EFFECT OF MENSTRUAL CYCLE ON THE PHARMACOKINETICS OF ENANTIOMERS OF IBUPROFEN

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ABSTRACT
The purpose of this study was to characterize the influence of the menstrual cycle on the disposition of the R(-) and S(+) enantiomers of ibuprofen in healthy female volunteers. Enrolment 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. Study protocol was submitted in writing and was presented before the institutional ethical committee and approval was obtained. Experimental design: 600 mg of IBU (Racemic mixture) was given to each subject after overnight fasting with 150 ml water and no food was permitted during the next 4hrs. 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, 12 and 24h after drug administration. Blood samples were centrifuged at 2000 g for 15 min. and plasma was separated. The plasma samples were stored at –800 C until HPLC analysis. The plasma samples were stored at –800 C until HPLC analysis. The samples were analyzed by Sensitive and Stereo specific high-performance liquid chromatographic method for ibuprofen in human plasma.

INTRODUCTION
Gender differences in drug pharmacokinetics and pharmacodynamics have been recognized for some time. For several reasons no two individuals can be considered identical and hence individualization of therapy is the current trend in treating the patients.

A common structural feature of 2-Arylpropionic Acid (2-APA) derivatives (profens) is a SP3 -hybridized, tetrahedral chiral carbon within the propionic acid side chain [1]. The extent of anti-inflammatory activity of profens depends upon the rate of inversion of R (-) ibuprofen inverted to S (+) Ibuprofen following separate oral administration of ibuprofen Enantiomers [2].

Racemic Ibuprofen or R(-) ibuprofen [3]. Erb et al. [4] reported that Peak concentration (Cmax) and Area under the curve (AUC) of the S-tiaprenolic acid (S-TPA) was greater than R-tiaprenolic acid (R-TP A) [5]. This was due to the chiral inversion of the R-TPA to S-TPA [4]. In a study it was found that S-Flurbiprofen has smaller volume of distribution and clearance compared to R-Flurbiprofen [6]. Elimination of half-life and mean residence time were shorter for S-carprofen than its antipode in calves [7].

MATERIALS AND METHODS
IBU was generously supplied by Acto Pharma Pvt. Ltd, Warangal, Andhra Pradesh, India. Flurbiprofen was a kind gift from Abbott India Limited, Goa: India n-Hexane, isoctane and 2-propanol were purchased from Merck India Pvt. Ltd, Mumbai, India.
**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 experiments.

**Inclusion criteria**

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

**The exclusion criteria**

1) Amennorhea
2) Use of Contraceptives

**Ethical Committee Approval**

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

**Experimental design**

600 mg of IBU (Racemic mixture) was given to each subject after overnight fasting 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 05, 0.75, 1, 1.5, 2, 3, 5, 8, 12 and 24h after drug administration. Blood samples were centrifuged at 2000 g for 15 min. and plasma was separated. The plasma samples were stored at -80°C until HPLC analysis.

**Table 1. Mean and (SD±) Pharmacokinetic parameters of R Enantiomer of IBU racemate following oral administration of 600 mg IBU during three phases. (n = 12)**

<table>
<thead>
<tr>
<th>Mean (±SD) Parameters</th>
<th>Follicular Phase (±SD)</th>
<th>Ovulatory (±SD)</th>
<th>Luteal (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (µg/ml)</td>
<td>22.7 (±16.50)</td>
<td>29.4 (±33.7)</td>
<td>22.0 (±6.8)</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.89 (±2.00)</td>
<td>1.35 (±0.44)</td>
<td>1.65 (±0.68)</td>
</tr>
<tr>
<td>t 1/2 (µg/ml)</td>
<td>4.06 (±3.27)</td>
<td>3.15 (±2.54)</td>
<td>2.47 (±2.05)</td>
</tr>
<tr>
<td>AUC εₐ (µg/ml/h)</td>
<td>54.7 (±32.0)</td>
<td>59.2 (±18.9)</td>
<td>2.47 (±2.05)</td>
</tr>
<tr>
<td>Vdarea/f (ml/kg)</td>
<td>1391.8 (±676.8)</td>
<td>916.0 (±862.1)</td>
<td>649.0 (±405.9)</td>
</tr>
<tr>
<td>Vssf (µg/ml)</td>
<td>1326.8 (±1017.8)</td>
<td>1071.3 (±930.2)</td>
<td>463.7 (±729.2)</td>
</tr>
<tr>
<td>CL/f (ml/kg/h)</td>
<td>309.8 (±207.0)</td>
<td>202.9 (±64.8)</td>
<td>207.0 (±106.5)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>6.97 (±5.82)</td>
<td>5.62 (±3.90)</td>
<td>3.16 (±0.37)</td>
</tr>
<tr>
<td>Ka (ha⁻¹)</td>
<td>2.72 (±1.78)</td>
<td>3.20 (±0.99)</td>
<td>2.98 (±1.16)</td>
</tr>
</tbody>
</table>

