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Comparison of Bioavailability and Pharmacokinetics of Diclofenac Sodium and Diclofenac Potassium in Healthy and *Escherichia coli* Induced Febrile Rabbits

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Abstract.- Diclofenac sodium and diclofenac potassium in tablet dosage form were tested for their bioavailability and disposition kinetics in a group of 18 rabbits in normal and *Escherichia coli* induced febrile condition with a washout period of 7 days. Biochemical and physiological parameters were measured in both normal and febrile state. Diclofenac levels in plasma were determined using a reversed-phase HPLC method. Primary kinetic parameters *i.e.* $AUC_{0-\infty}$, C_{max}, t_{max} and other disposition kinetics, were obtained with non-compartmental procedure. The values of physiological and biochemical parameter were significantly ($p<0.05$) low in febrile rabbits as compared to that of normal animals. The drug concentration levels and pharmacokinetics of orally administered diclofenac sodium and diclofenac potassium were changed during fever. The comparison of diclofenac sodium and diclofenac potassium in normal and febrile conditions showed significantly ($p<0.05$) increased level of diclofenac potassium in both conditions normal & febrile.

Keywords: Diclofenac sodium, diclofenac potassium, endotoxin induced fever, bioavailability, disposition kinetics.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are heterogeneous compounds often chemically unrelated (although most of them are organic acids), which nevertheless share certain therapeutic action and side effects. Their therapeutic activity appears to depend on large extent upon the inhibition of defined biochemical pathway responsible for the biosynthesis of prostaglandins and other related autacoids. NSAIDs have different spectrum of activity than do the analgesics. They have anti-inflammatory, antipyretic and analgesic effects. In general these agents are most effective in pain associated with inflammation such as arthritis (Martindale, 2002). Diclofenac possesses structural characteristics of arylalkanoic acid agents and display anti-inflammatory, analgesic and anti-pyretic properties (Dreve *et al.,* 2009). In the carrageen an induced rat paw edema assay, it is twice as potent as indomethacin and 450 times as potent as aspirin (Van Miert and Van Goghham, 1976). As an analgesic, it is 6 times more potent than indomethacin and 40 times more potent than aspirin in the phenyl-benzoquinone induced writhing assay in mice. As an anti-pyretic it is twice as potent as indomethacin and over 350 times as potent as aspirin in yeast induced fever assay in rats (Munir *et al*., 2007; Van Gogh and Van Miert, 1977). Fever is a condition in which body temperature is higher than normal *i.e.* 37°C (Roth *et al.,* 2006).

The cause of fever is pyrogens secreted by toxic bacteria or pyrogens released from degenerating tissues of the body (Chaudhary *et al*., 2001). When the set point of the hypothalamic thermostat becomes increased to higher level than normal, all the mechanisms for raising body temperature are brought into play, inducing heat conservation and increased heat production (Sharma *et al*., 2006). Fever induced by endotoxin has been used as an experimental tool to study the influence of fever on pharmacokinetics of in different subjects (Ahmad and Nawaz, 1995). Acute fever was produced within 30 minutes after injection of bacterial suspension (*E. coli*) and it persisted during the experiment. This study was designed to reveal

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any clinical variation in bioavailability and pharmacokinetics of diclofenac sodium and diclofenac potassium in febrile rabbits as compared to normal.

MATERIALS AND METHODS

Chemicals and reagents

Ammonium acetate, acetonitrile, glacial acetic acid and methanol were purchased from Merck, Germany. *E. coli* sample was provided by Pathological Laboratory, Quid-e-Azam Medical College, Bahawalpur, Pakistan. MacConkey's Agar was purchased from Oxoid, UK. Total protein and Albumin determination kits for plasma and serum were purchased from Randox Laboratories, Crumlin, UK, respectively. Total lipids and glucose determination kit for plasma and serum were purchased from Clonital, Carvico, Italy, respectively.

