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PHARMACOKINETICS OF THE NEW ANTIASTHMA AND ANTIALLERGY DRUG, AZELASTINE, IN PEDIATRIC AND ADULT BEAGLE DOGS

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ABSTRACT

The plasma concentrations and relative bioavailability of azelastine hydrochloride (AZ) and its desmethyl metabolite (DAZ) after a single and 10 once-a-day oral doses of 2·5 mg kg^{-1} AZ were determined in adult male and female and pediatric male and female beagle dogs. The pediatric and adult dogs were 4–6 weeks and 1–2 years of age, respectively. An analysis of variance (ANOVA) was performed on the bioavailability parameters among all groups and between the first and last doses. No statistically significant ($p<0·05$) sex-related differences in the bioavailability parameters were observed. The peak concentration (C_{max}) of AZ, especially after the first dose, was significantly higher in pediatric dogs than that in adult dogs, whereas following the multiple AZ administrations, the bioavailability parameters for the last dose were similar in the two age groups. The apparent volume of distribution (V_{ss}) of AZ suggested that the drug was extensively distributed into tissue in adult as well as in pediatric dogs. The V_{ss} was considerably smaller in pediatric dogs, which may explain the higher C_{max} in this age group. The bioavailability of the metabolite in adult dogs after multiple administration of AZ was higher than that in pediatric dogs. Although some differences in the parameters among the groups are statistically significant, they do not appear to have any biological significance. Therefore, similar AZ dosages may be required in pediatric and in adult populations to achieve optimum therapeutic drug levels.

KEY WORDS Azelastine Desmethylazelastine Pharmacokinetics Antiasthma Antiallergy Dogs Age/sex dependence

INTRODUCTION

Azelastine hydrochloride, 4-[(4-chlorophenyl)methyl]-(hexahydro-1-methyl-1H-azepin-4-yl)-1(2H)-phthalazinone, monohydrochloride (AZ), whose chemical structure is depicted in Figure 1, is a new long-acting antiallergy and antiasthma drug currently under clinical development[1].

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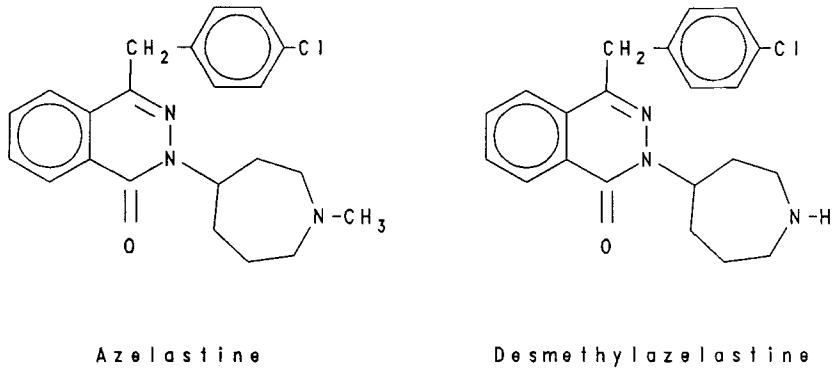

Figure 1. Structure of azelastine and desmethylazelastine

Preclinical pharmacology studies, mostly in the guinea pig, showed good antiallergic, antiasthmatic, and anti-inflammatory activities for AZ.[2,3] AZ was reported to be metabolized by *N*-demethylation to desmethylazelastine (DAS) in guinea pigs* and rats.† It has also been established that DAZ has pharmacological activity equivalent to the parent drug.* Since AZ is expected to be used in children for allergy and asthma treatments, determination of the age dependency of disposition of AZ and its pharmacologically active metabolite (DAZ) is necessary. No studies of AZ in pediatric animal models have been reported to date.

In the present study, the dog has been selected as an animal model for the evaluation of bioavailability of AZ. An earlier bioavailability study in adult dogs that received a single oral and a single intravenous AZ dose suggested that the bioavailability of AZ oral dose was about 17 per cent.[4] However, based on the estimated degree of first-pass metabolism of AZ, the authors concluded that the low bioavailability of the drug is attributed to the first-pass metabolism and the absorption of AZ oral dose is essentially complete. These results were also confirmed by another study in dogs administered 1 mg kg^{-1} oral dose of [^{14}C]AZ.†

This paper describes the results of a study to investigate age-related changes in relative bioavailability and pharmacokinetic parameters of AZ and its desmethyl metabolite in plasma of beagle dogs.

