Original Article

Mahidol Univ J Pharm Sci 2017; 44 (1), 32-39

Bioequivalence study of diacerein 50 mg capsules in healthy Vietnamese volunteers

G.T.H. Nguyen[1,], N.T.A. Le[1], H.T.T. Tran[1], V.N. Nguyen[1]*

[1] Institute of Drug Quality Control - Ho Chi Minh City, 200 Cobac Street, District 1, Ho Chi Minh City, Vietnam

Abstract

Diacerein is chemically 4, 5-diacetyloxy-9, 10-dioxo-anthracene-2-carboxylic acid and is a disease modifying antirheumatoid drug used in the treatment of Osteoarthritis and chronic inflammatory arthritis. Diacerein is entirely converted into its active metabolite rhein (4,5-dihydroxy -9,10-dioxoan thracene-2-carboxylic acid) before reaching the systemic circulation. This drug is administered orally as 50 mg twice daily and the the therapeutic concentration of rhein in blood plasma changes in a wide range of 1-10000 ng/mL, so a highly sensitive and accurate HPLC method was developed to quantify rhein in human plasma. This method successfully applied to the bioequivalence study of two formulations of diacerein 50 mg in healthy Vietnamese populations. After dosing, serial blood samples were collected for a period of 24 hrs. The power of all primary pharmacokinetic parameters were greater than 80 % indicating that the number of subjects was enough to confirm the bioequivalence of two formulations. No subject withdrew from our study, and no adverse events were found on analysis of vital signs or laboratory test results during the study. No abnormalities were found in clinical or biochemical parameters when comparing baseline versus end-of-study assessments. The 90% confidence intervals for the ratios of C_{max} (93.43 % - 102.80%), AUC_{0-t} (90.17 % - 113.76%) and $AUC_{0-\infty}$ (90.48 % - 113.32%) suggested that a single dose of the test and reference formulations of diacerein met the FDA regulatory requirements of bioequivalence.

Keyword:

1. INTRODUCTION

Diacerein (diacetylrhein, Fig.1A) is chemically 4, 5-diacetyloxy-9,10-dioxo-anthracene-2-carboxylic acid and is a disease modifying antirheumatoid drug used in the treatment of Osteoarthritis and chronic inflammatory arthritis. It is also known to inhibit interleukin-1. Diacerein is entirely converted into its active metabolite rhein (4,5-dihydroxy -9,10-dioxoan thracene-2 -carboxylic acid; Fig.1B) before reaching the systemic circulation. This drug is administered orally as 50 mg twice daily and the the therapeutic concentration of rhein in blood plasma changes in a wide range of 1-10000 ng/mL[1,2].

Literature survey revealed that various methods reported for determination of rhein in human plasma by HPLC includes UV detector applying precipitation technology[3-6], and mass spectrometry[7,8]. In the present study, a highly sensitive and accurate HPLC method was developed to quantify rhein in human plasma. Sample preparation was carried out by protein precipitation using diclofenac sodium as an internal standard.

Although the branded diacerein formulation was already marketed in Vietnam, the information regarding the pharmacokinetics of diacerein as well as the bioequivalence of these formulations in Vietnamese populations had seldom been reported to date. Therefore, the purpose of the present single-dose study was to determine the bioequivalence and to compare the pharmacokinetics of two formulations of diacerein (generic and innovator formula) in a fasting, healthy Vietnamese population.