Materials

Diclofenac sodium (Voltral® 50mg tablet, Novartis pharma Pakistan Ltd.); Diclofenac potassium (Caflam® 50mg tablet, Novartis pharma Pakistan Ltd.); Diclofenac sodium raw material (Novartis pharma); Diclofenac potassium raw material (Getz pharma).

Instrumentation

High performance liquid chromatography (HPLC, Perkin-Elmer); HPLC pump (Perkin-Elmer); Spectrophotometer variable detector (Perkin-Elmer); HPLC column ODS (C_{18}) (Perkin-Elmer); Data processing modular or interface (Perkin-Elmer); UV spectrophotometer (UV 1601 Shimadzu, Japan); Membrane filters 0.45µm (Sartorius, Germany).

Description of animals

For the investigation of bioavailability and disposition kinetics of diclofenac, 18 mixed breed rabbits were used. The animals were thoroughly examined before experimentation and were kept for 7 days to ensure their clinical conditions. They were fed with fresh green fodder thrice daily and water was provided *ad lib.* All these animals were housed under similar conditions.

Rabbits were distributed into 2 groups *i.e.* 9 rabbits in each group. Bioavailability and disposition kinetics of diclofenac were studied in normal and febrile. A washout period of at least 7 days was given between the normal and production of diseased condition (febrile state).

The study was approved by the Board of Advance Studies and Research, the Islamia University of Bahawalpur and was carried out according to the ethical principles.

Production of febrile condition

In animals fever was produced by intravenous injection of *E. coli*. Pure culture of *E. coli* was inoculated in nutrient broth and diluted it with sterile water to get a broth containing 130×10^7 count/mL. Neuobaur scale was used to count *E. coli* after serial dilution of the suspension. *E. coli* containing aqueous suspension was injected in the marginal ear vein of rabbits at a dosage rate of 0.01 mL/kg body weight. Rectal temperature was noted to confirm febrile condition (Ahmad *et al*., 2008).

Drug administration

Bioavailability and pharmacokinetics of diclofenac were studied in all the normal state of rabbits following an oral administration of 50 mg of both, diclofenac sodium and diclofenac potassium in different occasions. Each rabbit was further subjected to similar studies after administration of 50 mg of diclofenac sodium and diclofenac potassium through oral route following the induction of febrile condition.

Sampling procedure

The blood samples were drawn from the jugular vein of the rabbits held in the wooden box. The blood samples were collected at 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, and 8.0 hours after the oral drug administration in normal rabbits and after production of diseased condition in same rabbits. The blank blood samples were taken in all the animals. The blood samples were collected in heparinized plastic/glass centrifuge tubes. At each sampling time, 3 mL of blood was drawn with the help of disposable syringe plasma was separated and stored in freezer below -20°C until used for analysis of diclofenac.

Physiological and biochemical studies

Biochemical parameters were determined in normal and febrile state in rabbits *i.e*. body weight, blood pH by WTW Germany pH meter, packed cell volume by HERMLEZ-230HA Germany, total protein by Biuret reactions using testing kit of Randox Germany, albumin by bromocresol green (BCG) to form complex measured by spectrophotometer at 630nm using Randox testing kit Germany, globulin by subtracting concentration of albumin from the concentration of total proteins, blood glucose by Trinder method-end point using 1-Enzyme reagent, and free fatty acid by Zöllner and Kirsch method using testing kit by Clonital Italy, were measured (Ahmad *et al*., 2009).

Method of HPLC assay

An accurate High Performance Liquid Chromatographic (HPLC) method which was developed and validated in our laboratory was applied. A system (Perkin–Elmer), equipped with a UV detector (series 200 UV- visible set at 280nm) and Hypersil ODS C18 (5 µm) reversed phase column (250 × 4.6 mm I.D.) was used; HPLC interface (NC 1900) (Perkin-Elmer).The mobile consisted of a mixture of acetonitrile and 0.01M ammonium acetate buffer in the ratio of 60:40. The pH of mobile phase was maintained at 3.4 by glacial acetic acid. Optimum flow rate was 1.5 mL/min. Further, the method was validated by establishing standard curve for both diclofenac sodium and diclofenac potassium.

