Thiazides IV: Comparison of Dissolution with Bioavailability of Chlorothiazide Tablets

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Abstract □ Based on the initial dissolution rate profiles in water, a slow-dissolving, an intermediate-dissolving, and a fast-dissolving chlorothiazide 250-mg tablet were selected for the bioavailability and bioequivalence study. In addition, two marketed 500-mg chlorothiazide tablets were studied. The three 250-mg tablets were bioequivalent, as were the two 500-mg tablets. Therefore, the dissolution test conditions were modified to associate *in vitro* dissolution with *in vivo* performance of the product. Based on these results, it was concluded that a dissolution of 75% in 60 min by paddle method at 75 rpm in pH 7.4 phosphate buffer can be used as a quality assurance test for 250- and 500-mg chlorothiazide tablets.

Keyphrases □ Thiazides—dissolution–bioavailability comparison, chlorothiazide tablets □ Bioavailability—comparison to dissolution, thiazides, chlorothiazide tablets □ Dissolution—comparison to bioavailability, thiazides, chlorothiazide tablets □ Chlorothiazide—tablets, bioavailability–dissolution comparison, thiazides

A drug product such as a tablet or capsule consists of the active ingredient, the drug itself, and the inactive ingredients such as fillers, binding agents, lubricants, and other substances which, along with the process factors, influence the products' dissolution and its performance. The dissolution test has emerged as the single most important *in vitro* test for ensuring the quality of the solid oral dosage forms (tablets and capsules) and in many instances predicts the bioavailability of the product. In instances where the dissolution test results have been correlated with *in vivo* performance of the product, *in vitro* dissolution test criteria are used as part of bioequivalence requirements by the Food and Drug Administration. In the absence of *in vivo*-correlated *in vitro* results, the dissolution test can be used to ensure the characteristic of the batch.

The steps necessary to arrive at a meaningful correlation between *in vitro* dissolution and *in vivo* performance of a product include: (*a*) a dissolution survey of marketed multisource drug products by a method, (*b*) selection of drug products with a wide range of dissolution profiles for bioavailability study, (*c*) determination of apparent *in vitro–in vivo* correlation, and (*d*) refinement (if necessary) of the dissolution tests to reflect *in vivo* product performance and optimize the *in vitro–in vivo* correlation.

An *in vitro* dissolution test is reported for marketed chlorothiazide tablets which is associated with *in vivo* performance, and which could be utilized as a quality assurance test.

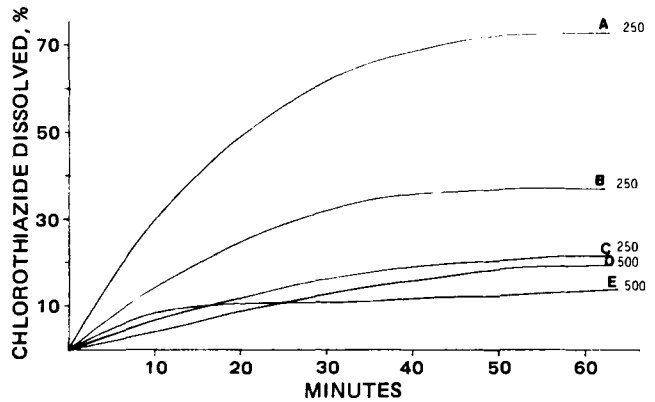

Figure 1—*Dissolution profiles of 250- and 500-mg chlorothiazide tablets in water by USP paddle method at 50 rpm.*

EXPERIMENTAL

Materials—The chlorothiazide tablets for the study were obtained directly from the manufacturers. The following products were used: 250 mg A, 250 mg B, 250 mg C, 500 mg D and 500 mg E[1].

***In Vitro* Dissolution Test**—The dissolution test was carried out by the procedure described in USP XX using the compendial Method II (paddle method) (1). Agitation speeds of 50, 75, and 100 rpm were used. The dissolution medium was distilled water, pH 7.4 or pH 8.0 phosphate buffer at 37 ± 0.2°.

