Journal of Antimicrobial Chemotherapy (1990) **25**, *Suppl. A*, 49–60

Pharmacokinetics of azithromycin in rats and dogs

Richard M. Shepard and Fred C. Falkner

Central Research Division, Pfizer Inc., Groton, CT 06340, USA

After intravenous or oral administration to rats and dogs, azithromycin was rapidly distributed into the tissues, where concentrations frequently exceeded those in serum by 100-fold or more within 24 h of a single dose. Tissue concentrations were proportional to the dose following single administrations of 10 to 40 mg/kg in rats and dogs. Tissue concentrations were higher after multiple dosing and became greater as the dose was increased from 10 to 40 mg/kg. Elimination half-lives were similar in most tissues and were about 40 h in rats after seven doses of 20 mg/kg and about 90 h in dogs after five doses of 30 mg/kg. Serum concentrations declined in a multi-exponential manner, reflecting initial rapid distribution into tissues and then slow return to serum from tissues. Azithromycin had good oral bioavailability in rats and dogs (46% and 97%, respectively). Rapid uptake of azithromycin by tissues from serum and slow redistribution from tissues to serum are apparently factors governing the pharmacokinetics of azithromycin in rats and dogs. Serum concentrations do not reflect the availability of azithromycin in tissues.

Introduction

For three decades erythromycin has been an effective and safe antibiotic for the treatment of respiratory, skin, and soft tissue infections in adults and children (Washington & Wilson, 1985*a*, *b*). The importance of erythromycin has been reinforced recently by its use as the primary or secondary therapeutic agent for legionnaires' disease, mycoplasma pneumonia, campylobacter diarrhoea, and chlamydial urethritis (Retsema *et al.*, 1987). However, the clinical usefulness of erythromycin has been limited by its inactivity against *Haemophilus influenzae* and *Neisseria gonorrhoeae* (Retsema *et al.*, 1987; Bright *et al.*, 1988) and low and erratic concentrations in serum (Wilson & van Boxtel, 1978; Washington & Wilson, 1985*a*), partly caused by inactivation at the pH of the stomach before absorption (Wilson & van Boxtel, 1978).

Azithromycin (CP-62,993; XZ-450) is the first of a class of antibiotics designated azalides (Bright *et al.*, 1988). It differs structurally from erythromycin by the insertion of a methyl-substituted nitrogen at position 9a in the lactone ring, to create a 15-membered macrolide. This modification causes a significant improvement in potency against Gram-negative bacteria in comparison with erythromycin with retention of the classical erythromycin activity against Gram-positive bacteria (Retsema *et al.*, 1987). Azithromycin is also much more stable in an acid environment than erythromycin (Fiese & Steffen, 1990, this Volume) and is well absorbed after oral administration to mice, rats, dogs, and monkeys (Girard *et al.*, 1987). Furthermore, the distribution of azithromycin in tissues is much more extensive, and elimination half-lives in tissues and serum are much longer than those of erythromycin (Girard *et al.*, 1987). To date,

0305-7453/90/25A049 + 12 $02.00/0

clinical experience with azithromycin (Pfizer Inc., data on file) has demonstrated excellent efficacy, which is best explained by tissue pharmacokinetics, rather than by serum concentrations. Because of the apparent importance of extravascular distribution in the efficacy of azithromycin, the pharmacokinetics of azithromycin in tissues of rats and dogs are described in this paper and compared with those of erythromycin.

Methods

All studies were conducted in Long-Evans rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) and beagle dogs (Marshall Farms, North Rose, NY). Azithromycin (Pfizer Central Research) was administered orally as the free base in suspension in 0·1% methylcellulose and intravenously as the citrate salt lyophile, dissolved in water. Multiple doses were administered every 24 h. Erythromycin (Upjohn Laboratories, Kalamazoo, MI) was administered orally as the free base in suspension in 0·1% methylcellulose and intravenously in solution in 100% ethanol.

For studies of serum pharmacokinetics, serial serum samples were collected by orbital sinus bleeding of rats and by puncture of the jugular vein of dogs. For studies of tissue pharmacokinetics, groups of three to ten rats or three to six dogs were killed and serum and tissues were collected. Samples were usually collected 24 h after the specified dose, except in the tissue half-life studies when samples were collected for up to 8 to 57 days after the last dose.