Assay procedure

Stock solutions (100 µg/mL) of diclofenac sodium and diclofenac potassium were prepared fresh daily by dissolving the drug in the acetonitrile. 2 mL of acetonitrile was added to 1mL of plasma samples to precipitate the proteins and vortexed for 1 minute. It was then centrifuged for 5 minutes at 3500 rpm. After centrifugation, supernatant layer was transferred to another Eppendorf tube and evaporated to dryness under nitrogen flux. The residue was then dissolved in 80µl of mobile phase and injected 20µl into the injection port. Serum diclofenac concentrations were measured by reversed-phase HPLC with ultraviolet detection (Said and Sharaf, 1998).

Pharmacokinetic analysis

Pharmacokinetic parameters of diclofenac sodium and diclofenac potassium were calculated by non-compartmental model of analysis. C_{max}, t_{max}, were determined by plasma concentration time profiles and $AUC_{0-\infty}$ was calculated by linear trapezoidal rule. Other parameters were calculated by MS-Excel.

Statistical analysis

Biochemical and pharmacokinetic parameters determined in normal and febrile rabbits were subjected to statistical analysis of paired t-test to observe the difference. Level of significance was set at 0.05. Mean values (n=9) and their standard error of mean (±SEM) were computed on statistical software SPSS version 12.0.

Table I.- Body weight, packed cell volume, and biochemical parameters of normal rabbits compared with febrile rabbits.

Parameters	Normal rabbits (n=12)	Febrile rabbits (n=12)
Body weight (Kg)	1.63 ± 0.14	1.62 ± 0.15 *
PCV (%)	34.5 ± 3.14	27.8 ± 2.50 **
pH	6.3 ± 0.13	6.4 ± 0.09 ^{ns}
Total lipids (mg/dl)	366.7 ± 17.57	326.8 ± 16.72 **
Total protein (g/dl)	6.84 ± 1.46	5.91 ± 2.07 **
Albumin (g/dl)	4.16 ± 1.65	3.26 ± 1.91 **
Globulin (g/dl)	2.42 ± 1.93	2.93 ± 1.85 *
A/G ratio	1.74 ± 1.88	1.13 ± 2.31 *
Glucose (mg/dl)	83.5 ± 9.12	88.3 ± 6.95 *
Body temperature (°C)	89.4 ± 1.61	104.5 ± 0.96 **

Mean±SEM; *, significant difference at $p<0.05$; **, highly significant difference at $p<0.01$; ns, non-significant difference at $p>0.05$.

RESULTS

The results of body weight, packed cell volume and other biochemical parameters of normal and febrile rabbits are compared in Table I. Plasma concentration versus time profile of diclofenac sodium and diclofenac potassium in normal and febrile conditions of rabbits after an oral dose of 50 mg tablet was compared and statistically evaluated (Fig. 1). Bioavailability and disposition kinetic parameters of diclofenac sodium and diclofenac

potassium in febrile rabbits were tested with the help of paired t- test (Tables II, III).

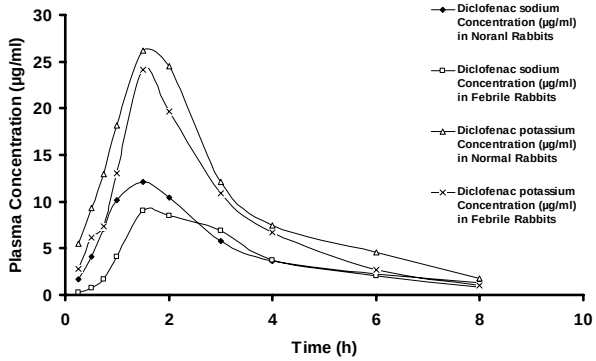

Fig. 1. Comparison of mean ± SEM plasma concentration of diclofenac sodium and diclofenac potassium in normal and febrile rabbits plotted on rectangular co-ordinate graph after an oral administration of 50 mg dose.

