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Pharmacokinetic Evaluation of Drug Interactions Between Co-Trimoxazole and Zidovudine in Rabbits

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Abstract: This study was conducted to determine the effects of concomitant administration of oral co-trimoxazole (120 mg kg^{-1}) and zidovudine (30 mg kg^{-1}) on their respective pharmacokinetics profiles in adult rabbits. The study was conducted in three phases, each separated from the other by a 2-week drug wash-out period. In the first phase, the animals received zidovudine (30 mg kg^{-1}, p.o.) alone; in the second phase, they received co-trimoxazole (120 mg kg^{-1}, p.o.) alone and in the third phase, both Zidovudine (30 mg kg^{-1}, p.o.) and co-trimoxazole (120 mg kg^{-1}, p.o.) were given concomitantly. Blood samples were withdrawn at intervals for 24 h and analysed for drug content. The concomitant administration of AZT and co-trimoxazole resulted in a non-significant ($p>0.05$) increase in peak plasma concentration, Area under Curve (AUC) and half-life ($t_{1/2}$) of AZT and a decrease in the elimination rate constant, absorption rate constant, clearance and apparent volume of distribution of AZT. The peak plasma concentrations of zidovudine (876.92±32.29 μg mL^{-1}), sulphamethoxazole (0.349±0.007 μg mL^{-1}) and trimethoprim (0.644±0.015 μg mL^{-1}) attained were increased non-significantly by 2.3, 9.17 and 5.59%, respectively. The apparent increase in serum concentration of co-trimoxazole, though not significant, resulted in a remarkable increase in the Reciprocal of Serum Inhibitory Titres (RSIT) against *B. subtilis* of up to 83.47% at the 2 h interval.The result of this study, suggests that co-trimoxazole does not cause major alterations in AZT pharmacokinetics in rabbits. In clinical practice, we could therefore infer that routine dosage adjustment may not be necessary when these 2 drugs are used concomitantly except in patients with co-existing hepatic or renal impairment where the risk of neutropaenia could be a major concern.

Key words: Acquired immunodeficiency syndrome, bioavailability, co-trimoxazole, HIV, pharmacokinetics, rabbits, zidovudine

INTRODUCTION

Pneumocystis Pneumonia (PCP) is a form of pneumonia caused by a yeast-like fungus, *Pneumocystis jirovecii*, formerly referred to as *Pneumocystis carinii* and previously thought to be a protozoa (Stringer *et al*., 2002; Redhead *et al*., 2006; Hawksworth, 2007). *Pneumocystis* Pneumonia (PCP) is one of the first opportunistic infections described in association with Acquired Immune Deficiency Syndrome (AIDS). Initial reports described it as the AIDS-defining diagnosis in approximately 60% of HIV-infected patients (Catteral *et al*., 1985; Engelberg *et al*., 1984).

Prior to the development of more effective treatments, PCP was a common and rapid cause of death in persons living with AIDS. Much of the incidence of PCP has been reduced by instituting a standard practice of using oral trimethoprim-sulphamethoxazole (TMP-SMX) combination (also known as co-trimoxazole) to prevent the disease in people with CD4 counts less than 200 mm^{-3} (Tarabey *et al*., 1993; May *et al*., 1994). Co-trimoxazole was first used successfully for the treatment of PCP in the mid-1970s (Hughes, 1981). Studies on immunocompromised children with alveolar PCP demonstrated efficacy for trimethoprim (20 mg/kg/day) plus sulphamethoxazole (100 mg/kg/day) orally. Because adverse events are few and rare, co-trimoxazole is regarded as the drug of choice in the treatment of PCP (Hughes, 1996). After several years of use, it is still effective in the management of opportunistic PCP in HIV patients and is therefore used in combination with antiretroviral drugs.

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One of such antiretrovirals that are frequently used in combination with TMP-SMX is Zidovudine (AZT), a nucleoside reverse transcriptase inhibitor. It is used to slow the progression of disease in patients infected with HIV and to prevent mother-to-child transmission. AZT is a potent inhibitor of the human immunodeficiency viruses and is widely used in the management of HIV infection. Because the most common side effects of AZT is myelosuppression which results in anaemia or neutropenia, it must be used with caution with other drugs used in HIV/AIDs that also cause neutropenia and anaemia such as gancyclovir and co-timoxazole (WHO, 1997; Moh *et al*., 2005).

