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Bioequivalence Assessment of Triamcinolone Tablets in Healthy Male Human Volunteers

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Abstract – The bioequivalence of two 4 mg triamcinolone tablets (Dong-Kwang Triamcinolone® *vs.* Wyeth Korea Ledercoat®) was assessed in healthy male volunteers after oral administration of 16 mg triamcinolone in a randomized crossover study. Blood samples were collected at specified time intervals, and plasma was analyzed for triamcinolone using a validated HPLC method. The pharmacokinetic parameters of T_{max}, C_{max}, $AUC_{0\rightarrow last}$, $AUC_{0\rightarrow inf}$, and $T_{1/2,\beta}$ were determined from plasma concentration-time profile of two formulations. The pharmacokinetic parameters were statistically compared to evaluate bioequivalence between two formulations, according to Korea Food and Drug Administration Guideline. The analysis of variance did not show any significant difference between the two formulations and 90% confidence limits fell within the acceptable range (80-120%) for bioequivalence. Based on these data it was concluded that the two products showed comparable pharmacokinetic profiles and that the Dong-Kwang triamcinolone® tablet is bioequivalent to the Wyeth Korea Ledercoat® tablet.

Key words: triamcinolone, bioequivalence, pharamcokinetics, HPLC, Dong-Kwang Triamcinolone®, Wyeth Korea Ledercoat®

Triamcinolone (9α-fluoro-16-hydroxyprednisolone), one of potent glucocorticoid steroids, has been widely used for the treatment of a wide spectrum of diseases such as adrenal insufficiency, inflammation, arthritis, asthma, leukemia, septic shock, and congenital adrenal hyperplasia (Schimmer and Parker, 2001; Carey, 1997; Rubin, 1997; Zelissen *et al.*, 1994). Glucosteroids transported by transcortin enter the target cell by diffusion and then form a complex with its cytosolic receptor protein. The steroid-receptor complex undergoes an irreversible activation process that results in its migration to the nucleus, binding to the hormone response elements (Schimmer and Parker, 2001; Mangeldorf *et al.*, 1995). This results in gene transcription and production of mRNA to modify protein synthesis.

Triamcinolone is similar in chemical structure to hydrocortisone, the endogenous glucocorticoid released from the adrenal gland. Triamcinolone, however, is more lipid soluble and has greater affinity for glucocorticoid receptor than hydrocortisone. Triamcinolone was metabolized mainly in the liver and kidney, resulting in inactivation by the reduction of the double bond at position 4-5, reduction of the ketone group at carbon-3, and hydroxylation at carbon-6 (Schimmer and Parker, 2001; Argenti *et al.*, 2000a).

Formulations of triamcinolone include tablets, suspension, pressurized inhaler (Argenti *et al.*, 2000b), and injection, which can be applied to patients parenterally, non-parenterally or topically. In patients receiving oral administration of 16 mg triamcinolone, terminal half-life ($T_{1/2,\beta}$) and T_{max} were reported to be 2.7 and 1.9 hr, respectively. C_{max} and $AUC_{0\rightarrow inf}$ were, respectively, 94.7 ng/ml and 557.9 ng · hr/ml. The volume of distribution of triamcinolone was twice the value of prednisolone and methylprednisolone that C_{max} values were higher than that of triamcinolone (Hochhaus *et al.*, 1990; Portner *et al.*, 1988). In the other study, half-life of triamcinolone was 2 hr with 23% of oral bioavailability and T_{max} was occurred at 1 hr, indicating rapid absorption of triamcinolone (Derendorf *et al.*, 1995). After intravenous administration of triamcinolone acetonide, only 1% of the dose was found in the urine as the parent form (Mollmann *et al.*, 1985). Chronic administration of triamcinolone results in significant reduction in basal serum cortisol level

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(Argenti *et al.*, 2000b).

The purpose of this work was to compare the bioavailability (rate and extent of absorption) of generic formulations of triamcinolone (4 mg Dong-Kwang triamcinolone® tablets) to the reference formulation (Wyeth Korea Ledercoat® tablets).

MATERIALS AND METHODS

Study Products

Test product is Dong-Kwang Triamcinolone® as a 4 mg tablet with batch number of Z003 and this product will be expired on June 13, 2004. It was prepared by Dong-Kwang Pharm. Co., Ltd. (Seoul, Korea). Ledercoat® tablets (4 mg triamcinolone) was used as a reference formulation. Its batch number is 1001 and this product will be expired on January 17, 2004. It was prepared by Wyeth Korea Inc.(Seoul, Korea).

