Table I—Precision Study on Spiked Plasma Samples [a]

Acetaminophen, μg/ml	Current, μamp	CV, %
41	22.7, 21.5, 19.3, 19.5, 17.9 16.9, 18.3, 19.3, 19.3[b]	9.23
101	50.3, 49.1, 51.5, 47.6, 49.9 53.1, 51.1, 53.8, 55.0, 55.4	4.99
202	118.1, 118.1, 114.1, 106.3, 107.0 105.1, 103.1, 101.5, 99.9, 96.8	6.84
403	191.1, 195.0, 191.1, 193.1, 200.9 199.0, 199.0, 195.0[c]	1.97

[a] The data in Table I can be fitted by the equation: current (microamperes) = [acetaminophen (μg/ml)] + 2.88, with $r = 0.9960$ and $S_{y \cdot x} = 5.93$. [b] One determination was rejected because of an irregular peak shape. [c] Two determinations were rejected on a statistical basis according to the method of Dean and Dixon (16).

the range of 20–400 μg/ml can be described by the equation: current (μamp) = 0.438 concentration (μg/ml) + 12.9, with a correlation coefficient of 0.991 and a standard error of the estimate ($S_{y \cdot x}$) of 7.76. Determination of multiple spiked samples gave the results presented in Table I.

Daily minor variations in the slopes of standard curves were attributed to aging of the electrode surface. Consequently, it was necessary to prepare standard curves daily. Plasma samples containing phenobarbital, salicylic acid, theobromine, theophylline, or aspirin at levels of 100 μg/ml gave voltammograms (+0.2–0.8 v) identical to those of blank plasma at a sensitivity setting that produced a half-scale response for acetaminophen at 100 μg/ml.

This method is sufficiently simple and sensitive for the rapid determination of acetaminophen in plasma at levels likely to be encountered in cases of toxic overdosage. Since no sample preparation is required and the instrumentation requires virtually no startup time, this method represents an extremely rapid method for acetaminophen. Since the measurements are made at potentials positive with respect to the reference electrode, dissolved oxygen does not interefere. Hence, sample degassing is not required.

Since the major biotransformation of acetaminophen in humans is direct conjugation with sulfate and glucuronic acid to form the sulfate and glucuronate metabolites (15), these metabolites should not interfere in this assay. In both of these metabolites, the phenolic group, which is oxidized at the carbon paste electrode, is conjugated and unavailable for electrochemical oxidation. Although the instrumentation required for this method is probably not available in many clinical laboratories, the recent introduction of a low-priced unit[5] will undoubtedly make differential pulse voltammetry a more common tool.

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[5] Model 364, Princeton Applied Research, Princeton, N.J.

COMMUNICATIONS

Simplified Derivation of Chiou–Hsu Equation for Rapid Estimation of Total Body Drug Clearance during Constant-Rate Intravenous Infusion

Keyphrases □ Total body drug clearance—rapid estimation during constant-rate intravenous infusion, equation derived □ Clearance, total body drug—rapid estimation during constant-rate intravenous infusion, equation derived □ Pharmacokinetics—rapid estimation of total body drug clearance during constant-rate intravenous infusion, equation derived

To the Editor:

An equation using two sets of plasma level data to estimate the total body clearance (*TBC*) of a substance during a zero-order input to the body was reported previously by Chiou and Hsu (1). The equation is:

$$TBC = \frac{2K_0}{C_{p_1} + C_{p_2}} + \frac{2V_d(C_{p_1} - C_{p_2})}{(C_{p_1} + C_{p_2})(t_2 - t_1)} \qquad \text{(Eq. 1)}$$

where K_0 is the zero-order input rate constant, V_d is the literature estimated apparent volume of distribution based on the linear one-compartment open model, and C_{p_1} and C_{p_2} are plasma levels of the substance at times t_1 and t_2 during the zero-order input, respectively.

This equation was originally proposed to estimate the total body clearance of endogenous creatinine and renal creatinine clearance in patients after a slight modification (1). It also was proposed recently to estimate rapidly the total body clearance of a drug in patients during a constant-rate intravenous infusion (2). Some precautions in using the equation for dosing individualization were discussed (2).

The derivation of Eq. 1 involved the Laplace transform

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method. However, the same equation can be derived in a much simpler manner, which will be the subject of this communication.

