Inhibition of human lung cancer cell growth by angiotensin-(1-7)

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Angiotensin-(1-7) [Ang-(1-7)] is an endogenous peptide hormone of the renin-angiotensin system with vasodilator and anti-proliferative properties. Human adenocarcinoma SK-LU-1 and A549 cells as well as non-small lung cancer SK-MES-1 cells were treated with serum in the presence and absence of Ang-(1-7), to determine whether Ang-(1-7) inhibits the growth of lung cancer cells. Ang-(1-7) caused a significant reduction in serum-stimulated growth in all three lung cancer cell lines. Treatment with Ang-(1-7) resulted in both a dose- and time-dependent reduction in serum-stimulated DNA synthesis in all three cell lines, with IC₅₀'s in the sub-nanomolar range. The Ang-(1-7) receptor antagonist [d-Ala⁷]-Ang-(1-7) blocked the attenuation of the serum-stimulated DNA synthesis of SK-LU-1 cells by Ang-(1-7), while neither AT₁ nor AT₂ angiotensin receptor subtype antagonists prevented the response to the heptapeptide. MAS mRNA and protein, a receptor for Ang-(1-7), was detected in the three lung cancer cell lines, suggesting that the anti-proliferative effect of Ang-(1-7) in the cancer cells may be mediated by the non-AT₁, non-AT₂, AT₇ receptors. Other angiotensin peptides [Ang I, Ang II, Ang-(2-8), Ang-(3-8) and Ang-(3-7)] did not attenuate mitogen-stimulated DNA synthesis of SK-LU-1 cells, demonstrating that Ang-(1-7) selectively inhibits SK-LU-1 cancer cell growth. Pre-treatment of SK-LU-1 cells with 10 nM Ang-(1-7) reduced serum-stimulated phosphorylation of extracellular signal-regulated kinase (ERK1) and ERK2, indicating that the anti-proliferative effects may occur, at least in part, through inhibition of the ERK signal transduction pathway. The results of this study suggest that Ang-(1-7) inhibits lung cancer cell growth through the activation of an angiotensin peptide receptor and may represent a novel chemotherapeutic and chemopreventive treatment for lung cancer.

Introduction

Angiotensin-(1-7) [Ang-(1-7)], a component of the renin-angiotensin system, is an endogenous, 7 aa peptide hormone with vasodilator and anti-proliferative properties (1-3). We showed that Ang-(1-7) attenuates vascular proliferation in vitro in cultured vascular smooth muscle cells (VSMCs) and in vivo, following vascular injury (2,4,5). The mitogen-stimulated growth of cultured rat aortic VSMCs was markedly reduced following treatment with Ang-(1-7) (4). The molecular mechanisms for the anti-proliferative response to Ang-(1-7) include stimulation of prostaglandin and cAMP production as well as inhibition of mitogen-activated protein (MAP) kinases (6,7). The heptapeptide also reduced vascular growth stimulated by balloon catheter injury to the rat carotid artery; the 2-fold increase in plasma Ang-(1-7) due to peptide infusion had no effect on heart rate or blood pressure (5,8). Loot et al. (9) reported that an 8-week infusion of Ang-(1-7) following coronary artery ligation prevented the deterioration of cardiac function, as indicated by a 40% reduction in left ventricular end-diastolic pressure. The Ang-(1-7)-mediated improvement in cardiac function was associated with a significant decrease in myocyte cross-sectional area, suggesting a role for Ang-(1-7) in the regulation of myocyte growth. In recent studies, we found that Ang-(1-7) reduced protein synthesis in myocytes as well as DNA and protein production in cardiac fibroblasts, indicating that the heptapeptide inhibits hypertrophy of cardiomyocytes and hyperplasia in cardiac fibroblasts (10). Taken together, these studies show that Ang-(1-7) serves as an endogenous regulator of cardiovascular cell growth.

