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Reactions of 2,2-Dimethylaziridine-Type Alkylating Agents in Biological Systems II: Comparative Pharmacokinetics in Dogs

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Abstract □ Pharmacokinetic studies in dogs were performed with a series of three 2,2-dimethylaziridine antineoplastic agents. These alkylating agents were relatively unstable in all aqueous media, with half-lives of 9–15 min in whole canine blood. This finding indicates that the aziridine compounds are capable of undergoing destruction in all biological tissues. A pharmacokinetic analysis of the plasma concentration *versus* time data was performed using a two-compartment open model, which includes drug elimination from both compartments. The three aziridine compounds showed similar pharmacokinetic behavior with respect to their loss from plasma, but they exhibited differences in their *in vivo* release of a potent alkylating intermediate (2,2-dimethylaziridine) and a second product, which was a strong inhibitor of pseudocholinesterase.

Keyphrases □ 2,2-Dimethylaziridines—reactions in biological systems, comparative pharmacokinetics in dogs □ Aziridines—2,2-dimethylaziridine-type alkylating agents, reactions in biological systems, comparative pharmacokinetics in dogs □ Antineoplastic agents—reactions of 2,2-dimethylaziridine-type alkylating agents in biological systems, comparative pharmacokinetics in dogs □ Pharmacokinetics—2,2-dimethylaziridine-type alkylating agents in biological systems, dogs

The 2,2-dimethylaziridine-type alkylating agents ethyl bis(2,2-dimethylaziridinyl)phosphinate (I), bis(2,2 - dimethylaziridinyl)phosphinylurethan (II), and tris(2,2-dimethyl-1-aziridinyl)phosphine oxide (III) have been shown to possess significant antitumor activity in a number of animal systems (1–3) and in humans (4–6). One characteristic chemical property is their unusually rapid hydrolysis with concomitant loss of their alkylating activity (7).

Since the effectiveness of an alkylating agent is probably a function of its reactivity toward various nucleophilic targets and of its local concentration and contact time with these targets, the *in vivo* activity of these particularly unstable alkylating agents is an interesting phenomenon and suggested that a study be made of the pharmacokinetic behavior of these drugs. The plasma concentration–time curves for such agents represent useful data for the scientific application of these drugs to the treatment of neoplastic diseases. Furthermore, a pharmacokinetic study of a series of agents with related structures could provide useful information for the design of new antineoplastic drugs.

This report describes the pharmacokinetic behavior of I–III in the dog following various routes of administration and the identification of 2,2-dimethylaziridine (IV) as part of the metabolic pathway of I and III in the plasma of dogs.

EXPERIMENTAL

Materials—The reagents used in the stability studies, pharmacokinetic studies, and analytical procedures were purchased and/or purified as described previously (7).

Analytical Procedures—The measurement of alkylating activity and the quantitative analysis of samples were performed with 4-(*p*-nitrobenzyl)pyridine, using the previously reported procedures (7).

Stability Studies—The rate of loss of I–III in various buffers, urine, plasma, and whole blood was studied. The methodology used was described previously (7).

Pharmacokinetic Studies—Male mongrel dogs, 20–28 kg, were fasted for approximately 18 hr before drug administration. The animals were lightly anesthetized with pentobarbital sodium, and one cephalic vein was catheterized for the collection of blood. Preliminary studies showed that the 2,2-dimethylaziridine compounds were stable in alkaline solution. Therefore, they were prepared for injection by dissolution in 5 ml of 0.05 *M* Na_2CO_3 (pH adjusted to 11.0) or 10^{-3} *M* KOH.

All three compounds were administered by intravenous injection, while II was also given by intramuscular injection into the gluteal muscle and by injection into the peritoneal cavity. Pretreatment with proadifen hydrochloride[1] [2-(diethylamino)-ethyl-2,2-diphenylvalerate hydrochloride] involved a dose of 20 mg/kg sc 1 hr before the administration of II.

Sample collection (sufficient blood to perform two to five determinations) was accomplished quickly (in about 20 sec) because of the rapid rate of change of plasma drug concentration. The time of sampling was assumed to be the midpoint of this brief collection interval. The catheter was maintained open by filling it with a heparin solution (100 units/ml in 0.9% NaCl). The contents of the catheter were discarded before each sample was drawn. The other mechanical features of sample handling were performed as described previously (7).

