Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2011; 3(1): 01-04

Research Article ISSN 0975 1556

Dermatopharmacokinetic Approach to Evaluate and Compare the Pharmacokinetic Profile of Marketed Preparations of Diclofenac Sodium

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ABSTRACT

In this single-dose-one arm, open label three way parallel design, pharmacokinetic study of three marketed formulations of Diclofenac Sodium using 12 healthy Indian male subjects the pharmacokinetic parameters of three marketed Diclofenac Sodium topical formulations were compared . Marketed Diclofenac Sodium topical formulations (A, B & C) were applied on the pre-marked forearms of the subjects as per the dosing schedule. Treatment sample C was used as a reference sample. Subjects received treatment A, treatment B & treatment C on both the arms simultaneously, following open label three way parallel design. Skin Stratum Corneum samples were collected in sterile glass test tubes during the study period. The samples were collected pre-dose and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, & 6.0 hours post-dose application. The Stratum Corneum samples were analysed for Diclofenac Sodium concentrations only. Pharmacokinetic parameters of Diclofenac sodium were calculated as C_{max}, $t_{max,}$ AUC (0-t) and AUC (0-∞) Diclofenac Sodium was estimated in Stratum Corneum using a validated Spectroscopic method. If the point estimate of the geometric mean ratio and the confidence intervals for the entire log transformed pharmacokinetic parameters [C_{max}, AUC (0-t) and AUC (0-∞)] were entirely included in the range of 80-125%, then the treatments were claimed to be bio-equivalent. A total of 12 subjects were enrolled in this study. The bioequivalence values of the test drug A were C_{max} of 23.767±2.398 µg/mL, t_{max} of 1.75 ±0.261 h, AUC_{0-t} of 100.507± 10.455 h. µg/mL, $AUC_{0-\infty}$ of 178.286± 22.859 h. µg/mL; of the test drug B C_{max} of 31.1± 2.742 µg/mL, t_{max} of 1.75 ±0.261 h, AUC_{0-t} of 103.555± 10.072 h. µg/mL, $AUC_{0-\infty}$ of 166.971± 47.627 h. µg/mL; and of test drug C C_{max} of 24.084± 2.216 µg/mL, t_{max} of 1.75 ±0.261 h, AUC_{0-t} of 100.586±11.15 h. µg/mL, $AUC_{0-\infty}$ of 179.887± 21.553 h. µg/mL.

Keywords: Dermatopharmacokinetic, Stratum Corneum, Diclofenac, Bioequivalence, Skin stripping.

INTRODUCTION

Bioequivalence is a relative term which denotes that the drug substance in two or more identical dosage forms, reaches the systemic circulation at the same relative rate and to the same relative extent i.e. their plasma concentration profiles will be identical without significant statistical differences. Thus in the case of topical formulations the drug has to penetrate through the layers of skin to reach the local site of action which is a complex process only due to the rate limiting barrier of the Stratum corneum. [1]

The penetration of a drug through the skin is a complex process typically rate-limited by the stratum corneum (SC). This external layer of the skin is composed of terminally differentiated corneocytes embedded in a complex lipid matrix comprising primarily ceramides, cholesterol, and free fatty acids. Delivery of drug by passive diffusion and the pharmacological effect elicited are dose-related: the better the drug permeates the skin, the greater the therapeutic effect. It follows, therefore, that formulation plays an important role in topical drug delivery as the composition of the vehicle will influence the partitioning and/or the diffusivity of the drug and hence the absolute amount delivered. [1-2]

The determination of the Bioequivalence of topical products involves the Dermatopharmacokinetic (DPK) approach. The DPK approach includes any measure of drug concentration in the skin, whether directly or indirectly related to the drug's therapeutic action, which can be determined continuously or intermittently for a period of time. This may include the measurement of either drug concentration in Stratum corneum over time and/or drug concentration in serial biopsy samples. The measurement of the change in the Stratum corneum drug concentration as a function of time is the objective of DPK approach and thus is a valid means of comparing a generic and innovator product for their ability to deliver drug to the deeper layers of the skin. [3]

