Does the Renin-Angiotensin System Participate in Regulation of Human Vasculogenesis and Angiogenesis?

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Abstract

Several lines of evidence suggest that hypertension and angiogenesis may be related phenomena but a functional link remains elusive. Here, we propose that the renin-angiotensin system (RAS), in addition to its central role in arterial hypertension, also regulates blood vessel formation during normal development and cancer. This mechanistic hypothesis is based on reports of biochemical, genetic, clinical, and epidemiologic data reviewed herein. Species differences between the RAS of rodents and humans likely account for why such a fundamental role in angiogenesis went unrecognized for so long. If proven correct, this hypothesis carries many implications for the medical practices of cardiology, oncology, and neonatology.

Introduction

Angiotensin-converting enzyme (ACE) inhibitor drugs are among the most widely prescribed antihypertensive agents. ACE inhibitors have shown particular efficacy in preventing adverse cardiac events in high-risk individuals, treatment of patients with congestive heart failure, and prevention of diabetic nephropathy. However, it has also been recognized for several years that these drugs are teratogenic when developing fetuses are subjected to prolonged exposure during the second and third trimesters of pregnancy (1). Congenital abnormalities associated with fetal exposure to ACE inhibitors include neonatal renal failure, oligohydramnios, and skeletal abnormalities, all of which were presumably related to decreased amniotic fluid production (2), with the proposed pathophysiology for these congenital abnormalities being attributed to reduction in fetal renal blood flow (3).

Because kidney development and urine production progress gradually and occur predominantly during the later stages of development (4), the major risk of congenital abnormalities due to ACE inhibitors was believed to be present in the second and third trimesters of pregnancy (5). However, in a retrospective analysis, Cooper and colleagues (6) found that infants born to women who took ACE inhibitors during the first trimester of pregnancy had an excess of major congenital malformations compared with unexposed women. No biological explanation was put forward to explain such striking findings (6) and the molecular mechanism for these observations was deemed unknown (7). Effects of ACE inhibitors on the developing kidney do not account for congenital abnormalities resulting from first-trimester exposure to ACE inhibitors, and it is notable that in the study by Cooper and colleagues (6), there was no significant increase in renal abnormalities after ACE inhibitor exposure in the first trimester.

In contrast, there were highly significant increased risks of developing malformations in the central nervous system (hazard ratio, 4.39) and cardiovascular system (hazard ratio, 3.79; ref. 6).

As we will review herein, findings based on clinical studies and further supported by data from preclinical studies suggest that the renin-angiotensin system (RAS) plays an essential role in developmental and pathologic blood vessel growth. These findings support the notion that inhibition of blood vessel formation may account for congenital abnormalities due to first-trimester ACE inhibitor exposure. The developing brain and heart are major sites of blood vessel formation (8), and the pattern of the recently reported malformations due to first-trimester ACE inhibitor exposure (4) is entirely consistent with impairment of vasculogenesis and early angiogenesis.

Angiogenesis and Vasculogenesis

Blood vessels develop, expand, and diminish through a complex cellular interplay (8). The initial assemblage of progenitor cells (angioblasts) into a primary network of small blood vessels and into the dorsal aorta and cardinal veins of the very young embryo is called vasculogenesis. These blood vessels then remodel through growth, migration, sprouting, and pruning to form a functional circulatory system by a process called angiogenesis—a simple name that hides a complex set of integrated chemical actions. For example, mouse embryos lacking the cell receptor Flk1 (also called VEGFR2) fail to form vessels at all or, if its ligand VEGF-A is mutated, die with vessels of severely reduced size. EphrinB2 is an early marker for arteries, and its receptor, EphB4, for veins. In chicks, although not yet tested in human embryos, some angioblasts already display neuropilin 1 [a vascular endothelial growth factor (VEGF) receptor restricted at subsequent stages to arteries], whereas others show neuropilin 2 (a receptor destined for veins). Remarkably, angioblasts with the pertinent markers migrate to the positions of future arteries and veins in advance of the onset of blood flow. In mammals, later stages of developmental angiogenesis as well as physiologic angiogenesis in the adult (wound healing, female reproductive cycling, and placenta formation) and pathologic angiogenesis—cancer and several major retinal and other diseases classifiable as "angiogenesis-dependent diseases" (9)—share some molecular mechanisms with the earlier vascular processes, but each also has unique biochemical features (10). Such features should, in principle, allow them to be individually targeted for therapeutic purposes.

