Determination of Pharmacokinetics of Flurbiprofen in Pakistani Population Using Modified HPLC Method

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Abstract

Pharmacokinetics of flurbiprofen has been studied in different populations, especially in Caucasian. However, there are very few studies reported from Eastern part of world. Previous studies suggested that genetic and environmental factors may cause inter-individual differences in flurbiprofen disposition, so we investigated the pharmacokinetics of flurbiprofen in Pakistani subjects. A single oral dose of 100 mg of flurbiprofen was administered to 22 healthy male Pakistani adults after overnight fasting for 10 h. Periodical blood sampling was done at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, and 24 h after dosing. Plasma concentration of flurbiprofen was determined by a modified high-performance liquid chromatography method, which was simple, sensitive, less time consuming and economical with ordinary internal standard. The method was validated according to ICH guidelines and was found to be sensitive, accurate and precise. The pharmacokinetic parameters observed in Pakistani subjects when compared with other populations (USA, UK, Canadian, French, and Indian) did not show considerable ethnic differences. However, one subject's data was suggestive of being poor metabolizer of flurbiprofen which supports the presence of CYP2C9 polymorphism contributing to inter-individual differences in flurbiprofen disposition. Pharmacogenomic studies are needed to verify this hypothesis.

Introduction

Flurbiprofen is a potent non-steroidal anti-inflammatory drug. It has been effectively used in the treatment of rheumatoid arthritis, osteoarthritis, gout, sunburn (1), ankylosing spondylitis (2), soft tissue trauma (3), acute tendonitis and bursitis (4), primary dysmenorrhea (5), and bone pain in cancer patients (6). Clinical trials are going on to measure its effectiveness in Alzheimer's disease and prostate cancer (7). It has demonstrated comparable efficacy to other NSAIDs (e.g., aspirin, indomethacin, ibuprofen, naproxen, and diclofenac) (8).

As flurbiprofen is absorbed rapidly and almost completely when given orally (9), systemic availability of racemic flurbiprofen has been reported to be 95–100% (10,11). The inter-individual variations in the absorption profile of a solid dosage form are attributable to the variations in gastric emptying rate among individuals (12). More than 99% of flurbiprofen is bound to plasma proteins (13). In humans flurbiprofen is eliminated primarily via oxidation and conjugation (10,11,14). Its metabolism is mediated by cytochrome P450 2C9 in a non-stereoselective manner (11,15,16). Due to this property, flurbiprofen has been used as an in vitro probe as well as an in vivo probe to estimate the CYP2C9 activity in humans (15–17). There are variations in expression and activity of CYP enzymes seen across the species and individuals due to genetics, dietary components, disease states, and environmental factors. Studies have shown existence of ethnic-related differences in the occurrence of the CYP2C9 genetic polymorphism (18,19). Several known genetic polymorphisms associated with the CYP2C9 enzyme have been shown to have a significant role in contributing inter-individual variability in flurbiprofen metabolism (20,21). The knowledge of such polymorphism helps in determining the optimal doses of selected CYP2C9 substrate drugs (22–26).

The pharmacokinetics of flurbiprofen has been studied in different populations, especially in Caucasians (10,11,13,27–33). However, there are very few studies reporting pharmacokinetic data from eastern part of the world (34). This study was designed to assess pharmacokinetic profile of flufbiprofen in Pakistani population and to provide regional data on pharmacokinetics of this widely used drug. The individual status of activity of drug metabolizing enzyme CYP2C9 which in turn is a method of phenotyping, was assessed using flurbiprofen as probe drug.

Various methods have been described in the literature for the detection of flurbiprofen in human blood (10,31–40). The reported techniques have varying sensitivities. Some of the methods are tedious and some require highly sophisticated instruments. The method used in the present study (41) was simple, reliable, and reasonably sensitive for accurate estimation of flurbiprofen in the plasma. The method was modified to use naproxen as internal standard instead of 2-fluoro-4-biphenylacetic acid. Various procedures were performed to validate the modified method according to International Conference on Harmonization (ICH) Guidelines on validation of analytical procedures (42).

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Experimental

Instrumentation and materials

The high-performance liquid chromatography (HPLC) system by PerkinElmer (Series 200) with autosampler and fluorescence detector was used. Chromatographic separation was done on Chromolith Performance RP-18 encapped column (4.6 × 100 mm, 5 µm particle size) provided by Merck Darmstadt, Germany. The chromatograms were recorded on connected computer.

