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The assessment of ACE activity in man following angiotensin I challenges: a comparison of cilazapril, captopril and enalapril

J. ESSIG, G. G. BELZ & A. WELLSTEIN
Zentrum für Kardiovaskuläre Pharmakologie, ZeKaPha GmbH, Wiesbaden und Zentrum der Pharmakologie der J. W. Goethe-Universität, Frankfurt, Main, Federal Republic of Germany

1 The aim of the studies was to develop a new methodology to estimate the pharmacodynamic properties and potency of angiotensin converting enzyme (ACE) inhibitors in man.
2 Angiotensin I dose-response curves were derived by continuous infusion of angiotensin I in increasing dose steps; steady state was reached within 3 min.
3 Interaction between angiotensin I (agonist) and ACE inhibitors (antagonist) was characterized according to Schild-plot methodology by measuring agonist dose-response curves using diastolic blood pressure in the absence and the presence of varying doses of the antagonist.
4 Cilazapril shifted the angiotensin I dose-response curves to the right. A twofold shift (apparent K_i-dose) was observed with approximately 0.6 mg cilazapril.
5 The effect of angiotensin I on diastolic blood pressure was determined before and up to 36 h after administration of 25 mg captopril, 10 mg enalapril, 4 mg cilazapril and a placebo orally. The pharmacodynamic half-life of captopril was about 2 h, whereas the effect of enalapril and cilazapril was about 4 h.
6 Angiotensin I dose-response curves are useful methods of investigating the pharmacodynamic properties of ACE-inhibitors in man.

Keywords kinetics pharmacodynamic properties angiotensin I ACE inhibitors

Introduction

Previous investigations of ACE inhibitors in humans have included methods for determining the inhibition of plasma ACE (Biollaz *et al.*, 1981; Bussien *et al.*, 1985; Given *et al.*, 1984) or measuring the reduction of blood pressure effects after bolus doses of angiotensin I. By analogy with the classic method used to assess β-adrenoceptor blockade when the heart rate response to a continuous isoprenaline infusion is measured (Wellstein *et al.*, 1988), we evaluated the degree of ACE inhibition by assessing the blood pressure response to a continuous infusion of angiotensin I.

In *in vitro* studies a competitive antagonism between angiotensin I and ACE inhibitors at the enzyme level is well known (Ondetti & Cushman, 1982; Strittmatter & Snyder, 1986) and should prove a useful technique in man.

The aim of these studies was to evaluate this new method of assessment of ACE inhibitors in man.

Correspondence: Professor Dr G. G. Belz, Alwinenstrasse 16, D 6200 Wiesbaden, FRG.

Methods

To establish angiotensin I dose-response curves in man, we used continuous infusions of angiotensin I in increasing doses rather than bolus injections. This was for reasons of safety and in order to achieve steady state levels.

Human angiotensin I (Senn Chemicals, Dielsdorf, Switzerland) was obtained in lyophilized form. A sterile stock solution of this substance containing 50 μg ml^{-1} in physiological saline was prepared and passed through a Millex GV filter (Millipore, Molsheim, France). Further dilutions were prepared with saline to reach final concentrations of 1.2 and 4 μg angiotensin I ml^{-1} for the infusion solution. Angiotensin I was infused using an automatic infusion pump in increasing doses (3 min each) of 0.1, 0.3, 0.9, 2.0, 3.9, 6.0, 9.0, 12.0, 15.0, and 18.0 μg min^{-1}.

All the studies were performed in healthy male volunteers aged 18 to 29 years after obtaining informed written consent. Each subject was healthy, as determined by history, physical examination, electrocardiogram and blood biochemical analysis. No restrictions concerning food were made but drugs other than the test drugs were not permitted during the studies.

