BIOPHARMACEUTICS & DRUG DISPOSITION, VOL. 5, 11–19 (1984)

PRELIMINARY OBSERVATIONS ON DISSOLUTION AND BIOAVAILABILITY OF TRIAMTERENE–HYDROCHLOROTHIAZIDE COMBINATION PRODUCTS

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ABSTRACT

The dissolution profiles of two brands of triamterene–hydrochlorothiazide (TRM–HCT) combination tablets and two brands of TRM–HCT combination capsules were studied using the USP paddle method at 100 rev min^{-1} in acid medium (0·1*N*). The tablets represent two products marketed in Germany, whereas the capsules represent the approved innovator's product and an unapproved generic product. The tablets dissolved almost 100 per cent in 15 min whereas the capsules dissolved less than 25 per cent in 60 min. A pilot bioavailability study was carried out in four normal healthy male volunteers. Urine samples were collected over a 48 h period and analysed for TRM, its major metabolite TRM-sulfate, and HCT using HPLC methods. The dissolution characteristics of TRM can be associated with the total drug excretion (absorption) of the product. On the other hand, the excretion (absorption) of HCT was independent of dissolution characteristics of the products. However, in TRM–HCT combination product, there appears to be a 50 per cent reduction in HCT excretion (absorption) when compared to the reported excretion (absorption) from a marketed single-entity product.

KEY WORDS Dissolution Bioavailability Triamterene–hydrochlorothiazide product

INTRODUCTION

The triamterene–hydrochlorothiazide (TRM–HCT) combination product represents a diuretic and antihypertensive drug product that combines two natriuretics. The combination product, a potassium-sparing diuretic, is used as adjunctive therapy in edema associated with congestive heart failure, cirrhosis of the liver, and nephrotic syndrome.[1] Recently, the Food and Drug Administration (FDA, Agency) recalled an unapproved generic TRM-HCT combination product because of a serious question of its safety, efficacy, and potential serious risk to patients. Using radioactive and non-radioactive triamterene (TRM), Pruitt *et al.*[2] have shown that TRM is incompletely

0142–2782/84/010011–09$01.00

Received 30 November 1982
Revised 5 April 1983

absorbed from the gastrointestinal tract, and that the drug undergoes extensive metabolism. In normal subjects, the bioavailability of TRM varies between 30 and 70 per cent. Tannenbaum *et al.*[3] compared the bioavailability of TRM–HCT combination products from tablets and capsules and found that, overall, the tablet formulation was more bioavailable than the capsule formulation.

Several research articles have appeared in the literature for the analysis of TRM and its metabolites in plasma and urine samples.[4–8]

This paper describes a new, improved HPLC method for determination of TRM and its major metabolite, triamterene sulfate (TRM-sulfate), in urine. It also compares the bioavailability of TRM and hydrochlorothiazide (HCT) from tablet and capsule dosage forms, and compares bioavailability with *in vitro* dissolution characteristics of the products.

EXPERIMENTAL

Materials

TRM and TRM-sulfate were obtained from Smith, Kline and French, Philadelphia, PA 19101. HCT and sulfadiazine were obtained as RS from USP. 3,5-Dibromosalicylic acid was obtained from Aldrich Chemical Co., Milwaukee, WI. Acetonitrile and methanol, distilled in glass, were obtained from Burdick and Jackson, Muskegan, MI. All materials were used as obtained without further purification.

The TRM–HCT combination products used in the study were:

A. Dyazide Capsules, Lot No. 2659E90; Smith, Kline and French, Philadelphia, PA.
B. Triam-thiazide Capsules, Lot No. 109024; Premo Pharmaceutical Laboratories, South Hackensack, NJ.
C. Tri-Thiazide Stada Tablets, Lot No. 472; Stada, Germany.
D. Dytide H Tablets, Lot No. 80130; Rohm Pharma, Germany.

Dissolution studies

The dissolution studies for TRM–HCT tablets and capsules were carried out using the paddle method, USP Method II,[9] at 100 rev min^{-1} in 0·1*N* hydrochloric acid. The amount of drug dissolved at 30 min and 60 min intervals was determined using a spectrophotometer. At the end of 30 min and 60 min, 10 ml of the dissolution medium was removed and filtered through a millipore filter. The absorption of the sample was measured at 272 nm (λ_{max} for HCT) and 358 nm (λ_{max} for TRM). From the absorbance reading, the percentage drug dissolved was calculated.

