Vitamin C and quinapril abrogate LVH and endothelial dysfunction in aortic-banded guinea pigs

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Bell, John P., Salah I. Mosfer, Derek Lang, Francis Donaldson, and Malcolm J. Lewis. Vitamin C and quinapril abrogate LVH and endothelial dysfunction in aortic-banded guinea pigs. Am J Physiol Heart Circ Physiol 281: H1704–H1710, 2001.—Left ventricular hypertrophy (LVH) is a cardiovascular risk factor. A possible role for endothelial dysfunction in this condition was investigated in a Dunkin-Hartley guinea pig aortic-banded pressure overload-induced model of LVH. Aortic banding produced significant elevation of fore- and hindlimb blood pressure (BP), heart-to-body weight ratios, plasma angiotensin II (ANG II), endothelin-1 (ET-1), tumor necrosis factor-α (TNF-α) levels, and coronary microvascular endothelial cell (CMEC) NAD(P)H-dependent superoxide (O2−) production, and a significant decrease in basal and stimulated CMEC cGMP levels. Treatment of aortic-banded animals with the angiotensin-converting enzyme inhibitor quinapril and the antioxidant vitamin C, either alone or in combination, did not affect BP but caused a significant inhibition of the increases in the heart-to-body weight ratio, ANG II, ET-1, and TNF-α levels, and O2− production and restored cGMP responses to levels comparable with sham-operated animals. These data suggest that quinapril and vitamin C are capable of inhibiting LVH development due to pressure overload via mechanisms that involve the inhibition of oxidative stress, an improvement in coronary endothelial function, and increased nitric oxide bioavailability.

ace inhibitors; antioxidant; left ventricular hypertrophy; endothelial function; oxygen-derived free radicals

There is now little doubt that left ventricular hypertrophy (LVH) is an independent cardiovascular risk factor leading to increased mortality in affected individuals. Although the nature of the underlying pathophysiological mechanisms that engender this risk remain elusive, several have been postulated (9), with impairment of both coronary blood flow and relaxation of resistance vessels as a consequence of endothelial dysfunction being high on the list. The question remains, however, as to the precise mechanisms involved in the induction of endothelial dysfunction. It has become increasingly well recognized that oxidative stress plays a crucial role in this process, with many of the diseases where reduced endothelium-derived nitric oxide (NO) activity has been demonstrated also being associated with increased production of oxygen free radicals, particularly superoxide anions (O2−) (6, 24, 30).

We (25) have previously demonstrated in a guinea pig model of pressure overload-induced LVH that this condition is also associated with increased production of O2− by coronary microvascular endothelial cells (CMEC). This excess production of oxygen free radicals would seem to result from upregulation of the NADH/NADPH oxidase system in these cells by angiotensin II (ANG II), as previously described for other cell types (12, 17, 32).

There is now compelling evidence showing ANG II to be a major factor involved in the development of essential hypertension (33) and cardiac hypertrophy induced by mechanical overload, both in vivo (3) and in vitro (16). It is our hypothesis that an ANG II-induced increase in O2− production and a subsequent decrease in NO bioavailability are fundamental to this process. Given that intracoronary administration of angiotensin-converting enzyme (ACE) inhibitors may improve active myocardial relaxation in those patients with hypertensive LVH (14) and that ACE inhibitors can prevent bradykinin degradation, leading to increased NO release from the coronary circulation (2), by implication suggest that NO activity is impaired in LVH. Additional support is provided by the observation that elevation of cGMP, the intracellular mediator of NO, has been shown to inhibit ANG II-induced growth of rat cardiac fibroblasts (10), cells that also play an important role in the development of cardiac hypertrophy (40).

Because it is also well known that CMEC-derived NO has profound beneficial effects on cardiac function (21, 35), improvement of endothelial function in LVH may have significant effects in reducing both the morbidity and mortality associated with this condition.

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The aim of the present study was, therefore, to investigate the effect of treatment with an ACE inhibitor, quinapril, and an antioxidant, vitamin C, on CMEC function in a guinea pig model of pressure overload-induced LVH.

