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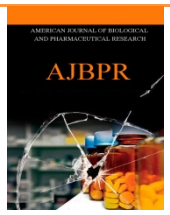

EFFECT OF MENSTRUAL CYCLE ON THE PHARMACOKINETICS OF FLURBIPROFEN ENANTIOMERS

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ABSTRACT

The purpose of this research was to characterize the influence of the menstrual cycle on the pharmacokinetics of Flurbiprofen (FLU) in healthy female volunteers. Gender differences in pharmacokinetics and pharmacodynamics of drugs have been recognized for some time. The large variations in hormone levels throughout the menstrual cycle could potentially have a significant effect on the metabolism of drugs. The 2-Aryl propionic acid (2-APA) derivatives are currently an important group of NSAIDS. A common structural feature of 2-APA NSAIDS is a SP^3–hybridized tetrahedral chiral carbon atom within the propionic acid side chain moiety with the S-(+) Enantiomer possessing most of the beneficial anti-inflammatory activity. Twelve healthy female volunteers participated in the study of age ranging from 16 to 25 years and weight in the range of 40 to 60 kg. The days were 3rd, 13th, 23rd, day of the menstrual cycle. A single oral dose of 100 mg of was given to each subject with 150 ml water. A predose blood sample served as an analytical blank. Subsequent blood samples were drawn at 0.5, 0.75, 1, 1.5, 2, 3, 5, 8, 12 hrs. Chiral columns were available for the separation of enantiomers of profens. The samples were analyzed by Sensitive and Stereo specific high-performance liquid chromatographic method for Flurbiprofen in human plasma.

INTRODUCTION

Gender differences in pharmacokinetics and pharmacodynamics of drugs have been recognized for some time. This issue has been ignored in clinical practice, despite there being ample evidence to suggest that gender can influence multiple aspects of pharmacokinetics. Female-specific issues such as pregnancy, menopause, oral contraceptive use, and menstruation may independently influence drug metabolism and serve as confounders to the interpretation of gender differences in drug handling or the effect.

Physically and hormonalchangesduringmenstrual cycle and difference in renal blood flow are several factors that may have some bearing on sex related differences in Pharmacokinetics for there more female species issues such as pregnancy, menopauses, oral contraceptives use and menstruation may independently influence drug metabolism.

Enantiomers are optically active compounds with one or more chiral centers and have identical physicochemical properties except the rotation of plane polarized light. Approximately 1 in 4 therapeutic agents are marketed as racemates; the individual enantiomers frequently differ in both their pharmacokinetic and pharmacodynamic profiles. These differences result when

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the drug molecule has an asymmetric interaction with a receptor, a transport protein or a metabolizing enzyme.

The purpose of this study was to characterize the influence of the menstrual cycle on the pharmacokinetics of FLU in healthy female volunteers.

MATERIALS AND METHODS

FLU was a kind gift from Abbott India Limited, Goa, India; IBU was generously supplied by Acto Pharma Pvt. Ltd, Warangal, Andhra Pradesh, India. n-Hexane, iso-octane, 2-propanol were purchased from Merck India Pvt. Ltd, Mumbai, India.

Study Protocol

Enrolment of subjects

Twelve healthy female volunteers have been included in the study after obtaining written informed consent. The age ranged from 16 to 25 years and weight was from 40 to 60 kg respectively. None of the participants had received any medication during two weeks prior to the study [1].

Inclusion criteria

1) Healthy as per the physical examination.
2) Non allergic to FLU selected
3) Without other medication
4) Written informed consent
5) Regular menstrual cycle

The exclusion criteria

1) Amenorrhea
2) Use of Contraceptives

Ethical Committee Approval

The study protocol was submitted in writing and presented before the institutional ethical committee and approval was obtained.

Experimental design: 100 mg of FLU (racemic mixture) was given to each subject with 150 ml water and no food was permitted during the next 4h. Blood samples (6ml) were drawn in to heparinized tubes from cubital vein. A predose blood sample served as an analytical blank. Subsequent blood samples were drawn 0.5, 0.75, 1, 1.5, 2, 3, 5, 8 and 12hr after drug administration. Blood samples were centrifuged at 2000 g for 15 min. and plasma was separated. The plasma samples were stored at -200^{0}C until HPLC analysis.

Analytical Procedure

The method involves extraction of drug and Ibuprofen (internal standard, I. S.) with a mixture of isooctane and 2-propanol. To 0.5 ml of plasma sample, 50 µl of I. S., 200 µl of sulphuric acid (0.06 M), and 4ml of isooctane: 2-propanol (95:5) were added. Samples were mixed thoroughly using vortex mixer and then centrifuged at 3000 rpm for 15 min. The organic phase was separated and evaporated under reduced pressure in a vacuum oven (Sheldon Mfg. Inc. USA). The residue was reconstituted in 50 µl of mobile phase and 20 µl were injected into HPLC column. The ratios of peak areas of drug to I.S. were calculated.

