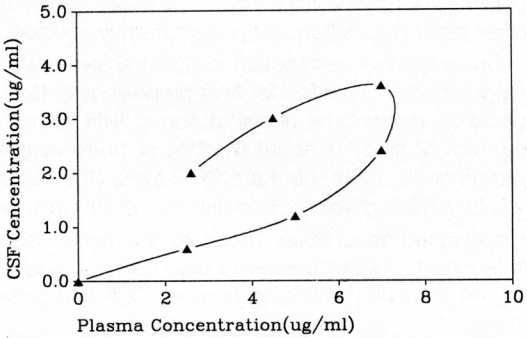


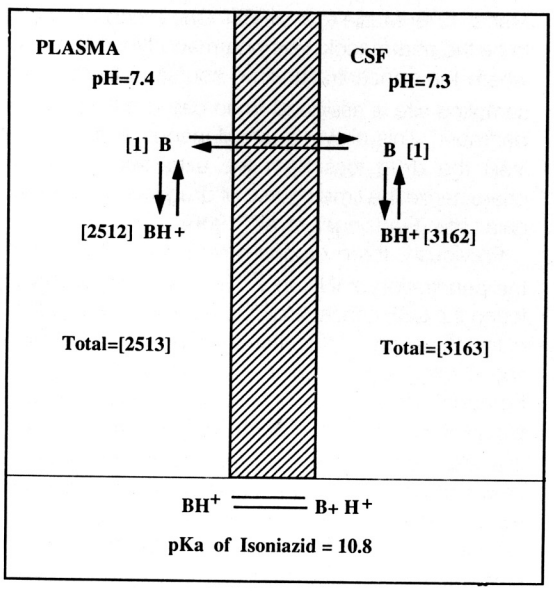
Fig. 5. Anticlockwise histeresis loop of plasma and CSF drug levels during non-equilibrium state owing to the equilibration delay between plasma and tissue site.

Previously, three reports have been published for the penetration of INH into CSF. Barclay et al. (1953) found the CSF concentration to be approximately 20% of that in serum 1 hour after a single dose administration. Contray to the finding, Elmendorf et al. (1952) and Forgan-Smith et al. (1973) recorded excellent penetration of INH (more than 90%) in CSF 3-6 hours after the last dose during multiple dose INH therapy. However, all the previous report calculated CSF/plasma partitions, at fixed time interval with scarce observations, which would give an inaccurate parameter for CSF partitioning and no information about fate of isoniazid in CSF. In this paper, a method is discribed to quantify the CSF penetration of isoniazid over the time in terms of the rate of disposition (keo) and equilibrium partition coefficient (ki). Except the assumption that negligible amount of INH is distributed from

plasma to CSF and from CSF to vascular pool, the other assumptions frequently used are physiological.

Our additional assumption in CSF transfer kinetic model should be justified for its appropriateness. Condsidering that volume of distribution of INH estimated from IV study is about 61±11% of body weight (Jenne et al., 1960), normal CSF volume (150 ml) is less than 0.5% of volume of distribution of INH in subject weighed about 60kg. Therefore, the model used in this study might have lesser than 0.5% of modelling error if INH partitioning between CSF and plasma is 1.

By the approach of modified pharmacokinetic-pharmacodynamic model, it is evident that INH disposition rate in CSF is faily slower than that in plasma, and INH concentrations last longer above concentration, which most strains of Mycobacterium tuberculosis were suceptible (1ug/mL; Holdiness, 1985) after the usual dose. Partitioning between CSF and plasma is shightly over the unity (ki; 1.17). It might be worthwile to explore the possible contributing factors for this unequal distribution of INH between CSF and plasma in tuberculous minigitis patients. To our knowledge, the governing factors of CSF/Plasma partitioning at equilibrium are protein binding in plasma and degree of ionization of the drug in plasma and CSF which determined by pH difference and pKa value of the drug. INH is a basic drug with pKa value of 10.8. Just assuming the pH of the CSF to be 7.3 and negligible protein binding of INH, partitioning of total INH between CSF and plasma at equilibrium could be predicted to be 1.25 by pH partitioning hypothesis (Fig. 6). However, the binding of INH to human plasma was reported to be quite variable from 4% to 30% within therapeutic range of concentrations (Israili et al., 1987). This factor might prohibit the favorable distribution into CSF. On the other hand, the acidic changes and elevation of lactic acid levels of CSF in tuberculous menigitis has been reported (Kopetzky et al., 1932, D'souza et al., 1978; Brook et al., 1978). The lowering of pH in CSF might profoundly increase the partitioning ratio of CSF/plasma by ion trapping in CSF site.



$$\frac{[CSF]}{[Plasma]} = \frac{1 + 10^{pKa - 7.3}}{1 + 10^{pKa - 7.4}} = 1.25$$

Fig. 6. Theoretical distribution of isoniazid at equilibrium based on assumptions of negligible protein binding of isoniazid and pH of CSF as 7.3.

