TIME DEPENDENT INFLUENCE OF DIAZEPAM ON THE PHARMACOKINETICS OF IBUPROFEN IN MAN

A. Thukaram Bapuji, D. Rambhau, P. Srinivasu, B. Ramesh Rao* and S.S. Apte

University College of Pharmaceutical Sciences, Kakatiya University, Warangal-506 009 (A.P.), India

SUMMARY

Circadian variation in the disease activity of rheumatoid arthritis has been established. Several nonsteroidal anti-inflammatory drugs have been studied for their chronokinetic behaviour. Time dependent influence of diazepam on the pharmacokinetics of diclofenac and naproxen has been reported. We report the time dependent influence of diazepam on the pharmacokinetics of ibuprofen in healthy subjects. Either ibuprofen or ibuprofen with diazepam was administered at 10.00 or 22.00 hours to eight healthy volunteers in a randomized crossover study. Serum ibuprofen levels were estimated by high performance liquid chromatography. There was a significant ($p<0.05$) increase in mean elimination half life (2.39 ± 0.42 to 3.59 ± 0.35 h) following ibuprofen and diazepam administration compared to ibuprofen alone administered at 22.00 hours. The mean clearance of ibuprofen was therefore lowered from 62.7 ± 8.9 to 41.7 ± 2.6 ml/h/kg under the influence of diazepam during the night. Such a time dependent influence of diazepam on the pharmacokinetics of ibuprofen may be due to circadian variation in the pattern of protein production in the liver and/or competitive protein binding of the two drugs during the dark period.

* Author for correspondence
e-mail: kakatiya@ap.nic.in

INTRODUCTION

Circadian rhythmicity in the disease activity of rheumatoid arthritis with an acrophase between 02.00 and 04.00 hours has been established /1,2/. Several nonsteroidal anti-inflammatory agents including aspirin /3/, indomethacin /4/, flurbiprofen /5/, ketoprofen /6/, naproxen /7/, diclofenac /8/ and ketorolac /9/, have been screened for their chrono-pharmacokinetic behaviour, with an eye towards designing the optimum therapeutic schedule. Ibuprofen is a member of the above group of drugs which has several advantages: better tolerance than aspirin and equipotency to aspirin /10/. The drug is commonly prescribed in treating inflammatory diseases.

Benzodiazepines are said to be capable of masking biological rhythms /11,12/, and are therefore under consideration for altering or resetting the circadian rhythm in individuals. Prescription of these drugs in combination with analgesics and anti-inflammatory agents is advocated in the treatment of rheumatoid arthritis /13/. Before prescribing such combinations, however, the possible pharmacokinetic and pharmacodynamic interactions need to be investigated. We have earlier reported the time dependent influence of diazepam on the pharmacokinetics of diclofenac /14/ and naproxen /15/ (and unpublished data). In this study we investigated the pharmacokinetics of ibuprofen after administration either alone or in combination with diazepam at 10.00 and 22.00 hours.

MATERIALS AND METHODS

Eight healthy male volunteers, weight 51-74 kg, height 158-180 cm and age 22-24 years, participated in the study. They were in good health documented by a complete medical examination by a physician, medical history and standard laboratory tests. Neither alcoholic beverages nor any medication were allowed for 2 weeks before the study and throughout the duration of the study.

The subjects were divided into four groups at random and were given the following treatments as per a randomized 4 x 4 Latin square design with a wash-out period of ten days between each treatment:

Treatment I: 400 mg of ibuprofen at 10.00 hours.
Treatment II: 400 mg of ibuprofen at 22.00 hours.

Treatment III: 400 mg of ibuprofen + 10 mg of diazepam at 10.00 hours

Treatment IV: 400 mg of ibuprofen + 10 mg of diazepam at 22.00 hours.

The study was conducted during the months of April and May. All the participants were briefed about the study and they had given written informed consent. The study was approved by the Institutional Ethical Committee.

The drug(s) was administered (as immediate release capsule(s)) with a glass of water after about 10 hours fasting. No food or drink was allowed to the subjects for three hours after drug administration. Regular meals were allowed before and after the stipulated time. Volunteers were confined to the laboratory during the study days.

Blood samples (2 ml) were collected from the median cubital vein at intervals of 0, 0.33, 0.66, 1.0, 1.5, 2, 3, 4, 5, 7, 10, 12 and 24 hours following drug(s) administration. The samples were allowed to clot, serum was separated, frozen and stored at -20°C until assayed.