DPK studies offer certain advantages as it is painless, the active drug substances (moieties) are protected from gastric enzymes, it avoids first pass effect, and it is simple to terminate if any adverse or undesired effect is observed. [2-4]

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Various Techniques and Methods Practiced in Dermatopharmacokinetic

There are many *in-vitro*, *in-vivo* methods for pharmacokinetic assessment of the dermal products, of which the most important and easy method is *in-vivo* tape stripping technique, which and some other techniques are as mentioned below:

- Tape Stripping Technique
- Microdialysis [2, 4]
- In Vitro Permeation Assessment [4, 6]
- Confocal Laser Scanning [4]
- Cadaver Skin Permeation [5]
- Vasoconstrictor Assay [5]

Tape Stripping Technique: The method consists of the standardized protocol of repeated applications and removal of adhesive tape on the skin surface, whereby consecutive layers of Stratum Corneum cells are sampled. As discussed by J Lademann *et al*; Tape stripping is a standard measuring method for the investigation of the Dermatopharmacokinetic of topically applied substances using adhesive films. These tape strips are successively applied and removed from the skin after application and penetration of topically applied substances; thus, the layers of the corneocytes and certain amount of topically applied substances are removed. The amount of the substances and the amount of Stratum corneum removed with the single tape strip is to be determined for calculation of the penetration profile. The topically applied substances removed from the skin can be thus determined by various analytical methods like HPLC, Mass Spectroscopy and other spectroscopic measurements. [4-5]

Diclofenac is an acetic acid no steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. Diclofenac is used to treat pain, dysmenorrhea, ocular inflammation, osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and actinic keratosis.Diclofenac has pharmacologic action similar to those of other prototypical NSAIAs. The drug exhibits anti-inflammatory, analgesic, and antipyretic activity. The exact mechanisms have not been clearly established, but many of the action appear to be associated principally with the inhibition of prostaglandins synthesis. Diclofenac inhibits the synthesis of prostaglandins in body tissues by inhibiting cyclooxgenase; at least 2 isoenzymes, cyclooxygenase-2 (COX-1) and -2 (COX-2) (also referred to as prostaglandin G/H synthase-1[PGHS-1] and [PGHS-2], respectively), have been identified that catalyze the formation of prostaglandins in the arachidonic acid pathway. The pharmacodynamic effect is thought to reduce prostaglandin E2 (PGE2) synthesis. [6]

SUBJECT AND METHOD

Study Subjects

Sufficient numbers of healthy Indian male human subjects was screened, out of those 09 male subjects were enrolled in the study and 03 male subjects were taken as standby. A total of 12 male subjects were applied with the study medication in the beginning of the study. The screening consent & study consent was taken respectively before drug application. Thereafter, subject's medical records were documented and physical examination was conducted. Inclusion eligibility was also based on successful completion of a clinical health evaluation , which consisted of a personal interview; a complete physical examination (BP, pulse, weight, temperature, and respiratory rate); diagnostic testing that included a 12-lead electrocardiogram and chest radiograph; a laboratory testing that included a complete blood cell count, metabolic and hepatic tests (alanine amino transferase [reference range, 5-55 U/L], aspartate amino transferase [5-34 U/L]), urine analysis, pregnancy test (for female subjects), blood chemistry for glucose (70-109 mg/dL), blood urea nitrogen (7-23 mg/dL), and creatinine (0.-1.3 mg/dL), as well as serologic tests for hepatitis (B and C), and HIV antibodies. Testing was performed by Central Pathology Laboratory, MGM Hospital, N-6 CIDCO, Aurangabad, (MS) INDIA 431005. Subjects were excluded if laboratory values were significantly above or below the reference range and/or if all tests had not been performed. In addition, the laboratory data were reviewed by the investigators of the clinical unit prior to the enrollment of the subjects. Subjects were compensated for participation.