**Table 2. Mean and (SD±) Pharmacokinetic parameters of S Enantiomers of IBU racemate following oral administration of 600 mg IBU during three phases. (n = 12)**

<table>
<thead>
<tr>
<th>Mean (±SD) Parameters</th>
<th>Follicular (±SD)</th>
<th>Ovulatory (±SD)</th>
<th>Luteal (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (µg/ml)</td>
<td>19.9 (±7.35)</td>
<td>25.8 (±13.0)</td>
<td>21.0 (±4.07)</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>2.041 (±1.05)</td>
<td>2.54 (±2.01)</td>
<td>1.75 (±0.58)</td>
</tr>
<tr>
<td>t 1/2 (µg/ml)</td>
<td>3.93 (±3.32)</td>
<td>3.06 (±1.73)</td>
<td>2.99 (±2.19)</td>
</tr>
<tr>
<td>AUC εₐ (µg/ml/h)</td>
<td>80.6 (±60.1)</td>
<td>76.0 (±36.1)</td>
<td>84.8 (±30.0)</td>
</tr>
<tr>
<td>Vdarea/f (ml/kg)</td>
<td>1022.6 (±505.1)</td>
<td>843.1 (±475.1)</td>
<td>647.0 (±387.4)</td>
</tr>
<tr>
<td>Vssf (µg/ml)</td>
<td>1084.3 (±704.8)</td>
<td>994.2 (±694.8)</td>
<td>746.9 (±562.9)</td>
</tr>
<tr>
<td>CL/f (ml/kg/h)</td>
<td>232.9 (±167.1)</td>
<td>192.1 (±101.2)</td>
<td>160.2 (±63.7)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>7.24 (±5.71)</td>
<td>6.09 (±3.2)</td>
<td>5.7 (±4.7)</td>
</tr>
<tr>
<td>Ka (ha⁻¹)</td>
<td>2.4 (±0.9)</td>
<td>2.5 (±0.92)</td>
<td>2.55 (±1.12)</td>
</tr>
</tbody>
</table>

**Analytical Procedure**

A sensitive and stereo specific High-Performance Liquid Chromatographic method in human plasma was employed for the determination of IBU described in chapter 2.

**Analysis of blank blood samples for hormones during IBUG Study**

The blank blood samples were analysed to know the blood concentration of hormones follicular stimulating hormones, leutinizing hormone, prolactin, by ELISA in VBR diagnostcs, Hanamkonda. Blood concentrations of oestrogen and progesterone were determined by the chemiluminisence method at M/S Vijaya Diagnostics, Hyderabad.

**Pharmacokinetic Data**

The pharmacokinetic parameters of Enantiomers of IBU were computed using a model independent method employing Winnenlinn. 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%.

DISCUSSION

Hormonal changes during the menstrual cycle are reported to influence the functioning of GIT, renal system, cardiovascular functions, metabolizing abilities and immune function variations. Estrogen and progesterone may delay gastric emptying and retard the GI transit. Progestrone has a smooth muscle relaxing effect and hence higher levels of progesterone in luteal phase could enhance the retention time of ingested material in small intestine [8]. Although such gastrointestinal influence of female sex hormones on the pharmacokinetics of drugs is yet to be thoroughly established. In the present study the rate and extent of absorption of ibuprofen, Sand R isomers have decreased during the luteal phase than during the other two phases particularly the follicular phase.

While Rudy et al. [9] were primarily interested in the influence of age on the pharmacokinetics of ibuprofen; their data also indicated that the influence of gender is not statistically significant.

The effects of gender and oral contraceptive steroids on the pharmacokinetics of R (-) ibuprofen were studied in groups of healthy adult males, females and oral contraceptive steroid (OCS) using females. The values of AUC, CLpo, t1/2 and Vss, app did not differ significantly between the groups [10]. Similarly, the percentage unbound of R (-) ibuprofen in pooled plasma from the three groups was not statistically different. Since chiral inversion is the major determinant of R (-) ibuprofen clearance in humans, it may be inferred from these data that gender- and oral contraceptives have little or no effect on conversion of R (-) ibuprofen to the pharmcologically active S-Enantiomer. Moreover, it is unlikely that hormonal factors influence the activity of the human hepatic long-chain fatty-acid: CoA ligase, the enzyme mediating the rate limiting step of R (-) Ibuprofen inversion. The enantioselective measurement of the plasma levels of both enantiomers allows to demonstrate the unidirectional nature of the inversion process [4]. In man, no inversion has been found Flurbiprofen for flunaxoprofen [11,12], ketoprofen [13] and tiaprofenic acid [14].

