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Effects of angiotensin (1-7) upon right ventricular function in experimental rat pulmonary embolism

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Summary. Right ventricular (RV) dysfunction contributes to poor clinical prognosis after pulmonary embolism (PE). The present studies evaluate the effects of angiotensin (1-7) (ANG (1-7)) upon RV function during experimental PE in rats. Circulating ANG II increased 8-fold 6 hr after PE (47±13 PE vs. 6±3 pg/mL, control, $p<0.05$). ACE2 protein was uniformly localized in the RV myocardium of control rats, but showed a patchy distribution with some cells devoid of stain after 6 or 18 hr of PE. RV function decreased 18 hr after PE compared with control treated animals (19±4 vs. 41±1 mmHg, respectively, $p<0.05$; 669±98 vs. 1354±77 mmHg/sec, respectively, $p<0.05$), while left ventricular function (LV) was not significantly changed. Animals treated with ANG (1-7) during PE showed improved RV +dP/dt and peak systolic pressure development to values not significantly different from control animals. Protection of RV function by ANG (1-7) was associated with improved arterial blood sO2, base excess and pH. Supplemental delivery of ANG (1-7) reduced the development of RV dysfunction, suggesting a novel approach to protecting RV function in the setting of acute experimental PE.

Key words: Renin-angiotensin system, Angiotensin converting enzyme 2, Angiotensin (1-7), Pulmonary embolism, Right ventricle

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Introduction

Pulmonary embolism (PE) is a major cardio-pulmonary disease with an incidence of 1 per 1000 individuals, hospitalizing approximately 150,000 Americans per year (Stein et al., 2003). Mortality rate exceeds 15% in the first 3 months (Piazza and Goldhaber, 2006; Tapson, 2008; Torbicki et al., 2008), but increases dramatically with the presence of right ventricular (RV) dysfunction (Kreit, 2004; Schoepf et al., 2004; Ten Wolde et al., 2004; Becattini and Agnelli, 2008; Watts et al., 2010). Our clinical studies show that approximately forty percent of normotensive PE patients have right ventricular (RV) dysfunction and ten percent have persistent exercise intolerance (Kline et al., 2006; Stevinson et al., 2007). These data suggest that it is important to understand and regulate RV damage following PE. Our rat studies show that moderate PE produces a 3-fold increase pulmonary vascular resistance (PVR) and injures the right ventricle (RV) by shear forces, stretch, increased work and by a neutrophil-mediated inflammatory response (Jones et al., 2003; Zagorski et al., 2003, 2007, 2008, 2009; Watts et al., 2006, 2008, 2009).

It has recently been discovered that several peptides once thought to be breakdown products of the renin-angiotensin system (RAS) possess biological activity, thus prompting renewed interest in the regulation of cardiovascular homeostasis via the balance of these peptides (Ferreira and Raizada, 2008; Reudelhuber, 2005; Varagic et al., 2008). Of particular interest is the counterbalance between the effects of angiotensin II (ANGII) and angiotensin (1-7) (ANG (1-7)) (Diz, 2008; Gallagher et al., 2008; Santos et al., 2008; Stewart et al., 2008; Varagic et al., 2008). Acting via the AT1 receptor (AT1R), ANG-II is prothrombotic, proinflammatory and causes vasoconstriction, which might exacerbate RV

injury from PE. Recent studies also indicate that ANG II can decrease ACE2 expression, which may diminish production of ANG (1-7) by this enzyme (Gallagher et al., 2006, 2008; Koka et al., 2008; Zhang et al., 2009). In contrast with ANG-II, ANG (1-7) acts via the Mas receptor, has mild vasodilatory effects, promotes nitric oxide synthesis and reduces ANG II signaling through the AT1R (Reudelhuber, 2006; Stewart et al., 2008).

Activation of the RAS and the effects of ANG (1-7) upon RV dysfunction in the setting of acute PE are, at present, unknown. The present study examines ANG-II formation and ACE 2 expression, following acute experimental PE in rats and evaluates the effects ANG (1-7) upon RV function during acute experimental PE.

Materials and methods

Experiments were performed using male Sprague-Dawley rats weighing between 350 and 400 g and were conducted with the approval of the Institutional Animal Care and Use committee of the Carolinas Medical Center in accordance with the Guide for the Care and Use of Laboratory Animals.

Pulmonary Embolism (PE) model and treatment groups

Microspheres (26±1 μm, 7525B, Thermo Scientific, Freemont, CA) were sterilized with 70% ethanol, washed with sterile 0.01% Tween 20, and resuspended in 0.01% sterile Tween 20 to produce a 10% suspension (13 million beads/ml). Animals were anesthetized using an intraperitoneal injection of xylazine (3 mg/kg) and ketamine (70 mg/kg) and placed on a warming pad. Microspheres (2.0 million beads/100 g body wt) were injected via the right jugular vein to produce PE. Control animals received all surgical treatments and the vehicle for the microspheres (0.01% Tween 20, 0.15 ml/100 g body wt). ANG (1-7) was injected intravenously 576 μg/kg), a dose that was previously described to be effective in reducing myocardial injury from diabetic hypertension (Benter et al., 2006, 2007, 2008).