The chromatographic system consisted of a Shimadzu LC– 10AT solvent delivery pump equipped with a 20 µl loop and rheodyne sample injector and SPD-10AVP dual wavelength UV-Visible detector.

Analysis of blank blood samples for hormones during FLU study

The blank blood samples were analyzed to know the blood concentration of hormones follicular stimulating hormones, leutinizing hormone, prolactin, by ELISA in VBR diagnostics, Hanamkonda. Blood concentrations of Oestrogen and progesterone were determined by the chemiluminescence method at MIS Vijaya Diagnostics, Hyderabad.

Pharmacokinetic Data

The pharmacokinetic parameters of enantiomers of FLU were computed using a model independent method employing WINNONLIN. The mean values of various pharmacokinetic parameters obtained in different subjects following the three treatments were compared using ANOVA and a difference was considered significant when the probability of chance explaining the results was reduced to 5%.

Table 1.Mean and (±SD) Pharmacokinetic parameters of R Enantiomers of FLU Racemate following oral administration of 100 mg FLU racemate during three phases. (n = 12)

Mean (±SD) Parameters	Follicular Phase (±SD)	Ovulatory Phase ((±SD)	Luteal Phase (±SD)
C_{max}(µg/ml)	4.93(±2.24)	5.41(±2.12)	5.17(±1.81)
T_{max}(h)	4.66(±1.87)	5.08(±2.50)	6.08(±2.57)
t ½(µg/ml)	8.81(±4.03)	8.52(±6.06)	8.00(±4.57)
AUC $_{0-\alpha}$µg/ml/h)	40.6(±19.8)	40.5(±20.1)	49.1(±17.2)
Vd area/f (ml/kg)	705.4(±220.1)	715.6(±395.6)	504.0(±183.8)
Vssf(µg/ml)	732.6(±238.3)	671.3(±467.3)	567.9(±207.6)

CL/f (ml/kg/h)	63.4(±29.4)	124.9(±230.6)	48.49(±20.9)
MRT(h)	14.8(±7.78)	15.1(±9.44)	0.816(±0.39)
Ka(ha^{-1})	1.08(±0.40)	0.90(±0.38)	0.816(±0.39)

Table 2.Mean and (±SD) Pharmacokinetic parameters of S Enantiomers of FLU Racemate following oral administration of 100 mg FLU Racemate during three phases. (n = 12)

Mean (±SD) Parameters	Follicular Phase (±SD)	Ovulatory Phase ((±SD)	Luteal Phase (±SD)
C_{max}(μg/ml)	4.26(±2.32)	3.34(±1.65)	4.82(±2.52)
T_{max}(h)	7.41(±7.71)	5.41(±2.84)	6.41(±2.23)
$t_{½}$(μg/ml)	9.43(±11.1)	7.73(±4.88)	5.71(±3.63)
AUC $_{0-\alpha}$(μg/ml/h)	37.6(±26.7)	34.5(±20.6)	41.8(±24.1)
Vd area/f (ml/kg)	747.8(±504.9)	734.0(±355.5)	456.9(±257.0)
Vssf(μg/ml)	744.6(±588.0)	827.3(±399.1)	544.2(±332.3)
CL/f (ml/kg/h)	77.9(±42.2)	78.05(±43.8)	58.9(±26.07)
MRT(h)	15.5(±17.2)	14.3(± 10.3)	11.5(±7.44)
Ka(ha^{-1})	0.98(±0.43)	1.00(±0.50)	0.73(±0.30)

DISCUSSION

The absorption of Flurbiprofen is rapid and almost complete when given orally. The area under the plasma concentration-time curve of Flurbiprofen is proportional to the dose administered to patients.The stereo selective metabolism and pharmacokinetics of the enantiomers of Flurbiprofen were investigated following the oral administration of the racemic-drug (100 mg) to four young and four elderly healthy volunteers (two males and two females per group). The findings suggest that age-related alterations in the disposition of Flurbiprofen could have significant implications for the use of the drug in the elderly [2]. Stereo selective disposition of Flurbiprofen in normal volunteers exhibits enantio selectivity at the level of protein binding and metabolite formation [3].

Jamali*et al*. [4] observed the dose-dependency of flurbiprofen enantiomer pharmacokinetics in the rat, The results are consistent with the hypothesis that the increasing amount of R (-) and (S)-flurbiprofen in the body causes displacement of flurbiprofen enantiomers from their protein binding sites, resulting in their increased total body clearance and volume of distribution. In our results also for the R-FLU the volume of distribution and clearance were increased in ovulatory phase than in the follicular and luteal phases, in case of S-FLU, the volume of distribution and clearance were increased in the follicular phase than in the ovulatory and luteal phases, but these results have not attained statistical significance.

