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Angiotensin II antagonism and plasma radioreceptor-kinetics of candesartan in man

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Aims The pharmacodynamic properties of the angiotensin II antagonist candesartan in humans were assessed from the rightward shifts of angiotensin II dose-effect curves (Schild regression technique). The pharmacokinetic characteristics were determined by radioreceptor assay (r.r.a.) and h.p.l.c.

Methods Twelve healthy male volunteers received single oral doses of 4, 8 and 16 mg candesartan cilexetil and placebo. Plasma was obtained for h.p.l.c. and r.r.a. (receptors: rat lung; radioligand: [^{125}I-Sar^{1}Ile^{8}]-angiotensin II). Before and up to 24 h post dosing angiotensin II was infused in ascending dose steps until blood pressure (systolic and/or diastolic) increased by +25 mmHg. Individual angiotensin II dose-effect curves were fitted according to an E_{max} model and dose ratios (DR) calculated from the antagonist induced rightward shifts.

Results Candesartan, the active metabolite of candesartan cilexetil, declined from peak concentrations at about 4 h with a $t_{1/2}$ of about 6 h. A linear relation (slope 1) between h.p.l.c. and r.r.a. data revealed that there is no other active metabolite. DR at 6–9 h post dosing reached a maximum of about 30 and at 24 h still amounted to 4–7, indicating the persistence of a relevant antagonistic effect *in vivo*. The apparent K_i-doses (derived from Schild regression plots) indicated a high potency (1.9 mg at 24 h) and slow decline of effect. Between plasma concentrations and antagonistic effect a counterclockwise hysteresis was visible.

Conclusions A longer persistence of the antagonistic effect at the receptor site than expected by the presence in plasma indicates a slow off-rate of candesartan cilexetil from *in vivo* receptors. This provides an additional rationale for the observed 24 h therapeutic activity of candesartan cilexetil.

Keywords: angiotensin II antagonists, candesartan dose-effect curves, radioreceptor assay, Schild regression technique

Introduction

Blockade of the renin-angiotensin-system (RAS) has been shown to be effective and useful in the treatment of hypertension and cardiac failure [1]. Angiotensin converting enzyme (ACE) inhibitors have been used for many years in these indications. These drugs have several additional mechanisms of action which besides their effect on angiotensin II include inhibition of bradykinin catabolism and interaction with prostaglandins [2]. The interaction with bradykinin metabolism is considered responsible for side effects like angioneurotic edema and the commonly reported dry cough [2, 3]. For a more specific inhibition of the RAS antagonizing angiotensin II at the receptor site should be effective. In addition, this mechanism might block angiotensin II in local RAS and angiotensin produced not by ACE but by chymase or other bypass enzymes [4]. Chymase dependent angiotensin formation seems to be important in the heart, where more than 80 percent of angiotensin formation was found to be mediated by this enzyme [5, 6]. Recent studies revealed widespread tissue distribution of human chymase indicating a probable significant influence in other tissues also [7].

In the class of orally active, non-peptide angiotensin II receptor antagonists, losartan is the first and most investigated substance. All currently developed compounds bind selectively to the angiotensin II receptor of the AT_1 subtype [8], antagonizing angiotensin II responses *in vivo* [9]. Candesartan cilexetil (TCV 116), developed as an angiotensin II antagonist with a long duration of action, is currently undergoing phase III clinical trials. Candesartan cilexetil is a prodrug with the active metabolite candesartan (CV-11974), which was found to have high selectivity and affinity to the AT_1 receptor [10]. Candesartan cilexetil shows potent and long-lasting antihypertensive effects in several animal models [11]. In patients with essential hypertension, 4–16 mg candesartan cilexetil significantly reduced blood

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pressure with maximum effects at 4–6 h and a sustained effect at 24 h after dose [12, 13]. Receptor antagonists may be quantitatively characterized *in vitro* by their interaction with the specific agonist. This standard procedure from experimental pharmacology—according to Arunlakshana & Schild—establishes agonist dose-effect curves in the presence of different doses of the antagonist (Schild regression technique [14]). It has been successfully applied *in vivo* in man to characterize duration and extent of the antagonistic properties of several drug groups and has been introduced as a testing tool for the renin-angiotensin system a decade ago [15–19].

