Correlation between plasma concentration of cilazapril and haemodynamic and hormonal effects in healthy man

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- 1 The haemodynamic and humoral effects of cilazapril, a new angiotensin converting enzyme (ACE) inhibitor, were evaluated in normotensive healthy volunteers.
- 2 Single oral doses of 1.25, 2.5, 5 and 10 mg of cilazapril inhibited ACE by $\geq 90\%$ and induced the expected pattern of changes of the renin-angiotensin-aldosterone-system.
- 3 Cilazapril had a long duration of action, since some ACE inhibition was still present 72 h after drug intake.
- 4 Cilazapril administered intravenously at doses of 5 and 20 $\mu\text{g kg}^{-1}$ for 24 h did not produce any significant effects.
- 5 During repeated administration of cilazapril for 8 days, no accumulation of cilazapril was observed and the clinical tolerance was excellent.
- 6 In normal volunteers, cilazapril administered orally acts as a potent inhibitor of converting enzyme.

Keywords cilazapril hormonal effects haemodynamic effects ACE inhibitor

Introduction

Since the original evaluation of the first angiotensin converting enzyme (ACE) inhibitor, captopril, several new compounds that inhibit angiotensin II formation have been synthesized and studied in healthy subjects or patients (Ferguson *et al.*, 1977; Brunner *et al.*, 1985). As new ACE inhibitors were developed, substances appeared that provided a longer duration of ACE inhibition. This efficacy and the good clinical tolerance of ACE inhibitors are now well established and due to these features, these compounds have become a major component of the treatment of hypertension (Gavras *et al.*, 1978; Case *et al.*, 1978; Veterans Administration, 1983) and congestive heart failure (Turini *et al.*, 1979; Ader *et al.*, 1980; Fouad *et al.*, 1982).

In the present article, the results obtained in normal healthy volunteers with cilazapril (Fasanella d'Amore *et al.*, 1987; Nussberger *et al.*, 1987), a recent ACE inhibitor, are summarized. The haemodynamic and humoral effects of cilazapril were examined after oral administration of single doses, during a 24 h infusion

and after repeated oral administration for 8 days. The pharmacokinetic data obtained during the same studies (Francis *et al.*, 1987) are included in another article of this issue (Williams *et al.*, 1989).

Methods

Study 1: Humoral and haemodynamic effects of single oral doses of cilazapril

The effects of single oral doses of cilazapril on blood pressure, heart rate and the various components of the renin-angiotensin-aldosterone system were studied first in 14 normotensive male volunteers (Fasanella d'Amore *et al.*, 1987). Each subject provided a medical history and underwent a complete physical examination before inclusion in the study. The protocol of the study was approved by the Hospital Ethics committee. After careful explanation of the nature and purposes of the study, written informed

consent was obtained from each volunteer. The safety controls included routine laboratory tests performed before and after each administration of cilazapril. Throughout the study, the subjects continued their usual free sodium diet.

The ability of cilazapril to block the generation of angiotensin II was first assessed in two volunteers by examining the effects of cilazapril on the blood pressure response to exogenous angiotensin I. An arterial catheter was placed in the radial artery to monitor blood pressure and heart rate continuously as described previously (Biollaz *et al.*, 1981). Thereafter, blood pressure dose-response curves to exogenous angiotensin I (Senn Chemicals, Dielsdorf, Switzerland) were established in each volunteer before and after oral administration of a single dose of 5 or 10 mg cilazapril. The blood pressure response to exogenous angiotensin I was completely inhibited by both doses of cilazapril. The peak inhibition occurred 30 and 45 min after drug intake and was still present 4 h later with both doses of cilazapril. These preliminary results already suggested that 5 mg p.o. may provide close to maximal ACE blockade.

In a second part of this study, 12 healthy subjects received, at 2 week intervals, two single doses of cilazapril. Four doses of the drug, i.e. 1.25, 2.5, 5 and 10 mg were tested in four groups of six subjects. The inhibitor was given p.o. with 200 ml water at 08.00 h, after an overnight fast. Blood was drawn regularly up to 72 h post-drug for the determination of plasma converting enzyme and renin activities, blood angiotensin I and plasma angiotensin II and aldosterone concentrations. The volunteers were asked to remain seated for the first 4 h of the study. They were allowed to rise to eat lunch. The subsequent samples were obtained with the subject returning to the research unit just 30 min before blood drawing.