*Wallace Laboratories, unpublished results.
†ASTA Pharma, Frankfurt, a.M., Germany, unpublished results.

MATERIALS AND METHODS

Materials

AZ was from Wallace Laboratories. DAZ HBr was obtained from ASTA Pharma, Frankfurt a.m., Germany. All other chemicals and supplies used were obtained from commercial sources.

Animal Procedures

The *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences was followed for the care and use of the animals.

Adult male and female beagle dogs (1–2 years old, weight range 8–13 kg) and pediatric male and female beagle dogs (4 to 5 weeks old, 1·0–3·0 kg) were purchased from White Eagle Laboratories, Inc., Doylestown, PA. Adult dogs were fed once a day at approximately 12 noon, while pediatric dogs were fed three times a day at approximately 12 noon, 4 pm, and 9 pm. Water was available *ad libitum*. Four groups of 16 each, male and female adult and pediatric dogs (total of 64) received a 2·5 mg kg^{-1} dose of AZ contained in a hard gelatin capsule at about 8 am on Study day 1. Following capsule administration, approximately 20 or 5 ml of water was administered to each adult or pediatric dog, respectively. There was no drug administration on Study day 2. The drug was administered again once a day to each dog from Study days 3 to 12 (10 consecutive doses).

Blood samples were withdrawn from either the cephalic, jugular or saphenous vein of each animal after the first and the last doses. Blood samples of 0·25 ml volume were drawn into a 0·5 ml Glaspak® syringe before the dose and at 0·5, 1·0, 1·5, 2, 3, 4, 6, and 8 h after the dose. Each sample was immediately transferred to a 0·25 ml Microcaps® capillary tube coated with sodium heparin and centrifuged at about 2800 rev min^{-1} for 25 min. A 0·100 ml aliquot of each plasma sample was transferred to a 15 ml capacity polypropylene tube. A larger blood sample of 2·5 ml volume was withdrawn at the 12, 16, and 24 h postdose intervals. After centrifugation exactly 1·00 ml aliquots of plasma were transferred to polypropylene tubes. A 7 ml blood sample was taken from each dog at the 24 and 30 h intervals following the last dose and the resultant plasma samples after centrifugation were each transferred to polypropylene tubes. In addition, blood samples of 0·25 ml were also taken from each dog at 2·5 h postdose on Study days 10 and 11 (doses 9 and 10) and processed as described above. All the plasma samples were immediately frozen and stored below −15 °, pending drug analysis.

Azelastine and desmethylazelastine HPLC assays

The plasma samples from each animal were analyzed for AZ and DAZ by a specific automated high performance liquid chromatography (HPLC) method.

Briefly, each plasma sample was extracted with 9 ml of *n*-hexane: 1-octanol (95 : 5) following the addition of 0·25 ml of 1N sodium hydroxide solution and 0·100 ml of the internal standard solution, 15 ng ml^{-1}. The organic phase was back extracted with dilute acetic acid and the acidic aqueous solution was injected onto a 2 × 250 mm Shandon Hypersil® CPS (5 μm) HPLC column. The mobile phase consisted of acetonitrile–water–ammonium phosphate–triethylamine adjusted to pH 3·0 with orthophosphoric acid. The flow rate was 0·45 ml mins^{-1} and the column temperature, 40 °. Fluorescence detection was used with 215 nm excitation and 360 nm emission filters. The linear quantitation range for both the AZ and DAZ was 0·312 to 320 ng ml^{-1}. For analysis a Waters model 712 WISP autoinjector (Milford, MA), a Waters model 590 pump, and an ABI model 980 fluorescence detector (Foster City, CA) were used. Data acquisition and processing was done on a Hewlett-Packard 3357 Laboratory Automation System (Avondale, PA).

Data analysis

The plasma AZ and DAZ concentration data obtained from the HP3357 LAS were processed using a commercial computer program, BIOPAK® .[5] The following bioavailability parameters were determined: peak concentration (C_{max}), time of peak concentration (t_{max}), area under the curve from time zero to time infinity (AUC), and elimination half-life ($t_{½}$). The ANOVA test was performed on the differences in bioavailability parameters obtained for all the groups. In the statistical analyses, $p < 0·05$ was considered significant.

