PHARMACOKINETICS AND DISPOSITION

R. Koytchev · R.-G. Alken · M. Vagaday · U. Kunter
V. Kirkov

Differences in the bioavailability of dihydrotachysterol preparations

Abstract The bioavailability of four preparations containing dihydrotachysterol (DHT_2) was tested in two separate trials with administration of single, oral doses of 1 mg per individual. The relative bioavailability of corresponding preparations (capsules vs capsules and oral solution vs oral solution) was tested in a randomised, crossover pattern within the same group of volunteers. Two different groups of 24 healthy volunteers took part in each trial. Solution and capsule bioavailability was also compared inter-individually. A new sensitive HPLC-method (quantification limit 0.5 ng · ml^{-1}) was used for the measurement of DHT_2 concentration in serum.

Three of the preparations tested had a similar bioavailability (mean AUC values of 195.5–223 ng · h · ml^{-1}); the bioavailability of the fourth preparation (A.T.10 oral solution) was considerably lower (mean AUC value 111.5 ng · h · ml^{-1}). The present dosage recommendations of all four preparations are identical.

A new dosage recommendation is thus required for the oral solution with low bioavailability (A.T.10).

Key words Dihydrotachysterol; bioavailability, pharmacokinetics, human, HPLC

Dihydrotachysterol (DHT_2) is a structural analogue of vitamin D_3. It was first isolated in 1930 by Holtz and Schreiber. If the A-ring of vitamin D_3 is rotated by 180° the C3-OH group simulates the C3-α-OH group of the active dihydroxy- metabolite of vitamin D_3. Such compounds are therefore called pseudo-1α-hydroxy-vi-

R. Koytchev (✉) · R.-G. Alken
Cooperative Clinical Drug Research and Development,
Waldstrasse 23–24, D-13403 Berlin, Germany

M. Vagaday · U. Kunter
Pharmacological Research Company „Biopharm" GmbH, Berlin,
Germany

V. Kirkov
Higher Medical School, Sofia, Bulgaria

tamin D analogues. Dihydrotachysterol and 5,6-*trans*-vitamin D belong to this group. Both substances are, on a molar basis, less active than vitamin D or 1,25(OH)$_2$vitamin D in stimulating calcium uptake into the intestine. The pseudo-1α-hydroxy-vitamin D analogues do not have to be hydroxylated in the kidney to an active form in order to exert their biological action. These compounds are therefore up to ten times more active in comparison to vitamin D when renal hydroxylation is impaired (hypoparathyroidism or chronic renal failure). The principal advantage of DHT_2 over other vitamin D analogues is its lower toxicity (Stanbury and Mawer 1978). The main biological action of DHT_2 is to raise the serum calcium level by increasing calcium absorption in the intestine and by mobilising calcium from bone tissue (Harrison et al. 1972, Remagen et al. 1975; Terepka and Chen 1962).

Like vitamin D, DHT_2 undergoes extensive metabolism. More than seven metabolites have been isolated and identified in rats (Proteous et al. 1988). The number of metabolites, combined with the interference of lipid components in serum, has made the quantification of DHT_2 in biological samples problematic. Only a few assays for DHT_2 have been described, and the results of these assays are inconsistent. Jongen et al. (1984) have reported that some unidentified metabolites of DHT_2 can interfere with the HPLC assay of 25-hydroxy-vitamin D. No trials have been conducted to compare the bioavailability of commercially available products. The dosage recommendations of most manufacturers are identical for capsules and oral solutions.

The aim of this trial was to compare the oral bioavailability of four oral formulations of DHT_2 using a new, selective and sensitive HPLC method.

Materials and methods

The DHT_2 formulations studied in the present trial were A.T.10 oral solution (preparation A, batch ZZ 701), A.T.10 oral capsules (preparation B, batch ZZ 691) (Bayer, Germany), Tachystin liqui-

dum (preparation C, batch 020492) and Tachystin capsules (preparation D, batch 031091) Ankerpharm, Germany). Two separate trials, each with a randomised, cross-over design, were conducted in order to achieve an intra-individual comparison of capsules on the one hand (trial 1) and solutions on the other (trial 2). An inter-individual comparison between capsules and solution was also performed. Each trial contained 24 healthy volunteers. The demographic data for both groups of volunteers were comparable. In both trials 14 female and 10 male volunteers were enrolled. The mean (SD) values for height, weight and age for trial 1 were: 169 (8.8), 66.4 (11.5) and 27.3 (5.8); and for trial 2 were: 169 (9.8), 68.8 (12.4) and 29.4 (6.1), respectivelly.

