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The pharmacokinetics of amlodipine in healthy volunteers after single intravenous and oral doses and after 14 repeated oral doses given once daily

J. K. FAULKNER & D. McGIBNEY
Department of Clinical Research, Pfizer Central Research, Sandwich, Kent

L. F. CHASSEAUD, J. L. PERRY & I. W. TAYLOR
Department of Metabolism and Pharmacokinetics, Huntingdon Research Centre, Huntingdon

1 Intravenous administration of amlodipine (single dose, 10 mg) to 12 volunteers gave a mean plasma half-life of 34 h, mean clearance of 7 ml min^{-1} kg^{-1} and a mean apparent volume of distribution of 21 l kg^{-1}.

2 Oral administration (single dose, 10 mg) to the same 12 volunteers gave a mean systemic availability of 64% and a mean plasma half-life of 36 h.

3 In a second study, repeated oral administration (once daily for 14 days, 15 mg) to 28 volunteers resulted in steady state plasma drug concentration being reached after seven doses, an accumulation of approximately threefold and a mean half-life of 45 h.

Keywords amlodipine pharmacokinetics single i.v./oral repeated oral

Introduction

Amlodipine is a dihydropyridine calcium channel blocking agent under evaluation in patients with essential hypertension and angina pectoris. The pharmacological properties of amlodipine in the dog, namely gradual onset and long duration of action, have been described by Dodd & Machin (1985). Its pharmacokinetic properties in this species have been reported by Beresford *et al.* (1985), the principal features being a terminal elimination half-life of about 30 h and oral bioavailability approaching 100%. In particular, the relatively long plasma elimination half-life of amlodipine distinguishes it from other calcium channel blocking agents which are already in widespread clinical use, and suggested the likelihood of a small difference between peak and trough plasma drug concentrations when amlodipine is given once daily.

The comparative pharmacokinetics of amlodipine after single intravenous (i.v.) and oral doses and its pharmacokinetics after repeated oral doses have been evaluated in healthy male volunteers. The studies were also conducted to investigate the safety of amlodipine, the dose levels chosen being those expected to be required in clinical practice (Jackson *et al.*, 1985).

Methods

Subjects and clinical procedures

(i) *Single dose intravenous* vs *oral comparison* This study was of balanced randomised cross-over design and involved 12 healthy male volunteers, mean age 25.8 ± 3.8 years, mean weight 66.6 ± 6.3 kg, who were studied on two occasions 34 days apart at the Clinique St Remi, Brussels. All subjects provided written informed consent to participate in the study. The protocol was approved by the independent ethics review committee of the Clinique St Remi and the study conducted in accordance with the provisions of the Declaration of Helsinki (revised Tokyo 1975).

On both occasions subjects fasted from the evening before drug administration but were allowed light carbohydrate meals 5 h and 9 h

after the dose. Tobacco, tea, coffee and other caffeine-containing beverages were not permitted. The control period was 1 h prior to the dose, during which subjects rested supine. An intravenous cannula was inserted into a forearm vein and measurements of the heart rate and blood pressure were obtained, and an ECG was recorded. After control measurements and blood sampling were complete, volunteers received either amlodipine 10 mg by infusion via the intravenous cannula (1 mg min^{-1}) or as 2 × 5 mg capsules orally with 100 ml water. Blood samples for plasma drug assay were obtained at the following times after dosing: i.v. route 0.1, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120 and 144 h; oral route 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, 96, 120 and 144 h. Subjects were restricted to the laboratory for the first 24 h period after the respective doses but were then allowed to leave and attend only for measurements and samples at the specified times up to 144 h after dose. Blood samples were collected in lithium heparin tubes and plasma was prepared immediately, frozen and stored at −20° C in glass tubes until analysed.

Measurements of blood pressure and heart rate were obtained in duplicate after subjects had been supine for 5 min and after standing for 2 min and were made at the following times after dosing: i.v. and oral routes, 2, 4, 6, 8, 12 and 24 h; i.v. only, also at 0.1, 0.25, 0.5 and 1 h. Measurements were made immediately preceding blood sampling, which was timed to coincide exactly with the protocol times. The blood pressure was measured with an automatic sphygmomanometer and the heart rate was calculated from the ECG.

Safety assessments consisted of routine haematological, serum and urinary tests carried out pre-dose and 24 and 120 h after dose on both occasions. ECG monitoring was conducted during intravenous infusion of drug and for 0.5 h afterwards. ECG recordings (12 lead) were also made at 1 h after intravenous dose and at 8 h after oral dose.