Assay

Ibuprofen in the serum samples was estimated by a modified high performance liquid chromatography method of Shimeki *et al.* /16/. To 0.5 ml of serum in a centrifuge tube, 0.5 ml of 1M sodium acetate buffer and 0.5 ml of internal standard solution (1 μg/ml naproxen in ethyl acetate) were added. To this 2 ml of ethyl acetate was added, mixed for one minute on a cyclomixer and centrifuged at 2,500 rpm for 5 minutes. The upper organic phase was transferred into a clean test tube and dried under vacuum. The residue was reconstituted with 100 μl of acetonitrile of which 25 μl was injected onto the column.

A Shimadzu high performance liquid chromatography unit equipped with SCL-6A module system controller, solvent delivery module LC-6A, column oven CTO-6A module, ultraviolet visible spectrophotometric detector SPD-6A module and C-R4A chromatopac data processor was used. An octadecyl silane reversed phase stainless steel column (150 x 4 mm) packed with porous silica spheres of 5 μm, surface modified with octadecyl groups was employed for chromatographic separation.

Chromatographic conditions

Mobile phase: acetonitrile: 0.1 M glacial acetic acid (60:40); flow rate: one ml per minute; UV detection at 254 nm, and detector sensitivity 0.01 aufs (absorbance units on full scale). The retention times were 5.8 and 10.4 minutes for internal standard (naproxen) and ibuprofen, respectively.

A standard graph was prepared by adding 0.1 ml of 20, 40, 60, 80, 100, 130 and 160 µg/ml solution of ibuprofen in acetonitrile to 0.5 ml of serum samples obtained from untreated volunteers. These samples were treated as described in the assay procedure. The peak height ratios obtained at different concentrations of the drug were plotted against the concentration of the drug. The slope of this plot, determined by least squares regression analysis, was used to calculate ibuprofen concentration in the unknown serum samples. The reproducibility of the assay was tested by analysing five samples each of spiked concentrations 1 µg/ml and 20 µg/ml. Recovery was found to be greater than 90% in all with a coefficient of variation less than 7%. The limit of detection for the drug was 0.05 µg and the lower limit of quantification in serum was 0.5 µg/ml.

Treatment of bioavailability data

Pharmacokinetic parameters, absorption rate constant (Ka), mean residence time (MRT), elimination half life ($t_{½}$), area under the serum concentration versus time curve (AUC), overall elimination rate constant (Ke), area under the first moment curve (AUMC), apparent volume of distribution for fraction of the dose absorbed (Vd/f) and systemic clearance (Cls/f) for ibuprofen, were calculated using a non-compartmental method.

The mean pharmacokinetic parameters of ibuprofen obtained after the four different treatments were compared using analysis of variance (ANOVA) and paired t-test to determine the significance of any time dependent interaction effects. A difference was considered significant when $p < 0.05$.

RESULTS

All the treatments were well tolerated by the subjects and no untoward incidents were reported. The mean serum ibuprofen

concentrations following the different drug treatments are shown in Fig. 1 and the means of various pharmacokinetic parameters under the treatments given are listed in Table 1.

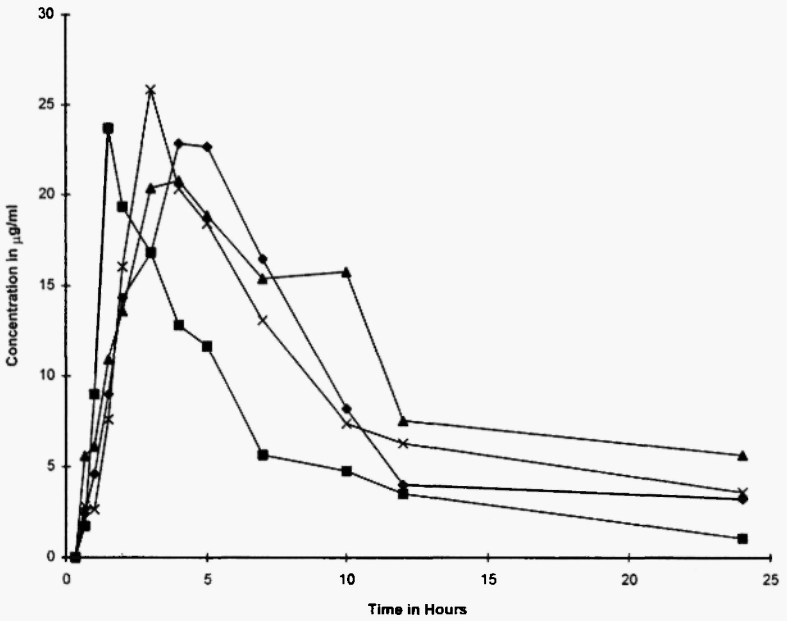

Fig. 1: The mean serum levels of ibuprofen after its oral administration either alone or in combination with diazepam at 10.00 hours or 22.00 hours. –◆– ibuprofen at 10.00 hours; –■–ibuprofen at 22.00 hours; –▲– ibuprofen + diazepam at 10.00 hours; –×– ibuprofen + diazepam at 22.00 hours.