Ang-(1-7) is produced primarily by the enzymatic breakdown of either angiotensin I (Ang I) or the vasoconstrictor angiotensin II (Ang II), as illustrated in Figure 1. Ang-(1-7) is formed directly from Ang I by multiple endopeptidases that cleave the Pro⁷-Phe⁸ bond, including neprilysin (NEP 24.11; EC 3.4.24.11), prolyl oligopeptidase (POP) and thimet oligopeptidase (TOP) (11-14). Ang-(1-7) is also produced by the metabolism of Ang II at the C-terminus by carboxypeptidases. While several enzymes expressing carboxypeptidase-like activity hydrolyze the Pro⁷-Phe⁸ bond to generate Ang-(1-7) (14), recent studies suggest that angiotensin converting enzyme 2 (ACE2) is the primary enzyme catalyzing this reaction. ACE2, although structurally similar to angiotensin converting enzyme (ACE) sharing ~42% sequence homology, has a distinct substrate specificity and ACE2 activity is unaffected by ACE inhibitors (15-18). While Ang-(1-7) is synthesized by the action of ACE2, the heptapeptide is metabolized by ACE to the 5 aa degradation product Ang-(1-5) (19). Inhibitors of ACE elevate Ang-(1-7) concentrations by both increasing Ang I, the substrate for Ang-(1-7) production, as well as by preventing Ang-(1-7) degradation.

Angiotensin peptides mediate their biological effects through interaction with specific, membrane receptors (as shown in Figure 1), which are distinguished using pharmacological agents. Ang II interacts with AT₁ angiotensin receptors, defined by their selectivity for the prototypical ligand losartan and similar antagonists (valsartan, L-158,809, etc.), while AT₂ receptors show selectivity for the antagonist PD123177 and PD123319 as well as the agonist CGP42112A.

Abbreviations: ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; Ang I, angiotensin I; Ang II, angiotensin II; Ang-(1-7), angiotensin-(1-7); [d-Ala⁷]-Ang-(1-7), [d-alanine⁷]-angiotensin-(1-7); EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; FBS, fetal bovine serum; MAP, mitogen-activated protein; PBS, phosphate-buffered saline; VSMC, vascular smooth muscle cells.
Measurement of thymidine incorporation

Dose response. Analysis of receptor subtype and response to angiotensin peptides. The incorporation of tritiated thymidine into quiescent cells growing in 24-well culture plates was measured in the following manner. Semi-confluent monolayers of cells in media with 10% FBS were made quiescent by a 48-h incubation in serum-free media. Cell monolayers were treated for 24 h in the presence and absence of various angiotensin peptides, receptor antagonists, or serum, as indicated below. During the last 4 h of treatment, 0.25 μCi of [3H]thymidine/mL culture medium was added to the growth media. Cell monolayers were washed with PBS and treated for 30 min with ice-cold 10% trichloroacetic acid. The acid-insoluble material was dissolved in 0.2% SDS in 0.1 N NaOH and the amount of [3H]thymidine incorporation was determined by liquid scintillation spectrometry in the presence of 5 μl of Ecolyme (ICN Biomedicals, Aurora, OH).

Dose response. Cells were treated with 1% FBS in the presence and absence of increasing amounts of Ang-(1-7) (10^-10 to 10^-7 M). The percent of DNA synthesis by Ang-(1-7) was calculated based upon the control in the presence of serum alone.

Analysis of receptor subtypes. Cells were treated with 100 nM Ang-(1-7), in the presence or absence of 1 μM of the AT1(-) receptor antagonist (D-Ala7)-Ang-(1-7), the AT2 receptor antagonist losartan, or the AT1R receptor antagonist PD123319. The percent of DNA synthesis by Ang-(1-7) in the presence of each antagonist was calculated based upon the control in the presence of antagonist alone.

Response to angiotensin peptides. Cells were treated with 100 nM Ang-(1-7), Ang II, Ang I, Ang-(2-8), Ang-(3-8) or Ang-(3-7), in the presence of 1% FBS. The percent of DNA synthesis by each peptide in the presence of serum was calculated based upon the control in the presence of serum alone.

