Coumermycin A_1—Biopharmaceutical Studies II

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Abstract □ Coumermycin monosodium salt in a 1:4 w/w mixture with *N*-methylglucamine is absorbed with an efficiency of about 20–25% in dogs and humans after oral administration. This is 5–15-fold the oral absorption efficiency of the drug alone. On intravenous or oral administration, blood levels decline exponentially after 3–4 hr. in dogs and after 4–6 hr. in humans; the half-lives of these declines are 8–9 hr. in dogs and 8–10 hr. in humans. Blood levels well over 1 mcg./ml. are readily achieved on oral dosage of 4–5 mg./kg. in dogs and humans.

Keyphrases □ Coumermycin A_1—biopharmaceutical characteristics □ Blood levels, coumermycin A_1—administration route effect □ Distribution—coumermycin A_1 □ Microbiological analysis—coumermycin A_1

The antibiotic coumermycin A_1, as the free acid or most simple salts, is poorly absorbed from the gastrointestinal (GI) tract of dogs and humans. In a previous paper, the authors reported that a mixture of the monosodium salt with *N*-methylglucamine (NMG) (1:4 by weight) in capsules enhanced blood levels on oral administration in dogs and humans by 5–15-fold (1). The present studies were made to determine the biopharmaceutical characteristics of this preparation *in vivo*.

EXPERIMENTAL

Materials and Methods—Oral and parenteral preparations were formulated in the following manners. Gelatin capsules of micronized coumermycin A_1 monosodium salt were prepared containing 50 mg. of drug and designated Capsules A. Capsules containing a mixture of 50 mg. of micronized coumermycin A_1 with 200 mg. of NMG were made and designated Capsules B. A fresh parenteral solution of coumermycin A_1, 50 mg./ml., was prepared in a solvent consisting of 30% propylene glycol, 10% ethyl alcohol, 1% benzyl alcohol, 0.5% NMG, and water for injection, with the pH adjusted to 9.4 by sodium hydroxide. In the parenteral solution, there is only a minimal amount of NMG, the ratio of drug to NMG being 1:0.1 or 1/40 of the ratio in the capsules.

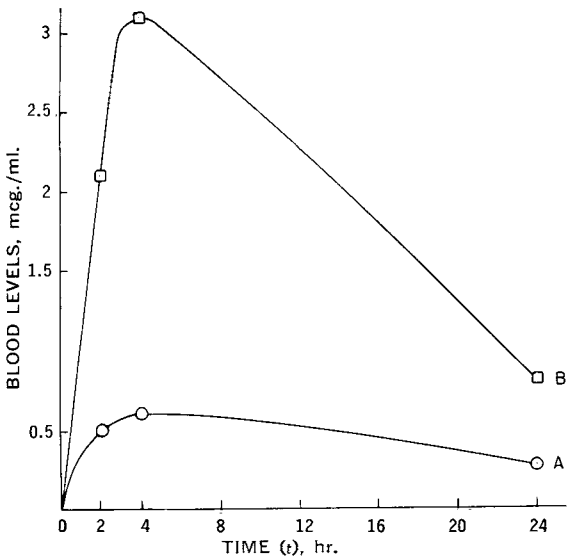

Figure 1—*Coumermycin blood levels in dogs after a single oral dose, 5 mg./kg., of: A, capsules of coumermycin alone; and B, capsules of coumermycin + NMG (1:4). Each group represents the average of six dogs.*

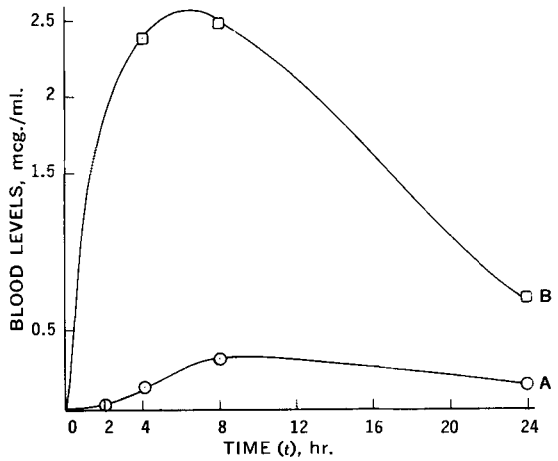

Figure 2—*Coumermycin blood levels in humans after a single oral dose, 4 mg./kg., of: A, capsules of coumermycin alone; and B, capsules of coumermycin + NMG (1:4). Each group represents the average of six subjects.*

Studies in Dogs—Dogs, each weighing about 10 kg., fasted 12–18 hr., were given capsules of drug formulation to provide the equivalent of about 5 mg./kg. Blood samples were taken at intervals and assayed microbiologically by a cup-plate assay for coumermycin concentration (1). Oral absorption efficiency was tested as follows: the parenteral formulation was administered intravenously at 0.5 and 2.5 mg./kg., respectively, to two dogs. Six other dogs were each given orally a single Capsule B. Blood samples were taken at intervals and measured for coumermycin concentration.

