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Research Article

Bioequivalence Studies of Two Calcitriol Capsules Formulation in Healthy Chinese Subjects under Fasting and Fed Conditions: The Randomized, Open-Label, Single-Dose, Crossover Studies

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ABSTRACT

Background: To evaluate the bioequivalence between test and reference formulations of calcitriol capsules under fasting and fed conditions and to assess their Pharmacokinetic (PK) and safety profiles.

Methods: A randomized, open-label, single-dose, crossover studies were conducted in healthy Chinese subjects. Test calcitriol capsules (T) or reference calcitriol capsules (R, 4.0 μg, 8 dosage units with the strengths of 0.5 μg per capsules) were randomly given to subjects under fasting (3-way 3-period crossover), with an administration sequence of T-R-R, R-T-R or R-R-T and fed (2-way 2-period crossover), with an administration sequence of T-R or R-T conditions, while each single administration was followed by a 14-day washout period. The plasma concentration and corresponding PK parameters of calcitriol were determined. The two formulations were considered to be bioequivalent if the 90% Confidence Intervals (CI) of the Geometric Mean (GM) ratio (T/R) for C_{max}, AUC_{0-t} and AUC_{0-inf} were all within the range of 80%-125%. Safety assessments including vital signs, physical examination, laboratory examination, 12-lead Electrocardiogram (ECG) and reports of Adverse Events (AE) were carefully documented.

Results: A total of 66 subjects (36 in fasting study and 30 in fed study) were randomized and all completed with the samples collected. 2 subjects (1 in fasting study and 1 in fed study) lost when follow up of AE, one month after the studies. The 90% CI of the GM ratio for C_{max}, AUC_{0-t} and AUC_{0-inf}, respectively, were 100.59%-112.86%, 99.79%-110.84%, and 99.30%-108.22% under the fasting condition and 99.25%-119.86%, 99.17%-111.58% and 97.83%-110.00% under the fed condition. In fasting study, 10(27.78%, n=36) experienced 15 AEs after the administration of T, and 25 (34.72%, n=72) experienced 34 AEs after the administration of R. In fed study, 14 (46.67%, n=30) experienced 24 AEs after the administration of T, and 5 (16.67%, n=30) experienced 7 AEs after the administration of R. Only one AE of no drug-related was grade 2. The incidence of AEs and drug-related AEs were similar between T and R (all P>0.05) and no serious AE occurred during the studies.

Conclusions: T and R were bioequivalent and well tolerated in healthy Chinese subjects under fasting and fed conditions.

Keywords: Plasma Concentration; Bioequivalence; Pharmacokinetics; Bioequivalent

INTRODUCTION

Calcitriol, known as 1,25-dihydroxyvitamin D3, is an important active metabolite of vitamin D3, which is converted from 25-hydroxyvitamin D3 in kidney [1]. People can generate 0.5 μg -1.0 μg calcitriol everyday usually, and it may increase slightly under the vigorous period of bone synthesis.

One of the most basic and important roles of calcitriol is the absorption of calcium and the regulation of bone calcification. Calcitriol can increase the sensitivity of parathyroid gland to extracellular calcium by binding to 1,25-dihydroxyvitamin

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D3 receptor, thereby weakening or inhibiting the secretion of parathyroid hormone. In addition, it can cooperate with parathyroid gland to stimulate osteoclasts to promote the transfer of calcium and phosphorus to blood circulation [2]. Another function of calcitriol is to enhance the reactivity and phagocytosis of monocyte macrophages to inflammatory substances, thereby enhancing immune function. Some studies reported that calcitriol can regulate the expression of B cells and maintain the homeostasis of B cells to regulate the immune function [3].

Numerous studies have confirmed the effectiveness in treating postmenopausal osteoporosis, renal osteodystrophy in hemodialysis patients, postoperative hypoparathyroidism, idiopathic hypoparathyroidism, pseudohypoparathyroidism, vitamin D-dependent rickets, low blood phosphorus vitamin D resistant rickets, etc. According to the pharmacological function of calcitriol, new indications are still in exploration. Some studies proved that calcitriol can be a treatment in reducing blood glucose and promoting insulin secretion in patients with type 2 diabetes, inducing appetite and weight loss, colitis, rheumatoid arthritis, autoimmune encephalopathy and cancer [4-11].

