Comparison of bioavailability and pharmacokinetics of diclofenac sodium and diclofenac potassium in normal and alloxan-diabetic rabbits

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Abstract: The present study was undertaken to compare the bioavailability and pharmacokinetic parameters of diclofenac sodium and diclofenac potassium in normal and experimentally induced diabetic state in 24 rabbits using a validated reversed phase HPLC method with a washout period of one week.

Biochemical and physiological parameters were measured in normal and diabetic rabbits. Primary kinetic parameters i.e. $AUC_{0-\infty}$, C_{max}, t_{max} and other disposition kinetics were determined with non-compartmental procedure.

A paired t-test for normal and alloxan treated rabbits revealed a significant decrease in packed cell volume and a significant increase in glucose, total lipids, total proteins, albumin and globulin were observed in diabetic (metabolically altered) rabbits. Plasma concentration of diclofenac (sodium) and diclofenac (potassium) decreased in diabetic condition. Significantly high concentration of diclofenac (potassium) was observed in normal and diabetic conditions as compared to diclofenac (sodium).

The variation in bioavailability and disposition of diclofenac sodium and diclofenac potassium in diabetic state will require adjustment of the dosage regimen prescribed for diabetics in clinical setting.

Keywords: Bioavailability, pharmacokinetics, diclofenac sodium, diclofenac potassium, diabetic state.

INTRODUCTION

Both salts of diclofenac i.e., diclofenac sodium and diclofenac potassium share almost the same physicochemical properties except their molecular weights. Diclofenac is available in 120 different countries and is perhaps the most widely used non-steroidal anti-inflammatory drug (NSAIDs) in the world, being the 8th largest selling drug overall. It was introduced in the U.S. in 1989, but was first marketed in Japan in 1974. It ranks among top 30 prescription drugs in the United States. Diclofenac possesses structural characteristics of the arylalkanoic acid agents and displays anti-inflammatory, analgesic, and anti-pyretic activity. Diclofenac is unique among the NSAIDs in that it possesses the mechanism of action for the inhibition of arachidonic acid cyclooxygenase system, lipooxygenase pathway, arachidonic acid release resulting in decreased production of prostaglandins and thromboxanes, leukotrienes and reduction of arachidonic acid, respectively (Martindale, 2002).

NSAIDs have a different spectrum of activity than do the narcotic analgesics. Diclofenac is mainly used in the relief of pain and inflammation in various conditions; musculoskeletal and joint disorders such as rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis.

Alteration in the biochemical environment of the body is likely to influence the disposition of drugs which are either weak acids or weak bases. Hyperglycemia, as an exemplary disease stat, can be produced without difficulty in experimental animals by chemical and surgical methods. Chemical induction of diabetes has been achieved with β-cell cytotoxic agents. Alloxan (mesoxylylurea) and streptozocin are the most extensively used agents for the induction of diabetes in experimental animals. Alloxan is a well known hyperglycemic agent (Lehninger 1975, Mohan *et al.,* 1980). Therefore, in the present study, diabetes was produced by administering alloxan to rabbits.

In the present study, before and after treatment with alloxan, the body weight, packed cell volume, pH of blood, glucose, total lipids, total proteins, albumin, and globulin alongwith bioavailability and disposition kinetic parameters of diclofenac, after its administration of its sodium and potassium salt were determined.

MATERIALS AND METHODS

Chemicals and reagents

Ammonium acetate, acetonitrile, glacial acetic acid and methanol were purchased from Merck, Germany. Alloxan monohydrate 99% (Research Chemical Ltd.), Total protein and Albumin determination kits for plasma and serum (Randox Laboratories Ltd., UK.) and Glucose and total lipids determination kits for plasma and serum (Clonital, Italy) were purchased through commercial sources.

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Materials

Diclofenac sodium (Voltral 50mg tablet, Novartis Pharma Pakistan, Limited), Diclofenac potassium (Caflam 50mg tablet, Novartis Pharma Pakistan, Limited), 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 (C18) (Perkin-Elmer); Data processing modular or interface (Perkin-Elmer); UV spectrophotometer (UV 1601 Shimadzu, Japan); Deep freezer (for storage of samples); Refrigerator (for solution storage); Nitrogen evaporator; Centrifuge; pH meter; Ultrasonic bath; Electric balance; Membrane syringe filter assembly; Vortex mixer; Vacuum pump; Distillation plant; Haematocrit (for packed cell volume determination); Mouth gag; micropipettes; Disposable syringe; Filtration assembly; Centrifuge tubes; Eppindrof tubes (2cc); Membrane filters 0.45 µm (Sartorius, Germany).

