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Zidovudine, Diclofenac and Ketoprofen Pharmacokinetic Interactions in Rats

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Abstract

Zidovudine (AZT) is widely used for the management of human immunodeficiency virus (HIV) infections. Non-steroidal anti-inflammatory drugs (NSAIDs) are often used for relief of non-specific fever and musculoskeletal pain in patients with HIV including those with AZT-induced myopathy. The effects of single oral doses of diclofenac and ketoprofen on AZT pharmacokinetics were studied in rats. The influence of AZT on the pharmacokinetics of diclofenac or ketoprofen was also investigated.

The administration of diclofenac ($3\,mg\,kg^{-1}$) or ketoprofen ($1\,mg\,kg^{-1}$) did not significantly alter AZT ($1{\cdot}5\,mg\,kg^{-1}$) pharmacokinetic parameters compared with administering AZT alone. There was no significant difference between the pharmacokinetics of ketoprofen given alone or in combination with AZT. However, the co-administration of AZT with diclofenac affected the pharmacokinetics of diclofenac. The C_{max} of diclofenac was significantly ($P < 0{\cdot}05$) increased by approximately threefold within a shorter time ($0{\cdot}6 \pm 0{\cdot}2\,h$). The mean AUC value for diclofenac was increased from 2·29 to $5{\cdot}04\,\mu g\,mL^{-1}\,h$ in the presence of AZT. AZT decreased the mean apparent clearance of diclofenac by 54%. The increase in diclofenac concentrations could be attributed to a decrease in its clearance or delay in its metabolite formation due to a competitive effect.

The results show that diclofenac and AZT should be given with caution because of the possible increase of diclofenac toxicity, in anticipation of follow-up clinical studies to examine this finding in man. AZT and ketoprofen could be a safe combination since no pharmacokinetic interaction was detected.

Optimum management of patients infected with the human immunodeficiency virus (HIV) and those living with the acquired immunodeficiency syndrome (AIDS) includes administration of many agents. The risk of adverse side effects and potentially significant drug–drug interactions are therefore increased considerably in these patients (Robertson-Dallas et al 1997). The antiretroviral zidovudine (AZT) was the first agent to be approved for the treatment of AIDS. A number of clinical benefits have been reported for patients with AIDS or AIDS-related complex receiving AZT, including increased survival and decreased opportunistic infections (Langtry & Campoli-Richards 1989; McLeod & Hammer 1992; Wilde & Langtry 1993). AZT undergoes extensive biotransformation by glucuronidation and reduction, which can be affected by either enzyme induction or inhibition (Sellers 1989; Eagling et al 1994). Glucuronidation of AZT is mediated through the uridine diphosphoglucuronsyl transferase (UDPGT) system (Resetar et al 1991). It is known that this system has many isoenzymes, some of which have an overlap in substrate specificity (Burchell & Coughtrie 1989; Tephly & Burchell 1990). Thus although a drug may be glucuronidated by an isoenzyme different than AZT, an interaction may still occur. Induction or inhibition of UDPGT enzymes would result in altered pharmacokinetic parameters causing therapeutic failure or toxicity, respectively. Interference with the conjugation of AZT or its renal excretion by other drugs could lead to enhanced AZT effect and/or increased toxicity.

Many drug interactions with AZT have been reported (Ameer et al 1992; Burger et al 1993; Gillum et al 1993; Morris 1994; Aiba et al 1995; Lee et al 1996; Taburet & Singlas 1996). AZT has a short half-life and adverse effects limit its clinical usefulness. To prevent adverse effects and to use AZT more effectively, it is necessary to investigate its pharmacokinetics and its interactions.

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgesic, and antipyretic activity (Brogden et al 1980). It undergoes first-pass metabolism, which decreases systemic bioavailability to 50%, producing inactive aromatic hydroxylated and conjugated metabolites. Diclofenac is eliminated primarily by the kidney as conjugated metabolites; little unchanged drug is detected in the urine (Willis & Kendall 1978).

Ketoprofen is an NSAID, a phenylpropionic acid derivative (Fakhreddin & Brocks 1990). It is metabolized in the liver with 60–75% of the dose appearing in the urine, primarily as an acyl glucuronide metabolite, and less than 10% of the dose is excreted as unchanged drug (Lewellen & Templeton 1976; Meunier & Verbeek 1999).