MATERIALS AND METHODS

Intervention protocol and aortic banding. Juvenile male Dunkin-Hartley guinea pigs (200–250 g) were randomized into the following five groups: 1) sham operated (28 animals), 2) untreated aortic banded (28 animals), 3) aortic banded plus quinapril (26 animals), 4) aortic banded plus vitamin C (26 animals), and 5) aortic banded plus quinapril and vitamin C (27 animals). Drug treatment with quinapril (3 mg·kg⁻¹·day⁻¹ by intraperitoneal injection) and/or vitamin C (57 mg·kg⁻¹·day⁻¹ in drinking water) was started 1 wk presurgery and continued for 6 wk postsurgery. The doses of quinapril and vitamin C were chosen on the basis of a preliminary study in which they were found to have no significant effect on blood pressure (BP) per se (unpublished data). Due to the variety of experiments included in this study, all parameters could not be measured in all animals. After 1 wk of pretreatment with the relevant intervention, pressure overload LVH was induced in the appropriate guinea pigs by placing a Week hemoclip (Pilling Week; preset internal diameter, 0.5 mm) around the subdiaphragmatic aorta, just above the renal arteries (25). All subsequent experiments were carried out 6 wk postsurgery. Indirect systolic BP was determined in the fore- and hindlimbs of restrained guinea pigs using an external inflator pulse cuff detection system (Harvard Apparatus), a process that required no anesthesia (22). Observations from a separate study (unpublished data) indicated that the systolic BP in untreated aortic-banded animals was significantly elevated 1 day postsurgery and remained consistently so for 6 wk. On this basis, in the present study, final mean BP values were calculated from the means of multiple readings taken from individual animals 6 wk postsurgery, providing a reliable estimate of the pressure load experienced by the left ventricle over the course of the study. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996).

Isolation of CMEC. Guinea pig CMEC were isolated as previously described (23). This earlier study also fully characterized these cells as being of microvascular origin. All experiments were undertaken on CMEC within 3 h of their isolation.

Measurement of CMEC cGMP levels. Six-well plates containing freshly isolated CMEC were first washed with fresh Krebs solution (composed of (in mM) 118 NaCl, 4.7 KCl, 1.2 NaH₂PO₄, 1.2 MgSO₄·7H₂O, 25 NaHCO₃, 11 glucose, 1.5 CaCl₂, and 0.01 indomethacin) before being incubated in a further 2 ml of Krebs solution at 37°C under an atmosphere of 5% CO₂ in air for 1 h. Drugs were then added at the concentrations and times indicated in RESULTS. Concentrations and exposure times were based on a previous study (23). At the end of the appropriate drug incubation period, the Krebs solution was aspirated off, and the reaction was terminated by the addition of 0.75 ml of ice-cold 65% (vol/vol) ethanol. The cells were scraped from the well, and the latter was washed with a further 0.75 ml of ice-cold ethanol. The combined volume was then centrifuged at 10,000 g for 5 min. The resulting supernatant was evaporated to dryness and assayed for cGMP content along with the protein content of the cell debris pellet (39).

Measurement of plasma ANG II, endothelin-1, and tumor necrosis factor-α levels. Guineapig plasma (all groups) was obtained, and the ANG II content was measured as previously described (25). The endothelin (ET)–1 and tumor necrosis factor (TNF)–α concentrations in the same plasma were measured as outlined below.

For the extraction of ET-1, 1 ml of plasma was thawed on ice and acidified with 1 ml of 1% (vol/vol) trifluoroacetic acid (buffer A). After centrifugation at 1,200 g for 20 min at 4°C, the supernatant was transferred to a 18C SEP column (Peninsula Laboratories) prevashed with 60% (vol/vol) acetonitrile in buffer A (buffer B; 3 ml twice). Buffer B (3 ml) was then used to slowly elute ET-1, and the resulting eluant was frozen immediately on a dry ice-methanol mixture. After freeze drying, samples were reconstituted in 0.25 ml of the appropriate assay buffer, and the ET-1 concentration was measured using a commercially available radioimmunoassay kit (Peninsula Laboratories).

Plasma concentrations of TNF-α were measured directly using a commercially available ELISA kit (BioSource International).

