Effects of enalaprilat on venoconstriction to norepinephrine: role of prostaglandins

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Received 27 September 2002; accepted 24 February 2003

Abstract

Objective: Most patients with cardiovascular disease continue to receive both aspirin and an angiotensin-converting enzyme (ACE) inhibitor. This is despite the fact that ACE inhibition also inhibits the enzyme kininase II and leads to accumulation of bradykinin which increases prostaglandins. We hypothesized that in normal veins, vasodilator prostaglandins contribute significantly to ACE inhibitor dilation of norepinephrine-induced venoconstriction, and this would be blocked by cyclooxygenase inhibition. Methods: The study was performed using the in vivo dorsal hand vein technique for measuring vascular responses directly. Venoconstriction to norepinephrine infusions (0.5–1024 ng/min) was assessed in eight normal subjects (46\textpm5 years, mean\textpmS.E.M.) during coinfusion of saline in one hand (control) and enalaprilat (1000 µg/min) in the contralateral hand. On a second morning (7\textpm1 days apart, mean\textpmS.E.M., random order), the same procedure was repeated with indomethacin (3 µg/min) coinfusion in both hands. Results: Enalaprilat shifted the norepinephrine dose–response curve to the right ($P=0.024$) and increased the norepinephrine log ED\textsubscript{50} (dose required to cause 50% venoconstriction) from 1.70\textpm0.08 to 2.31\textpm0.11 log ng/min ($P=0.001$). Indomethacin shifted the norepinephrine dose–response curve to the left ($P=0.018$) and decreased the norepinephrine log ED\textsubscript{50} from 1.70\textpm0.08 to 1.09\textpm0.18 log ng/min ($P=0.002$). In the presence of indomethacin, enalaprilat caused only a small but significant increase in the norepinephrine log ED\textsubscript{50}, from 1.09\textpm0.18 to 1.29\textpm0.18 log ng/min ($P=0.041$). Conclusions: The results suggest that vasodilator prostaglandins contribute significantly to the attenuation of sympathetic venoconstriction by enalaprilat. This may have clinical relevance in patients receiving aspirin and ACE inhibitors in the setting of increased sympathetic activity.

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Keywords: Clinical; Vasculature; Organism; Pharmacology

1. Introduction

Angiotensin-converting enzyme (ACE) inhibitors are now recommended in many patients with cardiovascular diseases and are first line therapy in patients with chronic heart failure [1,2]. Ischemic heart disease is the principal cause of heart failure in 60–70% of patients with systolic dysfunction [2]. It has also been shown in clinical trials that acetylsalicylic acid (ASA) reduces short-term mortality, reduces the incidence of recurrent myocardial infarctions, and reduces mortality from cardiovascular events. As a result, current practice is to use ASA routinely in many cardiovascular patients [3,4] unless there is a contraindication for its use. Thus, many patients with hypertension and the majority of patients with heart failure receive both ASA and an ACE inhibitor, although concerns have been raised about a possible adverse interaction between the two agents involving prostaglandins [5].

The acute benefit of ACE inhibitors in improving hemodynamics depends primarily on their ability to causevasodilation, often in an environment of increased sympathetic activity such as occurs in heart failure [6]. The facilitatory effects of angiotensin II on sympathetic activity...
are well known and the benefits of ACE inhibitors in reducing sympathetic responses may be mediated by their inhibitory effects on angiotensin II synthesis [7]. While the main benefit of ACE inhibitors is accepted as being through a reduction in angiotensin II levels, it has been proposed that a contribution also occurs from inhibition of endothelial ACE/kininase II, leading to accumulation of bradykinin, a potent endogenous vasodilator with effects mediated by factors which include prostacyclin and nitric oxide [8,9]. However, little is known about ACE inhibitor attenuation of sympathetic vasoconstriction at the post-synaptic vascular level. The ability of enalaprilat to mediate local dilation of preconstricted dorsal hand veins was demonstrated previously [10] and two other studies reported that ACE inhibitors attenuated vascular responses to sympathetic stimulation [7,11] but the mechanisms involved, including the role of prostaglandins, were not investigated. By facilitating accumulation of bradykinin [8,9], ACE inhibitors would be expected to have favorable effects on prostaglandin synthesis in veins where nitric oxide responses are reduced compared to arteries [12,13] and this may mean an increased role for prostaglandins.

