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COMMENTARY

Angiotensin Signaling and Apoptosis in the Neonatal Heart: Necessary Evils?

Commentary on the article by Choi *et al.* on page 325

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Angiotensin converting enzyme inhibitors such as captopril are potent and widely used antihypertensive agents that are frequently used as first-line therapy for chronic hypertension owing to their excellent safety profile. However, most authors recommend that these drugs not be used in the second and third trimester of pregnancy because of potential harm to the fetus (1, 2). To date, the mechanism of this reported fetal toxicity has not been extensively studied. The article by Choe *et al.* in this issue of *Pediatric Research* explores one possible mechanism for the deleterious effects of ACE inhibition, the prevention of angiotensin II-induced cardiac cell turnover and apoptosis.

Angiotensin converting enzyme (ACE) is a central element of the renin-angiotensin system (RAS), the most important regulator of blood pressure in land-dwelling mammals. ACE converts the decapeptide, angiotensin I, to the potent pressor octapeptide, angiotensin II (Ang II), which governs peripheral vascular tone and regulates glomerular filtration in the kidney. Ang II plays a critical role in the maintenance of fetal arterial pressure, and in the regulation of fetal GFR and renal blood flow (3). In addition to these direct actions on blood flow, Ang II also activates intracellular signaling pathways involved in growth and apoptosis in many cell types, including the myocytes and fibroblasts of the heart. Ang II promotes myocyte enlargement and protein synthesis, as well as hypertrophy-associated alterations in the cardiac gene expression program (4, 5), through specific cellular receptor subtypes AT1 and AT2. AT1 is the more abundant in the adult heart (6, 7), and has been linked to control of both hypertrophy and apoptosis in the cardiac myocyte (8, 9). The AT2 receptor in the heart is less well understood, but it has been proposed to act in opposition to AT1 based on its differential expression in various types of myocardial pathology (6, 10–13). The AT1 receptor is coupled through Gαq proteins to a variety of intracellular signals including the generation of oxygen free radicals (14, 15), to activation of Ras (16) and of the extracellular regulated kinase/mitogen-activated protein kinase (ERK/MAPK) protein kinase family (17). Ang II has also been shown to activate nuclear factor kappa B (NFκB)-dependent transcription in other cell types, possibly through its effects on cell redox state (18). ACE, Ang II, and its receptors are present from very early embryogenesis, implying that these Ang II-dependent signal pathways are also important during development (3, 19).

The significance of Ang II in modulating growth is highlighted by the fact that ACE inhibitors cause significant fetal toxicity during pregnancy. Although ACE inhibitors are not teratogens, they appear to inhibit aspects of fetal growth in the second and third trimesters, with dose-dependent decreases in implants and live births, reduced neonatal growth, and increased perinatal mortality across a wide range of species (20–22). In human babies, kidney function is frequently affected, resulting in oligohydramnios, renal tubular dysgenesis, and neonatal anuria (22). Abnormal growth of bone, lung or other organs, as well as mild to severe intrauterine growth retardation, persistent patent ductus arteriosus, fetal and neonatal death, have also been reported. A syndrome of renal tubular dysgenesis and severely underdeveloped calvarial bone has been linked to ACE inhibitor use in the second and third trimesters (23). The way in which ACE inhibitors cause these abnormalities is unclear, but could stem from hypotension, reduced blood flow, and chronic hypoxia, or alternatively through direct effects of Ang II on cell fate decisions in target tissues. Such cell fate decisions may take the form of growth or apoptosis, both of which are essential to normal fetal development.

In their manuscript, Choe *et al.* provide evidence that Ang II regulates a terminal wave of myocardial cell proliferation and apoptosis that has been previously shown to occur during the early postnatal period (24). Although the significance of apoptosis in this context is not clear, it coincides with the withdrawal of cardiac myocytes from the cell cycle and with the establishment of postnatal blood flow patterns. In the present study, treatment with the ACE inhibitor enalapril reduced the survival rate of neonatal rats, and also reduced both heart and

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body weights. Diminished heart size was associated with lowered cell proliferation rates, as well as reduced apoptosis, in both myocytes and interstitial cells (predominantly fibroblasts). Because proliferating cells outnumbered apoptotic cells 10:1, the net effect on heart growth was negative. However, the bulk of programmed cell death occurred in cardiac myocytes. None of these effects were seen with hydralazine at doses previously established to have equivalent effects on blood pressure. These experiments suggest that the effects of ACE (and ACE inhibition) on cardiac growth are mediated through direct cell signaling rather than through secondary hemodynamic effects.