Animals were euthanized 6 hours after treatment to measure plasma ANG-II (n=6 control, 10 PE) and ACE-2 immunohistochemistry (n=6) or after 18 hours to measure in vivo hemodynamics (n=10-12/group), cardiac function (n=12-18/group), ACE-2 immunohistochemistry (n=6/group) and blood chemistry (n=10-14/group).

Measurement of plasma ANG-II

PE-50 tubing was inserted into the right carotid artery and an aliquot of blood (5 ml) was removed into EDTA containing tubes and centrifuged (3,000 rpm, 10 minutes, 4°C). The plasma was then aliquoted and stored at -70°C until assay. Plasma samples (2 mL) were loaded on to Strata Phenyl (55 μm, 70A, 500 mg/3mL) solid phase columns (Phenomenex Inc., Torrence, CA) and ANG-II was eluted with methanol (500 μL). Angiotensin II concentration was determined using an ELISA assay (SPI-Bio Product #589301, Cayman Chemical, Ann Arbor, MI) according to the manufacturers instructions. Standards were linear from 1 to 125 pg/mL (r^2=0.9956).

ACE-2 immunohistochemistry

Hearts were perfused briefly via the aorta to remove blood from the coronary vasculature and ventricles. The outflow tract of the RV was isolated, fixed in 10% neutral buffered formalin, embedded in paraffin and cut in cross-section. Sections were de-parafinized, rehydrated and stained for ACE-2. The primary antibody was rabbit polyclonal antibody (ACE2, H-175: sc-20998, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), which was applied for 60 minutes (1:50 dilution). The secondary antibody was rabbit/mouse biotinylated link antibody (Dako, Carpentaria, CA) and sections were processed with peroxidase-conjugated streptavidin and diaminobenzidine/hydrogen peroxidase and were counter-stained using 0.03% aqueous light green stain (Polysciences, Warrington, PA).

Hemodynamics, in vitro heart function, and blood chemistry

Animals were anesthetized with xylazine (3 mg/kg) and ketamine (70 mg/kg) and placed on a warming pad filled with re-circulating water warmed to 105°F (Graymar solid-state T-pump; Orchard Park, NY) for the study of in vivo hemodynamics and blood chemistry. A 2-French Millar Mikro-Tip catheter transducer (SPR-249-A Millar Instruments, Houston, TX) was placed in the left carotid artery to monitor systemic pressures. A 2-French bent Millar catheter (SPR-513) was inserted into

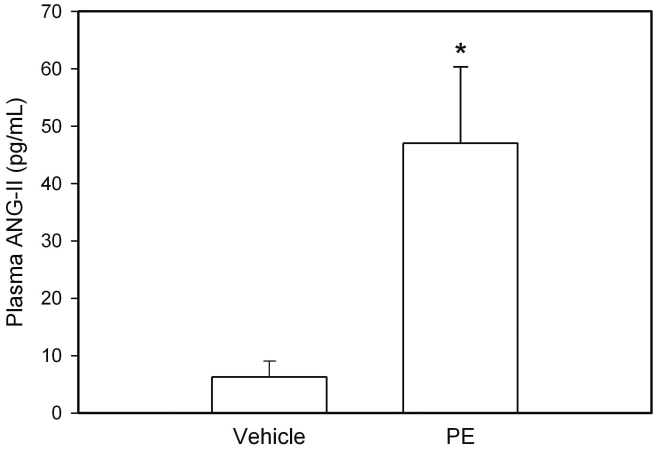

Fig. 1. Changes in circulating plasma levels of ANG-II seen with PE. Values are mean ± s.e., n=6-10, stars indicate significantly different values.

the right ventricle via the right jugular vein and intermittently advanced into the RV to monitor intraventricular pressures. The transducers were connected to a Transducer Balance Box, a UM 100A coupling box and an MP100 data acquisition unit, which was connected to a personal computer. Pressures were recorded using AcKnowledge software (BIOPAC Systems, Inc., Santa Barbara, CA). The arterial catheter was removed and PE-50 tubing was inserted to sample arterial blood (1 mL) for blood chemistry (i-STAT, MN300, Abbott Point of Care, Princeton, NJ) using CG4+ cartridges.

For the study of intrinsic cardiac function, hearts were removed from anesthetized rats via midline thoracotomy and placed in ice-cold saline. The aorta was perfused at 60 mm Hg in a non-recirculating, temperature-controlled (37°C) system. The perfusion solution, Krebs-Henseleit bicarbonate buffer, consisted of (in mmol/L): 118 NaCl, 4.7 KCl, 21 $NaHCO_3$, 1.25 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 11 glucose and .05 octanoate, which was gassed with 95% O_2/ 5% CO_2. Hearts were perfused to assess intrinsic function of right ventricle (RV) and left ventricle (LV) using two independent balloons, as previously described (Watts et al., 2006). Briefly, each latex balloon was tested to ensure that balloon volume was well-matched for ventricular volumes. Balloons were filled with saline and connected by PE tubing to Statham transducers (P23, Gould Electronics, Millersville, MD) via PE-60 tubing. Pressures were recorded using AcKnowledge software, as described above. Initial volumes of the RV and the LV balloons established 0 mm Hg end diastolic pressure (EDP). Hearts were then paced (300 beats/min, 5-ms duration, 5 volts) using a Grass SD9 stimulator (Astro-