***In Vivo* Tests**—The *in vivo* tests were carried out in 12 normal, healthy volunteers using a complete crossover Latin-square design. The urine samples were collected at intervals over a 72-hr period and were analyzed by a specific and sensitive HPLC method. The details of the study and the results are described elsewhere (2, 3).

RESULTS AND DISCUSSION

At present there is no dissolution test requirement for chlorothiazide 250- and 500-mg tablets in the compendia. The survey of dissolution profiles of marketed 250- and 500-mg tablets was carried out using a paddle method (USP XX, Method II) in water at 50 rpm. Several lots of the innovator's product were screened. The results of the survey (Table I, Fig. 1) suggest a wide variation in dissolution profiles under the test conditions. For 250-mg tablets, the dissolution varied from 15 to 81% in 30 min and from 21 to 88% in 60 min. Based on these results, three products with a wide range in dissolution profiles—slow dissolving product C, intermediate dissolving product B, and fast dissolving product A—were selected for the bioavailability–bioequivalence study. There are only two approved 500-mg chlorothiazide tablets on the market, and both showed very poor dissolution profiles. They were also used in the bioavailability–bioequivalence study.

The bioavailability–bioequivalence study was carried out in 12 healthy volunteers in a crossover fashion using 250-mg A, B, and C products. In another crossover study with 12 volunteers, 500-mg D and E products were used. The urine samples were collected at intervals over a 72-hr period and analyzed by a specific and sensitive HPLC method. The cumulative amount of drug excreted in 72 hr was calculated and used as a measure of the bioavailability of the product (Table II). The details of the *in vivo* studies are described elsewhere (2, 3).

From a 250-mg tablet, a mean of 42, 52, and 45 mg of chlorothiazide was recovered in 72 hr from C, B, and A tablets, respectively (Table II). The total amount of drug excreted in 72 hr was not significantly different between tablets. All products were bioequivalent.

In vitro dissolution results by the paddle method at 50 rpm in water could not be correlated with *in vivo* results because all the products showed approximately the same amount of drug elimination (absorption) in 72 hr and were bioequivalent. This suggests that the preliminary dis-

[1] 250 mg A: 250-mg chlorothiazide tablets from Danbury, batch no. 15069; 250 mg C: 250-mg chlorothiazide tablets from Merck, Sharp & Dohme, batch no. V1502; 250 mg B: 250-mg chlorothiazide tablets from Mylan, batch no. 066B; 500 mg E: 500-mg chlorothiazide tablets from Merck, Sharp & Dohme, batch no. 2762; and 500-mg D: 500-mg chlorothiazide tablets from Mylan, batch no. 054B.

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Table I—Dissolution of Chlorothiazide Tablets by Paddle Method Under Different Conditions [a]

Product	Water		pH 7.4 Phosphate Buffer						pH 8 Phosphate Buffer			
	50 rpm		50 rpm		75 rpm		100 rpm		50 rpm		100 rpm	
	30 min	60 min	30 min	60 min	30 min	60 min	30 min	60 min	30 min	60 min	30 min	60 min
250 mg C	16	22	27	35	73	91	92	98	32	48	108	111
	(3.4)[b]	(3.7)	(2.7)	(2.6)	(3.8)	(2.2)	(3.0)	(1.5)	(7.5)	(7.0)	(3.8)	(3.2)
250 mg B	32	37	37	55	71	82	73	83	51	76	76	86
	(5.5)	(6.6)	(2.5)	(2.4)	(2.1)	(2.6)	(2.3)	(2.5)	(5.7)	(9.0)	(1.3)	(1.1)
250 mg A	62	73	67	73	94	97	99	99	*NS*	*NS*	100	100
	(3.9)	(4.4)	(2.3)	(2.1)	(3.4)	(3.3)	(2.9)	(2.9)			(3.8)	(3.2)
500 mg E	11	14	26	34	79	96	95	98	31	41	103	105
	(4.2)	(4.8)	(2.1)	(4.6)	(4.0)	(3.1)	(2.6)	(1.4)	(3.6)	(4.1)	(3.6)	(2.6)
500 mg D	13	20	20	32	56	78	79	94	24	43	97	110
	(0.8)	(2.6)	(3.3)	(4.0)	(7.2)	(4.2)	(4.4)	(2.5)	(2.1)	(5.6)	(3.1)	(2.0)

