The effects of cilazapril alone and in combination with frusemide in healthy subjects

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1 In a fourway double-blind placebo controlled study, the effects of cilazapril, a new angiotensin converting enzyme inhibitor, on renal function and the responses to intravenous frusemide were studied in a group of twelve salt depleted male volunteers.

2 Cilazapril produced an increase in effective renal plasma flow and urinary output of prostaglandin E₂ metabolite (PGE₂-M) but no effect on sodium, potassium or water excretion.

3 Pretreatment with cilazapril antagonised the effects of frusemide on glomerular filtration, PGE₂-M and sodium excretion.

Keywords cilazapril frusemide effective renal plasma flow glomerular filtration

Introduction

Captopril and enalapril have been shown to increase renal blood flow acutely (Mimran *et al.*, 1979; McNabb *et al.*, 1986), an effect which depends on salt balance (McNabb *et al.*, 1986). The effect on the glomerular filtration rate is variable and depends on the experimental conditions (Mimran *et al.*, 1979; Hollenberg *et al.*, 1981; McNabb *et al.*, 1986; Navis *et al.*, 1986). Captopril and enalapril cause acute natriuresis (Brunner *et al.*, 1981; Hollenberg *et al.*, 1981; McNabb *et al.*, 1986; Navis *et al.*, 1986) which may be accompanied by potassium retention (Atlas *et al.*, 1979; Navis *et al.*, 1986) or in the case of enalapril by kaliuresis (McNabb *et al.*, 1985, 1986). Both drugs have been reported to increase prostaglandin levels (Swartz *et al.*, 1980; Oparil *et al.*, 1983) but there are no data for cilazapril.

Frusemide has acute effects on renal blood flow and glomerular filtration (Mackay *et al.*, 1984) which are unrelated to natriuresis. Frusemide causes acute increases in plasma renin activity (Frolich *et al.*, 1976; Johnston *et al.*, 1986) and prostaglandin excretion (Mackay *et al.*, 1984). Captopril has previously been shown to inhibit the diuretic responses to frusemide in animals, and in dogs there is evidence that captopril abolishes the acute

increases in renal blood flow produced by intravenous frusemide (Johnston *et al.*, 1984). There is little evidence concerning the effects of angiotensin converting enzyme inhibition on the acute effects of frusemide in man and this study was designed to correct that deficiency.

Methods

Twelve healthy male volunteers aged 20–31 were included in a double-blind, randomised, fourway cross over design after consent had been given, after the inclusion and exclusion criteria had been fulfilled and following approval of the study by the local Ethics Committee. This resulted in four treatment combinations—placebo/saline, cilazapril/saline, placebo/frusemide, cilazapril/frusemide. Each subject was studied on four separate occasions at least 1 week apart. For 7 days before the first study day and throughout the whole study period subjects consumed a diet containing 50–60 mmol of sodium and potassium during a 24 h period. 24 h sodium output was measured on day 4 to check compliance. If sodium excretion was greater than 60 mmol 24 h⁻¹, subjects were asked to restrict further their sodium

intake and 24 h urine collections were repeated until subjects were within the desired range.

On the day of each experiment subjects attended the Department of Therapeutics and Pharmacology having fasted from 23.00 h the previous night. At the same time subjects received 50 g potassium iodide by mouth to block the uptake of radioactive iodine by the thyroid gland. On arrival subjects assumed a semisupine position in which they remained throughout the study period except to pass urine.

Subjects were given 450 ml of water to drink on arrival and a fluid intake of 150 ml h^{-1} was given thereafter. An inulin bolus followed by an infusion was started through one of two indwelling cannulae which had previously been inserted into each arm. Ninety minutes later 5 mg cilazapril or identical placebo was administered after the second 45 min collection period.

Sixty minutes after oral therapy ^{131}I orthohippurate was injected and blood was sampled at frequent intervals over a 1 h period (Wagoner *et al.*, 1964). At 120 min after cilazapril or placebo a second injection of ^{131}I -iodohippurate was given. Blood was again sampled over a 1 h period. Correction for the residual activity from the first injection was made before calculation of the second effective renal plasma flow. Plasma renin activity (Haber *et al.*, 1969) and plasma angiotensin II (Nussberger *et al.*, 1984) were measured at 60 and 90 min (before cilazapril or placebo), 150 min (1 h later) and 210 min (before

and 215 and 240 min (after) frusemide or saline administration. Urinary prostaglandin E_2 metabolite was measured in each urine collection.

