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Dissolution Systems for Chloramphenicol Tablet Bioavailability

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Abstract □ The relationship between chloramphenicol (I) tablet bioavailability and *in vitro* dissolution rates was examined. The effect of solid food on the I tablet and powder bioavailability was also studied. Five tablets of I were selected for bioavailability testing on the basis of the dissolution rates of 18 I tablets (250 mg) determined by several methods. Compound I, 500 mg, was administered orally to five subjects, following overnight fasting, according to a crossover design. The bioavailability parameters were obtained from urinary I excretion. Among the five formulations studied, only one tablet (F) showed significantly poorer bioavailability. The dissolution rates at pH 1.2 did not give the same rank order as the bioavailability. The dissolution rate of Tablet F showed remarkable pH dependency. The dissolution rates at pH 4 showed good correlation with *in vivo* bioavailability data. The bioavailability of I powder was not affected by solid food. Tablet F, which had poor bioavailability in the fasting state, showed good bioavailability when administered just after the standard breakfast.

Keyphrases □ Chloramphenicol—various dosage forms, bioavailability correlated with dissolution rates □ Bioavailability—chloramphenicol, various dosage forms, correlated with dissolution rates □ Dissolution rates—chloramphenicol, various dosage forms, correlated with bioavailability □ Antibacterials—chloramphenicol, various dosage forms, bioavailability correlated with dissolution rates

The dissolution rates of 21 chloramphenicol (I) tablets manufactured in Japan were reported previously (1). In this paper, the *in vivo* bioavailability of selected I tablets was correlated with *in vitro* dissolution tests. The bioavailability of nine different I tablets available in Japan was determined previously by Watanabe *et al.* (2, 3), who showed significant correlation of *in vivo* bioavailability with disintegration time and dissolution rate as measured by a disintegration apparatus[1] using water as the medium.

The dissolution devices used in that study (beaker, rotating basket, oscillating basket, and disintegration[1] methods) all belonged to the stirred-tank reactor type (1). *In vivo–in vitro* correlation with rotating-flask type dissolution devices was not attempted. These investigators (2, 3) also reported better correlation of *in vitro* dissolution rate and *AUC* (area under plasma level–time curve) following oral administration of I tablets with water as the dissolution medium instead of the pH 1.2 solution recommended in JP IX. Chloramphenicol dissolution in an unbuffered medium could have complicated the system since the pH value changed as the tablet dissolved.

Comparative bioavailability studies of five I tablets are described in this report. These tablets were selected based on dissolution rates of 18 I tablets (250 mg) and I powder determined by seven methods (1). The relationship between *in vivo* bioavailability and *in vitro* dissolution was examined. The effect of food on the I bioavailability was also studied.

EXPERIMENTAL

Materials—The I tablets and powder were the same as those described previously (1), except for Tablets V and V′ which were of the same brand but different lot numbers.

***In Vitro* Studies**—The methods and procedures for determining the dissolution rate were the same as those reported previously (1): beaker (a), rotating basket (b), oscillating basket (c-II and c-III), rotating flask (d), solubility simulator (e)[2], and column (f). The dissolution medium pH was controlled by a pH stat. No corrections were made for acidic dissolution media adjusted at pH 1.2.

Bioavailability Studies—Six healthy adult male volunteers, 55–72 kg and 29–49 years old, participated after being informed about the study and the drug. All subjects received no barbiturates or other enzyme-inducing agents for 30 days before and for the duration of the studies. They also received no other medication or alcoholic beverages for 7 days before and for the duration of the studies.

Study I—The bioavailability of five I tablets was studied using a Latin square. Treatments were separated by 1 week. Subjects fasted for 10 hr prior to dosing and took two I tablets (total of 500 mg) with 300 ml of water. They took 200 ml of water at 2 hr and had lunch at 4 hr after administration. Urine samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 24, and 28 hr after dosing. The exact sampling times and the volume were recorded.

Study II—The effect of solid food on Tablet F and I powder bioavailability was studied with four subjects using a Latin square. Tablet F or I powder (500 mg) was taken either with 300 ml of water in the fasting state or immediately after a standard breakfast of 100 g of toast, 20 g of butter, 35 g of cucumber, 65 g of boiled egg, 200 ml of milk, and 100 ml of water. The urine collection procedure was the same as that for Study I.