Description of Animals

For the investigation of bioavailability and disposition kinetics of diclofenac, 24 mixed breed rabbits were purchased from market. 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. The study was approved by the Board of Advance Studies and Research, the Islamia University of Bahawalpur and was carried out according to the principles of Institutional Ethical committee for animal experiments.

Production of Diabetic Condition

For the induction of metabolic disorder, the rabbits were treated with a single intravenous dose of alloxan at a dosage level of 25 mg/kg as described previously (Nawaz *et al.* 1982).

Drug Administration

Bioavailability and pharmacokinetics of diclofenac were studied in normal rabbits after an oral administration of 50mg of diclofenac sodium and diclofenac potassium in different occasions. After a wash out period of one week, each rabbit was further subjected to similar studies after administration of 50 mg of diclofenac sodium and potassium through oral route after induction of diabetic 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 both study states. The blood samples were collected in heparinized 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 drug analysis.

Physiological and Biochemical studies

Biochemical parameters were determined in normal and diabetic 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 630 nm 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 (Clonital, Italy), and free fatty acid by Zollner & Krish method using testing kit by Clonital Italy, were measured (Doumas *et al.* 1971, Gornall *et al.* 1949, Said and Sharaf 1998).

HPLC Method

Method of assay

An accurate HPLC method, developed and validated in our laboratory, was applied. Above mentioned HPLC system was used with UV detector operated at 280 nm. Mobile phase consisted of a mixture of acetonitrile and ammonium acetate buffer 0.01 M with pH 3.4 adjusted by glacial acetic acid (60:40, v/v). Optimum flow rate was 1.5 ml/min.

Assay procedure

Fresh stock solutions (100 µg/ml) of diclofenac sodium and diclofenac potassium were prepared daily by dissolving the drug in the acetonitrile.

Extraction procedure

Two ml of acetonitrile was added to 1 ml of plasma samples to precipitate the proteins and vortexed for 1 minute. It was then centrifuged for 5 minutes at 4000 rpm. After centrifugation, supernatant layer was transferred to another Eppendorf (propylene) tube and evaporated to dryness under nitrogen flux. The residue was then dissolved in 80 µl of mobile phase and injected 20 µl into the injection port (Said and Sharaf 1998). The lower limit of detection and lower limit of quantification were 50 ng/ml and 125 ng/ml, respectively.

Pharmacokinetic Analysis

Pharmacokinetic parameters of diclofenac sodium and diclofenac potassium were calculated by non-compartmental model of analysis. C_{max} and 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 diabetic rabbits were subjected to statistical analysis of paired t-test to observe the difference. Mean values and their standard error of mean (SEM) were computed on statistical software SPSS 12.0. Level of significance was set at $P<0.05$.

RESULTS

Body weight, packed cell volume and other biochemical parameters were determined in all normal rabbits. Diabetic condition was produced by intravenous injection of alloxan monohydrate (25mg/kg). Blood glucose increased significantly ($P<0.01$) in diabetic (221.0 ± 16.26 mg/dl) than normal (89.58 ± 7.34 mg/dl) rabbits. Body temperature did not show any significant ($P>0.05$) difference between the two groups of animals (normal and diabetic).

The results of biochemical parameters in normal and diabetic rabbits are compared in table 1.

Plasma concentration versus time profiles of diclofenac (sodium) and diclofenac (potassium) in normal and diabetic condition of the rabbits after an oral dose of 50mg tablet is compared in figs. 1-4.

Bioavailability and disposition kinetic parameters of diclofenac sodium and diclofenac potassium in metabolically altered rabbits were tested with help of paired t-test and presented in tables 2 and 3.

Table 1: Mean ± SEM (n = 24) values of body weight, packed cell volume and biochemical parameters of normal rabbits compared with diabetic rabbits.

S. No.	Parameters	Normal	Febrile
1	Body Weight (Kg)	1.63 ± 0.14	1.60 ± 0.16 *
2	Packed Cell Volume (PCV, %)	25.76 ± 3.14	23.54 ± 2.71 **
3	pH	6.4 ± 0.13	6.3 ± 0.09 ^{ns}
4	Total Lipids (mg/dl)	373.0 ± 0.22	420.33± 0.29 **
5	Total Protein (g/dl)	6.47 ± 0.72	8.03 ± 0.38 **
6	Albumin (g/dl)	4.35 ± 1.21	4.91 ± 1.47 **
7	Globulin (g/dl)	2.25 ± 1.94	3.30 ± 2.45 *
8	A/G Ratio	1.93 ± 0.63	1.49 ± 0.60 *
9	Glucose (mg/dl)	89.58 ± 7.34	221.03 ± 16.26 **
10	Body Temperature (˚C)	98.4 ± 0.38	98.61 ± 0.37 **