In both trials all volunteers received 1 mg DHT_2 (1-ml solution or 2 capsules) as a single dose on two different occasions, at least 14 days apart, in a randomised, cross-over way within each trial. All volunteers gave their written consent to participate in the trials after being informed in detail by the investigator about possible risks and adverse effects in accordance with the Declaration of Helsinki. Each dose was administered with 200 ml water after a fasting period of at least 12 h. The intake of water and food during the first 12 h after administration was standardised. Blood samples were drawn 30 min, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 24, 36, 48 and 60 h post-dosing for pharmacokinetic measurements. The serum was separated by centrifugation not later than 30 min after sampling and all samples were stored at –20 °C until analysis.

The quantification of DHT_2 in serum was carried out using a newly developed HPLC method with UV-detection. Briefly, 1 ml serum was mixed with 2 ml internal standard solution (Vitamin D_2, 0.05 µg · ml^{-1}) in ethanol. After centrifugation (15 min at 1500 g) 2.5 ml of the supernatant was applied to activated solid phase extraction cartridges (Chromabond C18 ec). The cartridges were washed with 3 ml ethanol/0.5 M ammonium acetate (2:1) and 1 ml water and afterwards eluated with 5 ml acetonitrile. The eluate was dried under nitrogen at 45 °C. The dry residue was taken over in 90 µl acetonitrile. Fifty µl were applied on the column (Nucleosil 100–5 C18 AB, 250 × 3 mm). The mobile phase consisted of acetonitrile/water/acetic acid (950:50:50) and flow rate was 0.75 ml · min^{-1}. The detection wavelength was 252 nm. The retention times of DHT_2 and vitamin D_2 were 15.4 and 17 min respectively. The recovery of the internal standard and DHT was approximately 75 %. In Fig. 1 two chromatograms are presented using the same time scale. The dotted line is a chromatogram from serum with no DHT_2 or vitamin D_2 added the solid line is derived from a calibration sample, containing 20 ng · ml^{-1} DHT_2 and 100 ng · ml^{-1} vitamin D_2. The limit of determination was 0.5 ng · ml^{-1}. The method was linear between 0.5 and 50 ng · ml^{-1}. The average intra- and inter-assay variance was 4.7 % and 12.1 %, respectively. All samples from each volunteer in both trials were measured in one run in order to reduce the impact of the inter-assay variance.

The pharmacokineic evaluation was carried out using the „TopFit 2.0" software. The concentration maxima (C_{max}) and the time needed to achieve a maximum (t_{max}) were read directly from the concentration-time curve. No curve-fitting was performed. The following pharmacokinetic parameters were determined in a model-independent way: area under the curve according to the trapezoid rule (AUC) and extrapolated to infinity ($AUC_{0-\infty}$), using the parameters of the terminal monoexponential part of the concentration-time curve. In addition the elimination half-life ($t_{1/2}$) and the mean residence time (MRT) were calculated. The aim of the statistical evaluation was to determine if significant differences in bioavailability or in other pharmacokinetic parameters existed between the preparations. For this purpose the 90 %-confidence intervals for the intra-individual ratios (preparation C/A and D/B) for C_{max} and AUC were calculated using parametric (ANOVA, ANOVA-log.) and non-parametric (Wilcoxon; Hauschke et al. 1990) methods. The capsules and the solution were also compared inter-individually using ANOVA, Wilcoxon's test for unpaired samples and a Kolmogorov-Smirnoff two-sample test.
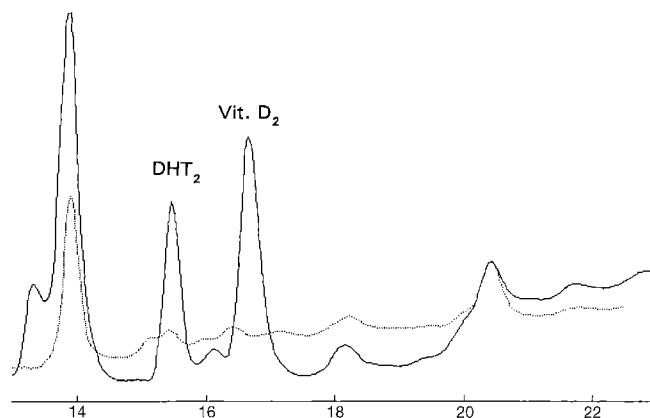

