Sulphydryl Angiotensin-Converting Enzyme Inhibitor Promotes Endothelial Cell Survival through Nitric-Oxide Synthase, Fibroblast Growth Factor-2, and Telomerase Cross-Talk

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ABSTRACT

The protective effect exerted by angiotensin-converting enzyme inhibitors (ACEI) in cardiovascular diseases caused by endothelial injury and aging has been attributed to the restoration of endothelial cell functions. Recently, we demonstrated a central role of the fibroblast growth factor-2 (FGF-2)/FGF receptor-1 system in mediating the acquisition of an angiogenic phenotype in coronary microvascular endothelium exposed to ACEI. Here, we report on the rescuing effect of ACEI on impaired endothelium and the intracellular signaling mechanisms that lead endothelial cells to enter apoptosis and to senesce. Conditions mimicking pathological cell damage (serum deprivation) lead to endothelial apoptosis as evidenced by increased caspase-3 activity. ACEI enhanced cell survival through activation of pro-survival and anti-aging signals involving Akt phosphorylation, endothelial nitric-oxide synthase (eNOS) expression and activation, FGF-2 and telomerase catalytic subunit (TERT) up-regulation, and delayed senescence. In microvascular endothelial cells exposed to ACEI, Akt/eNOS pathway-dependent FGF-2 was necessary for gene transcription of TERT. These protective effects were particularly evident for sulphydryl-containing ACEI (zofenoprilat), which were reported to exhibit potent antioxidant effects. In conclusion, ACEI with antioxidant properties up-regulate eNOS, FGF-2, and TERT mRNA, which favor endothelial cell survival and prolong their lifespan, thus restoring endothelial cell functions after vascular damage. These effects could explain the beneficial effects of these drugs in various cardiovascular diseases associated with endothelial injury and aging.

Since the introduction into clinical use of angiotensin-converting enzyme inhibitors (ACEI), our understanding of their mechanism of action has evolved from the widely held tenet that the therapeutic benefit of ACEI is exclusively associated with the decrease of vascular tone consequent to the inhibition of angiotensin II formation. This initial hypothesis has been disputed because of clinical evidence indicating that, even with marginal decreases of blood vessel tone, ACEI provide significant benefits (e.g., decreased mortality) in a large number of patients suffering from cardiac heart failure, acute myocardial infarction, and diabetes complications (Yusuf et al., 2000; Boss and Dawes, 2004; Kjeldsen and Julius, 2004; Brugts et al., 2009). The common thread linking these diverse diseases is endothelium dysfunction, widely recognized as one of the factors implicated in these pathologies. Reports in the literature describe improvement of damaged endothelium attributable to ACEI as well as the effects of ACEI on promoting vascular remodeling and diminishing the burden of cardiovascular risk factors (Galderisi and de Deytis, 2008). The underlying mechanism of the protection exhibited by ACEI has been attributed to their ability to influence the enzyme endothelial nitric-oxide synthase (eNOS), favoring the proper assembly of the enzyme complex and the engagement of its downstream effectors (Dim...
meler et al., 1997b). Through a complex set of intracellular signals, ACEI can restore the functioning of eNOS when it is impaired by molecular injuries inflicted by oxidative stress, a feature typical of diseased endothelium (Münzel and Keaney, 2001; Ignarro et al., 2002; Kohlstedt et al., 2004, 2005).

Our recent observations of the up-regulation of fibroblast growth factor-2 (FGF-2) by ACEI in coronary endothelium have contributed to delineating the pattern of molecules downstream of eNOS. FGF-2 overexpression seems to act in an autocrine mode, triggering the FGF-2/FGF receptor-1 system, hence promoting endothelial cell survival and the development of the angiogenic phenotype (Donnini et al., 2006).

The marked effect exerted by ACEI on the survival properties of coronary endothelium led us to investigate other signals that might contribute to the overall protection afforded by ACEI. We focused on Akt and telomerase reverse transcriptase (TERT) activities, both signals involved in the survival processes operating in a wide variety of cells (Breitschopf et al., 2001; Jakob and Haendeler, 2007). TERT is especially interesting in the context of diseased endothelium repair, because this reverse-transcriptase enzyme opposes the apoptotic drive of oxidative stress and delays vascular senescence (Murasawa et al., 2002; Brandes et al., 2005). Therefore, we performed a study on cultured postcapillary CVEC of bovine origin, cultured in an extremely low serum concentration (0.1% CS) analogous to the deprived nutrient conditions that are likely to occur in ischemic cardiac pathologies. We investigated functional endothelial parameters (survival and new capillary formation) and signals relevant to cell survival and death (Akt, NO/cGMP, FGF-2, TERT, and caspase-3) and the influence on these processes of a panel of ACEI bearing an SH group in their moiety (captopril, zofenoprilat) or devoid of an SH group (enalaprilat, lisinopril).