Hydrochlorothiazide does not exhibit any absorbance at the λ_{max} for triamterene. The amount of TRM was calculated from the standard absorbance of TRM. Triamterene exhibits some absorbance at the λ_{max} for HCT. The interference is directly proportional to the amount of TRM. A correction factor

was applied to the TRM value and subtracted from the reading at 272 nm to obtain the true absorbance reading for HCT. The amount of HCT was calculated from this corrected absorbance reading.

Bioavailability study

The study was carried out in four healthy male volunteers using four products in a crossover Latin square design with a 1-week washout period between dosage administration. The drug was administered after an overnight fast with 250 ml of water. Urine samples were collected before drug administration (time 0) and at 0–10, 10–24, 24–34, and 34–48 h intervals after drug administration. The urine samples were refrigerated during the collection interval. The volume of urine was measured and an aliquot of the sample was kept frozen until analysed.

Analysis of urine samples

Instrumentation: Urine samples were analysed using an HPLC system capable of flow programming under isocratic conditions. The HPLC system was equipped with a 30 cm × 4 mm i.d. micropak reverse-phase column (MCH-10), 254 nm fixed-wavelength uv detector (for hydrochlorothiazide) or a variable-wavelength fluorescence detector (for triamterene), and a strip chart recorder.

Hydrochlorothiazide

Exactly 1 ml of urine sample was spiked with 100 mcl of methanol containing 4 mcg of sulfadiazine (internal standard) and mixed. Exactly 30 mcl of the sample was injected on the HPLC column using a Valco loop injector. The mobile phase for the analysis of HCT consisted of 5 per cent acetonitrile in 0·1 per cent aqueous potassium dihydrogen phosphate. The flow rate was controlled using a flow program under isocratic conditions:

Time (min)	Flow (ml min^{-1})
0–27·0	1·0–1·9
27·1–30·0	1·9–1·0

The eluent was monitored using a 254 nm fixed-wavelength u.v. detector. The retention times for HCT and sulfadiazine were 18 and 21 min, respectively. The peak height ratio of HCT to sulfadiazine was used to calculate the amount of HCT from standard curve data.

The standard curve was prepared using blank urine samples spiked at concentrations of 0, 1, 2·5, 5, 10, 25, 50, and 100 mcg ml^{-1} of HCT and 4 mcg of sulfadiazine in 100 mcl of methanol. The standard curve was linear in the range studied ($r = 0·9989$). The method was precise (percentage C.V. was 8·5 at 1, 8·9 at 2·5, 4·0 at 5, 1·1 at 10, 4·0 at 25, 1·0 at 50, and 3·7 at 100 mcg ml^{-1}) and reproducible. Since the method did not involve any extraction, the recovery was complete. The limit of detection was 1 mcg ml^{-1}.

Triamterene

Exactly 1 ml of urine sample was spiked with 100 mcl of methanol containing 2 mg of 3,5-dibromosalicylic acid (internal standard) and mixed. Exactly 10 mcl of the sample was injected on the HPLC column using a Valco loop injector. The mobile phase for the analysis of TRM and its metabolites consisted of 45 per cent methanol in 0·1 per cent aqueous potassium dihydrogen phosphate. The flow rate was controlled using a flow program under isocratic conditions:

Time (min)	Flow (ml min^{-1})
0–10·0	1·0–3·5
10·1–12·0	3·5–1·0

The eluent was monitored using a fluorescence detector set at 365 nm for excitation and 440 nm for emission wavelengths. The retention time for triamterene sulfate (TRM-sulfate), 3,5-dibromosalicylic acid, and triamterene were 2·8, 4·5, and 8·8 min, respectively. The peak height ratios of TRM-sulfate to 3,5-dibromosalicylic acid and TRM to 3,5-dibromosalicylic acid were used to calculate the amounts of TRM-sulfate and triamterene from the respective sets of standard curve data.