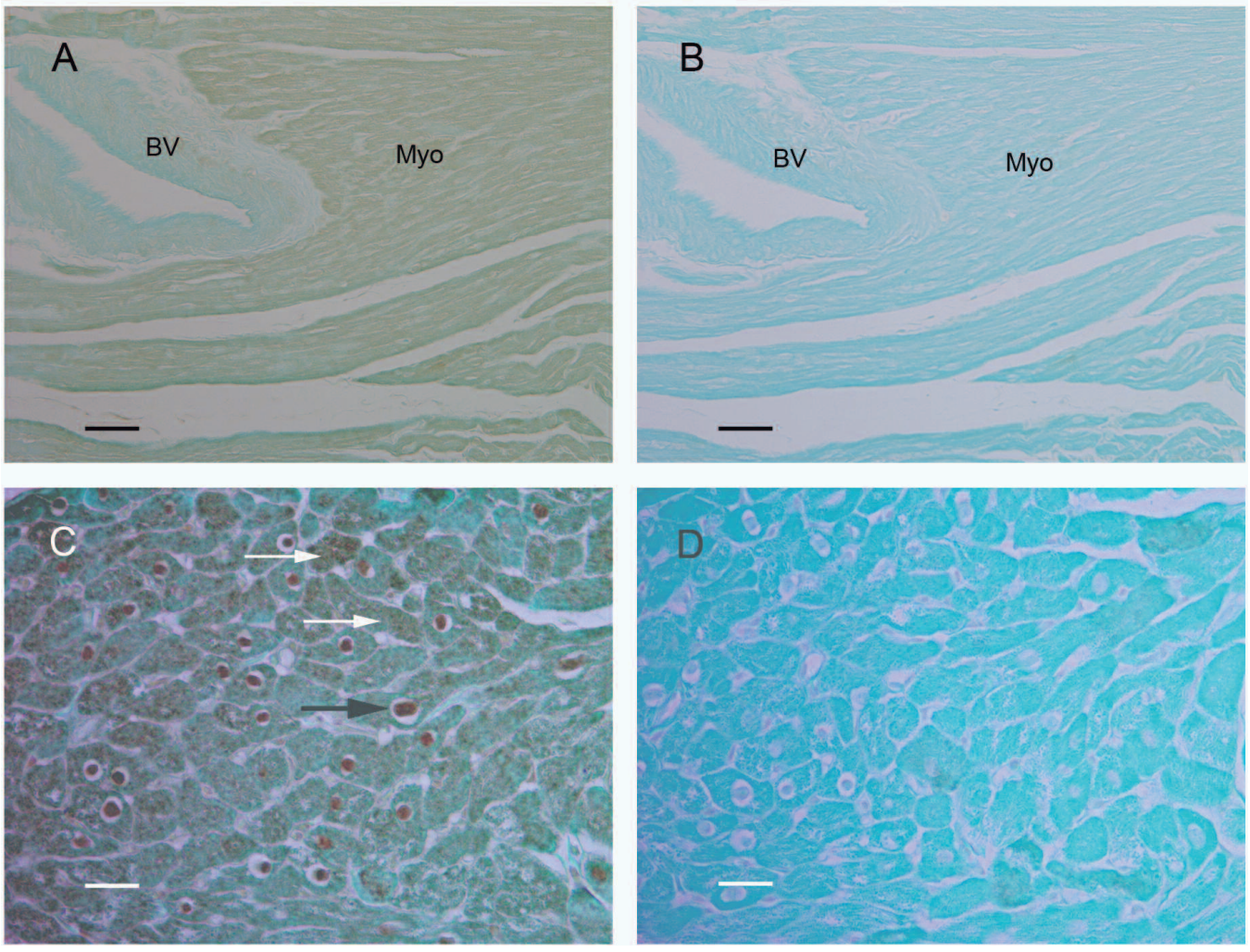

Fig. 2. Immunohistochemical localization of ACE2 protein in control hearts. **A.** Longitudinal view showing positive stain in the myocardium (Myo) and not in the blood vessel tissue (BV). **B.** Negative control. **C.** Cross-section view of the myocardium showing positive stain in the cytoplasm (white arrows) and nuclei (black arrow). **D.** Negative control. Bars: A, B, 40 μm; C, D, 20 μm.

Med, West Warwick, RI) and electrodes attached to the aortic cannula and the apex of the heart to allow direct comparison of pressure data among treatment groups. Heart perfusion was continued for each heart until stable heart function was obtained (10-15 min) before taking data for the heart. Heart function end points included peak systolic pressure (PSP) and maximum rate of pressure development (+dP/dt).

Statistical analyses

Values are presented as mean ± SE. Comparisons of three or more independent groups were made by ANOVA with Tukey's post-hoc testing with uneven sample sizes or Student-Newman-Keuls testing with equal sample sizes. Comparisons of two independent groups were made using Student's t-test. Significance was determined as $p<0.05$ using two-tailed testing.

Results

Plasma ANG II

Plasma ANG II levels increased 8-fold (Fig. 1) following 6 hours of PE, indicating that the rennin-angiotensin system is activated by the cardiovascular stress of PE.