The primary objective of the present study was to establish the quantitative pharmacodynamics following various single oral doses of candesartan cilexetil using this Schild regression technique. Secondary objectives were to further evaluate the pharmacokinetics of the active metabolite candesartan by conventional h.p.l.c. method and by a radioreceptor technique allowing global assessment of the biologically active substances *ex vivo in vitro*. Consequently the relationship between pharmacokinetics and pharmacodynamics was established.

Methods

The investigation was approved by the local ethics committee of the Medical State Council, Rhineland-Palatinate (Landesaerztekammer Rheinland-Pfalz) on October 26, 1994. All volunteers gave their consent in writing after being informed of the aims, nature and procedures of the trial.

Subjects

Twelve healthy male volunteers (as assessed from physical examination, ECG, haematological and clinical chemistry, urinalysis) participated in the study. Their mean age was 27 years (range 23–34 years), and their mean body weight was 77 kg (66–93 kg).

Study design and conditions

The study was carried out in a placebo-controlled, double-blind, four way crossover design with a 7 day washout period between the treatments. The treatment sequences were randomly allocated. Each volunteer received single oral doses of 4, 8, 16 mg candesartan cilexetil and placebo. The volunteers were studied after an overnight fast and continued fasting until 6.5 h after administration. Standardized food was served 6.5 h, 9.5 h and 12.5 h post administration. The volunteers abstained from xanthines and alcohol from 12 h before until the end of each study day. Tolerability was tested repeatedly by clinical examinations, standard laboratory and ECG.

Drug infusions

Before dosing and at 3 h, 6 h, 9 h, 12 h and 24 h after administration of study drug exogenous angiotensin II diluted with physiological saline solution was continuously infused in increasing dose steps at 3 min intervals (0.17 up to 20 $\mu g\,min^{-1}$) as described in detail previously [15, 17–19]. Three min after dose increase a steady state in blood pressure increase was achieved, as shown even for angiotensin I by Essig *et al.* [19]. The dosage of angiotensin II infusion was discontinued when systolic (SBP) and/or diastolic blood pressure (DBP) had exceeded the baseline value by +25 mmHg or the maximum infusion rate of 20 $\mu g\,min^{-1}$ had been reached. Under these conditions blood pressure was measured using a cuff mercury manometer and Korotkoff phase I and V sounds were used for the determination of SBP and DBP. All measurements were performed with the subjects lying in a relaxed recumbent position.

Drug assays

An indwelling cannula was fixed in a suitable antecubital vein of each subject and blood samples for pharmacokinetics were taken before and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 9.0, 12.0 and 24.0 h after medication. Concentrations of candesartan, the active metabolite of candesartan cilexetil, were determined using a validated column-switching reversed phase high performance liquid chromatography assay (h.p.l.c.) method) [20]. Sample preparation was done by solid phase extraction including the addition of an internal standard. Detection was carried out by u.v.-absorption at 210 nm. Quantification was performed according to the internal standard method. Precision and accuracy of quality control samples was between 2.9 and 6.9% and 95.0 and 99.6%, respectively, in the working range of the method.

An *ex vivo in vitro* radio receptor assay (r.r.a.) using angiotensin II receptors from lung tissue of rats was performed to evaluate the degree of radioligand displacement by active metabolites, including candesartan and any possible other active metabolite, measured as relative decrease of radioligand [^{125}I-Sar^{1}Ile [8]]-angiotensin II bound to the receptor. Membrane suspensions from homogenated lung tissue of rats were incubated with the plasma sample, radioligand and increasing concentrations of angiotensin II antagonist. Free radioligand was separated from the receptor bound fraction by filtration through glass fibre-filters. Remaining receptor bound radioactivity in the filters was counted in a γ-counter. Non-specific binding for the radioligand concentration range was evaluated with the addition of an excess of unlabelled ligand. In individual competition curves the percentage of receptor bound radioligand was plotted against the angiotensin II antagonist concentrations for each plasma sample and concentration equivalents ($n \times K_i$) were determined. (For principles of the assays used see reference [21]).