Time course. Quiescent confluent cell monolayers were removed from tissue culture flasks with trypsin/EDTA, diluted 1 to 5, and plated into 24-well tissue culture plates in the presence of 1% FBS. On days 1-6 after plating, cells were pulsed with [3H]thymidine for 4 h and its incorporation into acid-precipitable material was determined, as described above. The percent inhibition of DNA synthesis by Ang-(1-7) in the presence of 1% FBS was calculated based upon the control in the presence of serum alone, at each time point.

Measurement of extracellular signal-regulated kinase (ERK1/ERK2) activities. Quiescent SK-LU-1 cells were incubated for 10 min with serum-free media or media containing 1% FBS or were pre-incubated with 10 nM Ang-(1-7) for 30 min followed by a 10-min treatment with 1% FBS. Cell monolayers were washed with PBS containing 0.01 mM NaVO₃ to prevent protein dephosphorylation. Cellular proteins were solubilized in lysis buffer (10 mM NaCl, 50 mM NaF, 5 mM EDTA, 1% Triton X-100, 50 mM Tris–HCl, pH 7.4) containing 0.01 mM NaVO₃, 0.1 mM PMSF and 0.6 μM leupeptin for 30 min on ice. The supernatant was clarified by centrifugation (12,000 g for 10 min, 4°C) and the protein concentration was measured by the Lowry method (24). Solubilized proteins (15 μg/well) were separated on 10% polyacrylamide gels using the buffer system of Laemmli and transferred to polyvinyl membranes (Amersham Pharmacia, Piscataway, NJ) by electrophoresis. Non-specific binding to the membranes was blocked by incubation in 5% Blotto (5% evaporated milk, 0.1% Triton X-100 in Tris-buffered saline). Membranes were probed with a specific antibody to the activated phosphorylated form of the ERK1/ERK2 (P44/P42 MAP kinases; 1:1000 dilution), followed by incubation with goat anti-rabbit antibody (1:2000 dilution) coupled to horseradish peroxidase. Immunoreactive bands were visualized using enhanced chemiluminescence reagents and quantified by densitometry. Protein loading was visualized by incubation of stripped membranes with an antibody to actin.

Detection of MAS mRNA and protein by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and western blot hybridization. RNA was isolated from cultured human lung cancer cells, using the TRIZOL reagent (Gibco BRL), as directed by the manufacturer. The RNA concentration was determined by UV spectroscopy at 260 nm and an estimate of purity was assessed by the optical density ratio at 260/280 nm. RNA integrity was confirmed by ethidium bromide staining intensity of 28S and 18S ribosomal RNA following agarose gel electrophoresis. The isolated RNA was incubated with RQI DNase (Promega) to eliminate any residual DNA that will amplify during the PCR.
Inhibition of lung cancer cell growth by Ang-(1-7)

The effect of Ang-(1-7) on serum-stimulated cell growth was determined in human SK-LU-1, SK-MES-1 and A549 lung cancer cells, as shown in Figure 2. Cells were plated at low density into individual wells of a 24-well cluster dish, in the presence of 1% FBS. On day 1 following plating, the total number of cells per well was determined using a hemocytometer. Stimulation with 1% FBS caused a time-dependent increase in the total number of cells, reaching confluence between days 12 and 19. Ang-(1-7) was added each day to half of the wells, to a final concentration of 100 nM. The peptide was replaced daily, due to its rapid degradation (19). The total number of cells per well increased in the presence of 1% FBS and Ang-(1-7), but there was a significant reduction in cell number in the presence of Ang-(1-7). In addition, the total number of cells per well in the presence of FBS and Ang-(1-7) did not reach the same level as in the presence of FBS alone, suggesting that the Ang-(1-7)-mediated reduction in cell number may be due to inhibition in cell growth as well as a delay in cell cycle progression.