RESULTS

Stability Studies—The *in vitro* stability of I–III was measured in various buffers and biological media, using a broader varia-

Table I—Rate of Destruction of Compounds I–III in Various Media

Medium, Temperature, pH	Half-Life, min I	II	III
Heparinized human blood, 36°, 7.36[a]	17	11	7
Heparinized human blood, 25°, 7.36	—	—	39
Heparinized human plasma, 37°, 7.36	—	—	26
Citrated human plasma, 36°, 7.38	32	32	25
Citrated human plasma, 25°, 7.40	—	92	—
Heparinized canine blood, 36°, 7.48[a]	15	12	8
Heparinized canine plasma, 36°, 7.48	48	42	28
Human urine, 37°, 7.80[b]	44	31	22
Sodium phosphate, 0.066 *M*, 36°, 7.50	42	23	—
Sodium bicarbonate, 0.60 *M*, 25°, 7.80[a]	136	103	60
Sodium hydroxide, 0.001 *M*, 25°, 10.9	>153	>170	>60
Potassium hydrogen phthalate, 0.01 *M*, 25°, 5.0[b]	2.0	1.0	<0.5

[a] TLC examination of samples drawn at various times indicated that at least 95% of the alkylating species was parent compound. The trace alkylating substance occasionally observed after incubation had the chromatographic characteristics of 2,2-dimethylaziridine. [b] A few percent of alkylating activity remained after more than 10 half-lives of parent compound. In this case, TLC studies confirmed that 2,2-dimethylaziridine was the residual alkylating species.

tion of experimental conditions than in previous studies (7) to assess factors that might effect their *in vivo* destruction. As shown in Table I, they react rapidly in plasma, urine, and whole blood. The shorter half-time in whole blood as compared to plasma suggests that cells can provide nucleophilic targets for reaction with these agents.

The data from the experiments in buffer solutions show that compounds of this series behave similarly in that they are very unstable in acidic media (pH 5) but do not undergo measurable hydrolysis in 3 hr at pH 10.8. For this reason, these drugs were prepared for injection in alkaline vehicles and the blood samples were alkalinized quickly after collection.

Pharmacokinetic Studies—The time course of plasma concentrations of I following intravenous administration to three dogs is shown in Fig. 1. The results obtained in the individual animals are nearly identical and show rapid, biphasic disposition with a β-phase half-life of about 7.3 min. Compounds II and III behaved in a similar manner (Figs. 2 and 3).

The metabolic inhibitor[1] was also administered to a dog receiving II. As shown in Fig. 2, there was essentially no difference in plasma levels of II in pretreated *versus* unpretreated animals. This suggests that microsomal oxidative enzymes do not play a major role in the biotransformation of II. In fact, the stability data in various biological media (Table I) suggest that these aziridine compounds are eliminated primarily by hydrolysis and chemical reaction with cellular constituents.

One dog was given half of the usual dose of II (Fig. 2). The plasma concentrations of the drug were almost exactly 50% of those observed following the normal dose (12 mg/kg). This suggests that, over the clinically useful range (4–6), the pharmacokinetic behavior of this compound is not dose dependent in the mongrel dog.

Pharmacokinetic Analysis—The plasma concentration, C_p, *versus* time, t, data observed following intravenous administration of the drugs were computer fitted to the biexponential equation:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 1)}$$

by nonlinear least-squares regression analysis (8). The parameters

[1] SK&F 525-A.

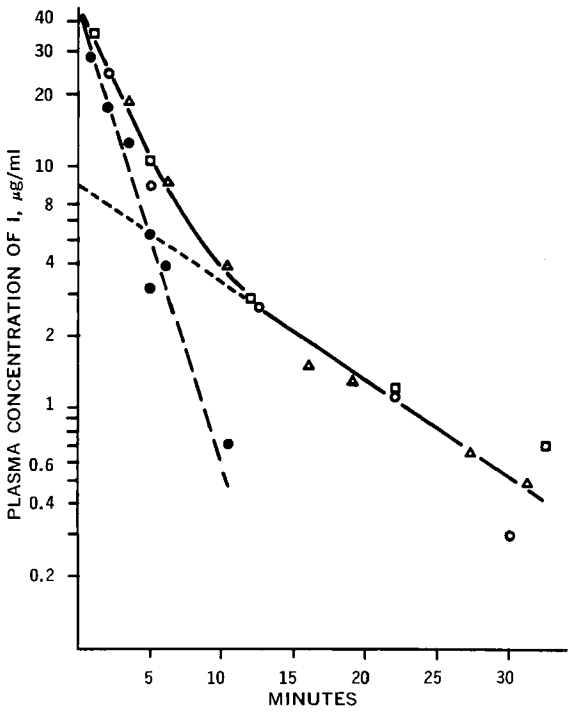


Figure 1—*Time course of I in plasma following rapid intravenous administration of 12 mg/kg to three dogs. Key: □, Dog 1; ○, Dog 2; △, Dog 3; and ●, points obtained by the method of residuals. The curves were obtained by computer least-squares regression of all data simultaneously.*

A and B are zero-time intercepts and α and β are disposition slope constants. The solid lines in Figs. 1–3 show the least-squares computer fit to the experimental data, and the calculated parameters are listed in Table II. The 95% confidence limits for the plasma half-life of I ($t_{1/2\beta}$ = 7.3 min) in the β-phase are 4.2 and 10.4 min.