Angiogenic Effects of Angiotensin-Modifying Peptidases

We reasoned that the RAS is an important, but generally unappreciated, regulator of developmental and pathologic
angiogenesis (Fig. 1). Over the past several years, extensive work from our group and others has uncovered proangiogenic roles of peptidases responsible for the downstream cleavage of angiotensin II to functionally active smaller proteins. The membrane-associated protease aminopeptidase A (APA; EC 3.4.11.7), a homodimeric type II membrane-spanning cell surface constituent that is expressed on vascular endothelial cells throughout the body, has only one well-defined role: cleaving the NH$_2$-terminal aspartate residue of the 8–amino acid angiotensin molecule to the 7–amino acid angiotensin III (Fig. 1; ref. 11). Angiotensin III has high affinity for angiotensin type I and type II receptors and shares many of the physiologic functions of angiotensin II in the cardiovascular system and in promoting production of chemokines and growth factors (12). We have shown that APA is up-regulated and enzymatically activated in the vascular endothelium of human tumors (13) and that mice lacking APA fail to mount the expected angiogenic response to hypoxia or growth factors (14). Furthermore, inhibitory peptides or monoclonal antibodies that block APA activity also potently inhibit angiogenesis in tumors (14) and in hypoxia-related pathologic angiogenesis in postnatal developing retina (15). These findings indicate that, like angiotensin II, angiotensin III also possesses proangiogenic activity.

Another agent, the membrane-bound zinc-dependent peptidase, aminopeptidase N (APN; CD13; EC 3.4.11.2), expressed in various epithelial cells and in macrophages, removes the NH$_2$-terminal arginine residue of angiotensin III to form the 6–amino acid angiotensin IV (Fig. 1). Angiotensin IV, unlike angiotensin II or III, does not bind with high affinity to the angiotensin type I or type II receptors but instead seems to bind to the recently characterized AT4 receptor. Notably, stimulation of the AT4 receptor strongly promotes endothelial cell growth, suggesting that angiotensin IV also possesses proangiogenic activity. We have shown that APN is highly expressed on tumor blood vessels and on other vessels undergoing pathologic angiogenesis, and have shown that APN antagonists are antiangiogenic in vivo (16). We have also generated APN-null mice genetically by homologous recombination and found that these mice are structurally and physiologically normal and can undergo physiologic angiogenesis but show a severely impaired angiogenic response under pathologic conditions (17). These biochemical and genetic lines of evidence indicate that peptides derived from the downstream cleavage of individual amino acids from angiotensinogen promote angiogenesis in a variety of settings and that inhibition of any of several peptidases within the RAS inhibits new blood vessel growth.

**Figure 1.** The role of the RAS in angiogenesis. Renin, produced primarily by the juxtaglomerular cells of the kidney, catalyzes the proteolytic cleavage of circulating angiotensinogen to angiotensin I. Blockade of downstream aminopeptidases involved in the further sequential proteolytic processing of the angiotensins also inhibits angiogenesis, suggesting a mechanism for the major congenital malformations seen in infants exposed to ACE inhibitors in the first trimester of pregnancy. In addition to the pharmacologic inhibition of the aminopeptidases (14, 16) depicted here, the genetic elimination of either APA (14) or APN (17) also results in severe angiogenesis impairment in mice. *, antangiogenic agent; #, antihypertensive agent.
VEGF as an Effector of Angiogenesis of the RAS

The angiotensin receptors are classic seven-transmembrane domain G protein–coupled receptors (GPCR). With a few notable exceptions (such as the thrombin receptor), GPCRs have not been implicated as direct modulators of angiogenesis but instead act indirectly to enhance blood vessel development and growth. Activation of angiotensin II receptors by peptides derived from RAS leads to potent induction of vascular endothelial growth factor (VEGF; refs. 18–20). The VEGF/VEGF receptor system plays a central role in vasculogenesis in embryonic development and in adult life (21). Enhanced VEGF production due to angiotensin II may be largely due to a potent angiotensin-dependent stabilization of the transcription factor hypoxia-inducible factor-1α (HIF-1α; ref. 22) through a recently characterized mechanism involving production of reactive oxygen species (23). Activation of angiotensin receptors also enhances transcription of HIF-1α via a protein kinase C–dependent mechanism (24). In addition to the effects on VEGF production, angiotensin II has been shown to enhance expression of the VEGF receptor KDR/Flik1 in a model of cultured endothelial cells (25), which may further amplify angiotensin-mediated angiogenesis.