Hence further studies are necessary to assess directly the role of prostaglandins in modulating vasoconstriction during ACE inhibition in order to further understand general hemodynamic effects which have been reported. The aim of this study therefore was to determine the influence of ACE inhibition on venoconstriction to NE, focusing in particular on the modulatory role of vasodilator prostaglandins. The study was designed to test the hypothesis that vasodilator prostaglandins contribute significantly to ACE inhibitor dilation of NE-induced venoconstriction and this would be blocked by cyclooxygenase inhibition.

2. Methods

2.1. Subjects

This investigation conforms with the principles outlined in the Declaration of Helsinki [14]. All subjects gave written informed consent to the study protocol approved by the University of Western Ontario’s review board for health sciences research involving human subjects. A total of 14 volunteers participated in the study but five were excluded because they were found to be hypercholesterolemic upon analysis of blood samples while one subject was excluded for having incomplete results. The results are of eight normal subjects, four females and four males (age 46±5 years, mean±S.E.M.; weight 65±5 kg). The subjects were normotensive, non-smokers, not taking any medications and in general good health according to history, physical examination and electrocardiogram. Subjects with sensitivity to any of the pharmacological agents or related compounds were excluded from the study. Subjects refrained from drinking alcohol or caffeine-containing beverages for at least 12 h prior to the study.

2.2. Experimental protocol

The study was performed on two mornings (7±1 days apart) in a quiet, temperature-controlled environment (22–24 °C). Subjects were allowed to rest for 30 min before starting the study during which time, if their lipid profile was not known, blood samples were collected for subsequent analysis. With the subject lying in the supine position, two 27-gauge butterfly needles (E-Z Set, Becton Dickinson, Sandy, UT, USA) were inserted in each hand into the straight portion of a dorsal hand vein with no immediate tributaries and continuous saline infusions (0.2 ml/min through each needle) were started using infusion pumps (Harvard, model 2400-003, South Natick, MA, USA).

Distension of dorsal hand veins was measured using the linear variable differential transformer technique [15] as previously described [16]. This technique has been evaluated and found to be highly reproducible as a means of studying venous responses repeatedly within subjects [15,17]. A sphygmomanometer cuff was placed on each upper arm and a mechanical cuff inflator (Hokanson, model G101, Winston-Salem, NC, USA) was used to intermittently inflate and maintain cuff pressure at 45 mmHg at which vein distension was measured. Both lower arms were rested on padded supports elevated to 30° from the horizontal to allow for emptying of hand veins during cuff deflation. The transducers of the linear variable differential transformers (Type 025 MHR, Schaevitz Engineering, Pennsauken, NJ, USA), housed in lightweight tripod stands, were placed over the veins with the central movable cores resting on the summit of the veins approximately 10 mm downstream from the tips of the most proximal needles and their positions were recorded continuously on chart recorders. Measurements of vein distension were made by inflating both cuffs for 2 min, during which stable plateau peaks were recorded, followed by deflation for 3 min. This was repeated until stable baseline measurements of vein distension had been recorded (at least two consistent peaks).

On one of the study days (randomly assigned), after obtaining stable baseline measurements of vein distension, the saline syringe for one of the distal needles (randomly selected) was replaced by an infusion of enalaprilat (1000 ng/min; Vasotec®, Merck Frosst, Kirkland, Quebec, Canada) which was maintained for the rest of the duration of the study. Fifteen minutes after the start of the enalaprilat infusion, graded doses of norepinephrine (NE; 0.5–1024 ng/min; Sabex, Boucherville, Quebec, Canada) were then infused through each proximal needle for 5 min at each dose level with vein distension measurements being made in the last 2 min. The same procedure was repeated on the other study day in the presence of a
coinfusion of indomethacin (3 μg/min; Merck Frosst) through the distal needles of both hands. Previous studies have established that NE dose–response curves on different hands are similar and reproducible on different days [17]. The difference between baseline distension and that obtained at each dose level of NE was expressed as a percentage of the baseline distension (% venoconstriction).