The standard curve was prepared using blank urine samples spiked at 0·05, 0·1, 0·25, 0·5, 1·0, 1·5, 2, 3, and 5 mcg ml^{-1} of TRM and TRM-sulfate and 2 mg of 3,5-dibromosalicylic acid in 100 mcl of methanol. The standard curve was linear in the range studied ($r = 0{\cdot}9969$ for TRM and $r = 0{\cdot}9996$ for TRM-sulfate). The method was precise (percentage C.V. was 4.4 at 0·05, 7·0 at 0·1, 8·9 at 0·25, 6·8 at 0·5, 1·3 at 1·0, 1·0 at 1·5 and 0·99 at 2 mcg ml^{-1} of TRM and 4·5 at 0·05, 8·0 at 0·1, 8·5 at 0·25, 2·2 at 0·5, 3·5 at 1·0, 2·8 at 1·5, 3·8 at 2, 7·4 at 3 and 7·1 at 5 mcg ml^{-1} of TRM-sulfate) and reproducible. Since the method did not involve any extraction, the recovery was complete. The limit of detection was 0·1 mcg ml^{-1} for both triamterene and triamterene sulfate.

RESULTS AND DISCUSSION

A simple HPLC method with flow programming under isocratic conditions was used for the analysis of urine samples. The method is simple, sensitive, accurate, and reproducible. It does not require any extraction, and both TRM and its major metabolite, TRM-sulfate, can be determined from a single injection. Flow programming under isocratic conditions thus offers several advantages: (a) drugs and metabolites, which otherwise might require different analytical conditions and more than one sample injection, can be analysed in a single injection; (b) the column is not exposed to different solvent conditions as in gradient elution, and is thereby not subjected to 'shocks' during a run; and (c) the column is always equilibrated with the solvent.

The dissolution profile of TRM–HCT combination products was determined using the paddle method at 100 rev min^{-1} in 900 ml of 0·1*N* hydrochloric acid.

Table 1. Dissolution profile of triamterene–hydrochlorothiazide products by paddle method at 100 rev min^{-1} in acid medium

Product	Triamterene % dissolved in 15 min	30 min	60 min	Hydrochlorothiazide % dissolved in 15 min	30 min	60 min
A*	—	4·2 (0·4)†	8·4 (0·8)	—	4·1 (0·5)	9·0 (0·9)
B*	—	8·3 (1·6)	20·2 (4·5)	—	12·3 (1·7)	27·8 (5·7)
C	97·9 (3·6)	99·0 (2·1)	—	98·6 (4·8)	99·6 (2·6)	—
D	71·0 (3·5)	97·5 (5·0)	—	68·0 (3·3)	96·7 (3·9)	—

* Almost 90–95 per cent dissolution was obtained as an infinity dissolution reading.
† Standard deviation.

Product A is the approved marketed capsule of the innovator firm in this country and Product B is the unapproved capsule of a generic firm in this country. Products C and D are tablets marketed in Germany. The dissolution characteristics of these products (Table 1) reveal that the tablets undergo rapid and complete dissolution (of both components) in 15 min, whereas the capsules exhibit very poor dissolution characteristics (for both components), even under very vigorous agitation. The tablets dissolved almost 100 per cent in 15 min, whereas the capsules dissolved only 25 per cent in 60 min. Of the two capsules, Product A was slower dissolving compared to Product B. The differences in dissolution between the two brands of capsules were almost twofold.

In the bioavailability study, the urine samples were analysed for TRM, TRM-sulfate, and HCT. Since the purpose of this pilot study was to compare the extent of bioavailability of the combination products and compare them with *in vitro* data, urine samples were not collected at small frequent intervals. The collection period was adjusted for convenience, as well as to obtain a rough estimate of drug elimination over time. For TRM and TRM-sulfate, the total amount eliminated in 48 h was determined. Bioavailability of TRM was estimated by multiplying the amount of TRM-sulfate by 0·725 (to convert it to an equivalent amount of TRM) and adding it to the amount of TRM. Although triamterene hydroxide has been reported as one of the metabolites of TRM, it could not be detected in urine samples in this study.

The results of the bioavailability study in four subjects are summarized in Tables 2 and Figures 1 and 2. The table shows the amount of TRM, TRM-sulfate, total TRM, and HCT eliminated in 48 h. Triamterene is extensively metabolized to sulfate ester. On an average, the amount of TRM-sulfate was 5–7 times the amount of the parent compound in urine. A total of 15–16 per cent of TRM was recovered in 48 h from the capsule dosage form (A and B). On

Table 2. Amount* (mean ± S.D.) of TRM, TRM-sulfate, and HCT eliminated in urine after oral administration of 50 mg TRM/25 mg HCT product