The safe dose range of vitamin D is wide, but the probability of calcitriol induced hypercalcemia is significantly higher than that of vitamin D. The most common adverse reaction of calcitriol is the increase of blood calcium and phosphorus in patients with renal function injury after medication [12]. Therefore, at the beginning of clinical application, it is necessary to carefully adjust the optimal dose of calcitriol according to the patient's blood calcium level and the minimum dose should be used. The blood calcium level should be monitored before increasing the dose to ensure the safety of patients.

The characteristics of calcitriol of low water solubility can speed and increase the absorption greatly. Tmax is basically between 2-6 hours after a single administration, and may be prolonged in the people with nephrotic syndrome and hemodialysis. The PK curve we referenced of the present studies is from a single dose bioequivalence study in Japan [13]. And Food and Drug Administration (FDA) review report of Rocaltrol {Roche Pharma (Schweiz), AG} showed that the maximum safe dose of this calcitriol is 6 μg, which can be used as a basis for the safety of overdose administration in the studies.

The present single-center, open-label, randomized, cross-over, bioequivalence studies under fasting and fed condition were conducted to evaluate the bioequivalence of test calcitriol capsule (Qingdao Haier Pharmaceutical Co., Ltd, Qingdao, China) and reference calcitriol capsule {Rocaltrol, Roche Pharma (Schweiz), AG} in healthy Chinese subjects under fasting condition (3-way 3-period crossover) and fed condition (2-way 2-period crossover). The tolerability and PK characterization of 4.0 μg (8 dosage units with the strengths of 0.5 μg per capsules) were also evaluated in the studies.

MATERIALS AND METHODS

Study design and subjects

The single-center, open-label, randomized, cross-over, bioequivalence studies were designed with a 14-day washout period between each application in both fasting and fed conditions and conducted from 24th Oct, 2020 to 28th Nov, 2020 and 31st Oct, 2020 to 22nd Nov, 2020 respectively. All subjects were in hospital 4 days before every application for a diet balance period and kept similar meals with low content of vitamin D during the whole studies. The subjects in the fasting study were randomly given calcitriol capsules (4.0 μg, 8 dosage units with the strengths of 0.5 μg per capsule) with 240 mL warm water in the sequence of T-R-R, R-T-R or R-R-T after fasting for at least 10 h overnight. The subjects in the fed study randomly received calcitriol capsules (4.0 μg) in the sequence of T-R or R-T after eating a high-fat, high-calorie diet for 30 min. The high-fat and high-calorie meal consisted of 240 g bacon, 115 g fried potato cake and 250 g soybean milk. All subjects received standard meals 4 h and 10 h after the administration of T or R and water intake were allowed up to 1 h before and 1 h after the administration.

Healthy subjects of both genders, aged between 18 and 65 years (inclusive), with a Body Mass Index (BMI) of 19-26 kg/m^2 (inclusive), the body weight of man more than 50 kg (inclusive) and that of woman more than 45 kg (inclusive), were enrolled in the studies. All subjects underwent a detailed medical physical examination, vital sighs, 12-lead ECG, chest X-ray, clinical laboratory (ALT, AST ≤ 1.2ULN, Cr ≤ ULN) and serological test (hepatitis B and C, treponema and human immunodeficiency virus). Demographic data and medical histories, as well as drug abuse and allergy histories were recorded.

The studies were approved by the clinical trial ethics committee of Huazhong University of Science and Technology (approval No. 2019-161), strictly in accordance with Good Clinical Practice (GCP) and the Declaration of Helsinki regarding medical research in humans. Written informed content was obtained from each subject prior to their enrollment. The register number in www.chinadrugtrials.org.cn is CTR20201512.

Randomization and sample size

The administration sequence for each subject was determined by a random table (seed=791210, block=6 in fasting study, and seed=792034, block=6 in fed study) which was generated by SAS software (version 9.4). The researchers of sample analysis were blinded.

