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The Pharmacokinetics of *N*-[^{14}C-Formyl]-Leurosine in Humans

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Abstract—*N-Formyl-leurosine labeled with ^{14}C in the formyl group was administered to six patients with malignant disease, and the pharmacokinetic behavior of the drug was determined. Chromatographic studies on plasma showed the presence of unchanged drug and 7 metabolites. No metabolites were found in the urine. The plasma decay curve of the unchanged drug was biphasic with half-life values of* $t_{1/2\alpha}$ = 18.6 min *and* $t_{1/2\beta}$ = 4.28 hr. *Within* 72 hr *only* 30–40% *of the radioactivity could be recovered in urine and feces in* 5 *patients. One patient consumed laxatives during the treatment. In this case* 95% *of the ^{14}C-dose was recovered. The contribution made by fecal elimination was* 80%.

INTRODUCTION

THE NATURAL alkaloids, vincristine (VCR) and vinblastine (VLB), or periwinkle *Catharantus roseus* G. Don (*Vinca rosea* L.) have gained much clinical popularity in the treatment of various tumors. Due to the absence of hematotoxicity VCR has been incorporated into a large number of combination protocols. Unfortunately the high neurotoxicity has severely limited the realization of its therapeutic potency. In an effort to develop a clinically useful but less neurotoxic derivative, *N*-formyl-leurosine (FLEU) was synthetized by Jovanovics and Szász [1] from the Chemical Works of Gedeon Richter Ltd., Hungary. The drug was chanelled into phase I clinical trials due to the absence of neurotoxicity and its broad antitumor spectrum in animal studies [2]. Dose escalation was stopped at 0.5 mg/kg FLEU in single dose regimes due to circulatory side effects similar to those observed with the naturally occurring Leurosine. The prevention of these manifestations is presently under investigation. In initial human studies the drug has proved to be effective against various forms of leukemia and lymphoma [3, 4].

The present study was undertaken to give insight into the pharmacokinetic properties of the drug in humans, to support the planning of effective treatment schedules and to try to correlate cardiorespiratory effects with plasma levels.

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MATERIALS AND METHODS

The sulphate salt of ^{14}C-FLEU used in our studies was prepared by G. Zólyomi *et al.* of the Research Institute for Pharmaceutical Chemistry, Budapest [5] (Fig. 1). The specific activity was 1.322 GBq/mM (35.73 mCi/mM) and the radiochemical purity was found to exceed 93% as determined by t.l.c. The ^{14}C-FLEU was sterilized by dissolving 11.1 MBq (300 mCi) portions in 10 ml of saline and passing it through a Millex® filter (Millipore Corp., Bedford). The labelled drug was then mixed aseptically with 200 ml saline containing unlabelled formyl-leurosine sulphate (Chemical Works of Gedeon Richter Ltd.) to achieve the desired dose. This solution was infused for 20–30 min.

Clinical conditions

Altogether six male and female patients with disseminated cancer and normal or close to normal liver and kidney function were selected for investigation (Table 1).

During the study no other antineoplastic treatment was given. Drugs for supportive therapy were administered according to the needs of the patients. Patient N.L. regularly

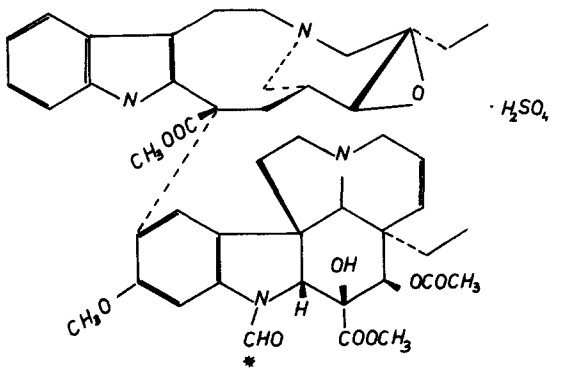


Fig. 1. Structure of N-(^{14}C-*formyl*)-*leurosine.*

homogenized in 1 litre of water. The samples were solubilized in Protosol® (New England Nuclear) and decolorized with H_2O_2 prior to mixing with Aquasol.

