Dose-dependent effects of angiotensin converting enzyme (ACE) inhibitors on glomerular prostanoid production by normotensive rats

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- 1 This study was designed to investigate whether the angiotensin converting enzyme (ACE) inhibitors, captopril, enalapril and fosinopril have a dose-dependent effect on the production of prostaglandin E₂ (PGE₂), prostaglandin I₂ (prostacyclin, PGI₂) and thromboxane A₂ (TxA₂) by glomeruli isolated from normotensive Wistar-Kyoto rats.
- 2 Measurements of glomerular prostanoid production were made under basal conditions and in the presence of excess exogenous arachidonic acid.
- 3 All three ACE inhibitors demonstrated dose-dependent effects upon glomerular prostanoid production which varied with the individual ACE inhibitor.
- 4 Enalapril induced a dose-dependent increase in the ratio of (PGE₂ + PGI₂)/TxA₂, from 2.17 ± 0.20 to 5.35 ± 0.84 and to 10.0 ± 1.16 with the low and high doses of enalapril respectively. In contrast, the high dose of captopril tended to reduce the ratio when compared to the low dose.
- 5 The results obtained in this study suggest that although all three ACE inhibitors appear to induce prostacyclin synthetase and/or modulate phospholipase A₂ (PLA₂) activity, these effects differ with the ACE inhibitor studied and the dose employed.
- 6 This study has demonstrated dose-dependent effects of three ACE inhibitors on glomerular prostanoid production which may be significant in modulating glomerular haemodynamics and growth characteristics of glomerular cells.

Keywords: Angiotensin converting enzyme inhibitors; isolated glomeruli; prostanoids; angiotensin converting enzyme

Introduction

Recent studies in this laboratory have demonstrated that certain angiotensin converting enzyme (ACE) inhibitors which differed in their molecular structure could modulate prostanoid production by glomeruli from both normotensive (WKY) and spontaneously hypertensive rats (SHR) (Harding *et al.*, 1991). Their effects on prostaglandin E₂ (PGE₂), PGI₂ and thromboxane A₂ (TxA₂) differed with the nature of the ACE inhibitor, but were independent of any effect on blood pressure and unrelated to the presence of a sulphydryl group in the molecular structure. It was suggested that their differing effects on glomerular prostanoid synthesis might be the result of variations in the glomerular concentration or bio-availability of each compound and that this might also explain the many discrepancies recently reported relating to the ability of different ACE inhibitors to modify the severity of injury in certain forms of experimentally-induced models of glomerulonephritis (Anderson *et al.*, 1986; Ohishi *et al.*, 1989). It was also felt that a similar explanation could account for the findings of Ikoma *et al.* (1991), in that the protection offered by the ACE inhibitor, enalapril, in an experimental model of glomerulonephritis was dose-related/dependent.

Although the mechanisms responsible for the effects of ACE inhibitors on glomerular function and morphology are not clearly understood, it is thought that they may be mediated through their influence on glomerular angiotensin II (AII) generation (Lo *et al.*, 1990), kinin degradation and/or prostanoid production (Galler *et al.*, 1982); since these compounds play a central role in determining changes in the biological activities of glomerular cells and certain aspects of

glomerular function, particularly those concerned with the maintenance of glomerular haemodynamics (Stork & Dunn, 1985; Nath *et al.*, 1987) and cell growth (Menè & Dunn, 1990; Paquet *et al.*, 1990). In this respect, it could be of relevance that the three ACE inhibitors previously studied all increased the ratio of (PGE₂ + PGI₂)/TxA₂, i.e. an increase in those prostanoids which are responsible for maintaining glomerular perfusion as opposed to those which compromise glomerular haemodynamics and also, those that inhibit cell growth compared with those prostanoids that have mitogenic properties.

Since initial experiments performed in this laboratory had demonstrated that doses of 60 mg kg⁻¹ day⁻¹ captopril, 15 mg kg⁻¹ day⁻¹ enalapril and 60 mg kg⁻¹ day⁻¹ fosinopril gave equivalent reductions in the blood pressure of SHR but produced differing effects upon glomerular prostanoid production (Harding *et al.*, 1991); the present study was designed to establish whether the effects of ACE inhibitors on glomerular prostanoid production might be dose-dependent as this would have important therapeutic implications as suggested by Ikoma *et al.* (1991). The influence of both 15 and 60 mg kg⁻¹ day⁻¹ doses of each ACE inhibitor on glomerular prostanoid production was therefore investigated.

Methods

Animals studied

Adult, 15 week-old, male WKY (Charles River, Margate, Kent) were studied. They were maintained on a diet containing 16.6% protein, 0.5% sodium and 0.7% potassium (Pilsbury, Birmingham), had free access to tap water and were housed in standard cages at a temperature of 20°C.