The pharmacokinetic model chosen for further analysis of these data is depicted in Scheme I. A two-compartment system was selected because the plasma concentration data were biexponential, showing both distribution and elimination phases. Drug loss from both the peripheral (rate constant k_r) and central (rate constant K_{el}) compartments was incorporated into this model because, as

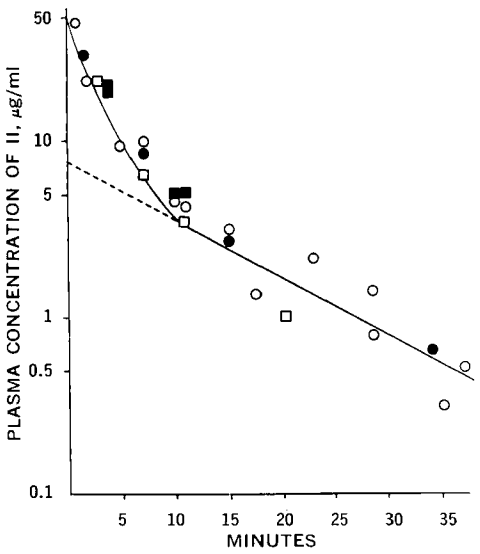


Figure 2—*Time course of II in plasma following intravenous administration of 12 mg/kg (○), 12 mg/kg following pretreatment with proadifen hydrochloride (□), 6 mg/kg normalized to 12 mg/kg (●), and multiple doses of 12 mg/kg (■) treated by the method of superposition.*

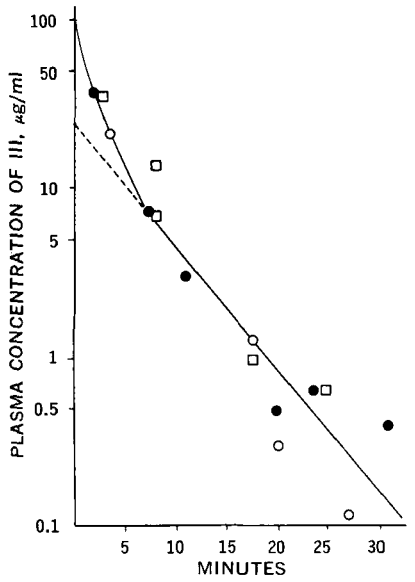


Figure 3—*Time course of plasma levels of III following intravenous administration of 24 mg/kg to three dogs. Key: □, Dog 1; ●, Dog 2; and ○, Dog 3. The curve represents the nonlinear least-squares computer fit of the data.*

shown in Table I, these aziridine compounds are destroyed in all aqueous media under physiological conditions.

A detailed solution to this type of model was evolved recently (9). Therefore, the *Appendix* contains only the equations that provide numerical parameters of interest with regard to the pharmacokinetics of I–III and of other alkylating agents (10–12).

The least-squares regression parameters, as well as some calculated pharmacokinetic constants, for I–III are listed in Table II. From these data, it can be seen that the volume of the central compartment, V_c, for all of these agents exceeds the plasma volume of the dog (about 0.05 liter/kg). This indicates that these compounds distribute rapidly out of the plasma into tissues. The total body clearance, Cl_B, of these agents is extremely rapid; for example, Compound III is cleared at a rate of about 2200 ml/min.

Further direct pharmacokinetic analysis of the data is not realistic, because it is not possible to quantitate the rates of loss of these compounds in the plasma and tissue compartments of the model separately. However, since a nonspecific mechanism of elimination appears to account for loss of I–III from the body, some relationship probably exists between k_r and K_{el}. The relative $t_{1/2}$ values for I–III in the media listed in Table I provide some indication of the rate of reaction of this agent with cellular components, but the *in vivo* situation is complicated by the possibility that hepatic and renal mechanisms are also involved in the removal of these drugs. Therefore, various k_r–K_{el} ratios were assumed in seeking an array of possible analytical solutions to the two-compartment model and to demonstrate the magnitude of error in such estimations.

The calculated transfer (k_{12} and k_{21}) and elimination (K_{el} and k_r) rate constants for I are listed in Table III. When k_r is allowed

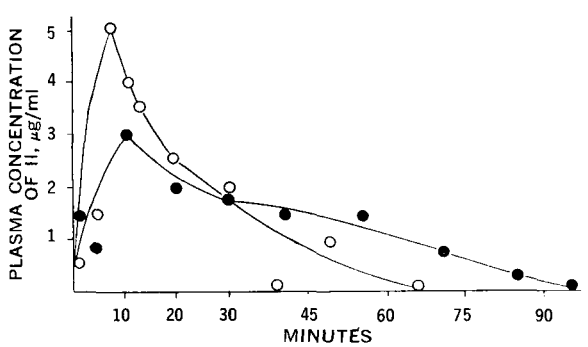
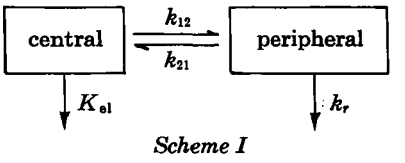