In fasting study, assuming the maximum value for an intra-individual Coefficient of Variation (CV) among C_{max}, AUC_{0-t} and AUC_{0-inf} of 45.00% considering the predicted GM ratios of 0.90, a 1-sided t-test error of 0.05, a power of 0.8 and a dropout rate of 20%, a total of 36 subjects were enrolled, with a 3-way 3-period design and Reference-Scaled Average Bioequivalence (RSABE) method. In fed study, assuming the maximum value for an intra-individual Coefficient of Variation (CV) among C_{max}, AUC_{0-t} and AUC_{0-inf} of 23.52% considering the predicted GM ratios of 0.95, a 1-sided t-test error of 0.05, a power of 0.8 and a dropout rate of 20%, a total of 30 subjects were enrolled, with a 2-way 2-period design and Average Bioequivalence (ABE) method.

Blood sampling and plasma concentration analysis

For subjects under fasting and fed conditions, venous blood samples (5 mL) were collected in vacuum anticoagulant tubes containing heparin sodium before the administration of 18 h (± 30 min), 12 h (± 30 min), 6 h (± 30 min) and 0 h (-1 h) and after the administration of 0.5 h (± 2 min), 1 h (± 2 min), 2 h (± 2 min), 2.5 h (± 2 min), 3 h (± 2 min), 3.5 h (± 2 min), 4 h (± 2 min), 5 h (± 10 min), 6 h (± 10 min), 7 h (± 10 min), 8 h (± 10 min), 10 h (± 10 min), 12 h (± 10 min), 24 h (± 10 min), 36 h (± 10 min), 48 h (± 10 min) and 72 h (± 10 min). The blood samples were collected and separated for plasma (centrifugation at 3000 rpm at 4 °C for 10

min within 1 h) under the yellow light avoiding the natural light. The plasma samples were stored with the Brown cryopreservation tube at a -70°C freezer immediately after separated.

The plasma concentrations of calcitriol were assayed using the validated liquid chromatography-tandem mass spectrometry (LC-MS/MS, Waters ACQUTY I CLASS & Xevo TQ-S). The linear range of the calibration curve for calcitriol was 20-800 pg/mL, with a lower limit of quantitation (LLOQ) of 20 pg/mL. The range of precision (CV%) and accuracy (recovery error %) was 5.3~5.5 and -1.5~1.0 in the sample analysis of fasting study, and separately, 2.9~6.2 and -2.1~1.0 in that of fed study. 2376 samples of fasting study and 1320 samples of fed study were tested. The data acquisition and integration were performed using UNIFY 1.7.1.022 & Excel 2021.

Outcomes

PK evaluation included plasma concentration and PK parameters of calcitriol, using the standard baseline reduced for baseline correction. As an endogenous substance, the average values before each administration were subtracted and determined by collecting blood samples at 0, -6, -12, and -18 h prior to each dosing. If a negative plasma concentration value results after baseline correction, this should be set to 0 prior to calculating the baseline corrected AUC. The PK parameters were calculated based on the actual sampling time with non-atrioventricular model, using WinNonlin software (version 8.2).

Safety evaluation included measurements of vital signs (body temperature, pulse and blood pressure), physical examinations and laboratory tests (routine blood and urine measurements, serum biochemistry and coagulation function), 12-lead ECG and reports of Adverse Events (AE). AE and drug-related AE were summarized using Medical Dictionary for Regulatory Activities (MedDRA) and the corresponding severity was graded using National Cancer Institute (NCI) Common Terminology Criteria Adverse Events (CTCAE) Ver. 5.0.

Statistical analysis

SAS (version 9.4) was used for statistical analyses. Continuous variables are presented as arithmetic means with the Standard Deviation (SD) and median with the maximum (Max) and minimum (Min), or GM with CV% presented as numbers of subjects. Count and level data provided the number and percentage of cases. All analyses were based on observational data, and data filling is not considered without outliers.