ACE-2 immunohistochemistry

ACE-2 protein was localized to the right ventricular myocardium and myocardial cell nucleus, but not the vascular tissue, in control animals (Fig. 2A,C). There was a uniform distribution of stain in the myocardium in

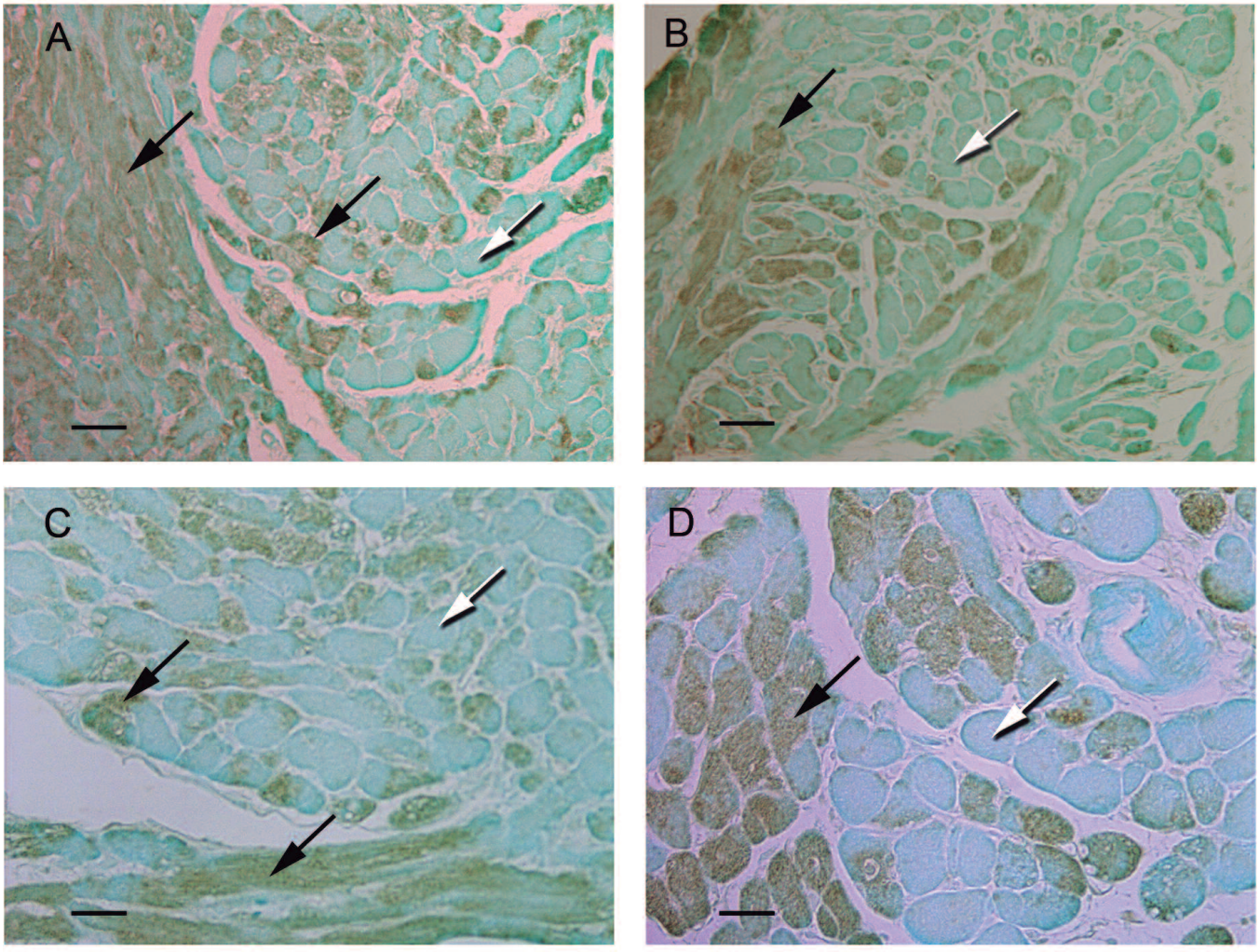

Fig. 3. Immunohistochemical localization of ACE2 protein in right ventricular tissue six hours after PE **(A and C)** and 18 hours after PE **(B and D)**. There is patchy distribution of stain in some myocardial cells (black arrows) with regions that are unstained (white arrows). Bars: A, B, 40 μm; C, D, 20 μm.

these animals. Stain was not observed when the primary antibody was omitted from the reaction (Fig. 2B,D). In contrast with the homogeneous stain observed in control animals, ACE2 distribution was patchy with some cells devoid of stain (white arrows) interspaced among cells with stain (black arrows) in the right ventricular myocardium of hearts examined 6 hours (Fig. 3A,C) or 18 hours (Fig. 3B,D) after PE.

Heart function

Hemodynamic measurements, made in vivo, indicated that experimental PE caused moderate pulmonary hypertension as indicated by increased right ventricular peak systolic pressure (47±2 mmHg PE, vs. 28±1 Control, $p<0.05$), and mild systemic hypotension with decreased mean arterial pressure (77±5 PE, vs. 123±6 Control, $p<0.05$, n=10-12/group) 18 hours after treatment.

Intrinsic right ventricular function, observed in isolated, perfused hearts, decreased following 18 hours of PE as evidenced by a 70% decrease in +dP/dt and a 50% decrease in systolic pressure compared with control animals (Fig. 4A,B). Treatment of rats with ANG (1-7) during PE resulted in a significant improvement in both +dP/dt and peak systolic pressure of the RV (Figure 4A,B). These values were not significantly different from those observed in control animals. In contrast with the RV, LV function was not significantly changed by PE or by treatment with ANG (1-7) (Fig. 4C,D).