Figure 4—*Plasma concentrations of II at various times following intraperitoneal injection of 12 mg/kg to two dogs.*



Scheme I

to vary in the range $0.25\ K_{el} \leq k_r \leq 2K_{el}$ (a realistic range), the transfer rate constants only change by a factor of about two. However, it is not possible to obtain unique numerical values for these parameters for any of the agents because of the uncertainty of the rate of loss of compound in extravascular fluids.

Effect of Administration Route—The intramuscular injection of II has been used clinically (4), and the intraperitoneal route has been used during animal experimentation (3). Therefore, blood level data following administration of II by these routes were obtained and are shown in Figs. 4 and 5.

The area under the plasma concentration–time curves *(AUC)* following intraperitoneal or intramuscular administration was substantially less than that observed following intravenous dosing. This incomplete drug availability is probably caused by destruction of the drug at the site of injection. The AUC_{ip}'s for two dogs were 58 and 62% of the AUC_{iv}, while the intramuscular route yielded 58 and 69% in two other dogs. The limited availability of this compound *via* the intramuscular route suggests that the dose of drug should probably be increased when this route is used clinically.

Decomposition Studies—Thin-layer chromatograms of plasma extracts from dogs treated intravenously with I–III were examined for the parent compound and other 4-(*p*-nitrobenzyl)pyridine-reactive substances, using a previously described spray reagent (7). 2,2-Dimethylaziridine (IV), a minor hydrolysis product of I–III (see Table I and Ref. 7), was observed in extracts from dogs treated with I and III, but it could not be identified unequivocally in the extracts from dogs treated with II.

The quantity of IV found after injection of III was more than could be expected on the basis of simple hydrolysis (estimated from the size and intensity of the spots on at least three TLC plates from each dog and compared with cochromatographed standards). Therefore, it is suspected that some III is metabolized to IV. The quantity of IV generated from I probably could have arisen *via* simple hydrolysis.

The extraction step of the TLC procedure for the identification of alkylating compounds recovers about 40% of IV added to a sample of canine whole blood and about 80% of I–III. The procedure for the quantitative estimation of alkylating agents in whole blood recovers about 95% of I and III and about 60% of II. However, only about 10% of IV is recovered by this method. Therefore, the curves presented in Figs. 1–3 may be viewed as representing plasma concentration of the parent compound with little or no interference from IV.

Since an appreciable amount of IV is formed from III and some is formed from I, it seemed worthwhile to obtain plasma concentration–time data for IV. The only animals that received IV had received an intravenous dose of another alkylating agent prior to the experiment. A dose of 16 mg/kg was used. At least 100 min was allowed to elapse between the doses of the two drugs. Blood sampling was confined to about the same postinjection period when the 2,2-dimethylaziridine phosphoramides were studied.

Because the extraction procedure recovers so little compound

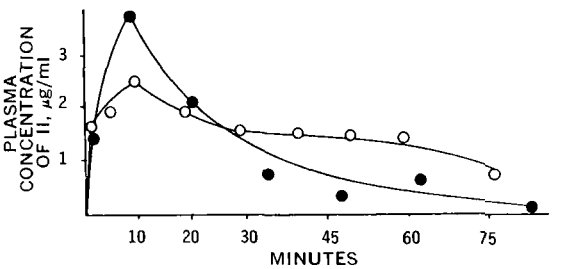


Figure 5—*Plasma concentrations of II at various times following intramuscular injection of a dose of 12 mg/kg to two dogs.*

Table II—Least-Squares Regression Parameters and Pharmacokinetic Constants for Compounds I–III

Parameter[a], units	I	II	III
A, $\mu g\ ml^{-1}$	42	46	41
α, min^{-1}	0.409	0.413	0.68
B, $\mu g\ ml^{-1}$	8.5	7.6	12
β, min^{-1}	0.095	0.074	0.14
V_c, liters kg^{-1}	0.24	0.225	0.25
Area, min $\mu g\ ml^{-1}$	191	214	142
C_p^0, $\mu g\ ml^{-1}$	50.5	53.6	53
Cl_B, ml min^{-1}	1530	1375	2200
$t_{1/2}\beta$, min	7.3	9.7	5.0

[a] Data normalized to a dose of 12 mg/kg.

(10%), it is necessary to interpret the results of these experiments conservatively. However, these experiments seemed worthwhile because they might help to show that IV does not cause interference in the blood level measurements following the administration of III. Comparison of Figs. 6 and 3 supports the view that the log-linear phase observed following the administration of III represents the plasma concentrations of III with little interference from IV, because the rates of decline of plasma alkylating activity between 15 and 40 min are very different following administration of III and IV.