Data analysis

Pharmacodynamic evaluation Diastolic blood pressure data collected during the angiotensin II infusion at 3 min after increase of each dose step were used to fit individual dose-effect curves (i.e. the curves representing the relation between the administered dose of the agonist angiotensin II and its effect on the diastolic blood pressure) using the sigmoid E_{max} model according to Hill: [22]

$$E_D = E_{min} + \frac{(E_{max} - E_{min})\ D^m}{ED_{50}{}^m + D^m}$$

where E_D denotes the effect at the corresponding angiotensin II dose D, E_{min} the baseline (pre-infusion) blood pressure, E_{max} the maximum blood pressure to be reached, m the slope of the dose-effect curve and ED_{50} the dose of angiotensin II at which the half-maximal effect is reached. Since E_{max} of blood pressure cannot be determined under *in vivo* conditions in humans, it was arbitrarily set to 500 mmHg. (It is noteworthy that calculation with E_{max} values between 300 and 2000 mmHg did not relevantly change the obtained dose ratios.) D, m, E_{min} and ED_{50} were estimated by an iterative procedure, minimizing the sum of squared differences between calculated and observed effect values. All 24 dose-effect curves of one subject were fitted simultaneously, providing the same slope at all curves. The rightward shift of each dose-effect curve was determined as individual dose ratio (DR) as follows:

$$DR = ED_{50,antag}/ED_{50,baseline}$$

where $ED_{50,antag}$ is the angiotensin II dose of the half-maximal effect in presence of the antagonist and $ED_{50,baseline}$ is that in absence of the antagonist (pre-dosage). The dose ratios at 3 h, 9 h and 24 h were plotted according to Arunlakhshana & Schild [14]: log(DR − 1) *vs* log(dose of candesartan cilexetil). From this correlation (assuming a slope of 1), we graphically derived the (fictive) doses of candesartan cilexetil, that would have been required to induce a twofold rightward shift of the angiotensin II dose response curves (DR − 1 = 1), providing the apparent K_i-doses at the respective time points.

Pharmacokinetic evaluation Individual and median plasma concentrations of candesartan were tabulated and presented graphically. Peak plasma concentrations (C_{max}) and time to reach peak concentrations (t_{max}) were tabulated. The plasma concentration profiles were described by a non-compartmental, model independent analysis by means of the TopFit program [23]: the terminal half-life $t_{1/2}$ was calculated from the terminal phase data regression. The area under the drug-concentration-time curves (AUC) was calculated over the sampling interval (24 h) using the log-linear trapezoidal rule.

Individual and median time courses of *ex vivo in vitro* radioligand receptor displacement were evaluated graphically.

The relation between pharmacokinetics and pharmacodynamics was analysed by correlating the antagonistic effect given as DR − 1 to plasma concentrations of candesartan (h.p.l.c.) as well as to the radioligand receptor occupancy. As the radioligand receptor displacement is a saturable function of the dose administered, whereas the plasma concentration is not, a concentration equivalent ($n \times K_i$) was calculated, in order to compare the two analytical methods as well as to compare r.r.a. results with the pharmacodynamic effect:
concentration equivalents:

$$n \times K_{i\ (h.p.l.c.)} = \text{results h.p.l.c.(ng ml}^{-1}\text{)}/440.46\ (10^{-6}/K_i$$

and

$$n \times K_{i\ (r.r.a.)} = \text{results r.r.a. (mol l}^{-1}\text{)}/K_i$$

(It should be mentioned that—in contrast to the concentration—the concentration equivalent is given without dimension.)