Radioactivity of tissue specimens was measured by a combustion system [7]. Radioactive compounds from plasma and urine were isolated by Amberlite XAD-2 resin (Serva). After removing residual water the drugs absorbed onto the resin were eluted with the following solvent systems: (1) chloroform : 2-propanol = 4 : 1; (2) chloroform : 2-propanol = 9 : 1; (3) 2 drops HCl in 100 ml

Table 1. Patients treated with ^{14}C-FLEU

Name	Sex	Age (yr)	Weight (kg)	Dose (mg/kg)	Diagnosis	Previous treatment
M.I.	M	42	76.5	0.49	Chronic. myeloid leukemia	Dibromomannitol
B.D.	M	63	55	0.48	Osteoclastoma mal.	Metothrexate
H.I.	M	24	53	0.48	Testicular tumor	Yes. Drug unknown
S.I.	F	70	45	0.51	Ovarian cancer	VP-16213 Metothrexate Cyclophosphamide
N.L.	F	56	68	0.176	Leiomyosarcoma	Vincristine Cyclophosphamide Metothrexate Actinomycin D Adriamycin
P.K.	F	20	41	0.288	Osteosarcoma	—

consumed senna tea and saline cathartics during the observation period. Patient M.I. died 2 weeks after FLEU treatment due to the progression of disease. Autopsy specimens of muscle, liver, brain, gut and lymph nodes were obtained.

Samples of blood were taken from the arm opposite to the injection site through an indwelling heparin lock into heparinized tubes at 0, 15, 30, 45, 60, 90 min and 2, 3, 4, 5, 6, 7, 8, 24, 48 and 72 hr following the termination of the infusion. Blood samples were centrifuged within 3 hr, and the plasma was kept at −20°C until further processing. Expired CO_2 was collected in hyamine at different time intervals [6]. Urine and feces were collected for at least 72 hr.

Analytical methods

Total radioactivity in samples of plasma, urine, feces and expired CO_2 was counted with a liquid scintillation spectrometer (LKB Wallac 8100) using Aquasol® universal cocktail (New England Nuclear). Quench correction was performed by external standardization. The total volume of feces collected was

MeOH. The eluents were evaporated to dryness under an N_2 stream and then redissolved in methanol. The samples were applied to precoated Kieselgel 60 plates (Merck). The solvent systems used were (1) benzene : chloroform : diethylamine = 40 : 20 : 3 (2) ethyl-acetate : ethanol = 3 : 1. The radioactive spots were located by radioscannin (Dünnschichtscanner II, Berthold). The urin samples were also tested after incubation wit β-glucuronidase arylsulphatase (Serva) fo 24 hr at 37°C. Further processing was carrie out as described above.

Calculation

A non-linear least-squares regression program (BMD × 85) was performed on computer K-20.

RESULTS

The plasma levels of total radioactivity an unchanged drug for a typical case are show in Fig. 2. Total radioactivity could be measured reliably in the plasma for up to 48 h The plasma decay curve of total radioactivit

