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Comparative Bioavailability Study of Two Brands of Terbutaline Sulphate Tablets in Healthy Human Volunteers

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Abstract

The purpose of this study was to evaluate the bioavailability of locally produced 2.5 mg terbutaline sulphate tablets (brand A) relative to a reference product, Bricanyl 2.5 mg tablets (brand B). The study was a single dose 5 mg randomized crossover one in 15 healthy volunteers in the fasting state. Urine was collected at intervals of 24 h. Total terbutaline excreted in urine as unchanged drug and as conjugates (sulphate and glucuronide) was determined by a developed and validated HPLC method. *In-vitro* characteristics of both brands were similar. Based on percent of the dose excreted in urine, the oral bioavailability ranged from 33.5% to 75.8% for both brands. Statistics were applied to judge bioequivalence according to USP 24 *in-vivo* bioequivalence guidance. Results indicated that brand A and B were bioequivalent and hence interchangeable in medical practice.

Keywords

Terbutaline sulphate; Tablet; Bioequivalence; Urine; HPLC analysis

Introduction

Terbutaline sulphate, 1-(3,5- dihydroxyphenyl)-2-(t-butylamin)ethanol sulphate is a β_2 adrenergic agonist used mainly in the treatment of asthmatics to produce bronchodilatation. The drug is administered orally, parenterally and by inhalation. The bioavailability of oral terbutaline ranges from 25 to 80% in different individuals

and is decreased by food. Around 60 % of the absorbed drug, following oral administration, circulates conjugated mainly to sulphate and less to glucuronide, both of which are pharmacologically inactive, and are formed presystemically in gut wall and liver. 90% of the absorbed drug including free and conjugated terbutaline is excreted in urine (1-4). These facts coupled with low plasma terbutaline levels in the ng mL^{-1} range (4), suggest that urinary excretion data of total terbutaline could be a possible substitute to blood level in comparative bioavailability studies.

The aim of the present study is to compare the bioavailability of locally produced 2.5 mg terbutaline sulphate tablets with a reference product in healthy volunteers, by measuring total free and conjugated terbutaline excreted in urine, in a single dose crossover study, using a sensitive, rapid and validated HPLC method.

Experimental

Subjects

Fifteen healthy male volunteers participated in the study after obtaining their signed informed consent. Their age ranged from 21 to 41 years (mean, 31.9), and their mass from 56 to 95 kg (mean, 73.7). They were judged to be healthy by a physician after physical examination and clinical laboratory tests. The study was performed in accordance with the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board and the Risk Involving Human Subject Committee of the FDA. The volunteers were instructed to abstain from taking any medication one week prior to and during the study week.

Materials

The dose administered was 5 mg terbutaline sulphate as either two tablets (brand A), each containing 2.5 mg terbutaline sulphate produced by Arab Drug Co.,

Egypt, or as two Bricanyl tablets (brand B), each containing 2.5 mg terbutaline sulphate produced by Astra, (Sweden, Batch No TF 225).

Other materials used include an ion-pairing agent for extracting free terbutaline from urine into chloroform, di-(2-ethylhexyl)-phosphate from Sigma Chemicals Co. (USA), acetonitrile far UV (Hiper Solv, BDH), chloroform (Analar, BDH), terbutaline sulphate powder supplied by the Arab Drug Co.,

Apparatus

The HPLC apparatus comprised a single piston pump (Waters model 501, Zaters associates, USA), a reversed phase C_{18} column; 150 ×3.9 mm i.d.; 5μm, particle size (Nova-Pak, Ireland), a tunable absorbance detector (Model 486, Waters Associates), a loop injector equipped with a 50 μl loop (Rheodyne, USA) and a data module integrator (Model 746, Waters Associates).