Chemicals

Authentic triamcinolone was provided by Dong-Kwang Pharam. Co., Ltd. (Seoul, Korea). Acetonitrile, diethyl ether and methanol were obtained from J. T. Baker (Phillipsburg, NJ, USA). Dexamethasone was purchased from Sigma (St. Louis, MO, USA). The other agents used for triamcinolone analysis were of analytical grade.

Study subjects

After approval of pre-planed proposal of the study by Korea Food and Drug Administration (KFDA), male volunteers who submitted the agreement to attend to this project were medically examined and 16 healthy volunteers were selected by a medical doctor in Chungbuk National University Hospital (Cheongju, Chungbuk, Korea), based on clinical examination including seropathological (hemoglobin, hematocrit, WBC, platelet), serochemical (blood urea nitrogen, creatinine, total protein, albumin, SGOT, SGPT, total bilirubin, cholesterol, glucose fasting, alkaline phosphatase), and urological (specific gravity, color, pH, sugar, albumin, bilirubin, RBC, WBC) data. The subjects were instructed not to take any medicine for at least 1 week prior to and during the study period. Informed consent was filled up by the subjects after explanation of the nature and aims of the work. They were accommodated to the same place one day before blood collection. They were fasted overnight before administration of the tablets. The study protocols were approved by the Institutional Review Board of Chungbuk National University Hospital.

Oral administration of triamcinolone tablets to human volunteers

A 21-gauge scalp-vein set was established on the arm vein of the volunteers, and 8 ml of blood for blank were collected. According to the prescription directed by the doctor, 4 tablets (16 mg) were orally taken to the designated group at random design (8 volunteers a group) with 150 ml of water. One group received the tablet for test, and the other for reference. No food was allowed until 4 hr after dose administration. Lunch and dinner were provided to volunteers according to a time schedule. Beverages and caffeine were not allowed during the study. These two groups were taken the formulation by the 2 × 2 Latin square crossover design after they have a wash-out period for one week. Blood was taken into heparin-treated Vacutainer tube (Becton Dikinson, Rutherford, NJ, USA) at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 8, and 12 hr after the oral administration. The time interval of blood sampling between volunteers was 2 min to consider blood collection time. Blood was centrifuged to obtain plasma at 4°C. The plasma was stored at −70°C until analyzed.

HPLC equipment

Triamcinolone concentrations in the plasma were determined by using a HPLC system (HP 1090 M, Hewlett Packard, DE, USA) equipped with DR5 solvent delivery system and an auto liquid sampler. Triamcinolone analysis was conducted at 246 nm using a diode array detector. The separation of triamcinolone was performed by using a Microbore Hypersil-ODS column (100 × 2.1 mm, I.D.; 5 μm particles). The flow rate of the column was 0.2 ml/min. Mobile phase was consisted of initially water: acetonitrile (80 : 20, v/v, %), and increased to water: acetonitrile (40 : 60, v/v, %) by 4% per min.

Preparation of calibration curve of triamcinolone

To a 15 ml of centrifuging tube, an aliquot of the thawed blank plasma (1 ml) was added. And the various concentrations of triamcinolone were spiked to make the final concentration of 10, 20, 30, 50, 100 and 200 ng/ml. Dexamethasone of 100 ng (10 μg/ml, 10 μl) was added as internal standard. After the tube was mechanically mixed on a vortex-mixer (Maxi Mix II, Thermolyne Co., Dubuque, IA, USA), 5 ml of diethyl ether was added. The tube was vigorously shaken on a shaker and the organic layer was separated by centrifugation (Triac, Clay Adams, Rutherford, NJ, USA). Extraction step with 5 ml of diethyl ether was repeated. The organic layer was evaporated on an evaporator under a vacuum condition. The residue was

dissolved in 100 μl of methanol and filtered with a membrane (pore size 0.45 μm; CE Minisart RC 4, Sartorius, Germany). The solution of 20 μl was applied to the HPLC system. Calibration curve was prepared from the area ratios of the peaks of triamcinolone to dexamethasone. Inter- and intra-day precisions and accuracy were obtained.

