TIME-DEPENDENT PHARMACOKINETIC INTERACTION BETWEEN ZIDOVUDINE AND RIFAMPICIN FOLLOWING ORAL ADMINISTRATION AT 10.00 AND 22.00 HOURS

Rajesh Karwa, Apte Shashank, Devraj Rambhau*, Devaraj Gopinath and Devraj Ravi[1]

University College of Pharmaceutical Sciences, Kakatiya University, Warangal-506 009 (A.P)
[1]Department of Pharmaceutical Sciences, Nagpur University, Nagpur, India

SUMMARY

Rifampicin, an antitubercular agent, is a known metabolic inducer. Previous studies have suggested that rifampicin may interfere with the pharmacokinetics of oral zidovudine when the two drugs are co-administered. Circadian variations in the pharmacokinetics of rifampicin have been reported. We report here a circadian influence on the pharmacokinetics of zidovudine in the presence of rifampicin when administered orally in rabbits. Either zidovudine or zidovudine with rifampicin was administered orally at 10.00 or 22.00 h to 12 healthy rabbits in a randomized cross-over study. Serum zidovudine was estimated by HPLC. A significant ($p < 0.05$) lowering of C_{max}, $t_{½}$, $AUC_{0\text{-}6h}$ and MRT was observed following zidovudine and rifampicin co-administration compared to zidovudine alone at 10.00 h. Accordingly clearance increased to a significant extent. However, such an interaction effect was masked following administration at 22.00 h. The time-dependent influence of rifampicin on the pharmacokinetics of zidovudine may be due to time-dependent changes in absorption and elimination of rifampicin, thus modifying its induction effect on the levels of UDP glucuronyl transferase and cytochrome P-450 content in liver which are responsible for metabolism of zidovudine.

* Author for correspondence
e-mail: gondevaraj@usa.net

KEY WORDS

rifampicin; zidovudine; pharmacokinetic interaction; time dependent changes

INTRODUCTION

Rifampicin, an antitubercular agent, is used to treat tuberculosis in conjunction with other antitubercular agents. Rifampicin is a known metabolic inducer. Previous studies have suggested that rifampicin may interfere with the pharmacokinetics of oral zidovudine (3′-azido-3′-deoxythymidine; AZT) when the two agents are administered simultaneously /1/. Since zidovudine is the drug most widely used in the treatment of patients with AIDS, the use of rifampicin in these patients may interfere with the beneficial effects of zidovudine.

Circadian variations in the pharmacokinetics of rifampicin have been reported /2/. Interestingly, Sothern *et al.* /3/ reported a significant within-day variation in levels of zidovudine in plasma and urinary excretion following steady-state dosing of patients with HIV infection. A potential circadian variation was suggested for zidovudine from this study with a limited population size.

The present study was designed to investigate the pharmacokinetic interaction between zidovudine and rifampicin administered orally in rabbits. The circadian influence on the pharmacokinetics of zidovudine in presence of rifampicin was also studied in the same animals.

MATERIALS AND METHODS

Twelve healthy male and female New Zealand breed rabbits weighing between 2.0 and 2.6 kg (average weight 2.3 kg) were used. The animals were purchased from National Institute of Nutrition (N.I.N.), Hyderabad, India, and were housed at 25 ± 1°C in separate cages. The animals were given a diet advised by N.I.N. and had free access to water.

The animals were divided into four groups at random and the study was conducted in a cross-over design allowing a washout period of seven days between each treatment. The study was conducted during the months of August and September. The following treatments were applied wherein zidovudine was dissolved in distilled water and a

single dose of 25 mg/kg body weight was administered orally with the help of a Ryle's tube, either alone or in combination with rifampicin suspension (25 mg/kg body weight). The drugs were administered orally after about 10 to 12 hours of fasting as follows:

Treatment I: Zidovudine (25 mg/kg) at 10.00 h

Treatment II: Zidovudine (25 mg/kg) + rifampicin suspension (25 mg/kg) at 10.00 h

Treatment III: Zidovudine (25 mg/kg) at 22.00 h

Treatment IV: Zidovudine (25 mg/kg) + rifampicin suspension (25 mg/kg) at 22.00 h

Blood samples (1 ml) were withdrawn from the left marginal ear vein at intervals of 0, 15, 30, 45, 60, 90, 120, 180, 240 and 360 min following drug administration. The samples were allowed to clot and then centrifuged to separate serum. The separated serum was frozen until analysis.