For the determination of pharmacokinetic parameters, mean plasma drug analyte concentration data for each group. i.e. adult male, adult female, pediatric male, and pediatric female, were subjected to exponential curve stripping and nonlinear least squares optimization by the RSTRIP® program.[6] The following equation was used:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-k_a t}$$

where C_p is the plasma concentration of the compound at time t; A, B, and C are the Y-intercepts of the exponential fitting; and k_a, α, and β are the first-order rate constants for the absorption, distribution, and elimination phases, respectively. The estimates of AZ absorption, distribution, and elimination half-lives, AUC, lag time for absorption (t_{lag}) and mean residence time (MRT) were obtained from the RSTRIP analyses. Similar parameters for the desmethyl metabolite were also determined. The total body clearance (CL) and volume of distribution at steady-state (V_{ss}) for AZ were calculated from equations:

$$\text{CL} = \frac{F \times \text{Dose}}{\text{AUC}}$$

$$V_{ss} = \text{CL} \times \text{MRT}$$

where F is the absolute bioavailability and AUMC is area under the first moment curve. F value of 0·17 was used in these calculations, as reported in the literature.[4]

RESULTS

The mean AZ plasma concentration–time curves for the adult and pediatric groups are presented in Figure 2. After the first dose, a higher C_{max} of AZ in the pediatric dogs than that in the adult dogs is evident. Nevertheless, the difference in C_{max} for the last dose after multiple AZ administrations was less