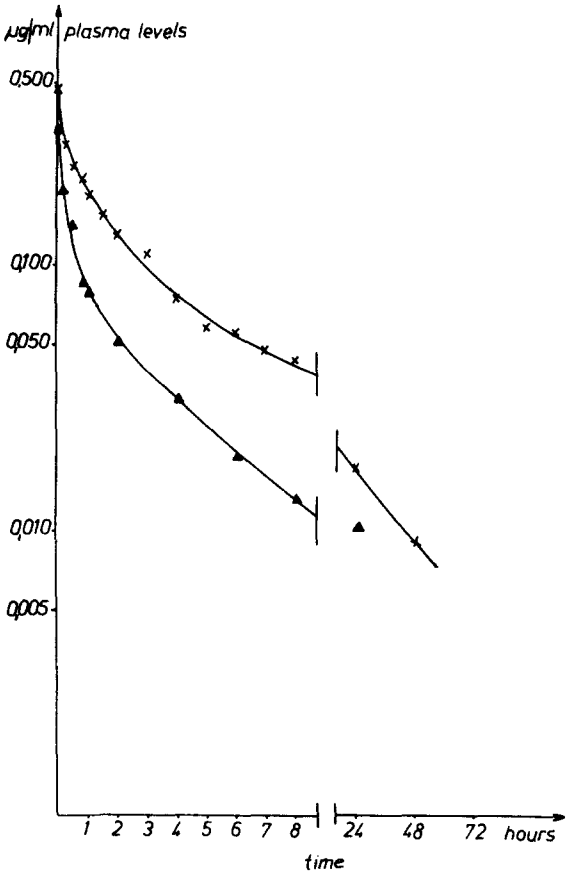


Fig. 2. Plasma decay curve of total radioactivity and unchanged N-(^{14}C-*formyl*)-*leurosine in patient B.D. receiving a single* 0.50 mg/kg *intravenous infusion.* +—+, *total radioactivity;* ▲—▲, *unchanged drug.*

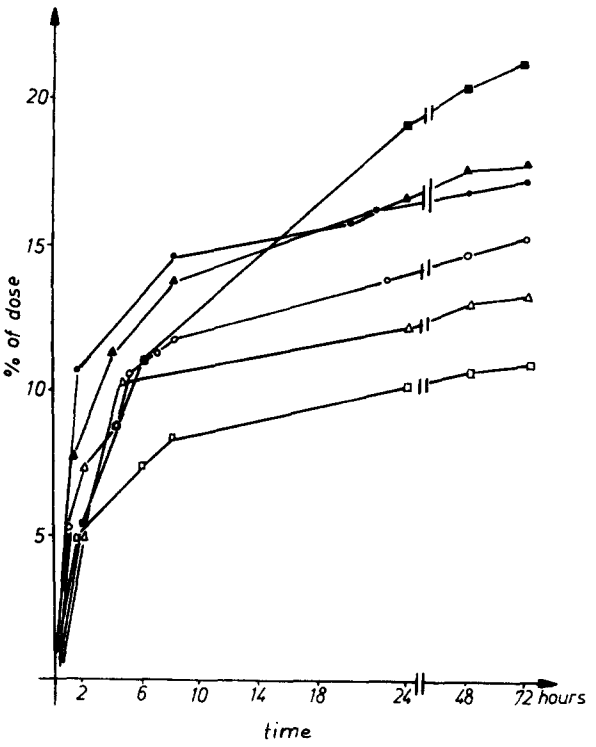


Fig. 3. Cumulative urinary excretion of radioactivity after i.v. infusion of ^{14}C-*FLEU in patients.* △—△, *M.I.;* ▲—▲, *B.D.;* □—□, *H.I.;* ■—■, *S.I.;* ○—○, *N.L.;* ●—●, *P.K.*

was multiphasic; at least 3 or 4 phases could be distinguished. Besides FLEU 7 radioactive metabolites could be measured. Their respective R_f values in the first solvent system were the following: 0.108, 0.183, 0.340, 0.420, 0.660, 0.792 ($R_{f\text{FLEU}} = 0.541$). The identification of these products is under investigation.

Approximately 11–21% of the dose was eliminated by the urine within 72 hr (Fig. 3). The urine contained primarily unchanged drug. There was no difference between the chromatograms of native and glucuronidase-treated urine samples. Therefore no conjugated metabolites were present. In the feces 5–20% of the dose was found in 5 patients within 72 hr. In 3 cases feces were collected up to 7 days, but no significant additional amounts of radioactivity could be found. The patient N.L. who took saline cathartics during the period of examination eliminated 83% of the dose in feces within 3 and 7 days, respectively. (Fig. 4.) The total recovery was 95% in her case. No activity was found in the expired CO_2.