Materials and Methods

Pseudocapillary Formation from Vessel Rings. Pseudocapillary sprouting from vessel rings of the human umbilical artery and characterization of the cells present in the pseudocapillaries were evaluated as described previously (Donnini et al., 2006).

Cell Cultures. CVEC were isolated and cultured as described previously (Schelling et al., 1988). FGF-2(-/-) endothelial cells were kindly provided by Prof. Paolo Mignatti and cultured as described previously (Yu et al., 2008).

Survival Studies. We resuspended 1000 cells per well (of a 96-well multiplate) in 10% CS, and after cell adherence (3–4 h), we replaced the supernatant with medium containing ACEI (0.01–10 μM) and 0.1% CS. Cells were kept in culture for 5 consecutive days, and medium with test substances was added freshly at 36 and 72 h. Cells were then fixed, stained, and randomly counted at 10× magnification (Donnini et al., 2006). Cell survival in response to ACEI was also assessed by the MTT test (Pezzatini et al., 2007). Data of absorbance units at 540 nm were reported as percentage of basal response.

Detection of Apoptosis. Apopain/caspase-3 activity was measured in cell lysates (Cantara et al., 2004). Cells (density of 1 × 10⁵ cells per 6-cm diameter dish) were serum-starved overnight. Stimulation was carried out for 6 h in medium containing 0.1% serum with 1 and 10 μM zofenoprilat in the absence or presence of 3 mM L-NMMA. Adherent and suspended cells then were pooled and lysed. Apopain/caspase-3 activity was monitored in vitro using the FluorAce apopain assay (Bio-Rad Laboratories, Milan, Italy) according to manufacturer instructions.

\textbf{Senescence-Associated β-Galactosidase Activity Assay.} CVEC were seeded in 6-well multiplates. After adhesion and serum starving, cells were exposed to the stimuli for 3 days. SA-β-gal activity was measured by use of the Cellular Senescence Assay Kit (Millipore Bioscience Research Reagents, Milan, Italy) according to the manufacturer instructions. A total of 400 cells were counted manually, and the percentage of senescent cells among the total cell number was determined.

Western Blotting for Phospho-Akt, Phospho-eNOS, and Cleaved Caspase-3. Western blotting was performed as described by Donnini et al. (2006). Cells were plated at 3 × 10⁵ in 6-cm diameter dishes. After adhesion, cells were serum-starved overnight and then exposed to ACEI or FGF-2 (10 min to 6 h). Electrophoresis (50 μg of protein per sample) was carried out in SDS with 10 or 15% polyacrylamide gels. Proteins were then blotted onto nitrocellulose membranes, incubated overnight with anti-phospho-Akt antibody, anti-phospho-eNOS (Ser1177), and caspase-3 (each at 1:1000), and then detected by use of an enhanced chemiluminescence system (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Results were normalized to those obtained by using an antibody against total Akt.

\textbf{NOS Activity and cGMP Production.} Sparse and synchronized CVEC were stimulated with various ACEI (1 μM, 18 h). cGMP accumulation was evaluated in cell lysates by enzyme immunoassay kits (Cayman Chemical Company, Milan, Italy) as described previously (Cappelli et al., 2004).

\textbf{eNOS, FGF-2, and TERT Expression of by Quantitative RT-PCR.} Subconfluent endothelial cells were allowed to adhere in 6-cm diameter plates. After 24 h of serum starving, CVEC were stimulated with ACEI (1–10 μM) for 6 h. Total RNA was extracted using an RNeasy kit (QIAGEN, Milan, Italy). Total RNA (1 μg) was reverse-transcribed using an iScript cDNA synthesis kit (Bio-Rad Laboratories), with the following thermal profile: 25°C for 5 min, 42°C for 30 min, and 85°C for 5 min. For all further analyses, the cDNA was diluted in water to obtain a final concentration of 30 ng/μl.