The Full Analysis Set (FAS) was defined as all randomized subjects who had received the investigational product at least once. The Safety Analysis set (SS) was defined as all randomized subject who had received the investigational product at least once with safety records after administration. PK Concentration Set (PKCS) was defined as all randomized subjects who had received the investigational product at least once with at least one blood concentration data. PK Parameter Set (PKPS) was defined as all randomized subjects who had received the investigational product at least once with at least one PK parameter data, and without the situation as bellowed: (1) three consecutive samples were found missed at any stage; (2) the subjects vomited within the time of twice the median value of T_{max}; (3) C_{max} was the first sample after administration. BE Set (BES) was defined as all randomized subjects who completed at least one period of the study and had at least one evaluable PK parameter.

As the evidence showed CV% >30% possibly in fasting study, the bioequivalence between the two formulation in fasting study was determined based on the main PK parameters (Cmax, AUC0-t and AUC0-inf) as follows: (1) ABE method, that is 90% CI of GM ratio (T/R) should fall within the range of 80-125%, if SWR<0.294 (CVW%<30%). (2) RSABE method, that is the upper limit of the unilateral 95% CI for (Y¯_T-Y¯_R)^2-θS_wR^2 should be ≤ 0 in addition to the same predesigned range of 80-125%, if SWR>0.294 (CVW%>30%).

In fed study, the bioequivalence between the two formulations was determined based on with ABE method that is 90% CI of GM ratio (T/R) should fall within the range of 80%-125%.

In both fasting and fed studies, the mixed effect model of the main PK parameters (C_{max}, AUC_{0-t} and AUC_{0-inf}) were converted and Analysis of Variance (ANOVA), and was used to calculate with sequence, treatment and period as fixed effects and subjects (sequence) as random.

RESULTS

Disposition of Subjects and their demographic characteristics

A total of 82 subjects were screened in the fasting study and 73 subjects in the fed study. Finally, 36 subjects (21 males and 15 females) were enrolled and randomized in the fasting study and 30 subjects (20 males and 10 females) in the fed study. All randomized subjects (n=66) completed the blood samples collected and were included in the FAS, SAS, PKCS, PKPS and BES. 2 subjects (1 in fasting study and 1 in fed study) lost when follow up of AE (Figures 1 and 2).

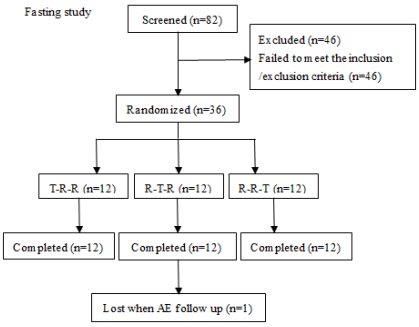

Figure 1: Disposition of subjects under fasting condition.

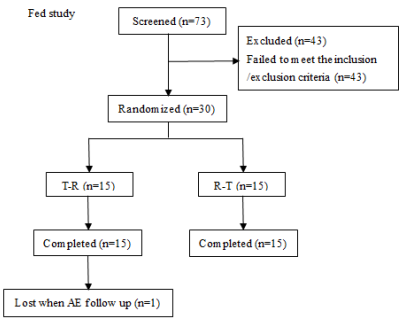

Figure 2: Disposition of subjects under fed condition.

The demographic and other baseline characteristics are presented in Table 1. Male subjects (62.12%) were in the majority in both the fasting and fed studies, and the mean (SD) age were 28.2 (6.1) and 27.4 (6.6) years, with a mean (SD) BMI of 21.92 (1.71) and 22.18

(1.67), respectively. All subjects had no medical histories, as well as drug abuse and allergy histories.

Table 1: Demographic and baseline characteristics.

	Fasting study (n=36)	Fed study (n=30)
Age (years)	28.2 (6.1)	27.4 (6.6)
Gender, n (%)	-	-
Male	21 (58.33%)	20 (66.67%)
Female	15 (41.67%)	10 (33.33%)
Height (cm)	1.6419 (0.0860)	1.6668 (0.0694)
Wight (kg)	59.34 (8.56)	61.72 (7.08)
BMI (kg/m^2)	21.93 (1.71)	22.18 (1.67)

Note: Data are presented as the mean (SD).