The chemicals and solvents used in this study were of HPLC grade. Acetonitrile, Hydrochloric Acid and Dipotassium hydrogen phosphate (K_2HPO_4) were purchased from Merck (Darmstadt, Germany, C/O MS Traders, Pakistan). Phosphoric acid was purchased from Sigma Aldrich, (Munich, Germany, C/O MS Traders, Pakistan). Naproxen (internal standard) was provided by Ind-Swift Pharmaceuticals (C/O Guddia International, Pakistan) and Rac-Flurbiprofen (external standard) from FDC Limited, India (C/O Guddia International, Pakistan). The formulation of flurbiprofen administered to the subjects was Tablet Ansaid (tablet containing 100 mg of flurbiprofen, batch No: 736029, manufactured by Upjohn Parke, Davis & Co., Ltd., Pakistan for Pharmacia Pakistan Pvt., Ltd).

Chromatographic conditions and standards preparation

The fluorescence detector was set at an excitation wavelength of 260 nm and an emission wavelength of 320 nm. The strength of acetonitrile used in mobile phase was 90% instead of 100% used by Hutzler (41). The mobile phase consisted of acetonitrile (90%)–20 m*M* K_2HPO_4 at pH 3.0 (40:60, v/v). The mobile phase was pumped at a flow rate of 2.5 mL/min. The volume of injection was fixed at 20 µL. All analyses were done at room temperature and column temperature was maintained at 25°C.

Flurbiprofen stock solution was prepared by dissolving it in 50:50 acetonitrile–water to make a 1 mg/mL solution. From this stock solution, standards were prepared to contain concentrations of 0, 0.25, 0.5, 1.0, 2.5, 5.0, 10, 15, and 25 µg/mL of flurbiprofen. All solutions were stored at 4°C. A stock solution of naproxen (IS) was prepared in 50:50 acetonitrile–water with a target concentration of 1 mg/mL. From this stock solution dilutions were prepared to make 50, 100, and 200 µg/mL working internal standard solutions. These solutions were stored at –20°C between uses. These working IS solutions were used for identification and selection of appropriate concentration of naproxen to be used as IS. The strength of 100 µg/mL was then used in plasma sample processing.

Calibration curve and sample processing

Calibration curve was constructed by spiking blank plasma with standard solutions of flurbiprofen and naproxen (IS). The calibration curve was generated by plotting the ratio of the peak area of flurbiprofen and naproxen (IS) against the flurbiprofen concentration in solution.

Plasma samples were processed by adding 100 µL acetonitrile, 200 µL of internal standard (100 µg/mL) and 40 µL of half-strength phosphoric acid (50:50 phosphoric acid–water) to 100 µL of plasma. The samples were vortexed for 30 seconds at speed of 2100 rpm and then centrifuged at 14000 g for 10 min. Afterwards 150 µL of supernatant was placed into autosampler vials for analysis. The autosampler was set to inject 20 µL onto the HPLC.

Method validation

QC samples were run as replicates of blank plasma spiked with a low concentration (0.5 µg/mL), a middle concentration (10 µg/mL), and a high concentration (25 µg/mL) of flurbiprofen along with a fixed concentration of internal standard.

The identification of flurbiprofen was made on the basis of retention time on chromatograms obtained from plasma samples spiked with standard solutions of flurbiprofen and comparing with plasma samples without spiking with flurbiprofen.

Linearity was assessed by calibration curve constructed using 8 standard solution concentrations covering the range of 0.25–25 µg/mL. Standard curves were analyzed in triplicate. The lower limit of quantitation (LLOQ) for flurbiprofen was selected as the lowest concentration of the standard curve. The lower limit of quantitation was the lowest flurbiprofen concentration at which the flurbiprofen peak was identifiable and discrete with suitable precision (coefficient of variation of less than 20%) and accuracy (determined concentration being within 20% variation of the nominal concentration).

The precision and accuracy of the plasma assay for flurbiprofen was determined by running quality control (QC) samples for 3 days. Intra-day assay variability was tested with 12 replicates of each QC concentration run on the same day. Inter-day assay variability was established by running 6 replicates of each QC concentration for 3 consecutive days.

To assure the stability of analyte and the analytical system, QC samples were run daily at the beginning of each run throughout the period of analysis. Analyte stability was also demonstrated by subjecting the samples of three concentrations to three freeze-thaw cycles.