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Protocol

Rats were randomly allocated into 7 groups, each containing 15 animals. Groups 1 and 2 received 15 or 60 mg kg⁻¹ day⁻¹ captopril, groups 3 and 4, 15 or 60 mg kg⁻¹ day⁻¹ enalapril and groups 5 and 6, 15 or 60 mg kg⁻¹ day⁻¹ fosinopril respectively whereas Group 7 received water alone i.e. control animals. The ACE inhibitors were administered in the drinking water for 10 days and water consumption measured on a daily basis, with appropriate adjustments to ensure that the dosage of ACE inhibitor remained constant. Systolic blood pressure was measured by the indirect tail-cuff method on the day before the start of treatment with the ACE inhibitor and on day 10 of treatment (Pfeffer *et al.*, 1971).

Preparation of isolated glomeruli

Preparations of isolated glomeruli in Krebs-Henseleit buffer, pH 7.4 were obtained by a mechanical sieving technique with glomeruli collected from the surface of a 75 µm sieve (Fond & Drummond, 1968). The purity of the glomerular suspension was assessed by light microscopy, with suspensions of >85% purity being used for the experiments. Glomerular protein content was determined by the method of Lowry *et al.* (1951) after digestion of the glomeruli with NaOH 2 mol l⁻¹.

Glomerular prostanoid production

Glomerular prostanoid production was determined by modifications of previously described methods (Schambelan *et al.*, 1985; Lefkowitz & Schreiner, 1987). Briefly, 0.9 ml portions of the glomerular suspension were incubated either alone or in the presence of 15 µmol l⁻¹ arachidonic acid (Sigma) for 0 and 10 min at 37°C to provide basal production and production in the presence of excess exogenous arachidonic acid. Reactions were terminated by the addition of 50 µl of 2.8 mmol l⁻¹ indomethacin followed by centrifugation at 1500 g for 3 min (4°C). The supernatants were then stored at -20°C before radioimmunoassay.

PGE₂, PGI₂ (assayed as the stable metabolite 6-keto PGF_{1α}) and TxA₂ (assayed as the stable metabolite TxB₂) were determined in duplicate with radioimmunoassay kits (New England Nuclear, Boston, MA, U.S.A.). The cross-reactivities for the antisera are claimed by New England Nuclear to be as follows: PGE₂ antiserum to PGF_{2α}, <0.01%; to 6-keto PGF_{1α}, <0.01% and to TxB₂, 0.02%. The TxB₂ antiserum to PGF_{2α}, 0.04%; to 6-keto PGF_{1α}, 0.01% and to PGE₂, 0.003%. The 6-keto PGF_{1α} antiserum to PGF_{2α}, 0.6%; to TxB₂, 0.4% to PGE₂, 0.4%. The results are expressed as ng of prostanoid produced mg⁻¹ glomerular protein 10 min⁻¹.

Statistical analysis

Data are given as means ± s.e.mean. The results were subjected to statistical analysis to determine whether the data were normally distributed. The significance of differences between the untreated (control) and experimental groups was

tested by a one-way analysis of variance followed by Dunnett's test for multiple comparisons. A *P* value of <0.05 was considered significant.

Results

Blood pressure (Table 1)

The ACE inhibitors had no influence on systolic blood pressure in either control animals (Group 7) or in Groups 1, 2, 3, 5 and 6. There was, however, a significant reduction in blood pressure from 137.8 ± 5.4 to 119.6 ± 5.1 mmHg, *P* < 0.05 in Group 4, i.e. rats receiving a high dose of enalapril.

Glomerular prostanoid production (Figure 1 and Table 2)

In the following section, all *P* values refer to differences between untreated and treated animals.

Captopril Under basal conditions, neither high nor low doses of captopril had any effect upon either glomerular PGE₂ or TxA₂ production whereas PGI₂ production was increased by the low dose captopril (*P* < 0.005).

Similarly, in the presence of arachidonic acid, only the low dose of captopril had any effect upon glomerular prostanoid production, affecting an increase in PGI₂ (*P* < 0.005).

Enalapril Treatment with low dose enalapril decreased the basal production of all three prostanoids (*P* < 0.05 for PGE₂, *P* < 0.01 for PGI₂ and *P* < 0.005 for TxA₂). In contrast the high dose affected an increase in the basal production of PGI₂ (*P* < 0.005). A decrease in glomerular TxA₂ production was also observed following a high dose of enalapril (*P* < 0.05).

In the presence of arachidonic acid, low dose enalapril reduced the production of TxA₂ (*P* < 0.05) and a high dose of enalapril increased PGI₂ production (*P* < 0.005).

Fosinopril Treatment with low dose fosinopril affected an increase in the basal production of PGI₂ (*P* < 0.01) and a decrease in TxA₂ (*P* < 0.05). The high dose had a similar effect upon PGI₂ production (*P* < 0.005) and, in addition, affected an increase in PGE₂ production (*P* < 0.01).