A control study was performed in a normal subject to confirm that enalaprilat infusion in one hand would not affect responses to NE in the contralateral hand. In one hand, venoconstriction to cumulative doses of NE (1–1024 ng/min) was measured first in the presence of a saline infusion in the contralateral hand. After a suitable washout period, during which baseline vein distension was restored, the venoconstriction to the same doses of NE was measured again at least 15 min after starting a continuous infusion of enalaprilat (1000 ng/min) in the contralateral hand. In each of another two subjects, two needles were inserted into a dorsal hand vein in one hand and the veins were preconstricted by approximately 50% of baseline vein distension with PGF2α (Upjohn Company, Don Mills, ON, Canada; 256 ng/min in one subject and 512 ng/min in the other), given through the distal needle. The PGF2α infusion was maintained for the rest of the study. After at least 15 min equilibration, an infusion of enalaprilat (1000 ng/min) given through the proximal needle was started and maintained for the rest of the study while vein distension was measured at similar intervals as before.

Skin temperature was continuously monitored in one hand by a temperature probe attached to a thermometer with digital readout display (YSI 409B, VWR Scientific, Mississauga, ON, Canada). Blood pressure and heart rate were measured throughout the study at 5 min intervals on the calf of the leg using an automated machine (Dinamap 846SX, Criticon, Tampa, FL, USA).

2.3. Data analysis

Data are presented as the mean±S.E.M. where appropriate. Non-linear curve fitting analysis over the dose range for infused NE was performed using a computer software program (Graphpad Inplot 4, H. J. Motulsky, San Diego, CA, USA). Comparisons of dose–response curves were made using analysis of variance for repeated measures. Comparisons of means were made using the paired Student’s t-test. Two-tailed P-values less than 0.05 were considered statistically significant.

3. Results

3.1. Subject characteristics

Baseline mean arterial pressures, heart rates and skin temperatures were similar on the two study days (Table 1) and did not change significantly with local norepinephrine infusions or over the duration of the studies. Baseline distension of the veins (Table 1) was not different between the two hands and did not differ between the study days.

3.2. Effects of indomethacin and enalaprilat on responses to NE

In the control study, enalaprilat infusion in one hand for the same duration and at the same concentration as during the actual studies did not affect responses to NE in the contralateral hand (Fig. 1). Neither enalaprilat nor indomethacin affected baseline vein distension prior to determination of NE dose–response curves.

Norepinephrine induced dose-dependent venoconstriction during the various treatments in all subjects (Fig. 2). Enalaprilat shifted the NE dose–response curve to the right

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control day</th>
<th>Indomethacin day</th>
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<tbody>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>92±2</td>
<td>91±3</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>57±4</td>
<td>56±3</td>
</tr>
<tr>
<td>Skin temperature (°C)</td>
<td>32.9±0.3</td>
<td>32.4±0.2</td>
</tr>
<tr>
<td>Hand vein distension (mm)</td>
<td>1.00±0.09 (S)</td>
<td>0.98±0.07 (Indo)</td>
</tr>
<tr>
<td></td>
<td>0.98±0.07 (Enal)</td>
<td>1.01±0.09 (Enal+Indo)</td>
</tr>
</tbody>
</table>

Indo, indomethacin; S, saline; Enal, enalaprilat; Enal+Indo, enalaprilat+indomethacin.
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Fig. 2. Effects of enalaprilat (Enal) 1000 ng/min on norepinephrine dose–response curves in the absence and presence of indomethacin (Indo) 3 μg/min in dorsal hand veins of eight subjects. *P=0.024 vs. saline control curve. **P=0.018 vs. saline control curve.

Fig. 3. Norepinephrine log ED50 (dose required to cause 50% vеноconstriction) in the presence of saline (○) or enalaprilat 1000 ng/min (▲) in dorsal hand veins of eight subjects. Mean values are shown with bars representing S.E.M. *P=0.001 vs. saline.