Study subjects

The study was conducted in accordance with the current Good Clinical Practices (43) and the Declaration of Helsinki (44). The study protocol was approved by Ethical Committee of Centre for Research in Experimental and Applied Medicine, Army Medical College, Rawalpindi. Twenty two healthy male subjects between the ages of 20–38 years participated in the study. Their weights were within 20% of normal body weight according to the Metropolitan Life Assurance tables. Each subject was evaluated to be healthy after a detailed medical history, physical examination and laboratory tests. Subjects with the history of smoking, drug abuse, any drug hypersensitivity, and illness were excluded. All subjects avoided taking any over-the-counter medicine for two weeks preceding the study. Subjects were informed of the nature, significance and consequence of the study and the investigational procedures. They gave informed consent by dated signature on the consent proforma.

Clinical procedure

Each subject received a single dose of one tablet of flurbiprofen (100 mg) after an overnight fast of 10 h. The drug was swallowed orally with 240 mL of drinking water. Liquids were permitted after one h of dosing. Standardized meal was served after 4 h of drug administration. The standardization of study environment and diet was observed. Subjects were permitted to be ambulatory during 24 h stay at study site.

Blood sampling was done over the period of 24 h. Each subject

was passed intravenous cannula for periodical blood sampling. A 5 mL blood sample was drawn immediately prior to and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, and 24 h after dosing. Each sample was transferred to heparinized tubes and was immediately centrifuged. Plasma was collected and frozen at –20°C until analysis.

Pharmacokinetic analysis

The pharmacokinetic parameters were derived using computer program, APO, MWPHARM version 3.60, a product of Mediware (Holland). The pharmacokinetic parameters were derived individually for each subject from the plasma concentration versus time data. A two-compartmental open model was assumed to derive the pharmacokinetic parameters. Peak drug concentration (C_{max}) and the time to peak drug concentration (T_{max}) were obtained directly from the data without interpolation. Area under the plasma concentration-time curve from time zero to time t (AUC_{0-t}) was calculated by trapezoidal rule, where t is the last measurable time point. The *AUC* extrapolated to $t - \infty$ was calculated using the relation Cm/β, where *Cm* is the final concentration of flurbiprofen and β is the slope of the least squares linear regression of the log concentration-time curve.

Results

Validation of HPLC method

The calibration curve of flurbiprofen in plasma using least square regression equation was linear within the range of 0.25–25 µg/mL. The equation of calibration was $y = 746634x - 62020$, r-square = 0.9992 (Figure 1).

The retention time for flurbiprofen was 6.5 min and for internal standard (naproxen) 3 min, making them well resolved (Figures 2 and 3).

The LOD of the flurbiprofen in plasma samples was 0.01 µg/mL. For flurbiprofen analysis in plasma by this method LLOQ was 0.25 µg/mL. The CV% for LLOQ was found to be 5.52%, which is well below the limits of 20%, and accuracy was found to be 95.73%.

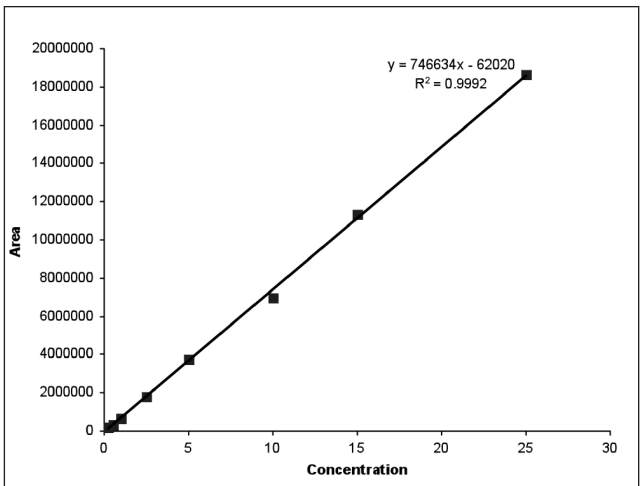

Figure 1. Calibration curve of flurbiprofen.

The method was shown to be precise and accurate. The intra-day variability ranged between 3.23–9.49% and inter-day variability ranged between 1.44–3.38%. The percent accuracy was in the range of 95–98% for intra-day assays and 97–99% for inter-day assays.