In the presence of arachidonic acid, neither the high nor the low dose of fosinopril had any effect upon glomerular TxA₂ production. The low dose increased the production of PGI₂ (*P* < 0.05) whereas the high dose increased PGE₂ (*P* < 0.01).

The ratio of (PGE₂ + PGI₂)/TxA₂ (Figure 2)

Since a change in the ratio of vasodilator to vasoconstrictor prostanoids and those prostanoids with the potential to limit cell proliferation as opposed to those concerned with promoting mitogenesis may have more functional significance than a change in the absolute value of any individual prostanoid, the effect of the three ACE inhibitors, upon the ratio of

Table 1 The effect of low and high doses of captopril, enalapril and fosinopril on mean systolic blood pressure

	Mean systolic blood pressure (mmHg)			
	15 mg kg ⁻¹ day ⁻¹		60 mg kg ⁻¹ day ⁻¹	
	Before	After	Before	After
Control	129.0 ± 4.0	126.2 ± 4.1	129.0 ± 4.0	126.2 ± 4.1
Captopril	122.2 ± 5.2	116.6 ± 6.9	128.5 ± 2.7	121.6 ± 2.1
Enalapril	128.0 ± 3.0	124.1 ± 3.1	137.8 ± 5.4	119.6 ± 5.1*
Fosinopril	116.2 ± 4.8	123.9 ± 6.4	129.1 ± 2.7	127.1 ± 4.0

Values are means ± standard error of the mean (*n* = 15) for each group. Animals received either 15 or 60 mg kg⁻¹ day⁻¹ captopril, enalapril, or fosinopril for 10 days. Untreated animals (control) received water alone.

Statistical significance: **P* < 0.05 compared to values before treatment.

Figure 1 Prostaglandin E_2 (PGE_2 , open columns), prostacyclin (PGI_2 , solid columns; assayed as 6-keto $PGF_{1\alpha}$) and thromboxane A_2 (TxA_2 , hatched columns; assayed as TxB_2) production by glomeruli from WKY rats after treatment with either 15 or 60 $mg\ kg^{-1}\ day^{-1}$ captopril (a), enalapril (b) or fosinopril (c) for 10 days. Control (Con) animals received water alone. Results are means $\pm$ s.e.mean (vertical bars). Statistical significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$ compared with respective values in control animals.

($PGE_2 + PGI_2$)/ TxA_2 has been determined. Low doses of all three ACE inhibitors increased the ratio of ($PGE_2 + PGI_2$)/ TxA_2 ($P < 0.01$ for captopril, $P < 0.005$ for enalapril and $P < 0.001$ for fosinopril). Although further increases in this ratio were noted with high doses of enalapril and fosinopril, this change achieved statistical significance only for enalapril ($P < 0.01$). However, there was no difference between the value of the ratio for high doses of enalapril and fosinopril.

Discussion

This study has demonstrated dose-dependent effects of three structurally dissimilar ACE inhibitors upon glomerular prostanoid production in normotensive Wistar Kyoto rats which vary for each ACE inhibitor. At low doses, captopril and fosinopril stimulated the basal production of PGI_2 . All three prostanoids were reduced by low dose enalapril whereas fosinopril also reduced TxA_2 . In contrast, the high dose of enalapril caused an increase in the basal production of PGI_2 and fosinopril caused an increase in both PGE_2 and PGI_2 . The high dose of captopril had no effect on glomerular prostanoid production.

Although it is appreciated that the preparation of isolated glomeruli by the sieving technique employed in this study may stimulate some prostanoid production, this would be

Figure 2 The effect of treatment with either 15 or 60 $mg\ kg^{-1}\ day^{-1}$ captopril ($\square$), enalapril ($\bullet$) or fosinopril ($\blacksquare$) for 10 days on the basal ratio of ($PGE_2 + PGI_2$)/ TxA_2 . Hatched bar represents the basal ratio in untreated animals. Results are means $\pm$ s.e.mean (vertical bars). Statistical significance: * $P < 0.01$; ** $P < 0.005$ and *** $P < 0.001$ compared to values in untreated animals.