Fig. 4. Norepinephrine log ED50 in the presence of saline (○) or indomethacin 3 μg/min (●) in dorsal hand veins of eight subjects. Mean values are shown with bars representing S.E.M. *P=0.002 vs. saline.

Fig. 5. Changes in norepinephrine (NE) log ED50 caused by enalaprilat 1000 ng/min in the absence (saline) and presence of indomethacin 3 μg/min in dorsal hand veins of eight subjects. *P=0.008 vs. saline.

Enalaprilat (1000 ng/min) infusion for 45 min did not cause dilation of veins preconstricted with PGF2α.

(P=0.024), resulting in an increase in NE log ED50 (NE dose causing 50% vеноconstriction; 2.31±0.11 vs. 1.70±0.08 log ng/min with saline, P=0.001; Fig. 3). Indomethacin shifted the NE dose–response curve to the left (P=0.018) with a decrease in NE log ED50 (1.09±0.18 vs. 1.70±0.08 log ng/min with saline, P=0.002; Fig. 4). In the presence of indomethacin, enalaprilat caused a small but significant increase in the NE log ED50 (1.29±0.18 vs. 1.09±0.18 log ng/min with indomethacin alone). The increase in NE log ED50 with enalaprilat was significantly reduced by indomethacin (delta +0.61±0.11 vs. +0.20±0.08 log ng/min, P=0.008, Fig. 5).

Enalaprilat (1000 ng/min) infusion for 45 min did not cause dilation of veins preconstricted with PGF2α.

4. Discussion

This study represents an in vivo direct mechanistic evaluation of ACE inhibitor dilation of NE-induced vеноconstriction at the local vascular level in normal subjects. Since the purpose of the study was to evaluate the contribution of vasodilator prostaglandins, intravenous indomethacin was used to block cyclooxygenase production of prostaglandins. The small doses of NE used caused only localized dose-dependent vеноconstriction and did not alter systemic blood pressure or heart rate. Enalaprilat infusion caused local dilation of NE-induced vеноconstriction, as evidenced by the significant shift to the right of the NE dose–response curve, again without evidence of systemic effects. Our results are consistent with others which have demonstrated attenuation of sympathetic vеноconstriction during ACE inhibition [7,11,18,19] although the contribution of prostaglandins was not assessed. With indomethacin, responsiveness to NE was increased during both saline and enalaprilat. Also, venuodilation with enalaprilat was significantly less during indomethacin coinfusion,
consistent with our hypothesis that vasodilator prostaglandins contribute significantly to ACE inhibitor attenuation of NE-induced venoconstriction.

The results in the present study have demonstrated the ability of indomethacin to modulate the actions of both NE and enalaprilat, suggesting a contributory role of vasodilator prostaglandins in human veins in vivo. Control reponsiveness to NE in this study was increased by indomethacin, confirming the importance of prostaglandins in modulating the NE-induced venoconstriction consistent with earlier studies using ASA [16] or other adrenoceptor agonists [20]. Similarly, vasodilation with enalaprilat was substantially reduced by indomethacin. Under the conditions of this study in healthy normals, this suggests most of the vasodilation to enalaprilat was mediated through prostaglandins, most likely due to accumulation of bradykinin [21]. Besides their stimulatory effects on prostaglandins, it has been suggested that ACE inhibitors may cause vasodilation by other ‘non-prostaglandin’ mechanisms. These include reduced synthesis of angiotensin II by a local vascular tissue renin–angiotensin system [22] and bradykinin stimulation of other endothelium-derived vasodilators such as nitric oxide [9]. There is no clear evidence from our study that these mechanisms play an important role in ACE inhibitor modulation of sympathetic venoconstriction to NE. However, there has been a recent suggestion of significant basal and stimulated nitric oxide activity in the quantitatively important smaller veins and venules [23] although the role of prostaglandins was not studied. Results of another study suggest that both prostaglandins and nitric oxide modulate venular tone [24], thereby raising the likelihood that our results in larger hand veins may also apply to the smaller veins and venules.