The parenteral preparation also was used to permit examination of the difference in blood levels achieved after intramuscular and intravenous administration, measured 1 hr. after injection, in comparison with the oral levels. The effect of repeated daily intravenous injection also was examined after administration at three different dosage levels.

Studies in Humans—Six human subjects were given a single oral dose of five Capsules A, representing a dose of approximately 4 mg./kg. of drug alone. Six other human subjects were given a single oral dose of five Capsules B, similarly representing a dose of approximately 4 mg./kg. of drug content, but in the presence of NMG. Blood samples were taken at intervals and measured microbiologically for coumermycin concentration. For the study of oral absorption efficiency in humans, a single human adult subject (L.R.) was injected intravenously with 50 mg. of coumermycin in parenteral solution; samples of blood were taken periodically for measurement of coumermycin concentration. Subsequently, the same subject was retested with a dose of 100 mg. i.v. In some experiments with dogs and humans, samples of urine and stools were also examined for the presence of excreted, unchanged antibiotic.

RESULTS AND DISCUSSION

The results of a single dose, oral administration, of antibiotic with and without NMG are presented for dogs in Fig. 1 and for humans in Fig. 2. The effect of NMG in enhancing the oral absorption, as indicated by elevation of blood levels, is clearly indicated. From the individual values and comparison of the area under the curves, the enhancement by NMG appears to be in the range of 5–15-fold over the drug alone in both dogs and man. The post-absorptive half-life is calculated to be about 10 hr.

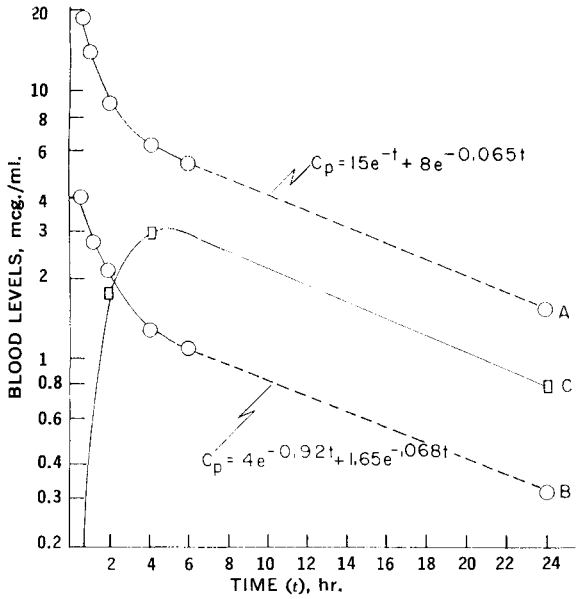

Figure 3—*Blood levels of coumermycin in dogs after 2.5-mg./kg. i.v. dose (A), 0.5-mg./kg. i.v. dose (B), and 2.5 mg./kg. p.o. (C). Data are presented in semilogarithmic form, the linear form of the corresponding equations being indicated in the two intravenous curves. The area under the oral curve is estimated in a linear graph (not shown).*

The efficiency of absorption of coumermycin in the NMG mixture can be calculated from the data of Fig. 3 in dogs and of Fig. 4 in humans. As pointed out by Riegelman *et al.* (2), a peripheral compartment may be detected only in the early phases subsequent to administration of a drug. By considering the present case a two-compartment, open-system model:[1]

$$\text{metabolism, elimination} \xleftarrow{k_{el}} \text{blood} \underset{k_{21}}{\overset{k_{12}}{\rightleftarrows}} \text{tissue} \quad \text{(Eq. 1)}$$

the equation governing the time-dependent concentration should be: $C_p = A \cdot \exp(-\alpha \cdot t) + B \cdot \exp(-\beta \cdot t)$, with a value extrapolated to zero time of $C_p^\circ = A + B$.

The parameters, A, B, α, and β, may now be evaluated from the curves by the feathering technique. The parameter values found are indicated in Figs. 3 and 4.

Once the parameters in the double-exponential decay curve are known, the forward and reverse diffusion constants k_{12} and k_{21} and the disposition constant k_{el} may be determined readily. Values of these for dog (from Fig. 3) and man (from Fig. 4) are listed in Table I.