Serum and tissue concentrations of azithromycin were determined by high performance liquid chromatography (HPLC) with electrochemical detection (Shepard, 1987) or by an agar well diffusion bioassay with *Micrococcus luteus* as the test organism (Girard *et al.*, 1987). For the HPLC assay, azithromycin and an internal standard (the 9a-N-propargyl analogue of azithromycin) were extracted from serum with methyl-*t*-butyl ether under alkaline conditions, back-extracted into aqueous citric acid, and re-extracted into methyl-*t*-butyl ether under alkaline conditions. Drug was solubilized from tissue by homogenization in acetonitrile containing internal standard, and aliquots of the acetonitrile layer evaporated to dryness and the residues reconstituted in alkaline buffer for the same isolation procedure employed for serum. The final ether extracts were concentrated to dryness and the residues reconstituted in acetonitrile/water (1/1) and washed with hexane. Aliquits of the aqueous phase were injected on to an HPLC reversed-phase γRP-1 alumina column (150 × 4·6 mm, 5 μm particle size; ES Industries) and eluted with a mobile phase of 70:30 0·02 M potassium phosphate:acetonitrile, adjusted to pH 11, at a flow rate of 1 ml/min. Detection was achieved by a LC-4B amperometric electrochemical detector (Bioanalytical Systems) at an oxidation potential of +0·8 V, and peak heights were measured with a SP-4200 computing integrator (Spectra Physics). Drug and internal standard eluted at approximately 8 and 10 min, respectively. Serum standard curves were prepared in concentration ranges of 0·02–0·2 and 0·2–2·0 mg/l, and tissue standard curves in ranges of 0·1–2, 1–10, 10–100, and 100–1000 mg/kg. All standard curves were linear with correlation coefficients greater than 0·990, and assay of quadruplicate standards produced relative standard deviations of less than 10% (R. M. Shepard, unpublished). The HPLC assay and bioassay produce similar results in serum and tissues. Serum concentrations of erythromycin were determined by HPLC with electrochemical detection (Duthu, 1984).

Elimination rate constants in serum and tissues were calculated by least squares regression of log concentration versus time. Half-lives were the quotient of 0·693 over

the elimination rate constant. The area under the serum concentration versus time curve (AUC) was calculated by the trapezoidal method and extrapolated to infinity by adding the ratio of the last concentration to the final elimination rate constant. C_{max} was the highest observed serum concentration and T_{max} the earliest time at which C_{max} occurred. After intravenous dosing, C_0 was estimated by extrapolation from serum concentrations at the first two time points after administration to the time of 0 h. Systemic clearance (Cl_s) was determined from the dose divided by the $AUC_{0-\infty}$ The volume of distribution at steady state (Vd_{ss}) was calculated as follows:

$$Vd_{ss} = \text{dose} \times \text{AUMC}/(\text{AUC})^2;$$

where AUMC is the area under the first moment of the serum concentration versus time curve. Oral bioavailability was estimated as the ratio of $AUC_{0-\infty}$ following oral administration to that following intravenous administration.

Protein binding of azithromycin and erythromycin in fresh dog, rat, and mouse serum was determined by equilibrium dialysis performed in an acrylic two-chambered apparatus (Fisher Scientific) of 1-ml capacity separated by a dialysis membrane (Spectra/Por 2). Serum was fortified with [^{14}C]azithromycin (Pfizer Central Research; 11·3–15·4 mC$_i$/mmol; 95–99% radiopurity) at concentrations ranging from 0·02 to 10 mg/l or with [^{14}C]erythromycin (DuPont NEN; 54·3 mCi/mmol; > 97% radiopurity) at a concentration of 0·5 mg/l and dialysed with mild agitation against an equal volume of 0·13 M pH 7·4 sodium phosphate buffer for 18 h at 25°C. Following dialysis the concentration of azithromycin or erythromycin in serum and buffer was determined by liquid scintillation counting and the percentage protein binding calculated as follows:

$$\%\ \text{protein binding} = \frac{(\text{serum concentration} - \text{buffer concentration})}{\text{serum concentration}} \times 100$$

Results

Tissue concentrations

After a single oral dose, mean tissue concentrations of azithromycin in several major organs were generally proportional to dose (10–40 mg/kg) in rats and dogs (Tables I and II). Tissue concentrations after a single dose of 20 mg/kg ranged from less than 1 mg/kg in fat of both species to 29 mg/kg in spleen of rats and 101 mg/kg in liver of dogs. Tissue concentrations were three- to six-fold greater in dogs than in rats after single oral doses of 10 to 40 mg/kg.

Two- to four-fold increases in tissue concentrations in rats and four- to seven-fold increases in dogs occurred upon daily oral administration of 20 mg/kg for seven days (Tables I and II). At this dose tissue concentrations were greatest after 170/190 days of treatment. Increases in tissue concentrations upon multiple dosing were greater at higher doses in both species. Tissue concentrations after multiple doses were 3- to 12-fold greater in dogs than in rats.