Bioequivalence and PK

Under fasting and fed conditions, the mean plasma concentration time curve (linear and semi-log) of calcitriol with baseline correction are shown in Figures 3 and 4, all with a similar trend during the treatment period. Table 2 summarizes the PK parameters with baseline correction in subjects after the administration of T and R under fasting and fed condition. The PK parameters of $AUC_{0\text{-}inf}$, λz and $t_{1/2}$ of two subjects at the second administration of R were not included to analysis because of their AUC% Extrap>20%. The results of variance analysis showed that there was no statistically significant difference among the administration sequences, formulations and periods with Ln (C_{max}), Ln ($AUC_{0\text{-}t}$) and Ln ($AUC_{0\text{-}inf}$) (P>0.05). There was an increased trend in mean C_{max} (168.928 vs. 181.104 pg/mL) and T_{max} (2.917 vs. 4.229 h) in the fed study compared with the fasting study.

As shown in Table 3, in the fasting study, the GM ratio (T/R) and corresponding 90% CI of calcitriol for C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ were 106.55 (100.59, 112.86 %), 105.17 (99.79, 110.84 %) and 103.66 (99.30, 108.22 %), respectively. And think of SWR (C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$)<0.294 (CVW%<30%), the 90% CI of GM ratio (C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$) all fell within the bioequivalence interval of 80%-125% under ABE method instead of RSABE. In the fed study, the GM ratio (T/R) and corresponding 90% CI of calcitriol for C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ were 109.07 (99.25, 119.86), 105.19 (99.17, 111.58) and 103.74 (97.83, 110.00), fell within the bioequivalence interval of 80%-125% under ABE method as well.

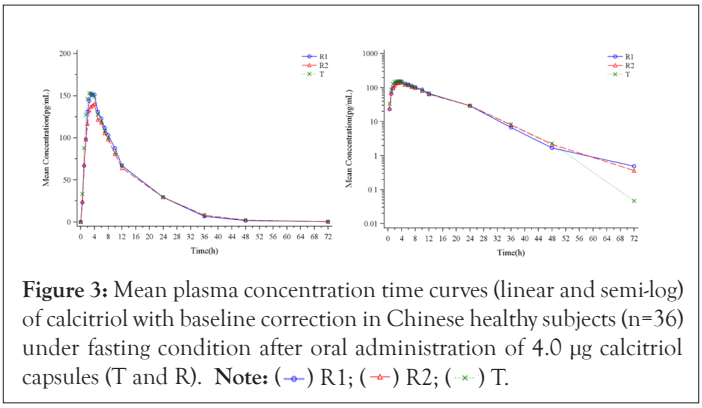

Figure 3: Mean plasma concentration time curves (linear and semi-log) of calcitriol with baseline correction in Chinese healthy subjects (n=36) under fasting condition after oral administration of 4.0 μg calcitriol capsules (T and R). **Note:** (-○-) R1; (-△-) R2; (-×-) T.

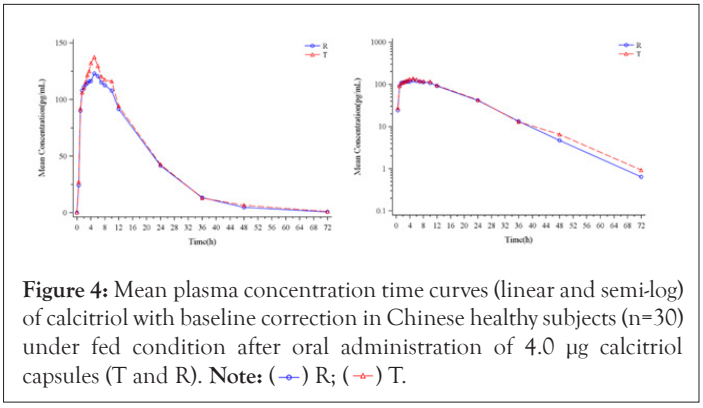

Figure 4: Mean plasma concentration time curves (linear and semi-log) of calcitriol with baseline correction in Chinese healthy subjects (n=30) under fed condition after oral administration of 4.0 μg calcitriol capsules (T and R). **Note:** (-○-) R; (-△-) T.