NADH/NADPH oxidase assay. NADH/NADPH oxidase activity and protein content in lysates of freshly isolated CMEC were measured as previously described (25). The integrals of the chemiluminescent responses were calculated over 30 min, expressed as volts × seconds, and normalized to sample protein content (V·s·mg protein⁻¹). That this technique specifically measures NADH/NADPH oxidase-derived O₂⁻ production was confirmed in a previous study (25) using cytochrome c reduction.

Statistics. All data are expressed as means ± SE. For analysis of all within-group data, ANOVA was followed by Dunnett’s multiple range test. For analysis of all between-group data, ANOVA was followed by Student-Newman-Keuls multiple range test. Significant differences are identified at the P < 0.05 level.

Chemicals and reagents. Most drugs and reagents were obtained from Sigma and Calbiochem. Quinapril was the kind gift of Parke-Davis. Tissue culture reagents were supplied by GIBCO-BRL. All drugs were dissolved in distilled water/buffer immediately before use except in the case of lucigenin (dissolved in DMSO) and phenylmethylsulfonyl fluoride and A23187 (both 100% ethanol).

RESULTS

Blood pressure. In untreated aortic-banded animals, final mean systolic BP was significantly elevated in both fore- (P < 0.01) and hindlimbs (P < 0.05, 98.6 ± 1.3 and 84.6 ± 0.8 mmHg, respectively, both n = 4) compared with the sham-operated animals (82.1 ± 2.1 and 77.7 ± 2.5 mmHg, respectively, both n = 4). These increases in BP were unaffected by treatment with either quinapril (98.8 ± 1.1 and 83.5 ± 0.7 mmHg, respectively, both n = 5) or vitamin C (100.6 ± 0.8 and 86.3 ± 1.2 mmHg, respectively, both n = 5) alone or in combination (100.5 ± 1.2 and 84.4 ± 1.4 mmHg, respectively, both n = 4).

Development and regression of LVH. At no point in the study did any of the animals exhibit either physical or clinical signs of heart failure. To assess the development of LVH, combined heart (wt wt)-to-body weight ratios (H/BW × 10², n ≥ 15) were measured in a sample (age matched) population of all groups of guinea pigs at a time point that coincided with CMEC...
banded animals were significantly (P < 0.05) lower than both the sham-operated and vitamin C-treated animals. No significant differences were observed between any of the other groups.

A significant (P < 0.01) increase in H/BW was observed in the untreated aortic-banded animals (0.453 ± 0.023) compared with the sham-operated group (0.320 ± 0.005). Treatment with either quinapril (0.408 ± 0.009) or vitamin C (0.389 ± 0.006) alone or in combination (0.404 ± 0.011), significantly (all P < 0.05) inhibited the increases in H/BW compared with the untreated aortic-banded animals.

**Measurement of CMEC cGMP levels.** CMEC cGMP levels were measured in this part of the study as an index of NO bioactivity. Exposure of CMEC isolated from sham-operated animals to either bradykinin or the calcium ionophore A23187 (both 1 μM for 90 s) resulted in significant (P < 0.01) increases in cGMP levels compared with baseline values (Fig. 1). In cells taken from untreated aortic-banded animals, baseline, bradykinin-, and A23187-stimulated levels of cGMP were significantly (P < 0.05) lower than those in sham-operated controls (Fig. 1). After treatment of aortic-banded animals with either quinapril or vitamin C alone, baseline, bradykinin-, and A23187-stimulated levels of cGMP in freshly isolated CMEC were not different from those in the cells from sham-operated controls (Fig. 1). Furthermore, after treatment of aortic-banded animals with quinapril and vitamin C in combination, both baseline and bradykinin-stimulated levels of cGMP were significantly (P < 0.05) elevated compared with those in sham-operated controls (Fig. 1).

Exposure of CMEC isolated from sham-operated and untreated aortic-banded animals to the exogenous NO donor sodium nitroprusside (1 μM for 90 s) resulted in significant (P < 0.01) increases in cGMP levels compared with baseline values (45.2 ± 5.4 vs. 8.2 ± 0.7 fmol/μg protein and 43.7 ± 4.8 vs. 4.1 ± 0.5 fmol/μg protein, respectively, all n = 6).

**Plasma ANG II, ET-1, and TNF-α levels.** Plasma levels of ANG II, ET-1, and TNF-α in untreated aortic-banded animals were significantly (P < 0.05) increased compared with sham-operated animals (Fig. 2). These increases were significantly (P < 0.05) inhibited in all treated groups of aortic-banded animals (Fig. 2).

