

Research Article

Influence of Menstrual Cycle on Pharmacokinetic Parameters of Carbamazepine in Epileptic Patients

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ABSTRACT

Epilepsy is a Characteristic disorder of recurrent seizures of cerebral origin, affects men and women equally. However, in female epileptic patients, especially during reproductive phase, phase of menstrual cycle has an interaction with the pharmacokinetics of antiepileptic drugs. The aim of our research work is to study the influence of menstrual cycle on the pharmacokinetic parameters of the Carbamazepine in patients with epilepsy. Salivary samples were collected from 20 female epileptic patients on long term oral carbamazepine monotherapy (not less than two years) with prescribed dosage regimen dose at the time points of 0, 1, 2, 3, 4, 6, 8 & 12 hours after dosing and analysed for drug content by HPLC method. Mean C_{max} of Carbamazepine was decreased by 20% in the ovulatory phase compared to that of follicular phase. The AUC_{0-∞} of Carbamazepine i.e, bioavailability was decreased in luteal phase by 20% than follicular phase. Volume of distribution of carbamazepine was significantly lower in follicular and luteal phases compared to ovulatory phase. Carbamazepine half-life was decreased and clearance was increased in luteal phase compared to follicular phase. Except V_{d/f} and V_{ss/f}, menstrual cycle phases did not significantly affect the pharmacokinetic profile of Carbamazepine in terms of C_{max}, T_{max}, AUC_{0-∞}, AUMC_{0-∞}, t_{1/2}, MRT, clearance and absorption rate constant. From the above observations it can be concluded that, menstrual hormonal changes influenced Carbamazepine pharmacokinetics to a lesser extent and V_{d/f} and V_{ss/f} were significantly altered. Mean salivary concentrations of Carbamazepine were higher in follicular phase than in ovulatory and luteal phases. Thus the epileptic seizures (related to menses) in catamenial epileptic patients may not be with low levels of drug but may be due to variation in the levels of estrogen and progesterone.

Keywords: Epilepsy – Menstrual cycle – Salivary samples – Carbamazepine.

INTRODUCTION

Epilepsy is defined as a disorder characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness caused by excessive abnormal discharge of brain neurons. Although it affects equally in men and women, female patients, especially during reproductive age, phase of menstrual cycle¹⁻³, pharmacokinetic changes during pregnancy^{4,7}, interaction of contraceptives with antiepileptic drugs⁸⁻¹⁰ influence convulsive activity. Catamenial epilepsy is a seizure disorder related to menstrual cycle characterized by an increase in seizure frequency at the time of menses. It affects up to 70% of women with epilepsy and catamenial seizures are common among women with focal or generalized epilepsy. Cyclical changes of ovarian hormones, estrogens and progesterones generate catamenial seizures. Generally, progesterone

has antiseizure effects, while estrogens facilitate seizure susceptibility¹¹.

Carbamazepine (CBZ) is the most widely used anticonvulsive drug for the treatment of generalized tonic-clonic and partial seizures. Because it induces hepatic microsomal enzymes, CBZ plasma concentrations are decreased by self-induction of microsomal enzymes after few weeks of therapy, which occasionally needs increased dosage to sustain effective therapeutic levels. It has narrow therapeutic index and complex pharmacokinetic properties, monitoring its concentration for better clinical management of patients is therefore required. The biological factors that influence the pharmacokinetics of CBZ include food, age, sex etc., in turn modify time to reach maximal plasma concentration, which alters its concentration at receptor sites and influences the pharmacodynamic action. Nonetheless, these factors play a significant role on drugs with narrow therapeutic range

either precipitates the adverse effects or masks the therapeutic action. Drug concentration maintenance plays a vital role in drugs with narrow margin of safety to overcome the therapeutic failure on one hand and to decrease the adverse effects on the other hand. Ovarian hormones alter physiological functions and thereby modify absorption, distribution, metabolism and excretion of drugs which in turn modulate pharmacodynamics.