Differences observed in right ventricular function among treatment groups were not due to heart rate (set at 300 bpm), coronary flow or balloon volume, which were not significantly different ($p>0.05$). Therefore, changes in +dP/dt and peak systolic pressure reflects differences in intrinsic contractile function among treatment groups.

Arterial blood chemistry

Pulmonary embolism also resulted in significant alterations in arterial blood chemistry with reduced sO2, negative base excess and acidosis (Fig. 5). Treatment with ANG (1-7) ameliorated these changes (Fig. 5), suggesting that improved cardiac function, observed in vitro, was associated with improved arterial blood chemistry in vivo.

Discussion

The present studies show that acute, experimental PE increased circulating ANG II and decreased right ventricular myocardial expression of ACE2 protein. In addition, right ventricular contractile function was depressed (decreased +dP/dt and peak systolic pressure),

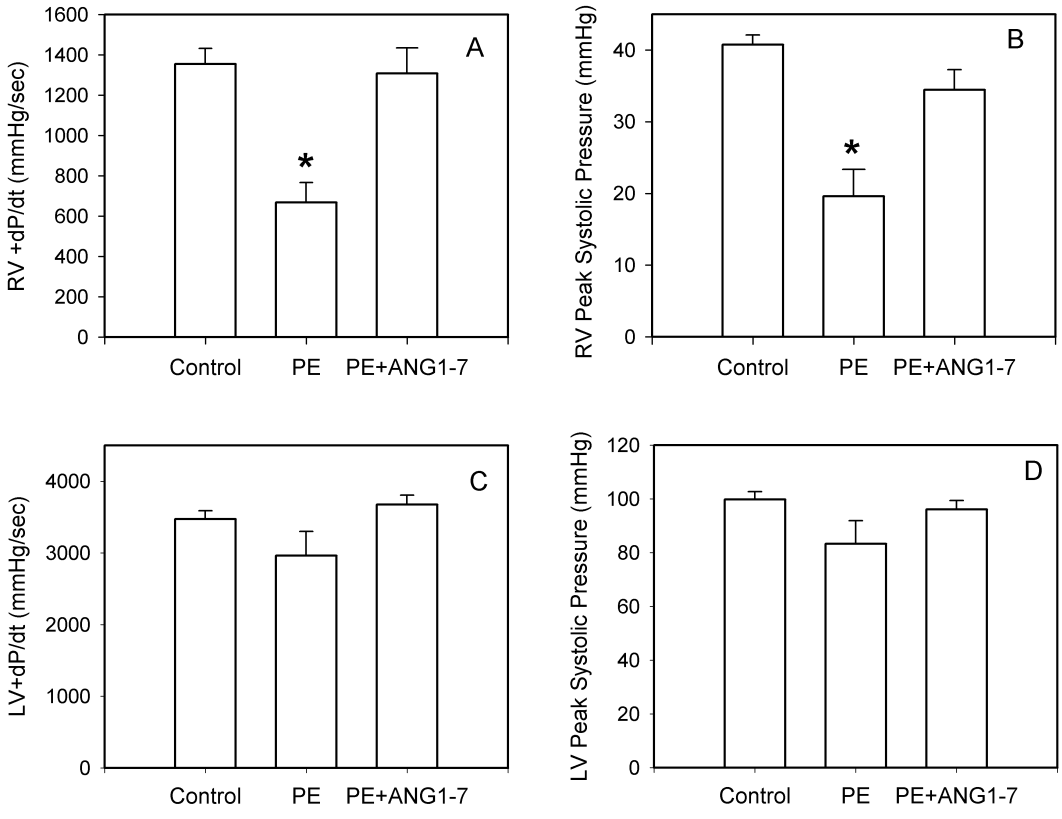

Fig. 4. Intrinsic cardiac contractile function determined in perfused hearts isolated 18 hours after treatment in vivo with control, pulmonary embolism (PE) or PE with ANG (1-7). **A.** RV +dP/dt. **B.** RV Peak systolic pressure. **C.** LV +dP/dt. **D.** LV Peak systolic pressure. Values are mean ± s.e., n=12-18/group. *: indicates a value significantly different from control and PE + ANG (1-7).

while LV function was unchanged following PE. The addition of ANG (1-7) during PE, improved both +dP/dt and peak systolic pressure in the right ventricle and improved arterial blood gas levels. Thus, the present studies suggest that supplementation with ANG (1-7) may be beneficial in the setting of acute PE.

ANG-II acts primarily upon AT1 receptors (AT1R) causing Ca^{++}- dependent smooth muscle contraction and pulmonary precapillary vasoconstriction, salt and water retention, inflammation, thrombosis, fibrosis, increased cyclooxygenase-2 expression and increased oxidative stress (Suzuki et al., 2003; Diz, 2008). ANG (1-7) opposes many of the actions of ANG-II by decreasing ANG-II signaling through AT_1R (Diz, 2008; Stewart et al., 2008), decreasing oxidative stress (Benter et al., 2006, 2007, 2008), activating beneficial levels of NO formation and Akt survival pathway (Giani et al., 2007; Sampaio et al., 2007), and reducing COX-2 expression, thereby lowering formation of constrictive prostaglandins and increasing formation of prostacyclin (Clark et al., 2003; Dartevelle et al., 2004; Bresser et al., 2006; Menon et al., 2007; Diz, 2008) Tipping the balance of ANG-II and ANG (1-7) towards the latter might therefore mitigate RV damage from PE. Recent clinical trials indicate that ANG (1-7) is well tolerated in humans and it appears to have antiproliferative capabilities and reduces cancer growth in a phase I trial (Petty et al., 2009), and beneficial effects in human diabetic nephropathy (Ferrario and Varagic, 2010) and chronic hypertension (Ferrario et al., 2010).

