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Bioavailability of isradipine in young and old rats: effect of mode of administration

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Abstract—The bioavailability of isradipine has been examined in 7- and 52-week-old rats after oral (12·5 mg kg^{-1}) or intravenous (2·5 mg kg^{-1}) doses as a solution and administration of various doses (1·8–85·5 mg kg^{-1}) in the diet. Serial plasma samples were obtained from each rat and the drug concentration was determined by radioimmunoassay. Absorption from the dose given by gavage was rapid but when administered in a drug-diet mixture, isradipine appeared in the plasma slowly and in a manner reflecting the feeding pattern. Its absolute bioavailability from the drug-diet mixture averaged 3% over the dose range tested. By gavage its bioavailability was enhanced to 5% of dose with peak plasma values approximately 7 times higher than from a comparable dose in the diet. The low oral bioavailability of isradipine in the rat was most likely due to extensive first-pass metabolism. The decline in plasma concentrations was biexponential, with a mean terminal half-life of 3·6–3·7 h after oral or intravenous dosing. The pharmacokinetic characteristics of isradipine examined were independent of the age of the rat, except that its volume of distribution decreased with age. The older rats also showed a greater inter-animal variability in isradipine bioavailability from the drug-diet mixture.

In most toxicological studies in the rat, the test drug is administered either by gavage or by incorporation into the diet. There is ample evidence to show that the absorption and/or metabolism of some drugs may be affected by the mode of oral administration used. For example, while the absorption and bioavailability of captopril were greater after gavage than given in the diet (Singhvi et al 1981), continuous dietary administration of cefatrizine yielded higher, albeit later, peak plasma concentrations and greater overall bioavailability than the same single daily dose by gavage (Van Harken & Hottendorf 1978). In contrast, the bioavailability of *N*-acetylprocainamide (Kamath et al 1981) and the extent of absorption of fluperlapine (Dain & Jaffe 1988) were unaffected when administered in the diet compared with gavage.

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Isradipine is a dihydropyridine derivative with a potent calcium channel blocking activity (Cortes et al 1983; Hof & Rüegg 1988). In the present study, its bioavailability in the rat has been evaluated after oral and intravenous dosing as a solution, as well as various doses mixed in with the diet. The study was intended to provide pharmacokinetic information in support of the toxicity trials in this species. Because of the subchronic or chronic nature of the toxicity studies, and the potential effect of age on the absorption and disposition of some drugs (Kapetanovic et al 1982a, b; Yacobi et al 1983), both 7- and 52-week-old rats were used.

Materials and method

Animals. Male Sprague-Dawley rats (Charles River) of two different age groups (49–50 days, ca 250 g and 52 weeks, ca 500 g) were housed individually in metabolism cages at room temperature (25°C) and allowed free access to water and food (Purina rat chow) or a drug-diet mixture.

Dosing and plasma collection. i) *Drug-diet mixture.* The intended

doses of isradipine were 2·5, 12·5, and 62·5 mg kg^{-1} day^{-1}. Therefore, based on an average daily food consumption of 20 g, mixtures of isradipine (Sandoz) and certified rat chow (Ralston-Purina) at drug:diet ratios of 1:32000, 1:6400, and 1:1280 were prepared by geometric dilution for the 7-week-old rats. Similarly, drug:diet ratios of 1:16000, 1:3200, and 1:640 were prepared for the heavier, older group. Lactose (1%) was added to the last batch to improve the palatability of the drug-diet mixture. Owing to reduced consumption of the apparently less palatable high dose, an additional group of 7-week-old rats was given a 1:640 drug:diet mixture in an attempt to deliver the intended high dose.