Plasma renin activity was determined by a radioimmunological microassay based on angiotensin I trapping by antibody as reported by Poulsen & Jorgensen (1974) and adapted in our laboratory (Nussberger *et al.*, 1987). Plasma converting enzyme activity was measured with a radioenzymatic method (Chen *et al.*, 1984). Radioimmunoassays were used for the determination of plasma aldosterone (Nussberger *et al.*, 1984b), immunoreactive angiotensin II and blood angiotensin I (Nussberger *et al.*, 1984a).

Study 2: Effects of 24 h intravenous infusion of cilazapril in normal volunteers

Since all doses of cilazapril in the range of 1.25 to 10 mg produced a marked ACE inhibition,

additional information was needed to assess whether even lower doses of cilazapril could result in considerable ACE inhibition. A study was therefore performed in which small doses of cilazapril were infused intravenously for 24 h in healthy volunteers to evaluate some humoral and haemodynamic effects.

Twelve healthy male subjects aged 22 to 30 years were included in this study. Informed consent and protocol approval were obtained as described above and the safety of the procedure was evaluated in identical fashion. All subjects were allowed a free sodium diet. On the day of infusion, the volunteers came to the outpatient clinic after an overnight fast and were installed on a bed in supine position. Two catheters were inserted in antecubital veins, one for the drug infusion and the other in the contralateral arm for blood sampling. The volunteers were asked to remain in supine position for the first 4 h and were then allowed to rise and to eat lunch. The subjects left the hospital at 22.00 h and returned at 07.30 h on the next day for the last determination.

Six subjects received at 1 week intervals and in a single-blind randomized manner either the placebo or 5 $\mu\text{g h}^{-1}$ cilazaprilat. The remaining six subjects received either the placebo or 20 $\mu\text{g h}^{-1}$ cilazaprilat. The agents were infused for 24 h at a constant rate using a portable pump (Injection MS 16A, Fresenius AG, Stans, Switzerland).

Supine blood pressure and heart rate were measured every 30 min for 3 h and again at times 4, 6, 8, 12, 14 and 24 h, using the classic stethacoustic method. The various parameters of the renin-angiotensin-aldosterone system have been determined at the same times as described above.

Study 3: Haemodynamic and humoral effects of cilazapril during repeat administration for 8 days

Based on the results of the preceding studies, it appears clear that cilazapril is a potent and longlasting converting enzyme inhibitor, effective at all doses ranging from 1.25 to 10 mg. However, because cilazapril has a long duration of action, it seemed particularly important and necessary to determine whether drug accumulation occurs during repeat administration and to establish the minimal effective dose that should be recommended for prolonged therapy. To answer these questions, a third protocol was designed to study the humoral and haemodynamic changes as well as the plasma cilazaprilat concentrations in normotensive volunteers during an 8 day administration of cilazapril.

Twelve normotensive healthy students aged 20 to 25 years and maintained on a free sodium diet participated in this third study. Informed consent and protocol approval were obtained again as described above. Safety laboratory and clinical evaluations were done at the beginning and on the last day of the study. During the 11 days of the study, the subjects were asked to come every morning at 07.00 h to the outpatient facility to receive their oral dose of cilazapril. Cilazapril was administered in a randomized double-blind fashion such that six of them received the dose of 1.25 mg and the other six 5 mg day⁻¹. Blood pressure, heart rate and plasma converting enzyme activity were measured each day prior to the administration of cilazapril. On the first and the eighth day, additional blood was sampled before and again 4 and 24 h following drug intake to determine plasma renin activity, blood angiotensin I, plasma angiotensin II and aldosterone concentrations. At the same time, blood was drawn for the determination of plasma cilazapril concentrations.