From 18.00 h on the preceding evening until the completion of the measurements on each study day no strenuous activities, caffeinated drinks, alcohol or nicotine were allowed. On the morning of the study days the subjects arrived at the institute in a fasting state. An indwelling venous cannula was inserted in the forearm and electrodes for ECG monitoring were attached. All cardiovascular parameters were recorded with the subjects in the supine position with the head elevated 15°. After a 15 min rest period, three baseline recordings were made 5 min apart. The angiotensin I infusion was started, and during the last 30 s of the infusion at each dose level the cardiovascular parameters were recorded. A mercury sphygmomanometer was used to measure systolic and diastolic blood pressure by noting Korotkov's sounds I and V. All measurements were made by the same observer.

Most of the studies presented used the new ACE inhibitor cilazapril, which in animal experiments has been shown to be highly potent and orally active (Holck *et al.*, 1986; Natoff *et al.*, 1985). In several experiments a placebo served as a negative control, while captopril and enalapril acted as positive controls.

In order to show that a continuous infusion of angiotensin I reached a steady state within 3 min (whether an ACE inhibitor was present or not) two experiments were performed. In a first study, six volunteers received a continuous infusion of 0.9 μg angiotensin I min^{-1} and blood pressure was measured at 1, 2, 3, 4, 5, 7, 10, 13, 16, 19, 22 and 25 min after the start of the infusion. In a second experiment with six volunteers the blood pressure response to angiotensin I infusion was measured 3 h after oral administration of 1.25, 3.75, 10 and 30 mg of cilazapril. At the highest angiotensin I dose level which produced an increase in diastolic blood pressure of about 20 mm Hg, the infusion was continued for another 15 min and blood pressure was then recorded.

In one experiment the specificity of the angiotensin I infusion method was tested in four volunteers. Angiotensin I dose-response curves were constructed as described above. After the blood pressure had returned to baseline (within 10 min), the same procedure was repeated, using angiotensin II-analogous angiotensin-amide (Hypertensin, Ciba Geigy, Basel, Switzerland). Immediately afterwards the volunteers received 30 mg cilazapril and 3 h later the infusions with angiotensin I and angiotensin II were repeated.

In another study in six volunteers the reproducibility of the method was tested by repeated angiotensin I infusions at the following time points after placebo: 3, 6, 9, 12, 24 and 36 h.

For an analysis of potency of cilazapril, dose-response curves of angiotensin I were determined in six volunteers before and 3 h after single oral doses of cilazapril (1.25 to 30 mg).

In a double-blind crossover study, time kinetics of single oral doses of 4 mg cilazapril, 10 mg enalapril, 25 mg captopril and placebo were evaluated by angiotensin I dose-response relationships up to 36 h after oral drug intake.

The basic principles and mathematical transformation of data used were those described previously (Belz *et al.*, 1987; Wellstein *et al.*, 1985, 1987a).

The calculations were done for each volunteer's results individually. Dose ratios (DR) were transformed according to Arunlakshana & Schild (1959):

$$\log (DR-1) = m \times \log I - \log K_i$$

and the mean apparent K_i-dose was derived from a weighted linear regression analysis, where m is the slope of the straight line, and I the dose of ACE inhibitor.

Results

Increasing doses of angiotensin I, given by continuous infusion, produced step-wise increases in blood pressure and peripheral resistance (Figure 1).

Continuous infusion of 0.9 μg angiotensin I min^{-1} yielded a mean plateau of about +17 mm Hg above baseline for diastolic blood pressure. (Figure 2). This plateau was reached within 3 min of the start of the infusion.

Equilibration time was not delayed in the presence of the ACE inhibitor cilazapril (Figure 3).

In the experiment to test the specificity of the angiotensin I infusion technique, the administration of a high dose of cilazapril did not reduce the effects of angiotensin II (right panel of Figure 4). Rather, a small 'sensitization' became apparent. In contrast, cilazapril administration abolished the effects of angiotensin I in the dose range up to 2.0 μg min^{-1}. Systolic blood pressure and peripheral resistance behaved similarly.