The results of freeze-thaw cycles showed no effect on stability of analyte as variability ranged between 1.11–1.78%. All these results are given in Table I.

Pharmacokinetic profile

Twenty-two healthy adult male subjects participated in this study. The age of the subjects ranged from 20–38 years (mean ± SE 29.4 ± 0.99). The weight of the subjects ranged from 50–94 kg (mean 70.09 ± 2.44) and height ranged from 165.1–180.3 cm (mean 170 ± 5.12). No adverse drug reaction was recorded in any subject during the study. The mean plasma concentration-time curve for flurbiprofen is plotted on linear and logarithmic scale

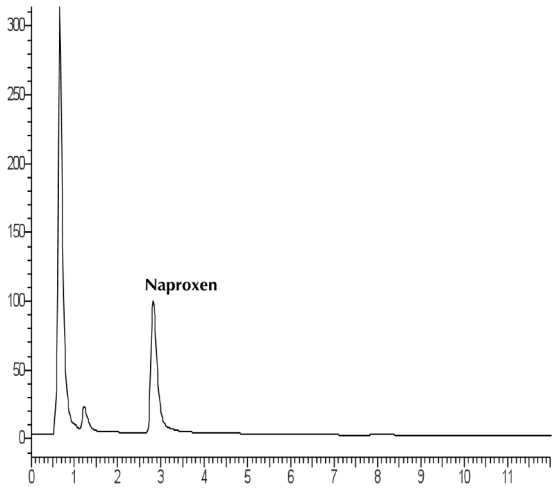

Figure 2. Chromatogram obtained from blank plasma sample spiked with internal standard.

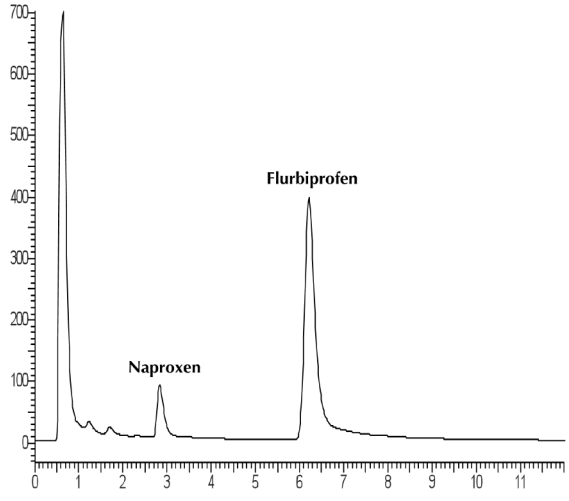

Figure 3. Chromatogram obtained from plasma sample spiked with internal standard and external standard of 5 µg/mL flurbiprofen.

as given in Figures 4 and 5, respectively. After oral administration, all subjects showed detectable amount of flurbiprofen in plasma after 15 min reflecting its characteristic of being rapidly absorbed from GIT. The results of mean pharmacokinetic data for single dose of 100 mg of flurbiprofen are given in Table II.

Table I. Intra- and Inter-day Precision and Accuracy of Flurbiprofen Assay

Assay Validation Procedures	Conc. Added (µg/mL)	Conc. Found (µg/mL) Mean ± SD	Coefficient of Variation (CV %)	Accuracy (%)
LLOQ	0.25	0.24 ± 0.013	5.52	95.73
Intra-assay	0.5	0.47 ± 0.045	9.49	94.83
Reproducibility	10.0	9.81 ± 0.317	3.23	98.08
	25.0	24.07 ± 1.202	4.99	96.27
Inter-assay	0.5	0.49 ± 0.017	3.38	98.50
Reproducibility	10.0	9.92 ± 0.254	2.56	99.19
	25.0	24.19 ± 0.348	1.44	96.76
Freeze-thaw	0.5	0.496 ± 0.006	1.11	99.27
Stability	10.0	10.58 ± 0.141	1.33	105.80
	25.0	25.78 ± 0.459	1.78	103.13

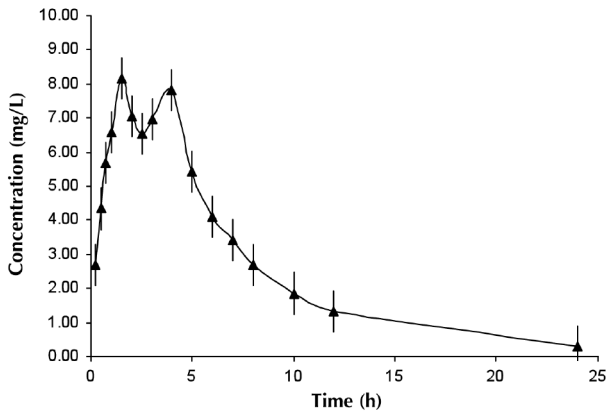

Figure 4. Plasma concentration-time curve of flurbiprofen on linear scale.