The blood pressure effect of the repeated cilazapril administration was also assessed with the ambulatory blood pressure recorder Remler M2000 (Remler Corp., San Francisco, CA,

USA). Each subject wore the recorder once before the study and a second time on the eighth day of ACE inhibition. The subjects were required to monitor their blood pressure at 30 min intervals for 11 h. An additional blood pressure recording was performed 1 month after the last dose of cilazapril in those subjects who received 5 mg day⁻¹ cilazapril.

Statistics

Statistical evaluation of the results was made using one-way or two-way analysis of variance and, when appropriate, the Student's *t*-test for paired data. Results are provided as mean $\pm$ s.e. mean.

Results and discussion

The time course of plasma ACE activity following oral administration of the various doses of cilazapril is presented in Figure 1. A marked inhibition of ACE activity was obtained with all doses of cilazapril and even the smallest dose of 1.25 mg cilazapril blocked ACE activity by more

Figure 1 Time course of plasma converting enzyme (ACE) activity following single oral administration of various doses of cilazapril (● 1.25 mg, ○ 2.5 mg, ▲ 5 mg and △ 10 mg) in normotensive volunteers, *n* = 6. From Fasanella d'Amore *et al.* (1987).

than 90%. As observed previously with other inhibitors, increasing the dose led to an earlier onset of inhibition and to a prolonged duration of the effect (Brunner *et al.*, 1981). In earlier studies, using the same techniques, it was shown that ACE activity has to be blocked by more than 90% to induce net blockade of the renin-angiotensin system (Brunner *et al.*, 1983). On this basis, cilazapril seems to effectively inhibit ACE at all doses tested in this study. Moreover, these results confirm the long-lasting effects of cilazapril, since 48 h after drug administration, ACE activity was still reduced to about 60% of its baseline activity.

As expected with any blockade of the angiotensin converting enzyme, blood angiotensin I and plasma renin activity rose in response to cilazapril administration (data not shown). Figure 2 shows the effects of the four doses of cilazapril on plasma aldosterone and angiotensin II concentrations. With the two highest doses of cilazapril, 5 and 10 mg, significant decreases in both aldosterone and angiotensin II levels were observed. It may seem surprising that in the face of an effective blockade of the renin angiotensin system plasma angiotensin II levels did not drop to unmeasurable levels. The persistence of some measurable angiotensin II after ACE inhibition is explained by the fact that immunoreactive angiotensin II levels have been determined with our older method which does not detect specifically the octapeptide angiotensin II. Thus, we

have shown recently that with this method, smaller peptides such as the heptapeptide angiotensin III, hexapeptide and pentapeptide angiotensin II represent an important fraction of the measured immunoreactive angiotensin II during ACE inhibition (Nussberger *et al.*, 1986). Furthermore, there is strong evidence that angiotensin II is generated in the tube during preservation thus contributing to increase the measurable levels of immunoreactive angiotensin (Nussberger *et al.*, 1986). More recently, plasma angiotensin II was determined during ACE inhibition using a very sensitive method detecting specifically the angiotensin II octapeptide (Nussberger *et al.*, 1985). In this study, true plasma angiotensin II levels virtually disappeared following ACE inhibition. Since comparable results were obtained when two dissimilar ACE inhibitors were evaluated, it is very likely that very low plasma angiotensin II concentrations would also be obtained after cilazapril administration if the same methodology would be applied.

The results of the second study show that cilazapril administered intravenously for 24 h at low doses had no major effect on blood pressure. Indeed, supine systolic and diastolic pressures remained stable throughout the study and only a slight but significant decrease in supine diastolic blood pressure was observed between the 14th and the 24th hour of infusion with both doses of cilazapril. No significant changes in

Figure 2 Effects (mean $\pm$ s.e. mean) of four doses of cilazapril ($\bullet$ 1.25 mg, $\circ$ 2.5 mg, $\blacktriangle$ 5 mg and $\triangle$ 10 mg) on plasma aldosterone and immunoreactive angiotensin II in six healthy volunteers. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs baseline. From Fasanella d'Amore *et al.* (1987).

heart rate were found with either doses of cilazaprilat.