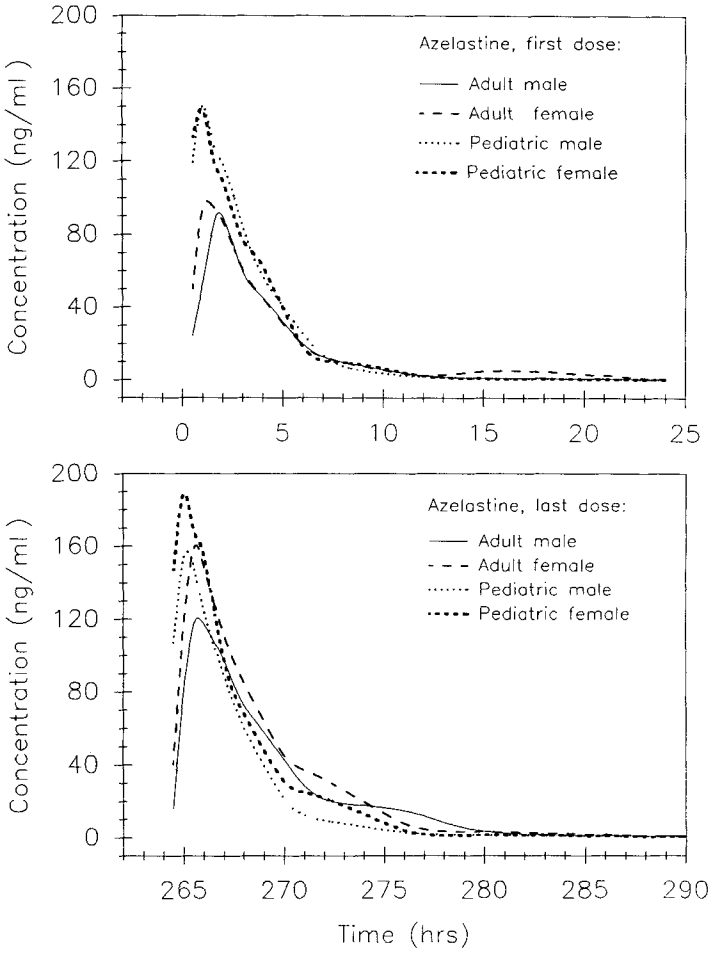

Figure 2. Mean plasma concentrations of AZ in adult and pediatric dogs

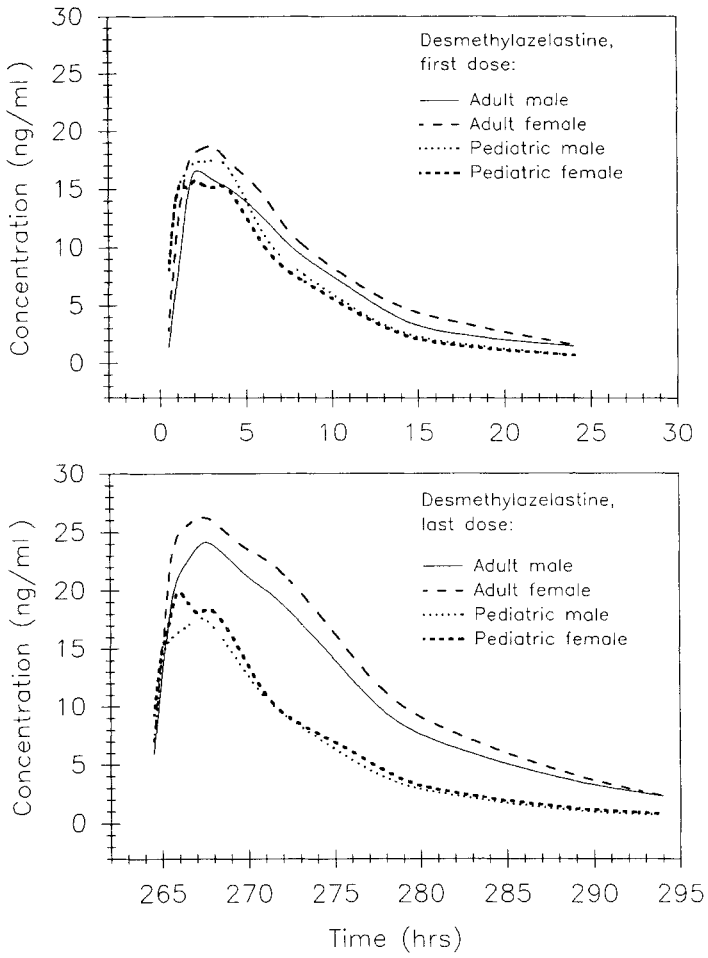

Figure 3. Mean plasma concentrations of DAZ in adult and pediatric dogs

apparent. The mean DAZ plasma concentrations for the two age groups, depicted in Figure 3, were similar after the first dose of AZ. However, the DAZ concentrations after the last dose of AZ in adult dogs were higher, while the concentrations remained the same in the pediatric dogs.

The ANOVA analysis showed no statistically significant sex-related differences in the bioavailability parameters for both age groups. Therefore, the bioavailability data for the male and female groups were combined for direct comparison of adult vs pediatric dogs. The mean values ($\pm$SD) of the combined bioavailability data for AZ are presented in Table 1. Relatively large SDs of the data suggested a greater level of interindividual variability. This large variability could be due to the very high first-pass metabolism of AZ in dogs

Table 1. Mean AZ bioavailability parameters in adult and pediatric dogs with statistical analysis ($n=32$)

Parameters	Adult		Pediatric	
Column	First (1)	Last (2)	First (3)	Last (4)
C_{max} (ng ml^{-1})	109·1	157·5	203·2$^{(1,3)}$	201·1$^{(2,4)}$
±SD	51·3	82·5	85·6	197·2
t_{max} (h)	1·7	2·0	1·4	1·2$^{(2,4)}$
±SD	0·7	1·3	0·9	0·7
AUC (ng h ml^{-1})	377	671$^{(1,2)}$	539$^{(1,3)}$	640
±SD	182	290	174	381
$t_{½}$ (h)	2·3	3·1$^{(1,2)}$	2·2	3·2$^{(3,4)}$
±SD	0·4	0·5	0·5	0·7

Note: Superscripts in parentheses identify statistical significance of the difference between the two columns for each parameter ($p<0·05$)

(up to 83 per cent) reported in a previous study.[4] In general, the bioavailability parameters of drugs that undergo extensive first-pass metabolism are reported to have high interindividual variability.[7]

In the adult group, the C_{max} of AZ for the last dose was about 40 per cent higher than that for the first dose. In contrast, the C_{max} in pediatric dogs following the first and last doses was similar. Furthermore, the C_{max} for the first dose in the pediatric group was about twice that in the adult group. For the last dose, however, the difference in C_{max} between the two groups was considerably smaller. The t_{max} was similar for the first and last doses in adult as well as in pediatric dogs. The AUC for AZ after the first dose in pediatric dogs was significantly higher (43 per cent) than that in adult dogs. Following the multiple AZ doses, however, the AUC for the last dose was similar in the two age groups. In adult dogs, the AUC for the last dose of AZ was increased by about 78 per cent compared to a 20 per cent increase in pediatric dogs, after the multiple dosing. The $t_{½}$ of AZ for the first dose, about 2 h, and for the last dose, about 3 h, was similar in adult and pediatric dogs. The $t_{½}$ in adult dogs is also very similar to that reported in an earlier study.[4] These half-lives suggested that the overall drug elimination was similar in the two age groups.