DISCUSSION

Body weight, packed cell volume and biochemical parameters were find out in 18 normal rabbits. Then same rabbits were used to produce hyperthermia induced by injection of *E. coli*. Body temperature was taken before and after injection of endotoxin and found to be 98.4±0.38°C and 104.5±0.985°C in normal and febrile conditions, respectively. The difference of temperature was highly significant ($p<0.01$) before and after the induction of fever. Packed cell volume, total lipids and total proteins showed a significant decrease in febrile rabbits when compared with normal animals. The decrease in packed cell volume may be due to increased cell destruction due to endotoxin in febrile rabbits. The concentration of albumin decreased highly significantly ($p<0.01$) in febrile rabbits that can be attributed to the catabolism and restricted protein anabolism in febrile state. Blood glucose showed a significant ($p<0.05$) difference between the two conditions. Rabbits when compared with normal animals. The concentration of albumin decreased significantly in febrile rabbits (Ahmad and Nawaz, 1995). Blood glucose showed a significant difference between the two conditions.

Table II.- Comparison bioavailability and disposition kinetics of diclofenac sodium and diclofenac potassium in normal rabbits after an oral administration of 50 mg dose.

Parameters	Diclofenac sodium (n=12)	Diclofenac potassium (n=12)
AUC (µg.h/ml)	32.029±0.296[a]	78.404±0.154**
AUMC (µg.h^2/ml)	117.746±0.157	206.545±0.10**
T_{max} (h)	1.625±2.102	1.750±2.494 ns
C_{max} (µg/ml)	10.548±0.430	31.648±0.250**
K_a (h^{-1})	0.362±2.332	0.862±2.494**
MAT (h^{-1})	3.856±0.615	1.199±2.068**
$t_{1/2\alpha}$ (h)	2.672±0.739	0.831±2.485**
MRT (h)	3.753±1.068	2.733±1.277*
V_d (L/kg)	6.586±0.596	2.159±0.995**
V_{SS} (L/kg)	0.472±2.537	0.288±2.949**
K_e (h^{-1})	0.394±4.307	0.384±3.373 ns
$t_{1/2\beta}$ (h)	3.753±0.567	1.979±1.534**
Cl (ml/h/kg)	1.725±1.385	0.770±1.0850**

[a]Mean±SEM; *, significant difference at $p<0.05$; **, highly significant difference at $p<0.01$; ns, non-significant difference at $p>0.05$.

$AUC_{0-\infty}$, total area under plasma concentration time curve; $AUMC_{0-\infty}$, total area under the first moment curve; K_a, absorption rate constant; C_{max}, maximum plasma drug concentration; Cl, total body clearance of diclofenac potassium; MAT, mean absorption time and $t_{1/2\alpha}$, absorption half life; T_{max}, time to reach maximum plasma drug concentration; MRT, mean retention time; K_e, elimination rate constant; $t_{1/2\beta}$, elimination half life; V_d, volume of distribution and V_{ss}, volume at steady state.

Plasma concentration versus time profile of diclofenac sodium and diclofenac potassium in normal and febrile conditions of rabbits after an oral dose of 50 mg tablet was compared and statistically evaluated (Fig. 1). Comparison of diclofenac sodium and diclofenac potassium showed a highly significant ($P<0.01$) difference between all the sampling times except at 8 hours where the difference is non-significant ($P>0.05$). The plasma concentration of diclofenac potassium was high in febrile condition as it was high in normal animals, when compared with plasma concentration of diclofenac sodium. This difference in plasma concentration of both of the salts may be due to the difference in their solubility and permeability. The plasma concentrations of diclofenac sodium and

diclofenac potassium in endotoxin induced febrile rabbits showed a significant decrease after drug administration as compared with normal rabbits. As globulin level increased in febrile rabbits, the diclofenac sodium and diclofenac potassium binding increased result in low plasma concentrations than in normal rabbits. A report has also been previously published involving decrease in plasma concentration level in febrile state (Dette *et al.*, 1982).