(ii) *Repeated oral administration* This study was a 14 day double-blind parallel group comparison of amlodipine 15 mg once daily with placebo in 56 healthy male volunteers, mean age 26.1 ± 36 years, mean weight 68.2 ± 7.0 kg. The study was conducted at the IPHAR Institute of Clinical Pharmacology, Munich. Ethical review of the protocol was by the independent committee appointed by the IPHAR Institute. All subjects provided written informed consent and the standards of conduct of the study were as described in the previous section. Subjects were studied in two consecutive groups of 28 and were resident in the Clinical Pharmacology Unit throughout the dosing period. Medication was provided as amlodipine 3 × 5 mg capsules or matching placebo capsules with 100 ml water at 09.00 h each day. Administration of amlodipine or placebo was randomised and balanced within each dosing group. Subjects were under close supervision when the daily dose was given to ensure complete compliance.

Measurements of heart rate and blood pressure and ECG readings were obtained prior to entry into the study and twice or three times daily throughout the dosing period. Venous blood samples for measurement of plasma drug concentration were collected pre-dose, at 4, 8, 12 and 24 h after the first dose, pre-dose on days 7, 10, 12 and 14, and at 4, 8, 12, 24, 72, 120 and 168 h after the final dose on day 14.

Drug safety assessments consisted of routine haematological, serum and urinary tests which were made at regular intervals during the study and 72 h after the final dose. A 12 lead ECG was obtained from each subject at 6 h post dose at intervals during the study.

Analysis of amlodipine in plasma

The method of analysis was based on the mass spectrometric method of Beresford & McCrae (1984, personal communication) but gas chromatography with electron capture detection was employed. To plasma (1 ml) was added 2 or 10 μl of a methanolic solution (0.5 μg base ml^{-1}) of UK-52,829 (the 2′,3′-dichlorophenyl analogue of amlodipine) to serve as the internal standard. After addition of 0.2 M borate buffer (2 ml), pH 9, plasma was extracted with *t*-butyl methyl ether (4 ml). The separated ethereal phase was then extracted with 0.1 M citric acid (2 ml) and the ethereal layer discarded. To the aqueous extract was added 1 M potassium carbonate solution (1 ml) followed, after brief mixing, with trimethylacetyl chloride (20 μl) to form the acyl derivative during 10 min of gentle mechanical mixing. The aqueous reaction mixture was extracted with *t*-butyl methyl ether (1 ml) and the organic phase evaporated to dryness. The residue was re-dissolved in *t*-butyl methyl ether (50 μl) of which 3 μl were injected into a gas chromatographic capillary column using the dropping needle technique.

The chromatography column was 25 m × 0.31 mm (i.d.) fused silica coated with crosslinked 5% phenylmethyl silicone gum (film thickness 0.17 μm) supplied by Hewlett-Packard. A 5880A Hewlett Packard gas chromatograph fitted with an electron capture detector was used, the carrier

gas (1 ml min^{-1}) and detector make up gas (35 ml min^{-1}) being nitrogen. The temperature was programmed from 280 to 320° C at 20° C min^{-1}, and then kept at 320° C for 9 min. The injection port and detector temperatures were maintained at 320° C. Under these conditions, the retention times of amlodipine and the internal standard were 4.4 and 5.4 min, respectively. All serial samples from an individual were analysed together and calibration standards and quality control test samples were included in every batch analysis. Approximately 10% of samples were analysed twice, the replicate sample being included at random. The stability of amlodipine in plasma stored at approximately −20° C was determined at 1, 2 and 6 months. The precision of the method of analysis of amlodipine in plasma (n = 5) was ±16.7%, ±12.5%, ±3.2% and ±5.6% at added concentrations of 0.2, 0.5, 2 and 12 ng ml^{-1}, respectively. The lower limit of reliable measurement was taken as 0.2 ng ml^{-1}, which was the lowest calibration point.