***Corresponding author:** nthgiang123@gmail.com, huonggiang@idqc-hcm.gov.vn

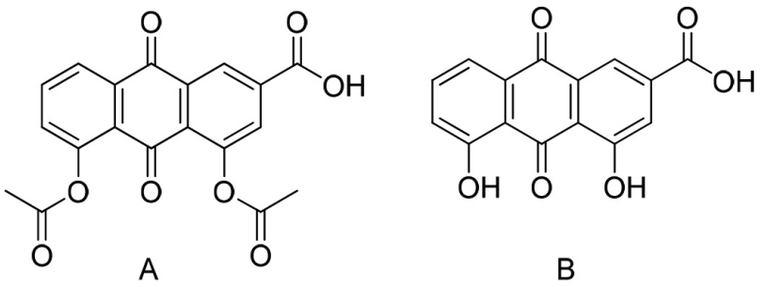

Figure 1. Chemical structure of (A) diacerein and (B) rhein

2. MATERIAL AND METHODS

2.1. Materials

2.1.1. Chemicals

Rhein (99.64%; Lot. 110M1180V) was obtained from Sigma-Aldrich, USA. Diclofenac sodium (99.50%; internal standard, Lot. QT 005080111) was obtained from Institute of Drug Quality Control Ho Chi Minh city, Vietnam. Methanol and acetonitrile (HPLC grade) were purchased from J.T.Baker, Malaysia. Acetic acid (PA grade) was a gift from Merck, Germany. Distilled water (Institute of Drug Quality Control Ho Chi Minh city, Vietnam) was filtered through 0.45 µm filters (Millipore, USA). Plasma sample were collected from HCMC Blood Transfusion and Hematology Hospital, Vietnam.

2.1.2. Drug

Test product (T) as Diacerein 50 mg capsule (ARTREIL) from DAVIPHARM Co., Ltd., Vietnam (Lot number 01012, Manufactured on November 2012, Expiry date November 2015). Reference product (R) was artrodar® capsule (Diacerein 50 mg) from TRB Pharma S.A, Argentina (Lot number 26022, Manufactured on June 2012, Expiry date June 2015).

The assayed drug content of the test product do not differ from the reference product by more than +/- 5 percent, at 99.90 % and 98.60 % respectively.

2.2. Analytical procedure

2.2.1. Extraction protocol

A 500 µL aliquot of human plasma was mixed 50 µL diclofenac in a concentration of 200 µg/mL, 50 µL of 0.1 M KOH and vortex-mixed for 15 second. Then 750 µL methanol was added and this mixture was vortex-mixed for 1 min and then centrifuged for 10 minutes at 16500 rpm and 5 ^{o}C. The supernatant was filtered and transferred to injection vials and 50 µL aliquots were injected into the HPLC system.

2.2.2. Chromatographic system and conditions

The HPLC system was the Shimadzu LC20A system (Shimadzu, Japan). Chromatogram was performed at ambient temperature on a steel, C_{18} (250 mm x 4.6 mm, 5 µm particle size) column (Gemini Phenomenex, USA). The mobile phase was a mixture of acetonitrile and pH 2.7 acetic acid buffer in a ratio of 60:40 (v/v). The flow rate was 1.0 mL min^{-1} and a UV wavelength of 256 nm was used.

2.3. Validation

The analytical method for rhein quantitation in plasma samples was validated according to international guidelines[14,15].

2.3.1. Selectivity

Analyte selectivity was ensured to avoid potential interferences by other endogenous components in plasma samples. Blank plasma samples from 6 different lots of volunteer plasma were checked for interfering peaks at, or close to, the retention times of rhein or IS.

2.3.2. Linearity

Calibration standards in plasma were

prepared 8 points at concentrations of 0.1; 0.3; 0.5; 1.0; 3.0; 5.0; 8.0 10.0 µg/mL. Linear was constructed by plotting peak area ratios of rhein to the IS. against concentrations in plasma. The correlation coefficient must be greater than 0.9900.

2.3.3. The lower limit of quantification (LLOQ)

The lower limit of quantification (LLOQ) was defined as the lowest concentration at which both precision and accuracy were less than or equal to 20 %, in replicates of 6.

2.3.4. Precision and accuracy

The measure of precision and accuracy, were determined at 0.1; 0.3; 5.0 and 8.0 µg/mL (LLOQ, LQC, MQC and HQC, respectively). Intra-day precision and accuracy was determined by repeated analysis of each QC sample on one day (n = 6), and inter-day was determined by repeated analysis on three consecutive days. The concentration of each sample was determined by linear on the same day. If they were within ± 15% of the actual value, except for the lower limit of quantification, for which ± 20% was regarded as satisfactory.