Interactions between NSAIDs and other drugs occur relatively frequently, because of the current use profile of NSAIDs, either as prescription or over-the-counter drugs. The NSAIDs are often used for relief of non-specific fever and musculoskeletal pain in patients with HIV including those with AZT-induced myopathy (Dalakas et al 1990), thus potentially increasing the risk of adverse events.

There are no clinical data available on the interaction of either diclofenac or ketoprofen with AZT. Therefore, this study has investigated the effect of these commonly used NSAIDs on the pharmacokinetics of AZT in rats. The rat model was chosen because Mays et al (1991) and Radwan et al (1995) reported that the rat model could be useful for predicting the effects of factors such as concurrently administered drugs, nutritional status and disease, on the disposition of AZT in man.

Materials and Methods

Materials

Diclofenac diethylamine was generously supplied by the Tabuk Pharmaceutical Manufacturing Co. (Tabuk, Saudi Arabia). Zidovudine was a courtesy donation of Burroughs Wellcome (Research Triangle Park, NC). Ketoprofen and flurbiprofen were purchased from Sigma Chemical Company (St Louis, MO). All other reagents and chemicals were analytical grade, and used as received.

Animals and dosing scheme

Fifty-four male Sprague–Dawley rats (180–220 g) were randomly divided into three groups (18 rats/group) and each group was subdivided into three subgroups for the different sampling times. Each subgroup ($n = 6$) was housed in one cage and marked. The study was conducted in a cross over design, in two phases, with a one-week wash-out period between phases. In phase I, to one group of the three groups of rats single doses of AZT, diclofenac or ketoprofen were given with oral tubing at 1·5, 3, and 1 mg kg^{-1}, respectively. Blood samples were collected from the orbital venous plexus from each group at 30 min and 6 h from the first subgroup, 2 and 8 h from the second subgroup, and 1, 3 and 12 h from the third subgroup after each drug administration. Therefore, each data point was the mean of six replicates. Only two to three blood samples were collected from each rat per day to avoid any damage to the eye. In phase II, two groups were given an oral dose of AZT (1·5 mg kg^{-1}) while diclofenac (3 mg kg^{-1}, p.o.) was given to the first group and ketoprofen (1 mg kg^{-1}, p.o.) was given to the second group. Blood samples were collected as described above. Food was withheld for 12 h before collecting blood samples for each phase but rats had free access to drinking water at all times during the experiment. Rats were lightly anaesthetized with ether only during blood sampling. Plasma samples were separated by centrifugation at 8000 rev min^{-1} for 15 min and stored at -20°C till assayed as described below.

Determination of AZT

AZT concentrations in plasma were measured using a sensitive HPLC assay (Radwan 1995). Briefly, 200 μL rat plasma samples were mixed with 20 μL of its internal standard (theophylline, 800 ng) in 10-mL centrifuged tubes. Isopropyl alcohol (50 μL) was added and vortexed for 30 s. The drug was extracted with 2 mL chloroform and vortexed at high speed for 1 min. After centrifugation at 1000 rev min^{-1} for 5 min, the organic layer was evaporated and the residue was reconstituted with 100 μL of the mobile phase. A 50 μL sample of the solution was injected into the HPLC for analysis. Acetonitrile (7·5%) in 0·2% acetic acid solution was used as the mobile phase. The column was a Novapak C18 column (3·9 × 150 mm) packed with 5-μm spherical particles. The sample run time was 8 min.

Determination of diclofenac and ketoprofen

Diclofenac and ketoprofen concentrations in plasma were measured using another sensitive HPLC assay. Briefly, 200 μL rat plasma samples were mixed with 20 μL of its internal standard (flurbiprofen, 1 mg L^{-1}) in a 1·8-mL disposable polypropylene microcentrifuge tube. The tube was vortexed for 30 s after the addition of each drug.

The solution was mixed with 600 μL acetonitrile, vortexed at high speed for 1 min, and centrifuged at 12 000 rev min^{-1} for 5 min. The supernatant was transferred to a 15-mL centrifuge tube, placed in a water bath (50°C) and evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 100 μL mobile phase before injection into the chromatograph for analysis. Acetonitrile (51%) in 0·2% acetic acid solution was used as the mobile phase. The column μ Bondapak C_{18} column (3·9 × 300 mm) was packed with 10-μm spherical particles. The flow rate was 1·5 mL min^{-1} and the sample run time was 10 min. The assay was fully validated. The percent intra-day relative standard deviation (r.s.d.) was < 6%, and inter-day r.s.d. was < 10% at three different plasma concentrations ($P < 0.05$). The mean percentage recovery of 0·1–10 μg mL^{-1} (n = 6) of diclofenac and ketoprofen were 94 ± 4 (r.s.d. = 7·3%) and 96 ± 3·5 (r.s.d. = 5·7%), respectively. The mean retention times of diclofenac, internal standard, and ketoprofen were 8·9, 7·4, and 5 min, respectively. The detection limit was 50 ng mL^{-1}.