For each screened gene, iQ SYBER Green Supermix (Bio-Rad Laboratories) and unlabeled primers designed with a Beacon design program (Bio-Rad Laboratories) and purchased from Applied Biosystems (Foster City, CA) were used: eNOS forward 5′-GCT TGA GAC CCT CAG TCA GC-3′, reverse 5′-GTT CTC CAG TCT TGA GCT GC-3′; FGF-2 forward 5′-TCA GCT CTT AGC AGA CAT TGG AAG AAA AAG-3′, reverse 5′-GGG GTG TGT GAC AGT AAC GCT ATG CTT ATG GGG-3′, and TERT forward 5′-ATG GGG ACA TGG AGA AGA AG-3′, reverse 5′-GTA GGG CTG CAG AAG AGC GTG-3′. β-Actin (forward 5′-TGA ATG GAC AGC CAT CAT GGA CCT CAG TCT AC-3′, reverse 5′-TAC CTC TCA GAG GAT GTA CAG CAG GAA ACG-3′) was used as a reference gene. We used 300 nM of each primer together with 20 μl of iQ SYBER Green Supermix (Bio-Rad Laboratories) and water in a total volume of 25 μl. After enzyme activation for 10 min at 95°C, 40 two-step cycles were performed (15 s, 95°C; 1 min, 60°C) in the iCycler detector (Bio-Rad Laboratories). Differentially expressed genes were quantified by using the threshold cycle (Ct) and the comparative method (2–ΔΔCt) as described previously (Livak and Schmittgen, 2001). Data are reported as -fold increase of basal condition (0.1% CS).

\textbf{Silencing Technique.} \textit{A} 21-nucleotide RNA [sense, r(ACG AAC UGG GCA GUA UAA A)dTdT; antisense, r(UUU AUA CUG CCC AGU UCG U)dTdT; FGF-2, GenBank accession number NM_002006] and control (nonsilencing) small interference RNA were chemically synthesized by QIAGEN. Transient transfection of small interference RNA in subconfluent endothelial cells and silencing efficacy were performed as described previously (Donnini et al., 2006).

Materials and Reagents. Cell culture reagents and captopril were from Sigma-Aldrich (Milan, Italy). Fetal calf serum (FCS) and CS were from HyClone (Logan, UT). VEGF and FGF-2 were from Peprotech (London, UK). NOC12, the NOS inhibitor l-NMMA, the guanylate cyclase inhibitor ODQ, the MAPK inhibitor U0126, and the PI3K inhibitor LY294002 were from Calbiochem.
(Nottingham, UK). Anti-phospho-Akt (Ser473), anti-Akt, anti-phospho-eNOS (Ser1177), anti-caspase-3, and neutralizing anti-FGF-2 antibodies were obtained from Cell Signaling Technology (Danvers, MA). Zofenoprilat, enalaprilat, and lisinopril were kindly provided by Menarini Ricerche.

**Statistical Analysis.** Results are expressed as means ± S.E.M. for (n) experiments performed in triplicate. Multiple comparisons were performed by one-way analysis of variance, and individual differences were tested by Fisher’s test after the demonstration of significant intergroup differences by analysis of variance.

**Results**

**Prosurvival Effect of ACEI on Microvascular Endothelium.** Given the favorable effect exerted by ACEI on endothelial dysfunction (Scribner et al., 2003), we investigated whether these positive activities could be observed in the survival of endothelial cells cultured under conditions of oxidative stress created by serum deprivation (Martin et al., 2005). We studied a panel of ACEI either bearing the SH group in their moiety (captopril and zofenoprilat) or devoid of this functional group (enalaprilat and lisinopril).

Exposure of endothelial cells for 5 consecutive days to increasing concentrations of the ACEI (0.01–10 μM) highlighted different prosurvival properties of the ACEI (Fig. 1A). MTT data demonstrated that zofenoprilat was the most effective, producing a maximal effect at 1 and 10 μM. Captopril was less potent than zofenoprilat, inducing maximal survival at 10 μM. The ACEI lacking the SH group were less effective, producing a bell-shaped dose-response curve with maximal effect at 0.1 μM (Fig. 1A).