Pharmacokinetic calculations

Data were analysed by model-independent methods. Inspection of the plasma drug concentration–time curves showed that in some cases, initial distribution phases were difficult to distinguish and accordingly the data were not analysed in terms of a multi-compartment model. Calculation of terminal elimination rate constants (k_{el}) was by least squares regression analysis of unweighted data, the times being usually from 6 or 8 h after dose until 144 h after dose. Areas under the drug concentration–time curve (AUC) were estimated using the log-linear trapezoidal rule. For intravenous data, time zero was taken as the end of infusion and the concentration at this time estimated by linear extrapolation using the first two available data points (0.1 and 0.25 h) to allow inclusion of the area prior to the first sample time. Drug clearance was calculated by dividing the intravenous dose by the AUC. The apparent volume of distribution (V [area]) was calculated by dividing the clearance by the elimination rate constant. For the comparison between the pharmacokinetics of amlodipine after oral and intravenous single doses, analysis of variance was carried out on log 10 transformed AUCs and terminal phase rate constants for individuals. For the multiple dose study, the elimination rate constants were used to calculate the theoretical extent of accumulation using the equation $R_{th} = 1/1-e^{-k\tau}$ where R_{th} is the predicted degree of accumulation, k the elimination rate constant and τ the dosage interval (Gibaldi & Perrier, 1982). The actual accumulation R was estimated using the equation R = AUC(0–24) fourteenth dose/AUC(0–24) first dose.

Results

Clinical aspects

Subjects in both studies completed their period of clinical evaluation without any untoward effects. There were no clinically important changes in blood pressure or heart rate after treatment with amlodipine, either in the comparison between single i.v. and oral doses or after repeated oral administration of amlodipine for 14 days with a placebo control group. There were no clinically significant changes in laboratory safety data attributable to amlodipine in either study.

Pharmacokinetics

Single intravenous and oral dose of amlodipine
Mean plasma drug concentration–time curves after i.v. and oral administration are shown in Figure 1. After i.v. administration, the mean concentration at 0.1 h was 144 ng ml^{-1} with a range of 41–451 ng ml^{-1}, at 2 h was 7.6 ng ml^{-1} with a range of 5.9–9.2 ng ml^{-1} and at 144 h was 0.44 ng ml^{-1} with a range of 0.2–0.7 ng ml^{-1}.

After oral administration, the mean ± s.d. of individual peak observed concentrations was 5.9 ± 1.2 ng ml^{-1} and the mean ± s.d. time to peak was 7.6 ± 1.8 h.

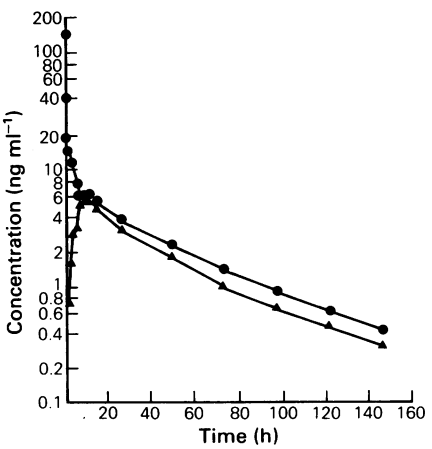

Figure 1 Mean drug concentrations in plasma after administration of 10 mg of amlodipine as an intravenous infusion (●) and orally (▲) as two capsules to 12 human subjects.

Table 1 Pharmacokinetic parameters of amlodipine after single i.v. and oral doses (mean ± s.d.)

	i.v.	*Oral*
Peak concentration (ng ml^{-1})	—	5.9 ± 1.2
Time of peak (h)	—	7.6 ± 1.8
AUC (0→∞) (ng ml^{-1} h)	371 ± 69	238 ± 53
Bioavailability (%)	—	64 (range 52–88)
Clearance (ml min^{-1} kg^{-1})	7.0 ± 1.3	—
V [area] (l kg^{-1})	21.4 ± 4.4	—
$t_{½}$ (h)	33.8 ± 5.3	35.7 ± 6.1
k_{el} (h^{-1})	0.021 ± 0.0032	0.020 ± 0.0036

Mean values of pharmacokinetic parameters, with s.d. or range of values as appropriate, are listed in Table 1.

Repeated oral dose of amlodipine The mean plasma drug concentration–time curve after administration of amlodipine once daily for 14 days is shown in Figure 2. Mean values (± s.d.) of calculated pharmacokinetic parameters are shown in Table 2. The theoretical accumulation ratio expected from a mean elimination rate constant of 0.016 h^{-1} is 3.12. When individual values of theoretical accumulation were correlated with observed accumulation, R, (determined from AUCs), there was no statistically significant difference between the slope of the regression line, forced through the origin, and unity ($P > 0.05$). The regression line had a slope of 1.06 with confidence limits of 0.91 and 1.20.

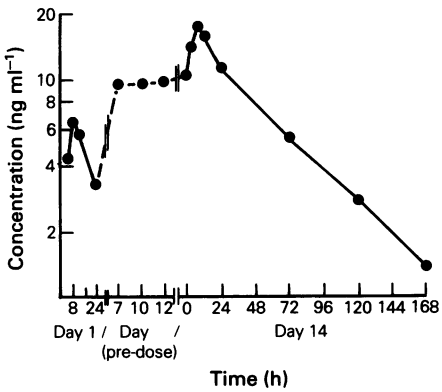

Figure 2 Mean plasma drug concentrations after daily oral administration of 15 mg of amlodipine for 14 days to 28 human subjects.