The oral bioavailability of Zidovudine is 60-80% with peak plasma concentration occurring at 30 min (Collins *et al*., 1989). Since, AZT is eliminated primarily by hepatic glucuronidation, inhibition of this pathway may require dose adjustment. Increased serum levels and toxicity may therefore occur with concomitant administration of drugs that are degraded in the liver, or that inhibit liver enzymes. Although the bioavailability of AZT is important with respect to its efficacy and toxicity as well as in the emergence of resistant strains of HIV; and AZT is often used in combination with TMP-SMX, there are scanty pharmacokinetics data on the effect of concomitant administration of these drugs on their individual pharmacokinetic profiles.

In this study, we carried out a preclinical investigation of possible pharmacokinetic interaction between zidovudine and co-trimoxazole since they are often used in combination in opportunistic *Pneumocystis* pneumonia infection in HIV patients.

MATERIALS AND METHODS

Drugs: Zidovudine tablets 300 mg (Zidovir®), Cipla, Mumbai) and co-timoxazole 480 mg tablet (Septrine ®), GlaxoSmithkline, Egypt) were used.

Culture medium and test organism: Muller Hinton broth (Biotec®, England) and typed culture of *Bacillus subtilis* were used for the microbiological studies. The prepared broth and other materials were autoclaved at 121°C for 15 min.

Animals: Five local strains of healthy adult male rabbits weighing between 1.5-2.3 kg were used in the experiment following our institutional ethical standards on the handling and use of experimental animals. The males were separated from the females and were kept in the animal house of the department of pharmacology and toxicology, University of Nigeria, Nsukka, for 3 weeks before the commencement of the experiment. The animals were fasted 12 h prior to the experiment but were allowed free access to food and water after withdrawing the 2nd h blood sample.

Preparation of drug sample: A 100 mg mL^{-1} suspension of each drug was prepared in 3% Tween 85 for the oral administration.

Beer-lambert's plot: Solutions of the drugs were prepared in distilled water and scanned between a range of 200 and 400 nm to determine the wavelength of maximum absorption for the drugs. Standard calibration curves were then plotted for zidovudine, sulphamethoxazole and trimethoprine at 266.6, 256.9 and 300 nm wavelengths. The absorbance of five different concentrations was plotted against the respective concentrations.

Determination of serum drug concentrations in rabbits: The rabbits were fasted 12 h before the experiment. The experiment was carried out in 3 phases allowing a 2-week wash-out time interval between phases. In the first phase, the rabbits were given 30 mg kg^{-1} of Zidovudine and then at predetermined time intervals of 0.5, 1.0, 2.0, 4.0, 8.0 and 24.0 h, blood samples were aseptically withdrawn from the marginal ear vein of each rabbit into a container. The blood samples were centrifuged for 10 min at 2500 x and the serum collected and stored under ice in a freezer until it was analysed. Thereafter, the rabbits were returned to the animal house and allowed free access to food and water for the next two weeks before the next phase.

In the second phase, 120 mg kg^{-1} of co-trimoxazole was given to each rabbit orally and then as before at predetermined intervals of 0.5, 1.0, 2.0, 4.0, 8.0 and 24.0 h blood samples were withdrawn and centrifuged and the serum was stored as previously described until analysed. Also, 0.5 mL of the plasma was used to determine the Serum Inhibitory Titre (SIT).

In the third phase, 30 mg kg^{-1} of zidovudine was given to each rabbit orally and immediately thereafter, 120 mg kg^{-1} of co-trimoxazole was also administered orally. Blood samples were then withdrawn at 0.5, 1.0, 2.0, 4.0, 8.0 and 24.0 h intervals and centrifuged and the serum was stored as before.

All the serum samples were diluted using distilled water and then analysed spectrophotometrically at 266.6, 256.9 and/or 300 nm wavelengths depending on the phase in which the samples were obtained. Samples obtained in the phase one of the study were analysed at 266.6 nm corresponding to the wavelength of maximum absorption (λmax) of zidovudine, phase 2 samples were analyzed at 256.9 and 300 nm corresponding to the λmax of SMX and TMP, respectively. Samples obtained in the phase 3 were analyzed at the 3 wavelenghts. Drug free serum taken from the rabbits was used as the blank.