Analysis

Zidovudine in serum samples was estimated by a high performance liquid chromatography (HPLC) method established in our laboratory. To 200 µl of rabbit serum in a centrifuge tube was added 100 µl of internal standard solution (20 µg/ml paracetamol in methanol). To this 0.9 ml of methanol was added and the contents were vortexed for 5 min and centrifuged. The methanol layer was transferred to a test tube and dried under vacuum. The residue was dissolved in 1 ml distilled water by vortexing and extracted with 6 ml of dichloromethane by mixing on a rotary shaker for 20 min. Following this the tubes were centrifuged at 2,500 rpm for 5 min to effect phase separation of the contents. The organic layer was separated and dried under vacuum. The resulting residue was reconstituted in 60 µl methanol, vortexed, and 20 µl were injected onto the HPLC column.

A Shimadzu high performance liquid chromatography unit consisting of system controller SCL 6A, solvent delivery module (LC-6A), column oven (CTO-6A), variable wavelength UV detector (SPD-6AV), chromatopac data processor (CR 4A) and an injector (Rheodyne) fitted with 20 µl capacity loop was used. An octadecyl silane reversed phase stainless steel analytical column (250 x 4.6 mm)

packed with porous silica spheres of 5 μm, surface modified with octadecyl groups, was employed for chromatographic separation.

Chromatographic conditions

Mobile phase: methanol:water (20:80) to which ammonium acetate was added to give a final concentration of 10 mM in the total mobile phase volume. pH was adjusted to 4.0 with acetic acid. Flow rate: 1 ml/min. UV detection was at 267 nm and separation was at ambient temperature. The retention times were 3.4 and 6.2 min for internal standard (paracetamol) and zidovudine, respectively.

A standard graph was prepared by adding 0.1 ml of 0.5, 1, 1.5, 2, 3, 4, 8, 12, 16 and 32 μg/ml solution of zidovudine in methanol to 0.2 ml serum samples. To this, 0.1 ml of paracetamol in methanol (containing 2 μg of the substance) was added as internal standard. These samples were treated as described in Analysis. 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 square regression analysis was used to calculate zidovudine concentrations in the unknown serum samples. The reproducibility of the assay was tested by analyzing five samples each of the spiked concentrations from 0.1 to 1.6 μg/ml. All mean recoveries were greater than 90% with coefficient of variation less than 7.9%. When rifampicin was injected with zidovudine, there was no effect on the retention time and peak height ratio of zidovudine, indicating that rifampicin did not interfere with the analysis.

Treatment of the bioavailability data

In the present investigation a model independent or non-compartmental pharmacokinetic approach was employed to determine pharmacokinetic parameters. Pharmacokinetic parameters - absorption rate constant (Ka), biological half-life ($t_{½}$), area under the concentration versus time curve (AUC), area under the first moment curve (AUMC), apparent volume of distribution for fraction of dose absorbed (Vd/f), and systemic clearance for fractions of the dose absorbed (Cls/f) for zidovudine - were obtained in each animal from serum concentration versus time data using a personal computer. Peak serum concentration (C_{max}) and the time to reach peak concentration (T_{max}) were obtained from the observed concentration versus time data in each animal.

Statistical analysis

The resulting means of the various pharmacokinetic parameters obtained in different animals following four treatments were compared using analysis of variance (ANOVA) to reveal any time-dependent changes. Paired pharmacokinetic parameters were compared by Student's t-test. A difference was considered significant when $p < 0.05$.

RESULTS

The HPLC chromatogram of zidovudine extracted from serum is shown in Figure 1. The mean serum concentrations of zidovudine following its administration either alone or in combination with rifampicin suspension at 10.00 h and 22.00 h are shown in Figures 2 and 3, respectively. The means of the various pharmacokinetic parameters under treatment are given in Table 1.