We then evaluated the effect of these compounds on the formation of pseudocapillaries from vascular explants of human umbilical artery in the presence of low serum concentrations (0.1% FCS) for up to 2 weeks in organ cultures. All compounds, tested at 1 μM, induced pseudocapillary sprouting, expressed as area occupied by capillaries at 10 days after continuous addition of drugs (Fig. 1B). Significant differences among the compounds were noted. The SH-containing compounds (captopril and zofenoprilat) produced the richest vascularization (2–3-fold increase relative to 0.1% FCS), whereas enalaprilat and lisinopril were much less efficacious. Evaluation of the endothelial marker CD31 in pseudocapillaries showed a time-related decline in those grown in low serum concentrations (from 15 ± 3% of CD31-positive cells at day 10 versus 10 ± 2% at day 15, n = 5), which was reversed by zofenoprilat (from 20 ± 2% CD31-positive cells at day 10 to 30 ± 4% at day 15, n = 5). Thus the drug promoted the development of pseudocapillaries rich in endothelial cells. In light of the above results, we selected zofenoprilat for further studies on cultured endothelial cells.

Apopain/caspase-3 activity, an early marker of cells entering apoptosis, was greatly increased in cells grown in low serum concentrations (0.1% CS) relative to cells grown in higher serum concentrations (Fig. 2A). Zofenoprilat (1–10 μM for 6 h) reduced caspase-3 activity, reaching significance (p < 0.01) at 10 μM (Fig. 2A). The protective effect of zofenoprilat on the apoptosis signal was linked to a functioning NOS pathway, because enzyme inhibition by L-NMMA returned caspase-3 levels to the basal activity (p < 0.05, Fig. 2B).

Because cell apoptosis and senescence are closely linked under conditions of growth factor deprivation (Brandes et al., 2005; Wang et al., 2007), we determined the effect of zofenoprilat (1–10 μM for 3 days) on senescence-associated SA-β-gal activity. The presence of SA-β-gal-positive endothelial cells, enhanced by serum deprivation, was markedly reduced by the drug (>50%), which exerted a protective effect comparable with that of the angiogenic/prosurvival growth factor FGF-2 (20 ng/ml) (Fig. 2C).

As a molecular target of endothelial survival/longevity, we examined TERT by measuring its mRNA expression. In preliminary experiments in CVEC exposed to zofenoprilat (10 μM), we determined that peak expression of the messenger occurred at 6 h (-fold increases of 1.8 ± 0.4, 6 ± 0.9 and 4.8 ± 0.3 at 1, 6, and 18 h, respectively, n = 3). After CVEC incubation with various ACEI (all at 10 μM), we observed varying degrees of TERT mRNA expression (Fig. 2D). Zofenoprilat increased TERT mRNA (6-fold), approaching the level reached by FGF-2 (20 ng/ml), whereas
enalaprilat appeared to be the weakest among the compounds tested. VEGF was devoid of any activity, as previously reported (Kurz et al., 2003) (Fig. 2D). Moreover, the effects exerted by zofenoprilat and enalaprilat were also observed in cultured human umbilical vein endothelial cells (above described conditions), in which TERT mRNA expression showed a fold increase of 5.4 ± 0.17 and 1.4 ± 0.34 for zofenoprilat and enalaprilat, respectively, over basal conditions.

**Activation of PI3K/Akt and eNOS Signaling in Endothelium Exposed to ACEI.** We extended the analysis to prosurvival signals related to FGF-2, because its up-regulation seems to be crucial for the protection exerted by ACEI on the endothelium (Donnini et al., 2006). We examined PI3K/Akt phosphorylation, eNOS expression and phosphorylation state/activity, and TERT mRNA expression in serum-deprived cultured coronary endothelial cells.

Zofenoprilat (10 μM) enhanced the phosphorylation of Akt (5–6-fold over control). This activation was slow but persistent (up to 6 h), whereas that produced by FGF-2 was rapid and declined with time (Fig. 3, A and B).

Assessment of eNOS activation downstream of Akt, measured by phosphorylation of eNOS in Ser1177 (Dimmeler et al., 1999), demonstrated that zofenoprilat increased eNOS activity (Fig. 3C). Furthermore, treatment of the cells with the PI3K inhibitor LY294002 obliterated the phosphorylation of Akt and eNOS produced by zofenoprilat, indicating that PI3K/Akt is the target of the ACEI.