The elimination half-lives of azithromycin in several tissues in rats following seven oral doses of 20 mg/kg were about 40 h (Figure 1). Following five oral doses of 30 mg/kg in dogs, elimination half-lives of about 90 h were observed in the liver, lymph nodes, and tonsil (Figure 2). Tissue elimination half-lives of about 170 h were noted in dogs after 190 daily oral doses at 20 mg/kg (Figure 3). Within each treatment group,

Table I. Mean azithromycin concentrations in rat tissues and serum after daily oral dosing

Tissue	Dose level (mg/kg)	Concentration (mg/kg or mg/l) at 24 h after dose Number of daily doses 1	5	10	170
Spleen	10	13	5·3	45	155
	20	29	108[a]	147	449
	40	36	60	374	—
Liver	10	13	4·1	24	34
	20	24	26[a]	70	109
	40	55	47	252	—
Kidney	10	4·8	2·9	17	23
	20	10	28[a]	54	81
	40	20	33	120	—
Lung	10	3·9	—	7·2	13
	20	7·0	18[a]	27	56
	40	17	—	110	—
Lymph nodes	10	4·5	—	11	11
	20	19	66[a]	31	47
	40	23	—	124	—
Eye	20	1·8	7·2[a]	—	—
Muscle	20	0·78	1·4[a]	—	—
Fat	20	0·51	2·2[a]	—	—
Serum	10	0·033	0·028	—	0·1
	20	0·063	0·09[a]	—	0·2
	40	0·12	0·12	—	—

[a]Concentrations are after seven doses.

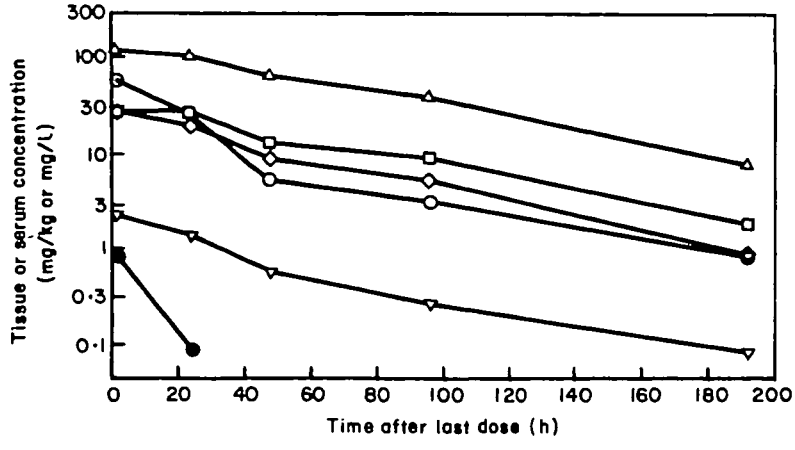

Figure 1. Mean azithromycin concentrations in tissues in rats after the last dose following seven daily oral doses of 20 mg/kg azithromycin. Azithromycin concentrations in spleen (△), kidney (□), lung (◇), liver (○), muscle (▽), and serum (●).

Table II. Mean azithromycin concentrations in dog tissues and serum after daily oral dosing

Tissue	Dose level (mg/kg)	Concentration (mg/kg or mg/l) at 24 h after dose Number of daily doses 1	5	190
Spleen	10	33	—	526
	20	48	326[a]	1110
	40	185	—	—
Liver	10	58	168	370
	20	101	457[a]	789
	40	238	817	—
Kidney	10	21	65	125
	20	30	116[a]	464
	40	88	192	—
Lung	10	22	—	154
	20	29	150[a]	443
	40	95	—	—
Lymph nodes	10	38	131	191
	20	42	189[a]	335
	40	134	484	—
Tonsil	10	—	70	—
	40	—	233	—
Retina	10	—	32	—
	40	—	131	—
Eye	20	3·7	23[a]	—
Muscle	10	2·1	—	—
	20	2·8	9·3[a]	—
	40	7·5	—	—
Fat	20	0·62	5·2[a]	—
Brain	30	—	1·2[b]	—
Serum	10	0·27	0·35	0·5
	20	0·40	1·0[a]	0·8
	40	0·90	0·98	—

[a]Concentrations are after seven doses.
[b]Mean of concentrations in cerebellum, cerebrum, and medulla.

the rates of decline of azithromycin concentrations in the spleen, liver, kidney, lung, lymph nodes, tonsil, and muscle differed by less than two-fold. Slower elimination rates were noted in the rat's eye and dog's retina and brain.

Serum concentrations

After oral and intravenous administration of azithromycin to rats (20 mg/kg) and dogs (250 mg, mean dose of 24 mg/kg) serum concentrations of azithromycin declined in a polyphasic manner with similar elimination patterns in the two species (Table III,

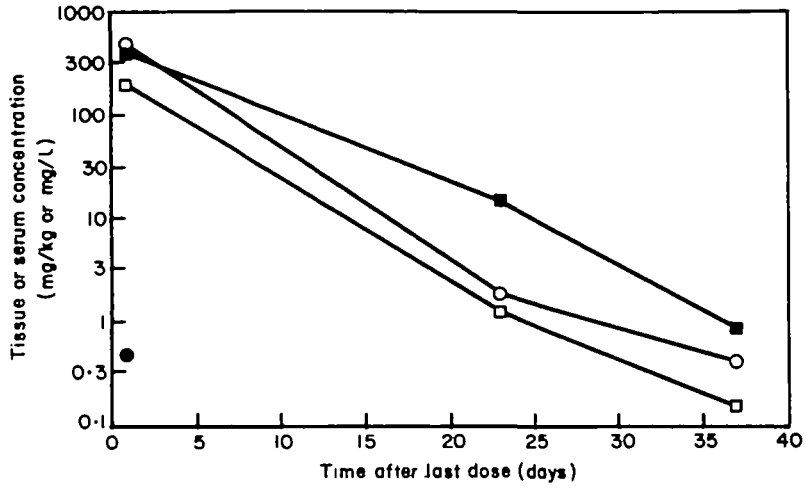

Figure 2. Mean azithromycin concentrations in tissues in dogs after the last dose following five daily oral doses of 30 mg/kg azithromycin. Azithromycin concentrations in lymph nodes (■), liver (○), tonsil (□), and serum (●).