Preparation of plasma samples

One ml of thawed plasma obtained from healthy human volunteers was added to the 15 ml centrifuging tubes, followed by addition of internal standard dexamethasone (10 μg/ml, 10 μl). The tube was treated as described above. Based on the calibration curve of triamcinolone, the plasma concentrations of triamcinolone were determined from peak area ratios of triamcinolone to dexamethasone.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined from the time-plasma concentrations of triamcinolone by non-compartmental analysis by using WinNonlin software (Scientific Consulting Inc., Cary, NC, USA). The highest concentration (C_{max}) and the time to reach the highest concentration (T_{max}) were read directly from the time-plasma concentration curves of triamcinolone. The area under the curve of time-plasma concentrations of triamcinolone until the infinitive time ($AUC_{0\rightarrow inf}$) was determined by the equation of $AUC_{0\rightarrow inf} = AUC_{0\rightarrow last} + C_{last}/\beta$, where β is the slope of the terminal phase of the time-log plasma concentration curve and C_{last} is the concentration at the last sampling time (EC, 1991).

Statistics

Data are presented as mean ± standard deviation. K-BE test® software (Seoul National University, Seoul, Korea) was used for the determination of bioavailability difference, minimum detection difference, the power (1−β) and 90% confidence limit (Lee *et al.*, 1998). Analysis of variance (ANOVA) was done by the general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC, USA) to determine F-values and probability. Two formulations are considered to be bioequivalent when 90% confidence limits of logarithmically transformed C_{max} and AUCs are ranged from log 0.8 to log 1.25 according to the recently revised KFDA guideline (KFDA, 2001) which is finally equal to European community and FDA guidelines (EC, 1991; FDA, 2001). In addition, it is also considered to be bioequivalent when statistical results for bioavailability difference, minimum detection difference and 90% confidence limit fall within 20% (KFDA, 1998; Lee *et al.*, 1998).

RESULTS AND DISCUSSION

Analytical method of the plasma concentration of triamcinolone was validated prior to the analysis of human plasma. Retention times of triamcinolone and dexamethasone in HPLC chromatograms were about 18.7 and 21.8 min, respectively (Fig. 1).

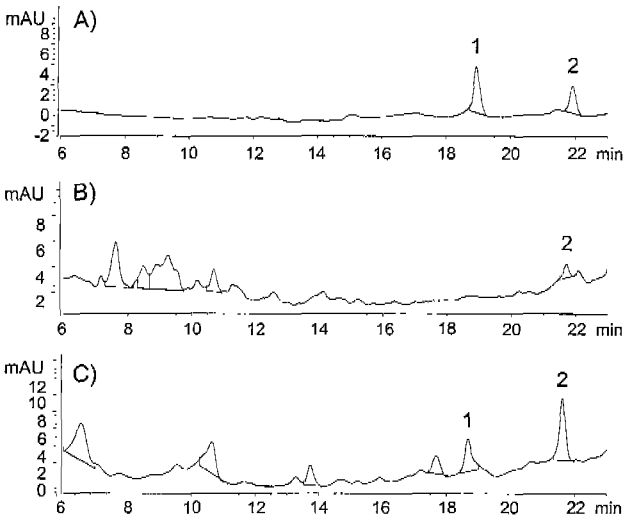

Fig. 1. The HPLC chromatograms obtained from A) standards of triamcinolone and internal standard dexamethasone (each, 100 ng/ml), B) blank plasma spiked internal standard, and C) plasma samples at 1 hr after oral administration of 16 mg triamcinolone to a human volunteer. Retention times of triamcinolone (peak 1) and dexamethasone (peak 2) were about 18.7 and 21.8 min, respectively.

Table I. Intra- and inter-day precision and accuracy for the determination of triamcinolone in the plasma of human volunteers.

Concentrations of triamcinolone (ng/ml)	Intra-day precision (CV %), n=3	Inter-day precision (CV %), n=3	Accuracy %
10	0.031 ± 0.006 (18.96)	0.026 ± 0.002 (8.64)	1.07
20	0.066 ± 0.002 (2.76)	0.064 ± 0.009 (14.31)	1.54
30	0.101 ± 0.006 (5.60)	0.094 ± 0.005 (5.64)	8.40
50	0.170 ± 0.005 (2.84)	0.187 ± 0.015 (7.96)	−2.63
100	0.351 ± 0.006 (1.57)	0.369 ± 0.016 (4.32)	2.16
200	0.706 ± 0.007 (0.92)	0.713 ± 0.056 (7.85)	6.96

Table II. Physical and pharmacokinetic parameters after administration of triamcinolone (4×4 mg tablets) to human volunteers involved in the bioequivalence study