The differential equation for describing the change of concentration of the substance with time during the zero-order input process is:

$$\frac{dC_p}{dt} = \frac{K_0}{V_d} - KC_p \qquad \text{(Eq. 2)}$$

where K is the apparent first-order elimination rate constant for the substance in the body. Equation 2 can be approximated to:

$$\frac{\Delta C_p}{\Delta t} = \frac{C_{p2} - C_{p1}}{t_2 - t_1} = \frac{K_0}{V_d} - KC_{p\,\text{mid}} \qquad \text{(Eq. 3)}$$

Since $C_{p\,\text{mid}}$ can be approximated to be equal to:

$$C_{p\,\text{mid}} = \frac{C_{p1} + C_{p2}}{2} \qquad \text{(Eq. 4)}$$

substitution of Eq. 4 into Eq. 3 results in the following relationship:

$$KV_d = \frac{2K_0}{C_{p1} + C_{p2}} + \frac{2V_d(C_{p1} - C_{p2})}{(C_{p1} + C_{p2})(t_2 - t_1)} \qquad \text{(Eq. 5)}$$

Since $TBC = KV_d$, Eq. 5 is the same as Eq. 1.

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Dose-Dependent Pharmacokinetics of 7-Chloro-1,3-dihydro-5-(2′-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one in Dogs

Keyphrases ☐ 1,4-Benzodiazepine, substituted—chlorodesmethyldiazepam, pharmacokinetics in dogs ☐ Metabolites—chlorodesmethyldiazepam, pharmacokinetics in dogs ☐ Chlorodesmethyldiazepam—pharmacokinetics in dogs ☐ Pharmacokinetics—chlorodesmethyldiazepam in dogs

To the Editor:

7-Chloro-1,3-dihydro-5-(2′-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one (chlorodesmethyldiazepam, I) is a major metabolite of 2-*o*-chlorobenzoyl-4-chloro-*N*-methyl-*N*′-glycylglycinanilide, a recently developed[1] minor tranquilizer. Compound I undergoes hydroxylation at the C-3 position and is converted to lorazepam (II) by liver microsomal enzymes of rats and mice (1).

When I was administered to dogs intravenously, its plasma level–time curves were dose dependent. No report has appeared concerning the dose-dependent metabolism and nonlinear pharmacokinetics of 1,4-benzodiazepines, in spite of many investigations on their metabolism and pharmacokinetics.

This communication describes a nonlinear pharmacokinetic model to explain the dose-dependent plasma concentration–time curve of I. Simulation values of parameters were derived from this model.

Two male dogs were injected with 0.5, 2.0, and 3.0 mg of I/kg iv. Plasma samples were collected from 10 min to 30 hr after injection. Compound I and free lorazepam were determined by GLC with an electron-capture detector[2]. Lorazepam glucuronide (III) in urine was determined after hydrolysis with glucuronidase–sulfatase. The plasma concentration–time curve following administration of 0.5 mg/kg declined linearly in two phases (distribution and elimination) on a semilogarithmic plot; the 2.0- and 3.0-mg/kg doses gave curves of convex shape after distribution had been completed, with a more pronounced curvature for the higher dose. However, the apparent plasma half-lives of I estimated from the terminal straight region of the curves were nearly equal for the three doses. The plasma concentration–time curves of I, as already mentioned, would exclude enterohepatic circulation as a main elimination pathway of I and/or a conjugate of I. In urine, the major metabolite was lorazepam glucuronide (35%), and only 0.05% of the unchanged drug (total of free and conjugated forms) was excreted over 54 hr. Other metabolites were not detected.

Ethyl alcohol (2) and phenytoin (3) show dose-dependent pharmacokinetics that fit a one-compartment model including the Michaelis–Menten equation.

The most simplified model for the plasma concentration–time curves of I is elaborated on the following assumptions: enterohepatic circulation of I and/or its glucuronide is negligible, and enzymes concerned with the metabolism of I have the same enzyme constants (V_m and K_m). Model equations employed for the plasma level–time curves of I are shown as Eqs. 1 and 2 but cannot be solved with the data of plasma levels only:

$$V_1 \frac{dC_1}{dt} = V_2 k_{21} C_2 - V_1 \left(k_{12} C_1 + \frac{V_m C_1}{K_m + C_1} \right) \qquad \text{(Eq. 1)}$$

$$V_2 \frac{dC_2}{dt} = V_1 k_{12} C_1 - V_2 k_{21} C_2 \qquad \text{(Eq. 2)}$$

Therefore, digital computer simulation was performed by the Runge–Kutta method using the kinetic parameters k_{12}, k_{21}, V_1, and V_2 estimated from the linear two-compartment open model at 0.5 mg/kg. The simulation curves produced a satisfactory fit for the 2.0- and 3.0-mg/kg data. The simulation values obtained were 0.58 and 1.30 μM/hr for V_m and 0.50 and 1.00 μM for K_m in Dogs 1 and 2, respectively. Ten- and one-tenthfold multiple transformations of the simulated V_m and K_m values gave curves significantly different from the experimental data.

These results support the kinetics in which the plasma decline of I obeys the two-compartment model, including

[1] Unpublished results.

[2] To be published.

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