Chronopharmacokinetics of zidovudine

The mean C_{max} value of zidovudine was higher when administered at 10.00 h treatment than at 22.00 h but the difference was not significant ($p > 0.05$). Comparison of the means of various pharmacokinetic parameters following drug administration at 10.00 and 22.00 h by ANOVA revealed no time-dependent changes in T_{max}, Ka, AUC_{0-6h}, $AUC_{0-\infty}$, AUMC, $t_{½}$, Cls/f, Vd/f or mean retention time (MRT).

Pharmacokinetic interaction between zidovudine and rifampicin following administration at 10.00 hours

Combined treatment with zidovudine and rifampicin at 10.00 h resulted in lowering of C_{max} by 13% (from 10.18 to 8.90 μg/ml, $t = 2.11$, $p < 0.05$), $t_{½}$ by 9% (from 72.16 to 65.82 min; $t = 2.74$, $p < 0.05$), AUC_{0-6h} by 21% (from 1088.32 to 858.8 μg/ml/min, $t = 2.63$, $p < 0.05$) and MRT by 8% (from 115.65 to 106.02 min, $t = 4.14$, $p < 0.05$) for zidovudine. Accordingly clearance increased to a significant extent by 25% (from 14.77 to 18.40 ml/min/kg, $t = 2.51$, $p < 0.05$). However, the means of the parameters T_{max}, Ka and Vd/f from serum levels versus time data of individual animals, by ANOVA and t-test, revealed no significant interaction effect ($p > 0.05$).

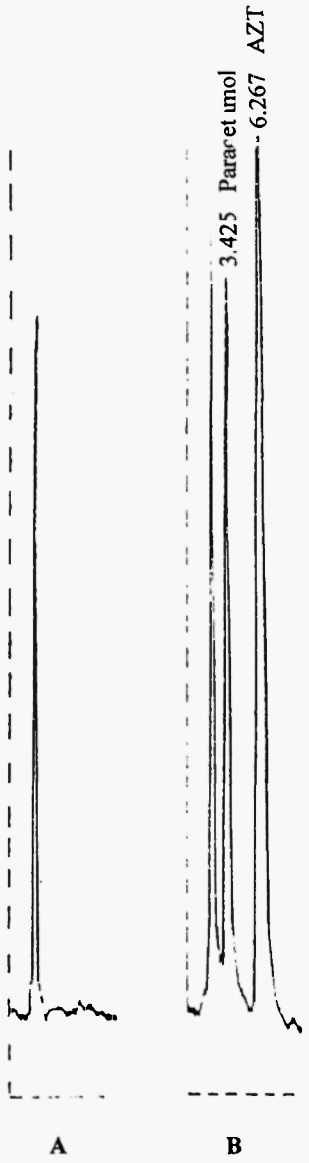

Fig. 1: HPLC chromatogram of (**A**) normal rabbit serum and (**B**) rabbit serum showing peaks of zidovudine (AZT) and internal standard (paracetamol).