Figure 4). Following an initial rapid distribution phase, the mean apparent half-life of elimination in the intermediate phase (2–6 h after dosing in rats, 1–4 h in dogs) was about 3–4 h in both species. The mean apparent elimination half-life from 6–48 h after dosing in rats and 24–72 h in dogs was about 30 h.

Serum concentrations of azithromycin were proportional to dose in rats and dogs after single oral doses of 10–40 mg/kg and after six months of daily dosing at a dose of 10–20 mg/kg (Tables I and II). Although increases in serum concentrations were small following daily administration for five to seven days, total increases in serum concentrations of two- to three-fold were noted in rats and dogs after six months of dosing.

The serum pharmacokinetics of azithromycin and erythromycin are compared in Table III. Peak azithromycin and erythromycin concentrations in serum after a single

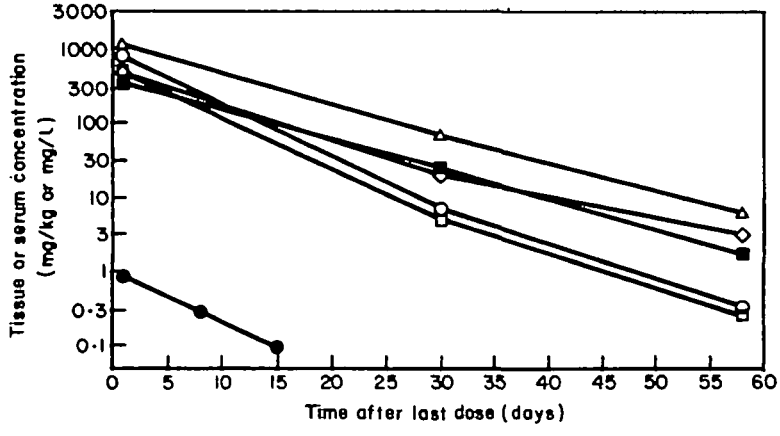

Figure 3. Mean azithromycin concentrations in tissues in dogs after the last dose following 190 daily oral doses of 20 mg/kg azithromycin. Azithromycin concentrations in spleen (△), lung (◇), lymph nodes (■), liver (○), kidney (□), and serum (●).

Table III. Serum pharmacokinetics of azithromycin and erythromycin after single intravenous and oral doses to rats and dogs

	Azithromycin		Erythromycin	
	rat	dog	rat	dog
Intravenous pharmacokinetics[a]				
C_0 (mg/l)	4·0	6·8	2·6	29
Elimination half-life (h):	3·5	3·5	1·2	1·2
(interval after dose)	(2–6 h)	(1–4 h)	(T_{max}-6 h)	(T_{max}-6 h)
	32	29	—	—
(interval after dose)	(6–48 h)	(24–72 h)		
$AUC_{0-\infty}$ h) (mg.h/l)	10	56	4·6	21
Cl_s (ml/min/kg)	34	6·0	73	16
Vd_{ss} (l/kg)	84	12	9·5	2·0
Oral pharmacokinetics[a,b]				
C_{max} (mg/l)	0·29	4·2	0·28	4·9
T_{max} (h)	2·0	0·33	1·7	0·50
Elimination half-life (h):	3·2	4·2	1·1	1·5
(interval after dose)	(2–6 h)	(1–4 h)	(T_{max}-6 h)	(T_{max}-6 h)
	22	35		
(interval after dose)	(12–48 h)	(24–72 h)		
$AUC_{0-\infty}$ h (mg.h/l)	4·1	56	0·67	11
Bioavailability (%)	46	97	15	52

[a]All data were normalized to a dose of 20 mg/kg for comparison. The following doses were used for azithromycin: rats −20 mg/kg iv and po, dogs −24 mg/kg iv and po. The following doses were used for erythromycin: rats −25 mg/kg iv and 50 mg/kg po, dogs −10 mg/kg iv and po.