The effects of repeated infusions of angiotensin I before and after oral placebo administration are presented in Figure 5. It is obvious that only small differences occurred in the response to angiotensin I, despite some diurnal variations in the baseline values. No significant changes in the dose-response curves were observed during the whole observation period of 36 h.

The influence of increasing single oral doses of cilazapril on the angiotensin I response are shown in Figure 6. Cilazapril reduced diastolic blood pressure in a dose dependent manner and shifted the angiotensin I dose-response curves to the right. Correlations between log dose and log dose ratio minus one (shift) resulted in a linear Schild-plot (Figure 7). The apparent K_i-dose (i.e. the dose inducing a two-fold shift of the angiotensin I dose-response curves and representing 50% inhibition of ACE activity) was approximately 0.6 mg, 3 h after cilazapril administration.

The time course of the effect of a single dose of 4 mg cilazapril on the angiotensin I response is shown in Figure 8a. The baseline response before administration of the ACE inhibitor was identical to the values registered with the placebo treatment. A reduction in blood pressure and a shift to the right of the angiotensin I dose-response curves occurred with a maximum effect 3 h after

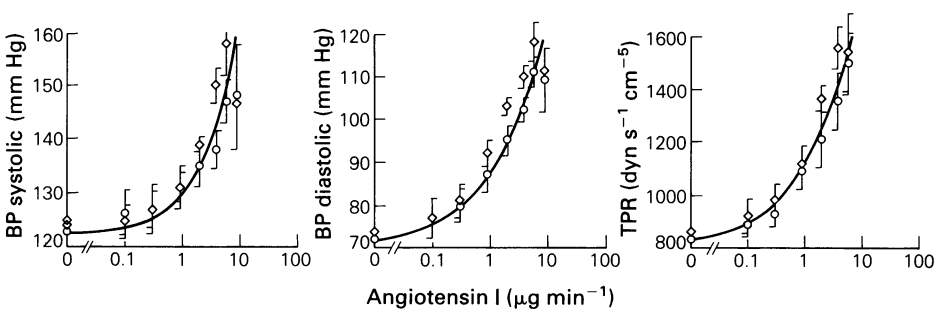

Figure 1 Influence of angiotensin I on systolic and diastolic blood pressures (BP), and total peripheral resistance (TPR). Presented are mean values ± s.e. mean (n = 6) (reprinted from Belz *et al.*, 1987).

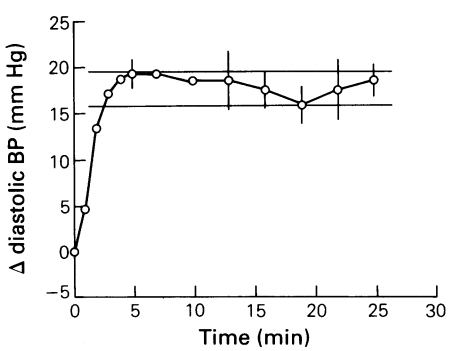

Figure 2 Increase of diastolic blood pressure during continuous infusion of 0.9 μg min^{-1} angiotensin I. Presented are mean ± s.e. mean values, n = 6.

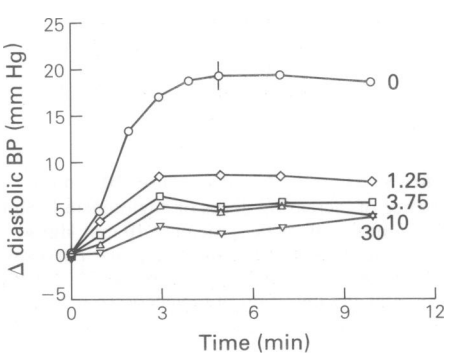

Figure 3 Equilibration time of the final dose steps of an angiotensin I infusion 0, 3 h after intake of 1.25, 3.75, 10 and 30 mg of cilazapril in n = 6 male volunteers.

