ADVERSE EVENT REPORTING FOR A DERMATOPHARMACOKINETIC STUDY OF DICLOFENAC SODIUM TOPICAL FORMULATIONS

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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. Diclofenac Sodium was estimated in Stratum Corneum using a validated Spectroscopic method and the treatments were claimed to be bio-equivalent.

The aim of this article was to report the occurrence of adverse events during this study. It was observed that only a single incidence of mild adverse event was reported in two volunteers, and it involved mild laceration on the right forearm. But, the event was found to be self resolving & with the relationship of the adverse event to study medication was "unlikely" but it could be due to the 'tape stripping method' employed for DPK analysis.

KEY WORDS: Dermatopharmacokinetic, Diclofenac, Tape stripping technique, Adverse events.

INTRODUCTION

Dermatopharmacokinetic (DPK)

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.

The determination of the Bioequivalence of topical products involves the Dermatopharmacokinetic (DPK) approach. The DPK approach involves the measure of any drug's 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.

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.[2,3,4]

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 the in vivo tape stripping technique, which and some other techniques are as mentioned below.

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

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 can be sampled. 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

Diclofenac, a phenyl acetic acid derivative, is a non 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[7].

Adverse Drug Reaction

All noxious and unintended responses to a medicinal product related to any dose are considered adverse drug reactions. The phrase responses to a medicinal product means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Need for Adverse Event (AE) reporting

The most frequent question that arises is the need to monitor the adverse reactions of the drugs, even though their safety profiles have already been studied adequately before their commercial release. The answer to this question is to make the drugs safer. In addition, the formal therapeutic trials are conducted in carefully controlled conditions; in highly selected and limited number of patients, so that the exact safety profile of the drug in the real life situations is not known. Children, pregnant women, and elderly are not included in clinical trials for ethical reasons. Therefore, the safety of the drug in these cases remains unknown until its release. Reporting of adverse drug reactions is done by mainly two methods - spontaneous and intensive. Though plagued by numerous problems like low yield of reports, sub-optimal quality and imperfect nature, these have often served to be a useful source of data or provided early warning signals for the drug related regulatory actions.[8] At the time a drug is approved knowledge about its risk is incomplete. Tests in animals are necessary and useful to discover toxic effects, but do not allow sufficient conclusions about human safety. Clinical studies focus on demonstrating efficacy statistically instead of comparing benefits and ADRs with those of existing drugs. The small number of patients involved in, and unsatisfactory length of, clinical studies limit the value of their findings. Thus, pre-approval clinical data include only information about the most common ADRs. In addition, specific doses are used and patients who may be at greater risk from ADRs are usually not studied during the development of a drug, e.g. young children, elderly people, pregnant or lactating women, patients concomitantly using other drugs or other therapies, patients with complicated disease conditions, sub-populations carrying known and relevant genetic polymorphism and patients of different racial and/or ethnic origins.[9] Thus, clinical studies give very limited information about risk and efficacy in real life conditions. Reporting of harm related data from clinical studies needs improvement.[10]

MATERIALS & METHODS

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; 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 as well as serologic tests for hepatitis (B and C), and HIV antibodies. 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.

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 reviewed 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 for the dosing / sampling procedure was obtained from each subject on admission to the clinical facility. The formulations used for the study were Diclofenac Gel (Defenac gel), Diclofenac Emugel (Voveran Emugel) & Diclofenac Spray (Duoflam Spray). Each of the marketed Diclofenac Sodium formulation [Test drug A-Diclofenac Gel B.P. 15 gm; Test drug B- Diclofenac Diethylamine BP 30 ml Spray; Duoflam Spra and Test drug C- Diclofenac Gel B.P. 30 gm; Voveran Emugel] were applied on the forearm of study subjects as per the dosing schedule. Treatment sample C was used as a reference sample. Subjects received a parallel treatment in the subsequent period of following dosing. 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

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 end time of collection to the nearest minute was recorded. Any deviation from the scheduled collection time was recorded promptly in the relevant raw data form.

- 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 -20^0c till analysed.
- A validated HPLC method was employed for the estimation of Diclofenac Sodium in human stratum corneum samples.
- The following Pharmacokinetic parameters of Diclofenac sodium were calculated: C max, T max, AUC (0-t) and t½ (apparent elimination half-life calculated as 0.693/Kel).
- Also, occurrence of any adverse drug reaction / adverse event was recorded in the adverse event form.

Pharmacokinetic Analysis

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 for the three treatments to be bio-equivalent.

RESULTS

The results of our study suggest that the treatment A and C formulations of Diclofenac were statistically indifferent in terms of their PK parameters (Cmax and AUC) considering that all 90% Class Intervals of the ratios of the PK parameters (Cmax and AUC) were found to be within the predetermined range (80% -125%). But, a considerable statistical difference was observed in terms of the PK parameters between treatment formulation A and C with treatment formulation B.

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.

Only one kind of adverse event was reported during the entire clinical study and it was observed in two volunteers. The adverse event was mild laceration on right forearm of the volunteers.

DISCUSSION

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. 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. Thus, the formulations of treatment A and C are bioequivalent while that of treatment A and C are not bioequivalent with the treatment B formulation. All formulations were generally well tolerated.

Two subjects had mild laceration on the right forearm on the dosing day of period I. The nature of the adverse event was "mild" and resolved on the same day without any concomitant medication. This adverse event, more than being associated with the formulations, was associated with the 'tape stripping method' of DPK analysis. Hence, the relationship of the adverse event to study medication was assumed to be "unlikely" but it could be due to the 'tape stripping method' employed for DPK analysis.

REFERENCES

1. Center for Drug Evaluation and Research, Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products - General Considerations. United States Food and Drug Administration 2003.
2. E Benfeld, Steen H Hansen, Aage Volund, Torkil Menne, Vinod P Shah. Bioequivalence of Topical Formulations In Humans: Evaluation By Dermal Microdialysis Sampling And The Dermatopharmacokinetic Method. Journal of Investigative Dermatology 2007; 127: 170-178.
3. Shah VP, GL Glynn, A Yacobi. Bioequivalence of Topical Dermatological Dosage Forms – Method of Evaluation of Bioequivalence. Pharmaceutical Research 1998; 15(2): 167-171.
4. Shah, Vinod P. Progress In Methodologies For Evaluating Bioequivalence Of Topical Formulations. American Journal of Clinical Dermatology 2001; 2(5):280.
5. Christophe Herkenne, Aarti Naik, Yogeshvar N Kalia, Jonathan Hadgraft, Richard H Guy, Ibuprofen Transport into and through skin from topical formulations: In vitro-In vivo Comparison. The society of investigative dermatology 2006.
6. Thomas J, Franz MD. Assessing The Validity Of Stratum Corneum Tape Stripping To Determine The Bioavailability / Bioequivalence Of Topical Drug Products , a letter to Roger Williams, FDA-CDER. 1999.
7. Bertram G. Katzung, Basic and clinical Pharmacology, 9th Edition, Lange Medical Boo, 2004.
8. National Pharmacovigilance Programme. Indian Journal of Pharmacology. Medknow Publication.2005; 37(6).
9. Bahri P, Tsintis P. Pharmacovigilance related topics at the level of the International Conference on Harmonization (ICH). Pharmacoepidemiology and Drug Safety. 2005; 14:377-387.
10. Definitions of Terms for Reporting Adverse Drug Reactions (IX): Nervous System and Psychiatric Disorders. Pharmacoepidemiology and Drug Safety. 1998; 7:39-49