These results confirm that both cell death and proliferation are components of the cardiac adaptation to growing hemodynamic demands in the young heart, and demonstrate that angiotensin signaling drives both phenomena. One major conclusion to be drawn from this study is that RAS-mediated cell turnover is an important component of the reshaping of the newborn myocardium to accommodate both increasing blood volume and new patterns of blood flow. The importance of Ang II in growth of the newborn heart parallels the prominent role of Ang II in cardiac hypertrophy in the adult. Together with the long and reasonably successful history of using neonatal cardiac myocytes to model adult heart growth (23, 25), these observations provide further evidence that the cell signaling pathways regulating normal cardiac growth and stress-induced hypertrophy may differ only in degree rather than representing qualitatively different processes.

An interesting parallel can be made with the phenomenon of myocardial remodeling in the adult. Remodeling is the end result of the complex cellular and molecular changes that occur in response to alterations in hemodynamic loading or other types of stress (20). Remodeling can occur in response to pressure or volume overload, excessive neurohormonal stimulation, or loss of myocardial tissue through infarction. It is reasonably well established that apoptosis, hypertrophy, and fibroblast (and to some extent, myocyte) proliferation are all components of the remodeling process, and that Ang II is an important mediator of these cellular phenomena. Ang II, mediated by the AT1 receptor subtype, has been shown to modulate cardiac myocyte growth (26–28) and apoptosis (13, 29–32), in response to stretch and pressure overload, in diabetic cardiomyopathy and after myocardial infarction. Prevention of Ang II-mediated remodeling is now an established therapeutic goal in the adult cardiac patient after acute coronary occlusion, and Ang II-mediated apoptosis may play an important role in the deterioration of cardiac function in heart failure patients (13, 33). Remarkably, Ang II appears to be an effector for both pediatric and adult remodeling, even though the consequences (normal growth *versus* pathologic decompensation) are diametrically opposed in the two cases. It will be important to identify the factors that render the pediatric myocardium resistant to decompensation in the face of chronic stress.

Another interesting finding of this study was that the rate of apoptosis in neonatal cardiac tissue was correlated with levels of the protein clusterin (variously known as apolipoprotein J, sulfated glycoprotein-2 (SGP-2), apoptor, glycoprotein 80 (gp80), glycoprotein III (GPIII), and testosterone-repressed prostatic message-2 (TRPM-2)), although not to levels of Bcl-x and Bcl-2, two other proteins implicated in apoptosis regulation. Clusterin is a ubiquitously expressed, secreted glycoprotein found in most biologic fluids. Expression of clusterin is induced in many tissue types undergoing involution, apoptosis or remodeling (reviewed in (34)). It has been variously postulated to act as a neuroprotectant during cellular stress, a mediator of cell-cell interactions, an anti-apoptotic signal, an antioxidant, a complement inhibitor, and a chaperone-like molecule that binds to partially denatured proteins and prevents their aggregation (35, 36). In the present study, a reduction in clusterin expression was observed in enalapril-treated rats, providing strong corroboration for the observed reduction in apoptosis. In contrast to clusterin, levels of Bcl-x and Bcl-2 did not differ appreciably between treated and untreated rats, suggesting that the total quantity of these proteins is not an important determinant of apoptotic rates in this particular setting. Other factors, such as phosphorylation state, subcellular localization, or the abundance of pro-apoptotic Bcl-2 family members, may be more important in governing the actions of these proteins during this apoptotic process (37, 38).

This study raises a number of interesting questions. Does the apoptosis in neonatal myocardium stem from the arrest of proliferative activity, or is it due to hemodynamic stress inherent in rapid postnatal growth? What are the long-term consequences of interfering with the postnatal remodeling process? Which, if any, of the cardiac effects of ACE inhibition account for its perinatal toxicity, and to what extent? How important is apoptosis in this setting? Certainly, apoptosis has a critical role in early fetal development; patterning of the heart, as well as of other organs and tissues, requires the precise deletion of specific cells. But it is not clear whether myocyte apoptosis during late fetal and early neonatal life has equivalent physiologic significance. It is also possible that other effects of ACE inhibition, for example reduced production of fibroblast-derived matrix molecules, share the blame for poor growth and development.