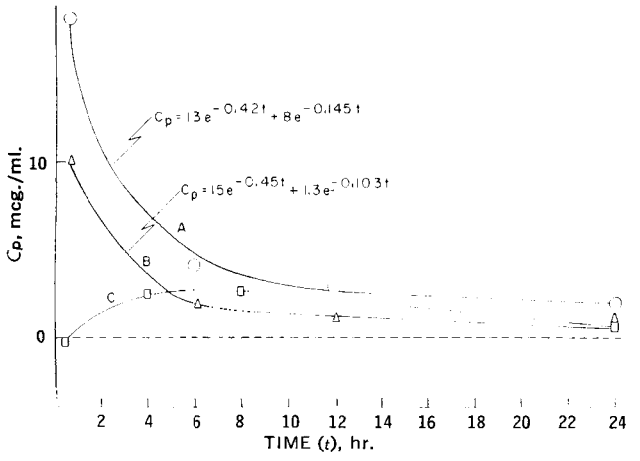

Figure 4—*Blood levels of coumermycin in man after 100-mg. i.v. dose (A), 50-mg. i.v. dose (B), and 250 mg. p.o. (C). The data are presented in linear form, and the appropriate least-squares equations indicated. The area under Curve C can be estimated by graphical means.*

[1] The notations of Riegelman *et al.* (2) are followed here.

The amount of drug in the tissue compartment (T) at time t is governed by the differential equation:

$$\frac{dT}{dt} = k_{12}[A \cdot \exp(-\alpha \cdot t) + B \cdot \exp(-\beta \cdot t)] - k_{21}T \quad \text{(Eq. 2)}$$

This is readily solved, using the integrating factor $\exp(k_{21}t)$ and imposing the initial condition that $T = 0$ at $t = 0$ yields the solution:

$$T = \frac{A \cdot k_{12}}{k_{21} - \alpha}[\exp(-\alpha \cdot t) - \exp(-k_{21}t)] + \frac{B \cdot k_{12}}{k_{21} - \beta}[\exp(-\beta \cdot t) - \exp(-k_{21}t)] \quad \text{(Eq. 3)}$$

This quantity integrated over time is used here as a measure of absorption, *i.e.*,

$$I = \int_0^\infty T dt = \frac{A \cdot k_{12}}{[k_{21} - \alpha]} \cdot \left[\frac{1}{\alpha} - \frac{1}{k_{21}}\right] + \frac{B \cdot k_{12}}{[k_{21} - \beta]} \cdot \left[\frac{1}{\beta} - \frac{1}{k_{21}}\right] \quad \text{(Eq. 4)}$$

Values of this measure of absorption (I) as found in dog and man are listed in Table I.

It is noted that, in the dog, absorption is linearly related to dose (188 = 5.42), whereas proportionality is not quite as apparent in man. In the latter case, however, I depends strongly on the accuracy of $k_{21} - \alpha$ and $k_{21} - \beta$. All dose-independent constants (k_{12}, k_{21}, k_{el}, α, and β) correlate well from low to high dosage. However, only a few points in time are used here, and the values in Table I, at best, should only be used for qualitative comparison. As reported by Wagner and Northam (3), the distribution volumes, when calculated in this fashion, are likely to be overestimates.

Equation 4 cannot be used for estimation of absorption by the oral route, but an insight into the physiological availability of the oral dosage form may be gained by use of the absorption definition of Wagner and Nelson (4), *i.e.*, by determining the area (a) under the curve in Fig. 4 (in linear presentation). In this manner, a value of 19 mcg.-hr./ml. is obtained for the 250-mg. p.o. dose. This is comparable to the 50-mg. i.v. data in Table I, implying a 20% efficiency *via* the oral route.

Similar figures are obtained in the case of the dog, where values are: A_∞ = 138 mcg.-hr./kg.-ml. for 2.5 mg. i.v.; A_∞ = 29 mcg.-hr./kg.-ml. for 0.5 mg./kg. i.v.; and 52 mcg.-hr./kg.-ml. for 5 mg./kg. p.o., so that 5 mg./kg. p.o. is equivalent to about 1 mg./kg. i.v., yielding an efficiency of about 20% *via* the oral route. A more straightforward procedure is to assume that the curve of the 5-mg./kg. p.o. dose, after 4 hr., is approximately equivalent to a hypothetical curve of the 1.25-mg./kg. i.v. dose, obtained by interpolation between 0.5-mg. and 2.5-mg./kg. i.v. doses, *i.e.*, 25% efficiency. Similar treatment of the data in Fig. 4 may be made,