The level of female hormones is phase specific and the pharmacokinetic parameters of drugs are altered by the cyclic changes in menstrual cycle. Variability in pharmacokinetics during different phases of menstrual cycle is demonstrated with Antipyrine¹², Methaqualone¹³, Theophylline¹⁴, Caffeine¹⁵, Zidovudine¹⁶ and Midazolam.

The aim of the present research work is to study the influence of menstrual cycle (i.e., follicular phase, ovulatory phase and luteal phase) on pharmacokinetic parameters of Carbamazepine in catamenial epileptic patients.

Saliva is used for the monitoring of systemic levels of drugs, as it offers distinctive advantages over serum^{17,18}. It is a readily available specimen, which is collected by non-invasive procedures and is helpful when multiple serial samples are needed, provides a cost-effective approach for the screening of large populations. The concentration of most drugs in saliva corresponds to the free or unbound plasma drug concentrations¹⁹⁻²².

A good correlation is observed between serum and salivary levels of Phenytoin, Phenobarbital and Carbamazepine²³. In our study, salivary levels of Carbamazepine in catamenial epileptic patients were estimated during three phases of menstrual cycle for the evaluation of pharmacokinetic parameters.

PATIENTS AND METHODS

20 female epileptic patients with their body weights ranging from 40 to 60kgs, height 140 to 160 cms and age 22 to 45 years were included in the study. The study protocol was approved by the Institutional Ethical Committee. Patients with regular menstrual cycle, not suffering from any other chronic disease except epilepsy (catamenial) and not using any other drug except Carbamazepine were included in the study. Patients with a history of cardiac, pulmonary, hepatic, renal, haematologic or endocrinologic disorders or having irregular menstrual cycles, suffering from amenorrhoea or women using contraceptive pills were excluded from the study.

Patient selection

The female epileptic patients were selected from the patients who visited Neurology department as out patients in the Guntur General Hospital, Guntur, after taking due permission from that department and written informed consent was obtained from all the patients who were willing to participate in the study. 20 female epileptic patients who complied inclusion criteria and on long term oral Carbamazepine monotherapy (not less than 2 years) with prescribed dosage regimen as per physician's prescription (200 mg morning and 200 mg in the night) were selected for the study. Salivary samples were collected from each patient prior to the morning dose (0 h) and at the time points of 1, 2, 3, 4, 6, 8 & 12 hours after dosing. Salivary samples were collected after cleaning the tongue debris and mouth every time before sampling, which were stored at -80°C until further analysis. Saliva samples containing carbamazepine were measured by HPLC method, on reverse phase C-18 column with a total analytical time less than 6.5 minutes²⁴.

Chromatographic conditions

Mobile phase consisting of methanol: water: glacial acetic acid (67: 33: 1 v/v/v) was prepared and mixed thoroughly, degassed and used for the HPLC analysis. 1.0 ml per minute flow rate was maintained throughout the analysis. The eluent was monitored using a UV-VIS detector set at 230 nm and sensitivity was set at 0.001 a.u.f.s.

Preparation of standard graph

Standard solutions

Stock solutions of 100 µg/ml each of Phenytoin and Carbamazepine were prepared in methanol. These solutions were further diluted with methanol to the required concentrations of each drug and stored at -4°C. For the preparation of standard graph 0.1, 0.5, 1, 5, 10, 50 and 100 µg/ml of Carbamazepine in saliva was used.

Patient saliva extraction procedure

To each 100 µl of saliva sample, 20 µl of internal standard (500µg/ml Phenytoin solution) was added and extracted with 1.7 ml of ethyl acetate, vortexed for 1 min and centrifuged at 13,000 rpm for 8 min. The supernatant was evaporated to dryness and the residue was reconstituted with 100 µl of mobile phase, vortexed for 1 min. and 20µl was injected onto HPLC. The standard solutions were also processed by similar extraction procedure. The retention times were 5.1 min. and 6.0 min. for Phenytoin and

Carbamazepine respectively. The peak area ratios obtained at different concentrations of the drug were plotted against the concentrations of the drug. The slope of this plot was calculated by least square regression analysis and was used to calculate Carbamazepine concentration in unknown saliva samples. Data was analysed for pharmacokinetic parameters by using RAMKIN software. Mean C_{max} and T_{max} values were obtained directly from concentrations and time data and various pharmacokinetic parameters for Carbamazepine were obtained in each patient from saliva concentration versus time data.