[a] Data represents mean dissolution of six tablets. [b] The standard deviation is given in parenthesis.

solution method for chlorothiazide tablets in water was overly discriminative. Therefore, this *in vitro* dissolution test was modified in order to arrive at a meaningful *in vitro* test.

Two types of *in vivo–in vitro* relationships are possible. The first type of relationship is where different *in vitro* dissolution results can be correlated with differences in observed *in vivo* parameters such as *AUC*, *Ae*, C_{max}, *etc.* The second type of relationship is where all products tested are bioequivalent, and *in vitro* dissolution data is associated with a minimum dissolution rate. In the case of chlorothiazide, where all three 250-mg products tested are bioequivalent (*i.e.*, no difference in amount of chlorothiazide recovered from urine in 72 hr), an attempt was made to obtain the latter type of *in vitro–in vivo* relationship. From the latter type of *in vitro–in vivo* relationship, and in the absence of any bioinequivalent product, the *in vitro* dissolution method will at least serve as a quality assurance test. Since several formulations made by different manufacturers were used in this study, one can be reasonably assured that a new product meeting this *in vitro* test will be bioequivalent with existing products.

Using USP Method II, the dissolution of 250-mg chlorothiazide tablets was carried out in pH 7.4 and 8.0 phosphate buffer at 50, 75, and 100 rpm (Table I). The dissolution results at 50 rpm were found to be slow and highly discriminative, whereas the agitation at 100 rpm was found to be so drastic and indiscriminative that it may not differentiate between the currently marketed bioequivalent products and a possible poorly bioavailable future generic product. The agitation at 75 rpm in pH 7.4 phosphate buffer was found to be appropriate and reasonable for all bioequivalent 250-mg products. Using pH 7.4 phosphate buffer medium and 75 rpm agitation, a dissolution of 70–94% in 30 min and 82–97% in 60 min was observed (Table II).

There are only two approved 500-mg chlorothiazide tablets on the market, and both showed poor dissolution in water at 50 rpm (Table I).

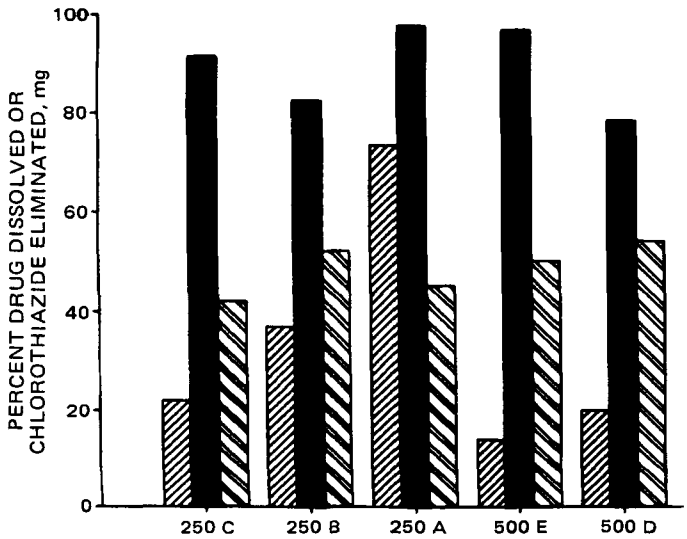

Figure 2—In vitro *dissolution (percent dissolved in 60 min) and* in vivo *cumulative elimination (milligrams in 72 hr) for 250- and 500-mg chlorothiazide tablets. Key: (▨) percent dissolved in water; (■) % dissolved in pH 7.4 phosphate buffer; and (▧) chlorothiazide eliminated in urine.*