Data analysis

All results are expressed as mean ± s.d. Pharmacokinetic parameters were estimated using model-independent methods (Gibaldi & Perrier 1982). The mean peak drug concentration (C_{max}) and the time to reach C_{max} (T_{max}) were derived directly from the individual plasma levels. The area under each drug concentration time curve (AUC, μg mL^{-1} h) to the last data point were calculated by the trapezoidal rule. The apparent oral clearance (CL/F) was calculated from Dose/AUC. Student's *t*-test was used to determine statistically significant differences ($P < 0.05$) of in-vivo data.

Results and Discussion

Figure 1 shows the mean AZT plasma concentration vs time profiles after oral dosing of AZT at 1·5 mg kg^{-1} alone, or when co-administered with diclofenac (3 mg kg^{-1}, p.o.) or ketoprofen (1 mg kg^{-1}, p.o.) in rats. The pharmacokinetic parameters of AZT, diclofenac, and ketoprofen in rats are summarized in Table 1. No significant ($P < 0.05$) change in AZT concentration–time profiles in the presence of either diclofenac or ketoprofen was detected. However, diclofenac administration with AZT resulted in a reduction in its C_{max} of approximately 18%, with a slight delay in its T_{max} (approximately 10 min), but the change was not significant ($P < 0.05$). Therefore, lack of an interaction with respect to the rate or extent of AZT was demonstrated.

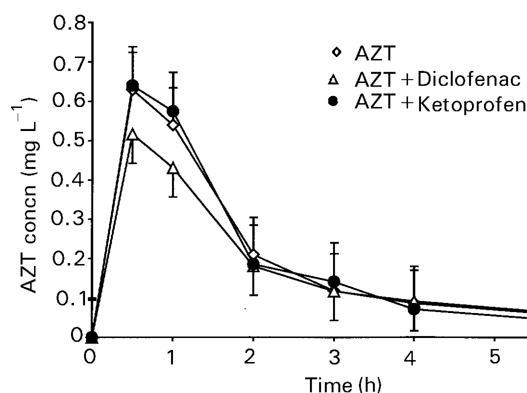

Figure 1. Mean plasma concentration–time profiles (± s.d.) after oral doses of 1·5 mg kg^{-1} AZT in the absence or the presence of either diclofenac (3 mg kg^{-1}, p.o.) or ketoprofen (1 mg kg^{-1}, p.o.) in three different groups of rats.

Diclofenac or ketoprofen co-administered with AZT produced no change in the apparent clearance of AZT. In this study the concentrations of AZT metabolites were not measured, but if AZT metabolites were changed, AZT concentrations would change also, but as no change in AZT was detected this was not the case. Similarly, indomethacin and naproxen have been reported to cause insignificant inhibition (10–30%) of AZT glucuronidation in AIDS patients (Barry et al 1993). This may reflect a lower sensitivity of AZT to selective inhibitions of parallel pathways of metabolism.

When initiating this study, the primary concern was possible alteration in the pharmacokinetics of AZT, while changes in the disposition of NSAIDs were of secondary concern. Figure 2 depicts the mean diclofenac plasma concentration–time profiles after oral dosing of 3 mg kg^{-1} alone or when co-administered with AZT (1·5 mg kg^{-1}, p.o.) in

Table 1. Pharmacokinetic parameters of AZT, diclofenac, and ketoprofen administered alone and when co-administered after oral doses of 1·5, 3, and 1 mg kg^{-1}, respectively.

Drug	Pharmacokinetic parameters		
	C_{max} (s.d.) (μg mL^{-1})	T_{max} (s.d.) (h)	AUC* (μg mL^{-1} h)
Diclofenac	0·60 (0·17)	1·67 (1·4)	2·29
Diclofenac + AZT	2·02 (0·59)	0·60 (0·2)	5·04
Ketoprofen	1·62 (0·60)	1·0 (1·1)	11·64
Ketoprofen + AZT	2·55 (0·84)	1·0 (1·0)	12·52
AZT	0·67 (0·11)	0·58 (0·20)	1·23
AZT + diclofenac	0·55 (0·22)	0·60 (0·22)	1·09
AZT + ketoprofen	0·67 (0·13)	0·75 (0·27)	1·21

*The area under concentration–time curve to the last data point.