Pharmacokinetics of ibuprofen after administration at 10.00 and 22.00 hours

The mean Cmax values of ibuprofen following administration at 10.00 and 22.00 hours were 27.22±3.16 and 38.84±5.93 µg/ml, respectively. Although lowered mean serum levels of ibuprofen were observed following administration at 10.00 hours compared to 22.00 hours the difference was not statistically significant ($p>0.05$). The mean Tmax values were 2.81±0.32 and 2.16±0.05 hours following administration at 10.00 and 22.00 hours, respectively. Thus, no significant difference ($p>0.05$) arising from the dosing time was observed in any of the pharmacokinetic parameters of ibuprofen.

TABLE 1

Mean (SEM) pharmacokinetic parameters from serum levels of ibuprofen following its administration at 10.00 and 22.00 hours either alone or with diazepam

	Ibuprofen		**Ibuprofen + Diazepam**	
	10.00 h	22.00 h	10.00 h	22.00 h
Cmax (mg/l)	27.22 (3.16)	38.84 (5.93)	29.60 (3.59)	30.69 (0.32)
Tmax (h)	2.81 (0.32)	2.16 (0.55)	4.12 (0.76)	2.30 (0.32)
Ka (h^{-1})	2.06 (0.25)	2.66 (0.49)	2.13 (0.24)	2.02 (0.21)
$t_{1/2}$ (h)	2.75 (0.45)	2.39 (0.42)*	4.96 (1.09)	3.59 (0.35)*
AUCo-t (mg/l/h)	123.4 (14.2)	110.8 (16.4)	137.3 (9.9)	131.9 (19.3)
AUCo-∞ (mg/l/h)	149.4 (20.9)	106.9 (14.2)	196.8 (31.7)	156.7 (20.1)
AUMC (mg/l/h)	748.7 (136)	500.1 (72)	929.6 (233)	1068.6 (187)
MRT (h)	5.01 (0.24)	5.05 (0.56)	7.60 (1.11)	5.62 (0.21)
Cls/f (ml/h/kg)	43.02 (4.8)	62.73 (8.94)*	38.50 (4.64)	41.75 (2.57)*
Vss/f (ml/kg)	215.0 (15.3)	233.0 (22.1)	246.0 (26.1)	286.0 (39.9)
Vd/f (ml/kg)	183.0 (17.8)	164.0 (15.7)	240.0 (3.1)	244.0 (45.1)

* $p < 0.05$

Pharmacokinetic interaction between ibuprofen and diazepam following administration at 10.00 hours

Concomitant treatment with diazepam at 10.00 hours prolonged the occurrence of peak serum levels (2.81±0.32 to 4.12±0.76 hours), increased the elimination half life (2.76±0.45 to 4.96±1.09 hours) and

MRT (5.01±0.24 to 7.6±1.11 hours) of ibuprofen. However, none of these changes attained statistical significance ($p>0.05$).

Pharmacokinetic interaction between ibuprofen and diazepam following administration at 22.00 hours

The mean Cmax value of ibuprofen decreased from 38.84±5.93 to 30.69±0.32 μg/ml following its administration in combination with diazepam at 22.00 hours. However, such a change was not statistically significant ($p>0.05$). The mean $t_{½}$ value increased from 2.39±0.42 hours to 3.59±0.35 hours following ibuprofen administration with diazepam at 22.00 hours, which is statistically significant ($p<0.05$). There was also a significant difference ($p<0.05$) in the mean clearance values (62.74±8.94 vs 41.75±2.57 ml/h/kg) following ibuprofen alone and ibuprofen with diazepam treatment at 22.00 hours. Thus, the influence of diazepam on the pharmacokinetics of ibuprofen is time dependent.

DISCUSSION

A time dependent influence of diazepam on the pharmacokinetics of naproxen /15/ (and unpublished data) and diclofenac /14/ has been reported.