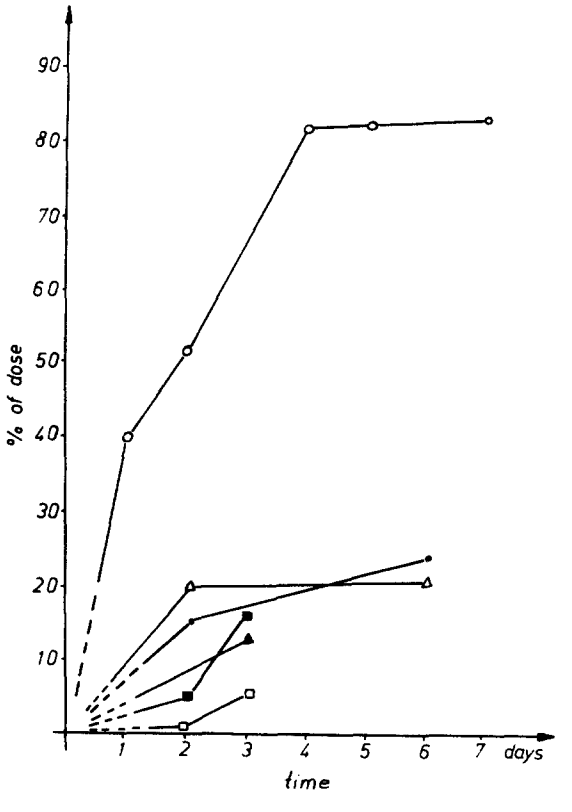
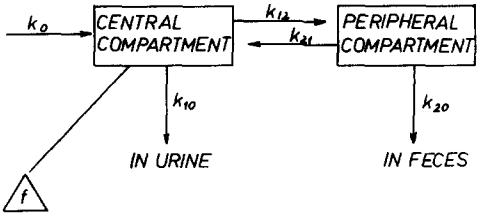


Fig. 4. Cumulative excretion of radioactivity in feces after i.v. infusion of ^{14}C-*FLEU in patients.*

In patient M.I. who died 2 weeks after treatment total radioactivity corresponding to 0.04 and 0.15 μg FLEU per wet weight was found in autopsy samples of brain and liver, respectively. In other tissues no appreciable activity could be detected.

The plasma level of the parent compound could be reliably measured only up to 8 hr. A two exponential plasma level curve was observed in 4 patients as exemplified for patient B.D. (Fig. 2). In two cases the curves contained 3 terms, but the error of the estimated rate constants was greater than 50%. The data suggest that the pharmacokinetic evaluation of unchanged drug would require min-



Scheme 1. *Two compartmental open-model pharmacokinetic system for* ^{14}C-*FLEU.*

imally a two compartment open-model system (Scheme 1). The equation derived for the curve is:

$$y_1 = Ae^{-\alpha t} + Be^{-\beta t},$$

where $y_1(t)$ is the concentration of unchanged drug in the central compartment at time t, A and B are the ordinate axis intercepts, and α and β are the first order rate constants. These values are summarized in Table 2.

The values of the parameters A, B, α and β and the amount of the drug excreted by the kidneys were used to calculate the individual rate constants of the model: k_{12}, k_{21} and k_{10}. These rate constants do not necessarily apply to any anatomically defined tissue compartments but are meaningful in defining the disposition of drug from the central compartment. The pharmacokinetic parameters of ^{14}C-FLEU obtained for each patient are summarized in Table 3.