Analysis of blank blood samples for hormones

The blank blood samples were collected from the patients before administration of the drug (0h) and analysed for concentrations of estrogen and progesterone hormones by the Chemiluminisence method.

Statistical analysis

All the results were expressed as mean \pm S.D, data was analysed using one way ANOVA, followed by Newman-Keuls multiple comparison test. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The mean salivary Carbamazepine levels versus time in three phases of menstrual cycle were shown in Fig.1, the salivary Carbamazepine levels were higher in the follicular phase than in ovulatory and luteal phases and its levels in the ovulatory phase were lower than follicular and luteal phase. Mean concentrations of estrogen and progesterone in three phases of menstrual cycle are summerised in Table 1. The mean levels of estrogen were 28.5 ± 16.5 , 98.5 ± 110.7 and 101.26 ± 85.7 pg/ml in follicular, ovulatory and luteal phases respectively. The mean levels of progesterone were 0.6 ± 0.4 , 4.5 ± 2.8 and 8.8 ± 7.2 ng/ml in follicular, ovulatory and luteal phases respectively. Mean values of various pharmacokinetic parameters of Carbamazepine obtained in three phases (Table 2) were compared with the values obtained in other two phases and for the calculation of percentage increase or decrease, follicular phase was treated as reference (Table 3). The mean Vd/f value was increased by 53.56% in the ovulatory phase and 9.31% in luteal phase compared to follicular phase. The difference between mean Vd/f values for follicular versus ovulatory phase ($P < 0.01$) and luteal versus ovulatory

phase ($P < 0.05$) was statistically significant. The mean $V_{ss/f}$ value was increased by 50.71% in the ovulatory phase and 6.3% in the luteal phase compared to follicular phase. The difference between mean $V_{ss/f}$ values for follicular versus ovulatory phase ($P < 0.01$) and luteal versus ovulatory phase ($P < 0.01$) was statistically significant.

Mean C_{max} of Carbamazepine was decreased by 20% in the ovulatory phase compared to that of follicular phase and was lowest of all the three values. Similar menstrual cycle phase dependent change in C_{max} was observed in case of alcohol with a lowest value at mid-cycle²⁵. The highest blood alcohol concentration was found in premenstrual phase of the cycle in women and its rate of absorption varied during the menstrual cycle and was lowest at mid-cycle²⁶. The mean C_{max} of Paracetamol was significantly lowered in the ovulatory phase than in the follicular phase²⁷ due to increased first pass metabolism during ovulatory phase.

Hormonal level variations and consequently physiological changes influence the pharmacokinetics of drugs by altering the properties such as gastrointestinal motility, thereby drug absorption, plasma protein levels as it alters protein binding, volume of distribution, drug metabolism and elimination. Higher levels of progesterone in luteal phase has smooth muscle relaxing effect, enhances the retention time of ingested material in small intestine, thereby it alters gastrointestinal transit and inturn drug absorption²⁸. Hormone induced alterations in gastrointestinal motility are frequently reported by women during the luteal phase of the menstrual cycle²⁹. Delayed gastrointestinal transit resulting from high progesterone levels potentially inhibit Lithium absorption, while the exaggerated gastrointestinal motility in the final days of the cycle due to decreased levels of progesterone lead to a reversal of the effect³⁰. In concurrence with this, in the present study also progesterone levels were significantly high during luteal phase and the $AUC_{0-\infty}$ of Carbamazepine i.e, bioavailability was decreased in luteal phase by 20% than follicular phase.