[b]Oral erythromycin data from D. Girard (unpublished data) and from Girard *et al.* (1987).

oral dose (with all serum concentrations corrected to a dose of 20 mg/kg) were similar in the rat and also in the dog, as were T_{max} values. However, serum $AUC_{0-\infty}$ values for azithromycin were two- to six-fold greater than those for erythromycin after oral and

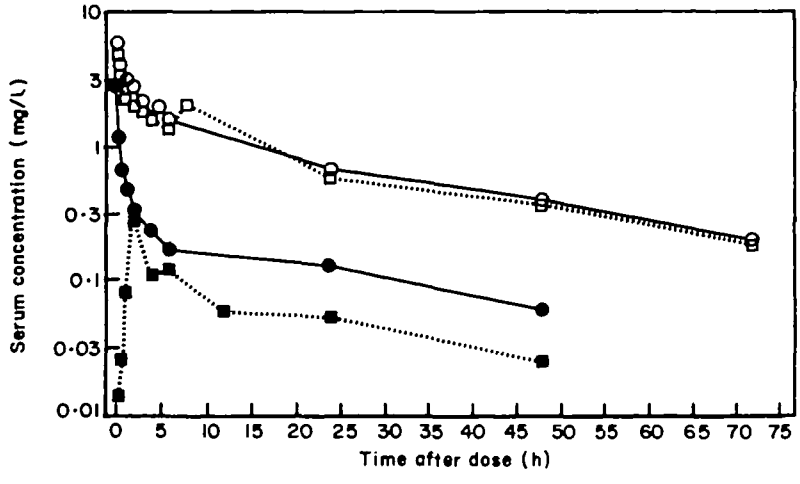

Figure 4. Mean azithromycin concentrations in serum in rats and dogs after intravenous and oral administration of azithromycin. Rat serum concentrations after a 20 mg/kg dose: iv (●), po (■). Dog serum concentrations after a 24 mg/kg dose: iv (○), po (□).

Table IV. Protein binding of azithromycin and erythromycin in dog, rat, and mouse serum[a]

Serum concentration (mg/l)	Protein binding (%) dog	rat	mouse
Azithromycin			
0·020	26·3	29·3	—
0·050	28·2	16·4	19·9
0·10	24·3	16·4	—
0·20	21·5	15·7	—
0·50	26·3	15·8	7·2
1·0	19·5	15·5	—
2·0	12·5	14·2	—
5·0	18·9	12·8	7·7
10·0	16·4	14·1	—
Erythromycin			
0·50	78·1	17·5	19·0

[a]Mean of triplicate determinations.

intravenous dosing in rats and dogs. The oral bioavailability of azithromycin (46% in rat, 97% in dog) was two- to three-fold greater than erythromycin (15% in rat, 52% in dog). Serum elimination half-lives of erythromycin in rats and dogs (about 1·2 h) were much shorter than those of azithromycin. Systemic clearance rates of azithromycin in rats and dogs were two- to three-fold slower than those of erythromycin, and apparent volumes of distribution at steady state were six- to nine-fold greater.

Protein binding of azithromycin was low to moderate in dog, rat, and mouse serum, with values of 26%, 16%, and 7·2%, respectively, at a concentration of 0·5 mg/l (Table IV). Protein binding increased with decreasing concentrations of azithromycin in serum, reaching maximum values of between 20% and 30% at concentrations at or below 0·05 mg/l.

Discussion

The affinity of azithromycin for tissues in both rats and dogs is demonstrated by the very high tissue to serum concentration ratios, which frequently exceeded 100 to one after a single dose. Azithromycin concentrations were tissue-dependent, with high concentrations in spleen, liver, kidney, lung, lymph nodes and tonsil, and ten-fold lower concentrations in muscle and fat. However, even concentrations in the latter tissues were much greater than serum concentrations. Although the penetration of most antibiotics into the eye and brain is usually poor, azithromycin levels in these tissues exceeded serum levels by 20- and 1·2-fold, respectively, and this suggests unusual potential for treatment of some organ-specific infections.

The slow egress of azithromycin from tissues was consistent with its affinity for tissue and explained the increases in tissue concentrations with multiple daily dosing in both rats and dogs. The elimination half-lives in most tissues within the treatment groups were similar. Tissue elimination half-lives appeared to increase with duration of dosing. In rats the 40-h elimination half-lives after seven doses were somewhat greater than those of about 23 h (range 12–37), reported after a single dose (Girard *et al.*, 1987),

while in dogs the 90-h half-lives after five doses were less than those of about 170 h after 190 doses.

The polyphasic serum pharmacokinetics of azithromycin in both rats and dogs were consistent with a drug being distributed rapidly and extensively in tissue and then redistributed slowly from tissue, thereby producing high tissue levels and modest but prolonged serum levels. The apparent elimination half-life between 1–2 and 4–6 h after dosing of about 3–4 h from serum is attributed to a combination of distribution of drug into tissues and clearance by excretion and metabolism. The rate of elimination then slowed considerably to a half-life of about 30 h at 48–72 h after dosing. This phase is attributed to rate-limiting slow release of azithromycin from tissue into serum, accompanied by excretion and metabolism. In rats after a single dose, the slow serum elimination half-life of about 27 h was similar to the half-life of about 23 h reported in tissues (Girard *et al.*, 1987). After multiple doses, serum concentrations were below the limit of detection during the time interval used to determine tissue elimination half-lives, preventing a comparison of serum and tissue half-lives. As in tissues, azithromycin concentrations in serum increased upon multiple dosing but to a lesser extent. Ratios of tissue to serum concentrations of azithromycin were therefore higher after multiple doses than after a single dose. The low binding of azithromycin to serum protein, and presumably also to tissue protein, make this factor unimportant in determining the extent of azithromycin penetration into tissue. Rather the lipophilicity and the basic pK_a of azithromycin may be important determinants of tissue distribution (Wise, 1986). Protein binding of erythromycin in dog and mouse serum (78% and 19%, respectively) was much greater than that of azithromycin, but was similar in rat serum (18%).