The mean values (±SD) of the bioavailability parameters for DAZ are presented in Table 2. No major differences in the C_{max} (20 ng ml^{-1}) and the t_{max}(2–3 h) following the first dose were observed between the two age groups. In adult dogs, the C_{max} for the last dose was about 40 per cent higher than that after the first dose, and in pediatric dogs it was similar. In the adult dogs, the AUC of DAZ for the last dose was increased by about twofold when compared to that for the first dose. In the pediatric dogs, the AUC was similar for the first and last doses. The $t_{½}$ was similar in adult and pediatric dogs, except the slightly longer $t_{½}$ in adult dogs for the last dose.

Table 2. Mean DAZ bioavailability parameters in adult and pediatric dogs with statistical analysis ($n = 32$)

Parameters	Adult		Pediatric	
	First (Column 1)	Last (Column 2)	First (Column 3)	Last (Column 4)
C_{max} (ng ml^{-1})	19·4	26·9$^{(1,2)}$	20·8	20·0$^{(2,4)}$
±SD	5·3	6·9	7·2	7·8
t_{max} (h)	2·6	3·6$^{(1,2)}$	2·1	2·5$^{(2,4)}$
±SD	1·2	1·8	1·3	1·0
AUC (ng h ml^{-1})	194	380$^{(1,2)}$	154$^{(1,3)}$	180$^{(2,4)}$
±SD	77	143	58	100
$t_{½}$ (h)	5·7	6·9$^{(1,2)}$	4·5$^{(1,3)}$	5·0$^{(2,4)}$
±SD	1·4	1·6	1·2	1·1

Note: Superscripts in parentheses identify statistical significance of the difference between the two columns for each parameter ($p < 0·05$)

The mean AZ and DAZ concentration data for each group were fitted to a three-exponential equation using RSTRIP. The mean (±SEM) concentrations with the corresponding fitted curves for the first and last doses are shown in Figures 4 and 5, respectively. As can be seen a good fit of observed concentration data to the triexponential equation values was obtained for both compounds.

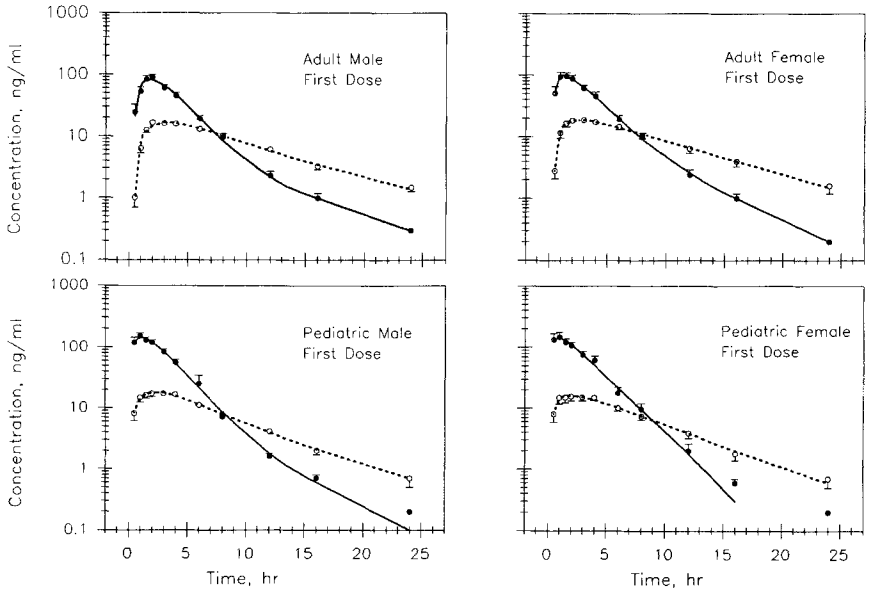

Figure 4. Observed AZ (●) and DAZ (○) plasma concentrations after the first dose with the corresponding best-fit curves for AZ (—) and DAZ (---)