2.3.5. Recovery

Recovery was determined at LQC, MQC and HQC of rhein. Recovery was evaluated by comparing the peak area of extracted samples to the peak area of unextracted samples.

2.3.6. Stability

The stability of rhein in human plasma was evaluated at two concentrations (0.3 and 8.0 µg/mL) under different conditions, simulating the same conditions that occurred during study sample analysis. The short-term stability study was established after 6 hours stored at room temperature, after 24 hours pretreatment stored at 10^{o}C temperature in autosampler and freeze-thaw stability. The long-term stability was stored at -20 ^{o}C in 15, 35 and 60 days. Each level performed six replicates

2.3. Bioequivalence study

2.3.1. Study design

This was a single-center, randomized, open-label, single-dose, crossover, two-period, two-formulation study conducted to determining the bioequivalence study between the generic product and the reference product after oral administration of single dose of diacerein 50mg in healthy Vietnamese volunteers. This study was designed and conducted at Institute of Drug Quality Control, Ho Chi Minh City, Ministry of Health - Vietnam, in July 2013.

The study was conducted according to the principles of the Declaration of Helsinki and its amendments for biomedical research involving human subjects and the principles of the Good Clinical Practice guidelines. The clinical trial followed the guidance provided in Regulations for Clinical Trials of the Ministry of Health of Vietnam, and the clinical protocol and the informed consent form were approved by the local Ethics Committee (approval No. [2013]61/QyĐ-HĐĐĐ). All eligible subjects were informed of the aim and risks of the study by the clinical investigators and provided written informed consent before participation.

2.3.2. Study subjects

14 subjects were selected from healthy Vietnamese volunteers to participate in this study. Before study entry, subjects were interviewed and underwent a routine physical examination, including vital sign monitoring to ensure that they were healthy enough to participate in the study. Subjects met the following inclusion criteria: healthy; age between 18 and 45 years; body mass index (BMI) between 18 and 25 kg/m^2; do not abuse tobacco product, drug, alcohol; no clinically significant medical history of hypertension, diabetes, respiratory illness, gastrointestinal illness, renal or liver impairment, hereditary disease, tuberculosis; no known allergy to study medication or similar drugs; no current infection; negative HIV/AIDS, HBsAg and anti HCV.

2.3.3. Blood sampling

A total of thirty 6 mL blood samples (total volume of 180 mL) were obtained by venipuncture from each subject during the course of the study for evaluation of rhein. Blood samples were collected at 0 (pre-dose), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 10, 15 and 24 hours after study drug administration. The blood samples for rhein determinations were centrifuged within 20 minutes of blood collection. Immediately following centrifugation, plasma was transferred to plastic 5 mL labeled storage tubes using clean pipettes. The plasma samples were stored at or below -20 °C until analysis.

2.3.4. Pharmacokinetic and statistical analysis

The pharmacokinetic parameters were determined for all subjects using non-compartmental techniques. Excel software 2013 with an appropriate validated program was used for the calculation of pharmacokinetic parameters. To evaluate the bioequivalence of the test and reference formulations, ANOVA for the crossover design was conducted on ln-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ by Equiv Test PK software (Ireland). Statistical test was also conducted on T_{max} using Wilcoxon test at the significance level of 0.05. The two formulations were considered bioequivalent if the 90% confidence intervals for the ratios of the averages (population geometric means) of the measures of AUC_{0-t}, $AUC_{0-\infty}$, C_{max} of test and reference formulations were within a range of 80.00 % to 125.00 %[11,12].

3. RESULTS AND DISCUSSION

HPLC chromatograms of plasma rhein were shown in Fig. 2. The retention time of rhein was approximately 5.9 minutes. The HPLC method was validated in terms of linearity, range, precision, accuracy, recovery and stability. The calibration range was selected on the basis of expected concentration of rhein in human plasma samples. And the summary of validation results of the analyte and internal standard were shown in Table 1; 2.