Study III—One subject participated in a study of the relationship

[1] Erweka.

[2] Sartorius-Membranfilter GmbH, Göttingen, West Germany.

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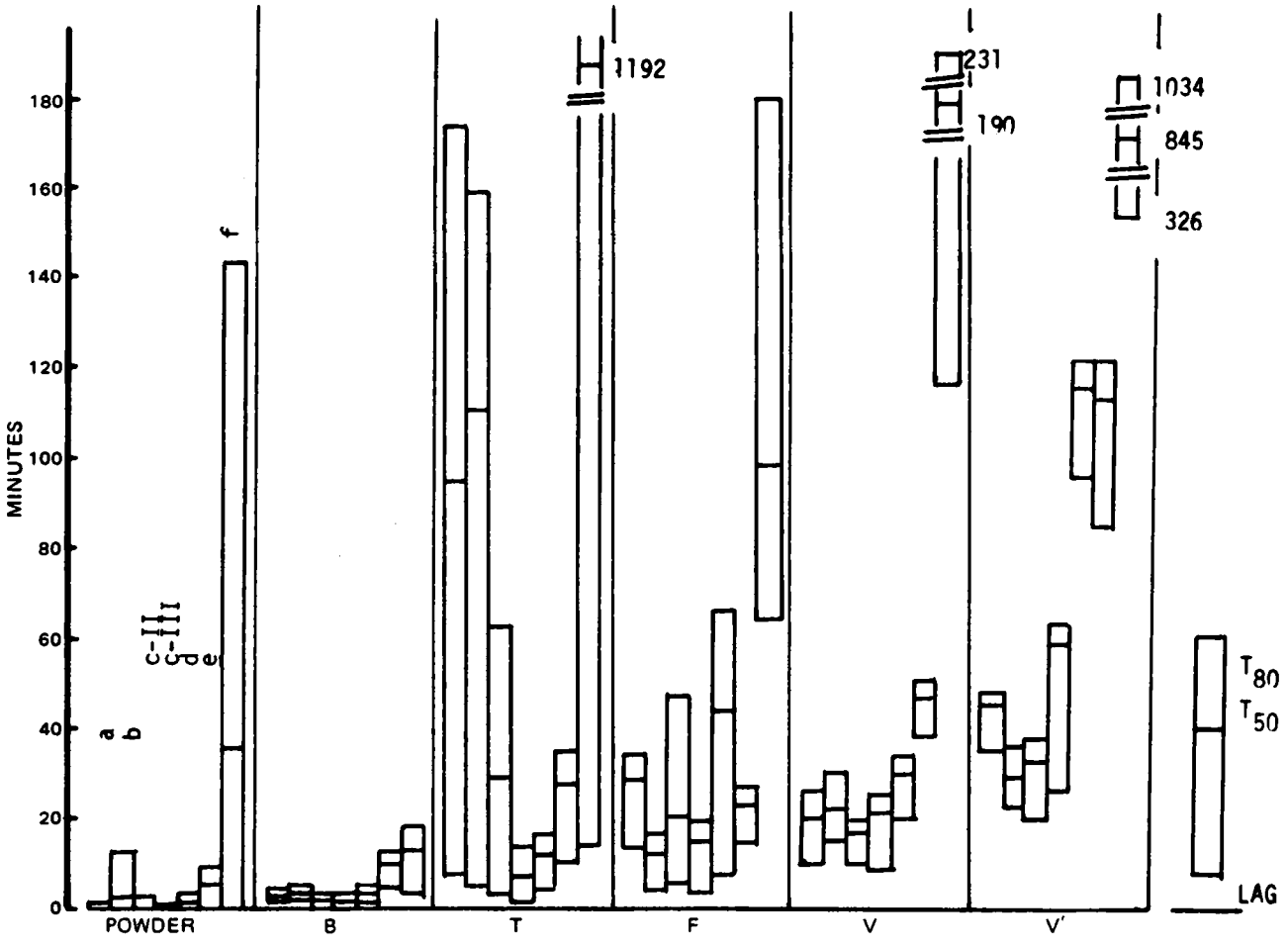

Figure 1—*Dissolution rates of six I formulations.*

between urinary recovery and dose. Oral I doses of 155, 250, and 500 mg of powder were given according to the protocol described for Study I.