Table 1: Treat A Gel

Time	0	0.5	1	1.5	2	3	4	6
N	12	12	12	12	12	12	12	12
Mean	0	13.184	16.822	21.854	22.592	19.893	17.194	11.948
SD	0	2.643	2.724	3.384	2.512	2.761	3.114	0.882
Min	0	8.007	12.78	16.428	19.23	16.208	10.972	10.23
Max	0	17.16	22.245	26.241	25.96	24.63	21.234	13
CV%	Missing	20.04	16.19	15.49	11.12	13.88	18.11	7.38
Geometric Mean	Missing	12.916	16.62	21.608	22.462	19.716	16.906	11.917

Table 2: Treat B Spray

Time	0	0.5	1	1.5	2	3	4	6
N	12	12	12	12	12	12	12	12
Mean	0	13.952	21.449	28.297	27.521	19.003	13.886	11.232
SD	0	2.024	3.423	5.053	3.78	3.705	2.593	2.206
Min	0	11.23	15.476	18.037	21.36	14.56	10.024	8.23
Max	0	16.705	26.35	34.85	34.23	25.321	18.36	14.68
CV%	Missing	14.51	15.96	17.86	13.74	19.5	18.67	19.64
Geometric Mean	Missing	13.815	21.189	27.842	27.285	18.687	13.666	11.027

Table 3: Treat C Emulgel

Time	0	0.5	1	1.5	2	3	4	6
N	12	12	12	12	12	12	12	12
Mean	0	13.661	16.773	21.728	22.918	19.342	17.372	11.917
SD	0	2.608	2.747	3.381	2.933	2.659	3.423	1.199
Min	0	8.45	11.95	16.89	18.32	15.62	11.25	10.03
Max	0	17.66	21.965	26.25	26.607	23.54	22.56	13.5
CV%	Missing	19.09	16.38	15.56	12.8	13.75	19.71	10.06
Geometric Mean	Missing	13.414	16.56	21.481	22.739	19.172	17.03	11.859

Study Design

This study was carried out as per the ICH (Step 5), 'Guidance for Good Clinical Practices (GCP)' and the principles of Declaration of Helsinki (Scotland, October 2000).The

Independent Ethics Committee shall review the protocol and the informed consent form for this study. A single-dose-one arm, open label three way parallel design was used.

Subjects were admitted and housed in the clinical facility at least 2 hour before the application of the dose during the study. Informed consent (Appendix 5) for the dosing / sampling procedure was obtained from each subject on admission to the clinical facility. Each of the marketed Diclofenac Sodium formulation [Test drug A- Diclofenac Gel B.P. 15 gm; Defenac Gel Lic. No.: JK/01/07-08/131 Batch No.: ADN902, Mfg. Date: 02/2009, Exp. Date: 01/2012, Mfg. By: LUPIN LTD,Mumbai.; Test drug B- Diclofenac Diethylamine BP 30 ml Spray; Duoflam Spray, Lic. No.: AD/248-A, Batch No.: 8001, Mfg. Date: 04/08, Exp. Date: 04/2011, Mfg. By: SVIZERA HEALTH CARE, Mumbai and Test drug C- Diclofenac Gel B.P. 30 gm; Voveran Emulgel, Lic. No.: KTK/25/460/2001, Batch No.: 8Z099T, Mfg. Date: 12/2008 Exp. Date: 11/2011, Mfg. By: Novartis India Ltd., Bangalore] was applied on the forearm of the study subjects as per the dosing schedule. Treatment sample C was used as a reference sample [7]. The dosing procedure was as mentioned below:

- Both the forearms were washed with mild soap and copious amount of water and dried in air.
- Both the forearms were marked for total of 08 application sites of 1 sq.cm area each.
- 5 mm length product (semisolid dosage forms) or sufficient amount of drug sample was applied on all the sites so that the product completely and smoothly covers the site area (Spray dosage forms).
- The stratum corneum samples were collected from the sites on the desired pre decided time.

Stratum Corneum Sampling

Skin Stratum Corneum samples were collected in sterile glass test tubes during the study period. The samples were collected pre-dose and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0 hours post-dose application. The stratum corneum samples were analysed for Diclofenac Sodium concentrations only.

For each subject the total number of blood draws were 02 (01 for screening and another during post study assessment); the total volume of blood withdrawn (10 ml for the pre-study evaluation and 10 ml for the post study) through the vein puncture were not exceed 20 ml.

Procedure

Study samples were collected as follows. The pre-dose samples were collected within one hour prior to drug application. The post-dose samples were collected within 2 minutes of the scheduled time where the end time of collection to the nearest minute would be recorded.