Table II—Relationship Between *In Vitro–In Vivo* Parameters for Chlorothiazide Tablets

Product	*In Vitro* Dissolution Data[a]				*In Vivo* Cumulative[b] Excretion Data, mg mean ± *SD*	
	Water		pH 7.4 buffer			
	20 min	60 min	30 min	60 min		
250 mg C	16	22	73	91	42	15
250 mg B	32	37	71	82	52	25
250 mg A	62	73	94	97	45	19
500 mg E	11	14	79	96	50	13
500 mg D	13	20	56	78	54	15

[a] *In vitro* dissolution data obtained by paddle method in water at 50 rpm and pH 7.4 phosphate buffer at 75 rpm. [b] Represents mean cumulative excretion in 12 subjects.

Both products showed the same extent of absorption and elimination, and were bioequivalent. The dissolution of these two 500-mg products was studied under various test conditions. A dissolution of 56–79% in 30 min and 78–96% in 60 min was achieved by the paddle method at 75 rpm in pH 7.4 phosphate buffer.

As seen in Table II, approximately the same amount of drug was eliminated in urine in 72 hr from both 250- and 500-mg chlorothiazide tablets. There is no statistical difference in the bioavailability of these two products in normal healthy volunteers. The implications of this bioavailability finding are discussed separately (2–4). Both 250- and 500-mg tablets achieved approximately the same percent of dissolution in pH 7.4 phosphate buffer by the paddle method at 75 rpm. Figure 2 shows the relationship between *in vivo* and *in vitro* data. For comparison, *in vitro* dissolution data in water are also included. The figure clearly shows that the amount of drug eliminated in urine and the amount of drug dissolved in phosphate buffer are nearly the same in all five formulations studied.

In order to evaluate the appropriateness of the dissolution method and the acceptance limits as a routine quality control test, additional batches of chlorothiazide tablets were tested. These products[2] represented four manufacturers and were different from those that were used in the bioequivalency study. The results showed that all products dissolved >75% in 60 min, thus suggesting that these products would be bioequivalent.

A dissolution test which will ensure the quality of chlorothiazide tablets has been established. The product should dissolve not less than 75% in 60 min when the dissolution test is carried out by USP paddle method in 900 ml of pH 7.4 phosphate buffer at 37° and agitation of 75 rpm. On the basis of these findings, it is reasonable to expect that a product meeting such a dissolution criterion will be bioequivalent to the marketed product.

REFERENCES

(1) United States Pharmacopeia, XX, United States Pharmacopeial

[2] Bolar's 250-mg chlorothiazide tablet, batch no. 15319, 83% dissolution; Mylan's 250-mg chlorothiazide tablet, batch no. B027A, 77% dissolution; Danbury's 250-mg chlorothiazide tablet, batch no. 112935, 100% dissolution; and Merck, Sharp & Dohme's 500-mg chlorothiazide tablet, batch no. V1911, 83% dissolution.

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Effect of Skin Binding on Percutaneous Transport of Benzocaine from Aqueous Suspensions and Solutions

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Abstract □ Various aqueous suspensions of benzocaine containing nonionic surfactants exhibited the same rate of *in vitro* penetration through hairless mouse skin. Saturated solutions yielded a lower rate of benzocaine penetration due to depletion of drug from the donor portion of the diffusion cell. Extensive skin binding was responsible.