Assay—Unchanged and total urine nitro I compounds were determined by colorimetry as described by Maruyama and Suzuki (4) with slight modifications.

Total Nitro I Compounds—Urine, 1 ml, was diluted to 100 ml with water, and 4 ml of 0.25 *N* NaOH and 0.3 ml of 2% $Na_2S_2O_4$ were added to 1 ml of the solution and mixed well. After the solution stood for 15 min at room temperature, 0.5 ml of 2.5% $NaNO_2$ and 1.0 ml of 4 *N* HCl were added and mixed well. After 5 min, 0.5 ml of 5% sulfamic acid was added; the solution was shaken and allowed to stand for 5 min. Then 0.5 ml of 0.5% Tsuda's reagent solution was added, and the mixture was allowed to stand for 90 min at 37°. The solution absorbance was determined spectrophotometrically[3] at 650 and 560 nm.

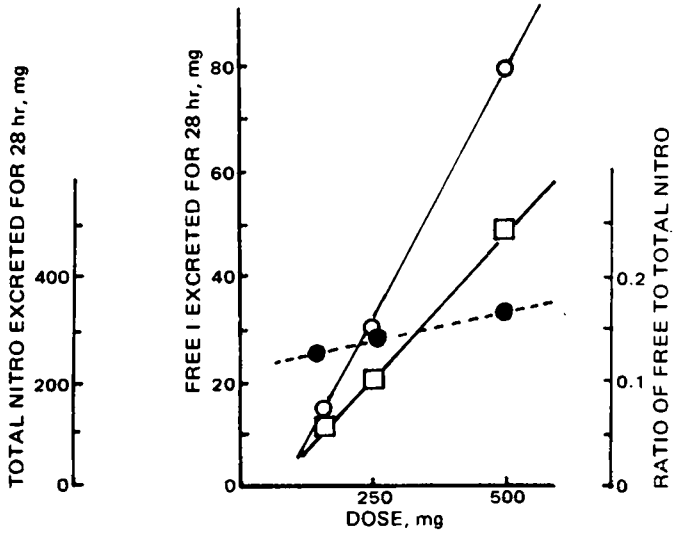

Figure 2—*Dose dependency of urinary excretion of free and total nitro following oral I powder administration. Key:* ○, *free I;* □, *total nitro compounds; and* ●, *ratio of free to total nitro.*

Unchanged I—An aliquot of urine was diluted 20 times with water, 4 ml of pH 6.0 phosphate buffer (0.2 *M*) and 12 ml of ethyl acetate were added to 2 ml of the solution, and the solution was shaken for 10 min mechanically. After centrifugation, the aqueous layer was removed and 10 ml of fresh phosphate buffer (pH 6.0) was added. The mixture was shaken for 10 min and centrifuged. Ten milliliters of the organic layer was evaporated to dryness under reduced pressure in a boiling water bath. Water (4.5 ml) was added, and the solution was heated for 5 min to dissolve I completely. After the solution cooled, 0.5 ml of 1.0 *N* NaOH and 0.3 ml of 2% $Na_2S_2O_4$ were added. The assay then followed the procedure described for total nitro compounds.

Aqueous solutions of 2% $Na_2S_2O_4$ and 2.5% $NaNO_2$ were prepared just prior to addition. The absorbance due to the urine composition was almost constant and very small in comparison with other colorimetric methods (5, 6). The urine sample collected just prior to dosing could be used as the urine blank with very slight error.

RESULTS AND DISCUSSION

***In Vitro* Studies**—Figure 1 shows the relative dissolution rates of the five I tablets and powder as reflected by lag time, time for 50% dissolution (T_{50}), and time for 80% dissolution (T_{80}). Each formulation showed a distinguishing dissolution rate profile. Tablet B had very fast dissolution rates by all seven methods. The powder dissolution behavior was similar to that of Tablet B in all dissolution methods except Method f. Tablet T showed especially slow dissolution rates in Methods a, b, and f, which have poor dispersing intensity (1).