The pharmacokinetics of azithromycin were qualitatively similar in the rat and dog. In both species the affinity of azithromycin for tissues resulted in high tissue and low serum concentrations, long elimination half-lives, and increases in tissue and serum concentrations upon multiple dosing. The dog had higher tissue and serum concentrations of azithromycin than the rat, possibly owing to a combination of greater oral bioavailability and slower clearance in the dog. The two- to three-fold higher tissue to serum ratios in rats than in dogs after a single oral dose were consistent with the larger volume of distribution in the rat. Tissue elimination half-lives were somewhat longer in the dog (approximately 90 h after five doses) than in the rat (40 h after seven doses), consistent with the generally greater increases in tissue concentrations of azithromycin noted in dogs upon multiple dosing.

The importance of rapid tissue uptake and slow redistribution to serum as factors governing the pharmacokinetics of azithromycin is illustrated by the differences in the serum pharmacokinetics of azithromycin and erythromycin observed in the present study. Consistently with azithromycin's greater penetration and persistence in tissues, in comparison with erythromycin (Girard *et al.*, 1987), azithromycin had a larger volume of distribution and slower systemic clearance which resulted in a longer serum half-life than was seen with erythromycin.

While the ability of erythromycin and other macrolide antibiotics to penetrate tissues and extravascular fluids is well recognized (Osono & Umezawa, 1985), the much greater and more wide-ranging tissue penetration of azithromycin in both animals and humans is noteworthy. For example, ratios of tissue to serum concentrations *in vivo* in the range 0·5–10 have been reported for erythromycin in a variety of tissues in rats and dogs (Lee, Anderson & Chen, 1956; Girard *et al.*, 1987) and in humans (Dette, 1979;

Brun *et al.*, 1981; Falchi *et al.*, 1985) and for roxithromycin in humans (Bergogne-Bérézin, 1987; Puri & Lassman, 1987; Wise *et al.*, 1987). However, the ratios of tissue to serum concentrations for azithromycin ranged from 10 to greater than 100 for most tissues, some 10- to 20-fold greater than those for erythromycin and roxithromycin. *In vitro*, erythromycin and roxithromycin were extensively concentrated in phagocytes (Prokesch & Hand, 1982; Hand *et al.*, 1984; Carlier, Zenebergh & Tulkens, 1987) and a variety of tissue culture cells (Martin, Johnson & Miller, 1985; Villa *et al.*, 1988). However, azithromycin uptake by phagocytic cells is five- to 15-fold greater than that of erythromycin (Gladue *et al.*, 1989). Also noteworthy is the great persistence of azithromycin in tissues compared with that of erythromycin. Elimination half-lives in rat tissues after a single oral dose were about 23 h for azithromycin and only about 1 h for erythromycin (Girard *et al.*, 1987).

The macrolide and azalide antibiotic uptake into tissues is apparently due, at least in part, to extensive uptake by lysosomes, as observed for erythromycin (Carevic, Prpic & Sverko, 1975; Carlier *et al.*, 1987), roxithromycin (Carlier *et al.*, 1987; Villa *et al.*, 1988), and azithromycin (R. M. Shepard and D. E. Amacher, unpublished results). Intracellular localization of drug in lysosomes is a characteristic of many basic drugs and dyes and results from the ability of lipophilic, weak organic bases to become concentrated in acidic, membrane-bound vesicles (de Duve *et al.*, 1974; Ohkuma & Poole, 1981). Azithromycin is structurally distinct from erythromycin in that it is an azalide with two rather than one strongly basic tertiary amine (cationic) centres. Thus, the much greater affinity of azithromycin relative to erythromycin for tissues is attributed to the additional basic amine centre. Spiramycin (Osono & Umezawa, 1985; Bergogne-Bérézin, 1988) and dirithromycin (Bozler *et al.*, 1988), two other macrolide antibiotics with a second basic amine group in the molecule, also appear to have pharmacokinetics characterized by extensive tissue penetration and slow release from tissues.