Table III.- Comparison of bioavailability and disposition kinetics of diclofenac sodium and diclofenac potassium in febrile rabbits after an oral administration of 50 mg dose.

Parameters	Diclofenac sodium (n=12)	Diclofenac potassium (n=12)
AUC (μg.h/ml)	31.268±0.228[a]	53.703±0.217 *
AUMC (μg.h^2/ml)	71.837±0.162	145.271±0.134**
T_{max} (h)	1.583±1.547	1.438±1.641 ^{ns}
C_{max} (μg/ml)	15.081±0.331	27.123±0.318 **
K_a (h^{-1})	0.565±2.336	1.085±3.662 **
MAT (h^{-1})	1.939±1.314	0.925±4.105 **
$t_{1/2\alpha}$ (h)	1.344±1.578	0.641±4.931 **
MRT (h)	2.566±1.070	2.797±1.211 ^{ns}
V_d (L/Kg)	6.835±0.374	3.038±0.810 *
V_{SS} (L/Kg)	0.882±1.486	0.384±2.822 *
K_e (h^{-1})	0.430±2.575	0.377±2.686 ^{ns}
$t_{1/2\beta}$ (h)	2.566±0.514	2.797±0.403 ^{ns}
Cl (ml/h/Kg)	2.335±0.790	1.051±1.688 **

[a]Mean±SEM; *, significant difference at $p<0.05$; *, highly significant difference at $p<0.01$; ns, non-significant difference at $p>0.05$.
For abbreviations see Table II.

Bioavailability and disposition kinetic parameters of diclofenac sodium diclofenac potassium in febrile rabbits were tested with the help of paired t-test (Tables II, III). Total area under plasma concentration time curve ($AUC_{0-\infty}$), total area under the first moment curve ($AUMC_{0-\infty}$), C_{max}, absorption rate constant (K_a), maximum plasma drug concentration (C_{max}) and total body clearance (Cl) of diclofenac potassium were higher statistically ($p<0.01$) when compared with diclofenac sodium in febrile animals. Mean Absorption Time (MAT) and absorption half life ($t_{1/2\alpha}$) were found significantly ($P<0.01$) higher for diclofenac sodium. Time to reach maximum plasma drug concentration (T_{max}), mean retention time (MRT), elimination rate constant (K_e), elimination half life ($t_{1/2\beta}$), volume of distribution (V_d) and volume at steady state (V_{ss}) for diclofenac sodium and diclofenac potassium were found to be non-significantly ($P<0.05$) different in febrile rabbits. Drug concentration level and pharmacokinetics of orally administered diclofenac sodium and diclofenac potassium are changed during fever, as shown by lower drug levels and a greater volume of distribution. Comparison of diclofenac sodium and diclofenac potassium in normal and febrile conditions shows significantly increased level of diclofenac potassium in both of the conditions that may be due to increased solubility and permeability of diclofenac potassium. The $AUMC_{0-\infty}$ shows a significantly ($p < 0.05$) lower value due to its low plasma concentration in febrile rabbits.

The volume of distribution at steady state (Vss), which is a sum of volumes of central and tissue compartments, is greater in febrile rabbits than in normal ones. This change in the value of Vss could be explained on the basis of significantly lower plasma concentrations of diclofenac sodium and diclofenac potassium in the febrile condition. The drug concentration in the deeper tissues will be substantially higher in the febrile condition which may have toxic effects. This higher drug concentration at the site of action may sometimes be beneficial though this concentration is above the minimum effective concentration of the drug and well within the therapeutic window.

CONCLUSIONS

It is concluded that fever has potential of modifying the absorption, distribution, protein binding and metabolic disturbances leading to change in the bioavailability and disposition kinetics of drugs. This study will be helpful in dosage adjustment of diclofenac sodium and diclofenac potassium in *E. coli* induced febrile patients and monitoring the toxicity during prolonged use in clinical practice.

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