DISCUSSION

The rate constants associated with rapidly equilibrating sites in the peripheral compartment ranged from 1.01 to 4.69 hr^{-1} for α. These values correspond to half life ranges of 8.82–41.16 min. The overall elimination rate constant β falls in a range of 0.077–0.282 hr^{-1}, corresponding to an apparent half-life range of 2.46–9.00 hr. The magnitude of the two hybrid rate constants α and β reflects fairly rapid initial distribution and elimination of the unchanged drug.

The individual rate constants k_{12} and k_{21} reflect the rate of transfer into and out of the peripheral compartment, respectively. The mean ratio of k_{21}/k_{12} of 0.194 suggests drug binding to tissue sites in this compartment.

By using the rate constants obtained following i.v. infusion (Tables 2 and 3), the drug levels in the central and peripheral compartments were simulated for each patient (Fig. 5). These simulations clearly show that the peripheral compartment contains more unchanged drug than the central compartment. The mean ratio of AUC_2/AUC_1 is 3.27. The pharmacokinetic profile indicated that the k_{21}, i.e., the rate of return of the drug from peripheral compartment, is the rate controlling factor in the elimination of FLEU from the body.

Sugar *et al.* [8] demonstrated a significant accumulation of drug in the liver of animals. The liver cell membrane bound ^{14}C-FLEU with a higher specific activity in comparison to other organs [9]. According to our preliminary observations on the binding characteristics of ^{14}C-FLEU to different proteins by the method of equilibrium dialysis, the drug binds to human serum albumin with an association constant (K_a) of 2.54×10^2 M^{-1}, corresponding to binding sites (N) of 0.72 Similarly, FLEU has a weak affinity to γ-globulin ($K_a = 2.22 \times 10^2$ M^{-1}, $N = 1.26$). On the other hand, it is bound to low density lipoproteins (LDL) more strongly ($K_a = 3.2$ $\times 10^4$, $N = 5.15$), probably by hydrophobic apolar bonds. The prolonged retention of radioactivity observed in patients indicates a strong binding of the drug to tissue elements from where the total radioactivity corresponding to the parent drug and its products are released very slowly with a terminal half life of several days. The fact that 2 weeks after drug administration radioactivity could be detected in liver and brain (patient M.I.) also supports this conclusion.

Among the naturally occurring vinca alkaloids the pharmacokinetic characteristics of vinblastine (VLB) and vincristine (VCR) have also been studied in humans. In the plasma, the elimination curve of VLB was biphasic [10] while VCR exhibited three phases [11]. In both cases the terminal half-lives of the unchanged drugs were similar to tha

Table 2. Estimated exponential parameters of the plasma concentration function and the amount of drug excreted in urine $[y_3(\infty)y_1 = Ae^{-\alpha t} + Be^{-\beta t}]$

	M.I.	B.D.	H.I.	S.I.	N.L.	P.K.	Mean ± S.D.
A D%/litre	43.5 ± 2.4	84.2 ± 13.4	88.5 ± 13.4	67.9 ± 4.8	175.0 ± 13.2	58.4 ± 45.7	86.25 ± 46.53
B D%/litre	26.1 ± 1.5	19.0 ± 3.9	29.4 ± 3.3	18.0 ± 4.3	40.4 ± 8.3	18.3 ± 6.6	25.20 ± 8.80
α hr^{-1}	4.15 ± 0.48	4.69 ± 1.19	4.62 ± 0.87	1.62 ± 0.24	2.47 ± 0.48	1.01 ± 0.83	3.09 ± 1.61
$T_1 = 0.693/\alpha$ hr	0.167 ± 0.019	0.147 ± 0.037	0.150 ± 0.028	0.428 ± 0.063	0.281 ± 0.055	0.687 ± 0.564	0.31 ± 0.21
β hr^{-1}	0.195 ± 0.02	0.261 ± 0.05	0.282 ± 0.04	0.164 ± 0.05	0.186 ± 0.040	0.077 ± 0.025	0.19 ± 0.07
$T_2 = 0.693/\beta$ hr	3.56 ± 0.40	2.66 ± 0.51	2.46 ± 0.35	4.23 ± 1.29	3.73 ± 0.80	9.00 ± 3.51	4.28 ± 2.41