The study was conducted using four rats for each dose group. At 2000 h on the day of study, each rat was presented with a feeder cup with sufficient drug-diet mixture for 24 h (one feeding cycle). At 24 h, the amount of mixture remaining in the cup, and spillage, were determined. The drug-diet mixture was then replaced with standard rat chow. Serial blood samples (250 μL) were collected in heparinized micropipettes (Drummond) via the tail vein of each rat just before dosing and at 3, 6, 9, 12, 15, 18, 21, 24, 36, 44, 60, and 66 h after presenting the dose. Plasma was separated by centrifugation and immediately frozen until analysis.

ii) *Gavage and intravenous doses.* A 2·5 mg mL^{-1} solution of isradipine in water containing 2·5% DMSO and 3·5% Tween 80 was prepared. Four rats from each age group received 5 mL kg^{-1} of the solution orally while another four received 1 mL kg^{-1} as an injection, via the jugular vein exposed surgically under light ether anaesthesia. Plasma was collected immediately before and at 0·25, 0·5, 1, 2, 3, 4, 6, 8, 12, 24, 32, and 48 h after dosing. From the intravenously dosed rats, an additional sample was obtained 5 min postdose.

Radioimmunoassay for isradipine. Plasma concentrations of isradipine were determined by a specific radioimmunoassay (RIA) (Clifton et al 1988). Analysis using 20 μL aliquots was done on undiluted or diluted (up to 1:100 v/v with assay buffer) rat plasma to ensure that for the wide concentration range encountered (ca 0·1–5000 ng mL^{-1}), most measurements would fall within the optimal range of the standard curve (20–80% of blank response, equivalent to 1–400 ng mL^{-1} in undiluted plasma, and showing within and between assay coefficients of variation of 3 and 10%, respectively, in this concentration range). Acceptable assay variability and recovery (< 20% CV) were obtained with concentrations as low as 0·25 ng mL^{-1}.

Data treatment. The area under the concentration-time curve (AUC) of isradipine was calculated by the trapezoidal rule. The elimination half-life was estimated by linear regression of the terminal log-linear phase of the concentration-time profile. Statistical comparisons of these parameters between age groups were performed using a two-tailed *t*-test.

Results and discussion

The doses of isradipine administered to rats through a drug-diet mixture are summarized in Table 1. Due to a smaller diet