Significant changes in endogenous sex hormone concentrations occur during the menstrual cycle and pregnancy, leading to alterations in protein binding, distribution and clearance³¹. Follicular phase has higher circulating levels of α -1 acid glycoprotein (AAG), influence protein binding and changes free drug concentration, AAG concentrations are higher during the first week of the menstrual cycle than at other times³². It is

expected that a decrease in available binding protein reduces the extent of drug protein binding, increases free drug fraction only for highly bound drugs. Alterations in protein binding affect steady-state unbound drug concentration, volume of distribution and drug half-life in a complex non-linear fashion that depends on hepatic extraction ratio and baseline parameters³³. Specifically, highly bound drugs with high extraction ratios (eg. Tricyclic Antidepressants, Verapamil) are expected to have increased free fraction, increased volume of distribution and increased biological half-life (for drugs with large volume of distribution). In the present study, volume of distribution was significantly lower in follicular and luteal phases compared to ovulatory phase could be due to increased protein binding of drug, as Carbamazepine is highly plasma protein bound drug i.e., about 75-85%. The binding of Baclofen to neocortical membranes was varied as a function of the estrous cycle, with the lowest binding during the estrous stage in adult rat³⁴. Although such menstrual cycle related drug distribution changes in humans are yet to be investigated, in this study, the mean apparent volume of distribution and volume of distribution at steady state were increased by 53.76% and 50.71% respectively during the ovulatory phase compared to follicular and luteal phases and these differences were statistically significant.

Mean AUC and C_{max} of Carbamazepine were decreased and clearance was increased in luteal phase compared to follicular phase. In the previous study with Ranitidine similar changes were observed³⁵.

Large fluctuations in hormone concentration throughout the menstrual cycle potentially impact hepatic enzyme activity and affect the metabolism of drugs. Progesterone inhibit and induce hepatic enzyme activity³⁶⁻³⁸. Estrogens inhibits the metabolism of many drugs by inhibiting liver microsomal enzymes and androgens stimulate microsomal enzymes³⁸. Non specific CYP substrates exhibit higher clearance and lower AUC at ovulation with prolonged clearance in the luteal phase³⁹. Sex differences in drug metabolism and elimination are mainly related to steroid hormonal levels. CYP3A4, which is responsible for the metabolism of over 50% of drugs exhibit higher activity in women compared to men^{40,41}. Inter individual variability in Methyl prednisolone disposition among young women also attributed to menstrual cycle variability in CYP3A4 activity⁴². In contrast, the similarity in Alfentanil disposition on day 2, 13 and 21 suggest that hepatic cytochrome P4503A4

activity does not vary significantly with hormonal changes during the menstrual cycle⁴³. Menstrual cycle changes in uterine P4503A4 content, apparently do not influence Alfentanil clearance as it metabolises Alfentanil to a lesser extent when compared with liver enzymes⁴⁴. Similarly, Midazolam clearance was not significantly different during three major phases of the mensrual cycle, strongly suggests that hepatic CYP3A4 activity does not vary significantly with hormonal changes⁴⁵ during the menstrual cycle atleast on day 2, 13 and 21. Though non-significant, Theophylline clearance was increased and half-life was decreased in luteal phase compared to follicular phase⁴⁶. In our study, Carbamazepine half-life was decreased and clearance was increased in luteal phase compared to follicular phase.

Ovulatory phase has higher clearance and smaller AUC and $t_{1/2}$ of Antipyrine due to estrogen-progesterone surges in mid-cycle¹². Similar observations in the plasma metabolism of Methaqualone were reported⁴⁶. Higher clearance and smaller $AUC_{0-\infty}$ of Carbamazepine in ovulatory phase compared to follicular phase was observed might be due to estrogen-progesterone surge.