Inhibition of DNA synthesis by Ang-(1-7)

The effect of Ang-(1-7) on serum-stimulated [3H]thymidine incorporation was examined in human lung SK-LU-1, A549 and SK-MES-1 cancer cells. Semi-confluent cell monolayers were made quiescent by a 48-h incubation in serum-free media. Subsequently, cells were treated with increasing amounts of Ang-(1-7) for 28 h, in the presence of 1% FBS. DNA synthesis was determined by the incorporation of [3H]thymidine into acid-precipitable DNA. FBS (1%) caused a significant increase in [3H]thymidine incorporation in all three cell lines (SK-LU-1, 266.6 ± 44.5%, n = 4; A549, 346.6 ± 83.7%, n = 3; and SK-MES-1, 425.9 ± 138.8%, n = 6), while Ang-(1-7) treatment resulted in a dose-dependent reduction in serum-stimulated thymidine incorporation, with IC50’s in the sub-nanomolar range (Figure 3). The IC50 was defined as the concentration of the peptide producing one-half of the maximal Ang-(1-7)-mediated inhibition of [3H]thymidine incorporation. The attenuation of human lung adenocarcinoma SK-LU-1 cell DNA synthesis was dependent on the dose of Ang-(1-7) over the range of 10 pM–100 nM. A maximal reduction of 58.8 ± 9.1% of DNA replication was observed using 10 nM Ang-(1-7) with an IC50 of 0.05 nM. Ang-(1-7) similarly attenuated mitogen-stimulated thymidine incorporation of human lung adenocarcinoma A549 cells with a maximal inhibition of 60.3 ± 14.9% with 100 nM Ang-(1-7) and an IC50 of 0.11 nM as well as non-small cell lung cancer SK-MES-1 cells with a maximal inhibition of 65.9 ± 4.3 by 10 nM Ang-(1-7) and an IC50 of 0.04 nM. These results suggest that Ang-(1-7) inhibits mitogen-stimulated DNA replication of human lung cancer cells in a dose-dependent manner with IC50’s similar to the concentration of Ang-(1-7) measured after treatment of rats with an ACE inhibitor (27,28).
The inhibition of DNA synthesis by Ang-(1-7) was also dependent upon the time of treatment. For these experiments, actively growing cells in 1% FBS were treated with 100 nM Ang-(1-7) daily, beginning one day after plating. The incorporation of \(^{3}H\)thymidine into SK-LU-1, A549 and SK-MES-1 lung cancer cells in the presence of 1% FBS was progressively reduced following the daily addition of Ang-(1-7), as shown in Figure 4. Ang-(1-7) was renewed each day due to the endogenous degradation of the peptide (19). A 4–5 day treatment with Ang-(1-7) reduced serum-stimulated growth by 42.9 ± 10.6% in SK-LU-1 cells, 53.2 ± 7.8% in A549 cells and 38.1 ± 7.5% in SK-MES-1 cells.

**Effect of angiotensin receptor antagonists on the inhibition of DNA synthesis by Ang-(1-7)**

The subtype of angiotensin peptide receptor involved in the inhibition of serum-stimulated \(^{3}H\)thymidine incorporation by Ang-(1-7) was determined using selective angiotensin receptor antagonists. Inhibition of the serum-stimulated growth of SK-LU-1 cells by 100 nM Ang-(1-7) was blocked by 1 mM \([D-Ala\text{7}]-\text{Ang-(1-7)}, the selective antagonist for the Ang-(1-7) receptor (Figure 5). Neither 1 mM losartan nor 1 mM PD123319, compounds, which selectively block the AT\(_1\) and AT\(_2\) subtypes of angiotensin receptors, respectively, was effective in attenuating the anti-proliferative effect of Ang-(1-7) in SK-LU-1 cells. These results show that the anti-proliferative actions of Ang-(1-7) in lung cancer cells are mediated by a non-AT\(_1\), non-AT\(_2\) AT\(_{\text{1/2}}\) receptor.