Table 2: Summary of PK parameters of calcitriol with baseline correction after oral administration of 4.0 μg calcitriol capsules (T and R) in Chinese healthy subjects using BES.

PK parameters	Mean ± SD (CV%)	Median (min, max)	Mean ± SD (CV%)	Median (min, max)
Fasting	T (n=36)		R (n=36*)	
C_{max} (pg/mL)	168.928 ± 50.205 (29.72)	186.089 (46.11, 231.79)	160.521 ± 49.735 (30.98)	169.637 (52.01, 236.85)
$AUC_{0\text{-}t}$ (h*pg/mL)	2058.236 ± 479.417 (23.29)	2114.121 (891.51, 2852.54)	1984.423 ± 503.590 (25.38)	2084.053 (767.20, 2767.88)
$AUC_{0\text{-}inf}$ (h*pg/mL)	2143.274 ± 483.167 (22.54)	2229.980 (904.12, 2852.68)	2073.929 ± 487.397 (23.50)	2165.583 (1049.28, 2914.02)
T_{max} (h)	2.917 ± 1.467 (50.28)	2.505 (1.50, 10.00)	3.465 ± 1.217 (35.11)	3.250 (1.50, 6.50)
$t_{1/2}$ (h)	7.868 ± 2.252 (28.63)	7.883 (3.83, 13.26)	8.113 ± 2.410 (29.70)	7.715 (5.05, 16.68)
λz (1/h)	0.097 ± 0.033 (33.76)	0.088 (0.05, 0.18)	0.094 ± 0.023 (24.18)	0.093 (0.04, 0.14)

Fed	T (n=30)		R (n=30)	
C_{max} (pg/mL)	181.104 ± 42.112 (23.25)	183.367 (81.77, 256.95)	165.849 ± 39.360 (23.73)	161.428 (89.55, 242.36)
AUC_{0-t} (h*pg/mL)	2550.181 ± 664.054 (26.04)	2457.057 (1169.35, 4073.03)	2414.802 ± 552.846 (22.89)	2421.945 (858.00, 3416.12)
AUC_{0-inf} (h*pg/mL)	2618.552 ± 673.087 (25.70)	2517.847 (1323.08, 4080.09)	2512.104 ± 560.016 (22.29)	2518.743 (960.73, 3603.16)
T_{max} (h)	4.229 ± 2.878 (68.06)	4.000 (1.00, 10.04)	4.400 ± 3.341 (75.92)	3.500 (0.99, 12.00)
$t_{1/2}$ (h)	8.025 ± 2.586 (32.22)	7.800 (3.31, 14.51)	8.445 ± 2.183 (25.85)	8.179 (4.79, 14.21)
λz (1/h)	0.096 ± 0.034 (35.52)	0.089 (0.05, 0.21)	0.087 ± 0.022 (25.20)	0.085 (0.05, 0.14)

Note: *The PK parameters of R in two periods were averaged before analysis.

Table 3: Summary of bioequivalence based on C_{max}, AUC_{0-t} and AUC_{0-inf} of calcitriol following administration of T and R in Chinese healthy subjects using BES.

PK parameters	GM		GM ratio (%)	90% CI	CV of R (%)	SWR	Power (%)	The upper limit of the unilateral 95%
Fasting	T (n=36)	R (n=72)						
C_{max} (pg/mL)	159.17	149.4	106.55	100.59, 112.86	19.42	0.192	99.84	-0.01
AUC_{0-t} (h*pg/mL)	1191.65	1893.71	105.17	99.79, 110.84	18.57	0.184	99.99	-0.01
AUC_{0-inf} (h*pg/mL)	2078.14	2004.70*	103.66	99.30, 108.22	16.94	0.168	100	-0.01
Fed	**T (n=30)**	**R (n=30)**						
C_{max} (pg/mL)	175.71	161.11	109.07	99.25, 119.86	-	-	77.44	-
AUC_{0-t} (h*pg/mL)	2463.04	2341.49	105.19	99.17, 111.58	-	-	99.93	-
AUC_{0-inf} (h*pg/mL)	2532.62	2441.32	103.74	97.83, 110.00	-	-	99.99	-

Note: *For AUC_{0-inf}, two subjects data at the second administration of R were not included to analysis because of their AUC% Extrap>20% (n=70).