**NADH/NADPH-dependent superoxide anion production in CMEC.** The cytosolic fraction of the freshly isolated cells failed to produce a chemiluminescent response either in the absence or presence of NADH or NADPH (data not shown). The following data therefore describe the responses of the particulate fraction. NADH- and NADPH-dependent O2 generation were significantly (P < 0.01 and P < 0.05, respectively) increased in aortic-banded animals compared with those in the sham-operated group (Fig. 3). Treatment of aortic-banded animals with quinapril and vitamin C, either alone or in combination, resulted in NADH- and NADPH-dependent O2 production returning to levels similar to those in the sham-operated controls (Fig. 3). No chemiluminescent response was demonstrated by
any CMEC fraction in the absence of NADH or NADPH (data not shown).

DISCUSSION

The data described in the present study demonstrate that pressure overload-induced LVH in the guinea pig is associated with a significant degree of endothelial dysfunction. Indeed, CMEC, which may have important effects on myocardial contraction, were shown to display a significant loss of both basal and stimulated NO activity as measured indirectly by cGMP accumulation. This endothelial dysfunction is shown to be associated with significant increases in both plasma levels of ANG II, ET-1, TNF-α, and NADH/NADPH-dependent O₂⁻ production in CMEC lysates. That the impaired CMEC cGMP response is due to decreased NO activity is supported by the fact that CMEC from both sham-operated and untreated aortic-banded animals responded to exogenous NO with similar increases in cGMP levels. The latter would suggest that there is no impairment in the soluble guanylate cyclase or cGMP-dependent phosphodiesterase activities of these cells and that they are equally capable of responding to NO.
Treatment of aortic-banded guinea pigs with the ACE inhibitor quinapril and the antioxidant vitamin C, either alone or in combination, was shown to have significant effects on the parameters mentioned above. A significant improvement in endothelial function was observed after the various treatment interventions. In the presence of either quinapril or vitamin C alone, both basal and stimulated levels of cGMP in freshly isolated CMEC returned to levels comparable with those seen in sham-operated animals. Furthermore, in the presence of quinapril and vitamin C in combination, both basal and bradykinin-stimulated levels of cGMP were significantly elevated above those seen in sham-operated animals. Similarly, increases in both plasma levels of ANG II, ET-1, and TNF-α and NADH/NADPH-dependent O₂ production in untreated aortic-banded animals were inhibited after all interventions. These improvements in the presence of the treatments were accompanied by a significant inhibition of LVH development in the absence of any change in BP.

There have been many studies in both animal models and humans in which ACE inhibitors have been shown to prevent the development of LVH (39). This effect has been largely attributed to the decrease in BP induced by these agents rather than the concomitant improvement in endothelial function. However, the present study describes an improvement in endothelial function and a significant reduction in LVH development after treatment with quinapril in the absence of a fall in BP. Therefore, it is possible that the improvement in endothelial function observed after quinapril treatment may play a significant role in preventing LVH.

ACE inhibitors have been shown to improve endothelial function partly through the potentiation of bradykinin-induced NO production. However, they may also exert a positive effect via the inhibition of ANG II production, given that the latter is known to upregulate the production of damaging O₂⁻ from CMEC (25) as well as other cell types (12, 17, 32). The data in the present study indeed demonstrate that quinapril treatment is associated with a decrease in plasma ANG II levels and a concomitant decrease in NADH/NADPH-dependent O₂⁻ production. This effect is associated with a significant increase in NO bioavailability, as measured by CMEC cGMP content. These data support a recent study (20) in patients with coronary artery disease in which quinapril was demonstrated to selectively improve endothelium-dependent vasodilator responsiveness via increased NO bioactivity.