* = Significant difference at $p < 0.05$, ** = Highly significant difference at $p < 0.01$, ns = Non-significant difference at $p > 0.05$

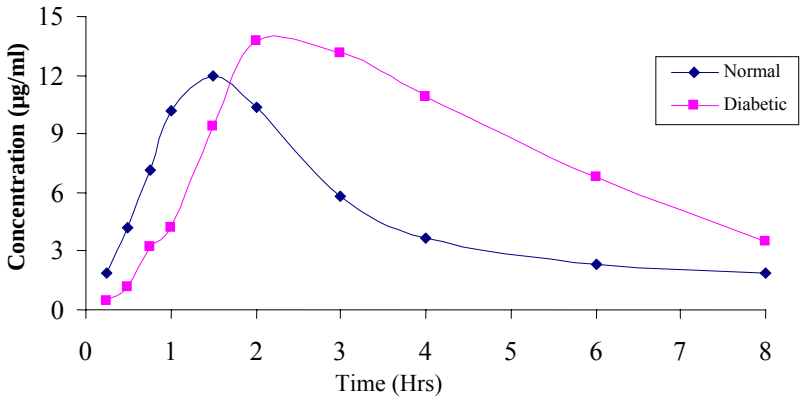

Fig. 1: Comparison of mean (n=24) plasma concentration of diclofenac sodium in normal and diabetic rabbits plotted on rectangular co-ordinate graph after an oral administration of 50 mg dose.

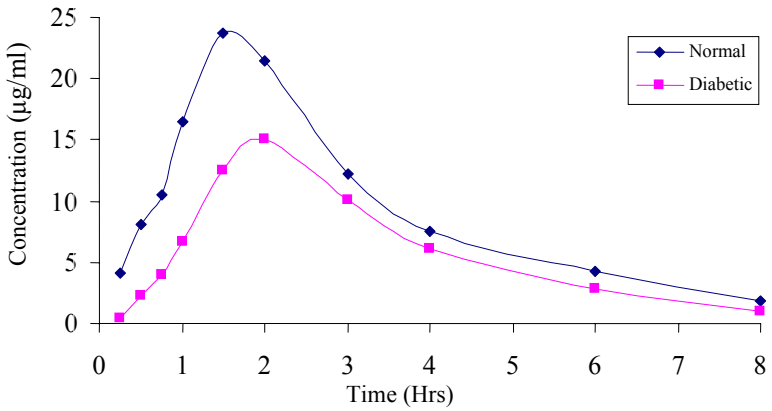

Fig. 2: Comparison of mean (n=24) plasma concentration of diclofenac potassium in normal and diabetic rabbits plotted on rectangular co-ordinate graph after an oral administration of 50 mg dose.

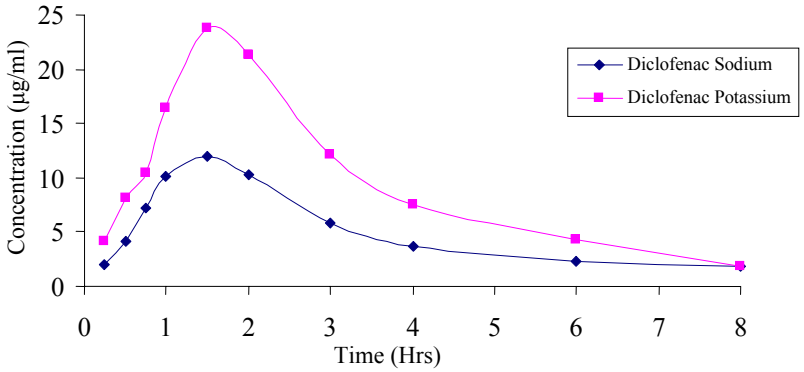

Fig. 3: Comparison of mean± SEM (n=24) plasma concentration of diclofenac sodium and diclofenac potassium in normal rabbits plotted on rectangular co-ordinate graph after an oral administration of 50 mg dose.