Safety

In fasting study, 10 (27.78%, n=36) experienced 15 AEs after the administration of T, and 25 (34.72%, n=72) experienced 34 AEs after the administration of R. In fed study, 14 (46.67%, n=30) experienced 24 AEs after the administration of T, and 5 (16.67%, n=30) experienced 7 AEs after the administration of R. In both the fasting and fed studies, the common AEs were attributed by laboratory test and ECG results such as elevated alanine aminotransferase, positive of urine leukocyte, part of electrocardiographic wave prolongation, Q-T prolongation, ST segment abnormality, sinus bradycardia, elevated blood triglyceride, elevated blood uric acid and decreased blood pressure. Almost all AEs were reported to be related to T or R. In fasting study, 10 (27.78%, n=36) experienced 15 Drug-related AEs as Adverse Drug Reaction (ADR) after the administration of T, and 24 (33.33%, n=72) experienced 33 AEs after the administration of R. In fed study, 13 (43.33%, n=30) experienced 23 ADR after the administration of T, and 5 (16.67%, n=30) experienced 7 AEs after the administration of R.

In fasting study, one AE (skin laceration of left leg) was grade 2 with medicine and medical treatment and no drug-related, and others were all grade 1 and drug-related. In fed study, the severity of all AEs was grade 1, and one AE (conjunctival hemorrhage of left eye) was no drug-related. There was no significant different in the incidence of AEs and ADRs between T and R (P>0.05). No death, serious AE or serious drug-related AE occurred during the study. 2 subjects (1 in fasting study and 1 in fed study) lost when follow up of AE, one month after the studies.

DISCUSSION

The randomized, open-label, single-dose, crossover studies under fasting and fed conditions in Chinese healthy subjects were conducted to establish the bioequivalence of two calcitriol formulations, and in agreement with the 90% CI of the GM ratio (T/R) of PK parameters, all fell within the pre-specified range of 80%-125%, values which complied with the bioequivalence requirement of the National Medical Products Administration (NMPA) for generic drug [14].

In the studies, the mean $t_{1/2}$ of calcitriol for T and R were7.868 h and 8.113 h under fasting condition and 8.025 h and 8.445 under fed condition, respectively. The washout period was 14 days, which was much higher than 7 times $t_{1/2}$. In addition, the plasma concentration of calcitriol was recorded as Beneath Limit of Quantification (BLQ) in all subjects before the second period (and third period in fasting study) under baseline correction.

In both fasting and fed studies, the incidence of AEs and drug-related AEs were all with no significant difference between T and R (P>0.05), but higher than the data in label or the previous research. The over dose of calcitriol and a long period with the meals of low content of vitamin D might be the reason, but all AEs were followed and return to normal level, except two subjects lost at one month after the administration. It was evaluated as the slight, temporary and effect-less.

As an endogenous substance, the detectable concentration of calcitriol in blood is on pg level, which brings considerable difficulties in PK studies. Early PK studies have used enzyme-linked immunosorbent assay to determine the blood concentration of calcitriol, whose lower limit of quantification is 50 pg/mL [15]. With the popularization of LC-MS/MS, the lower limit of quantification can reach 20 pg/mL and 5 pg/mL with UPLC-MS/MS, which leads more accurate PK characteristics of calcitriol [16-18].

A limitation of the present studies is the sample size of the fasting study, which was estimated as a high variation with the RSABE method. In case of the possibility of low variation, we made the pre-definition of RSABE or ABE before the study conducted and used ABE finally according to the value of SWR.

CONCLUSION

In summary, T and R were bioequivalent both under fasting and fed condition, with similar PK characteristics. Overall, the two formulations were well tolerated with a slight, temporary and affect-less incident of AEs. The BE studies can be regarded as pieces of evidence that the test drug can be used as the genic drug of the reference one.

DECLARATION OF COMPETING INTEREST

The authors report no declarations of interest.