Product	Time	TRM mean (mg)	±S.D.	TRM-sulfate† mean (mg)	±S.D.	Total TRM mean (mg)	±S.D.	HCT mean (mg)	±S.D.
A	0–10	1·34	1·08	4·50	1·28	5·84	2·3	5·47	3·37
	10–24	0·17	0·09	1·05	0·59	1·22	0·7	1·36	0·93
	24–34	0·09	0·10	4·60	0·30	0·55	0·4	0·44	0·64
	34–48	0·01	0·01	0·15	0·09	0·16	0·1	0·62	0·84
	Total (0–48)‡	1·62	1·20	6·15	2·09	7·77	3·1	7·89	3·88
B	0–10	1·13	0·31	4·65	1·88	5·78	2·2	4·65	1·17
	10–24	0·26	0·38	1·23	1·53	1·49	1·9	2·41	1·88
	24–34	0·05	0·35	0·37	0·44	0·42	0·5	1·24	0·97
	34–48	0·02	0·01	0·16	0·08	0·18	0·1	0·94	1·22
	Total (0–48)	1.46	0·42	6·41	2·20	7·87	2·6	9·18	4·18
C	0–10	3·47	0·94	12·04	2·02	15·51	2·4	5·17	1·12
	10–24	0·07	0·02	0·51	0·29	0·58	0·3	1·70	0·20
	24–34	0·05	0·03	0·51	0·32	0·56	0·3	0·95	0·66
	34–48	0·05	0·02	0·27	0·22	0·32	0·3	0·20	0·40
	Total (0–48)	3·66	0·91	13·31	1·91	16·97	2·2	8·02	1·08
D	0–10	2·63	0·59	13·82	5·27	16·45	5·7	4·23	0·46
	10–24	0·21	0·27	0·90	0·70	1·11	1·0	3·73	5·53
	24–34	0·07	0·12	0·25	0·46	0·32	0·6	0·40	0·59
	34–48	0·01	0·02	0·10	0·12	0·11	0·1	0·00	0·00
	Total (0–48)	2·92	0·70	15·07	4·50	17·99	4·8	8·36	5·65

* Data represents mean value of four subjects.
† Expressed as equivalent amount of triamterene.
‡ 0–48 h time interval.

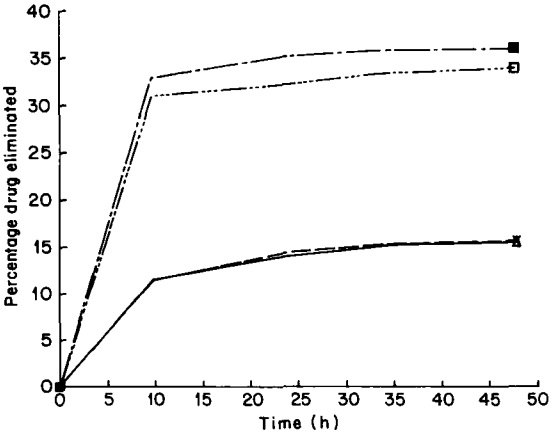

Figure 1. Percentage cumulative urinary excretion of triamterene after administrating 50 mg TRM/25 mg HCT. Key: △, A-cap; x, B-cap; □, C-tab; ■, D-tab

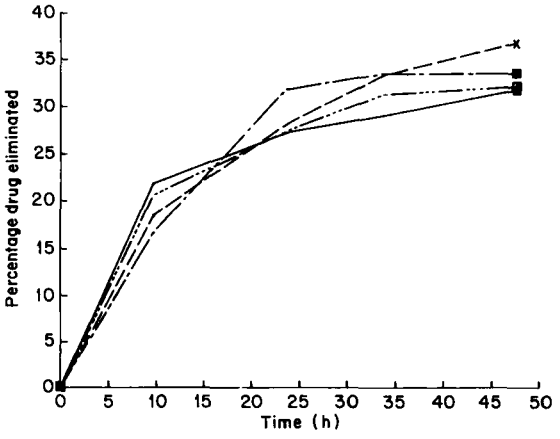

Figure 2. Percentage cumulative urinary excretion of hydrochlorothiazide after administering 50 mg TRM/25 mg HCT. Key: △, A-cap; X, B-cap; □, C-tab; ■, D-tab

the other hand, about 35 per cent of TRM was recovered in 48 h from the tablet dosage form. Thus, the data indicate that TRM is about twice as available from the tablet dosage form (Figure 1) as from the capsule dosage form. This observation is consistent with the findings of Tannenbaum *et al.*[3] The data also show high intersubject variability. The tablets show good dissolution characteristics and exhibit better bioavailability, whereas the capsules show poor dissolution characteristics and exhibit poor bioavailability (Figure 3). Thus, the bioavailability of the TRM component of the combination product can be associated with its dissolution. This is consistent with the observations of Stuber *et al.*[10] Stuber *et al.* used TRM tablets, coated TRM tablets, and TRM capsules in their study and had carried out the dissolution studies using paddle method, basket method, and flow through apparatus. They showed that the dissolution