Statistics

Standard descriptive statistics were applied to summarize pharmacodynamic as well as pharmacokinetic data. For the dose ratios of diastolic blood pressure a comparative analysis between active treatments and placebo was performed by analysis of variance (ANOVA) with effects for volunteers, treatment and sequence. Level of statistical significance was set at $\alpha = 0.05$.

Results

Pharmacodynamics

Figure 1 depicts an example of the simultaneously fitted angiotensin II dose-effect curves on diastolic blood pressure of one individual volunteer before and up to 24 h following placebo and 16 mg candesartan cilexetil. Almost identical dose-effect curves without shift are depicted after placebo administration (a) and a significant rightward shift is shown at the various time points after dosage of 16 mg of candesartan cilexetil (b).

After placebo administration no relevant effect was discernible, demonstrating the good reproducibility of the method. The time courses of median rightward shifts of the angiotensin II dose-effect curves (DR − 1) for diastolic blood pressure after different doses of candesartan cilexetil are shown in Figure 2. A maximum effect is yielded at 6 h after administration and subsequently a similar decline of effect can be seen following all dosages applied. A 'half-life of pharmacodynamic effect' of about 7.5 h (7.55 h, 6.12 h and 7.54 h for doses of 4, 8 and 16 mg of candesartan cilexetil, respectively) was derived by linear regression of the log-transformed DR − 1. For DR − 1 of diastolic blood pressure significant treatment effects were found ($P < 0.002$), whereas no significance was observed for subject or period effects.

In Figure 3 the candesartan cilexetil doses are correlated with the rightward shifts of the angiotensin II dose-effect curves in a double logarithmic plot (Schild-plot). The relatively narrow range of doses (factor 4) does not allow definite conclusions about the slope of the relation, but the assumed steepness factor of 1 appears to well describe the underlying correlations. The apparent K_i-doses (i.e. the doses which would have induced a twofold rightward shift of angiotensin II dose-effect curve, indicating occupancy of 50% of the functional receptors) were derived for the various time points, resulting in 0.92, 0.49 and 1.94 mg of candesartan cilexetil after 3, 9 and 24 h, respectively.

Pharmacokinetics

Figure 4 shows the plasma concentration time courses of candesartan determined by h.p.l.c. following 4, 8 and 16 mg candesartan cilexetil. The corresponding pharmacokinetic data are given in Table 1. Figure 5 depicts the time course of radioligand receptor occupancy (given as percentage of radioligand [^{125}I-Sar^{1}Ile^8]-angiotensin II bound to the receptor). As visible from Figure 6 there is no hysteresis loop discernible between plasma concentration of candesartan from h.p.l.c. and the concentration equivalent ($n \times K_i$) determined from the radioligand receptor assay.

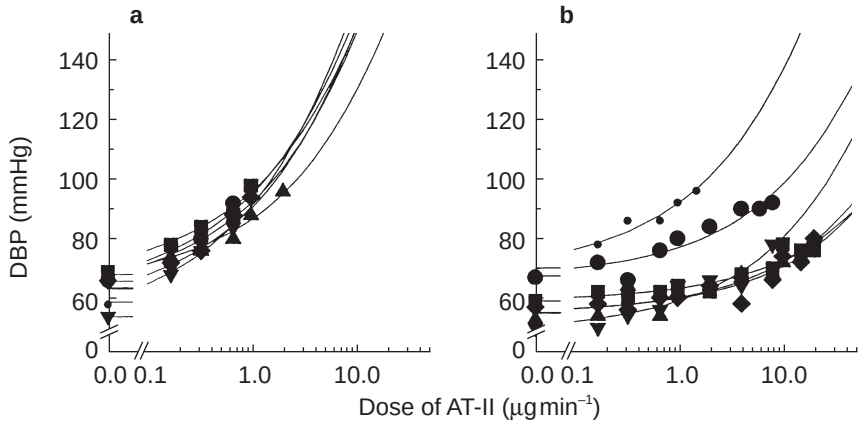

Figure 1 Examples of angiotensin II dose-effect curves for diastolic BP in one subject before administration (• = 0 h) and at various time points (■ = 3 h, ◆ = 6 h, ▼ = 9 h, ▲ = 12 h and ● = 24 h after administration) following placebo (a) or a single oral dose of 16 mg candesartan cilexetil (b). Symbols represent the observed blood pressure values, lines indicate the fitted sigmoid E_{max} model.