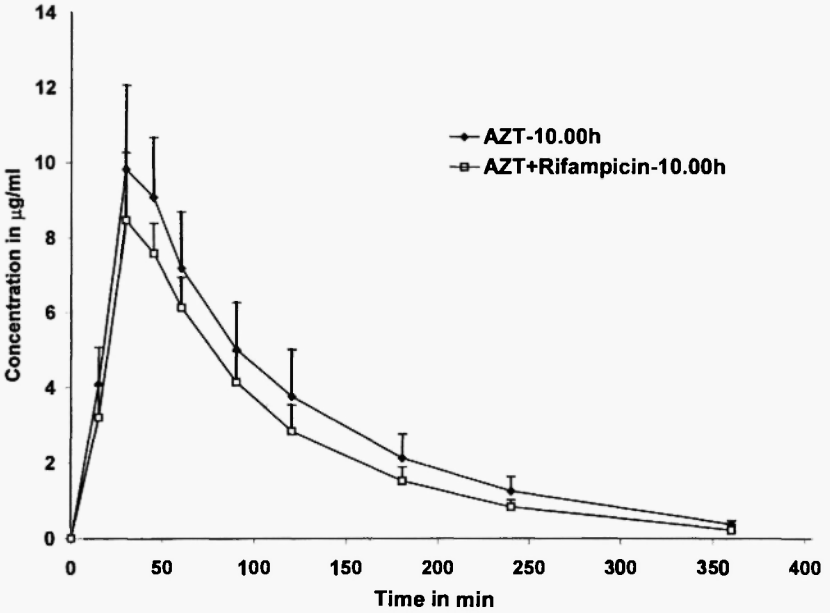

Fig. 2: Serum levels of zidovudine (AZT) after its oral administration either alone or in combination with rifampicin at 10.00 h (means ± SD, n = 12).

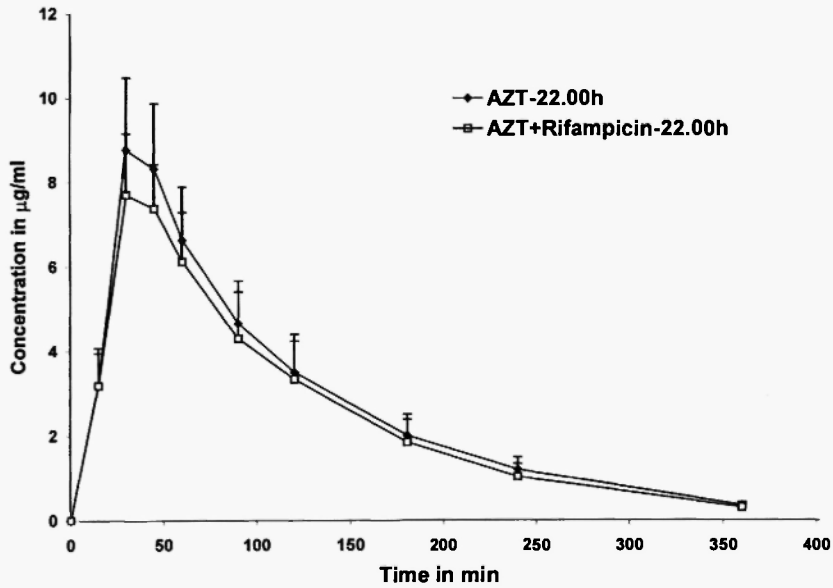

Fig. 3: Serum levels of zidovudine (AZT) after its oral administration either alone or in combination with rifampicin at 22.00 h (means ± SD, n = 12).

TABLE 1

Pharmacokinetic parameters [mean (SEM)] obtained from serum levels of zidovudine following its oral administration at 10.00 and 22.00 hours either alone or with rifampicin

	Zidovudine		Zidovudine + Rifampicin	
	10.00 h	**22.00 h**	**10.00 h**	**22.00 h**
C_{max} (mg/ml)	10.18 (1.73)*	9.60 (1.31)	8.90 (1.19)*	8.11 (1.09)
T_{max} (min)	32.5 (5.83)	35.0 (7.38)	33.75 (6.78)	35.0 (7.38)
Ka (min^{-1})	1.46 (0.14)	1.40 (0.81)	1.43 (0.17)	1.40 (0.18)
$t_{½}$ (min)	72.16 (4.71)*	71.81 (7.71)	65.82 (6.47)*	67.65 (5.69)
AUC_{0-t} (µg/ml/min)	1088.32 (262.31)*	998.70 (191.60)	858.80 (148.05)*	918.74 (197.10)
AUC_{∞} (µg/ml/min)	1126.92 (270.7)	1034.71 (195.44)	880.20 (151.00)	936.86 (203.03)
AUMC (µg.min^2/ml)	112964.30 (31232.08)	105330.06 (23279.50)	83856.65 (17048.20)	94938.15 (24369.70)
Cls/f (ml/min/kg)	14.77 (3.74)*	15.71 (2.91)	18.40 (3.31)*	17.64 (4.37)
Vd/f (ml/kg)	1676.37 (411.29)	1837.72 (324.00)	1930.35 (306.49)	1980.62 (381.57)

n=12. * $p < 0.05$ (t-test).