Statistical analyses were carried out using an analysis of variance for a balanced Latin square design to determine the effects of each drug alone and together using placebo/saline study day as the comparator. The results are expressed as the mean $\pm$ s.e. mean and the level of significance was chosen at the 5% level.

Results

The effect of cilazapril on renal function

Cilazapril had no effect on the glomerular filtration rate during the first two periods (Figure 1) but between 120 and 180 min after cilazapril administration an increase was observed at all three collection periods which almost achieved statistical significance ($P < 0.07$). Effective renal plasma flow during the second hour following placebo was $616 \pm 38 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$. The corresponding figure for cilazapril was significantly higher at $716 \pm 34 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$, $P < 0.05$ (Figure 2). At 2–3 h, cilazapril again increased effective renal plasma flow significantly as compared with placebo ($725 \pm 29 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$ vs $608 \pm 36 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$, $P < 0.05$).

The effect of cilazapril on the excretion of prostaglandin E_2 metabolite is shown in Figure 3. Prostaglandin E_2 metabolite excretion was

Figure 1 Glomerular filtration rate (mean $\pm$ s.e. mean, $n = 12$) following placebo (hatched boxes) or cilazapril 5 mg (clear boxes).

Figure 2 Effective renal plasma flow (mean $\pm$ s.e. mean, $n = 12$) during the 1–3 h period following placebo (hatched boxes) or cilazapril 5 mg (clear boxes). * $P < 0.05$ significant difference from placebo.

increased at all time periods as compared with placebo; 30–75 min $2006 \pm 396 \text{ pg min}^{-1} \text{ vs } 1232 \pm 326 \text{ pg min}^{-1}$, $P < 0.05$; 75–100 min $1694 \pm 300 \text{ pg min}^{-1} \text{ vs } 913 \pm 179 \text{ pg min}^{-1}$, $P < 0.01$; 120–140 min $2099 \pm 350 \text{ pg min}^{-1} \text{ vs } 1336 \pm 16 \text{ pg min}^{-1}$, $P < 0.05$; 140–160 min $2021 \pm 479 \text{ pg min}^{-1} \text{ vs } 1297 \pm 317 \text{ pg min}^{-1}$, $P < 0.05$; 160–

180 min $2129 \pm 325 \text{ pg min}^{-1} \text{ vs } 1254 \pm 271 \text{ pg min}^{-1}$.

The changes in plasma renin activity and angiotensin II concentrations are shown in Figure 4. Plasma renin activity was unchanged by placebo, but in the presence of cilazapril, plasma renin activity was increased at all time periods compared with baseline ($P < 0.05$) and placebo ($P < 0.05$, Figure 4). Values doubled from baseline $2.0 \pm 0.2 \text{ ng AI ml}^{-1} \text{ h}^{-1}$ to $4.0 \pm 0.8 \text{ ng AI ml}^{-1} \text{ h}^{-1}$ at 1 h and almost doubled again at 2 h ($7.5 \pm 1.5 \text{ ng AI ml}^{-1} \text{ h}^{-1}$). The value at 2 h was $9.0 \pm 2.0 \text{ ng AI ml}^{-1} \text{ h}^{-1}$. Levels of angiotensin II were lower at 2 h after cilazapril ($5.7 \pm 0.8 \text{ fmol ml}^{-1}$, $P < 0.01$) and 2.5 h ($7.3 \pm 1.1 \text{ fmol ml}^{-1}$, $P < 0.05$) as compared with baseline ($11.0 \pm 1.3 \text{ fmol ml}^{-1}$). However angiotensin II values during placebo were not significantly lower than the values during cilazapril.