At the end of the cilazaprilat infusion, ACE activity had decreased to 70% of its baseline activity with the lower dose of cilazapril and to 20% with the 20 $\mu\text{g h}^{-1}$ infusion (Figure 3a). Thus, despite its prolonged administration, cilazaprilat at these doses did not result in what would be considered an effective ACE inhibition. The measurements of the other components of the renin-angiotensin system also showed no significant changes with either dose. In particular, there were no increases in plasma renin activity and angiotensin I levels and angiotensin II remained unchanged during the 24 h infusion.

The plasma cilazaprilat concentrations measured during continuous infusion of 5 and 20 $\mu\text{g h}^{-1}$ are presented in Figure 3b. As shown, plasma cilazaprilat concentrations were still increasing between 14 and 24 h and steady state drug concentrations may not have been reached at the end of the infusion. Moreover, with the highest dose of infusion (20 $\mu\text{g h}^{-1}$), plasma concentrations of 2 ng ml^{-1} were hardly obtained. These low plasma cilazaprilat concentrations certainly explain the lack of any signifi-

cant humoral changes discussed above. Indeed, pharmacokinetic studies have shown previously that cilazaprilat concentrations of about 3 ng ml^{-1} correspond to 90% ACE inhibition (Francis *et al.*, 1987). Thus, it is obvious that the doses of cilazapril evaluated in this study were too low to induce an effective inhibition.

Whereas no subjective side-effects were reported during placebo or the low dose cilazaprilat infusion, two subjects receiving the 20 $\mu\text{g h}^{-1}$ infusion complained of symptoms that led to the interruption of the infusion. In the first volunteer, marked orthostatic hypotension occurred, and in the second, the infusion was discontinued because of malaise accompanied by nausea and vomiting. The occurrence of side-effects during cilazaprilat infusion contrasts with the excellent tolerance observed when the drug was administered orally as single or even repeated doses. These side-effects were observed in the two subjects studied on the same day and whether these effects have been caused by cilazaprilat or not is difficult to determine. In any case, for safety reasons, the infusion that had been started in a third volunteer the same day, was stopped although he did not present any symptoms. The development of orthostatic hypotension during

Figure 3 a) Effects of intravenous cilazaprilat on plasma converting enzyme (ACE) activity in normotensive volunteers. b) Plasma cilazaprilat concentrations obtained during intravenous infusion of cilazaprilat in normal subjects. $\circ$ placebo ($n = 12$), $\triangle$ cilazaprilat 5 $\mu\text{g h}^{-1}$ ($n = 6$) and $\diamond$ cilazaprilat 20 $\mu\text{g h}^{-1}$ i.v. (— $n = 6$, --- $n = 3$). $\blacktriangle$ $\blacklozenge$ $P < 0.05$ vs placebo. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs time 0. Values are mean $\pm$ s.d.

ACE inhibition with cilazapril is somewhat surprising since this side-effect is rarely reported during ACE inhibition (Edwards *et al.*, 1985). Moreover, if this side-effect could possibly happen in the presence of low angiotensin II levels, repeated measurements of angiotensin II in this particular volunteer showed absolutely no change throughout the study. Thus the mechanism of this orthostatic hypotension remains unclear. Orthostatic hypotension persisted for 6 h after drug infusion and was reversed by saline administration.

During repeat administration for 8 days, cilazapril 1.25 mg day⁻¹ did not produce any significant change in blood pressure or heart rate. The blood pressure profiles obtained in subjects given 5 mg day⁻¹ are presented in Figure 4. As reported previously (Bidiville *et al.*, 1987) pooling the results obtained with three ACE inhibitors, no consistent decrease in blood pressure was observed in these normotensive subjects. However, with cilazapril some isolated significant differences were observed between pressures recorded before and during treatment. When the average of all blood pressures recorded during the 11 h was considered, a significant decrease in diastolic blood pressure was reached during cilazapril treatment (75 ± 2 mm Hg before and 66 ± 2 mm Hg during cilazapril, $P < 0.01$), but there was no change in systolic blood pressure. This drop in diastolic

blood pressure during ACE inhibition with cilazapril is actually the first significant blood pressure change observed by our group in normotensive volunteers receiving ACE inhibitors. Indeed, in contrast to other groups who reported significant drops in blood pressure during ACE inhibition in normotensive salt-repleted subjects (McGregor *et al.*, 1979, 1981), we have not been able to demonstrate significant pressure variations in response to angiotensin converting enzyme inhibitors even when the compounds were administered for longer periods (Bidiville *et al.*, 1987). Nevertheless, even this significant decrease in diastolic blood pressure observed with cilazapril has to be interpreted with caution, since diastolic blood pressure on the eighth day of cilazapril was not different from that measured one month later (post control).