We found a significant elevation in circulating ANG-II and decreased ACE2 protein expression following experimental PE. Thus, the balance of these two factors is in favor of ANG II. Several factors may reduce the expression of ACE2 during PE. Increased input by ANG II to AT_1R decreased ACE2 protein expression and ANG (1-7) formation in several systems (Gallagher et al., 2006, 2008; Koka et al., 2008; Zhang et al., 2009). We have also previously shown that acute PE induces expression of HIF-1α in the RV of rats (Zagorski et al., 2008). Increased HIV-1α upregulates ACE activity and ANG II formation, while decreasing ACE2 protein expression in hypoxic pulmonary artery smooth muscle cells (Zhang et al., 2009). Thus, there are at least two pathways reducing ACE2 protein expression, thereby tipping the balance toward ANG-II.

Administration of exogenous ANG (1-7) improved +dP/dt and peak generated systolic pressure in the setting of acute experimental PE. It has previously been reported that ANG (1-7) decreases chronic hypertension, but has little effect on systemic pressure in normal animals (Chappell et al., 1998). Pulmonary vasodilation would be beneficial during PE, but excessive systemic vasodilation could be detrimental, especially if right ventricular preload were reduced out of proportion to pulmonary vascular relaxation. Increased ANG (1-7) production via fusion protein in rats caused increased cardiac output with decreased total peripheral resistance and decreased vascular resistance to the lung, and other organs, suggesting a physiological role in tonic control of regional blood flow (Sampaio et al., 2003; Botelho-Santos et al., 2007). ANG (1-7) has also been shown to counteract the ANG-II mediated vasoconstriction, increased vascular permeability and hydrostatic edema formation in acute respiratory distress syndromes

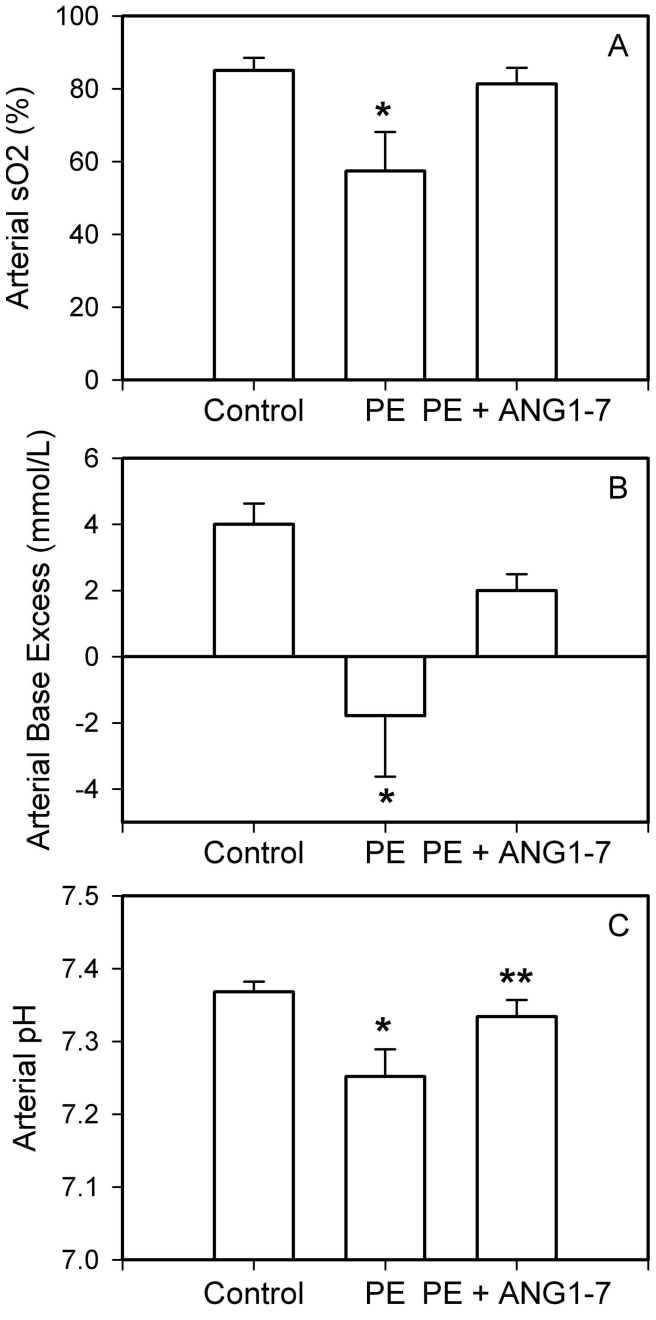

Fig. 5. Arterial blood hemoglobin saturation sO2 **(A)**, base excess **(B)** and pH **(C)** observed 18 hours after control treatment, PE, or PE + ANG (1-7). Values are mean ± s.e., n = 10-14/group. *: indicates a value significantly different from control and PE + ANG (1-7).