- Before sampling the drug remained on the site was removed by mild force using three cotton swabs to ensure the complete removal of residual drug from the site.
- The pre cut (1 sq. cm) adhesion tape was applied on the site and the mild force was applied to ensure the proper adhesion of the tape on the site area. The tape was removed and discarded.
- Eight adhesion tape pieces were applied on the site area in the same manner and each tape was removed from the site before the next one is applied. The removal was done using the forceps and the removal should be done by one stroke to ensure the complete removal of stratum corneum.
- All 8 samples tapes were collected in a single test tube which were then sealed and stored in the refrigerator at -20°C till analysed.

Analytical Method

A validated UV spectroscopic method was employed by using Chemito-Spectroscan UV 2600, Double Beam UV-Visible Spectrophotometer for the estimation of Diclofenac Sodium in human stratum corneum. This method involves the extraction of the Diclofenac Sodium form sample by using methanol and measuring the absorbance at 285nm. The concentration of Diclofenac Sodium in sample is determined from calibration curve.

The standard stock solution of Diclofenac sodium was prepared by weighing 50mg of Diclofenac Sodium powder and shaking it with 60 ml of methanol in a 200-ml volumetric flask which was then diluted with methanol. From this solution 4ml was diluted up to 100ml with methanol, to get the solution with concentration of 10µg/ml. The test solution was prepared by taking 1, 2, 4, 6, 8, 10 ml from the standard stock solution in six different labelled (1 µg/ml, 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml) test tubes and making volume up to 10ml by adding methanol. (Note: no need to add methanol in last µg/ml sample). Methanol was used as blank solution.

Calibration Curve was prepared by using various dilutions (1 µg/ml-10µg/ml) as-transfer required quantity of blank solution in to the cuvette and the absorbance was seen, take the first test tube (1 µg/ml) transfer the required quantity of the test solution into the cuvette then the absorbance at 285 nm was measured and was recorded, the steps 2 and 3 for remaining dilutions were repeated. Finally the graph of concentration versus absorbance (OD) was plotted.

Pharmacokinetic Analysis

Individual plasma concentration– time curves were constructed; Cmax and Tmax were directly obtained from these curves. AUC from time 0 (baseline) to 6 hours (AUC0–6) was calculated using the trapezoidal rule (Chow and Liu, 2000; Chow and Liu, 2007). From the terminal log-decay phase, elimination rate constant (ke) was estimated using linear regression, and t½ was estimated using the following equation: t½ = ln2/ke where ln was defined as the natural logarithm. Extrapolation of AUC from baseline to infinity (AUC0–∞) was calculated as follows: AUC0-∞ = AUC0–6 + (C6/ke) where C6 was defined as concentration at 6 hours.

To compare the bioavailability of the formulations tested, Cmax, AUC from baseline to time t (AUC0–t), and AUC0–∞ was carried out for each study. Ratios of Cmax, AUC0–t, and AUC0–∞ for all formulations were calculated and 90% CIs were obtained. The 90% CIs for the corresponding ratios of Cmax, t_{max}, AUC_0–t, and AUC0–∞ should be within the 80% to 125% range.

RESULTS

Twelve subjects were enrolled in the comparison between three formulations of Diclofenac (mean age, 25.16 years).

The bioequivalence values of the test drug A were C_{max} of 23.767±2.398 µg/mL, t_{max} of 1.75 ±0.261 h, AUC_{0-t} of 100.507± 10.455 h. µg/mL, $AUC_{0-\infty}$ of 178.286± 22.859 h. µg/mL; of the test drug B C_{max} of 31.1± 2.742 µg/mL, t_{max} of 1.75 ±0.261 h, AUC_{0-t} of 103.555± 10.072 h. µg/mL, $AUC_{0-\infty}$ of 166.971± 47.627 h. µg/mL; and of test drug C C_{max} of 24.084± 2.216 µg/mL, t_{max} of 1.75 ±0.261 h, AUC_{0-t} of

100.586±11.15 h. µg/mL, $AUC_{0-\infty}$ of 179.887± 21.553 h. µg/mL.