The effects of pretreatment with cilazapril on the renal responses to frusemide

Glomerular filtration rate was increased during the 20 min after frusemide administration with placebo ($\Delta = 37.9 \pm 4.3 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$, $P < 0.05$, Figure 5). The increase in glomerular filtration observed with frusemide in the cilazapril pretreated group failed to achieve statistical significance during this time period ($\Delta 18.9 \pm 6.3 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$, $P < 0.01$). Values returned to baseline over the 1 h period after

Figure 3 Excretion of urinary $\text{PGE}_2\text{-M}$ (mean $\pm$ s.e. mean, $n = 12$) following placebo (hatched boxes) or cilazapril (clear boxes). * $P < 0.05$, ** $P < 0.01$ significant difference from placebo.

Figure 4 Plasma renin activity and angiotensin II levels (mean $\pm$ s.e. mean, $n = 12$) after placebo (●—●) or cilazapril 5 mg (○---○). * $P < 0.05$, ** $P < 0.01$ significant difference from baseline, † $P < 0.05$ significant difference from placebo

frusemide (Figure 5). The acute increases in glomerular filtration rate were significantly less in the cilazapril pretreated group than in the placebo treated group ($P < 0.05$, Figure 5). Although frusemide produced acute increases in effective renal plasma flow, these were not statistically significant and there were no differences between the placebo and cilazapril pretreated groups.

The increments in renal prostaglandin E₂ metabolite excretion after cilazapril did not achieve significance in the time period before frusemide was given. In the presence of placebo, frusemide produced an increase in prostaglandin excretion at 0–20 min (120–140 min, Figure 6, $\Delta 660 \pm 306$ pg min⁻¹). These changes were significantly lowered by cilazapril pretreatment at the first and second time periods after frusemide ($P < 0.05$, Figure 6).

The results of the effects of cilazapril and frusemide on plasma renin activity and plasma angiotensin II levels are demonstrated in Figure 7. Pretreatment with cilazapril resulted in an increase in baseline plasma renin activity when compared with placebo ($P < 0.01$). In the presence of placebo, frusemide increased plasma renin activity at 5 min (130 min, Figure 7, $P < 0.05$). Plasma renin activity was also increased after frusemide when the subjects were pretreated with cilazapril but these were significantly greater than in the placebo treated group

Figure 5 Glomerular filtration rate (mean $\pm$ s.e. mean, $n = 12$) before and after frusemide 20 mg intravenously in volunteers pretreated with placebo (clear boxes) or cilazapril (hatched boxes). * $P < 0.05$ significant difference from baseline, † $P < 0.05$ significant difference from change over baseline produced by frusemide: placebo treated vs cilazapril pretreated.

Figure 6 Urinary prostaglandin E₂ metabolite excretion (mean $\pm$ s.e. mean, $n = 12$) following frusemide 20 mg intravenously in volunteers pretreated with placebo (clear boxes) or cilazapril 5 mg (hatched boxes). * $P < 0.05$ significant difference from change over baseline produced by frusemide: placebo pretreated vs cilazapril pretreated.

Figure 7 Plasma renin activity and angiotensin II levels (mean $\pm$ s.e. mean, $n = 12$) following frusemide 20 mg intravenously in subjects pretreated with placebo (●—●) or cilazapril 5 mg (○—○). * $P < 0.05$, ** $P < 0.01$ significant difference from baseline, † $P < 0.05$, †† $P < 0.01$ significant difference from placebo.

($P < 0.01$, Figure 7). In subjects pretreated with placebo, frusemide increased angiotensin II at 5 min ($\Delta 11.8 \pm 3.8$ fmol ml⁻¹ 130 min, Figure 7, $P < 0.01$). This was completely blocked by pretreatment with cilazapril (Figure 7).

The effects of frusemide on urine flow rate and on urinary sodium and potassium excretion are shown in Figure 8. As expected, frusemide produced an increase in water, potassium and sodium excretion. Although cilazapril pretreatment appeared to reduce water, potassium and sodium excretion following frusemide, the differences were only significant for sodium excretion during the first two urine collection periods after diuretic administration.

Discussion

Cilazapril, like other angiotensin converting enzyme inhibitors, increased plasma renin activity and reduced angiotensin II concentrations. Cilazapril also increased the excretion of prostaglandin E₂ metabolite which may reflect increased prostaglandin synthesis. An increase in prostaglandin E₂ has been reported with captopril (Swartz *et al.*, 1980; Moore *et al.*, 1981) and enalapril (Oparil *et al.*, 1983) and has been attributed to increased kinin levels (Swartz *et al.*, 1980) or a direct effect of the drug on phospholipase activity (Swartz *et al.*, 1980).