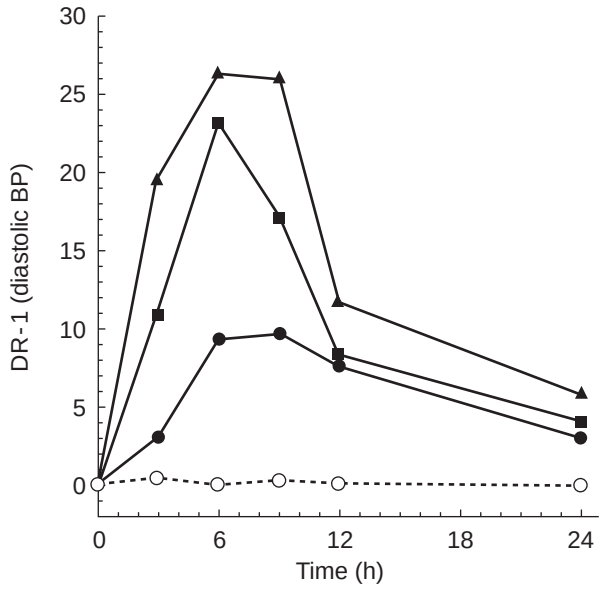

Figure 2 Time course of shift of the ANG II dose-effect curves for diastolic blood pressure (BP) (dose ratio −1 = DR−1) following oral doses of candesartan cilexetil (median values; ● = 4 mg, ■ = 8 mg, ▲ = 16 mg, ○ = placebo).

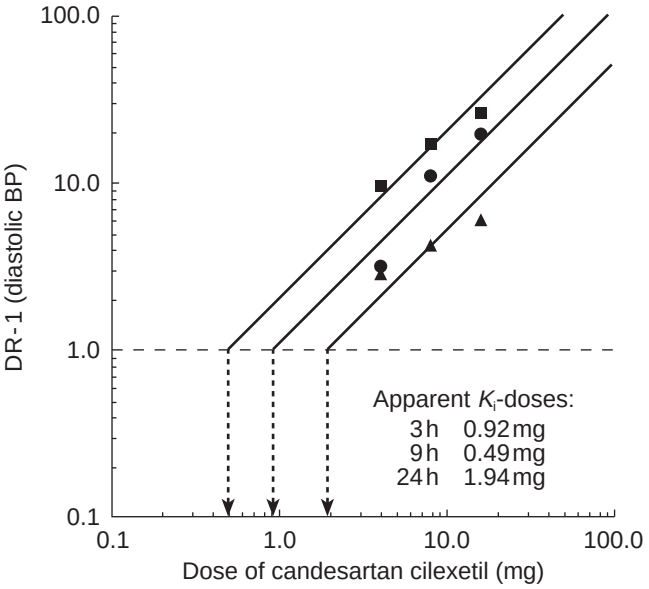

Figure 3 Regression plot according to Schild (DR-1 of diastolic BP *vs* administered dose of candesartan cilexetil (4,8,16 mg)) for several time points (median values; ● = 3 h, ■ = 9 h, ▲ = 24 h, — = regression function with assumed slope = 1). The dotted line ..... indicates the line of DR − 1 = 1, from which the fictive apparent K_i-doses can be determined (dotted arrows).

Pharmacokinetics/Pharmacodynamics (PK/PD)

The *in vivo* antagonistic effect, measured from the shift of the angiotensin II dose-effect curves as DR − 1, was correlated to the plasma pharmacokinetics, measured from h.p.l.c. (*cf.* Figure 7) and from radioreceptor assay (*cf.* Figure 8). Both correlations show counterclockwise hysteresis loops, indicating a delay and longer persistance of effect than to be expected from the plasma concentration time course. As the receptor binding is saturable in contrast to h.p.l.c. and DR − 1, concentration equivalents ($n \times K_i$) have been used to eliminate this source of error.