Determination of pharmacokinetic parameters: We assumed a one compartment model after a single oral administration. The following pharmacokinetic properties were determined for each animal in every phase before mean values were taken.

Area Under the Curve (AUC): The average serum concentration of Zidovudine and Co-trimoxazole obtained each phase of the study was plotted against time and the AUC calculated using the trapezoid rule.

Absorption rate constant (K_a): Absorption rate constant was derived by the method of back feathering from the graph of average serum concentration against time (absorption phase), using the relationship:

$$\text{Slope} = -0.43K_a$$

Elimination rate constant (K_{el}): The elimination rate constant was obtained from the slope of the least square regression analysis for the apparently linear portion of the log serum concentration versus time curve using the relationship:

$$\text{Slope} = -0.43K_{el}$$

Half life ($t_{1/2}$): Assuming a first order elimination rate, the $t_{1/2}$ of the drug in the serum obtained from each phase was determined using the relation: $t_{1/2} = 0.693K_{el}$.

Peak serum Concentration (C_{max}) and time to peak serum concentration (T_{max}): These were obtained from the serum concentration versus time curve.

Serum Clearance rate (Cl_T): The rate of clearance of Zidovudine and Co-trimoxazole from the serum in all the phase of the study was determined using the relation:

$$CL_T = K_{el}V_d$$

Where, V_d is the volume of distribution

Volume of distribution: This was calculated using the relationship:

$$V_d = Db/K_{el} * (AUC)$$

Db is the amount of drug administered

Preparation of inoculum: A pure culture of *Bacillus subtilis* was used as test organism. The microorganism was maintained and cultivated by sub-culturing on nutrient broth, stored at 4°C after incubating for 24 h at 37°C. The organism was activated before use by successive subculturing in 10 mL Muller Hinton nutrient broth and then incubating for 24 h.

A 24 h old culture was used in each occasion. The culture was standardized to contain 10^5 CFU mL^{-1} using MacFaland standard, of which 0.1 mL was used for the test.

Susceptibility testing: A stock solution of Co-trimoxazole (0.64 mg mL^{-1}) was prepared and was diluted two-fold using nutrient broth (Muller-Hinton broth). The minimum inhibitory concentration was determined using the broth dilution method.

Determination of Serum Inhibitory Titre (SIT): A 0.4 mL of nutrient broth was pipetted into a set of text tubes arranged in rows. Then 0.1 mL of serum sample was pipetted into the first tube in the row and mixed thoroughly. From the resulting mixture, 0.1 mL was again transferred to the next test tube and the 5 fold serial dilution continued until the last test tube in the row from which 0.1 mL of the mixture was discarded.

Each tube was now inoculated aseptically with 40 μL of the standard *B. subtilis* and incubated at 37°C for 24 h. This process was repeated for all the serum samples collected at different time intervals in all the phases of the investigation.

Serum sample containing no drug and sterile nutrient broth was used as control. The absence or the presence of growth in the tubes was noted after the incubation period and the least dilution which showed no microbial growth in the broth was recorded for each tube. The Reciprocal of mean Serum Inhibitory Titre (RSIT) was calculated from the highest dilution that inhibited the growth of the *B. subtilis* and used as an index to compare the antimicrobial activity of the serum samples collected over a 24 h period.

Statistical analysis: Results were analysed using one way Analysis of Variance (ANOVA) and the data subjected to LSD post hoc test. Differences between treatments were regarded as significant at $p<0.05$.

RESULTS AND DISCUSSION

Concurrent use of two or more drugs is often essential and indeed sometimes mandatory in order to achieve a desired therapeutic objective. A potential drug-drug interaction refers to a possibility that that one drug may alter the pharmacological effects of another drug administered concurrently. The net result may either be an enhanced or reduced effects of either or both drugs, or even an appearance of a new effect that is not peculiar to or observed with either of the drugs given alone (Aguwa, 2004).

Although, AZT has continued to demonstrate good clinical efficacy with decreased mortality in patients with asymptomatic and advanced HIV infections (Cooper *et al*., 1993), it is associated with significant toxicity in advanced HIV disease which is most commonly manifested as anaemia and neutropaenia (Richman *et al*., 1987). Prior to the development of more effective treatments, *Pneumocystis* Pneumonia (PCP) was a common and rapid cause of death in persons living with AIDS. The incidence of PCP has been reduced by a standard practice of using oral Co-trimoxazole to prevent the disease in people with CD4 counts less than 200 mm^{-3}.