Furthermore, IV appears to possess pharmacokinetic characteristics that are quite different from those of I–III. This may have some importance in the future design of 2,2-dimethylaziridines. That is, given the bis(2,2-dimethylaziridinyl)phosphine oxide structure, the nature of the group attached to the R position seems to have very little influence upon the pharmacokinetic characteristics of the compound. Since dimethylaziridine seems to act quite differently, it might be worthwhile to examine the effect of linking 2,2-dimethylaziridine to groups other than the phosphoryloxy moiety.

Preliminary data concerning the generation of a potent cholinesterase inhibitory intermediate from II were reported (13), and a comparative study of I–III was performed previously (14). These data show that the hydrolysis products of I cause substantially more cholinesterase inhibition (measured as inhibition of the procaine esterase activity of horse serum cholinesterase) than do the hydrolysis products of II and that III produces very little inhibitory intermediate(s). Since some clinical toxicity caused by II (5, 15) has been attributed to cholinesterase inhibition, and since it has been suggested that this intermediate may be pharmacologically important, it seems essential to consider the effects of the parent compound, IV, and the cholinesterase inhibitor intermediate(s) in the pharmacology and toxicology of I–III.

DISCUSSION

Chemotherapeutic Implications—A tumor could behave as a portion of the highly perfused central compartment (possible examples are renal and bronchogenic carcinomas) or as a portion of the lesser perfused peripheral compartment (perhaps carcinoma of the breast), or it might not be visualized at all by compartment modeling (possibly tumors of the central nervous system). Thus, it is of interest to examine the time course of drug levels outside of the central compartment. Because the three agents studied have similar pharmacokinetic properties, only the data for I (Fig. 1) will be analyzed.

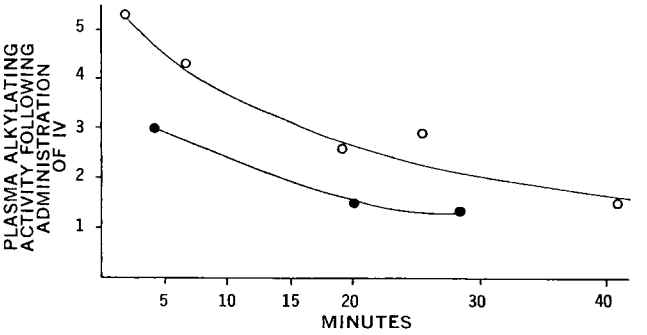


Figure 6—*Plasma levels of IV at various times following intravenous administration of a dose of 16 mg/kg to two dogs. The plasma concentrations of dimethylaziridine have been converted to alkylating activity corresponding to micrograms of III per milliliter.*

By using Eqs. A9 and A10 *(Appendix)*, the time course of drug in the central and peripheral compartments was calculated, using the various possible pharmacokinetic rate constants in Table III. As shown in Fig. 7, the quantity of drug in the peripheral compartment reaches a maximum (X_t^{max}) in about 5 min. The time required to reach this maximum is independent of k_r. However, an interesting phenomenon occurs with respect to the total amount of drug passing through the peripheral compartment. As the value of k_r increases, the value of X_t increases while the amount–time profile of drug in the central compartment remains constant.

Thus, as the ability for destruction of the drug increases in the peripheral compartment, it appears that more drug is shunted into this site in order for an identical plasma drug concentration curve to be seen. This is evidenced by the concomitant increase in k_{12}, and the decrease in k_{21}, and also by the increase in V_d^{ss} and tissue clearance, Cl_T, as k_r increases (Table III). In view of the unusually rapid hydrolysis of I–III at pH below 7.5 (Table I), it seems reasonable to assume that the magnitude of k_r largely reflects the rate of hydrolysis of the drug. This should be particularly the case if tumor tissues, which are known to be relatively acidic (pH ~6), form part of the peripheral compartment.

These pharmacokinetic considerations (Table III and Fig. 7) suggest that, given two drugs producing identical plasma concentrations at equal doses or infusion rates, the agent that hydrolyzes more rapidly may be more effective against tumors of the peripheral compartment because more of the drug will be located in the tissues at a given time. Although only limited data are available (1, 2), III does seem to be more effective than I in this type of tumor.

There is another interesting aspect of this interpretation. It was reported previously from this laboratory (16) that the ED_{90} values for a large series of alkylating agents correlate well with the rates of hydrolysis of these agents when the tumor studied was probably acting as a portion of the peripheral compartment (Walker carcinoma 256, transplanted subcutaneously in the flank region of male rats). Thus, the rate of hydrolysis of these compounds may influence both their chemotherapeutic activity and their pharmacokinetic behavior and could be a major correlative parameter in structure–activity studies.