REFERENCES

1. Mason RS. Vitamin D: A hormone for all seasons. Climacteric. 2011;14(2):197-203.
2. Lal H, Pandey R, Aggarwal SK. Vitamin D: Non-skeletal actions and effects on growth. Nutr Res. 1999;19(11):1683-1718.
3. Chen S, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. J Immunol. 2007;179(3):1634-1647.
4. Eftekhari MH, Akbarzadeh M, Dabbaghmanesh MH, Hasanzadeh J. Impact of treatment with oral calcitriol on glucose indices in type 2 diabetes mellitus patients. Asia Pac J Clin Nutr. 2011;20(4):521-526.
5. Trinko JR, Land BB, Solecki WB, Wickham RJ, Tellez LA, Maldonado-Aviles J, et al. Vitamin D3: A role in dopamine circuit regulation, diet-induced obesity, and drug consumption. eNeuro. 2016;3(2):122-125.
6. Du J, Wei X, Ge X, Chen Y, Li YC. Microbiota-Dependent Induction of Colonic Cyp27b1 Is Associated with Colonic Inflammation: Implications of Locally Produced 1, 25-Dihydroxyvitamin D3 in Inflammatory Regulation in the Colon. Endocrinology. 2017;158(11).4064-4075.
7. Li C, Yin S, Yin H, Cao L, Zhang T, Wang Y. Efficacy and Safety of 22-Oxa-Calcitriol in Patients with Rheumatoid Arthritis: A Phase II Trial. Med Sci Monit. 2018;24:9127-9135.
8. Mayne CG, Spanier JA, Relland LM, Williams CB, Hayes CE. 1,25-Dihydroxyvitamin D3 acts directly on the T lymphocyte vitamin D receptor to inhibit experimental autoimmune encephalomyelitis. Eur J Immunol. 2011;41(3):822-832.
9. Waddell A, Zhao J, Cantorna MT. NKT cells can help mediate the protective effects of 1,25-dihydroxyvitamin D3 in experimental autoimmune encephalomyelitis in mice. Int Immunol. 2015;5:237-244.
10. Dixon KM, Tongkao-On W, Sequeira VB, Carter SE, Song EJ, Rybchyn MS, et al. Vitamin D and death by sunshine. Int J Mol Sci. 2013;14(1):1964-1977.
11. Shintani T, Rosli SN, Takatsu F, Choon YF, Hayashido Y, Toratani S, et al. Eldecalcitol (ED-71), an analog of 1α,25-dihydroxyvitamin D3 as a potential anti-cancer agent for oral squamous cell carcinomas. J Steroid Biochem Mol Biol. 2016;164:79-84.
12. Food and Drug Administration. Label of ROCALTROL[EB/OL]. 1998.
13. Pharmaceuticals and Medical Devices Agency. Calcitriol (Chugai Pharmaceutical Co., Ltd.) IF [EB/OL]. 2022.
14. National Medical Products Administration. Technical Guidelines for Bioequivalence Study of Chemical Generic Drugs Which Evaluated with Pharmacokinetic Parameters as the Endpoint [EB/OL]. 2018.
15. Jin SE, Park JS, Kim CK. Pharmacokinetics of oral calcitriol in healthy human based on the analysis with an enzyme immunoassay. Pharmacol Res. 2009;60(1):57-60.
16. Kissmeyer AM, Kim S. Sensitive analysis of 1a, 25-dihydroxyvitamin D3 in biological fluids by liquid chromatography-tandem mass spectrometry. J Chromatogr A. 2001;935(1):93-103.
17. Patankar S, Pudage A, Pradhan V, Joshi N, Vaidya V. A liquid chromatography/electrospray ionization tandem mass spectrometric method for the quantification of calcitriol in human plasma: Application to pharmacokinetic study in human subjects. Int J Bioassays. 2013;2(11):1498-1507.
18. Weinstock-Guttman B, Zivadinov R, Qu J, Cookfair D, Duan X, Bang E, et al. Vitamin D metabolites are associated with clinical and MRI outcomes in multiple sclerosis patients. J Neurol Neurosurg Psychiatry. 2011;82(2):189-195.