High tissue penetration and long elimination half-lives from tissues are important for the in-vivo efficacy of azithromycin (Girard *et al.*, 1987; Girard, Girard & Retsema, 1990, this Volume; Retsema *et al.*, 1990, this Volume). The most important parameter in the efficacy of antibiotics is the delivery of adequate amounts of antibiotic to the site of infection, whether within tissues, interstitial fluid, abscesses, or cells. That concentrations of azithromycin in tissues are more reliable predictors of in-vivo efficacy than those in serum has recently been demonstrated. Azithromycin was very effective in Gram-positive and Gram-negative models of localized infections in mice and rats, even though serum concentrations were consistently below the MIC (Girard *et al.*, 1987; Girard *et al.*, 1990, this Volume; Retsema *et al.*, 1990, this Volume). Furthermore, clinical experience with azithromycin (Pfizer Inc., data on file) has demonstrated excellent efficacy against sexually transmitted diseases and infections of the upper and lower respiratory tracts, despite serum concentrations below the MICs of infecting organisms. Thus, tissue azithromycin is apparently available to inhibit micro-organism growth. This was consistent with the observation that azithromycin, following uptake by phagocytic cells, can later be released by these cells (Gladue *et al.*, 1989).

The affinity of azithromycin for tissues has been confirmed in humans (Foulds, Shepard & Johnson, 1990, this Volume). Since doses in humans for the treatment of most infections are expected to be about 5–10 mg/kg/day (250–500 mg/day) for a maximum of five days, the animal tissue data at 10 mg/kg for one to five or seven days presented in this paper are expected to anticipate the human experience. The tissue pharmacokinetics of azithromycin suggest that a single dose or a short course of once-

daily dosing may be very effective in the oral treatment of sexually transmitted diseases, upper and lower respiratory tract infections, and skin and skin structure infections in humans.

Acknowledgements

We acknowledge with pleasure the technical assistance of Richard A. Ferraina, Michelle A. Mullins, and Janis A. Gregoire in the dosing of animals and the collection and HPLC-EC analysis of samples. The assistance of Drs James J. Burgun and James T. Mayne in the dosing of animals and collection of samples and that of Dr T. C. Soli in the microbiological analysis of azithromycin are gratefully acknowledged. Discussions with Drs George Foulds and Donald C. Hobbs during the preparation of this manuscript were greatly appreciated.

References

Bergogne-Bérézin, E. (1987). Tissue distribution of roxithromycin. *Journal of Antimicrobial Chemotherapy* **20**, *Suppl. B*, 113–20.

Bergogne-Bérézin, E. (1988). Spiramycin concentrations in the human respiratory tract: a review. *Journal of Antimicrobial Chemotherapy* **22**, *Suppl. B*, 117–22.

Bozler, G., Heinzel, G., Lechner, U., Schumacher, K. & Busch, U. (1988). Pharmacokinetic properties and metabolic behavior of dirithromycin determine its high tissue penetration in man. In *Program and Abstracts of the Twenty-eighth Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, 1988*. Abstract 924, p. 274. American Society for Microbiology, Washington, DC.

Bright, G. M., Nagel, A. A., Bordner, J., Desai, K. A., Dibrino, J. N., Nowakowska, J. *et al.* (1988). Synthesis, in-vitro and in-vivo activity of novel 9-deoxo-9a-aza-9a-homoerythromycin A derivatives; a new class of macrolide antibiotics, the azalides. *Journal of Antibiotics* **41**, 1029–47.

Brun, Y., Forey, F., Gamondes, J. P., Tebib, A., Brune, J. & Fleurette, J. (1981). Levels of erythromycin in pulmonary tissue and bronchial mucus compared to those of amoxycillin. *Journal of Antimicrobial Chemotherapy* **8**, 459–66.

Carevic, O., Prpic, V. & Sverko, V. (1975). Correlation between erythromycin and acid phosphatase in mouse liver. *Biochimica et Biophysica Acta* **381**, 269–77.

Carlier, M.-B., Zenebergh, A. & Tulkens, P. M. (1987). Cellular uptake and subcellular distribution of roxithromycin and erythromycin in phagocytic cells. *Journal of Antimicrobial Chemotherapy* **20**, *Suppl. B*, 47–56.

de Duve, C., de Barsy, T., Poole, B., Trouet, A., Tulkens, P. & Van Hoof, F. (1974). Lysosomotropic agents. *Biochemical Pharmacology* **23**, 2495–531.

Dette, G. A. (1979). Vergleich der Gewebegangigkeit von Erythromycin. *Infection* **7**, 129–45.

Duthu, G. S. (1984). Assay of erythromycin from human serum by high performance liquid chromatography with electrochemical detection. *Journal of Liquid Chromatography* **7**, 1023–32.

Falchi, M., Teodori, F., Carraro, A., Cioce, C., Scaglione, F., Braga, P. C. *et al.* (1985). Penetration of erythromycin into tonsillar tissue. *Current Medical Research and Opinion* **9**, 611–5.

Fiese, E. F. & Steffen, S. H. (1990). Comparison of the acid stability of azithromycin and erythromycin A. *Journal of Antimicrobial Chemotherapy* **25**, *Suppl. A*. 39–47.