Discussion

The main pharmacokinetic property of amlodipine relevant to its therapeutic use is its relatively long elimination half-life of 45 h after repeated doses. This results in accumulation with steady state peak, average and minimum drug concentrations during the dosage interval being approximately three-fold higher than the corresponding concentrations observed after a single dose. Steady state was apparently reached by the seventh dose, as judged by the pre-dose amlodipine concentrations on days 7, 10, 12 and 14 (Figure 2), which is in accord with the general rule of three to four plasma drug half-lives being required to reach steady state. After the 14th dose, the mean concentration of amlodipine 24 h after the dose was 66% of that of the mean peak concentration. This small degree of fluctuation

Table 2 Pharmacokinetic parameters of amlodipine after 14 repeated oral doses given once daily (mean ± s.d.)

	Day 1	*Day 14*	*Ratio Day 14/Day 1*
Peak concentration (ng ml^{-1})	6.9 ± 2.6	18.1 ± 7.1	2.6(0.9–5.7)
Time of peak (h)	8.9 ± 3.7	8.7 ± 1.9	—
Trough concentration (ng ml^{-1})	3.3 ± 1.2	11.8 ± 5.3	3.6(1.6–11.7)
Average concentration* (ng ml^{-1})	4.5 ± 1.6	14.5 ± 5.8	3.2(1.2–7.4)
$t_{½}$ (h)	—	44.7 ± 8.6	—
k_{el} (h^{-1})	—	0.016 ± 0.0034	—

*Average concentration is for the dose interval and is AUC (0–24)/24.

between peak and trough amlodipine concentrations favours the attainment and maintenance of a uniform therapeutic response in patients.

The elimination rate constants determined for individuals after the final dose of repeated oral administration had a unimodal distribution in the group of 28 subjects studied (Figure 3) and a range which was not unduly large (c.v. ± 21%). It appears that amlodipine accumulates in the plasma of individuals to a degree which can be predicted from the rate constant for many, but not all individuals. The close agreement between the predicted accumulation, calculated from the mean elimination rate constant, and the actual accumulation of amlodipine for the group is indicative of the model having some utility in calculations of dose size and frequency, for example, loading dose.

Comparative pharmacokinetics after single i.v. and oral dose show that the systemic availability of oral amlodipine is moderately high, 64%, and not subject to excessive inter-subject variability (range 0.52–0.88). From the low plasma clearance of 7 ml min^{-1} kg^{-1}, it could be expected that the systemic availability would be approximately 75%, assuming complete absorption of oral doses and elimination solely by hepatic metabolism.

The apparent volume of distribution (V [area]) of amlodipine is comparatively high at 21.4 l kg^{-1}, indicating that a large proportion of the body load of drug is in the tissues rather than in the blood. After i.v. dose, the initial decrease in plasma concentrations is rapid and movement of drug from the blood and into tissue is complete or nearly complete, depending on the individual, at 0.75–2 h. After oral administration, absorption, is detectable within 0.5–1 h but is slow as the peak drug concentration is not reached until a mean time of approximately 8 h. The slow absorption is a property of the drug itself rather than its formulation in capsules as amlodipine is apparently absorbed equally slowly from a solution formulation (Chasseaud & Taylor, 1984, unpublished data).

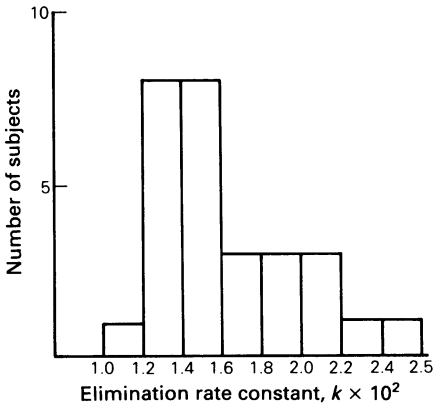

Figure 3 Frequency histogram of elimination rate constant of amlodipine for 28 subjects after repeated oral dose.

The long half-life of amlodipine has a potentially beneficial influence on the steady state profile of circulating drug when drug is taken once daily. It is likely that patients can be treated with amlodipine once daily and that the drug's overall pharmacokinetic properties will contribute to a gradual onset of action and consistency over 24 h in therapeutic response.

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