of toxins A, B and C. Toxin C differs from toxin B only in having –Ala–28, –Ile–32, and –Lys–49 in place of –Gly–28, –Ser–32, and –Arg–49 residues. Also, toxin C differs from toxin A in having –Ala–28 and –Lys–49 in place of –Gly–28 and –Arg–49.

The primary structure of toxin C thus elucidated was the same as that of *Naja naja siamensis* neurotoxin 3, which is a major neurotoxin in the venom.[4] At present, it is not known whether the presence of toxin C in the venom of *Naja naja* is due to contamination of the venom of *Naja naja siamensis* during the milking or whether the venom of *Naja naja* originally contains toxin C as one of the neurotoxin components.

Acknowledgement The authors are indebted to Professor I. Yamashina of our laboratory for valuable discussions and encouragement in the course of the study. Thanks are also due to Dr. H. Nishitani of the Department of Neurology of Utano National Hospital for encouragement and Dr. Yokoyama of Takara Shuzo Co., Ltd. for providing acid carboxypeptidase isolated from the culture fluid of *Penicillium janthinellum*. This work was aided in part by a grant from the National Center for Nervous, Mental and Muscular Disorders of the Ministry of Health and Welfare, Japan.

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[Chem. Pharm. Bull. 29(5)1462—1466(1981)]

A Model Independent Approach to describe the Blood Disappearance Profile of intravenously Administered Drugs

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(Received June 23, 1980)

A model independent approach is proposed to describe the blood disappearance profile of intravenously administered drugs. This approach requires a new definition of the total volume of distribution as a function of time. The total volume of distribution is regarded as the sum of V_1 and V_2. V_1 represents the initial volume of distribution after the intravenous administration of drugs. V_2 represents the additional distribution volume where the drug is distributed after the initial rapid distribution has ceased. To characterize V_2, two parameters, the maximum value of V_2, $(V_2)_{max}$, and the distribution constant, K_d, which is equal to the time when V_2 is equal to one-half its maximum, are adopted. It has been suggested by a simulation study that these two parameters, $(V_2)_{max}$, and K_d, are the major determinants of the rapid initial disappearance (*i.e.* distribution

phase) of drugs from the blood stream after intravenous administration. The blood disappearance profile of warfarin was analyzed on the basis of this approach.

Keywords——pharmacokinetics; distribution phase; model independent approach; total volume of distribution as a function of time; simulation study; warfarin kinetics

In general, the serum or plasma drug concentration decays exponentially after the intravenous administration of drugs. When a plot of the logarithm of serum drug concentration against time shows a concave curve, a two- or three-compartment model has been used.[1] For example, to represent the blood disappearance profile of digoxin, two exponential terms with four parameters, A, B, α, and β, have been used.[2] In addition, the plasma disappearance profile of warfarin is thought to be composed of at least three exponential terms with six parameters such as P, A, B, π, α, and β,[3] and a three-compartment model was assumed.[4] However, it is difficult to compare the parameter values of different pharmacokinetic models.

In this paper, a new approach is proposed to overcome this problem and to describe blood concentration *vs.* time profile in terms of a model independent approach.

Theoretical

Upon intravascuclar administration drugs enther the blood stream immediately and leave there very rapidly. Usually the drug distributes in a few minutes into the extravascular fluid of highly perfused tissues. Therefore, the initial volume of distribution designated as V_1 is defined as a proportionality constant to correlate the initial blood concentration C_0 and the injected dose X_0. Thereafter, the drug distributes more slowly into less perfused tissues, and the total volume of distribution of the drug increases gradually. This increasing portion after the initial rapid distribution has ceased is defined as "additional distribution volume" and is designated as V_2. When the drug has become distributed into almost all of the tissues, V_2 reaches the maximum value. Therefore, the following equation, which is a well-known one in pharmacokinetics and which represents such a time-dependency of V_2, was used to represent V_2 as a function of time.

$$V_2 = \frac{(V_2)_{\max} \cdot t}{K_d + t} \tag{1}$$

where K_d is defined as a "distribution constant" and is equal to the time when the value of V_2 is equal to one-half its maximum, $(V_2)_{\max}$.

As the total volume of distribution V is the sum of V_1 and V_2, V is also a function of time. Then,

$$V = V_1 + \frac{(V_2)_{\max} \cdot t}{K_d + t} \tag{2}$$

where t is the time after intravenous administration.

On the other hand, the amount of drug in the body at any time after the administration is given by multiplying the blood concentration C by the total volume of distribution V. In addition, by assuming that the drug is eliminated from the blood stream in a "first-order" manner, the material balance in such a system can be represented by the following equation:

$$\frac{d(V \cdot C)}{dt} = -k(V \cdot C) \tag{3}$$

Differentiation of Equation 3 and rearrangement give

$$\frac{dC}{dt} = -kC - \frac{C}{V}\frac{dV}{dt} \tag{4}$$

This equation is equivalent to Equation (4—13) of Smith[5] which represents the reaction

rate of material in a chemical reactor with changing volume and with a first-order reaction process.