Table I—Biopharmaceutical Parameters of Coumermycin A_1 on Intravenous Injection

Figure	3	3	4	4
Curve	B	A	B	A
B, mcg./ml.	1.65	8.0	8.0	1.3
β, hr.$^{-1}$	0.068	0.065	0.145	0.103
A, mcg./ml.	4	15	13	15
α, hr.$^{-1}$	0.92	1	0.42	0.45
C_p°, mcg./ml.	5.65	23	21	16.3
Dose, D[a]	0.5	2.5	100	50
Distribution volume, $V_p = D/C_p^\circ$, l.	0.89	0.108	4.75	3.3
A/α	4.35	15	55.2	33.3
B/β	24.2	123	31	12.6
C_p°/k_{el}	28.6	138	86	45.9
k_{el}, hr.$^{-1}$	0.20	0.17	0.24	0.36
$\alpha\beta/k_{el} = k_{21}$, hr.$^{-1}$	0.32	0.39	0.25	0.13
$\alpha + \beta - k_{21} - k_{el} = k_{12}$, hr.$^{-1}$	0.47	0.53	0.08	0.06
I, mcg.-hr./ml.	42	188	28	20

[a] Doses in the first two columns refer to mg./kg., in the last two simply to mg. The distribution volume, therefore, refers to l./kg. and liters, respectively.

Table II—Blood Levels in Dogs after Intramuscular and Intravenous Administration

Dose, mg./kg.	Route	Blood Level, 1.0 hr. after Dose, mcg./ml.[a]
10	Intramuscular	26
20	Intramuscular	74
10	Intravenous	85

[a] Each value represents the average of two dogs.

indicating that in humans the oral absorption efficiency of the coumermycin in the mixture with NMG is approximately 20–25%. These figures, of course, are estimates since the two compartments are actually not in steady state of equilibrium throughout the cited period.

The intravenous curves in humans (Fig. 4) show that the early (0.5 hr. after dose) values are essentially dilutions of the drug in the blood volume. Thus, 50 mg. i.v. in a human adult with about 5 l. of blood and fluid in equilibrium with it calculates to 10 mcg./ml. which is what was obtained. A similar calculation for the 100-mg. case yields a value of 20 mcg./ml. as compared to the 18 mcg./ml. actually found. It is noted that it requires 3–4 hr. in dogs and 4–6 hr. in humans before the $A \cdot \exp(-\alpha \cdot t)$-term becomes negligible; after this period the blood level, of course, declines in a conventional first-order fashion. As seen from the limited data here, the overall half-life in humans on 50- and 100-mg. intravenous dose appears somewhat longer than the overall half-life in dogs.

Table II summarizes the data obtained with blood levels in dogs after intramuscular and intravenous administration as measured 1 hr. after the dose. The results suggest some retardation of movement of the drug from the intramuscular site. Thus, it appears that at least twice the dose by the i.m. route is required to achieve the same blood levels as those obtained 1 hr. after i.v. dosing.

The effect of repeated daily i.v. injections of coumermycin A_1 on blood levels in dogs is shown in Table III. Levels were measured 1 hr. after the first, fifth, and tenth daily injection. These data suggest that on i.v. administration, increasing dosage increases blood levels after the first dose but not linearly. After the initial dose, the subsequent increase in blood levels after repeated dosage is greater than the multiple of dose increase. This suggests decreased migration of the drug out of the blood into tissue stores with increased repeated dosage, possibly approaching saturation of these tissues at higher dosage.

Bioassay of urine samples from dogs or humans, even after high blood levels were achieved, indicated the presence of little or no intact coumermycin A_1. On the other hand, substantial amounts of drug were found in the feces of dogs and humans. These data will be reported later.

Table III—Blood Levels in Dogs after Intravenous Administration of 1, 5, and 10 Daily Doses

Dose, mg./kg.	Blood Level, mcg./ml.,[a] 1.0 hr. after Dose Day 1	Day 5	Day 10
1	4.0 ± 0.4	5.4 ± 0.6	6 ± 1.2
3	17.5 ± 0.5	24 ± 6	27 ± 6
9	71 ± 15	135 ± 18	121 ± 14

[a] Each value represents the average of four dogs.

SUMMARY AND CONCLUSIONS

Coumermycin monosodium salt in a 1:4 w/w mixture with NMG is absorbed with an efficiency of about 20–25% in dogs and humans on oral administration, based on the assumption of a two-compartment open model. This is 5–15-fold the oral absorption efficiency of the drug alone. On intravenous or oral administration, blood levels decline exponentially after 3–4 hr. in dogs and 4–6 hr. in humans; the half-lives of these declines are 8–9 hr. in dogs and 8–10 hr. in humans. Blood levels well over 1 mcg./ml. are readily achieved on oral dosage of 4–5 mg./kg. in dogs and humans.

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