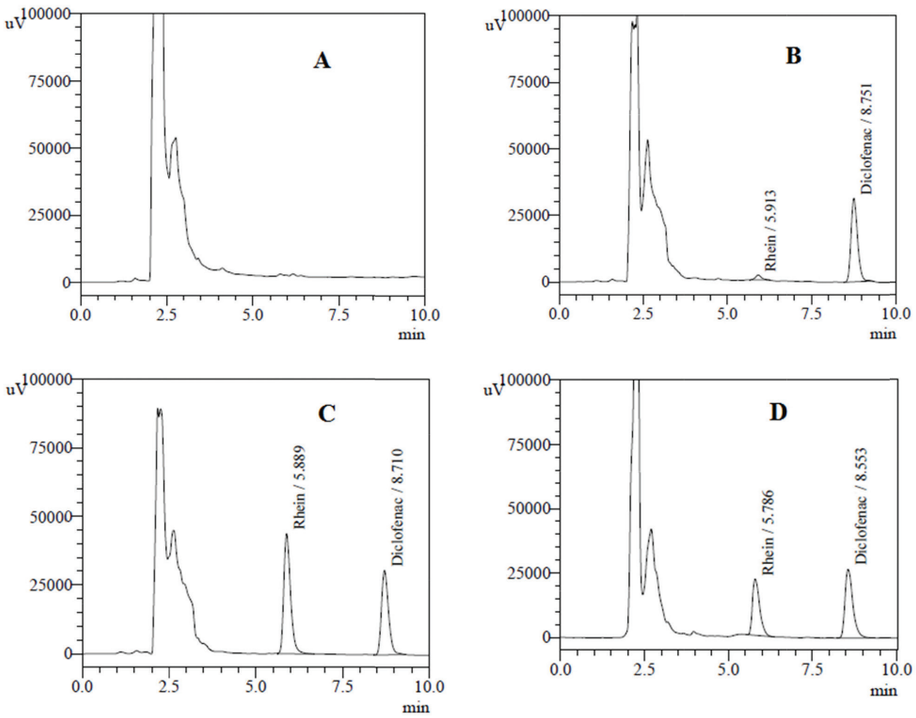

Figure 2. Chromatograms obtained from (A) blank plasma; (B) plasma spiked with 0.1 and 200 µg/mL of rhein and diclofenac; (C) plasma spiked with 8.0 and 200 µg/mL of rhein and diclofenac, respectively; (D) blood sample of volunteer was collected at 2 hours.

Table 1. The summary of validation results

Information requested	Data
Selectivity	No interference at the retention time and transition of analyte and internal standard.
Linearity (Range)	0.1 to 10 µg/mL
Coefficient of determination (r^2)	0.9978
Lower limit of quantification (LLOQ)	0.1 µg/mL
Precision	
• Intra-day precision	1.12 % to 2.56 %
• Inter-day precision	1.34 % to 3.52 %
Accuracy	
• Intra-day precision	102.05 % to 107.43 %
• Inter-day precision	101.98 % to 106.72 %
Recovery	
• Analyte	91.96 % to 99.20 %
• Internal standard	95.12 % to 99.44 %

Table 2. Stability samples result for rhein in human plasma

Stability test	QC (spiked conc. µg/mL)	Relative response (%)
Room temperature for 6 hours	0.3	98.10
	8	100.08
Freeze - thaw stability	0.3	95.30
	8	101.04
After 24 hours pretreatment	0.3	100.91
(10 oC)	8	98.23
Long term stability	0.3	92.68
(at - 20 oC for 15 days)	8	98.13
Long term stability	0.3	103.44
(at - 20 oC for 35 days)	8	98.03
Long term stability	0.3	104.43
(at - 20 oC for 60 days)	8	102.87

Bioequivalence study results

A total of 4 female and 10 male subjects (mean [SD][range]) age, 24.2 [3.7][21-35] years; body mass index (BMI), 21.1 [2.3][18.1 - 24.6] kg/m^2 were enrolled in the study. All subjects completed both treatment periods.