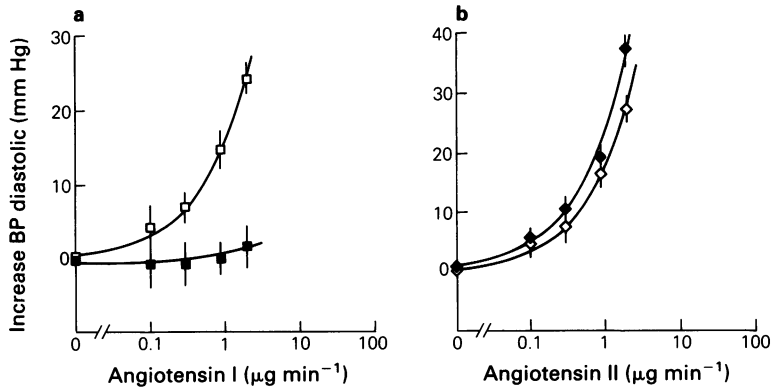

Figure 4 Comparison of the effects of cilazapril (30 mg orally) on angiotensin I (a) and angiotensin II (b) effects. Presented are mean changes (± s.e. mean; $n = 4$) as compared with baseline. Symbols: open circles before cilazapril, closed circles 3 h after cilazapril (reprinted from Belz *et al.*, 1987).

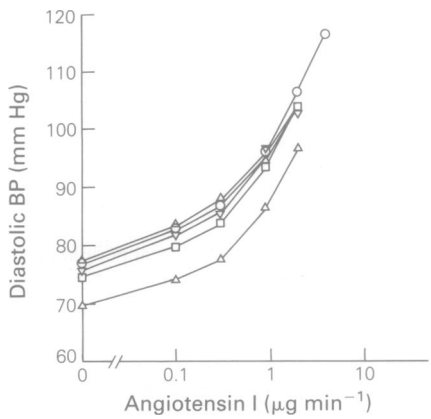

Figure 5 Diastolic blood pressure (BP) in the presence of increasing doses of angiotensin I. Shown are the mean values (s.e. mean < 10 mm Hg; $n = 6$). The various angiotensin I dose-response curves were derived before (○) and at 3 (◇), 6 (△), 9 (▽) and 12 (□) h after the ingestion of a placebo (reprinted from Wellstein *et al.*, 1987a).

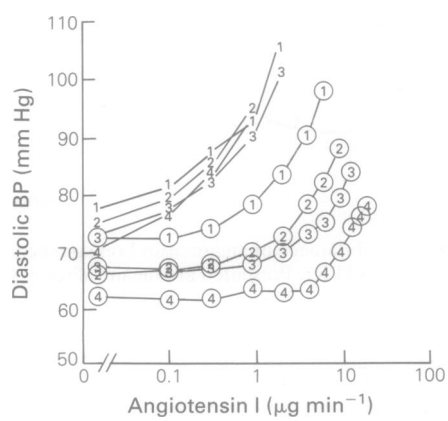

Figure 6 Diastolic blood pressure during continuous infusion of increasing doses of angiotensin I and the antagonism to the response of increasing doses of cilazapril. Control responses before cilazapril: 1 = 1.25 mg, 2 = 3.75 mg, 3 = 10 mg and 4 = 30 mg; responses 3 h following cilazapril in circles (reprinted from Wellstein *et al.*, 1987b).

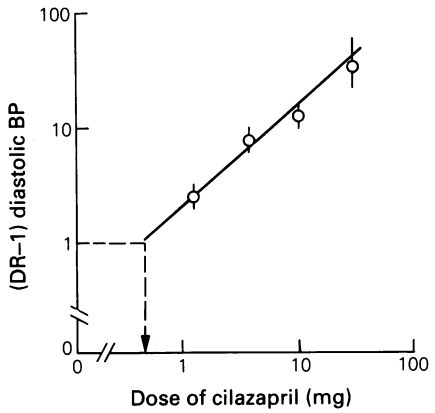

Figure 7 Schild-plot of the relationship between log dose of the ACE inhibitor cilazapril and the log dose ratio minus one (DR−1) of angiotensin I response for diastolic blood pressure (BP). Presented are means with 95% confidence intervals. The arrow indicates the apparent K_i-dose of cilazapril (reprinted from Wellstein *et al.*, 1987b).