Plasma Vasopressin, Aldosterone concentrations and renin activity are significantly higher in the luteal phase than in the follicular phase of the menstrual cycle when plasma estrogen levels are highest⁴⁷. Urinary kallikrein excretion is also greater in the luteal phase⁴⁸. Another study found urinary sodium excretion to decline in the periovulatory phase. These changes havc the potential to alter the distribution and excretion of medications. Both volume of distribution and clearance of Carbamazepine were increased in luteal phase compared to follicular phase in our study. Inter and intra-patient variation in the AUC might be due to variation in estrogen levels. A significant negative relationship was found between AUC and estradiol levels suggesting that Zidovudine glucuronidation change in relation to the menstrual cycle phase⁴⁹. Similar results i.e. negative relationship between $AUC_{0-\infty}$ of Carbamazepine and estradiol levels observed in ovulatory and luteal phases in our study.

Except $V_{d/f}$ and $V_{ss/f}$, menstrual cycle phases did not significantly affect the pharmacokinetic profile of Carbamazepine in terms of C_{max} , T_{max} , $AUC_{0-\infty}$, $AUMC_{0-\infty}$, $t_{1/2}$, MRT, clearance and absorption rate constant. This is in consistent with studies using Nitrazepam⁵⁰, Alprazolam⁵¹ and Triazolam⁵².

During the follicular phase of the menstrual cycle, the levels of estrogen and progesterone

are relatively low, whereas during the mid-luteal phase, ovarian steroid hormone levels are high⁵³. Mean C_{max} , $t_{1/2}$ and AUC of Cocaine were decreased in luteal phase compared to follicular phase after intravenous administration. After intranasal Cocaine, women had higher peak plasma levels during the follicular phase than during the luteal phase⁵⁴. The C_{max} , $t_{1/2}$ and $AUC_{0-\infty}$ of Carbamazepine were decreased in luteal phase compared to follicular phase in the present study.

CONCLUSION

From the above observations it can be concluded that, phase specific menstrual

hormonal changes influenced Carbamazepine pharmacokinetics to a lesser extent and $V_{d/f}$ and $V_{ss/f}$ were significantly altered. Mean salivary concentrations of Carbamazepine were higher in follicular phase than in ovulatory and luteal phases. Thus the epileptic seizures (related to menses) in catamenial epileptic patients may not be with low levels of drug but variation in the levels of estrogen and progesterone.

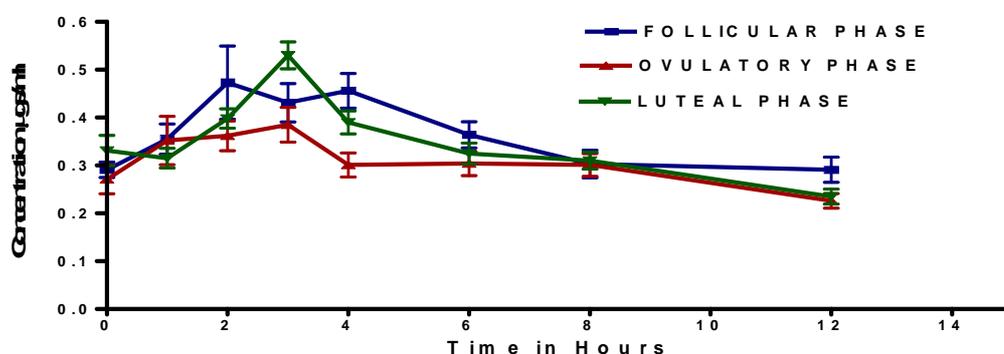


Fig. 1: Mean salivary concentration versus time profile of Carbamazepine during three phases of menstrual cycle in epileptic patients

Table 1: Mean levels of hormones in epileptic patients using Carbamazepine in three phases of menstrual cycle (n=20)

Hormone	Follicular phase	Ovulatory phase	Luteal phase
Estrogen (pg/ml)	28.5 ± 16.5	98.5 ± 110.7	101.26 ± 85.7
Progesterone (ng/ml)	0.6 ± 0.4	4.5 ± 2.8	8.8 ± 7.2

Table 2: Pharmacokinetic parameters of Carbamazepine during three phases (n=20)