The mean plasma concentrations of rhein versus time after oral administration of test and referent products was shown in Table 3. Table 4 summarized the pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}) the ratio $AUC_{0-t}/AUC_{0-\infty}$ results and the statistical comparison results of rhein. The mean plasma rhein concentrations were showed in Fig.3.

Table 3. The mean (±SD) plasma concentration of rhein versus time after oral administration of test and referent products (N = 14).

Time (hr)	Mean (±SD) plasma concentration for test product (µg/mL)	Mean (±SD) plasma concentration for reference product (µg/mL)
0.0	0.00 ± 0.00	0.00 ± 0.00
0.5	0.60 ± 0.23	0.75 ± 0.35
1.0	1.32 ± 0.70	1.45 ± 0.80
1.5	1.76 ± 0.87	2.35 ± 1.39
2.0	2.85 ± 1.74	3.51 ± 1.99
2.5	3.57 ± 2.54	4.07 ± 2.54
3.0	3.70 ± 2.90	3.94 ± 2.35
3.5	4.00 ± 2.87	4.02 ± 2.67
4.0	3.95 ± 2.65	3.65 ± 2.42
4.5	3.73 ± 2.39	3.53 ± 2.34
5.0	2.68 ± 1.33	2.75 ± 1.87
6.0	1.89 ± 0.97	1.87 ± 1.38
10	1.02 ± 0.57	0.95 ± 0.65
15	0.45 ± 0.27	0.42 ± 0.26
24	0.18 ± 0.12	0.18 ± 0.12

Table 4. Summary pharmacokinetic parameters of rhein

Product	Test (mean ± SD)	Reference (mean ±SD)	Geometric mean ratio T/R	90% confidence interval (%)
AUC_{0-t} (µg.h/mL)	30.00 ± 8.80	29.62 ± 7.70	101.28	90.17 - 113.76
$AUC_{0-\infty}$ (µg.h/mL)	31.50 ± 9.13	31.11 ± 8.37	101.26	90.48 - 113.32
C_{max} (µg/mL)	5.36 ± 0.88	5.47 ± 1.10	98.00	93.43 – 102.80
T_{max} (hour)	3.11 ± 1.08	2.57 ± 0.87		
AUC_{0-t} / $AUC_{0-\infty}$ ratio (%)	95.29	94.95		
$T_{1/2}$ (hour)	5.32	5.72		
K_e	0.1303	0.1212		

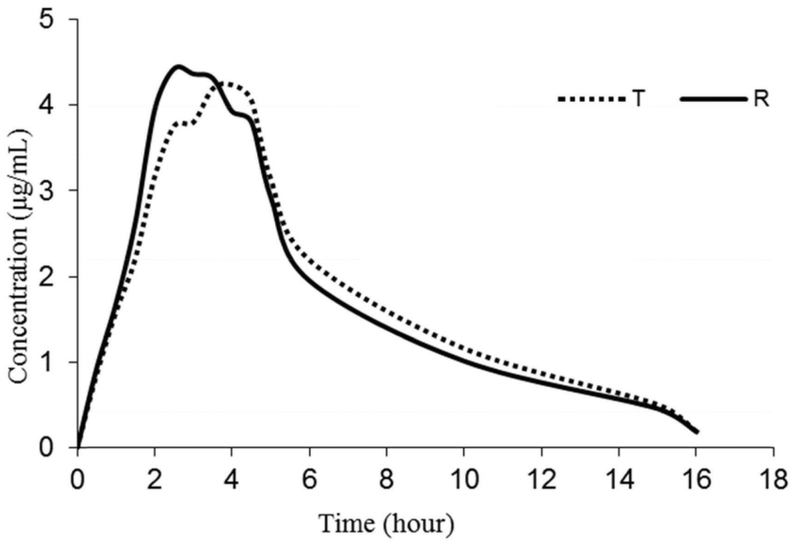

Figure 3. Mean plasma rhein concentration-time curve

The power of all primary pharmacokinetic parameters were greater than 80 % indicating that the number of subjects was enough to confirm the bioequivalence of two formulations.