Table 3. Calculated pharmacokinetic parameters

	M.I.	B.D.	H.I.	S.I.	N.L.	P.K.	Mean ± S.D.
k_{10} (hr^{-1})	0.096	0.281	0.177	0.145	0.146	0.050	0.149 ± 0.079
k_{12} (hr^{-1})	3.06	3.94	3.91	1.23	2.01	0.762	2.48 ± 1.36
k_{20} (hr^{-1})	0.227	0.259	0.296	0.168	0.192	0.084	0.204 ± 0.075
k_{21} (hr^{-1})	0.962	0.473	0.515	0.238	0.312	0.191	0.448 ± 0.282
Ratio k_{21}/k_{12}	0.314	0.120	0.131	0.193	0.155	0.251	0.194 ± 0.076
V_c^a (litre/kg)	0.93	0.54	0.38	0.89	0.34	1.14	0.70 ± 0.33
AUC_1^b (D%/litre/hr)	130.	58	64	150	110	290	140 ± 79
AUC_2^c (D%/litre/hr)	331	302	310	432	410	680	411 ± 142
Ratio $AUC_2 AUC_1$	2.55	5.21	4.84	2.88	3.73	2.34	3.26 ± 0.93
$k_{10} \cdot V_c^a$ (litre/kg/hr^{-1})	0.088	0.152	0.067	0.129	0.050	0.057	0.091 ± 0.041
D%/AUC_1/(litre . hr$^{-1/e}$)	0.77	1.72	1.56	0.67	0.91	0.34	1.00 ± 0.54
$V_\beta^f = D\%/AUG_1 . \beta$ litre	3.949	6.590	5.532	4.085	4.892	4.416	

a = Volume of distribution in the central compartment; b = area under the concentration–time curve of the central compartment; c = area under the concentration–time curve of peripheral compartment; d = renal clearance; e = total body clearance; f = volume of distribution at the β phase.