Keyphrases □ Benzocaine—effect of skin binding on percutaneous transport from aqueous suspensions and solutions □ Skin binding—effect on percutaneous transport of benzocaine from aqueous suspensions and solutions □ Percutaneous transport—effect of skin binding, of benzocaine from aqueous suspensions and solutions

The effect of surfactants on drug penetration through skin is an important aspect of formulation of topical drug products. The influence of these additives depends on the rate-limiting step in penetration, so that the type of preparation and conditions of application to the skin are important considerations. Penetration from aqueous solutions containing a fixed benzocaine concentration depended on the extent of benzocaine solubilization by surfactant included in the formula (1). Penetration flux was directly proportional to the unbound benzocaine concentration. A series of surfactant solutions saturated with benzocaine had the same rate of benzocaine penetration. No alteration of membrane permeability was found. This work was extended to aqueous gel suspensions to determine the rate limiting step in penetration and the influence of nonionic surfactants in these systems. The suspensions were similar to those utilized in previous studies of benzocaine release (2, 3).

EXPERIMENTAL

Materials and apparatus used for the *in vitro* penetration experiments were reported previously (1). Surfactants used in the formulations were commercially available polyoxyethylene nonylphenols[1]. The membrane was whole hairless mouse skin. The receptor solution (normal saline containing 0.25% chlorobutanol) was maintained at 37° and stirred at 500 rpm. The donor compartment was not stirred. Further details concerning experimental procedure appeared previously (1). All donor systems were studied in triplicate.

All the suspensions were prepared on a w/v basis. Accurately measured surfactant stock solution was transferred to a wide mouth glass jar containing a magnetic stirring bar. To that solution, the required amount of benzocaine was added and stirring was continued for ~15 min until all lumps were broken. A preweighed quantity of gelling agent was sprinkled on the top and stirring was continued for ~3 hr to yield a smooth homogeneous suspension. A control suspension without the drug was prepared for every formulation. All the suspensions were analyzed for benzocaine content and were stored at 30° until needed.

[1] Igepal CO series, GAF Corp., N.Y.

RESULTS AND DISCUSSION

Details of the suspension formulations are listed in Table I. In the case of all the formulations studied, when the cumulative amount of benzocaine in the receptor compartment was plotted against time, a linear relationship with a small lag time was observed. A typical plot is shown in Fig. 1. The gradual rise in penetration rate (Fig. 1) until steady state was achieved is typical of membrane controlled diffusion. When transport through the vehicle is rate limiting, the flux is initially high and then gradually decreases with time. A second indication that diffusion across the stratum corneum was the slow step in benzocaine transport is high flux values obtained from the suspension formulations, which will be discussed.

The flux values were calculated for all formulations (Table I). ANOVA showed that drug concentration, gelling agent, and surfactant concentration had no statistically significant effect on flux. No influence of the surfactants on skin membrane integrity was evident.

These results are similar to those with saturated benzocaine solutions (1). However, there is a surprising difference in the magnitude of the penetration values when saturated solutions are compared to suspensions. Mean benzocaine flux from saturated solutions was ~60 $\mu g\ hr^{-1}\ cm^{-2}$ (1), whereas the average flux for suspensions was 101 $\mu g\ hr^{-1}\ cm^{-2}$. This difference was unexpected since the free benzocaine concentration, which provides the driving force for diffusion, should have been the same in both types of preparation.

In penetration experiments, the amount of drug transported during the course of an experiment is usually small enough to justify the assumption that the donor concentration is constant. In conducting experiments on benzocaine solutions, there had been no reason to question the validity of this basic assumption. However, in view of the difference in benzocaine flux from saturated solutions as compared to suspensions, it was hypothesized that depletion of drug from the donor must have been taking place in the solution systems during the course of the penetration experiments.

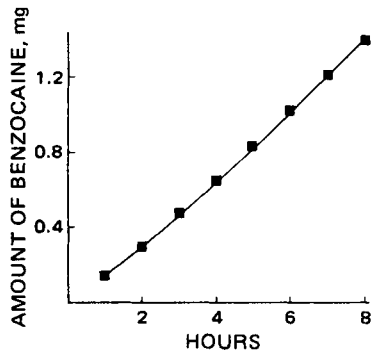

Figure 1—*Penetration of benzocaine through hairless mouse skin from a 5% suspension containing 0.0227 M polyoxyethylene (15) nonylphenol.*

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