The dose of enalaprilat was chosen to achieve local concentrations at the study site approximating systemic levels attainable clinically in vivo [25], assuming a peripheral venous blood flow of approximately 1 ml/min [26]. Enalaprilat infusion in one hand did not alter responses to NE in the contralateral hand as demonstrated in the control study, thereby ruling out any confounding effects of enalaprilat on the control NE dose–response curve with saline. Furthermore, enalaprilat did not alter baseline vein distension during the 15 min equilibration period, consistent with the veins being in a state of near to maximal dilation [26] under the environmental temperature and relaxed, quiet conditions of the study.

Contrary to its effects on NE-induced venoconstriction, enalaprilat did not dilate veins which were preconstricted with PGF₂α, consistent with a previous study [11]. The observation that the effects of indomethacin on NE log ED₅₀ were greater in the presence of enalaprilat suggests an additive effect of prostaglandins stimulated by both NE and enalaprilat. Since PGF₂α does not appear to stimulate modulatory prostaglandin synthesis [20], the prostaglandins stimulated by enalaprilat alone may not be sufficient to affect venoconstriction to PGF₂α.

Indomethacin, available for intravenous use in humans, was used to achieve local inhibition of cyclooxygenase as earlier studies found comparable effects of indomethacin [20] and orally administered ASA [16] on sympathetic-mediated venoconstriction. Although there have been reports of non-specific effects of indomethacin [27,28], these were reported at concentrations much higher than those used in this study. Indomethacin did not alter baseline vein distension, thereby ruling out any direct venoconstrictor effects. This also suggests that any basal release of vasodilator prostaglandins is very small and does not play a significant physiologic role.

Our current findings appear to implicate prostaglandins as the principal mediators of ACE-inhibitor-mediated dilation in normal human veins constricted with NE. This may have clinical implications in patients with cardiovascular disease who are on concurrent treatment with ASA and ACE inhibitors. Some studies have reported adverse effects of the inhibition of prostaglandin synthesis on the beneficial hemodynamic effects of ACE inhibitors in patients with coronary heart disease and heart failure [29–32], but the findings have not been consistent [33–35]. So far, available data suggest that the interaction between ACE inhibitors and ASA may be clinically relevant only at higher (>300 mg daily) and not lower doses of ASA which are used for prevention of thrombosis [31,35]. We used one intravenously infused dose of indomethacin and the clinical implications of our findings need to be validated in prospective studies in which the ASA treatment is randomized and different doses of ASA are given concomitantly with ACE inhibitors.

Bradykinin B₂-receptors are constitutively expressed and regulate vascular function in normal animal physiology [36]. Bradykinin mediation of some ACE inhibitor effects has been demonstrated in a number of in vivo and in vitro animal and human studies [8,9,36]. The contribution of bradykinin to the observed responses with enalaprilat was not directly determined in the current study. As chronic ACE inhibition may induce renal and vascular B₁-receptors [36], the magnitude of the acute changes we observed may not be identical to those seen during chronic therapy. The effects of ACE inhibition were evaluated in healthy subjects and further studies are indicated in patients with cardiovascular disease where the endothelium, an important modulator of vascular tone and source of prostaglandins, may be dysfunctional [37,38]. In heart failure, there is potential for expression of inflammation-induced B₁-receptors which have also been shown to cause vasodilation that may involve prostaglandins [36]. The interactions of endothelial dysfunction, upregulation of B₁-receptors, and prostaglandins requires further study.

In conclusion, in healthy normal subjects, enalaprilat causes dilation of hand veins acutely constricted with increasing doses of NE and this action may be principally mediated by vasodilator prostaglandins. This could have pathophysiological relevance in circumstances of sympa-
thetetic activation if cyclooxygenase inhibitors are also clinically used.

Acknowledgements

We gratefully acknowledge the assistance of Ruth Miles RN and Gord Machiori PhD during the studies. The studies were supported by the Heart and Stroke Foundation of Ontario and the Medical Research Council of Canada. Ms. Dzekka was supported by the Canadian Commonwealth Scholarship Program.

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