Next, we evaluated both the expression of eNOS (through RT-PCR) and its enzyme activity by measuring...
Fig. 3. Molecular mechanisms activated in microvascular endothelium by ACEI: role of PI3K/Akt and the eNOS pathway. A and B, Akt phosphorylation by FGF-2 and ACEI. A representative blot is reported. The bar graphs represent the optical densities related to the ratio phospho-Akt/Akt (n = 3), *p < 0.05 versus time 0. C, role of PI3K activity on Akt and eNOS phosphorylation. Cells were incubated with 1 μM LY294002 and then stimulated with 10 μM zofenoprilat for 10 min. FGF-2 (20 ng/ml) was used as positive control. Phospho-Akt and phospho-eNOS (Ser1177) were then detected by Western blotting. The blot shown is representative of three experiments with overlapping results. D, PI3K activity controls FGF-2 mRNA...
cGMP accumulation in response to the compounds under study in serum-deprived endothelial cells. Among the compounds, only zofenoprilat induced a strong increase in eNOS expression (16-fold), whereas both lisinopril and enalaprilat failed to up-regulate eNOS expression (Table 1). Likewise, zofenoprilat (1 μM) nearly doubled the amount of cGMP produced by cells, whereas neither lisinopril nor enalaprilat was effective on cGMP accumulation, providing evidence that the enhanced enzyme expression levels translated into increased NO production. Indeed, the effect of zofenoprilat on eNOS enzymatic activity (measured by conversion of L-arginine in L-citrulline after 15 min of incubation) was sensitive to the inhibitory action of 1-NAME (30 ± 5 and 5 ± 2% increase over basal NOS activity by 1 μM zofenoprilat in the absence and presence of 2 mM L-NAME, respectively).

Consistent with the role of PI3K/Akt and eNOS pathways on the senescence and survival signals activated by the ACEI, PI3K inhibition abolished FGF-2 and TERT up-regulation (Fig. 3, D and E) as well caspase-3 activity (Fig. 3F). Furthermore, simultaneous treatment of the cells with zofenoprilat with the MAPK inhibitor U0126 (10 μM each, 6 h) resulted in obliteration of TERT up-regulation (1.8- ± 0.6-fold increase, n = 3), indicating a role for ERK1–2 activated by ACEI in TERT expression.

The eNOS/cGMP axis exerted a tight control on zofenoprilat-induced telomerase overexpression, evident because its blockade with 1-NAME and ODQ abolished the activation of the messenger formation (Fig. 3G). Conversely, incubation of cells with the NO donor drug NOC12 (10 μM) was freshly readded at 36 and 72 h after the treatment with zofenoprilat in control siControl cells. B, mRNA for TERT was measured by conversion of L-arginine in L-citrulline after 15 min of incubation. The NO donor drug NOC12 (10 μM) was used to check assay specificity (n = 3).

**TABLE 1**

<table>
<thead>
<tr>
<th>ACEI Preincubation</th>
<th>eNOS Expression</th>
<th>Stimuli for cGMP</th>
<th>cGMP Levels</th>
<th>pμmol/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.00</td>
<td>None</td>
<td>0.2 ± 0.05</td>
<td>100.0</td>
</tr>
<tr>
<td>Enalaprilat</td>
<td>1.50</td>
<td>NOC12</td>
<td>0.83 ± 0.08**</td>
<td>200.0</td>
</tr>
<tr>
<td>Lisinopril</td>
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<td>None</td>
<td>0.33 ± 0.02</td>
<td>100.0</td>
</tr>
<tr>
<td>Zofenoprilat</td>
<td>16.79</td>
<td>ODQ</td>
<td>0.30 ± 0.05</td>
<td>100.0</td>
</tr>
<tr>
<td>Zofenoprilat</td>
<td>16.79</td>
<td>ODQ</td>
<td>0.59 ± 0.01*</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* p < 0.05
** p < 0.01 vs 0.1% CS medium.
# p < 0.05 vs zofenoprilat alone.

Central Role of Endogenous FGF-2 in ACEI Effects on Survival and Senescence. Because ACEI up-regulate FGF-2 formation (Fig. 3D and Donnini et al., 2006) necessary for ACEI-mediated cell survival (Fig. 4A), we wondered whether the overexpression of the growth factor might induce the observed activation of TERT mRNA and long-term survival. When the zofenoprilat-induced FGF-2 increase was prevented through FGF-2 silencing, we detected no increase of TERT mRNA in response to the drug, suggesting a causal link between these events (Fig. 4B).

Because FGF-2 stored in the extracellular matrix can be rapidly released by different stimuli (Finetti et al., 2008;
Data are reported as percentage of basal response (nFGF-2 antibody and stimulated for 6 h with zofenoprilat (10°C), transcription was measured in CVEC treated with a neutralizing anti-ACEI effect in FGF-2-null endothelial cells (Murakami et al., 2008), we assessed its specific role in the ACEI-mediated endothelial protective actions exerted by ACEI on a wide range of human pathologies (Yusuf et al., 2000; Kjeldsen and Julius, 2004; Napoli et al., 2008) led us to investigate signaling involved in cell survival (FGF-2) and the senescence process (TERT). We showed that, among the ACEI examined here, those bearing an SH group in their moiety (captopril, zofenoprilat) exhibit a distinct prosurvival effect, whereas those devoid of SH (enalaprilat, lisinopril) are much less active.