The Tablet T dissolution lag time was small. Tablet V' appeared to

[3] Hitachi 156.

Table I—Mean Chloramphenicol Bioavailability Parameters from the Five Formulations

Parameter[a]	Formulation B	T	F	V	V′	Results of ANOVA	Tukey's Allowable Difference
Excretion lag time, hr	0.2[b]	0.4	5.2	1.2	1.4	$p < 0.05$	B < T < V < V′ < F[c]
Maximum urinary excretion rate, mg/hr	15.42	14.34	3.44	14.24	13.64	$p < 0.05$	B > T > V > V′ > F
Time of maximum urinary excretion rate, hr	1.46	2.30	15.45	2.15	2.50	$p < 0.05$	B < V < T < V′ < F
Cumulative amount excreted in urine for 28 hr, mg	77.12	73.36	41.76	78.12	71.34	$p < 0.05$	V > B > T > V′ > F
Ratio of free to total nitro excreted for 28 hr	0.159	0.164	0.156	0.159	0.152	NS[d]	

[a] Obtained from the urinary excretion of unchanged compound. [b] Mean of five subjects. [c] Formulations not underscored by the same line differ significantly in their mean values for the parameter ($p < 0.05$). [d] Not significant ($p > 0.05$).

Table II—Chloramphenicol Dissolution Rates in Two Different Media

Formulation	Time of 50% Dissolution, min Method d SJ[a]	Method d DW[b]	Method c-III SJ	Method c-III DW
Powder	1.1	1.5	0.3	1.0
Tablet B	3.6	4.2	1.6	3.2
Tablet T	12.6	10.9	7.3	12.6
Tablet F	44.4	>1140	15.0	114.0
Tablet V	29.0	170.0	21.1	34.5
Tablet V′	116.0	1022.0	59.5	49.4

[a] Simulated gastric juice without enzyme, pH 1.2. [b] Distilled water.

have a strongly coated film; its lag time was very long, but dissolution after the lag time was comparatively fast. The rotating vessel reactor dissolution methods (d and e) showed especially poor destructive intensity against Tablet V′. Tablet V showed fast dissolution after the lag time by all methods except Method f. Tablet F showed especially poor dissolution by Method d but comparatively good dissolution by other methods.

Dose Dependency of I Urinary Excretion—Figure 2 shows the relationship between dose and the cumulative amount excreted in 28 hr following oral administration of I powder in one subject (Study III). The excreted free and total nitro I compounds were proportional to the dose administered, but the ratio of free to total nitro compounds increased slightly with dose from 0.128 to 0.162. This result suggested that urinary I excretion in humans can be treated by linear pharmacokinetics within the dose range studied.

Bioavailability—The bioavailability parameters following five different treatments are summarized in Table I. Based on ANOVA (analysis of variance), statistically significant differences were found among the treatments in all parameters except the ratio of free to total nitro I compounds. Tablet F showed significant differences from the other tablets in all parameters except the ratio of free to total nitro compounds excreted. Tablet F showed an excretion lag time of about 5 hr. This tablet took the longest time to reach the maximum excretion rate, which was also the smallest value among the treatments. The total amount excreted in 28 hr after dosing with Tablet F was about one-half of the amount excreted after the other tablets. The poor Tablet F bioavailability, however, appeared not to be due to *in vitro* dissolution rates (Fig. 1).

All dissolution tests in Fig. 1 were carried out at pH 1.2. The *in vitro* dissolution was reexamined using water as the dissolution medium as suggested by Watanabe *et al.* (3).

Table II showed that, with Method d, the Tablet F dissolution rate in water was remarkably slower than that determined at pH 1.2, as were the dissolution rates of Tablets V and V′. With Method c-III, only Tablet F showed a slower dissolution. With water as the dissolution medium, the pH changed as dissolution progressed; the change was dependent on the formulation.