Subjects	Sex	Age (Years)	Weight (kg)	Height (cm)	Ledercoat			Dong-Kwang		
					$AUC_{0\rightarrow last}$ (ng/ml· hr)	C_{max} (ng/ml)	T_{max} (hr)	$AUC_{0\rightarrow last}$ (ng/ml· hr)	C_{max} (ng/ml)	T_{max} (hr)
A1	M	21	65	181	399.1	94.4	2..0	435.5	96.1	2.0
A2	M	25	70	170	500.2	107.4	2.0	479.6	128.6	2.0
A3	M	25	85	176	344.7	101.1	3.0	299.2	103.9	3.0
A4	M	25	68	178	388.0	139.7	2.0	306.0	85.6	2.0
A5	M	25	58	173	448.2	83.3	2.5	402.3	83.3	2.0
A6	M	26	69	176	370.3	98.1	2.5	364.5	89.5	2.5
A7	M	24	69	169	405.1	112.7	2.5	342.6	95.0	2.5
A8	M	23	61	169	282.4	90.0	3.0	288.0	94.9	2.5
B1	M	28	70	180	469.4	107.1	2.0	439.7	115.0	2.0
B2	M	23	60	171	310.4	72.6	1.5	234.4	106.0	1.5
B3	M	27	63	170	530.3	126.0	1.5	452.8	98.4	2.0
B4	M	27	71	173	587.2	156.1	2.0	681.3	155.1	2.0
B5	M	25	58	169	572.8	101.6	2.0	510.6	100.8	2.0
B6	M	28	56	163	368.5	126.8	2.0	459.2	136.4	1.5
B7	M	26	73	168	364.5	103.6	1.5	443.9	91.5	2.0
B8	M	24	65	177	499.5	153.5	2.0	521.3	158.3	2.0
Mean	_	25.1	66.3	172.7	427.5	110.9	2.1	416.3	108.7	2.1
S.D.		1.9	7.3	4.9	91.3	23.9	0.5	110.4	23.6	0.4

Detection limit of triamcinolone obtained from 1 ml of the plasma by this HPLC method was 10 ng/ml. Calibration curve showed good linearity at concentrations from 10 to 200 ng/ml of triamcinolone ($y=0.00358 \times 0.004530$, $r^2 = 0.9997$). CV% for intra-day and inter-day precisions were ranged from 0.9% to 19% and from 4.3% to 14.3%, respectively. Accuracy was less than 8.4%. These results were showed in Table I.

All 16 volunteers have participated in the study to the end with discontinuation. The mean age of the volunteers was 25.1 years, ranging from 21 to 28 years. The mean body weight was 66.3 kg with the ranges from 56 to 85 kg, and the height of the volunteers was ranged from 163 to 181 cm with the mean value of 172.7 cm. These physical parameters for each volunteers was showed in Table II. The seropathological, serochemical and urological data in the volunteers were showed a normal value (data not shown), indicating that no subjects have hepatic, renal or hematological deficiency or malfunction.

Oral dosage of triamcinolone was 16 mg, based on the report of pharmacokinetic studies (Hochhaus *et al.*, 1990; Portner *et al.*, 1998) with using a 4 mg tablet unit for the purpose of comparing bioavailability of two formulations of 4 mg tablets. The principal pharmacokinetic parameters obtained from the time-plasma concentrations of triamcinolone (Figure 2) were showed in Table II. T_{max}, C_{max}, and $AUC_{0\rightarrow last}$ of the test formulation were not statistically different from those of the reference ($Pr > 0.05$). The mean T_{max} was 2.09 hr for the test formulation and 2.13 hr for the reference formulation. The

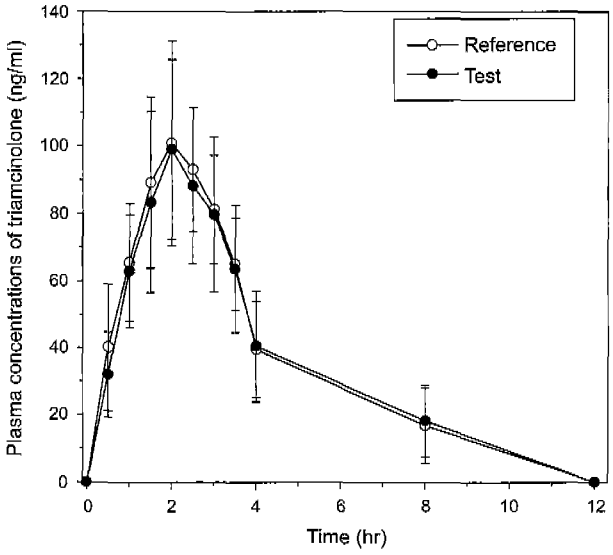

Fig. 2. The plasma concentration-time curves of triamcinolone after oral administration of two different formulations (as 4×4 mg tablets) of triamcinolone in human volunteers. Mean values (± SD) of plasma triamcinolone concentrations of 16 volunteers for test or reference formulation were represented.