It is a common practice to use AZT and Co-trimoxazole concurrently in people living with HIV who have opportunistic infection of PCP. It is therefore important to assess the possibility of pharmacokinetic drug interactions between these agents which are individually associated with neutropaenia and anaemia (Richman *et al*., 1987; Moh *et al*., 2005). Drug interactions involving antiviral agents mostly reflect shared toxicity with other agents (Morris, 1994). The concentrations of both drugs achieved in the serum are also very important for their respective efficacy and in the management of possible emergence of resistant strains.

The wavelengths of maximum absorption of the drugs in distilled water which was determined and used in the studies are 266.6, 256.9 and 300 nm for Zidovudine, Sulphamethoxazole and Trimethoprim, respectively. The Beer-Lambert's calibration equations for the drugs are shown in Table 1. The effect of concurrent administration of AZT and TMP-SMX on the mean serum concentrations of Zidovudine, Sulphamethoxazole and Trimethoprim at various time intervals are shown in Table 2.

Concomitant administration of AZT and TMP-SMX produced some changes in pharmacokinetic profile of the each of the drugs. The AUC for Zidovudine, Sulphamethoxazole and Trimethoprim was increased by 2.96, 2.39 and 5.23%, respectively which were not significantly different at $p<0.05$ form the values when the drugs were taken alone (Table 3). The maximum plasma Concentration attained (C_{max}) of these drugs increased by 2.3, 9.17 and 5.59%, respectively.

The half life of Zidovudine and Trimethoprim were increased by 12.07 and 1.47 5, respectively while that of suphamethoxazole was decreased by 15.56% (Table 3). The elimination rate constant of Zidovudine and Trimethoprim were decreased by 41.67 and 6.58%, respectively, while that of suphamethoxazole was increased by 9.78%. Concurrent administration of the drug caused a decrease in the rate of clearance by 24.59 and 8.15% for Zidovudine and Trimethoprim, respectively, but resulted in an increase of 7.51% for Sulphamethoxazole. There was no appreciable change in the volume of distribution for Zidovudine, but the volume of distribution for Sulphamethoxazole and Trimethoprim was slightly lowered by 2.44 and 1.99%, respectively.

The MIC of the strain of *Bacillus subtilis* use in this study was 0.04 μg mL^{-1}. Apparently, the serum inhibitory concentration of co-trimoxazole against *Bacillus subtilis* was decreased by the concomitant administration with zidovudine. Therefore, a higher reciprocal of serum inhibitory titre was recorded for serum collected at similar time intervals (Table 4). This effect was more remarkable at the 2 h interval where an increase of 83.47% in the reciprocal of serum inhibitory titre was recorded.

The results of this study conducted in rabbits did not show any significant changes ($p<0.05$) in the pharmacokinetic parameters of zidovudine or co-trimoxazole when the 2 drugs were used together. This may translate to a safe practice in the concomitant use of these drugs in patients. The possibility of significant pharmacokinetic interactions in actual clinical practice may not be completely ruled out because of idiosyncratic

Table 1: The wavelengths of maximum absorption and calibration curves of Zidovudine, Sulphamethoxazole and Trimethoprim

Drug	*Wavelength of maximum absorption (nm) in distilled water	Beer-Lambert's regression equation
Zidovudine	266.6	Y = 0.0325X (0.9918)
Sulphamethoxazole	256.9	Y = 73.792X (0.9822)
Trimethorprim	300.0	Y = 12.617 (0.9830)

* Scanned between 200 and 400 nm; Y is the absorbance while X is the drug concentrations in μg mL^{-1}, Correlation coefficients is shown in parenthesis

Table 2: The effect of concurrent administration of Zidovudine and Co-trimoxazole on mean serum concentrations of the each drug