Table III—Effect of Extravascular Elimination on the Calculated Pharmacokinetic Constants for Compound I

z, Ratio of $k_r:K_{el}$	k_{12}, min^{-1}	k_{21}, min^{-1}	K_{el}, min^{-1}	k_r, min^{-1}	V_d^{ss}, liters[a]	X_t^{max}, mg[a]	C_t^{max}, mg/liter[b]	Cl_p, ml/min	Cl_T, ml/min
0	0.093	0.148	0.263	0	9.52	44	11.9	1531	0
0.1	0.108	0.123	0.249	0.0249	10.05	52	11.9	1449	82
0.2	0.133	0.103	0.223	0.0446	11.05	63	11.9	1298	233
0.25	0.145	0.095	0.211	0.0527	11.52	68	11.9	1228	303
0.5	0.199	0.069	0.157	0.0786	13.63	93	11.9	913	618
1.0	0.261	0.053	0.0951	0.0951	16.08	122	11.9	553	978
2.0	0.304	0.045	0.0515	0.103	17.75	142	11.9	300	1231

[a] Calculated using Eq. A10. [b] Calculated from: $C_t^{max} = X_t^{max}/(V_d^{ss} - V_c)$.

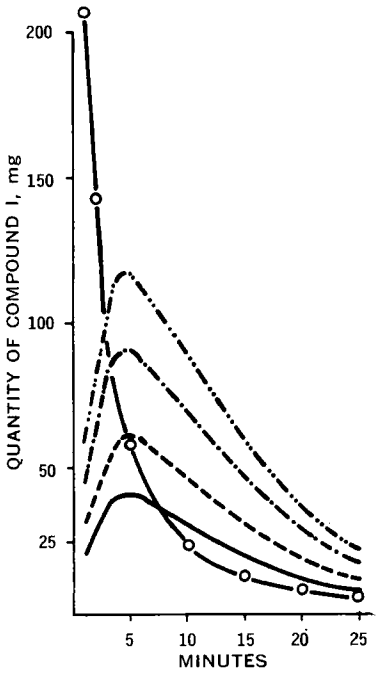


Figure 7—*Amount of I in the central compartment* (O——O) *and in the peripheral compartment as a function of time assuming* $k_r = 0$ (——), $k_r = 0.2\ K_{el}$ (– – – –), $k_r = 0.5\ K_{el}$ (–·–·–·), *and* $k_r = K_{el}$ (–· · –· · –).

APPENDIX

The differential equations that describe the two-compartment model shown in Scheme I are:

$$\frac{dX_c}{dt} = k_{21}X_t - k_{12}X_c - K_{el}X_c \qquad \text{(Eq. A1)}$$

and:

$$\frac{dX_t}{dt} = k_{12}X_c - k_{21}X_t - k_rX_t \qquad \text{(Eq. A2)}$$

where the initial conditions are X_c^0 = dose and $X_t^0 = 0$.

These equations can be solved using LaPlace transforms and matrix algebra. Equations for the various rate constants were provided by Suzuki and Saitoh (9) as follows:

$$k_{21} = \frac{A(\beta - \alpha)}{A + B} + \alpha - k_r \qquad \text{(Eq. A3)}$$

$$K_{el} = \frac{\alpha\beta - k_{12}k_r}{k_{21} + k_r} \qquad \text{(Eq. A4)}$$

$$k_{12} = \alpha + \beta - k_{21} - k_r - K_{el} \qquad \text{(Eq. A5)}$$

$$k_r = \frac{B(\alpha - \beta)}{(A + B)} + \beta - k_{21} \qquad \text{(Eq. A6)}$$

Values for the biexponential parameters A, α, B, and β are obtained by fitting the experimental data to Eq. 1. In addition, the volume of the central compartment, V_c, and the steady-state volume of distribution can be calculated as follows:

$$V_c = \text{dose}/C_p^0 = \text{dose}/(A + B) \qquad \text{(Eq. A7)}$$

$$V_d^{ss} = V_c\left(1 + \frac{k_{12}}{k_{12} + k_r}\right) \qquad \text{(Eq. A8)}$$

The amounts of drug in the central, X_c, and peripheral, X_t, compartments as a function of time are:

$$X_c = \frac{X_c^0(k_{21} + k_r - \alpha)}{\beta - \alpha}e^{-\alpha t} + \frac{X_c^0(k_{21} + k_r - \beta)}{\alpha - \beta}e^{-\beta t} \qquad \text{(Eq. A9)}$$

$$X_t = \frac{-k_{12}X_c^0}{\alpha - \beta}e^{-\alpha t} + \frac{k_{12}X_c^0}{\alpha - \beta}e^{-\beta t} \qquad \text{(Eq. A10)}$$