Tolerability

No relevant adverse events were seen during the investigation.

Table 1 Pharmacokinetic parameters (mean ± s.d.) of CV-11974 after various single oral doses of candesartan cilexetil (h.p.l.c.).

	Candesartan cilexetil		
Variable	*4 mg*	*8 mg*	*16 mg*
C_{max} (ng ml^{-1})	28.4 ± 5.6	52.8 ± 17.9	98.9 ± 39.26
t_{max} (h)	4.8 ± 0.9	4.3 ± 1.1	4.1 ± 0.9
$t_{1/2,z}$ (h)	6.2 ± 2.1	6.9 ± 1.9	5.4 ± 2.0
MRT (h)	11.3 ± 2.5	11.9 ± 2.7	10.1 ± 2.9
AUC(0,∞) (ng ml^{-1} h)	264.3 ± 85.6	541.9 ± 242.6	891.3 ± 398.1

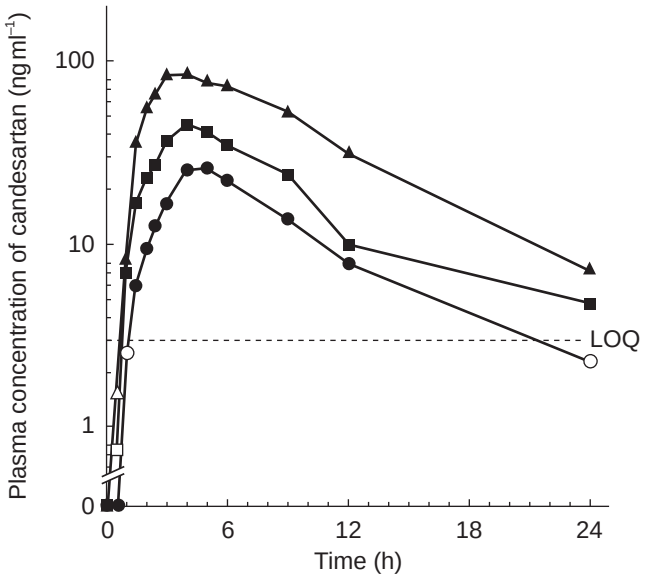

Figure 4 Plasma concentrations of candesartan, the active metabolite of candesartan cilexetil, after oral doses of candesartan cilexetil (h.p.l.c.; median values; ● = 4 mg, ■ = 8 mg, ▲ = 16 mg, ... = limit of quantification [LOQ = 3 ng ml^{-1}]; observed values below LOQ were treated as LOQ/2, symbols of median values below LOQ are unfilled).

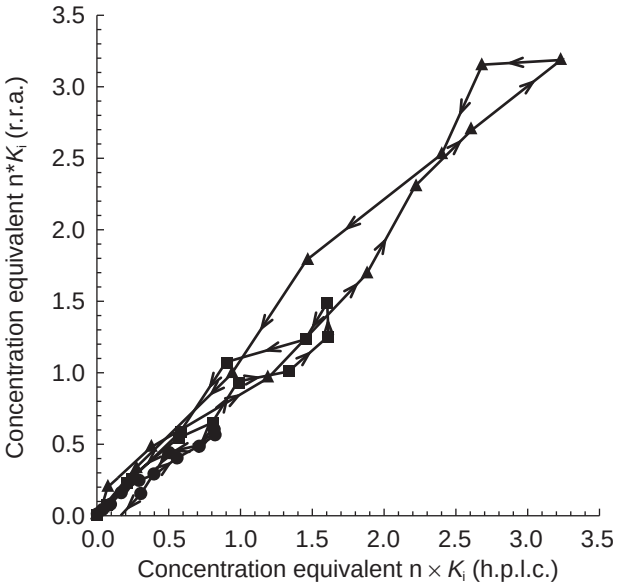

Figure 6 Relation between *in vitro* radioligand receptor binding (given as concentration equivalent $n \times K_i$) and concentration of candesartan (h.p.l.c., given as concentration equivalents $n \times K_i$) after oral doses of candesartan cilexetil (mean values; ● = 4 mg, ■ = 8 mg, ▲ = 16 mg; ↗ indicating the direction of hysteresis loop).