(ARDS) (Imai et al., 2008). ANG (1-7) also decreases thrombus formation (Kucharewicz et al., 2002). Thus, ANG (1-7) appears to have positive effects upon multiple homeostatic pathways that should be beneficial in the setting of PE. The present studies show that supplemental delivery of ANG (1-7) reduces the development of RV dysfunction and enhances blood gas exchange.

The present studies show that plasma ANG II levels increase markedly during experimental PE and ACE-2 expression is reduced, suggesting an imbalance favoring ANG II over ANG (1-7) during PE. The supplemental delivery of ANG (1-7) reduces the development of RV dysfunction, suggesting a novel approach to protecting RV function in the setting of acute PE.

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References

Becattini C. and Agnelli G. (2008). Predictors of mortality from pulmonary embolism and their influence on clinical management. Thromb. Haemost. 100, 747-751.

Benter I.F., Yousif M.H., Anim J.T., Cojocel C. and Diz D.I. (2006). Angiotensin-(1-7) prevents development of severe hypertension and end-organ damage in spontaneously hypertensive rats treated with L-NAME. Am. J. Physiol. Heart Circ. Physiol. 290, H684-H691.

Benter I.F., Yousif M.H., Cojocel C., Al Maghrebi M. and Diz D.I. (2007). Angiotensin-(1-7) prevents diabetes-induced cardiovascular dysfunction. Am. J. Physiol. Heart Circ. Physiol. 292, H666-H672.

Benter I.F., Yousif M.H., Dhaunsi G.S., Kaur J., Chappell M.C. and Diz D.I. (2008). Angiotensin-(1-7) prevents activation of NADPH oxidase and renal vascular dysfunction in diabetic hypertensive rats. Am. J. Nephrol. 28, 25-33.

Botelho-Santos G.A., Sampaio W.O., Reudelhuber T.L., Bader M., Campagnole-Santos M.J. and Souza dos Santos R.A. (2007). Expression of an angiotensin-(1-7)-producing fusion protein in rats induced marked changes in regional vascular resistance. Am. J. Physiol. Heart Circ. Physiol. 292, H2485-H2490.

Bresser P., Pepke-Zaba J., Jais X., Humbert M. and Hoeper M.M. (2006). Medical therapies for chronic thromboembolic pulmonary hypertension: an evolving treatment paradigm. Proc. Am. Thorac. Soc. 3, 594-600.

Chappell M.C., Iyer S.N., Diz D.I. and Ferrario C.M. (1998). Antihypertensive effects of angiotensin-(1-7). Braz. J. Med. Biol. Res. 31, 1205-1212.

Clark M.A., Tallant E.A., Tommasi E., Bosch S. and Diz D.I. (2003). Angiotensin-(1-7) reduces renal angiotensin II receptors through a cyclooxygenase-dependent mechanism. J. Cardiovasc. Pharmacol. 41, 276-283.

Dartevelle P., Fadel E., Mussot S., Chapelier A., Herve P., de Perrot M., Cerrina J., Ladurie F.L., Lehouerou D., Humbert M., Sitbon O. and Simonneau G. (2004). Chronic thromboembolic pulmonary hypertension. Eur. Respir. J. 23, 637-648.

Diz D.I. (2008). Future directions in cardiovascular pharmacology: examples from the Renin-Angiotensin system. Mol. Interv. 8, 222-225.

Ferrario C.M. and Varagic J. (2010). The ANG-(1-7)/ACE2/mas axis in the regulation of nephron function. Am. J. Physiol. Renal Physiol. 298, F1297-F1305.

Ferrario C.M., Ahmad S., Joyner J. and Varagic J. (2010). Advances in the renin angiotensin system focus on angiotensin-converting enzyme 2 and angiotensin-(1-7). Adv. Pharmacol. 59, 233.

Ferreira A.J. and Raizada M.K. (2008). Are we poised to target ACE2 for the next generation of antihypertensives? J. Mol. Med. 86, 685-690.

Gallagher P.E., Chappell M.C., Ferrario C.M. and Tallant E.A. (2006). Distinct roles for ANG II and ANG-(1-7) in the regulation of angiotensin-converting enzyme 2 in rat astrocytes. Am. J. Physiol. Cell Physiol. 290, C420-246.

Gallagher P.E., Ferrario C.M. and Tallant E.A. (2008). Regulation of ACE2 in cardiac myocytes and fibroblasts. Am. J. Physiol. Heart Circ. Physiol. 295, H2373-H2379.

Giani J.F., Gironacci M.M., Munoz M.C., Pena C., Turyn D. and Dominici F.P. (2007). Angiotensin-(1 7) stimulates the phosphorylation of JAK2, IRS-1 and Akt in rat heart in vivo: role of the AT1 and Mas receptors. Am. J. Physiol. Heart Circ. Physiol. 293, H1154-H1163.

Imai Y., Kuba K. and Penninger J.M. (2008). The discovery of angiotensin-converting enzyme 2 and its role in acute lung injury in mice. Exp. Physiol. 93, 543-548.

Jones A.E., Watts J.A., Debelak J.P., Thornton L.R., Younger J.G. and Kline J.A. (2003). Inhibition of prostaglandin synthesis during polystyrene microsphere-induced pulmonary embolism in the rat. Am. J. Physiol. Lung Cell. Mol. Physiol. 284, L1072-L1081.