Fig. 1 Chromatograms from human serum with no DHT_2 and vitamin D_2 added (*dotted line*) and from a sample with DHT_2 (20 ng · ml^{-1}) and vitamin D_2 (100 ng · ml^{-1}) added (*solid line*)
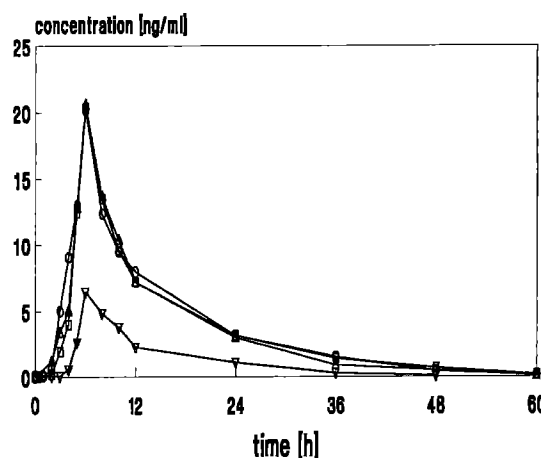

Fig. 2 Serum DHT_2 concentrations after oral administration of 1 mg DHT_2 (data are means). —▽— preparation A; —▤— preparation B; —⊖— preparation C; —△— preparation D

Results

The mean concentration-time curves of all preparations are represented in Fig. 2. It is evident that the concentration of preparation A [6.3 (4.2) ng · ml^{-1}] was considerably lower than that of preparations B [21.8 (8.4) ng · ml^{-1}] C [21.3 (9.3) ng · ml^{-1}] and D 22.3 (10.8) ng · ml^{-1}]. In two cases (volunteers 18 and 24) the concentration maxima after preparation A were below the limit of detection (0.5 ng · ml^{-1}). In four other cases (volunteers 3, 11, 12 and 16) after the same preparation the concentrations were so low that no pharmacokinetic evaluation was possible. The $AUC_{0-\infty}$ values after all preparations tested are presented in Table 1. When reading the table it should be noted that different volunteers took part in both trials. The values of preparations B, C and D did not differ significantly, whereas preparation A led to considerably lower values.

Some other pharmacokinetic parameters having no direct impact on bioavailability (t_{max}, $t_{1/2}$ and MRT) are

represented in Table 2. The results demonstrate no significant differences between the preparations.

The intra-individual comparison between similar galenic preparations (capsules vs capsules and solution vs solution) is given in the form of 90 %-confidence intervals in Table 3. The results demonstrate a 2–3-fold difference between the two oral solutions and only a slight difference between the capsules.

The inter-individual statistical comparison of C_{max} and $AUC_{0-\infty}$ values between different preparations (capsules compared to oral solution) demonstrated significant differences only between preparation A and all other preparations, the values of the former being significantly lower ($P < 0.001$) by means of all statistical tests (ANOVA, Wilcoxon's test for unpaired samples and the Kolmogorov-Smirnoff two sample test). The bioavailability of both preparations from one manufacturer (C and D) was practically identical. More than a 2-fold difference was found between the capsules and the oral solution from the other manufacturer (preparations A and B).

Discussion

Little published information is available concerning the pharmacokinetics of DHT_2 in man under normal or pathological conditions. Taylor et al. (1988) measured DHT_2 levels in six volunteers in the course of continuous administration of daily doses of between 0.4 and 0.8 mg (according to body weight) for 8 days. Four hours after administration of the daily dose concentration maxima were between 5 and 10 ng · ml^{-1}. No considerable changes in serum electrolyte levels (particularly hypercalcaemia) were reported in the same trial. Harrison et al. (1967) reported hypercalcaemia in patients with hypocalcaemic disorders treated with doses of DHT_2 higher than 1 mg per day. Normocalcaemia in the same group of patients was maintained with doses of less than 1 mg per day, suggesting a possible correla-

Table 1 $AUC_{0-\infty}$ values for four different DHT_2 preparations

Volunteer	Solution		Capsules	
	A	C	D	B
1	81.3	238.3	303.3	582.7
2	144.1	241.9	251.4	227.5
3	**	374.2	57.8	110.3
4	18.7	73.1	452.7	202.4
5	94.1	167.8	125.8	178.0
6	205.8	183.2	143.7	295.8
7	30.1	218.5	98.5	124.8
8	88.1	471.7	76.6	146.0
9	45.9	173.3	94.4	42.5
10	53.6	268.4	274.0	221.9
11	**	353.5	388.0	250.6
12	**	158.4	302.8	423.3
13	123.4	203.2	421.0	190.0
14	590.5	122.7	205.3	212.2
15	51.9	78.8	140.5	22.1
16	**	289.8	280.2	112.4
17	115.7	562.0	80.2	38.3
18	**	85.3	159.2	308.9
19	27.9	105.9	198.2	155.0
20	93.6	174.5	197.1	91.6
21	10.0	75.8	255.3	298.2
22	119.8	279.3	216.7	112.1
23	111.7	312.7	92.3	147.2
24	**	140.4	284.3	199.0
Mean	111.5	223.0	212.5	195.5
SD	129.6	125.7	111.8	125.5
Median	90.9	193.2	201.7	184.0