Evidence against an accumulation of cilazapril during repeat administration has been provided by the results of plasma ACE activity measured in these two groups of subjects treated for 8 days. As shown in Figure 5, a marked decrease in ACE activity was observed on the first and 8th days when it was measured 4 h after administration of 1.25 and 5 mg cilazapril. On the other days, when ACE activity was only determined just before the daily dose of cilazapril, it was reduced in dose-dependent fashion. Most important, the levels of ACE activity were ex-

Figure 4 Ambulatory blood pressure profiles obtained in six normotensive volunteers before (control, $\blacktriangle$), at the end of an 8 day course of cilazapril (5 mg p.o. once daily, $\bullet$), and 1 month after stopping therapy (post-control, $\circ$). From Bidiville *et al.* (1987). * $P < 0.05$, ** $P < 0.01$ vs control.

Figure 5 Plasma angiotensin converting enzyme (ACE) activity (mean $\pm$ s.e. mean) determined in two groups of six subjects receiving either 1.25 ($\blacktriangle$) or 5 mg ($\triangle$) cilazapril for 8 days. Measurements were done on each day prior to administration of the drug. From Nussberger *et al.* (1987).

Table 1 Plasma concentrations of cilazaprilat (ng ml^{-1}) in normal volunteers

Dose	Day 1			Day 8		
	0 h	4 h	24 h	0 h	4 h	24 h
<i>Cilazapril 1.25 mg</i>						
Mean	0.05	13.4	1.3	1.5	15.5	1.6
s.e. mean	0.05	2.0	0.1	0.1	0.6	0.1
<i>Cilazapril 5 mg</i>						
Mean	0	41.5	1.6	1.8	38.7	1.9
s.e. mean	0	3.8	0.2	0.2	2.6	0.2

tremely stable from the second day on, suggesting the absence of any accumulation of cilazaprilat.

The lack of cilazapril accumulation during repeat administration is further confirmed by the plasma concentrations of cilazaprilat obtained in these subjects on days 1 and 8. As shown in Table 1, a clear relationship between the dose administered and the plasma concentrations of cilazaprilat measured 4 h later was seen on both days. In addition, no difference was found between drug concentrations determined 24 h post-drug on the first and the last day. Thus, both the hormonal and the pharmacological measurements confirm the conclusion of the pharmacokinetic analysis performed during the

acute administration of cilazapril (Francis *et al.*, 1987). It was suggested that the free drug is eliminated with a relatively short half-life and that the prolonged presence of cilazaprilat is due mainly to drug bound to the converting enzyme. Under these circumstances, accumulation of cilazapril would not be expected unless there is a major decrease in the clearance of the free drug as, for example, during renal failure.

The humoral changes induced by repeated doses of cilazapril were very similar to those reported above during single dose administration, i.e. blood angiotensin I and plasma renin activity were markedly increased at 4 h whereas plasma angiotensin II and aldosterone

concentrations decreased. These changes were not significant with the lowest dose of 1.25 mg day⁻¹ cilazapril.

In contrast to the intravenous infusion and despite its prolonged administration, no subjective or objective side-effects were reported during the 8 days of cilazapril treatment. In particular, none of the volunteers complained of orthostatic hypotension or gastrointestinal discomfort. These results were in agreement with our first impression that the clinical tolerance of cilazapril seemed to be good even during prolonged oral administration, a fact that mean-

while has been confirmed by a large number of patients treated with cilazapril.

In summary, we have presented in this article the results of three studies in which cilazapril was evaluated in normotensive healthy subjects. The blood pressure and the hormonal effects of this agent were assessed during single dose as well as prolonged administration. Our results demonstrate that cilazapril is a potent and longlasting converting enzyme inhibitor. Despite its long duration of inhibition, no accumulation of the drug occurred.

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