The G-protein coupled, seven transmembrane protein encoded by the \(\text{MAS}\) gene was identified as an Ang-(1-7) receptor (22). We identified \(\text{MAS}\) mRNA in SK-LU-1, SK-MES-1 and A549 lung cancer cells by RT-PCR using gene-specific primers to generate a 410 bp fragment, as shown in Figure 6. Additionally, an ~48 kDa protein was detected by western blot hybridization using a \(\text{MAS}\)-specific antibody.
lung cancer cells. SK-LU-1 cells were treated for 24 h with various angiotensin peptides and DNA synthesis was assessed following a 4-h pulse with \[^3\text{H}\]thymidine. As shown in Figure 7, only Ang-(1-7) significantly reduced serum-stimulated DNA synthesis in human lung cancer cells. Neither Ang II, Ang I, Ang-(2-8), Ang-(3-8) or Ang-(3-7), in the presence of 1% FBS. Cells were treated for 24 h and DNA replication was determined following a 4-h pulse with \[^3\text{H}\]thymidine. The data are presented as the percentage of serum-stimulated incorporation. \(n = 4-12\), in triplicate. *\(P < 0.05\) as compared with control, in the absence of any angiotensin peptide.

**Inhibition of ERK1 and ERK2 activities by Ang-(1-7)**

ERK1 and ERK2 activities were measured in quiescent SK-LU-1 lung cancer cells stimulated with 1% FBS to determine whether Ang-(1-7) inhibition of SK-LU-1 cell growth was due to a reduction in MAP kinase activities. Growth stimulation by mitogens is mediated, at least in part, through activation of the MAP kinases ERK1 and ERK2 to induce early response genes and increase transcription. Ang-(1-7) may inhibit cultured lung cancer cell growth by preventing the phosphorylation and activation of MAP kinases in response to mitogen stimulation. FBS (1%) caused a significant increase in the phosphorylation of both ERK1 (228.5 ± 15.2% of control, \(n = 3\)) and ERK2 (216.6 ± 20.0% of control, \(n = 4\)) in SK-LU-1 cells. As shown in Figure 8, a 30-min pre-treatment with 10 nM Ang-(1-7) caused a marked decrease in the serum-stimulated phosphorylation of ERK1 and ERK2. An ~68% reduction of ERK1 activity was observed in the lysates from lung cancer cells incubated with the heptapeptide, while ERK2 activity was attenuated by ~65%. These results suggest that the Ang-(1-7)-mediated anti-proliferative effects occur through inhibition of the ERK signal transduction pathway.

**Discussion**

In this study, we showed that Ang-(1-7) caused a marked decrease in DNA synthesis and cell proliferation of cultured human lung cancer cells, as observed by reduced serum-stimulated \[^3\text{H}\]thymidine incorporation, decreased cell number and attenuated ERK1/ERK2 activities. Our experiments provide the first evidence that Ang-(1-7) inhibits the proliferation of human lung cancer cells and support the hypothesis that Ang-(1-7) serves as an endogenous regulator of cell growth. Ang-(1-7) reduced the cell number of all three human lung cancer cell lines and the maximal cell number did not reach the levels achieved in the control. These results suggest that the molecular mechanism of Ang-(1-7)-mediated inhibition of cell growth involves both a reduction in cellular proliferation and the induction of apoptosis. This is supported by our preliminary studies using a gene array with 1776 genes implicated in carcinogenesis. A number of genes involved in
tumor suppression, apoptosis and cell cycle inhibition were upregulated in serum-stimulated SK-LU-1 cells treated with Ang-(1-7), while several oncogenes, enzymes involved in MAP kinase signaling, and cell cycle progression genes were down-regulated. Current studies are underway to confirm these results. Reports from the Uhal laboratory show that Ang II induces apoptosis in human A549 lung cancer cells (29,30). The induction of apoptosis by Ang II was blocked by losartan, indicating that this effect is mediated by an AT1 receptor. Taken together, our studies and those by the Uhal laboratory on the inhibition of lung cancer cell growth with angiotensin peptides suggest that the renin-angiotensin system may play an important role in the regulation of aberrant cell growth.