In our study, no period, treatment and sequence effects for any pharmacokinetic property were found using ANOVA in all subjects. The ratio $AUC_{0-t}/AUC_{0-\infty}$ results indicate that a washout period of 24 hours was adequate for total elimination of the drug between the 2 administration periods (no less than 90%). No subject withdrew from our study, and no adverse events were found on analysis of vital signs or laboratory test results. No abnormalities were found in clinical or biochemical parameters when comparing baseline versus end-of-study assessments.

This study assessed the bioequivalence of diacerein capsules. There were no significant differences between formulations in pharmacokinetic properties in the small, selected, fasting, healthy Vietnamese population.

4. CONCLUSION

The assay reported in this paper is rapid, specific and sensitive for quantification of rhein in human plasma and was fully validated according to commonly acceptable FDA guidelines. Our study found that the test and reference formulations of diacerein met the regulatory criteria for bioequivalence in healthy Vietnamese volunteers.

5. ACKNOWLEDGMENTS

This research was sponsored by DAVIPHARM Co., Ltd., Vietnam.

REFERENCES

1. Falgarone G, Dougados M. Diacerein As a Disease-modulating Agent in Osteoarthritis. Curr Rheumatol Rep. 2001;3(6): 479-83.
2. Nicolas P, Tod M, Padoin C, Petitjean O. Clinical Pharmacokinetic of Diacerein. Clin Pharmacokinet. 1998;35(5): 347-59.
3. Zhu W, Wang XM, Zhang L, Li XY, Wang BX. Pharmacokinetic of Rhein in Healthy Male Volunteers Following Oral and Retention Enema Administration of Rhubarb Extract: A Single Dose Study. Am J Chin Med. 2005;33(6):839-50.

4. Chakrabarty US, Mandal U, Bhaumik U, Chatterjee B, Ghosh A, Bose A, Pal TK. Bioequivalence study of two capsule formulations containing diacerein 50 mg in healthy human subjects. Arzneimittel-forschung. 2008;58(8): 405-09.
5. Ojha A, Rathod R, Padh H. Simultaneous HPLC-UV determination of rhein and aceclofenac in human plasma. J Chromatogr B. 2009;877(11-12):1145-48.
6. Yaroshenko IS, Ya. Khaimenov A, Grigoriev AV, Sidorova AA. Determination of Rhein in blood plasma by HPLC with UV detection and its application to the study of bioequivalence. J Anal Chem. 2014;39(8): 793-99.
7. Layek B, Kumar TS, Trivedi RK, Mullangi R, Srinivas NR. Development and validation of a sensitive LC-MS/MS method with electrospray ionization for quantitation of rhein in human plasma: application to a pharmacokinetic study. Biomed Chromatogr. 2008;22(6):616-24.
8. Jiang JY, Yang MW, Qian W, Lin H, Geng Y, Zhou ZQ, Xiao DW. Quantitative determination of rhein in human plasma by liquid chromatography-negative electrospray ionization tandem mass/mass spectrometry and the application in a pharmacokinetic study. J Pharm Biomed Anal. 2012;57:19-25.
9. US Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for industry: bioanalytical method validation; 2001. Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation /Guidances/ucm070107.pdf.
10. State Food and Drug Administration, Center for Drug Evaluation. Guidance for Bioavailability and Bioequivalence Studies for Chemical Drug Products in Humans; 2005. Available from: http://www.cncro.com/fgwjDetail_28.html.
11. US Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for industry: bioavailability and bioequivalence studies for orally administered drug products - general considerations; 2003. Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070124.pdf.
12. US Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for industry: statistical approaches to establishing bioequivalence; 2001. Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070244.pdf.