In conclusion, our approch of the modified pharmacokinetic-pharmacodynamic model for drug transfer into CSF allows simulation plus evaluation of INH concentration in plasma and CSF for optimum design of proper INH dose regimens in the management of tuberculous meningitis. This could contribute to extend the scope of pharmacokinetic modelling for the evaluation of CSF penetration of the drugs especially with large volume of distribution.

REFERENCES

Advenier C, Saint-Aubin A, Gobert C, Houin G, Albengress E: Tillement JP: *Pharmacokinetics of isoniazid in the elderly. Br J Clin Pharmacol 10: 167-169, 1980.*

Barclay WR, Ebert RH, LeRoy GV, Manthei RW, Roth LJ: *Distribution and excretion of radioactive isoniazid in tuberculous patients. J Am Med Ass 151: 1384-1388, 1953.*

Brook I, Bricknell KS, Overturf GD, Finegold SM: *Measurement of lactic acid in cerebrospinal fluid of patients with infections of the central nervous system. J Infect Dis 137: 384-390, 1978.*

Cronberg S: *A simple mathematical model for diffusion of drug into an infection site. Scand J Infect Dis Suppl 14: 100-104, 1978.*

D'Sonza E, Mandal BK, Hooper J, Parker L:*Lactic-acid concentration in cerebrospinal fluid and differential diagnosis of meningitis. Lancet i: 579-580, 1978.*

Ellard GA, Gammon PT: *Pharmacokinetics of isoniazid metabolism in man. J Pharmacokinet Biopharm 4: 83-113, 1976.*

Elmendorf DF, Cawthon WU, Muschenheim C, McDermott M: *The absorption, distribution, excretion and short term toxicity of isonicotinic acid hydrazide(Nydrazid) in man. Am Rev Tuberculosis 65: 429-442, 1952.*

Forgan-Smith R, Ellard GA, Newton D, Mitchison DA: *Pyrazinamide and other drugs in tuberculosis meningitis. Lancet 2: 374, 1973.*

Ganzienger U, Schiel H, Georggopoulos A, Gumbold G: *The*

utility of diffusion chambers as models for the description of drug disposition. J Antimicrob Chemother 18: 93-102, 1986.

Holdiness MR:*Cerebrospinal fluid pharmacokinetics of the antituberculosis drugs. Clin Pharmacokinet 10: 532-534, 1985.*

Holford NHG, Sheiner LB: *Understanding the dose-effect relationship; Clinical application of pharmacokinetic-pharmacodynamic models. Clin Pharmacokinet 6: 429-453, 1981.*

Israili ZH, Rogers M, El-Attar H: *Pharmacokinetics of antituberculosis drugs in patients. J Clin Pharmacol 27: 78-83, 1987.*

Jenne JW: *Studies of human patterns of isoniazid metabolism using an intravenous fall-off technique with a chemical method. Am Rev Resp Dis 81: 1-8, 1960.*

Koretzky SJ, Fishberg EH: *Changes in distribution ratio of consitituents of blood and spinal fluid in meningitis. J Lab Clin Med 18: 796-800, 1932.*

Mattie H: *Pharmacokinetic aspects of tissue penetration. Scand J Infect Dis Suppl 14: 125-126, 1978.*

Mattie H, Hoogeterp JJ, Hermans J: *The relation between plasma and tissue concentrations of antibiotics, description of a method. J Pharmacokinet Biopharm 15: 191-202, 1987.*

Metzler CM. PCNONLIN and NONLIN 84: *Software for the statistical analysis of nonlinear models. Am Statistician 40: 52, 1986.*

Olson WA, Dayton PG, Israili ZH, Pruitt AW: *Spectrophotofluorometric assay for isoniazid and acetylisoniazid in plasma adapted to pediatric studies. Clin Chem 23: 745-748, 1977.*

Reidenberg MM, Levy M, Drayer DE, Zylber-Katz E, Robbins WC: *Acetylator phenotype in idiopathic systemic lupus erythematosus. Arthritis Rheum 23: 569-573, 1980.*

Scott M, Wright RC, Weaver DD: *The discrmination of phenotypes for rate of disappearance of isonicotinyl hydrazide from serum. J Clin Invest 48: 1173-1176, 1969.*

Stuben J: *Ablauf der Konzentrations veranderung verschiedener sulfonamide in serum, im Gewebssaft der Haut und in Dentinliquor. Dtsch Zahn Mund Kieferheilk 26: 177-205, 1957.*

Weber WW, Hein DW: *Clinical pharmacokinctics of isoniazid. Clin Pharmacokinet 4: 401-422, 1979.*