The greatest differences were observed in the ability to promote eNOS mRNA expression and to stimulate NO production. In fact, whereas zofenoprilat induced a large increase of eNOS expression (16-fold), the increases elicited by enalaprilat and lisinopril were barely larger than control (2-fold). Correspondingly, we observed varying degrees of enhanced NO production in cells exposed to ACEI, which in the case of zofenoprilat was higher (3-fold) than in control. The magnitude of these signaling changes had an impact on cell functions. Examples are the promotion of endothelial survival and the acquisition of the angiogenic phenotype, demonstrated by the rich capillary sprouting after zofenoprilat subchronic exposure. Thus, a correlation might be drawn between the up-regulation of the eNOS pathway and endothelial protective functions by SH-containing drugs. It is noteworthy that the reversal of endothelial dysfunction by ACEI, a concept proposed 2 decades ago (Rajagopalan and Harrison, 1996), continues to be a matter of study, as shown by a recent clinical trial in moderately hypertensive patients, which demonstrated significant improvements, attributable to zofenoprilat, in parameters related to oxidative stress (Napoli et al., 2008).

The rapid and steady activation of the PI3K/Akt pathway, a signal related to eNOS activation, observed mainly for zofenoprilat, is of interest because it may promote the alignment of the eNOS molecule in a conformation conducive to efficient NO production, thus contributing to overcoming stress-induced enzyme uncoupling. The ACEI-induced enhancement of Akt phosphorylation and of NO production has been reported to suppress the prevailing apoptotic program present in serum-deprived endothelial cells.
cells, as in this study (Dimmelser et al., 1997a; Mital et al., 2002).

The other salient finding of this work concerns the ability of ACEI, particularly zofenoprilat, to reverse early signs of cell replicative senescence produced by the hostile nutrient-deprived environment. Indeed, application of ACEI to endothelial cells promoted TERT overexpression (3–6-fold), concomitant with a reduction of SA-β-gal activity accumulation. The mechanism whereby ACEI promote TERT, in the present conditions, seems to be related to the activation of the FGF-2 pathway, because its silencing or neutralization abrogates TERT expression. Although the evidence indicating down-regulation of telomerase by oxidative stress is overwhelming (Haendeler et al., 2004; Vogel et al., 2008; Erusalimsky and Skene, 2009), interventions that favor its up-regulation are limited to NO donor drugs (Vasa et al., 2000), statins (Haendeler et al., 2004), low-dose aspirin (Hu et al., 2008), estrogen (Grasselli et al., 2008), and FGF-2 (Kurz et al., 2003). The direct stimulation of human TERT expression by NO, either endogenously produced or delivered by NO donor agents as shown here, has recently been disputed (Erusalimsky and Skene, 2009). These discrepancies, albeit important, have little influence on our interpretation, because we propose that the endothelial telomere system is regulated by the steady FGF-2 output, the effects of which are antagonized by inhibitors of Akt, NO, and MAPK pathways.

The mechanism we outline in the present study and our previous work (Donnini et al., 2006) illustrates the central action of FGF-2 in counteracting the arrest of endothelial replicative capacity through the up-regulation of the telomere system. This study further reinforces the necessity of the endogenous FGF-2, both released for the prestored pools and synthesized de novo, for endothelial survival and functioning. These ACEI-induced protective effects, not accounted for by the inhibition of ACE, are class-related, although SH-bearing drugs are far more efficacious pro- tendon agents. Other signaling pathways have been described recently (Münzel and Keaney, 2002; Ryan and Sigmund, 2004; Kohlstedd et al., 2004, 2005). These reported studies have demonstrated the presence in the vascular endothelium of a specific ACE pathway, with numerous ramifications, including enhanced synthesis of the cardioprotective prostacyclin (Kohlstedd et al., 2005), and the results of these studies may concur as to the overall beneficial effects of ACEI.

In light of these novel findings, which provide a rationale for the ACEI-promoted benefits, the question arising is whether the current ACEI therapeutic regimens are adequate to sustain the full expression of the above mechanisms in terms of dosage and kinetic profile.

Acknowledgments

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