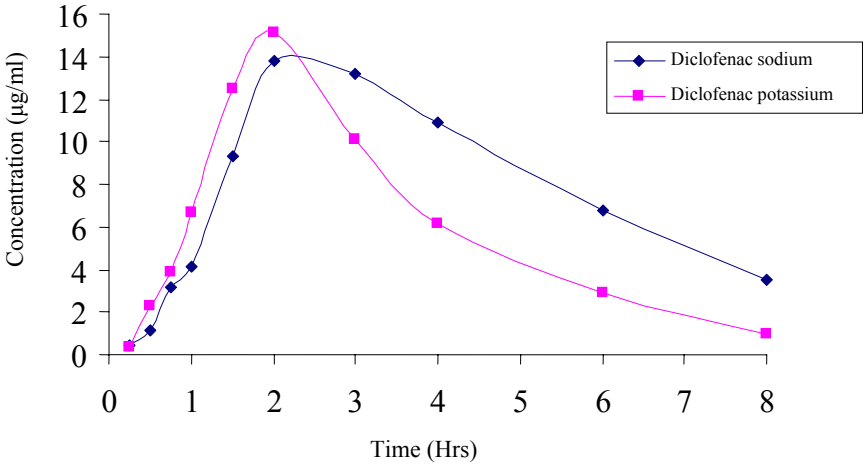

Fig. 4: Comparison of mean ± SEM (n=24) plasma concentration of diclofenac sodium and diclofenac potassium in diabetic rabbits plotted on rectangular co-ordinate graph after an oral administration of 50 mg dose.

Table 2: Comparison of (mean ± SEM) bioavailability and disposition kinetics of diclofenac sodium and diclofenac potassium in normal rabbits after an oral administration of 50 mg dose

S. No.	Parameters	Diclofenac sodium	Diclofenac potassium
1	AUC (µg.h/ml)	37.397 ± 0.210	80.734 ± 0.159 **
2	AUMC (µg.h^2/ml)	104.084 ± 0.108	225.778 ± 0.103 **
3	T_{max} (h)	1.667 ± 1.501	1.708 ± 1.971 ^{ns}
4	C_{max} (µg/ml)	13.644 ± 0.382	26.830 ± 0.313 **
5	K_a (h^{-1})	0.501 ± 2.479	0.862 ± 2.494 *
6	MAT (h^{-1})	2.176 ± 1.266	1.199 ± 2.068 **
7	$t_{1/2\alpha}$ (h)	1.508 ± 1.521	0.831 ± 2.485 *
8	MRT (h)	2.679 ± 1.446	2.911 ± 1.149 ^{ns}
9	V_d (L/Kg)	4.713 ± 0.627	2.159 ± 0.995 **
10	V_{SS} (L/Kg)	0.678 ± 1.798	0.288 ± 2.950 **
11	K_e (h^{-1})	0.385 ± 3.112	0.630 ± 3.688 *
12	$t_{1/2\beta}$ (h)	1.857 ± 1.737	2.017 ± 1.381 ^{ns}
13	Cl (ml/h/Kg)	1.767 ± 1.088	0.721 ± 2.016**

* = Significant difference at $p < 0.05$, ** = Highly significant difference at $p < 0.01$, ns = Non-significant difference at $p > 0.05$

Table 3: Comparison of (mean ± SEM) bioavailability and disposition kinetics of diclofenac sodium and diclofenac potassium in diabetic rabbits after an oral administration of 50 mg dose

S. No.	Parameters	Diclofenac sodium	Diclofenac potassium
1	AUC (µg.h/ml)	49.950 ± 0.218	50.731 ± 0.194 ^{ns}
2	AUMC (µg.h^2/ml)	150.965 ± 0.112	160.366 ± 0.10 ^{ns}
3	T_{max} (h)	2.148 ± 1.173	1.854 ± 1.377 ^{ns}
4	C_{max} (µg/ml)	12.843 ± 0.409	18.130 ± 0.419 *
5	K_a (h^{-1})	0.425 ± 1.960	1.068 ± 4.463 **
6	MAT (h^{-1})	3.911 ± 0.516	0.938 ± 4.923 **
7	$t_{1/2\alpha}$ (h)	2.711 ± 0.620	0.650 ± 5.923 **
8	MRT (h)	2.918 ± 1.645	2.992 ± 1.411 ^{ns}
9	V_d (L/Kg)	3.409 ± 0.853	3.681 ± 0.716 ^{ns}
10	V_{SS} (L/Kg)	0.396 ± 2.562	0.473 ± 1.708 ^{ns}
11	K_e (h^{-1})	0.341 ± 5.074	0.394 ± 4.164 *
12	$t_{1/2\beta}$ (h)	2.057 ± 1.975	2.073 ± 1.676 ^{ns}
13	Cl (ml/h/Kg)	1.155 ± 1.506	1.350 ± 1.111 ^{ns}

* = Significant difference at $p < 0.05$, ** = Highly significant difference at $p < 0.01$, ns = Non-significant difference at $p > 0.05$