Significant changes in endogenous sex hormone concentrations occur during the menstrual cycle and during pregnancy, leading to alterations in protein binding, distribution and clearance [5]. Gender specific pharmacodynamic data suggests the existence of sex related differences [6].

Comparison of data obtained in the follicular phase with those obtained in the luteal phase revealed differences in most pharmacokinetic parameters, which is seemingly indicative of the characteristic physiological changes associated with the luteal phase that largely affect the kinetics and availability Ranitidine [7]. Although it has been postulated that hormonal fluctuation within the menstrual cycle phase is the primary cause of documented gender differences in the pharmacokinetics and pharmacodynamics of drugs. Further study of related factors is required to understand how gender and menstrual cycle rhythms affect the pharmacokinetic process in their entirety [8].

The S-enantiomer of carprofen and flurbiprofen showed higher AUC and t1l2 and these enantiomers are highly bound to proteins [9].Ketoprofen enantiomers showed small differences in protein binding whereas R-2-phenylpropionic acid was eliminated faster than S-antipode leading to greater AUC for S-isomer [10]. In case of indoprofen, the R-enantiomer is more bound and rapidly eliminated than its antipode[11] whereas repeated administration f1urbiprofen caused accumulation of S (+) enantiomer [12].Greenblatt*et a*l., 1980,Robbert*set at*. [13]studied the stereo selective disposition of flurbiprofen in uraemic patients and concluded that adjustment of flurbiprofen dosing rate in uraemic patients is not indicated on the basis of pharmacokinetics.

Sex differences in drug metabolism and elimination are mainly related to steroid hormone levels. CYP3A4, which is responsible for the metabolism of over 50% of therapeutic drugs, exhibits higher activity in women than in men. Onetheless, the absence of a sex difference has been reported by some workers. The activity of several other CYPCYP2CI9, CYP2D6, CYP2EI) isozymes and. the conjugation (glucuronidation) activity involved in drug metabolism may be higher in men than in women [14].

Gislinger G*et al*. [15] reported that the R (-) enantiomer had higher AUC, lower clearance data and

higher e-max values than the S-enantiomer after oral administration of different doses of the ketoprofenracemate.

Tia profenic acid (TPA) is a 2-APA NSAID which possess single chiral carbon atom, therefore exists as two enantiomers.Daveis*et al.*[16], reported that theC_{max} and AUC of the S-TPA was greater than R-TPA following intra peritoneal administration. This is due to the chiral inversion of the R-TPA to S-TP A [17]. Knadler*et al.* [18] results show that S-ketoprofen inhibits the carragenan induced edema and induces the production of intlammatorycytokinine and interleukin-l effectively. The racemic ketoprofen exhibits little stereo selectivity in its pharmacokinetics.

Knadler*et al.* [19]concluded that the binding of racemic flurbiprofen in elderly and obese volunteers and patients with liver disease was not significantly different from normal subjects; but binding was less in hypoalbuminic patients and patients with renal impairment. (Fletcher*et al*. [20] investigated the enantiomeric interaction of flurbiprofen in rat and reported that interaction is a result of displacement from plasma protein binding sites of one enantiomer by the other.

Walle*et al.*(1989) [21]concluded that in 23 young women there was no significant association between the circulating levels of either estradiol or testosterone and any of the clearances of propranolol. In the nine women, the binding did not change with fluctuating plasma oestradiol concentrations during the menstrual cycle.

In the present study the changes in estrogen levels during ovulatory phase have not shown any influence on AUC_{o-t} of S-FLU unlike R-FLU. Only AUC_{o-t} of R-FLU showed an increasing trend with increasing levels of estrogen in ovulatary phase, but not in other phases. Even though the FSH levels differed significantly among volunteers during different phases FSH does not seem to influence overall pharmacokinetic behavior of both R & S-FLU during different phases. The present study indicated only the trend that the hormone levels may influence the pharmacokinetic behavior of the two isomers. In order to understand the possible quantitative influence of different phases implying different hormone levels are disposition of the two isomers of FLU perhaps it is necessary to minimize the inter individual variability of different hormones. A study employing a large number of subjects has to be under taken. Alternately a population pharmacokinetic study may be under taken.

Conclusions

In the present study the changes in hormones have not shown any influence on pharmacokinetics significantly among volunteers during different phases. They do not seem to influence overall pharmacokinetic behavior of both R&S-flurbiprofen during different phases.

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