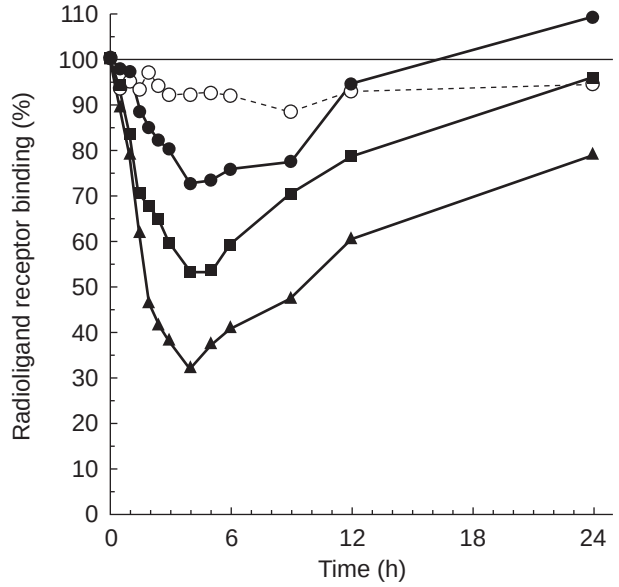

Figure 5 *In vitro* radioligand receptor binding (percentage of radioligand bound) after addition of *ex vivo* human plasma obtained following oral doses of candesartan cilexetil (median values; ● = 4 mg, ■ = 8 mg, ▲ = 16 mg, ○ = placebo).

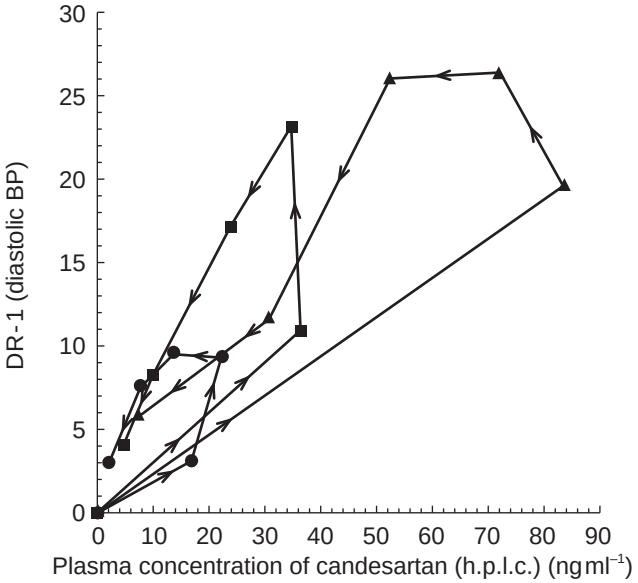

Figure 7 Relation between shift of the ANG II dose-effect curve (DR − 1, diastolic BP) and concentration of candesartan (h.p.l.c.) after oral doses of candesartan cilexetil (median values; ● = 4 mg, ■ = 8 mg, ▲ = 16 mg; ↗ indicating the direction of hysteresis loop).

Discussion

In this study a classic approach from experimental *in vitro* pharmacology [14, 24], i.e. the assessment of dose-effect curves of an agonist in the presence of different doses of an antagonist, was used to characterize the quantitative pharmacodynamic properties of the new angiotensin II antagonist candesartan cilexetil in man. This method, using the regression technique of Schild, has to be considered to be very useful for the quantitative evaluation of the competitive action of selective antagonists also in humans, though it has only rarely been used to characterize substances *in vivo* in humans. A further purpose of the present study on candesartan cilexetil was to investigate whether in man a quantitative relationship between *in vivo* angiotensin II blockade on the one side and chemical detection as well as *ex vivo in vitro* receptor occupancy on the other side could be established.