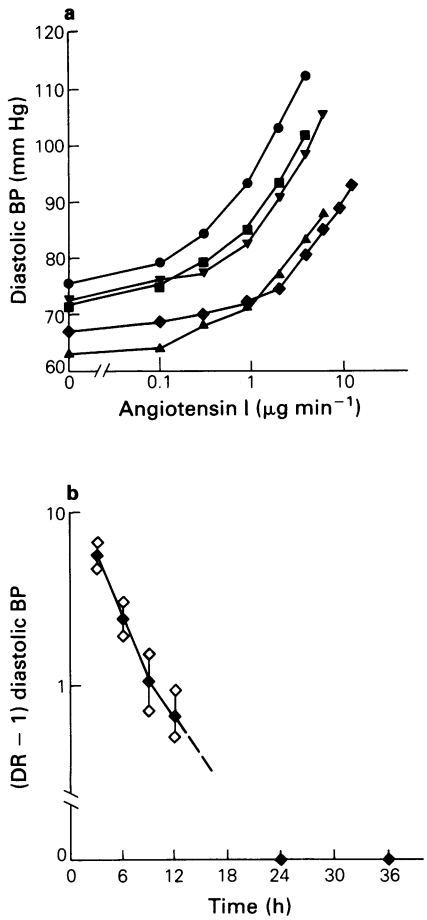

Figure 8 Diastolic blood pressure in the presence of increasing doses of angiotensin I. a) The various angiotensin I dose-response curves were derived before (●) and at 3 (◆), 6 (▲), 9 (▼), and 12 (■) h after the oral administration of 4 mg cilazapril. b) Shift of the angiotensin I dose-response curve (DR−1) at different times after ingestion of 4 mg cilazapril (mean (◆) ± 95% confidence interval (◇); $n = 6$). Data points on the abscissa refer to an effect not different from placebo (reprinted from Wellstein *et al.*, 1987a).

cilazapril ingestion (Figure 8b). Thereafter a time dependent reduction was observed, and 24 and 36 h after cilazapril the baseline angiotensin I response was restored. The effect declined with an apparent half-life of about 4 h. The same was found for 10 mg of enalapril (Figure 9), while the effects produced by captopril declined more rapidly with a half-life of 2 h (Figure 10).

Discussion

Steady state of angiotensin I response was reached within 3 min of the start of the infusion whether an ACE inhibitor was present or not. This is one of the main prerequisites for the Schild approach.

The analysis according to Schild allowed the potency of the inhibitor (K_i-dose) and the degree of antagonism in man to be compared with *in vitro* results (Ondetti & Cushmann, 1982; Strittmatter & Snyder, 1986). The Schild analysis with a slope of 1 indicated competitive antagonism between angiotensin I and the ACE inhibitor cilazapril. The high potency of cilazapril in man was in accord with results from *in vitro* and *in vivo* animal experiments (Holck *et al.*, 1986; Natoff *et al.*, 1985).

One could speculate that the apparent K_i-dose derived from the Schild-plot and the drug half-life characteristics could be used to determine the correct therapeutic dose.

The pharmacodynamic half-life of captopril as assessed by angiotensin I antagonism was similar to the half-life of about 2 h determined for this drug in pharmacokinetic experiments (Kubo *et al.*, 1985). The half-life of cilazapril was almost identical with that of enalapril which was about 4 h. These half-lives appear shorter than those derived from pharmacokinetic data (Francis *et al.*, 1987). At present the reasons for these discrepancies are unresolved.

In conclusion we demonstrated that angiotensin I dose-response curves are useful methods of assessing the pharmacodynamic properties of ACE inhibitors in man.