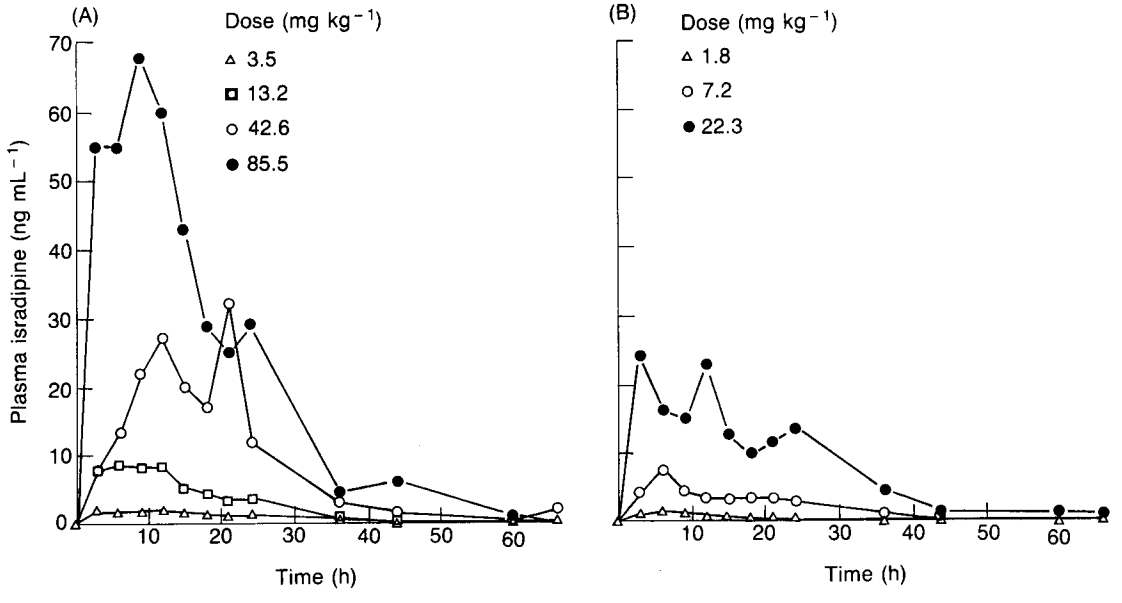

FIG. 1. Mean plasma concentrations of isradipine in (A) 7-week-old and (B) 52-week-old rats administered a drug-diet mixture.

Table 1. Doses of isradipine in rats administered a drug-diet mixture.

	7-week-old				52-week-old		
Drug:diet ratio	1:32000	1:6400	1:1280	1:640	1:16000	1:3200	1:640
Rat weight (g)	215±11[1]	222±10	232±5·9	250±22	459±12	441±14	560±30
24 h drug-diet consumption (g)	24·1±0·5	18·6±4·7	12·6±5·7	13·7±1·8	12·9±5·9	10·2±1·7	7·8±4·6
Dose of isradipine (mg kg^{-1})	3·5±0·2	13·2±3·7	42·6±19·5	85·5±11·0	1·8±0·9	7·2±1·1	22·3±13·4

[1] mean ± s.d., n = 4.

Table 2. Comparison of dose-normalized C_{max} and AUC values between 7- and 52-week-old rats.

Dose	Parameter*	7-week-old	52-week-old	*t*-test
Drug-diet				
Mixture	C_{max}/Dose	0·8±0·3 (16)[1]	1·3±0·6 (12)	$P<0·01$
	AUC/Dose	15±2·8 (16)	19±7·6 (12)	$P<0·05$
Gavage	C_{max}/Dose	5·3±1·6 (4)	9·6±2·0 (4)	$P<0·05$
	AUC/Dose	23±3·0 (4)	40±14 (4)	$P<0·10$
Intravenous	C_{max}/Dose	648±524 (4)	2257±1082 (4)	$P<0·05$
	AUC/Dose	478±136 (4)	786±212 (4)	$P<0·05$

[1] Mean±s.d. (n).
* C_{max} (ng mL^{-1})/Dose (mg kg^{-1}).
AUC (ng h mL^{-1})/Dose (mg kg^{-1}).

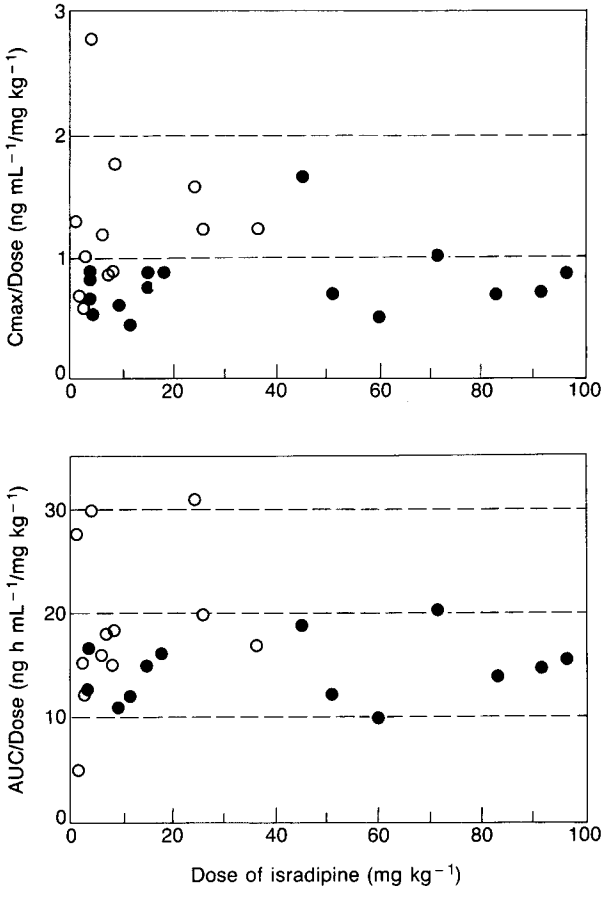

FIG. 2. Dose-normalized C_{max} and AUC values of individual rats (●, 7-week-old; ○, 52-week-old) administered isradipine as a drug-diet mixture.