Study design

The study was random two-way crossover design (Table1). Each subject received a single dose of either brand A or B with 200 mL of water after an overnight fast. Subjects continued fasting for further 4 h but drank water regularly. A standard meal was served 4 h after dosing. Urine was collected just before dosing and at 2, 4, 6, 8 and 24 h. Urine volume was recorded and an aliquot was frozen (-18° C for a maximum of 2 weeks). After a period of 7 days, the study was repeated in the same manner to complete the crossover design. No drug could be detected by HPLC in urine collected 24-48 h in a pilot experiment.

Chromatographic conditions

Urine samples were analyzed for terbutaline according to a sensitive selective and accurate high performance liquid chromatography (HPLC) method.

The method of extraction of terbutaline from urine samples was adapted with some modification from a method reported for the extraction of salbutamol from plasma

(5) as terbutaline is very close chemically to salbutamol. The method was developed and validated before the study in our laboratory.

Chromatographic conditions were as follows: The mobile phase consisted of 5% acetonitrile and 1% methanol in 0.5% acetic acid adjusted to pH 4.0. Injected sample volume was 50 μl, flow rate of degassed mobile phase 1 ml. min^{-1} at ambient temperature, UV detector set at 276 nm at an attenuation of 0.04 aufs. No interfering peaks from urine samples was observed. Detection of terbutaline was achieved by monitoring the absorbance at a wavelength of

Table 1 Study design

Group	Number of volunteers	Treatment	
		Period 1	Period 2
I	(7)	A	B
II	(8)	B	A

276 nm; the peak area was measured and the concentration was calculated using the corresponding linear regression equation relating peak areas of the standards to their concentrations. The retention time of terbutaline was 5.3 min. Each analysis required a maximum of 8 min. The method was validated by following international guidelines (6).

Processing of urine samples and standards

Fifty μL of 10 mol L^{-1} HCL were added to one mL of each urine sample and the tubes were incubated at 40 °C overnight to hydrolyze terbutaline conjugates (1). Hydrolysed urine samples were neutralized with 50 μL 10 mol L^{-1} NaOH. One mL of phosphate buffer (0.5 mol L^{-1}, pH 7.2) was added together with 6 mL of 0.1 mol L^{-1} diethylhexyl phosphate in chloroform. The tubes were vortexed for 5 minutes and

centrifuged at 3000 rpm for 20 minutes. The urine layer was discarded and the remaining chloroform layer washed with 3 mL water which was separated and discarded, and the drug in chloroform was extracted into 500 μL 0.5 mol L^{-1} HCL. An aliquot of 50 μL of this acidic extract was injected onto the HPLC column. Recovered standards, 2-6 μg. mL^{-1} terbutaline sulphate in blank urine were processed with each set of samples.

In vitro studies

Samples of the two brands were tested according to the USP 24 (7) compendial requirement with respect to their mass variation, content uniformity and dissolution profile was performed USP apparatus I, 100 rpm, 900 mL of water at 37 ± 0.5° C) Samples were withdrawn at designated time intervals and assayed by HPLC. Six μL 10 mol L^{-1} HCL were added to each mL sample before injection to ensure peak sharpness. HPLC conditions were as detailed above.

Statistical analysis

Two-way analysis of variance (ANOVA, GLM procedure; using Win Nonlin computer program (8) for crossover design was used to asses the effect of formulation, periods, sequences and subjects within sequence on percent of the dose excreted in urine (9). Sequence effects were tested against the mean square term for subjects within sequence. All other effects were tested against the mean square error term. The difference between two brands was considered statistically significant for p-values equal to or less than 0.05. Parametric 90% confidence intervals (10) based on the ANOVA of the mean difference (A-B) of % terbutaline excreted values were computed.

Results and Discussion

Results of compendial in vitro tests for brand A & B (Table 2) indicated that both brands met specifications required by USP 24.