Effects of Drug Inhibitors of Angiotensin Signaling on Tumor Angiogenesis

The proangiogenic effects of angiotensin described above provide mechanistic support to the notion that pharmacologic blockade of angiotensin signaling may interfere with tumor angiogenesis (26). In support of this approach are studies of human tumors that correlated the extent of angiotensin receptor expression with tumor VEGF expression, tumor angiogenesis, and/or tumor invasiveness (27–29). Emerging data suggest that in addition to systemically produced angiotensin, the tumor environment contains all of the necessary components of the RAS to produce angiotensin locally and contribute importantly to tumor angiogenesis and tumor progression (26, 29, 30), further implicating the RAS as a molecular mediator of tumor angiogenesis. Indeed, studies in mouse models show that captopril and other ACE inhibitors reduce tumor growth in mouse models by blocking angiotensin (31, 32). In these experimental models, ACE inhibitor administration is associated with a profound decrease in circulating levels of vascular endothelial growth factor (VEGF), a finding consistent with the established ability of angiotensin II to enhance VEGF production by endothelial cells (23) and vascular pericytes (33). Further confirmation comes from recent findings showing that drugs that directly inhibit function of angiotensin receptors inhibit tumor angiogenesis and cancer growth in vivo (34–36). Intriguingly, and consistent with the findings in preclinical models, a retrospective cohort analysis of patients in an antihypertensive agent trial revealed a decreased cancer incidence in patients on ACE inhibitor drugs (37), suggesting that long-term use of ACE inhibitors may protect against cancer and that the antiangiogenic properties of these agents may have substantial clinical relevance (20, 38). More recently, long-term treatment with ACE inhibitors or angiotensin receptor blockers was shown to decrease cancer incidence in individuals with ACE gene polymorphisms associated with elevated serum ACE levels (39).

RAS Knockout Mice and the Thalidomide Paradox

In light of the excess congenital abnormalities seen in human fetuses exposed to ACE inhibitors during the first trimester, when blood vessel formation is of major importance, one might predict that animals lacking ACE would also possess profound congenital abnormalities. Yet, mice lacking ACE show no evidence of congenital abnormalities such as those seen in human fetuses exposed to ACE inhibitors (40). Similarly, our group has shown that mice genetically lacking either APA or APN show no overt evidence of congenital abnormalities (14, 17). However, the paucity of developmental abnormalities in rodents lacking ACE does not preclude the possibility that ACE inhibition causes developmental abnormalities by inhibiting early vessel formation in humans. It is well established that rodents exposed to the antiangiogenic agent thalidomide show no developmental abnormalities, yet humans are highly sensitive to its teratogenic effects (41). The sensitivity of humans to blood vessel inhibitors during the first trimester of pregnancy suggests either a more refined regulation of the developing vascular endothelium in humans or a greater redundancy of effective vasculogenic and angiogenic agents in rodents.

Open Questions

Despite the significant amount of data outlined above in support of the hypothesis that the RAS is an important regulator of vasculogenesis and angiogenesis, the precise roles of the different constituents of this system remain poorly understood. By use of standard knockout and back-crossing technology, mouse models can be engineered in which several of the enzymes regulating conversion of angiotensin to its downstream peptides can be eliminated; for instance, study of double-null mice (i.e., APA−/−/APN−/−) or triple-null mice (i.e., ACE−/−/APA−/−/APN−/−) may clarify mechanisms of developmental abnormalities due to impaired angiogenesis. Because complete and specific pharmacologic blockade of angiotensin-associated peptidases may also be required to understand fully the developmental abnormalities caused by impaired angiogenesis that this hypothesis would predict, one may have to seek new peptidase inhibitors (42).

Moreover, the findings that biochemical or genetic interference with components of the RAS inhibits angiogenesis in experimental models (14–17, 31) fit well with the intriguing (and almost decade old) epidemiologic data showing that ACE inhibitors are associated with decreased incidence of human cancer (37); in their seminal “hypothesis-generating” Lancet report, Lever and colleagues (37) left open the question of molecular mechanism. We feel that the time has come for tumor vascular targeting and angiogenesis inhibition to be strongly considered as therapeutic factors to exploit (43). The relative expression of receptors of the angiotensin system within vascular endothelium in different tumors and at varying stages of carcinogenesis, although still poorly understood, is clearly relevant to the putative role of angiotensins in promoting tumor angiogenesis.

Manipulation of the RAS may be an important tactic particularly for the treatment of markedly angiogenic tumors. Ideally, disruption of the RAS through new agents that selectively inhibit ACE or other peptidases within the RAS may not only prevent cancer (37, 39) but could also conceivably augment the efficacy of established chemotherapeutic agents. Because the safety profile of ACE inhibitors (outside of pregnancy settings) is well established, and because hypertension is a fairly common side effect of many cancer therapeutic agents, addition of ACE and/or other peptidase inhibitors to selected therapeutic cancer regimens would perhaps serve “double duty” by simultaneously providing inhibition of tumor angiogenesis and beneficial effects on the cardiovascular...
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