Foulds, G., Shepard, R. M. & Johnson, R. B. (1990). The pharmacokinetics of azithromycin in human serum and tissues. *Journal of Antimicrobial Chemotherapy* **25**, *Suppl. A*, 73–82.

Girard, A. E., Girard, D., English, A. R., Gootz, T. D., Cimochowski, C. R., Faiella, J. A. *et al.* (1987). Pharmacokinetic and in-vivo studies with azithromycin (CP-62,993), a new macrolide with an extended half-life and excellent tissue distribution. *Antimicrobial Agents and Chemotherapy* **31**, 1948–54.

Girard, A. E., Girard, D. & Retsema, J. A. (1990). Correlation of the extravascular pharmacokinetics of azithromycin with in-vivo efficacy in models of localized infection. *Journal of Antimicrobial Chemotherapy* **25**, *Suppl. A*, 61–71.

Gladue, R. P., Bright, G. M., Isaacson, R. I. & Newborg, M. F. (1989). In-vitro and in-vivo uptake of azithromycin (CP-62,993) by phagocytic cells: possible mechanism of delivery and release at sites of infection. *Antimicrobial Agents and Chemotherapy* **33**, 277–82.

Hand, W. L., Corwin, R. W., Steinberg, T. H. & Grossman, G. D. (1984). Uptake of antibiotics by human alveolar macrophages. *American Review of Respiratory Diseases* **129**, 933–7.

Lee, C.-C., Anderson, R. C. & Chen, K. K. (1956). Distribution and excretion of radioactivity in rats receiving N-methyl-C^{14}-erythromycin. *Journal of Pharmacology and Experimental Therapeutics* **117**, 265–73.

Martin, J. R., Johnson, P. & Miller, M. F. (1985). Uptake, accumulation, and egress of erythromycin by tissue culture cells of human origin. *Antimicrobial Agents and Chemotherapy* **27**, 314–9.

Ohkuma, A. & Poole, B. (1981). Cytoplasmic vacuolation of mouse peritoneal macrophages and the uptake into lysosomes of weakly basic substances. *Journal of Cell Biology* **90**, 656–64.

Osono, T. & Umezawa, H. (1985). Pharmacokinetics of macrolides, lincosamides, and streptogramins. *Journal of Antimicrobial Chemotherapy* **16**, *Suppl. A*, 151–66.

Prokesch, R. C. & Hand, W. L. (1982). Antibiotic entry into human polymorphonuclear leukocytes. *Antimicrobial Agents and Chemotherapy* **21**, 373–80.

Puri, S. K. & Lassman, H. B. (1987). Roxithromycin: a pharmacokinetic review of a macrolide. *Journal of Antimicrobial Chemotherapy* **20**, *Suppl. B*, 89–100.

Retsema, J., Girard, A., Schelkly, W., Manousos, M., Anderson, M., Bright, G. *et al.* (1987). Spectrum and mode of action of azithromycin (CP-62,993), a new 15-membered-ring macrolide with improved potency against gram-negative organisms. *Antimicrobial Agents and Chemotherapy* **31**, 1939–47.

Retsema, J. A., Girard, A. E., Girard, D. & Milisen, W. B. (1990). Relationship of high tissue concentrations of azithromycin to bactericidal activity and efficacy *in vivo*. *Journal of Antimicrobial Chemotherapy* **25**, *Suppl. A*, 83–9.

Shepard, R. M. (1987). High pressure liquid chromatography (HPLC) assay for azithromycin (CP-62,993/XZ-450) in serum and tissues. In *Program and Abstracts of the Twenty-seventh Interscience Conference on Antimicrobial Agents and Chemotherapy, New York, 1987.* Abstract 238, p. 138. American Society for Microbiology, Washington, DC.

Villa, P., Sassella, D., Corada, M. & Bartosek, I. (1988). Toxicity, uptake, and subcellular distribution in rat hepatocytes of roxithromycin, a new semisynthetic macrolide, and erythromycin base. *Antimicrobial Agents and Chemotherapy* **32**, 1541–6.

Washington, J. A. & Wilson, W. R. (1985*a*). Erythromycin: a microbial and clinical perspective after 30 years of clinical use, part 1. *Mayo Clinic Proceedings* **60**, 189–203.

Washington, J. A. & Wilson, W. R. (1985*b*). Erythromycin: a microbial and clinical perspective after 30 years of clinical use, part 2. *Mayo Clinic Proceedings* **60**, 271–8.

Wilson, J. T. & van Boxtel, C. J. (1978). Pharmacokinetics of erythromycin in man. *Antibiotics and Chemotherapy* **25**, 181–203.

Wise, R. (1986). The clinical relevance of protein binding and tissue concentrations in antimicrobial therapy. *Clinical Pharmacokinetics* **11**, 470–82.

Wise, R., Kirkpatrick, B., Ashby, J. & Andrews, J. M. (1987). Pharmacokinetics and tissue penetration of roxithromycin after multiple dosing. *Antimicrobial Agents and Chemotherapy* **31**, 1051–3.