Table 2 In vitro data for terbutaline sulphate tablets

USP 24 tests	Brand A	Brand B
Mass variation (10 individual tablets, mean mass± SD)	182.29 ± 1.61	183.86 ± 1.80
Content uniformity (10 individual tablets, mean content as % of labeled amount ±SD)	104.10 ± 0.44	98.40 ± 0.31
Dissolution limit	100% within 15 minutes	100% within 10 minutes

The cumulative amount (%) of terbutaline dissolved in vitro from both brands of test tablets and reference tablets is plotted as a function of time in Fig. 1. The results are within the range of USP 24 dissolution requirements; not less than 75% of the labeled amount of terbutaline is dissolved in 45 min.

A representative standard line of terbutaline sulphate within concentration range of 2-6 μg. mL^{-1} resulted in R of 0.9978.

Assay recovery values, obtained by comparing peak areas of recovered standards with direct injection of standards in 0.5 mol L^{-1} HCL were 63.3 % ±17.1 (n=23) at2μg.mL^{-1}, 64.7 % ±12.4 (n=30) at 4 μg .mL^{-1} and 69.8% ± 12.7 (n=32) at 6 μg. mL^{-1}

The intra-day precision of the assay (Table 3) was evaluated by replicate (n=3) analyses of urine samples containing terbutaline sulphate at three different concentrations. The relative standard deviation ranged from 1.2 to 8.3%. The inter-day precision of the assay was assessed by performing several standard curves using three different concentrations over a range of 2-6 μg mL^{-1} in blank urine and were repeatedly analyzed on different days over a period of 32 days. The inter-day relative standard deviation varied from 4.3 to 12.6%.

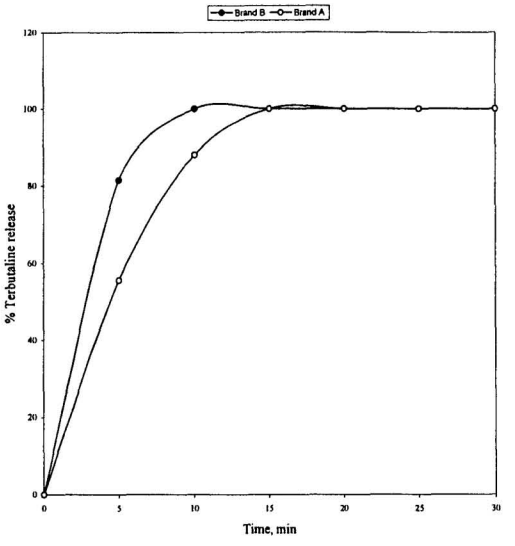

Figure 1: Dissolution rate of (o) brand A, (•) brand B tablets according to USP 24 test, each point is the mean of 6 tablets.

Table 3: Inter-day and intra-day precision of terbutaline sulphate in blank urine

Terbutaline sulphate added ($\mu g.mL^{-1}$)	Inter-day RSD (%)[a]	Intra-day RSD (%) [b]
2	12.6	8.3
4	5.7	4.2
6	4.3	1.2

Number of independent analyses: [a] $n = 32$, [b] $n = 3$

Table 4 gives mean cumulative excretion ± SD obtained for each brand at different times. Mean excretion rates are shown in Figure 2. Eight of thirty individuals excretion rate profiles showed double peaks; the earlier peak appearing at 1 h and later one at 5-7 h. similar double peaks in serum