	Mean plasma concentration±SEM (μg mL^{-1}) (Percentage change in parenthesis)					
Time (h)	Zidovudine Alone×10^{-2}	Zidovudine+Co-trimoxazole×10^{-2}	Sulphamethoxazole alone	Sulphamethoxazole +Zidovudine	Trimethoprim alone	Trimethoprim +Zidovudine
0.5	728.59±9.32	731.53±12.36 (0.40)	0.319±0.019	0.335±0.02 (5.02)	0.411±0.019	0.446±0.021 (8.52)
1.0	729.59±5.92	721.62±7.03 (-1.15)	0.344±0.009	0.348±0.01 (1.16)	0.517±0.018	0.528±0.019 (2.13)
2.0	737.88±10.89	739.94±12.22 (0.28)	0.341±0.014	0.344±0.014 (0.88)	0.565±0.011	0.596±0.024 (5.49)
4.0	876.92±32.29	897.11±6.44 (2.30)	0.349±0.007	0.381±0.015 (9.17)	0.644±0.015	0.680±0.036 (5.59)
8.0	561.56±12.61	688.87±14.70 (22.67)	0.323±0.0032	0.0326±0.0065(0.93)	0.633±0.013	0.658±0.037 (3.95)
24.0	586.18±39.41	624.15±13.01 (6.65)	0.275±0.0196	0.279±0.022 (1.45)	0.446±0.037	0.480±0.069 (7.62)

Table 3: Changes in the pharmacokinetic parameters of Zidovudine, Trimethoprim and Sulphamethoxazole when administered concurrently

Treatment	AUC ($\mu g.h\ mL^{-1}$)	C_{max} ($ug\ mL^{-1}$)	T_{max} (h)	Half-life (h)	Absorption rate constant (K_a)	Elimination rate constant (K_{el})	Clearance (mL/kg/hr)	Volume of distribution (V_d) (mL/kg body weight)
AZT alone	159.36±9.54	8.77±0.32	4±0.0	76.34±3.81	0.98±0.84	0.0113±0.0034	0.0061±0.00017	0.54±0.05
AZT +TMP-SMX	164.07±68.49	8.97±0.84	4±0.0	85.58±7.85	0.87±0.65	0.00851±0.008	0.0046±0.0005	0.54±0.063
	(2.96)	(2.30)	(0.00)	(12.07)	(-10.79)	(-41.67)	(-24.59)	(0.00)
SMX alone	7.33±0.19	0.349±0.01	4.0±0.0	106.7±30.71	3.68±0.98	0.0092±0.0029	4.53±1.49	480.49±20.16
SMX+AZT	7.50±0.25	0.381±.015	4.0±0.0	90.12±20.42	2.38±0.96	0.0101±0.0029	4.87±1.56	469.74±20.16
	(2.39)	(9.17)	(0.00)	(-15.56)	(-35.17)	(9.78)	(7.51)	(-2.24)
TMP alone	13.18±0.48	0.644±0.015	4.0±0.0	110.39±21.41	1.78±1.04	0.0076±0.0018	2.33±0.52	305.74±13.59
TMP+AZT	13.87±1.08	0.68±0.036	4.0±0.0	112.01±19.82	1.76±1.18	0.0071±0.0012	2.14±0.41	299.65±5.49
	(5.23)	(5.59)	(0.00)	(1.47)	(-1.12)	(-6.58)	(-8.15)	(-1.99)

Values in parenthesis are percentage changes in the respective parameter. AZT = Zidovudine; TMP-SMX = Co-trimoxazole; TMP = Trimethoprim; SMX = Sulphamethoxazole

Table 4: Serum inhibitory titre of Co-trimoxazole against *Bacillus subtilis* when given alone and when given concomitantlywith Zidovudine

Time (h)	Mean reciprocal serum inhibitory titre RSIT±SEM: Co-trimoxazole alone	Co-trimoxazole±Zidovudine (% Change in parenthesis)
0.5	47.25±1.89	60.94±1.88 (28.97)
1.0	76.26±1.71	103.42±1.58 (35.62)
2.0	100.00±2.47	183.47±1.47 (83.47)
4.0	239.63±1.48	309.0±1.39 (28.95)
8.0	248.91±1.54	241.82±1.50 (-2.85)
24.0	34.67±2.04	45.45±1.94 (31.09)

N = 5; RSIT = Reciprocal serum inhibitory titre

responses of different subjects. An earlier study has reported an effect of the combination on B cell immune response in PCP (Feola and Garvy, 2006).

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