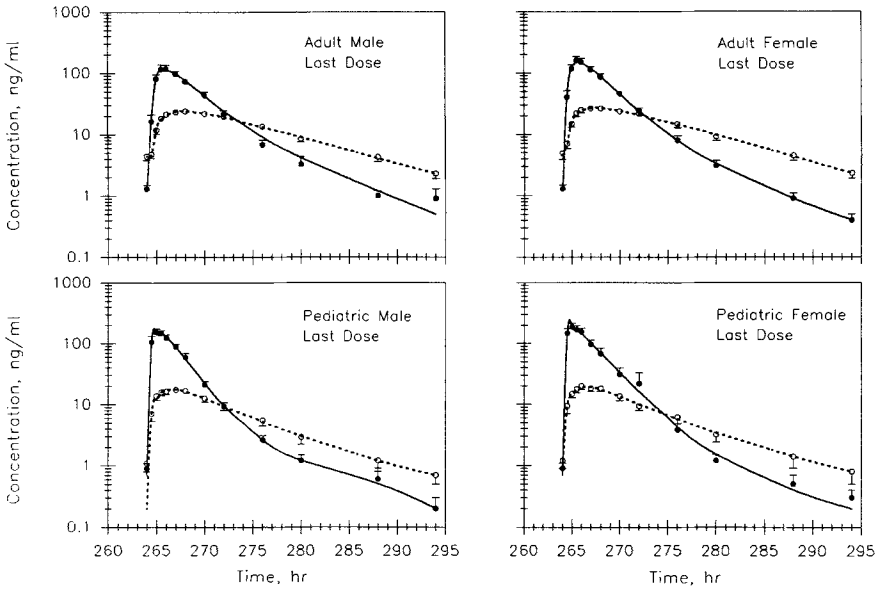

Figure 5. Observed AZ (●) and DAZ (○) plasma concentrations after the last dose with the corresponding best-fit curves for AZ (—) and DAZ (---)

Table 3. Parameters obtained by fitting of plasma AZ concentration data in dogs to a triexponential function using 'RSTRIP'

Parameters	Adult				Pediatric			
	Male		Female		Male		Female	
	FD	LD	FD	LD	FD	LD	FD	LD
C_{max} (ng ml^{-1})	89·7	117·7	95·5	158·8	150·5	153·9	148·1	189·0
t_{max} (h)	2·0	2·0	1·5	1·5	1·0	1·0	1·0	1·0
t_{lag} (h)	0·35	0·43	0·00	0·37	0·00	1·10	0·00	0·16
AUC (ng h^{-1} ml^{-1})	350	620	420	713	542	558	541	696
Rate constant (h^{-1})								
k_a	0·783	1·245	0·910	1·396	1·143	1·529	0·913	2·858
α	0·631	0·379	0·536	0·389	0·517	0·509	0·529	0·383
β	0·146	0·138	0·194	0·137	0·151	0·106	0·144	0·085
$t_{½}$ (h)								
k_a	0·89	0·56	0·76	0·50	0·61	0·45	0·76	0·24
α	1·10	1·84	1·29	1·78	1·34	1·36	1·31	1·81
β	4·76	5·06	3·57	5·07	4·58	6·51	4·82	8·14
CL (l h^{-1} kg^{-1})	0·58	0·35	0·68	0·28	0·37	0·30	0·36	0·20
V_{ss} (l kg^{-1})	2·12	1·72	2·27	1·20	1·07	1·00	1·05	0·67
MRT (h)	3·66	4·91	3·36	4·24	2·92	3·31	2·89	3·40

FD: first dose, LD: last dose.

The best-fit optimized pharmacokinetic parameters of AZ are presented in Table 3. The C_{max}, t_{max}, AUC, and $t_{½}$ values from the RSTRIP analyses, based on fitted curve estimations, were slightly different than those obtained using BIOPAK (Tables 1 and 2). Yet both sets were well within the ranges defined by the mean $\pm$SD for each parameter. The absorption, distribution and elimination rates of AZ appear to be similar in the two age groups. The mean residence times (MRT) for AZ in adult and pediatric dogs were approximately 4 and 3 h, respectively. The CL of AZ after multiple doses was similar for both age groups. The estimate of CL in adult dogs in this study is also very similar to that determined in an earlier study.[4] The apparent volume of distribution at steady-state (V_{ss}) was considerably larger in adult dogs than that in pediatric dogs. These V_{ss} values suggested extensive distribution of AZ into tissue.