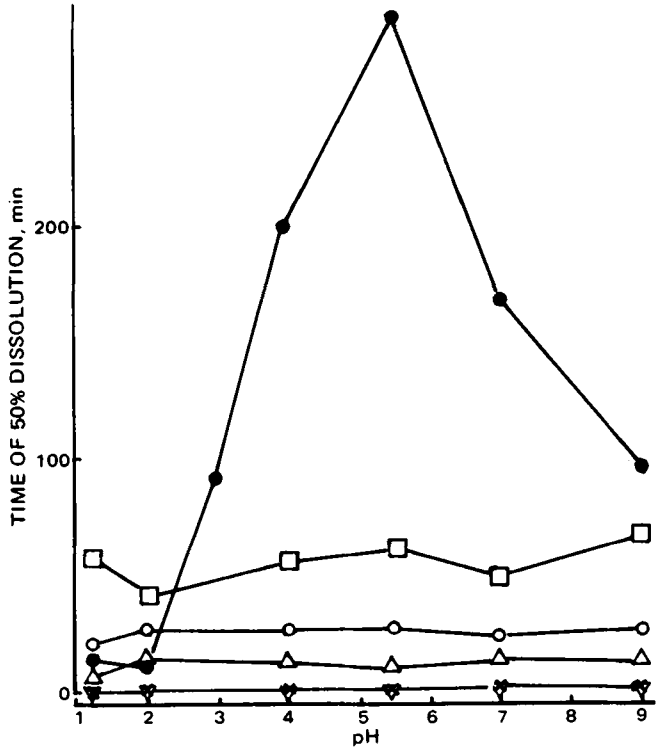

Figure 3—*Effect of pH on time of 50% I formulation dissolution. Key:* ▽, *powder;* ×, *Tablet B;* △, *Tablet T;* ●, *Tablet F;* ○, *Tablet V; and* □, *Tablet V′.*

Figure 3 represents the dissolution rates determined by Method c-III when the medium pH was controlled *via* pH stat. Tablet F was the only tablet whose dissolution showed a remarkable pH dependency. Since the powder dissolution rate was not a function of pH, the pH dependency shown by Tablet F might have been caused by the white shellac tablet coating.

Figure 4 shows the relationship between *in vivo* bioavailability and *in vitro* dissolution of the five tablets tested. The *in vitro* dissolution rates were determined by Method c-III at pH 4.0. From Fig. 4, there appeared to be a critical dissolution rate point distinguishing the equivalent from

Table III—Effect of Food on Chloramphenicol Powder and Tablet F Bioavailabilities

Parameter[a]	Powder Fasting	Powder Nonfasting	Powder Paired *t* Test	Tablet F Fasting	Tablet F Nonfasting	Tablet F Paired *t* Test
Excretion lag time, hr	0.13[b]	0.50	NS[c]	5.20	2.60	NS
Maximum urinary excretion rate, mg/hr	15.00	15.15	NS	3.44	14.95	$p < 0.025$
Time of maximum urinary excretion rate, hr	1.63	2.53	NS	15.45	4.06	$p < 0.025$
Cumulative amount excreted for 28 hr, mg	76.73	89.55	NS	41.76	82.85	$p < 0.05$

[a] Obtained from the urinary excretion of unchanged compound. [b] Mean of four subjects. [c] Not significant ($p > 0.05$).

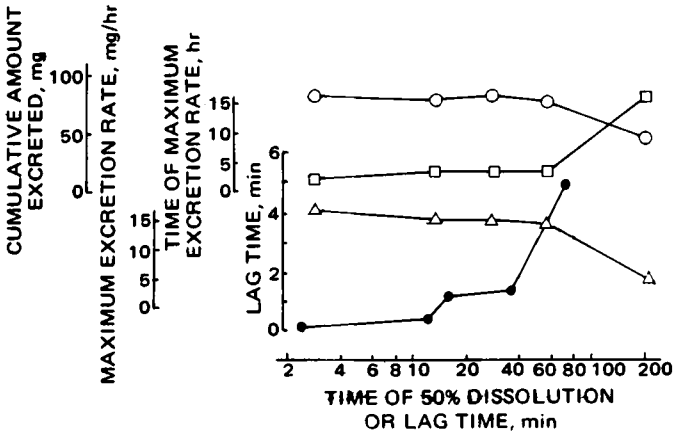

Figure 4—*Correlation of* in vitro *parameters (time of 50% dissolution and lag time) with urinary excretion* in vivo. *Key:* ●, *urinary excretion lag time;* △, *maximum urinary excretion rate;* □, *time of maximum urinary excretion rate; and* ○, *cumulative amount excreted in urine for 28 hr. These values were estimated by means of the urinary excretion of unchanged compound.*