While the precise molecular pathways involved in the growth-modulating properties of Ang-(1-7) are not yet elucidated, we showed that treatment of cultured human lung cancer cells with the heptapeptide significantly reduced the phosphorylation and activation of ERK1 and ERK2. The ERK cascade is activated in response to stimuli, such as growth factors, cytokines or DNA damaging agents, by the MAP kinase kinases, which are dual-specificity kinases that generally recognize only certain MAP kinases as substrates. Phosphorylation by MAP kinase kinases is regulated by the upstream MAP kinase kinases. Thus, the results from our study suggest that Ang-(1-7) either inhibits or down-regulates: (i) ERK1 and ERK2 directly; (ii) the MAP kinase kinases, which phosphorylate ERK1 and ERK2; or (iii) the MAP kinase kinase kinase that activates MAP kinase kinase. Alternatively, Ang-(1-7) may stimulate or up-regulate a MAP kinase phosphatase, which would result in a decrease in active MAP kinase. In recent studies, we showed that the Ang-(1-7)-mediated inhibition of VSMC growth was prevented by pre-treatment with protein phosphatase inhibitors, suggesting that Ang-(1-7) may up-regulate a MAP kinase phosphatase to inhibit cell growth (31). Future experiments will elucidate the precise regulation of this MAPK signal transduction pathway following treatment of human lung cancer cells with Ang-(1-7).

Activation of ERKs plays an important role in the transformation and proliferation of lung cancers. ERK1 and ERK2 activities were increased in chemically induced lung tumors in mice (32-34) with a significant allelic loss of wild-type Ras (33,34). Similarly, enhanced cell proliferation concomitant with phosphorylation of the epidermal growth factor (EGF) receptor and ERK2 was observed in cultured human adeno-squamous carcinoma cells treated with EGF (35). EGF plays a major role in lung cancer cell autocrine growth, and genistein, a tyrosine kinase inhibitor, abolished the EGF-mediated cell proliferative effects. Specific inhibition of the ERK cascade by PD98059, a MAP kinase kinase inhibitor, also blocked EGF-stimulated growth (36). Cultured human lung cancer cells treated with anti-proliferative glucocorticoids or FR901228, a histone deacetylase inhibitor that induces growth arrest, exhibited lower ERK1 and ERK2 activities, suggesting that regulation of this signal transduction pathway may be a mechanism for the anti-growth activity (37,38). Inhibition of ERK by a conditionally expressed phosphatase demonstrated that ERK activity was necessary for Ras effector mutant-transformed cells to express matrix-degrading activity in vitro and tissue invasiveness in vivo (39). These studies indicate that pharmacological agents, such as Ang-(1-7), that reduce the ERK signal transduction pathway, may provide potent chemotherapeutic and chemopreventive therapy for lung carcinogenesis.