To derive the equation for C as a function of time t, it is easier to integrate Equation 3 than Equation 4, and the result is:

$$(V \cdot C) = (V \cdot C)_0 \, e^{-kt} \tag{5}$$

where $(V \cdot C)_0$ is equal to the initial amount of drug in the body, namely the administered dose, X_0. Then, rearrangement of equation **5** gives:

$$C = \frac{X_0}{V} e^{-kt} \tag{6}$$

Substitution of Equation 2 into Equation 6 gives:

$$C = \frac{X_0}{V_1 + \dfrac{(V_2)_{max} \cdot t}{K_d + t}} e^{-kt} \tag{7}$$

This equation represents the blood concentration *vs.* time profile of a drug administered intravenously. At time 0, namely immediately after the intravenous administration, t is equal to zero. Then, the blood concentration at time zero C_0 is given by:

$$C_0 = \frac{X_0}{V_1} \tag{8}$$

At a sufficient time after the intravenous dose, namely $t \simeq \infty$, the total volume of distribution V reaches the steady state, $V = V_1 + (V_2)_{max}$. Then, Equation 7 reduces to:

$$C = \frac{X_0}{V_1 + (V_2)_{max}} e^{-kt} \tag{9}$$

Equation 9 shows that the blood concentration declines monoexponentially after the distribution of the drug into the tissues has reached the steady state (*i.e.* at a sufficient time after the intravenous administration).

Results and Discussion

The preceding considerations indicate that the intravenously administered dose X_0, initial volume of distribution V_1, maximum value of the additional distribution volume $(V_2)_{max}$, distribution constant K_d and elimination rate constant k are interrelated with respect to their effect on the blood concentration *vs.* time profile. First, the effect of distribution constant K_d on the blood disappearance profile of a drug that is eliminated from the blood stream very slowly (the value of the first-order elimination rate constant k is 0.0693 hr^{-1}) was studied and the results are shown in Fig. 1; the other parameter values are given in the legend. When the value of K_d was extremely small, $K_d = 0.001$ hr, the blood level of the drug reached the steady state soon after the administration. However, as the value of K_d increased, the time when the blood concentration *vs.* time profile reaches the steady state was delayed and the blood level *vs.* time curve became less steep at the initial distribution phase.

In addition, a similar simulation study was carried out with a drug that is eliminated rapidly from the blood stream (the value of the first-order elimination rate constant k is 0.693 hr^{-1}) and the same result was obtained.

Next, the effect of the maximum value of V_2 on the blood concentration *vs.* time profile of a drug eliminating very slowly from the blood stream was studied and the simulated result is shown in Fig. 2. From this figure, it is suggested that the initial disappearance rate of the drug from the blood stream became greater as the value of $(V_2)_{max}$ increased. In addition, the steady state blood levels became lower as $(V_2)_{max}$ increased. However, $(V_2)_{max}$ did not affect the terminal slope of the blood concentration *vs.* time profile. Therefore, the elimination

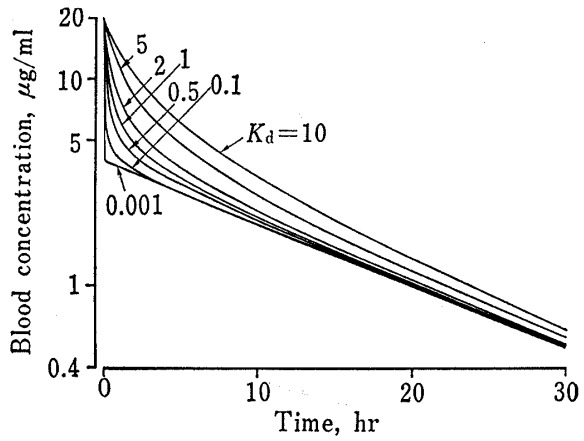

Fig. 1. The Effect of Distribution Constant K_d on the Blood Level *vs.* Time Profile of a Drug eliminating very slowly from the Blood Stream

The parameter values used are:
X_0(dose)=100 mg
V_1=5 *l*
$(V_2)_{max}$=20 *l*
k(elimination rate constant)=0.0693 hr^{-1}