Numerical Evaluation of Rate Constants—Equations A3–A6 allow the calculation of k_{12}, k_{21}, and K_{el} when given known values of k_r. This is not feasible in the present study; thus the values of the various rate constants must be approximated by relating the value of k_r to that of K_{el}. In the present situation, it is assumed that k_r is proportional to K_{el} by the relationship:

$$k_r = zK_{el} \qquad \text{(Eq. A11)}$$

where z is a nonnegative real number. Solution of Eq. A4 by substituting Eqs. A11, A3, and A5 yields:

$$K_{el} = \frac{\alpha\beta - (zK_{el})\left[\alpha + \beta - K_{el} - \dfrac{A\beta + B\alpha}{A + B}\right]}{\dfrac{A\beta + B\alpha}{A + B}} \qquad \text{(Eq. A12)}$$

Crossmultiplying and equating the entire expression to zero yield:

$$0 = \alpha\beta + \left[-z\alpha - z\beta + (z - 1)\frac{A\beta + B\alpha}{A + B}K_{el} + zK_{el}^2\right] \qquad \text{(Eq. A13)}$$

Solution of this quadratic equation for any z yields one positive root for K_{el}. The values of k_r, k_{21}, and k_{12} can then be obtained from Eqs. A11, A3, and A5, respectively.

Clearance Parameters—The body clearance, Cl_B, is a model-independent parameter which can be calculated from:

$$Cl_B = \text{dose/area} \qquad \text{(Eq. A14)}$$

where area is the total area under the plasma concentration *versus* time curve obtained either by trapezoidal approximation or by integration of Eq. 1. The plasma clearance, Cl_p, is usually considered to reflect the sum of processes such as renal and hepatic clearances of drug from the plasma or central compartment and is obtained from:

$$Cl_p = (K_{el})(V_c) \qquad \text{(Eq. A15)}$$

The body and plasma clearance terms are often used interchangeably, but they are actually identical only when elimination does not occur outside of the central compartment. When elimination takes place in the peripheral compartment, the body clearance should obviously exceed the plasma clearance. It is, therefore, useful and appropriate to define the difference between the two values as the tissue clearance. The calculation involves:

$$Cl_T = Cl_B - Cl_p \qquad \text{(Eq. A16)}$$

Comparison of such clearance values provides an indication of the relative contribution of central and peripheral compartment clearance mechanisms to overall elimination of the drug from the body.

REFERENCES

(1) A. Munson, H. L. Babbitt, Z. F. Chmielewicz, T. J. Bardos, and J. L. Ambrus, *J. Surg. Oncol.*, **1**, 167(1969).

(2) A. Munson, H. L. Babbitt, A. Segaloff, Z. F. Chmielewicz, T. J. Bardos, and J. L. Ambrus, *Cancer Chemother. Rep.*, **51**, 253(1967).

(3) W. Regelson and O. Pierucci, *Radiat. Res.*, **22**, 368(1964).

(4) L. Stutzman, D. V. Razis, J. E. Sokal, J. L. Ambrus, and C. A. Ross, *Cancer Chemother. Rep.*, **18**, 31(1962).

(5) H. A. Velasco, C. A. Ross, J. H. Webster, J. E. Sokal, L. Stutzman, and J. L. Ambrus, *Cancer*, **17**, 841(1964).

(6) J. H. Webster, J. Sokal, C. A. Ross, H. A. Velasco, J. L. Ambrus, and L. Stutzman, *Cancer Bull.*, **22**, 59(1970).

(7) D. Lalka and T. J. Bardos, *J. Pharm. Sci.*, **62**, 1294(1973).

(8) D. W. Marquardt, NLIN 2: Share Program No. SDA-3094-01, 1966.

(9) T. Suzuki and Y. Saitoh, *Chem. Pharm. Bull.*, **21**, 1458(1973).
(10) L. B. Mellett and L. A. Woods, *Cancer Res.*, **20**, 518(1960).
(11) *Ibid.*, **20**, 524(1960).
(12) N. V. Nadkarni, E. G. Trams, and P. K. Smith, *Cancer Res.*, **19**, 713(1959).
(13) T. J. Bardos and J. L. Ambrus, *Proc. Int. Cong. Chemother., 3rd, 1964*, 1036.
(14) D. Lalka, Ph.D. dissertation, State University of New York at Buffalo, Buffalo, N.Y., 1973.
(15) C. A. Ross, J. L. Ambrus, J. E. Sokal, H. A. Velasco, L. Stutzman, and D. V. Razis, *Cancer Chemother. Rep.*, **18**, 27(1962).
(16) T. J. Bardos, N. Datta-Gupta, P. Hebborn, and D. J. Triggle, *J. Med. Chem.*, **8**, 167(1965).