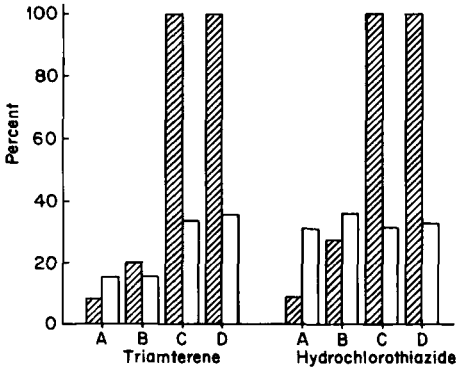

Figure 3. *In vitro–in vivo* relationship for triamterene–hydrochlorothiazide. Key: ▨, % dissolved; □, % excreted

results by the paddle method correlated best with the bioavailability (AUC and C_{max}) of the product. In the present study, we were able to associate the dissolution results of capsules and tablets by the paddle method with the extent of bioavailability (Ae) of the products.

TRM–HCT drug administration requires continuous patient titration when used in the treatment of edema or hypertension. A significant increase in rate and extent of absorption (i.e. bioavailability) of TRM (due to differences from brand to brand) can result in electrolyte imbalance (particularly hyperkalemia) in susceptible patients. Especially at risk are elderly patients with compromised kidney function resulting from diabetes or hypertension. Abnormal elevation of serum potassium can result in sudden death from cardiac irregularities.[11,12] It is therefore essential that all marketed TRM–HCT preparations be bioequivalent to preclude such adverse effects.

In the data submitted to the Agency for the generic Product B, the C_{max} values of the TRM were reported to be 7–8 fold higher than those achieved by the innovator's product. This might be due to a difference in the observed dissolution rates of Products A and B. The observed difference in C_{max} values between the two products raised a serious question of the safety and potential risk of the generic unapproved TRM–HCT product. This is why that product was recalled from the market.

Table 2 also shows the amount of HCT recovered (absorbed and eliminated) from urine in 48 h. The amount recovered in 48 h represents about 32–37 per cent of the drug administered. The amount was nearly the same from capsules and tablets, and could not be associated with the dissolution characterstics of the HCT component (Figure 3). This indicates that for HCT absorption, the dissolution of the product is not the rate-limiting step. The bioavailability of the HCT component observed here is approximately half the bioavailability reported after administration of HCT as a single entity.[13,14] The low recovery (absorption) of HCT from TRM–HCT combination products observed here is consistent with the observation of Tannenbaum *et al.*[3] It is also of interest to note that Brodie *et al.* observed a significant reduction in the bioavailability of another thiazide diuretic, bemetizide, when administered with triamterene.[15]

This observation of low HCT recovery raises the possibility of an interaction between TRM and HCT resulting in lower bioavailability of the HCT component. Although formulation factors would normally be suspected as the cause of decreased HCT bioavailability, they are unlikely, since both tablets and capsules result in lower HCT bioavailability. It appears that somehow, in the presence of TRM, HCT may not be well absorbed, at least when administered in the ratio of 2 : 1. If this is true, then the question of administering TRM–HCT combination product in the ratio of 2 : 1 to patients must be addressed.

A clinical study using TRM and HCT in different combination ratios and arriving at the optimum ratio has not been reported. Heath and Freis[16] reported a study where TRM and HCT were used in the ratio of 2 : 1 and found to be clinically effective, based on sodium and potassium elimination. Other

combinations have not been reported. In addition, drug absorption–elimination characteristics were not evaluated in their study. It is of interest to note that in Finland,[17] the combination product contains 75 mg TRM and 50 mg HCT (ratio of 1·5 : 1). A new study would be required to determine whether the purpose or effect of the proportionately higher amount of HCT is to overcome the interference in absorption of HCT in the presence of TRM.

The possibility of interaction between TRM and HCT, and the optimum combination ratio, are currently under investigation.

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