Answering this last question will be challenging. With existing models, it is not possible to block early neonatal cardiac remodeling selectively without impact on the function of other organs, especially the kidney and lungs. Consequently it is difficult to identify the source of greatest risk to the fetus and newborn from late-term ACE inhibitor use. Although nothing is known about the functional significance of either proliferation or apoptosis in the late-term, it is possible to speculate that the remodeling process is advantageous rather than harmful at this stage of cardiac development. Working this out will require targeting anti-apoptotic strategies to the fetal and newborn myocardium, most likely through transgenic approaches.

REFERENCES

1. Barr MJ 1994 Teratogen update: angiotensin-converting enzyme inhibitors. Teratology 50:399–409
2. Mastrobattista JM 1997 Angiotensin converting enzyme inhibitors in pregnancy. Semin Perinatol 21:124–134
3. Lumbers ER 1995 Functions of the renin-angiotensin system during development. Clin Exp Pharmacol Physiol 22:499–505
4. Lijnen P, Petrov V 1999 Renin-angiotensin system, hypertrophy and gene expression in cardiac myocytes. J Mol Cell Cardiol 31:949–970

5. Jin H, Yang R, Awad TA, Wang F, Li W, Williams SP, Ogasawara A, Shimada B, Williams PM, de Feo G, Paoni NF 2001 Effects of early angiotensin-converting enzyme inhibition on cardiac gene expression after acute myocardial infarction. Circulation 103:736–742
6. Haywood GA, Gullestad L, Katsuya T, Hutchinson HG, Pratt RE, Horiuchi M, Fowler MB 1997 AT1 and AT2 angiotensin receptor gene expression in human heart failure. Circulation 95:1201–1206
7. Serneri GG, Boddi M, Cecioni I, Vanni S, Coppo M, Papa ML, Bandinelli B, Bertolozzi I, Polidori G, Toscano T, Maccherini M, Modesti PA 2001 Cardiac angiotensin II formation in the clinical course of heart failure and its relationship with left ventricular function Circ Res 88:961–968
8. Cigola E, Kajstura J, Li B, Meggs LG, Anversa P 1997 Angiotensin II activates programmed myocyte cell death in vitro. Exp Cell Res 231:363–371
9. Kajstura J, Cigola E, Malhotra A, Li P, Cheng W, Meggs LG, Anversa P 1997 Angiotensin II induces apoptosis of adult ventricular myocytes in vitro. J Mol Cell Cardiol 29:859–870
10. Gullestad L, Haywood G, Aass H, Ross H, Yee G, Geiran O, Kjekshus J, Simonsen S, Bishopric NH, Fowler M 1998 Angiotensin II receptor subtype expression after heart transplantation. Cardiovasc Res 38:340–347
11. Busche S, Gallinat S, Bohle RM, Reinecke A, Seebeck J, Franke F, Fink L, Zhu M, Sumners C, Unger T 2000 Expression of angiotensin AT(1) and AT(2) receptors in adult rat cardiomyocytes after myocardial infarction. A single-cell reverse transcriptase-polymerase chain reaction study. Am J Pathol 157:605–611
12. Goette A, Arndt M, Rocken C, Spiess A, Staack T, Geller JC, Huth C, Ansorge S, Klein HU, Lendeckel U 2000 Regulation of angiotensin II receptor subtypes during atrial fibrillation in humans. Circulation 101:2678–2681
13. Barlucchi L, Leri A, Dostal DE, Fiordaliso F, Tada H, Hintze TH, Kajstura J, Nadal-Ginard B, Anversa P 2001 Canine ventricular myocytes possess a renin-angiotensin system that is upregulated with heart failure. Circ Res 88:298–304
14. Griendling KK, Ushio-Fukai M 2000 Reactive oxygen species as mediators of angiotensin II signaling. Regul Pept 91:21–27
15. Wang HD, Xu S, Johns DG, Du Y, Quinn MT, Cayatte AJ, Cohen RA 2001 Role of NADPH oxidase in the vascular hypertrophic and oxidative stress response to angiotensin II in mice Circ Res 88:947–953
16. Sadoshima J, Izumo S 1996 The heterotrimeric G q protein-coupled angiotensin II receptor activates p21 ras via the tyrosine kinase-Shc-Grb2-Sos pathway in cardiac myocytes. EMBO J 15:775–787
17. Aoki H, Richmond M, Izumo S, Sadoshima J 2000 Specific role of the extracellular signal-regulated kinase pathway in angiotensin II-induced cardiac hypertrophy in vitro. Biochem J 347 Pt 1:275–284