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Cycloalkanones V: Synthesis, Distribution, and Effects on Triglyceride Metabolism

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Abstract □ The ^{14}C-labeled 2,8-dibenzylcyclooctanone was synthesized to study its absorption, distribution, and excretion in rats. Maximum drug absorption from the GI tract occurred between 12 and 14 hr after administration. The major organs possessed maximum amounts of the drug in 1 hr, with the liver concentrating the most with 6.56% ^{14}C and the muscle mass reaching a maximum of 41% ^{14}C after 14 hr. The drug remained in the GI tract over the first 6 hr and was associated with the lipid and glycogen fractions. Eighty-seven percent was eliminated in the feces after 72 hr. 2,8-Dibenzylcyclooctanone caused a significant reduction *in vitro* of dihydroxyacetone phosphatase acyltransferase and *sn*-glycerol-3-phosphate acyltransferase, which is the proposed mechanism for the observed *in vivo* reduction of hepatic, intestinal, and serum triglycerides and total glycerolipids. *In vivo* administration of the drug resulted in a depression of liver acid phosphatidyl phosphatase, acid phosphatase and lipase, and adipose lipase. The drug increased the rates of excretion of exogenous cholesterol, palmitic acid, and progesterone.

Keyphrases □ Cycloalkanones—synthesis of radiolabeled 2,8-dibenzylcyclooctanone, tissue distribution, effects on triglyceride metabolism, rats □ 2,8-Dibenzylcyclooctanone, radiolabeled—synthesis, distribution to tissues, effects on triglyceride metabolism, rats □ Triglyceride metabolism—effects of 2,8-dibenzylcyclooctanone □ Hypolipidemic activity—2,8-dibenzylcyclooctanone

Previously, the synthesis and hypolipidemic activity of cyclooctanone derivatives were reported (1). In these studies, 2,8-dibenzylcyclooctanone (I) lowered serum cholesterol by 50%, triglyceride by 42%, and glycerol by 31% in Sprague–Dawley rats. The structure–activity relationship of some derivatives was reported (2), delineating the minimum requirement for hypocholesterolemic activity in this series of compounds.

In a continuing effort to study the mechanism of action, I was labeled with ^{14}C and studies were conducted on absorption, distribution, and excretion. Studies also were performed on the effects of I on triglyceride metabolism.

EXPERIMENTAL

Organic Synthesis—The chemical method used for isotopic labeling of I was derived from that of Piantadosi *et al.* (1). Sodium (0.5 g) was dissolved in 12.5 ml of absolute ethanol, and the solution was cooled to room temperature. Four milliliters of this solution was added to a mixture of benzaldehyde (650 mg, 6.13 mmoles) and cyclooctanone (386.8 mg, 3.065 mmoles) in a 10-ml round-bottom flask equipped with a condenser protected by a drying tube. The mixture was stirred at room temperature for 4 hr. At this time, 5 ml of water was added and the mixture was extracted three times with 10 ml of benzene.

The combined extracts were evaporated to yield a viscous residue, and the residue was purified by silica gel column chromatography (35 g, 2.4 × 30 cm). Benzene was used as an eluent, and the first 130 ml of eluent contained no product. The desired 2,8-dibenzylidenecyclooctanone was obtained in the next 60 ml, and this eluate was evaporated to yield 320 mg (35%) of pure 2,8-dibenzylidenecyclooctanone as a pale-yellow solid, mp 108–110° [lit. mp 108–110° (1) and mp 111° (3)].

Ten percent palladium-on-carbon (50 mg) and sodium methylate (30 mg) were added to a solution of 2,8-dibenzylidenecyclooctanone (320 mg, 1.06 mmoles) in 25 ml of ethyl acetate. The mixture was hydrogenated until exactly 2.12 mmoles of hydrogen was absorbed. The catalyst was filtered and washed with 5 ml of hot ethyl acetate. The filtrate was evaporated to yield a viscous residue, which was purified by silica gel column chromatography (12 g, 1.2 × 40 cm), using benzene as the elution solvent. Fractions of 2 ml were collected. After a forerun of 56 ml, 32 ml was collected and evaporated to dryness. All fractions were shown to contain only I by TLC [R_f 0.72 in benzene–chloroform (95:5)]. The yield was 190 mg (62%), mp 82–83° [lit. (1) mp 82–83°].

^{14}C-2,8-Dibenzylidenecyclooctanone and ^{14}C-2,8-Dibenzylcyclooctanone—The identical procedure as described for the preparation of unlabeled I was utilized. For the first run, 581 mg of benzaldehyde and 69.15 mg (1.5 mCi) of benzaldehyde-(carbonyl-^{14}C)[1] (2.25 mCi/mmole) were used. The labeled compound obtained was identical to the unlabeled compound in R_f value. The ^{14}C-labeled I (192 mg) (22% yield based on the starting material) was obtained with a specific activity of 460 μCi/mmole (1.5 μCi/mg).

For the second run, 31.3 mg (2 mCi) of benzaldehyde-(carbonyl-

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