This was demonstrated for the time kinetics of ACE inhibitors as well as for the analysis of their potency according to Schild's method.

For the new ACE inhibitor cilazapril we confirmed the results from animal experiments and demonstrated the oral activity and high potency of this drug.

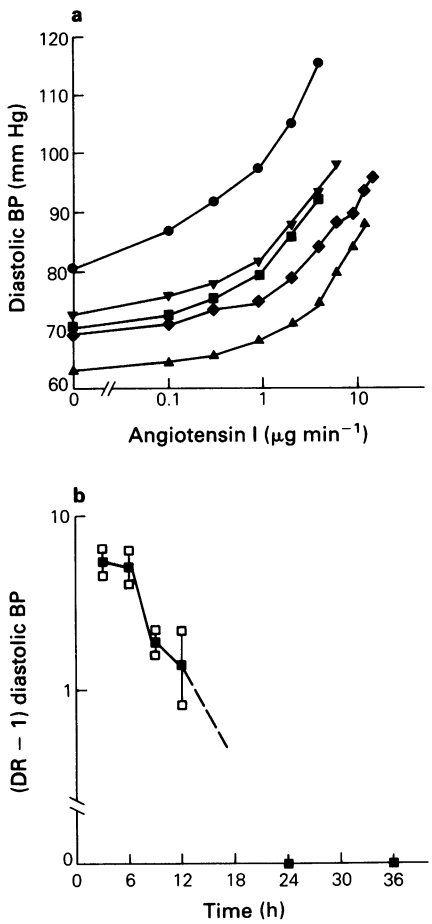

Figure 9 Diastolic blood pressure in the presence of increasing doses of angiotensin I. a) The various angiotensin I dose-response curves were derived before (●) and at 3 (◆), 6 (▲), 9 (▼), and 12 (■) h after the oral administration of 10 mg enalapril. b) Shift of the angiotensin I dose-response curve (DR−1) at different times after ingestion of 10 mg enalapril (mean (■) ± 95% confidence interval (□); $n = 6$). Data points on the abscissa refer to an effect not different from placebo (reprinted from Wellstein *et al.*, 1987a).

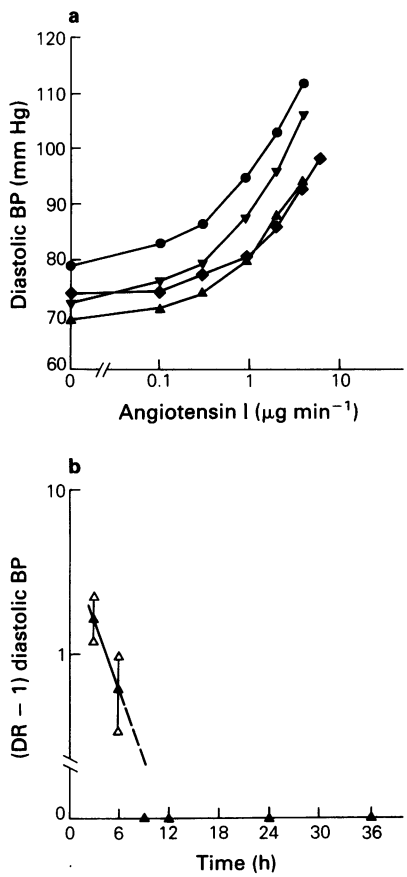

Figure 10 Diastolic blood pressure in the presence of increasing doses of angiotensin I. a) The various angiotensin I dose-response curves were derived before (●) and at 3 (◆), 6 (▲), 9 (▼), and 12 (■) h after the oral administration of 25 mg captopril. b) Shift of the angiotensin I dose-response curve (DR−1) at different times after ingestion of 25 mg captopril (mean (▲) ± 95% confidence interval (△); $n = 6$). Data points on the abscissa refer to an effect not different from placebo (reprinted from Wellstein *et al.*, 1987a).

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