18. Brasier AR, Jamaluddin M, Han Y, Patterson C, Runge MS 2000 Angiotensin II induces gene transcription through cell-type-dependent effects on the nuclear factor-kappa B (NF-kappaB) transcription factor Mol Cell Biochem 212:155–169
19. Schutz S, Le Moullec JM, Corvol P, Gasc JM 1996 Early expression of all the components of the renin-angiotensin-system in human development. Am J Pathol 149:2067–2079
20. Francis GS 1998 Changing the remodeling process in heart failure: basic mechanisms and laboratory results. Curr Opin Cardiol 13:156–161
21. Morisco C, Zebrowski D, Condorelli G, Tsichlis P, Vatner SF, Sadoshima J 2000 The Akt-glycogen synthase kinase 3beta pathway regulates transcription of atrial natriuretic factor induced by beta-adrenergic receptor stimulation in cardiac myocytes. J Biol Chem 275:14466–14475
22. Khaper N, Singal PK 2001 Modulation of oxidative stress by a selective inhibition of angiotensin II type 1 receptors in MI rats. J Am Coll Cardiol 37:1461–1466
23. Schaub MC, Hefti MA, Harder BA, Eppenberger HM 1997 Various hypertrophic stimuli induce distinct phenotypes in cardiomyocytes. J Mol Med 75:901–920
24. Kajstura J, Mansukhani M, Cheng W, Reiss K, Krajewski S, Reed JC, Quaini F, Sonnenblick EH, Anversa P 1995 Programmed cell death and expression of the protooncogene bcl-2 in myocytes during postnatal maturation of the heart. Exp Cell Res 219:110–121
25. Bishopric NH, Andreka P, Slepak TI, Webster KA 2001 Molecular mechanisms of apoptosis in the cardiac myocyte. Curr Opin Pharmacol 1:141–150
26. Baker KM, Chernin MI, Wixson SK, Aceto JF 1990 Renin-angiotensin system involvement in pressure-overload cardiac hypertrophy in rats. Am J Physiol 259:H324–H332
27. Sadoshima J, Izumo S 1993 Molecular characterization of angiotensin II–induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. Critical role of the AT1 receptor subtype. Circ Res 73:413–423
28. Sadoshima J, Xu Y, Slayter HS, Izumo S 1993 Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. Cell 75:977–984
29. Leri A, Claudio PP, Li Q, Wang X, Reiss K, Wang S, Malhotra A, Kajstura J, Anversa P 1998 Stretch-mediated release of angiotensin II induces myocyte apoptosis by activating p53 that enhances the local renin-angiotensin system and decreases the Bcl-2-to-Bax protein ratio in the cell. J Clin Invest 101:1326–1342
30. Liu Y, Leri A, Li B, Wang X, Cheng W, Kajstura J, Anversa P 1998 Angiotensin II stimulation in vitro induces hypertrophy of normal and postinfarcted ventricular myocytes. Circ Res 82:1145–1159
31. Anversa P, Olivetti G, Leri A, Liu Y, Kajstura J 1997 Myocyte cell death and ventricular remodeling. Curr Opin Nephrol Hypertens 6:169–176
32. Goldstein S, Sharov VG, Cook JM, Sabbah HN 1995 Ventricular remodeling: insights from pharmacologic interventions with angiotensin-converting enzyme inhibitors. Mol Cell Biochem 147:51–55
33. Kang PM, Izumo S 2000 Apoptosis and heart failure: A critical review of the literature. Circ Res 86:1107–1113
34. Koch-Brandt C, Morgans C 1996 Clusterin: a role in cell survival in the face of apoptosis? Prog Mol Subcell Biol 16:130–149
35. Calero M, Rostagno A, Matsubara E, Zlokovic B, Frangione B, Ghiso J 2000 Apolipoprotein J (clusterin) and Alzheimer's disease Microsc Res Tech 50:305–315
36. Poon S, Easterbrook-Smith SB, Rybchyn MS, Carver JA, Wilson MR 2000 Clusterin is an ATP-independent chaperone with very broad substrate specificity that stabilizes stressed proteins in a folding-competent state. Biochemistry 39:15953–15960
37. Jarpe MB, Widmann C, Knall C, Schlesinger TK, Gibson S, Yujiri T, Fanger GR, Gelfand EW, Johnson GL 1998 Anti-apoptotic versus pro-apoptotic signal transduction: checkpoints and stop signs along the road to death. Oncogene 17:1475–1482
38. Bossy-Wetzel E, Green DR 1999 Apoptosis: checkpoint at the mitochondrial frontier. Mutat Res 434:243–251