It should be noted that even in the presence of quinapril treatment and normalized plasma ANG II and ET-1 levels, a significant increase in BP was still observed. The exact reason for the persistent hypertension is unknown, but the physical presence of the aortic band itself is likely to have made a contribution. Given the fact that the increase in BP is greatest in the forelimb, which is proximal to the stenosis, this explanation would seem plausible. However, even though plasma levels of ANG II and ET-1 were normalized, it is not clear what changes occurred in the concentration of these agents at a tissue level. Isolated production of these agents in vascular smooth muscle cells, for example, would have profound effects on BP. Because experiments have demonstrated that higher concentrations of quinapril do indeed normalize the aortic banding-induced increase in BP (unpublished observations), this would suggest that tissue generation of ANG II and ET-1 may indeed play a role in BP control. Further speculation as to a specific mechanism for this effect is inappropriate because further studies would be required to address this issue.

An unexpected finding of the study was the slight, but significant, reduction in body weight of the aortic-banded quinapril-treated group. We can provide no adequate explanation for this finding other than its occurrence by chance because no similar finding was observed in the aortic-banded animals treated with quinapril together with vitamin C.

There is a significant body of evidence in the literature to suggest that vitamin C can markedly improve endothelial function and NO bioavailability in various cardiovascular disease states (8, 37). Moreover, a recent study (7) also suggests that vitamin C may be useful to lower BP in hypertensive patients. Although the latter effect was not demonstrated in the present study, a significant decrease in NADH/NADPH-dependent O₂⁻ production and a significant increase in CMEC NO bioavailability were evident in the presence of this antioxidant.

It would also appear from the present study that there is a positive synergistic effect of quinapril and vitamin C on endothelial function, suggesting that this improvement is attained via more than one mechanism. It is interesting to note that both interventions, either alone or in combination, were associated with significantly decreased plasma levels of ANG II and ET-1 compared with those seen in untreated animals. ACE inhibitors, as well as reducing plasma ANG II levels, have also been shown to inhibit ET-1 release from endothelial cells via the accumulation of endogenous bradykinin (28). Furthermore, it has also been reported that ANG II is involved in the stimulation of ET-1 production from various cell types (11, 16, 31, 36).

As mentioned above, an ANG II-induced increase in O₂⁻ production may lead to decreased NO bioavailability. Because inhibition of NO synthesis can lead to increased release of ET-1 from endothelial cells (4, 34), it is not surprising that a decrease in ANG II levels in the presence of quinapril is associated with a decrease in ET-1.

The observed effect of vitamin C on plasma ANG II and ET-1 levels is more puzzling, although it may simply involve increased NO bioavailability resulting in reduced ET-1 release from endothelial cells (4, 34) and inhibition of ACE activity (1).

As previously mentioned above, the improvement in endothelial function was associated with a significant inhibition in LVH development in the absence of a change in BP. It is possible that the increased bioavailability of CMEC-derived NO has a direct effect on cardiac growth. This response may be via the de-
increased action/activity of the potent growth promoters ANG II, ET-1, and TNF-α. However, it may also be via a direct growth-inhibiting effect of NO. The fact that bradykinin-released NO has been shown to inhibit cardiac fibroblast extracellular matrix development (18) and that cGMP has directly been demonstrated to inhibit ANG II-induced growth of rat cardiac fibroblasts (10) would tend to lend weight to this hypothesis.

Cardiac hypertrophy and heart failure are frequently accompanied by elevated plasma levels of TNF-α (26, 38), the latter playing an important role in the pathophysiology of these conditions. For instance, TNF-α is thought to contribute to cardiac myocyte hypertrophy via the stimulation of reactive oxygen species (29) and to hypertension through the release of ET-1 (5, 15, 27). TNF-α has also been shown to enhance ANG II-mediated effects by upregulating the expression of AT1 receptors (13). It has also been suggested that ANG II itself may cause the release of TNF-α from the kidney (19). It is therefore possible that quinapril and vitamin C inhibit the accumulation of TNF-α and its effects via converging mechanisms involving the inhibition of ANG II production and increased NO bioavailability.

In summary, the results of the present study demonstrate that this guinea pig model of pressure overload-induced LVH is associated with endothelial dysfunction, elevated plasma levels of ANG II, ET-1, and TNF-α, and increased CMEC NADH/NADPH-dependent O₂ production, changes that were reversed by treatment with quinapril and vitamin C, either alone or in combination, in the absence of any change in BP. It is possible that the observed inhibition of pressure overload-induced LVH development may be via mechanism(s) that involve the inhibition of oxidative stress, the improvement of coronary endothelial function, and increased NO bioavailability.

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