The best-fit optimized pharmacokinetic parameters of DAZ are presented in Table 4. The rate of DAZ appearance in plasma was similar in adult and pediatric dogs. The lag time for the metabolite appearance in plasma was similar to that of AZ absorption. This similarity suggested that the rate of AZ absorption could be the limiting factor for its conversion to the desmethyl metabolite. The MRT for DAZ in adult and pediatric dogs were approximately 11 and 8 h, respectively.

DISCUSSION

Many drugs administered to children exhibit differences in absorption, distribution, metabolism, and excretion from those observed in adults. These

Table 4. Parameters obtained by fitting of plasma DAZ concentration data in dogs to a triexponential function using 'RSTRIP'

Parameters	Adult				Pediatric			
	Male		Female		Male		Female	
	FD	LD	FD	LD	FD	LD	FD	LD
C_{max} (ng ml^{-1})	16·5	23·9	18·7	26·1	17·4	17·5	15·7	19·9
t_{max} (h)	2·0	4·0	3·0	3·0	3·0	3·0	2·0	2·0
t_{lag} (h)	0·44	0·28	0·39	0·22	0·04	0·08	0·23	0·02
AUC (ng h^{-1} ml^{-1})	183	357	200	385	155	181	141	199
Rate constant (h^{-1})								
k_{app}	0·628	0·911	1·149	0·845	0·762	0·752	3·221	0·856
α	0·216	0·462	1·094	0·184	0·268	0·221	0·826	0·189
β	0·084	0·098	0·124	0·111	0·124	0·088	0·163	0·073
$t_{½}$ (h)								
k_{app}	1·10	0·76	0·60	0·82	0·91	0·92	0·22	0·81
α	3·21	1·50	0·63	3·76	2·59	3·13	0·84	3·67
β	8·26	7·11	5·57	6·27	5·60	7·85	4·26	9·46
MRT (h)	9·97	11·96	8·95	11·41	7·33	9·22	6·96	9·67

FD: first dose, LD: last dose.
k_{app}: Metabolite appearance in plasma.

can be attributed to pharmacogenetic factors, age, and growth. The age-related differences in pharmacokinetics are well documented for several drugs.[8] For safe and effective use of a drug in children, the pharmacokinetic behavior of the drug in children should be characterized. For AZ there are presently no pharmacokinetic data available in very young children. Therefore, in this study the relative bioavailability and pharmacokinetics of AZ and its equally active desmethyl metabolite were determined in pediatric and adult dogs to provide needed data in animal models.

The only striking difference in bioavailability parameters between the two age groups was the C_{max} of AZ in pediatric dogs. It was about twice that in adult dogs after the first dose. The higher C_{max} in pediatric dogs could be due to the lower volume of distribution of AZ and/or the faster absorption of the drug in this group when compared to that in adult dogs. Indeed, in pediatric dogs, the V_{ss} of AZ was about one-half that in adult dogs. This lower volume of distribution in pediatric dogs could be due to the relatively lower proportion of adipose tissue mass to the body weight when compared to that in adult dogs. However, in children older than 1 year, the proportion of adipose tissue to the body weight is expected to be similar to that in man.[9] Therefore, the higher C_{max} of AZ observed in pediatric dogs may not occur in children. The rate of AZ absorption, based on the estimated absorption rate constants, was similar in both age groups.

The AUC of the active desmethyl metabolite for the last dose in adult dogs was about twofold larger than that for the first dose. This value was also twofold larger than that for the last dose in the pediatric dogs. One plausible reason for this larger AUC value could be due to the difference in V_{ss} for DAZ between the two age groups. However, in this study, the V_{ss} for DAZ could not be calculated since this requires the knowledge of the extent of metabolic conversion of AZ to DAZ in dogs.

In summary, the relative bioavailability of AZ after multiple drug administrations was similar in pediatric and adult dogs, except for the higher C_{max} in pediatric dogs. After the multiple dosing of AZ, there was no excessive increase in plasma concentrations of the drug or its active metabolite in the pediatric and adult dogs. No major changes in the kinetic behavior of the drug or its equally active desmethyl metabolite were observed after multiple dosing of AZ in pediatric dogs. There were no biologically significant differences in the bioavailability parameters of the drug between the adult and pediatric dogs, although some statistically significant differences in some of the parameters were found. The potential clinical relevance of our findings is that the same dosages of the drug could be used in children and in adults to achieve optimal drug therapy.

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