The pharmacokinetic properties, derived from the present study, confirm previous results in respect to plasma concentrations of the active metabolite candesartan, C_{max} at about 4 h and a decline with a half-life of about 6 h [25]. It should be noted that 24 h after administration plasma

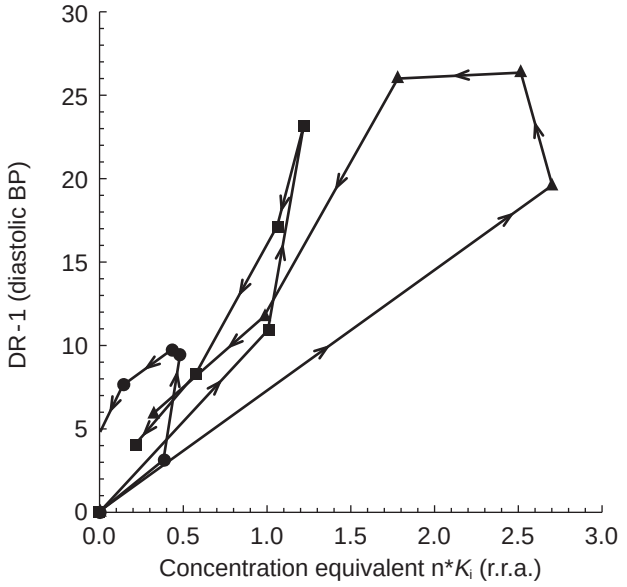

Figure 8 Relation between shift of the ANG II dose-effect curve (DR −1, diastolic BP) and *in vitro* radioligand receptor binding (given as concentration equivalent $n \times K_i$) after oral doses of candesartan cilexetil (median values of DR −1, mean values of $n \times K_i$; ● = 4 mg, ■ = 8 mg, ▲ = 16 mg; ↗ indicating the direction of hysteresis loop).

concentrations of candesartan had decreased to the limit of quantification.

A linear relation with a slope of unity was found between candesartan plasma concentrations measured by chemical detection (h.p.l.c.) and *ex vivo in vitro* radioligand receptor displacement, given as concentration equivalents ($n \times K_i$). This is a clear proof for the absence of other active metabolites in the central compartment.

As expected, angiotensin II dose-effect curves were shifted to the right after administration of candesartan cilexetil in a dose dependent manner. The maximal antagonistic effect observed is characterized by a dose ratio −1 of about 25–30, i.e. after a therapeutic dose of candesartan cilexetil the dose of angiotensin II has to be increased 25- to 30-fold to obtain the same DBP increase as in the absence of the antagonist.

The apparent K_i-doses (i.e. the dose of candesartan cilexetil which would induce a twofold shift of the angiotensin II dose-effect curve) amounted to 0.92 and 1.94 mg at 3 and 24 h after administration, respectively, confirming the high potency of the substance. From these data, as well as from the persisting rightward shift 24 h after administration, it is obvious, that the effect of candesartan at the angiotensin II receptor site *in vivo* lasts longer than one would expect from the pharmacokinetic properties in plasma; for other angiotensin antagonists much faster decay of effects had been observed [26]. The results are supported by hysteresis loops (*cf.* Figures 7,8), demonstrating that there is no simple linear relation between pharmacodynamic and pharmacokinetic behaviour. These phenomena indicate a slow off-rate of candesartan from the receptor site *in vivo* and offer an explanation for 24 h activity as found in clinical studies in spite of a moderate pharmacokinetic half-life and low plasma concentration levels at 24 h after administration. Our finding of an apparent K_i-dose of 1.94 mg 24 h p.a. well corresponds with results from clinical studies in hypertension, where therapeutic doses between 4 and 16 mg once daily candesartan cilexetil had been established [27].

We conclude that candesartan cilexetil is a highly potent, orally active angiotensin II antagonist in humans with a long duration of action providing a 24 h antagonistic activity after a single daily dose.

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