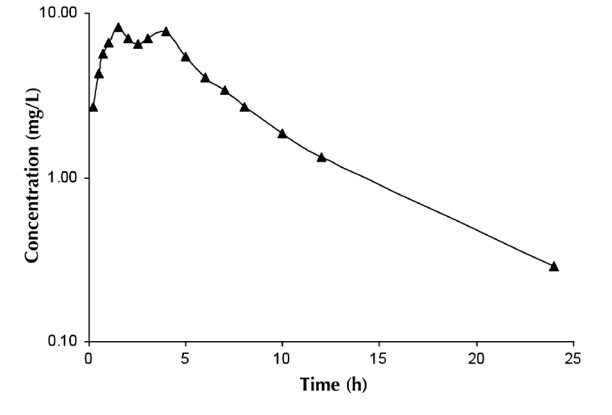

Figure 5. Plasma concentration-time curve of flurbiprofen on a logarithmic scale.

Discussion

The modified analytical method showed good specificity, sensitivity, linearity, precision and accuracy over the entire range of clinically significant concentrations in plasma. Modifications were advantageous as internal standard (naproxen) used was cheaper and easily available. Instead of limiting the pressure setting at 67 bars (972 psi) as by Hutzler (41), in this study the analysis was carried out through pressure range of 800–1200 psi making analysis more accommodative. It also accommodated the higher standard concentration of 25 µg/mL. Due to early retention times, the run time for assay was reduced leading to economy of resources.

All the subjects showed detectable concentration of flurbiprofen after 15 min of drug administration. This is in line with the established absorptive characteristics of flurbiprofen, which make it rapidly absorbed drug after oral administration (9,12,27,28,37). In this study the lag time was found to be 0.10 h which confirms the reported characteristic of rapid absorption of orally administered flurbiprofen preparation. The study showed double peak behavior in flurbiprofen plasma concentration-time profile. Previous study has also shown such behavior (12). The incidence of double peak phenomena has been attributed to the effect of gastric emptying on the flurbiprofen absorption, in which a part of the dose dissolves and empties relatively early with the ingested water and the rest of it remains in the stomach until the next inter-digestive migratory movement complex (IMMC) activity occurs, then it is emptied and rapidly absorbed (12).

The comparison of pharmacokinetic parameters determined in Pakistani subjects was done with data reported in other studies carried out after administration of 100 mg tablet of flubiprofen as shown in Table III. The data suggests the absence of any considerable difference in pharmacokinetic parameters of flurbiprofen among different ethnic groups. Although there may be differences in genetic make up and environmental conditions of Pakistanis and their foreign counter-parts, the reported data is

Table II. Pharmacokinetic Parameters of Flurbiprofen*

Pharmacokinetic Parameters	Mean ± SD
Lag Time (h)	0.10 ± 0.09
Absorption $t_{1/2}$ (h)	1.48 ± 1.0
Ka (L/h)	0.96 ± 1.23
Vd (L)	12.07 ± 4.3
C_{max} (mg/L)	12.04 ± 3.21
T_{max} (h)	2.43 ± 1.28
$AUC_{(0-24)}$ (h.mg/L)	61.31 ± 21.31
$AUC_{(0-\infty)}$ (h.mg/L)	62.15 ± 38.19
$t_{1/2}$ (h)	6.85 ± 7.89
Kel (L/h)	0.92 ± 1.56
Cl (L/h)	1.89 ± 0.63

* *Ka* = absorption rate constant; *Vd* = apparent volume of distribution; C_{max} = peak plasma concentration; T_{max} = time to achieve peak plasma concentration; AUC_{0-24} = area under the curve from time 0 to 24 h; $AUC_{0-\infty}$ = area under the curve from time 0 to infinity; $t_{1/2}$ = elimination half life; *Kel* = elimination rate constant; *Cl* = Clearance