Figure 8 Urinary water, sodium and potassium excretion following frusemide 20 mg intravenously in subjects pretreated with placebo (clear boxes) or cilazapril 5 mg (hatched boxes). * $P < 0.05$ significant difference from change over baseline produced by frusemide: placebo pretreated vs cilazapril pretreated.

The increase in vasodilator prostaglandins could contribute to the increases in renal blood flow seen in the present study. This association between increases in renal blood flow and increased output of prostaglandins has been described for captopril and enalapril (Mimran *et al.*, 1979; Brunner *et al.*, 1980; Hollenberg *et al.*, 1981; McNabb *et al.*, 1986; Navis *et al.*, 1986). Previous studies have not demonstrated an increase in glomerular filtration with angiotensin converting enzyme inhibitors (Mimran *et al.*, 1979; Brunner *et al.*, 1981; Bauer, 1984). An increase in glomerular filtration rate is often seen in animals (Navar & Rosivall, 1984) and in this study the increases as compared with placebo almost achieved statistical significance at the 5% level. These differences could well be related to differences in salt balance. In the salt depleted setting it has been suggested that the predominant effect of angiotensin II is at the efferent arteriole to maintain glomerular filtration. If this is correct then a reduction in angiotensin II would be expected to decrease glomerular filtration. The probable increase

which we observed in glomerular filtration rate may be explained by a reduction of angiotensin II activity on the afferent and efferent arterioles (Navar & Rosivall, 1984) or by a combined effect of the increase in vasodilator prostaglandins and reduction in angiotensin II to increase glomerular surface area and so alter the glomerular ultrafiltration coefficient and thus glomerular filtration.

Frusemide produced the expected diuresis, natriuresis and kaliuresis. The ability of frusemide to stimulate the renin angiotensin system was evident from 5 min and reached maximum at 10 min which is consistent with previous reports (Frölich *et al.*, 1976; Johnston *et al.*, 1986; Mackay *et al.*, 1984). The increases in renal plasma flow following frusemide have been reported before (Mackay *et al.*, 1984) and are critically dependent on salt balance. The effect is seen only when the kidney is in a salt retaining state and the renin angiotensin system is activated (Gerber, 1983). An increase in glomerular filtration rate has not always been observed with frusemide (Tilstone *et al.*, 1977)

but has been described in other studies (Vorbürger, 1964; Mackay *et al.*, 1984). It occurred in this study but was short-lived as was the increase in urinary prostaglandin E₂ metabolite. The increase in urinary vasodilator prostaglandins following frusemide have been described before (Mackay *et al.*, 1984). Pretreatment with cilazapril abolished the increases in prostaglandin E₂ metabolite and attenuated the increases in glomerular filtration and sodium excretion. The increases in angiotensin II following frusemide were also abolished by cilazapril.

Some of these results are probably related to changes in baseline produced by cilazapril. The magnitudes of the changes are then relatively lower when compared with placebo. This is similar to our previous observations in dogs (Johnston, 1984). The increase in glomerular filtration rate seen during the first time period after frusemide was attenuated by pretreatment with cilazapril. This undoubtedly reflects increases in baseline values produced by cilazapril. Cilazapril also reduced the frusemide induced changes in prostaglandin E₂ metabolite. Since

vasodilatory prostaglandins are involved in the renal vascular and diuretic responses to frusemide (Gerber, 1983) this decrease could account for the changes in effective renal plasma flow (not significant) and natriuresis produced by frusemide after cilazapril administration.

The increment in plasma renin activity produced by frusemide was more marked in the presence of cilazapril. These results reflect the combination of acute stimulation by frusemide and removal of the negative feedback inhibition of angiotensin II. The increases in angiotensin II following frusemide were blocked by cilazapril and demonstrate effective inhibition of angiotensin converting enzyme activity in the presence of acute renin release.

Overall the acute administration of cilazapril increased renal blood flow and the output of prostaglandin E₂ metabolite but failed to have a significant effect on sodium, potassium or water excretion. Pretreatment with cilazapril antagonised the effects of frusemide on glomerular filtration and on prostaglandin E₂ metabolite and sodium excretion.

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