Xiaotao and Hall (1993) [15] showed that pivalic acid inhibits the formation of R (-) ibuprofenoyl-CoA in isolated rat hepatocytes; pivalic acid (released from the ampicillin prodrg pivampicillin) is known to bind with intracellular CoA by forming long-lived thioesters, In this case no reduction of inversion was observed. Using the same biological model, they found that the inversion of R (-) IBU is inhibited by fatty acids, and they demonstrated that this inhibition is due to a transient depletion of the CoA pool [13,14]. Clofibric acid, a hypolipidemic agent, is known to induce a number of enzymes involved in fatty acid metabolism and causes, in addition, an increase in the total amount of hepatic CoA [15]. The effect of clofibrate on the chiral inversion of R (-) ibuprofen in humans was examined by Davies et al. [1]. In their randomized, crossover study, clofibrate pretreatment not only increased the clearance by inversion but also interacts with the oxidative metabolism of IBU enantiomers. Studies in isolated rat hepatocytes showed that xenobiotics interacting with the oxidative metabolic pathways can indirectly influence the extent of inversion [2]. Pretreatment with phenobarbital, an inducer of cytochrome P450, increased the metabolism by noninversion pathways and consequently reduced the fraction inverted of R (-) IBU. Not all xenobiotics whose metabolism is CoA-dependent interfere with the chiral inversion of R (-) ibuprofen. Thus, the presence of benzoic acid or salicylic acid, which undergo glycine conjugation via activation by CoA, dose not affect the chiral inversion of R (-) IBU in rat hepatocytes [3]. In our study also ibuprofen R-enantiomer C-max values are lesser than the S-enantiomer in three phases. These findings indicate that hormonal factors probably do not affect the activity of the human hepatic long-chain fatty-acid: CoA ligase, the enzyme mediating the rate limiting step of R (-) IBU inversion.

Avergerinos and Hutt [16] investigated the plasma disposition of ibuprofen enantiomers following oral administration of the racemic drug in 24 healthy male volunteers. They observed that the plasma elimination of R (-) IBU was more rapid than that of the S-enantiomer, 64 % of the total area under the plasma conc. time curves (AUC) was due to the pharmacologically active enantiomer. Furthermore, the influence of dose (200-800mg) on the pharmacokinetic characteristics of the enantiomer of ibuprofen was investigated in 3 subjects. The dose-normalized AUe values and oral clearance showed a dose dependence in the disposition of R (-) IBU.

The influence of increasing doses of rac-ibuprofen on the pharmacokinetics of its individual enantiomers was further investigated by Oliary et al. [5]. In this study, rac-ibuprofen (200, 400,800 and 1200 mg p.o.) was given on 4 occasions to 4 healthy male volunteers (age 23-28yr, weight 64-78kg). For all 4 doses, the AUC for both the total and unbound fraction in plasma was significantly greater for the S (+) enantiomer. With increasing rac-ibuprofen dose, there was a less than proportional increase in the total AUC (based on the total plasma concentration) for the enantiomers, which was statistically significant for R (-) IBU. These results were similar to those observed by other [6]. Avergioshutt Iwakawa et al [16] went a step further and administered rac-ibuprofen and then each enantiomer separately in order to evaluate the potential of enantiomer- enantiomer interaction as well as to determine the rate and the extent of systemic inversion in 12 healthy males and concluded that the kinetics of R (-) IBU are not altered by the concurrent administration of S (+) IBU.

A study revealed that age per se is associated with a 2-fold increase in the unbound concentration of S-IBU probably due to an impairment of its metabolism. Indeed,
in the elderly subjects, a modest but significant reduction in the tabolite formation clearance was noted particularly for S-IBU glucuronide and to a lesser extent for S-hydroxy IBU [7].

More recently Paliwal et al. [17] observed stereo selective differences between ibuprofen enantiomers in their binding affinity and to a lesser extent in their competitive inhibitory potential. The intrinsic binding of R (-) IBU was greater than S (+) IBU and the unbound fraction was greater for S (+) enantiomers than for the R (-) enantiomer after a given dose of R (-)IBU or racemate.

CONCLUSION

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- IBU 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 IBU perhaps it is necessary to minimize the inter individual variability of different hormones. A study employing a large number of subjects has to be undertaken. Alternately a population pharmacokinetic study may be under taken.

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REFERENCES