Table III. Comparison of Pharmacokinetic Profile of Flurbiprofen in Different Ethnic Population

Source	AUC_{0-24} (h.mg/L)	$AUC_{0-\infty}$ (h.mg/L)	C_{max} (mg/L)	T_{max} (h)	$t_{1/2}$ (h)	*Vd* (L)	*Cl* (L/h)
Pakistani	61.31 ± 21.31	62.15 ± 38.19	12.04 ± 3.21	2.43 ± 1.28	6.85 ± 7.89	12.07 ± 4.3	1.89 ± 0.63
USA Caucasians (33)	–	59 ± 17	12.9 ± 3.7	–	3.3 ± 0.8	–	1.83 ± 0.49
USA Caucasians (28)	–	76.6 ± 21.2	12.6 ± 3.1	2.0 ± 1.7	5	–	–
USA Caucasians (32)	71 ± 15	–	12.1 ± 1.0	–	4.3 ± 0.5	–	1.836 ± 0.264
USA Caucasians (11)	82.19 ± 20.1	82.74 ± 20.4	14.2 ± 4.23	1.9 ± 1.51	7.41 ± 1.28	–	1.28 ± 0.27
UK Caucasians (27)	76.2 ± 21.3	–	16.52 ± 3.93	1.7 ± 1.15	5.52 ± 1.4	9.1	1.2
Canadian Caucasians (30)	–	58.3 ± 12.1	11.7 ± 3.9	2.3 ± 1.6	6.2 ± 1.6	15.8 ± 4.7	1.77 ± 0.31
Canadian Caucasians (29)	80.50 ± 25.12	–	16.74 ± 3.69	1.32 ± 0.66	–	–	–
French Caucasians (31)†	37.37 ± 7.98	–	5.99 ± 1.3	2 ± 1	7.5 ± 1.9	–	1.39 ± 0.28
USA Caucasians (41)†	–	43.8 ± 13.8	10.5 ± 2.9	1.5 ± 0.9	3.7 ± 1	6.2 ± 1.2	1.23 ± 0.32
USA Caucasians (13)†	–	50.6 ± 17.6	9.4 ± 2.5	2.3 ± 1	4.3 ± 1.6	6.12 ± 1.11	1.09 ± 0.35
Indians (34)‡	94.11 ± 20.81	–	9.47 ± 1.84	4	6.46 ± 1.25	–	–

* AUC_{0-24} = area under the curve from time 0 to 24 h; $AUC_{0-\infty}$ = area under the curve from time 0 to infinity; C_{max} = peak plasma concentration; and T_{max} = time to achieve peak plasma concentration; $t_{1/2}$ = elimination half life; *Vd* = apparent volume of distribution; Cl = Clearance;
† Data from 50 mg tablet
‡ Data from 200 mg sustained release tablet

suggestive of no considerable influence of environmental factors on the pharmacokinetic profile of flurbiprofen. As regard the genetic factors are concerned, there is an example of one of Pakistani subjects whose pharmacokinetic profile was somewhat different from rest of the individuals. He had a long elimination half-life of 38.29 h and a low clearance of flurbiprofen of 0.45 L/h. The calculated $AUC_{0-\infty}$ was 222.4 h.mg/L and AUC_{0-24} was 138.53 h.mg/L in this subject. The C_{max} was observed to be 19.06 mg/L achieved at T_{max} of 1.5 h. All these parameters are suggestive of this subject having a CYP2C9 "poor metabolizer" genotype. Due to absence of data regarding genotype of the subject, it was not possible to investigate this individual further. A previous study has also shown such exceptional case (32). However it appears that genetic factor does not play any significant role in causing variations in pharmacokinetic disposition of flurbiprofen in different ethnic groups, though there may be differences among individuals in different populations.

Conclusion

The data obtained from other ethnic groups reported in various studies and in this study suggest the absence of any considerable difference in pharmacokinetic parameters of flurbiprofen among different ethnic groups. Although there may be differences in genetic make up and environmental conditions of Pakistanis and their foreign counter-parts, the reported data is suggestive of no considerable influence of environmental factors on the pharmacokinetic profile of flurbiprofen. Regarding the genetic factors concerned, there are inter-individual differences which are attributable to several known genetic polymorphisms associated with the CYP2C9 enzyme but it appears that they do not play any significant role in causing variations in pharmacokinetic disposition of flurbiprofen in different ethnic groups.

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