DISCUSSION

Bioavailability and pharmacokinetic parameters of diclofenac sodium and potassium were evaluated with the help of plasma drugs concentrations. A non-significant ($P>0.05$) difference in plasma levels of alloxan treated rabbits was observed. The difference in plasma concentration of protein before and after alloxan treatment may be due to an impairment of protein metabolism in liver which is supported by other investigators also (Mohan *et al.,* 1980, Iqbal *et al.,* 1989). A decrease in body weight of diabetic rabbits was noted in the present study. This may be attributed to the enhanced catabolic activities. After the alloxan treatment of rabbits, blood glucose increased two to three fold showing an average of 221.03 ± 16.26 mg/dl and a range of 205 to 254 mg/dl. The increase in blood glucose level was due to the administration of alloxan which destroys β-cells of the pancreas, and therefore, interfering with the insulin production. Similar results are explained in the literature (Nawaz *et al.,* 1982, Said and Sharaf, 1998).

An increase in the plasma level of total lipids was observed in diabetic rabbits which may be attributed to the mobilization of lipids for energy purpose, also mentioned in literature (Guyton, 1971).

It was observed that there is a highly significant ($P<0.01$) increase in the plasma concentration of diclofenac potassium when compared with diclofenac sodium in normal state. This may be due to an increased solubility and permeability of the diclofenac potassium.

In diabetic rabbits, a variable pattern was seen in the concentration of diclofenac potassium being significantly increased at 30 min, 1.0, 1.5 and 2.0 h. At 3.0, 4.0, 6.0 and 8.0 h, there was a significant ($P<0.05$, 0.01) decrease in the plasma concentration of diclofenac potassium when compared with diclofenac sodium. However, it needs further exploration on the basis of biochemical and physiological basis.

Similar pattern of diclofenac (sodium) was observed in normal and diabetic states. Whereas, diclofenac

(potassium) plasma concentration was highly significantly ($P<0.01$) low in diabetic state compared to normal state. The reason for its low plasma concentration may be due to reduced absorption of the drug from gastrointestinal tract or a reduction in blood pH might have favored passage of drug across the biomembranes. Similar results were reported by Iqbal *et al.* (1989).

All parameters studied were found statistically non-significantly different ($P>0.05$) except C_{max}, absorption rate constant, elimination rate constant, absorption half-life and MAT which were found significantly different ($P<0.05$, 0.01). It was observed that the values of significantly different parameters of diclofenac potassium were higher when compared with diclofenac sodium.

It is clear from tables 2 and 3 that significant ($P<0.05$) increase in the area under the plasma concentration time curve (AUC) and area under the first moment curve (AUMC) occurred in diabetic rabbits as diabetes may reduce the absorption of diclofenac (sodium) into general circulation. Moreover, decreased values of AUC and AUMC for diclofenac (potassium) were found in diabetic rabbits compared to diclofenac (sodium). A higher absorption half life value in the diabetic rabbits further suggests a slower absorption of diclofenac (sodium).

The elimination rate constant decreased significantly ($P<0.05$) in the diabetic animals. A significant ($P<0.05$) increase in total body clearance of diclofenac (potassium) was observed in alloxan treated rabbits compared with normal controls. The possible cause of this increase may be excessive urination by the diabetic animals (less than 1% of the dose of diclofenac is excreted unchanged in urine) (Kumar *et al*., 2002). These results are similar to the observations made by Nawaz *et al.* (1982). In alloxan treated and metabolically altered rabbits, a decrease in pH is favorable for the conjugation of drugs (diclofenac and its metabololites) (Bort *et al.,* 1999, Tang 2003, Grillo *et al.,* 2003). The conjugated drug can easily cross the biomembranes of drug eliminating organs (glomerular filtration in the kidney). Assuming non-significant ($P>0.05$) alteration in pH, higher flow of urine prevented any possible tubular back diffusion and caused greater part of renal elimination in total body clearance.

CONCLUSIONS

Plasma concentration of diclofenac sodium and diclofenac potassium decreased in diabetic condition. A significantly higher concentration of diclofenac potassium was observed in normal and diabetic rabbits when compared with diclofenac sodium. Comparison of bioavailability and disposition kinetics showed that diclofenac potassium absorbed at faster rate and to a larger extent so it could be a fast acting analgesic as compared to diclofenac sodium. The present study shows that the metabolic alteration of the alloxan induced diabetes after oral administration of diclofenac sodium and diclofenac potassium influenced the disposition kinetics which apprise of the need for an adjustment of dosage regimen under such conditions. Clinical implications of this study await verification in "real" diabetic condition.

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