Table 4: Ratios, 90% CIs of natural log-transformed data, the probability of exceeding the limits of acceptance (80%-125%)

Dependent	Test	FormRef	RefGeoLSM	TestGeoLSM	Ratio[%Ref]	CI_90_Lower	CI_90_Upper
Ln(AUCINF_obs)	A	C	178.64	176.96	99.06	87.73	111.85
Ln(AUClast)	A	C	100.01	100.01	100	93.03	107.48
Ln(Cmax)	A	C	23.99	23.65	98.6	92.24	105.4
Ln(AUCINF_obs)	B	C	178.64	161.96	90.66	80.29	102.37
Ln(AUClast)	B	C	100.01	103.11	103.1	95.92	110.82
Ln(Cmax)	B	C	23.99	30.99	129.18	120.85	138.09

Pharmacokinetic parameters

Mean and SD values of tmax, Cmax, AUC0–T, and AUC0–∞ for each formulation are shown in Table 1-3 and depicted in Fig. 1.

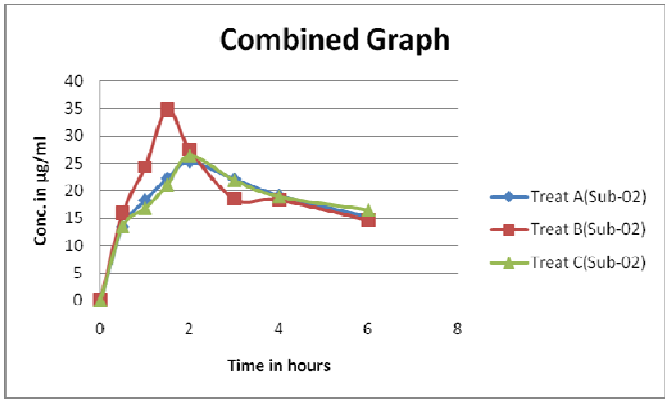

Fig. 1: Combined graph

Table 4 shows the 90% CIs of the ratios (three formulations) for the transformed values of Cmax (as an index of rate of absorption), AUC0–t, and AUC0–∞ □□(as an index of the extent of absorption); The 90% CIs for the corresponding ratios of Cmax, AUC0–6, and AUC0–∞ were within the 80% to 125% range. Tmax values were obtained, but there were not compared in the bioequivalence analysis because this parameter is not considered as a bioequivalence criteria.

DISCUSSION

The results of our study suggest that the treatment A and c formulations of diclofenac were not statistically different in terms of their PK parameters (Cmax and AUC). Considering that all 90% CIs of the ratios of the PK parameters (Cmax and AUC) were found to be within the predetermined range (80% -125%). But the statistical difference in terms of the PK parameters was observed between treatment A and C with treatment formulation B.

Based on the above observations, this study suggests that the formulations of treatment A and C are bioequivalent while that of treatment A and C are not bioequivalent with the treatment B formulation.

No moderate or serious AEs were reported by the investigators. Potential recall bias of AEs in this study was not likely because only one dose of each formulation was administered during each treatment; subjects were under medical surveillance in the clinical unit.

CONCLUSION

This study has demonstration that all the pharmacokinetic parameters calculated for test formulations A were close to those of the reference formulation C and there were no statistically significant difference between the two formulations. On the other hand the pharmacokinetic parameters of test formulation B were statistically different from reference formulation C. Test formulation A and Reference formulations C were bioequivalent with respect to the rate and extent of Diclofenac absorption, which was expressed by similar values for Cmax, AUC0-t and AUC0-∞ which clearly indicated that these values were within the acceptable bioequivalence limits of 80-125%. Thus, it can be assumed that the two formulations were therapeutically equivalent and interchangeable in clinical practice. The test formulation B gives different values for Cmax, AUC0-t and AUC0-∞ than that of reference formulation C which demonstrated that both were not bioequivalent with each other. All formulations were generally well tolerated.

In summary, as the measurement of the change in the Stratum Corneum drug concentration as a function of time is the objective of DPK approach and thus from the above results it is clear that DPK is a valid means of comparing a test and reference product for their ability to deliver drug to the deeper layers of the skin.

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