Pharmacokinetic interaction between zidovudine and rifampicin following administration at 22.00 hours

Co-administration of rifampicin with zidovudine at night resulted in a significant ($p < 0.05$) lowering of the C_{max} of zidovudine by 16% (from 9.60 to 8 μg/ml, $t = 3.02$). Comparison of the means of various other pharmacokinetic parameters of zidovudine following its co-administration with rifampicin at 22.00 h revealed no other significant differences ($p > 0.05$).

DISCUSSION

Temporal variations in the pharmacokinetics of several chemotherapeutic agents have been reported /2,4-8/. Di Santo *et al.* /9/ studied the chronokinetics of erythromycin in 24 healthy adult males by administering 250 mg of the drug orally every six hours for 3 days at 02.00, 08.00, 14.00 and 20.00 h, and observed greatest peak plasma concentration at 11.30 h, shortest time to peak at 20.00 h, and highest AUC at noon. Sothern *et al.* /3/ described a significant within-day variation in the levels of zidovudine in plasma and urinary excretion following steady-state dosing of patients with HIV infection. They found a peak plasma zidovudine level during the sleep span (00.00 to 08.00 h).

Zidovudine is rapidly and completely absorbed from the gastrointestinal tract following oral administration, but undergoes extensive first-pass metabolism /10/. In humans and animal models, following either oral or intravenous administration of zidovudine, the most abundant metabolite found in plasma and in urine is the 5′-glucuronide (GZDV) /11/. 5′-Glucuronidation, which appears to represent the major metabolic pathway of zidovudine in humans and animals, is catalyzed by the enzyme UDP glucuronyl transferase, which has been reported to show circadian variations in activity /12/. Recently, Stagg *et al.* /13/ detected two novel catabolites of zidovudine, 3′-amino-3′-deoxythymidine (AMT), and its 5′-glucuronide (GAMT). Metabolism of zidovudine to AMT is correlated with the cytochrome P-450 content in the liver /14/. Diurnal variations in the activity of the cytochrome P-450 enzyme system are well documented /12,15-19/.

In the present study, no significant difference was observed in any of the pharmacokinetic parameters of zidovudine when administered

alone at 10.00 or 22.00 h. However, when zidovudine was co-administered with rifampicin, the combined treatment resulted in the lowering of C_{max}, $t_{½}$, AUC_{0-6h} and MRT, resulting in enhancement of clearance, at 10.00 h, but not at 22.00 h. Glucuronyl transferase activity has been reported to be much higher at 21.00 h than at 09.00 h in rats /20/. In humans, glucuronidation of paracetamol is higher at 14.00 h than at 06.00 h /21/. Rifampicin is a known inducer of drug metabolizing enzymes. Continued administration of rifampicin causes proliferation of smooth endoplasmic reticulum with concomitant increase in hepatic cytochrome P-450, β-glucuronidase, *p*-nitrophenol glucuronyl transferase, β-*N*-acetylglucuronidase and corticosteroid hydroxylase /22/. In order to induce metabolism, rifampicin must be available in liver microsomes in sufficient quantity. Thus the extent of rifampicin availability in the liver would determine the extent of enzyme induction. Rifampicin undergoes enterohepatic circulation. Twenty-five percent of administered rifampicin undergoes biliary excretion and recycling /23/. Avachat *et al.* reported a lower absorption rate constant of rifampicin upon administration at 00.00 h than at 06.00, 10.00 or 18.00 h /2/. The mean elimination half-life of rifampicin was significantly lower after administration at 22.00 h than after administration at 10.00 h /24/. Thus rifampicin shows lower absorption and faster elimination in the rest phase than in the active phase. These time-dependent changes in the disposition of rifampicin could possibly be responsible for the reduced metabolism and elimination of zidovudine when co-administered with rifampicin at 22.00 h.

This significant lowering of the bioavailability of zidovudine following its co-administration with rifampicin during the morning may have therapeutic implications. Maximal induction of the hepatic microsomal enzyme system following rifampicin administration needs about 7 days continuous administration. Thus, such an interaction should be further tested following subchronic dosing with rifampicin.

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