the nonequivalent tablets. Since only one tablet was detected as nonequivalent in this study, the critical point could not be determined clearly. However, an approximate critical T_{50} point appeared to be more than 50 min. The critical value occurred surprisingly late, even though the *in vitro* rates were determined by the dissolution method that had the most vigorous agitating, dispersing, and destructive intensities among the seven methods studied.

Effect of Food on Bioavailability—The relative bioavailability of the powder and Tablet F was studied under fasting and nonfasting conditions. Table III shows the bioavailability parameters compared by the paired t test. The powder showed equivalent bioavailability with the tablets, except for Tablet F under fasting condition, and did not show any bioavailability difference between fasting and nonfasting states. Tablet F, which had poor bioavailability after fasting, became equivalent in bioavailability with the other formulations when it was administered just after the standard breakfast. Possibly, the more violent movement of the tablet in the GI tract after food might have increased drug absorption.

CONCLUSIONS

Film-coated I tablet absorption in humans is related to dissolution. The dissolution device that correlated well with bioavailability was one with a comparatively violent destructive force for the coated tablet film and high test medium agitation. The test medium pH must also be considered because dissolution of certain tablet coatings was pH dependent.

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Antitumor Agents: Structure–Activity Relationships in Tenulin Series

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Received May 25, 1978, from the **Department of Chemistry, University of Tennessee at Chattanooga, Chattanooga, TN 37401*, and the ‡*Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514.* Accepted for publication November 21, 1978.

Abstract □ Systematic structural modifications were performed on the natural sesquiterpene lactone tenulin to define those groupings essential to, or significant in, its *in vivo* antitumor activity. Accordingly, the following tenulin analogs were prepared: dihydrotenulin, 2,3-epoxytenulin, isotenulin, dihydroisotenulin, 2,3-epoxyisotenulin, and tetrahydrodeacetylisotenulin. Both the cyclopentenone and the hemiketal units in tenulin were necessary for high *in vivo* activity.

Keyphrases □ Tenulin—antineoplastic activity, structure-activity relationships, *in vivo* study □ Antineoplastic activity—tenulin, structure-activity relationships, *in vivo study*

The sesquiterpene lactone tenulin (I) is a major bitter principle in several species of the plant genus *Helenium* (Family Compositae) (1–6). High *in vivo* tenulin antitumor activity toward the Ehrlich ascites and Walker 256 carcinosarcoma screens and, to a lesser extent, toward P-388 leukemia has been demonstrated (7, 8). The tenulin cyclopentenone unit may act as a Michael acceptor in cellular enzyme essential sulfhydryl group alkylation.

Tenulin forms a Michael addition product with L-cysteine to give the zwitterionic compound II (7, 8). The adduct II, containing the cyclopentanone group, showed 84% activity loss toward the Ehrlich ascites screen, suggesting that a cyclopentenone unit is important for high activity. Low II activity also might be the result of lower solubility in lipid membranes.

In the present study, the tenulin structure was modified in ways not significantly affecting drug transport. This structure–*in vivo* antitumor activity work reveals those tenulin features necessary for high activity.

RESULTS AND DISCUSSION

Chemistry—Tenulin (I), isolated after extraction of *Helenium amarum* (6, 7), was subjected to catalytic hydrogenation conditions to give dihydrotenulin (III) (1). A new compound, 2,3-epoxytenulin (IV), was prepared in quantitative yield from tenulin upon brief treatment with cold, basic hydrogen peroxide solution. 2,3-Epoxytenulin, $C_{17}H_{22}O_6$ (by exact mass measurement), had a 194–197° melting point and IR ab-

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