Pharmacokinetic parameter	Follicular phase Mean ±SD	Ovulatory phase Mean ±SD	Luteal phase Mean ±SD	Statistical significance	p-value
C_{max} (ug/ml)	0.61 ± 0.28	0.49 ± 0.18	0.56 ± 0.09	NS	0.1817
T_{max} (hrs)	3.25 ± 1.12	3.3 ± 1.89	3.1 ± 0.45	NS	0.8792
AUC_{0-t} (ug/ml/hr)	4.30 ± 1.53	3.65 ± 1.09	4.03 ± 0.83	NS	0.2312
$AUC_{0-\infty}$ (ug/ml/hr)	11.09 ± 5.65	8.12 ± 2.48	8.90 ± 3.44	NS	0.0652
$AUMC_{0-\infty}$ (ug/ml/hxh)	301.27 ± 237.31	193.09 ± 81.03	208.15 ± 149.47	NS	0.0983
$t_{1/2}$ (hrs)	15.21 ± 7.07	14.9 ± 4.98	13.6 ± 5.64	NS	0.6564
$V_{d/f}$ (ml/kg)	17583.87 ± 8562.70	27002.78 ± 12436.76	19222.6 ± 6404.20	F vs O < 0.01** L vs O < 0.05*	0.0058
$V_{ss/f}$ (ml/kg)	19380.16 ± 8749.11	29208.55 ± 13470.34	20606.94 ± 6179.62	F vs O < 0.01** L vs O < 0.01**	0.0051
CLs/f (ml/hr/kg)	965.33 ± 670.09	1151.87 ± 429.09	1091.78 ± 432.89	NS	0.5195
MRT (hr)	23.99 ± 10.00	23.43 ± 7.47	20.9 ± 7.64	NS	0.4869
K_a (h^{-1})	0.132 ± 0.081	0.119 ± 0.045	0.165 ± 0.121	NS	0.2611

Values are expressed as Mean ± SD, *P<0.05 is considered as statistically significant

Table 3: Percentage changes in pharmacokinetic parameters of Carbamazepine in different phases of menstrual cycle

Pharmacokinetic parameter	Follicular phase	Ovulatory phase	Luteal phase	Statistical significance	p-value
C _{max} (ug/ml)	0.61 Reference	0.49 19.67% ↓	0.56 8.2% ↓	NS	0.1817
T _{max} (hrs)	3.25 Reference	3.3 1.54 % ↑	3.1 4.6 % ↓	NS	0.8792
AUC _{0-t} (ug/ml/hr)	4.30 Reference	3.65 15.11% ↓	4.03 6.27 % ↓	NS	0.2312
AUC _{0-∞} (ug/ml/hr)	11.09 Reference	8.12 26.78 % ↓	8.90 19.74 % ↓	NS	0.0652
AUMC _{0-∞} (ug/ml/hxh)	301.27 Reference	193.09 35.9% ↓	208.15 30.9% ↓	NS	0.0983
t _{1/2} (hrs)	15.21 Reference	14.9 2.03% ↓	13.6 10.6 % ↓	NS	0.6564
Vd/f (ml/kg)	17583.87 Reference	27002.78 53.56 % ↑	19222.6 9.31 % ↑	Fvs O < 0.01** L vs O < 0.05*	0.0058
Vss/f (ml/kg)	19380.16 Reference	29208.55 50.71% ↑	20606.94 6.3 % ↑	F vs O < 0.01** L vs O < 0.01**	0.0051
CLs/f(ml/hr/kg)	965.33 Reference	1151.87 19.32 % ↑	1091.78 13.09 % ↑	NS	0.5195
MRT(hr)	23.99 Reference	23.43 2.3% ↓	20.9 12.88% ↓	NS	0.4869
Ka (h ⁻¹)	0.132 Reference	0.119 9.84% ↓	0.165 25 % ↑	NS	0.2611

F = Follicular phase; O = Ovulatory phase; L= Luteal phase.

Values are expressed as Mean ± SD; *P<0.05 is considered as statistically significant

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