** Pharmacokinetic evaluation not possible because of extremely low levels in serum

tion between dosage and biological action of DHT_2 in the normal therapeutic dose ranges.

The results of the present trial demonstrate significant differences between the DHT_2 preparations studied. A direct intra-individual comparison of AUC and C_{max} values was made between the corresponding formulations (solution vs solution and capsules vs capsules) of two different manufacturers, indicating that

Table 2 Other pharmacokinetic parameters after DHT_2 administration [means (SD)]

Parameter	Solution		Capsules	
	A	C	D	B
t_{max} [h]	6.6 ± 1.0	6.1 ± 1.3	6.0 ± 0.7	6.2 ± 1.2
$t_{1/2}$ [h]	7.7 ± 9.9	7.9 ± 4.4	8.2 ± 4.2	6.6 ± 3.4
MRT [h]	9.8 ± 3.5	10.4 ± 3.9	11.9 ± 3.9	9.9 ± 2.9

Table 3 90 %-confidence intervals for the intra-individual ratios of $AUC_{0-\infty}$ and C_{max} determined by means of different statistical methods

	Method			
	ANOVA	ANOVA(log)	Wilcoxon	Hauschke et al. (1990)
Preparation C compared to preparation A				
$f_{C/A}$ ($AUC_{0-\infty}$)	1.27–2.67	1.77–3.66	1.98–3.74	1.88–4.72
$f_{C/A}$ (C_{max})	2.78–3.91	2.91–5.29	2.67–5.07	2.64–5.43
Preparation D compared to preparation B				
$f_{D/B}$ ($AUC_{0-\infty}$)	0.72–1.13	0.67–1.08	0.68–1.11	0.72–1.12
$f_{D/B}$ (C_{max})	0.84–1.21	0.78–1.19	0.78–1.26	0.78–1.26

no significant differences could be seen between the capsules although the formal regulatory requirements for bioequivalence (90 %-confidence intervals for log-normally distributed AUC ratios within the 80–125 % limits) were not fulfilled. A considerable (2–3-fold) difference was seen between the two oral solutions, which made an indirect (inter-individual) comparison between the capsules and the solution of the same manufacturer necessary. The results of this comparison, both in the form of confidence intervals for the inter-individual ratios for AUC and C_{max} and of different parametric and non-parametric statistical tests, confirmed that the difference was significant. It should be taken into consideration that the magnitude of the difference is certainly underestimated, since no pharmacokinetic data was available in 6 out of 24 volunteers who took preparation A because of extremely low levels of DHT_2. This means that the 6 volunteers with the lowest AUC values could not be subjected to statistical evaluation. A strong positive bias is therefore present for the AUC and C_{max} values of preparation A. The difference between the preparations can not be explained by differences between the two groups of volunteers, since both groups were similar demographically and since no difference could be seen between the other three preparations no matter whether intra- or inter-individual comparisons were carried out (Fig. 2). Preparation A had a two to three times lower bioavailability than the other three preparations.

A possible explanation for this phenomenon is the lipid base used for the production of the different formulations. Like vitamin D, DHT_2 is practically insoluble in water but very soluble in lipids. The only preparation in which a different lipid base (middle-chained triglycerides) was used was preparation A. In all other cases peanut oil or peanut oil and glycerin were used as solvents. The extremely high lipid solubility of DHT_2 could lead to absorption together with the lipid solvent. Thus, differences in the rate or extent of absorption between the different lipids could lead to differences in bioavailability.

The findings of this trial have considerable implications for the clinical use of different DHT_2 formulations. A new dosage recommendation in accordance with the findings of this trial seems appropriate for preparation A, which is available in several countries.

Usually, therapy with DHT_2 is carried out under strict control of the main target parameter – serum levels of calcium. Under such circumstances no particular risk for the patient is present. Generally, no changes of the formulation during therapy should be recommended unless comparative data concerning its bioavailability is present.

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