The growth inhibitory effect of Ang-(1-7) in cultured human SK-LU-1 lung cancer cells was blocked by [d-Ala7]-Ang-(1-7). This antagonist is selective for the Ang-(1-7) receptor, since it reduces responses to Ang-(1-7) and inhibits [125I]Ang-(1-7) binding to its receptor (2,3,40,41). The protein encoded by MAS, a G-protein coupled, seven transmembrane protein, binds [125I]Ang-(1-7) and both renal and vasodilatory responses to Ang-(1-7) are reduced following MAS ablation (42), suggesting that MAS mediates the Ang-(1-7) responses. In addition, we recently showed that either antisense oligonucleotides or siRNA’s to mas blocks the inhibition of MAP kinase activity in cultured VSMCs (23), suggesting that it mediates the anti-proliferative response to Ang-(1-7) in vascular cells. Neither the AT1 receptor antagonist losartan nor the AT2 receptor antagonist PD123319 prevented the anti-proliferative effect of Ang-(1-7) in SK-LU-1 cells, indicating that the growth inhibition by the heptapeptide was coupled to a pharmacologically distinct AT1(-7) receptor present on human lung cancer cells. We identified both the mRNA for MAS and MAS protein in SK-LU-1, SK-MES-1 and A549 lung cancer cells, suggesting that MAS may mediate the anti-proliferative response to Ang-(1-7) in lung cancer cells. These results are supported by our previous reports showing that the inhibition of mitogen-stimulated VSMC growth by Ang-(1-7) was not prevented by the AT1 antagonist L158,809 or the AT2 antagonist PD123177. However, [Sarcosine1-Threonine8]-Ang II, a non-selective angiotensin peptide antagonist, or [d-Ala7]-Ang-(1-7) effectively blocked vascular growth inhibition by Ang-(1-7) (42). Besides rat aortic VSMCs, we identified previously a specific AT(-17) receptor on bovine aortic endothelial cells, canine coronary artery endothelial cells and rat mesenteric VSMCs (1,2,43). This study represents the first report of a functional AT1(-7) receptor on any type of tumor cell.

The anti-proliferative property of Ang-(1-7) was not shared by other angiotensin peptides, including Ang I, Ang II, Ang-(2-8), Ang-(3-7) or Ang-(3-8). Ang I is a substrate for Ang II and Ang-(1-7) production but does not interact with membrane receptors. Ang II- and Ang-(2-8)-mediated effects occur via the pharmacologically defined AT1 and AT2 receptors (44,45) while Ang-(3-8) and Ang-(3-7) both interact with the AT1 receptor (46). These results provide additional evidence that the growth modulating effects of Ang-(1-7) are mediated by a specific AT1(-17) receptor. This specificity confers multiple treatment options. The heptapeptide could be administered singly or in a therapeutic modality combining Ang-(1-7) or compounds such as ACE inhibitors that elevate the concentration of the peptide and other chemopreventive agents could be used to provide synergistic protection. Since elevation in the circulating levels of Ang-(1-7) does not increase body weight, heart rate or blood pressure or cause deleterious side effects (5), increased levels of the peptide are tolerated, an important property of a pharmacological agent.

The anti-proliferative responses to Ang-(1-7) were both dose- and time-dependent with IC50’s in the sub-nanomolar range, similar to the endogenous Ang-(1-7) levels after treatment of rats with an ACE inhibitor (47,48). ACE inhibitors, currently in widespread use for the treatment of hypertension, cause a reduction in Ang II and a significant elevation in both tissue and circulating Ang-(1-7) (47-51). Our results suggest that the reduced cancer risk observed in patients after administration of ACE inhibitors may be due, at least in part, to the elevated levels of Ang-(1-7). In a prospective study of 5207
patients in Scotland, the relative risks of incident and fatal cancer among the 1559 patients treated with ACE inhibitors were significantly reduced, to 0.72 and 0.65, respectively. The relative risk was lowest in patients with lung or sex-specific cancer. In fact, the relative risk of lung cancer in patients treated with ACE inhibitors was 0.34, significantly reduced as compared with treatment with other antihypertensive drugs, where the relative risk was 0.98. Yoshiji et al. (53) showed that treatment of mice with ACE inhibitors significantly reduced the proliferation of a heptacellular carcinoma allograft, while AT1 receptor antagonists were ineffective. These results suggest that the anti-growth properties of ACE inhibitors are not due to a reduction in circulating concentrations of the mitogenic peptide Ang II but may result from an increase in serum Ang-(1-7). Thus, administration of Ang-(1-7) or compounds that elevate the circulating levels of the peptide may be effective chemotherapeutic and chemopreventive agents with significantly reduced side effects needed to treat lung cancer.

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References


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