According to Rao and Rambhau /15/ the pharmacokinetics of naproxen was altered when it was administered with diazepam at 10.00 hours but not after treatment at 22.00 hours (unpublished data). Diazepam lowered the mean peak serum naproxen concentrations (83.4 to 64.5 μg/ml), prolonged the time to peak concentration (1.36 to 2.0 hours) and decreased the absorption rate (4.07 to 2.42 h^{-1}) following the combination treatment at 10.00 hours; no such changes were noted after the same treatment at 22.00 hours. In contrast to this, Nagamahender *et al.* /14/ observed a more pronounced effect of diazepam on the pharmacokinetics of diclofenac during the night than during the day. Under the influence of diazepam the Cmax of diclofenac was raised by 112% after treatment at 22.00 hours and 68% after treatment at 10.00 hours. Furthermore, a 60% increase in AUC, 36% decrease in clearance and 24% decrease in Vss/f of diclofenac was noted due to the concomitant treatment with diazepam at 22.00 hours only. The authors attributed such a time dependent influence of

diazepam on the pharmacokinetics of diclofenac to the chrono-effectiveness of diazepam.

Most plasma proteins such as albumin and alpha-1-acid glycoproteins, which are highly involved in drug transport, are reported to exhibit circadian rhythmicity in humans /17/. This may be because of the 24-hour pattern of protein production by the liver /18/. The difference between the diurnal crest and the nocturnal trough in protein concentration amounts to only 10% in young adults, but it may be as great as 20% in the elderly /18/. This age-related increase of the circadian amplitude of this rhythm results from a large nocturnal dip in plasma proteins between 00.00 to 04.00 hours. The elderly are more likely to be prescribed drugs than younger people, and more likely to be taking a mixture of drugs. It can be anticipated that the free fractions of certain drugs at night might be larger in the elderly than in young adults.

Bruguerolle *et al.* /19,20/ have shown that the free fraction of several medications is dependent on the time of dosing in rats. The greatest protein binding of lidocaine /20/ occurs during the active span. The unbound fraction of these drugs varied from 10 to 28% as a function of different dosing times over a 24-hour period due to the circadian rhythm of plasma protein binding.

Angeli *et al.* /21/ have reported that the binding of plasma transcortin exhibits circadian rhythmicity in humans. The lowest binding capacity occurs during the night, around 04.00 hours, when cortisol secretion is physiologically nil or quite low, and the highest binding capacity occurs around 16.00 hours. This means that higher free and thus effective plasma corticosteroid levels are achieved during the morning, the time of peak cortisol secretion.

Circadian changes in plasma protein binding have been reported in humans for drugs including diazepam /22/ and valproic acid /23/. For these drugs the free fractions reach their respective crests in the early morning.

We observed a significant decrease in Cls/f and increase in $t_{½}$ of ibuprofen when it was administered with diazepam at 22.00 hours. This could be because of a decrease in plasma protein concentration. According to earlier reports /17/, although there is only a 10% decrease in plasma protein concentration during 00.00 to 04.00 hours, it is not known to what extent this could alter albumin specific binding sites. Ibuprofen is reported to undergo capacity limited protein binding.

Secondly, due to administration at night, the concentration of diazepam in plasma builds up slowly and this may result in a competitive protein binding interaction between ibuprofen and diazepam. In the event of such an interaction the free fraction of ibuprofen should be increased. Since ibuprofen is not excreted as such to any significant extent, the free drug so released from binding sites may distribute into the extravascular compartment, thereby resulting in persistence of the drug in the body, and hence decreased clearance and increased plasma half life and volume of distribution (although not found to be significant in this study).

If such a mechanism occurs, the presence of diazepam will facilitate the extravascular distribution of ibuprofen, which appears to be a prerequisite for its therapeutic effect. Thus, the time dependent pharmacokinetic interaction between ibuprofen and diazepam noted in this study may be of clinical relevance in the context of increased disease activity of rheumatoid disorders during the night and general concomitant administration of diazepam-like drugs at bedtime.

ACKNOWLEDGEMENTS

We thank all the volunteers for their enthusiastic participation in the study. We thank M/S Dr. Reddy Laboratories, Hyderabad, India, for their gift sample of ibuprofen. Mr. A. Thukaram Bapuji is thankful to UGC, New Delhi for financial assistance in the form of a fellowship.