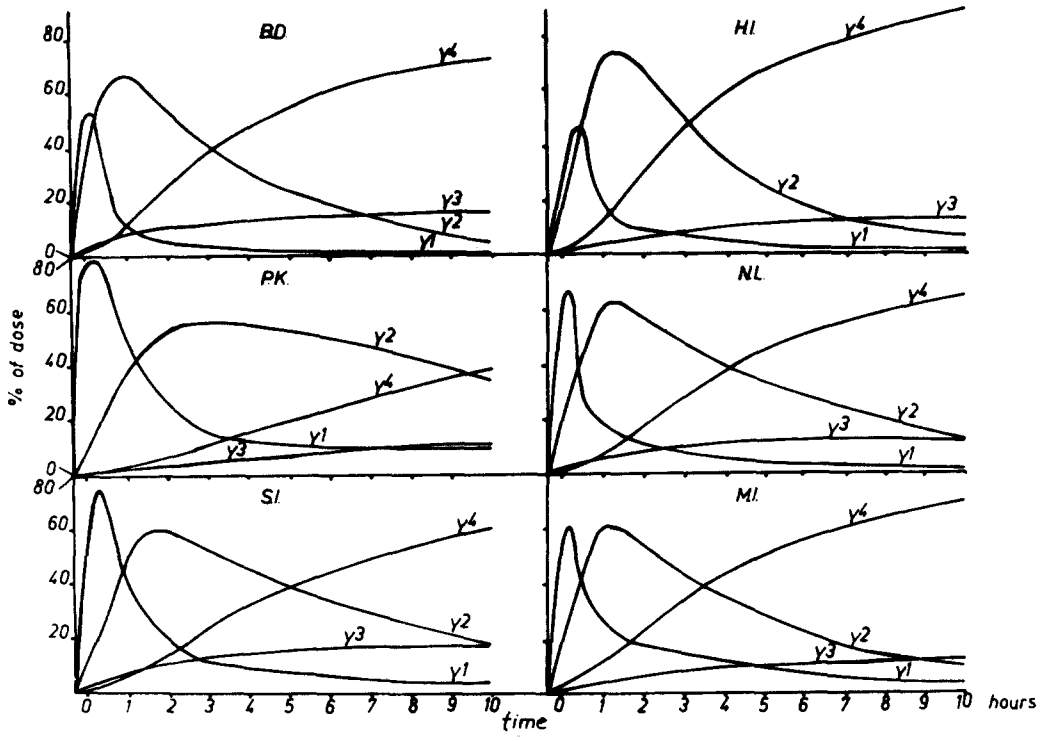


Fig. 5. Simulated compartmental profiles of unchanged ^{14}C-FLEU in patients, y_1, central compartment; y_2 peripheral compartment; y_3, urine; y_4, feces.

observed in the case of FLEU, i.e., between 2 and 9 hr. In all three cases a considerable amount of drug remained in the body for a prolonged time. The functional significance of this phenomenon is unknown. Evidently, the widely different optimal dose schedules determined clinically thus cannot be rationally supported by pharmacokinetic data. Neither do the pharmacokinetic data explain, at present, the differences in the type and time course of toxic side effects caused by various vinca alkaloids and their derivatives.

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APPENDIX

In the case of two compartment open model the plasma concentration after terminating the infusion during time τ is given by the sum of two exponentials:

$$f(t)=Ae^{-\alpha(t-\tau)}+Be^{-\beta(t-\tau)},$$

where

$$f(\tau)=A+B \qquad t\geqq\tau.$$

τ is the duration of the infusion.

Fitting the function $f(t)$, the parameters A, B, α and β can be estimated, but all the four rate constants cannot be determined from the exponential parameters. However, knowing the excreted amount of drug in urine the transport rate constants can be calculated.

Introduce the $K=k_{20}+k_{21}$, which can be got from the exponential parameters

$$K=\frac{\alpha\cdot\beta(a+b)}{\alpha a+\beta b},$$

where

$$a=A(1-e^{-\beta\tau}) \quad \text{and} \quad b=B(1-e^{-\alpha\tau}).$$

Knowing K, k_{10} can be obtained

$$k_{10}=\frac{y_3(\infty)}{k_0u+\dfrac{A\beta+B\alpha}{\alpha\beta(A+B)}y_1^{(\tau)}},$$

where

$$u=K\left[\frac{\tau}{\alpha\cdot\beta}-\frac{e^{-\alpha\tau}-1}{\alpha^2(\alpha-\beta)}+\frac{e^{-\beta\tau}-1}{\beta^2(\alpha-\beta)}\right]-\frac{e^{-\alpha\tau}-1}{\alpha(\alpha-\beta)}$$

$$-\frac{e^{-\beta\tau}-1}{\beta(\alpha-\beta)}$$

$$y_1(\tau)=k_0\left\{K\left[\frac{1}{\alpha\cdot\beta}+\frac{e^{-\alpha\tau}}{\alpha(\alpha-\beta)}-\frac{e^{-\beta\tau}}{\beta(\alpha-\beta)}\right.\right.$$

$$\left.\left.+\frac{1}{\alpha-\beta}(e^{-\beta\tau}-e^{-\alpha\tau})\right\}\right.$$

$y_3(\infty)$: amount of drug excreted in urine.

From the values of K and k_{10} the values of all the other constants can be determined:

$$k_{12}=\alpha+\beta-k_{10}-K$$

$$k_{20}=Kk_{10}/k_{12}$$

$$k_{21}=K-k_{20}.$$

The volume of the central compartment (V_c) is obtained by

$$V_c=\frac{y_1(\tau)}{A+B}.$$

The given algorithm is easy to program and can be used on a pocket calculator, such as HP-65.

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