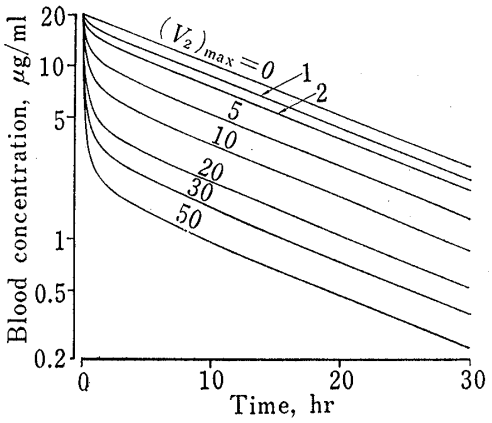

Fig. 2. The Effect of Maximum Value of Additional Distribution Volume $(V_2)_{max}$ on the Blood Level *vs.* Time Profile of a Drug eliminating very slowly from the Blood Stream

The parameter values used are:
X_0=100 mg
V_1=5 *l*
K_d=0.5 hr
k=0.0693 hr^{-1}

rate constant *k* is independent of $(V_2)_{max}$. A similar simulation study was carried out with respect to a drug that is eliminated from the blood steram rapidly, and the same result was obtained.

Estimation of the Parameter Values——When blood concentration *vs.* time data are plotted on a semilogarithmic paper and a concave curve is obtained, the value of V_1 may be obtained from the initial concentration C_0 and the injected dose X_0 by means of the relationship represented by Equation 8. In addition, the sum of V_1 and $(V_2)_{max}$ can be calculated from the intercept by extrapolation of the blood level *vs.* time curve at the steady state to time zero. By subtracting the value of V_1 from this sum, the value of $(V_2)_{max}$ may be obtained.

To determine the value of K_d, Equation 7 should be rearranged. Consequently,

$$K_d = \frac{[(V_1+(V_2)_{max})C - X_0e^{-kt}]t}{X_0e^{-kt} - V_1C} \qquad (10)$$

As the value of first-order elimination rate constant *k* is obtained from the terminal slope of the blood concentration *vs.* time curve, the value of K_d may be calculated by substituting the observed blood concentration *C* at time t during the distribution phase into Equation 10.

As an example, the pharmacokinetic data of warfarin,[3] given in Table 1, were analyzed by this method. The tri-exponential equation giving the best fit to these data was as follows[3]:

$$C = 0.963e^{-4.53t} + 0.485e^{-0.398t} + 0.679e^{-0.0488t} \qquad (11)$$

The values of the two parameters, V_1 and $(V_2)_{max}$, were determined as described above. Namely, V_1=43.1 ml, and $(V_2)_{max}$=91.9 ml. By substituting these values into Equation 10, K_d was calculated from each data point at the distribution phase and the results are also listed in Table I. Therefore, the estimated value of K_d is 1.04 hr. By introducing this value, even though it is a rough estimate, and the other parameter values into Equation 7, the calculated values of plasma warfarin concentration were determined (Table I).

In classical pharmacokinetics,[2] several volumes of distribution such as V_1, V_2, Vd_β, Vd_{area}, Vd_{ss}, and Vd_{extrap} have been used to analyze a blood concentration *vs.* time profile showing two-exponential decay. However, this means that distribution volume of drugs will increase gradually after the administration. Therefore, by making the volume of distribu-

Table I. Time Course of Warfarin Disappearance from Rat Plasma, Estimated Values of K_d and the Calculated Plasma Warfarin Concentrations

Time (hr)	Plasma concentration (μg/ml)	K_d (hr)	Calculated plasma concentration (μg/ml)
0.083	1.81	0.974	1.83
0.25	1.40	0.824	1.48
0.50	1.17	0.883	1.23
0.75	1.01	0.804	1.08
1.0	0.971	0.963	0.990
1.333	0.958	1.30	0.906
1.666	0.880	1.23	0.847
2.0	0.819	1.15	0.802
2.5	0.797	1.42	0.751
3.067	0.725	1.22	0.706
3.5	0.647	0.736	0.678
4.033	0.663	1.23	0.648
5.0	0.591	0.868	0.602
7.0	0.524	0.949	0.529
11.0	0.406	a)	0.421
23.0	0.237	a)	0.228
29.0	0.154	a)	0.169
35.0	0.116	a)	0.125
47.25	0.0651	a)	0.0686

Average $K_d = 1.04 \pm 0.21$ (S.E.).

a) K_d was not calculated because these data belong to the terminal phase.

tion as a function of time, we attempted to represent the blood concentration *vs.* time profile in terms of a model independent method. The results of a simulation study indicate that this new approach is indeed applicable to the pharmacokinetic analysis of drugs.

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[Chem. Pharm. Bull. 29(5)1466—1469(1981)]

The Properties of Water of Crystallization of Sodium Prasterone Sulfate

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(Received September 5, 1980)

The properties of water of crystallization of sodium prasterone sulfate (DHA·SO_3Na·$2H_2O$) were investigated by thermometric measurements. The anhydrous form (DHA·