REFERENCES

1. Swannell AJ. Biological rhythms and their effect in the assessment of disease activity in rheumatoid arthritis. Br J Clin Practice 1983; 38 (Suppl 33): 16-19.
2. Kowanko IC, Knapp MS, Pownall R, Swannell AJ. Domiciliary self measurement in rheumatoid arthritis and demonstration of circadian rhythmicity. Ann Rheum Dis 1982; 41: 445-452.
3. Markiewicz A, Semenowicz K. Time dependent changes in the pharmacokinetics of aspirin. Int J Clin Pharmacol Biopharm 1979; 17: 409-411.
4. Clench J, Reinberg A, Dziewanowska Z, Ghata J, Smolensky MH. Circadian changes in the bioavailability and effects of indomethacin in healthy subjects. Eur J Clin Pharmacol 1981; 20: 359-369.
5. Kowanko IC, Pownall R, Knapp MS, Swannell AJ, Mahoney PGC. Circadian variations in the signs and symptoms of rheumatoid arthritis and

in the therapeutic effectiveness of flurbiprofen at different times of the day. Br J Clin Pharmacol 1981; 11: 477-484.
6. Queneau P, Ollagnier M, Decousus H, Cherrah A, Perpoint B. Ketoprofen chronokinetics in human volunteers. Ann Rev Chronopharmacol 1984; 1: 353-356.
7. Rao BR, Rambhau D, Rao VVS. Pharmacokinetics of single dose administration of naproxen at 1000 and 2200 hours. Chronobiol Int 1993; 10: 137-142.
8. Mustafa M, Suryawati S, Dwiprasto I et al. The relative bioavailability of diclofenac with respect to time of administration. Br J Clin Pharmacol 1991; 32: 246-247.
9. Srinivasu P, Rambhau D, Rao BR. Chronopharmacokinetics of ketorolac in healthy volunteers. Clin Drug Invest 1995; 10: 110-116.
10. Busson M. Update on ıbuprofen: Review article. J Int Med Res 1986; 14: 53-62.
11. Seidel WF, Roth T, Roehrs T, Zorick F, Dement WC. Treatment of a 12 hour shift of sleep schedule with benzodiazepines. Science 1984; 224: 1262-1274.
12. Reinberg A, Smolensky M, Labrecque G. The hunting of a wonder pill for resetting all biological clocks. Ann Rev Chronopharmacol 1988; 4: 171-200.
13. Read AE, Baritt DW, Hewer LR, eds. Modern Medicine, 3rd Ed. English Language Book Society. Avon: Churchill Livingstone, 1986.
14. Nagamahender V, Rambhhau D, Rao BR, Rao VVS, Venkateshwarlu G. Time dependent influence of diazepam on the pharmacokinetics of orally administered diclofenac sodium in human subjects. Clin Drug Invest 1995; 10:296-301.
15. Rao BR, Rambhaau D. Influence of diazepam on the pharmacokinetic properties of orally administered naproxen. Drug Invest 1992; 4: 416-421.
16. Shimeki JL, Rao NGS, Khalil SKW. High performance liquid chromatographic determination of ıbuprofen in plasma. J Pharm Sci 1980; 70: 514-516.
17. Bruguerolle B, Levi F, Arnand C, et al. Alteration of physiologic circadian time structure of six plasma proteins in patients with advanced cancer. Ann Rev Chronopharmacol 1986; 3: 207-210.
18. Touitou Y, Touitou C, Bogdan A, et al. Differences between young and elderly subjects in seasonal and circadian variations of total plasma proteins and blood volume as reflected by hemoglobin, hematocrit and erythrocyte counts. Clin Chem 1986; 32: 801-804.
19. Bruguerolle B, Bouvenet G, Bartolin R. Temporal and sex related variations of lorazepam kinetics. Ann Rev Chronopharmacol 1984; 2: 21-25.
20. Bruguerolle B, Jadof G. Influence of hour of administration of lidocaine on its intraerythrocytic passage in the rat. Chronobiologia 1983; 10: 295-297.
21. Angeli A, Frajria R, De paoli R, Fonzo D, Ceresa F. Diurnal variation of prednisolone binding to serum corticosteroid binding globulin in man. Clin Pharmacol Ther 1978; 23: 47-53.
22. Naranjo CA, Sellers EM, Giles HG, Abel JG. Diurnal variation in plasma diazepam concentrations associated with reciprocal changes in free fractions. Br J Clin Pharmacol 1980; 9: 265-272.

23. Patel IH, Venkatraman R, Levi RH, Vishwanathan C, Ojemann LM. Diurnal oscillations in plasma protein binding of valproic acid. Epilepsia 1982; 23: 282-290.