Table 2 The effects of captopril, enalapril and fosinopril on glomerular prostanoid production in the presence of excess arachidonic acid

	PGE_2		PGI_2		TxA_2	
	15 $mg\ kg^{-1}$	60 $mg\ kg^{-1}$	15 $mg\ kg^{-1}$	60 $mg\ kg^{-1}$	15 $mg\ kg^{-1}$	60 $mg\ kg^{-1}$
Con	4.46 $\pm$ 1.02	4.46 $\pm$ 1.02	1.31 $\pm$ 0.19	1.31 $\pm$ 0.19	0.81 $\pm$ 0.18	0.81 $\pm$ 0.18
Cap	4.14 $\pm$ 0.31	5.16 $\pm$ 0.43	2.78 $\pm$ 0.20***	1.04 $\pm$ 0.15	1.15 $\pm$ 0.23	0.66 $\pm$ 0.12
Ena	4.23 $\pm$ 0.63	4.33 $\pm$ 0.58	1.36 $\pm$ 0.28	2.74 $\pm$ 0.34***	0.33 $\pm$ 0.09*	0.36 $\pm$ 0.06
Fos	3.28 $\pm$ 0.11	8.82 $\pm$ 0.82**	2.20 $\pm$ 0.23*	1.75 $\pm$ 0.18	0.81 $\pm$ 0.14	0.42 $\pm$ 0.15

Values represent mean prostanoid production $\pm$ s.e.mean (ng prostanoid mg^{-1} glomerular protein 10 min^{-1}) in the presence of 15 $\mu mol\ l^{-1}$ arachidonic acid by glomeruli isolated from rats treated with either 15 or 60 $mg\ kg^{-1}\ day^{-1}$ of captopril (Cap), enalapril (Ena) or fosinopril (Fos) for 10 days. Con are control animals which received only water.

Statistical significance: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.005$ compared to values in untreated animals.

likely to affect both untreated (control) and treated groups of rats and would therefore be taken into account in assessing any influence of an ACE inhibitor on glomerular prostanoid production. The alternative of measuring urinary prostanoid production is known to be a poor reflection of glomerular production because it relates to total renal production and to some prostanoid production from extra-renal sites. In addition, studies performed in this laboratory have shown that the *in vitro* incubation of isolated glomeruli with various concentrations of ACE inhibitors has no effect on glomerular prostanoid production.

Although there are no previous reports of studies designed to examine the influence of different doses of ACE inhibitors *in vivo* on prostanoid production, the present findings complement those reported by Nakagawa *et al.* (1987) in which a dose-dependent inhibitory effect of captopril on prostacyclin generation by cultured human vascular endothelial cells was demonstrated.

In the present study, untreated animals demonstrated a profile of basal glomerular prostanoid production consistent with that described by us and other investigators ($\text{PGE}_2 > \text{TxA}_2 = \text{PGI}_2$) (Folkert & Schlondorff, 1979; Schlondorff *et al.*, 1980). The addition of arachidonic acid to isolated glomeruli produced a greater increase in PGE_2 production than PGI_2 , suggesting that endoperoxide E-isomerase has a greater affinity for PGH_2 than prostacyclin synthetase. In contrast, a decrease in thromboxane production was noted in the presence of excess arachidonic acid. Whilst an increase might have been anticipated, the reduced thromboxane suggests that thromboxane synthetase is substrate inhibited: a finding in agreement with the work described by Uehera *et al.* (1988) in studies using rat vascular smooth muscle cells.

The present findings suggest the induction of prostacyclin synthetase by all three ACE inhibitors under both basal conditions and in the presence of excess arachidonic acid, such that PGH_2 was preferentially metabolized to PGI_2 rather than PGE_2 . The fact that low doses of captopril and

fosinopril increased PGI_2 production under both assay conditions without any change in PGE_2 production also suggests that they increase prostacyclin synthetase activity rather than having an effect on phospholipase A_2 (PLA_2). In contrast, low dose enalapril reduced the synthesis of all three prostanoids under basal conditions, presumably by an inhibition of PLA_2 . At the high dose, enalapril affected an increase in PGI_2 synthesis without any apparent effect on PLA_2 as the total sum of basal prostanoid production remained constant. This effect contrasts with the apparent stimulation of PLA_2 activity induced by high dose fosinopril. On the available evidence, one cannot exclude the possibility that some changes in glomerular prostanoid production might have resulted from an inhibition of one enzyme with the subsequent redirection of prostanoid synthesis.

All three ACE inhibitors produced increases in the ratio of $(\text{PGE}_2 + \text{PGI}_2)/\text{TxA}_2$. Although the maximum effects induced by enalapril and fosinopril were similar, only enalapril exhibited a dose-dependent effect. Whilst it is appreciated that the influence of ACE inhibitors on prostanoid production may differ between epithelial, mesangial and endothelial cells of the glomerulus, one could nevertheless speculate that an increase in the ratio of $(\text{PGE}_2 + \text{PGI}_2)/\text{TxA}_2$ may have beneficial influences on glomerular haemodynamics, cell growth and mesangial matrix expansion; all of which are considered to be important in the pathogenesis of progressive glomerular injury. Indeed, one might suggest that changes in glomerular prostanoid production induced by enalapril provide a possible explanation for the observations of Ikoma *et al.* (1991), that this ACE inhibitor limits experimentally-induced glomerular injury in a dose-dependent manner.

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