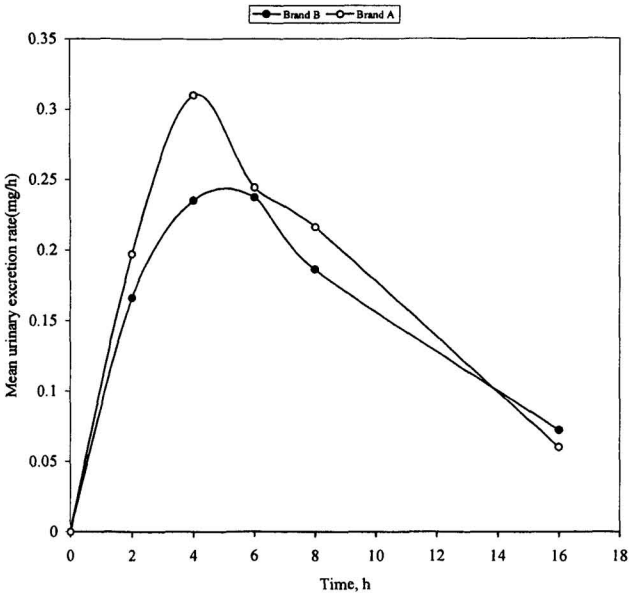

Figure 2. Mean urinary excretion rate of terbutaline expressed as terbutaline sulphate, following the oral administration of 5 mg terbutaline sulphate to 15 volunteers, (o) brand A, (•) brand B

Table 4 Mean cumulative total terbutaline excreted in urine 24 h following 5 mg oral dose of terbutaline sulphate

Time (h)	Mean cumulative excretion, mg (±SD)[a]	
	Brand A	Brand B
2	0.332 (0.18)	0.395 (0.17)
4	0.802 (0.29)	1.016 (0.42)
6	1.274 (0.39)	1.505 (0.48)
8	1.647 (0.41)	1.937 (0.47)
24	2.794 (0.52)	2.897 (0.54)

[a] Calculated as terbutaline sulphate.

terbutaline profiles following oral administration have been reported (4).

Percent of the dose excreted as unchanged terbutaline and as conjugates in 24 h is shown in Table IV. The values obtained reflect percent absorbed and ranged from 33.5% to 76.6 in agreement with reported value for bioavailability of terbutaline after one application ranging form 25 to 80 % (1, 2). On the basis of the % of dose excreted in the 15 volunteers, the bioavailability of brand A relative to B ranged from 76.9% to 115.5 with a mean of 97.4 % ± 12.8 (Table 5).

ANOVA results are given in Table 6. Effects of sequence, period and treatment proved insignificant difference between the two formulations. The 90% confidence interval for the mean difference in percent excreted between A and B fell within the conventional bioequivalence FDA acceptable range of 80- 125% (Table 7), and indicated that the two brands were bioequivalent and thus considered therapeutically equivalent.

Table 5 Percent of the dose excreted in urine and percent relative bioavailability

Volunteer code	Percent of the dose excreted in urine in 24 h		Percent relative bioavailability, (A/ B) × 100
	Brand A	Brand B	
1	75.8	76.6	98.5
2	41.1	53.4	76.9
3	47.2	42.1	112.1
4	59.9	65.5	91.5
5	60.8	64.4	94.3
6	59.8	65.2	91.7
7	58.2	50.4	115.5
8	47.5	59.6	79.8
9	49.0	60.0	81.7
10	67.4	58.6	114.9
11	65.1	69.3	93.9
12	47.8	52.7	90.7
13	38.2	33.5	114.1
14	56.9	54.8	103.8

15	64.9	63.7	101.8
Mean bioavailability			97.4 ± 12.8

Table 6 Analysis of variance applied to percent of the dose excreted in urinein 24 h

Source	Degree of freedom	SS	MS	F
Sequence	1	325.79	325.79	1.73 NS
Subjects within sequence	13	2444.53	188.04	
Period	1	16.25	16.25	0.63 NS
Treatment	1	31.29	31.29	1.22 NS
Error	13	334.90	25.76	
Total	29	3152.76		

SS= sum of square

MS= mean square

F = MS/EMS

EMS= error mean square

Table 7 Confidence interval for the difference in percent excreted between brand A & B

Parameter	Value
Mean percent excreted, brand A	55.9 ± 10.26
Mean percent excreted, brand B	57.9 ± 10.34
90% Confidence interval expressed as percent relative bioavailability*	92.3% ↔ 100.7

*Express as: Mean $\pm t \{ \frac{SD}{\sqrt{n}} \}$

Conclusion

The present study documents urinary excretion data following administration of two brands of terbutaline tablets to healthy volunteers.

Analysis of variance and confidence intervals permitted judgment of bioequivalence. Both brands can be considered equally effective in medical practice.

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