consumption than anticipated, the mean doses in the 52-week-old rats were somewhat lower than those intended. In rats given the drug-diet mixtures, isradipine appeared in the plasma slowly, thus reflecting the usual pattern of feeding (Fig. 1). Although peak plasma concentrations were observed at approximately 9–12 h, the curves effectively showed a plateau between 3 and 24 h, by when dosing had stopped; then isradipine levels declined to below or near the detection limit of the RIA by 44 h. As infrequent blood sampling took place in the post-dosing phase, elimination half-lives were not calculated in rats receiving the drug-diet mixtures. To evaluate the dose-bioavailability relationship, the dose-normalized C_{max} (peak plasma concentration) and AUC values for individual rats are plotted against the dose of isradipine in Fig. 2. In the 7-week-old rats, these parameters were consistent between animals and demonstrated a linear dose-bioavailability relationship. The 52-week-old rats showed no definitive dose-related trend in either C_{max}/Dose or AUC/Dose, although both parameters reflected a relatively large inter-animal variability. Statistical comparisons showed that the mean C_{max} and AUC, normalized by the dose, were significantly greater in the 52-week than in the 7-week-old rats (Table 2).

In contrast to dosing in the diet, the absorption rate of isradipine after gavage was rapid. In both age groups of rats, the peak plasma concentration was reached at 0·5 h postdose (Fig. 3) and, after normalizing by the dose, was approximately 7 times higher than that obtained from a drug-diet mixture (Table 2). Drug levels subsequently declined to $<10\%$ of the peak value within 12 h, the terminal half-life being 3·7±0·4 (s.d.) h in the 7-week-old rats and 3·6±1·1 (s.d.) h in the 52-week-old rats. The mean AUC/Dose after gavage was 1·5 and 2·1 times that of the drug-diet mixtures in the 7-week and 52-week-old rats, respectively (Table 2). Thus, the rate of absorption as well as the overall bioavailability, of isradipine were substantially greater from gavage than from diet administration. The increased bioavailability observed with dosing by gavage was probably related to the more rapid presentation of isradipine to the liver, which could have led to partial saturation of presystemic metabolism (Shargel & Yu 1980). Alternatively, it is also possible that the actual amount of isradipine absorbed from the diet was smaller than that from the oral solution due to potential drug-diet interactions or binding. As shown in Table 2, the dose-normalized values of C_{max} and AUC were again greater in the 52-week- than in the 7-week-old rats. Therefore, in this respect, the rat is similar to humans. Previous investigators (Schran et al 1988) have found significantly higher plasma levels of isradipine (by 25%) in elderly, normal male subjects than in young normal males.

Compared with the oral doses, the intravenous dose yielded much higher initial plasma concentrations, the mean value at 5 min being 1541 and 5638 ng mL^{-1} in the 7-week- and 52-week-old rats, respectively (Fig. 3). Drug concentrations declined biexponentially and the terminal half-life (7-week: 3·7±1·6 h; 52-week: 3·6±1·4 h) was identical to that observed after oral dosing in the same age group. Comparison of the dose-normalized AUC values after oral and intravenous dosing indicated that the absolute bioavailability of isradipine was ca 3% for the drug-diet mixture and 5% for the gavage solution in both age groups. Since orally administered isradipine is almost completely absorbed in the rat (P. Tanner, M. Azria, unpublished data), the relatively low bioavailability is most likely due to extensive first-pass metabolism. This notion is