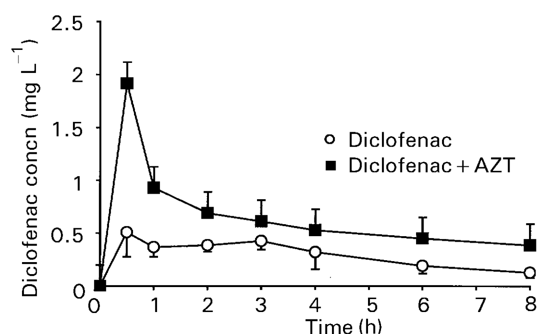

Figure 2. Mean plasma concentration–time profiles (± s.d.) after multiple oral doses of 3 mg kg^{-1} diclofenac alone or in the presence of AZT (1·5 mg kg^{-1}, p.o.) in the same group of rats.

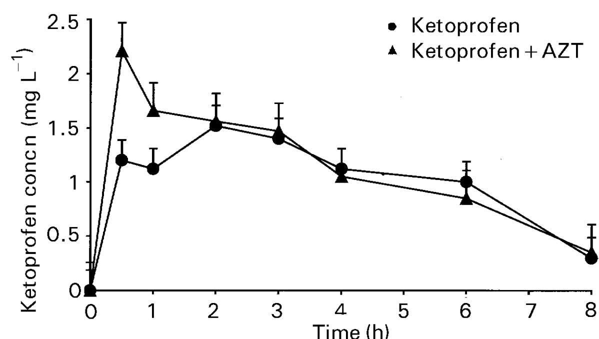

Figure 3. Mean plasma concentration–time profiles (± s.d.) after oral doses of 1 mg kg^{-1} ketoprofen alone or in the presence of AZT (1·5 mg kg^{-1}, p.o.) in the same group of rats.

rats. Interestingly, the pharmacokinetic parameters of diclofenac were significantly altered by co-administration of AZT. The mean C_{max} of diclofenac was significantly ($P < 0{\cdot}05$) increased by approximately threefold (2·02 ± 0·59) in a shorter time (0·6 ± 0·2 h). The mean AUC of diclofenac was more than doubled after AZT administration. AZT decreased the mean apparent CL of diclofenac by 54% (0·6 vs 1·3 L h^{-1} kg^{-1}). This indicates that AZT interfered with diclofenac metabolism. Kovarik et al (1997) reported similar results with diclofenac when co-administered with cyclosporin in rheumatoid arthritis. They showed that diclofenac AUC was doubled in the presence of cyclosporin. They speculated that the pharmacokinetic interaction, which is unique to diclofenac, was caused by the inhibition in the first-pass metabolism of diclofenac by cyclosporin. On the other hand, Derendorf et al (1986) detected a slight increase (approximately 20%) in the C_{max} of diclofenac after intramuscular administration of a combination of diclofenac and triamcinolone acetate.

Figure 3 shows the mean ketoprofen plasma concentration–time profiles after oral dosing of 1 mg kg^{-1} alone or when co-administered with AZT (1·5 mg kg^{-1}, p.o.) in rats. Although the administration of AZT increased the C_{max} of ketoprofen from 1·6 ± 0·6 h to 2·55 ± 0·84 h, it was not a significant effect ($P < 0{\cdot}05$). There was no significant change in AUC or apparent CL of ketoprofen due to AZT co-administration. Therefore, ketoprofen and AZT could be a safe combination since no pharmacokinetic interaction was detected. The results showed a lack of pharmacokinetic drug interaction with respect to AZT, but because the data were derived from single-dose administrations care should be followed in extrapolation to steady-state situations. The results suggest that ketoprofen can be co-administered with AZT without significant pharmacokinetic interaction. However, there is an indication that AZT interferes with diclofenac metabolism in rats. In AIDS patients with liver damage, the administration of diclofenac may result in glucuronidation impairment. Therefore, as a precaution to minimize its hepatotoxicity, the dose of diclofenac should be reduced if administered with AZT.

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