mean C_{max} was 108.7 and 110.9 ng/ml for the test and reference formulations, respectively. The mean $T_{1/2,\beta}$ of triamcinolone tablets orally administered were 4.56 hr for the test formulation and 3.99 hr for the reference. Hochhaus *et al.* (1990) had reported the half-life of 2.8 hr, C_{max} value of 94.65 ± 23.03 ng/ml and T_{max} of 1.90 ± 0.52 hr in human volunteers orally taken 16 mg of triamcinolone tablets. In the other studies, the half-life

Table III. Statistics for pharmacokinetic and bioequivalence parameters of triamcinolone formulations

	C_{max}		$AUC_{0\rightarrow last}$		T_{max}	
Drug, F (1, 30)[a]	F	Pr>F	F	Pr>F	F	Pr>F
	0.01	0.9213	0.12	0.7303	0.04	0.8358
Bioavailability difference (%)[b]	−1.99		−1.75		−1.46	
Power (1−β)[c]	>0.9		>0.9		>0.9	
Minimum detection difference (Δ%)[d]	13.65		11.81		9.90	
90% Confidence limit						
Untransformed (δ%)[e]	−9.98≤δ≤5.98		−8.65≤δ≤5.15		−7.26≤δ≤4.32	
Log-transfomed (μ_T/μ_R)	$0.90 \le (\mu_T/\mu_R) \le 1.02$		$0.93 \le (\mu_T/\mu_R) \le 1.06$		–	

[a]Obtained from the general linear procedure of SAS.
[b-e]Obtained by using K-BE test Software.
[d]α=0.05, 1-β=0.8.

of triamcinolone was reported to be 4 hrs (Saito *et al.*, 1979). $AUC_{0\rightarrow last}$ for two formulations was about 82% of $AUC_{0\rightarrow inf}$. By definition, bioequivalent drug products are pharmaceutical equivalents that have similar bioavailability (i.e., are not significantly different with respect to rate and extent of absorption) when given in the same molar dose and studied under similar experimental conditions. If the drug products are demonstrated to be bioequivalent, then the efficacy of these drugs is assumed to be similar (Shargel and Yu, 1993).

Analysis of variance was also carried out using logarithmically transformed $AUC_{0\rightarrow last}$, $AUC_{0\rightarrow inf}$, and C_{max} and non-transformed T_{max}. There were no significant differences between the formulations in $AUC_{0\rightarrow last}$, $AUC_{0\rightarrow inf}$, and C_{max}. The point estimates and 90% confidence intervals for $AUC_{0\rightarrow last}$ (parametric), $AUC_{0\rightarrow inf}$ (parametric), and C_{max} (parametric) were 0.96 (0.90 to approximately 1.02), 0.97 (0.87 to approximately 1.07), and 0.99 (0.93 to approximately 1.06), respectively, satisfying the bioequivalence criteria of Korea Drug Administration Guideline (KFDA, 2001), and these results, concomitantly, satisfied the bioequivalence criteria of European Community and the United State FDA guidelines (EC, 1991; FDA, 2001).

Bioavailability differences % of C_{max}, $AUC_{0\rightarrow last}$ and T_{max} between two formulations were 1.99, 1.75 and 1.47, respectively. Minimum detection difference % for C_{max}, $AUC_{0\rightarrow last}$ and T_{max} were 13.65, 11.81 and 9.90, respectively. 90% confidence limits for these parameters were located within ±20% (Table III). C_{max} and $AUC_{0\rightarrow last}$ satisfied the bioequivalence acceptance criteria described in the Korea KFDA guideline (KFDA, 1998).

All taken together, the statistical comparison of $AUC_{0\rightarrow last}$ and C_{max} indicated no significant difference in the two brands of 4 mg triamcinolone tablets. 90% confidence intervals for $AUC_{0\rightarrow last}$ and C_{max} were satisfied the bioequivalence criteria as suggested in the Korea or United State Food and Drug Administration. Based on the pharamcokinetic and statistical data, we conclude that the Dong-Kwang triamcinolone® tablet is bioequivalent to the Ledercoat® tablet produced by Wyeth Korea, and the two products can be considered interchangeable in medical practice.

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