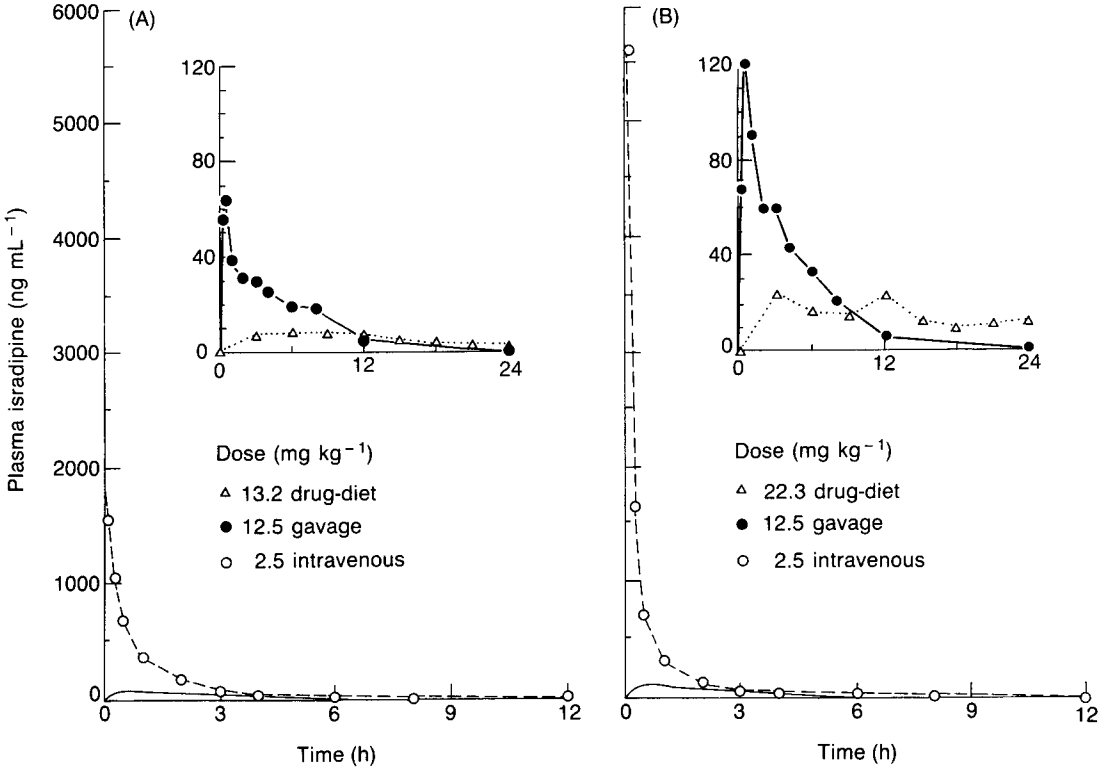

FIG. 3. Mean plasma concentrations of isradipine in (A) 7-week-old and (B) 52-week-old rats after oral and intravenous dosing.

supported by a rapid plasma clearance (Dose/AUC = 35 mL min^{-1} kg^{-1} in the 7-week-old rats) which is virtually identical to the normal liver plasma flow rate of 33 mL min^{-1} kg^{-1} in the rat (Bischoff et al 1971). The finding is also similar to previous observations in humans, in which the bioavailability of isradipine was estimated to be only 17% due to first-pass metabolism (Tse & Jaffe 1987). As was the case in the oral dose studies, the C_{max} and AUC of isradipine following intravenous administration were greater in the 52-week-old than in the 7-week-old rats, despite similar half-lives in the two groups. Therefore, it appears that the volume of distribution of isradipine in the 52-week-old rats might be smaller than that in the younger rats, as suggested by a 3·7-fold difference in the initial plasma concentration between the two groups. Similar decreases in distribution volume with age have been documented for numerous drugs in humans, partially due to a significant decrease in total body fluid in the elderly (Lamy 1980).

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