Kline J.A., Hernandez-Nino J., Rose G.A., Norton H.J. and Camargo C.A. Jr (2006). Surrogate markers for adverse outcomes in normotensive patients with pulmonary embolism. Crit. Care Med. 34, 2773-2780.

Koka V., Huang X.R., Chung A.C., Wang W., Truong L.D. and Lan H.Y. (2008). Angiotensin II up-regulates angiotensin I-converting enzyme (ACE), but down-regulates ACE2 via the AT1-ERK/p38 MAP kinase pathway. Am. J. Pathol. 172, 1174-1183.

Kreit J.W. (2004). The impact of right ventricular dysfunction on the prognosis and therapy of normotensive patients with pulmonary embolism. Chest 125, 1539-1545.

Kucharewicz I., Pawlak R., Matys T., Chabielska E. and Buczko W. (2002). Angiotensin-(1-7): an active member of the renin-angiotensin system. J. Physiol. Pharmacol. 53, 533-540.

Menon J., Soto-Pantoja D.R., Callahan M.F., Cline J.M., Ferrario C.M., Tallant E.A. and Gallagher P.E. (2007). Angiotensin-(1-7) inhibits growth of human lung adenocarcinoma xenografts in nude mice through a reduction in cyclooxygenase-2. Cancer Res. 67, 2809-2815.

Petty W.J., Miller A.A., McCoy T.P., Gallagher P.E., Tallant E.A. and Torti F.M. (2009). Phase I and pharmacokinetic study of angiotensin-(1-7), an endogenous antiangiogenic hormone. Clin. Cancer Res. 15, 7398-7404.

Piazza G. and Goldhaber S.Z. (2006). Acute pulmonary embolism: part I: epidemiology and diagnosis. Circulation 114, e28-e32.

Reudelhuber T.L. (2005). The renin-angiotensin system: peptides and enzymes beyond angiotensin II. Curr. Opin. Nephrol. Hypertens. 14, 155-159.

Reudelhuber T.L. (2006). A place in our hearts for the lowly angiotensin 1-7 peptide?. Hypertension 47, 811-815.

Sampaio W.O., Nascimento A.A. and Santos R.A. (2003). Systemic and regional hemodynamic effects of angiotensin-(1-7) in rats. Am. J. Physiol. Heart Circ. Physiol. 284, H1985-H1994.

Sampaio W.O., Souza dos Santos R.A., Faria-Silva R., Mata Machado L.T., Schiffrin E.L. and Touyz R.M. (2007). Angiotensin-(1-7) through receptor Mas mediates endothelial nitric oxide synthase activation via Akt-dependent pathways. Hypertension 49, 185-192.

Santos R.A., Ferreira A.J. and Simões e Silva A.C. (2008). Recent advances in the angiotensin-converting enzyme 2-angiotensin(1-7)-Mas axis. Exp. Physiol. 93, 519-527.

Schoepf U.J., Kucher N., Kipfmueller F., Quiroz R., Costello P. and Goldhaber S.Z. (2004). Right ventricular enlargement on chest computed tomography: a predictor of early death in acute pulmonary embolism. Circulation 110, 3276-3280.

Stein P.D., Hull R.D., Ghali W.A., Patel K.C., Olson R.E., Meyers F.A. and Kalra N.K. (2003). Tracking the uptake of evidence: two decades of hospital practice trends for diagnosing deep vein thrombosis and pulmonary embolism. Arch. Intern. Med. 163, 1213-1219.

Stevinson B.G., Hernandez-Nino J., Rose G. and Kline J.A. (2007). Echocardiographic and functional cardiopulmonary problems six months after first-time pulmonary embolism in previously healthy patients. Eur. Heart J. 28, 2517-2524.

Stewart J.A. Jr, Lazartigues E. and Lucchesi P.A. (2008). The angiotensin converting enzyme 2/Ang-(1-7) axis in the heart: a role for MAS communication? Circ. Res. 103:1197-9.

Suzuki Y., Ruiz-Ortega M., Lorenzo O., Ruperez M., Esteban V. and Egido J. (2003). Inflammation and angiotensin II. Int. J. Biochem. Cell Biol. 35, 881-900.

Tapson V.F. (2008). Acute pulmonary embolism. N. Engl. J. Med. 358, 1037-1052.

Ten Wolde M., Sohne M., Quak E., Mac Gillavry M.R. and Buller H.R. (2004). Prognostic value of echocardiographically assessed right ventricular dysfunction in patients with pulmonary embolism. Arch. Intern. Med. 164, 1685-1689.

Torbicki A., Perrier A., Konstantinides S., Agnelli G., Galie N., Pruszczyk P., Bengel F., Brady A.J., Ferreira D., Janssens U., Klepetko W., Mayer E., Remy-Jardin M., Bassand J.P., Vahanian A., Camm J., De Caterina R., Dean V., Dickstein K., Filippatos G., Funck-Brentano C., Hellemans I., Kristensen S.D., McGregor K., Sechtem U., Silber S., Tendera M., Widimsky P., Zamorano J.L., Zamorano J.L., Andreotti F., Ascherman M., Athanassopoulos G., De Sutter J., Fitzmaurice D., Forster T., Heras M., Jondeau G., Kjeldsen K., Knuuti J., Lang I., Lenzen M., Lopez-Sendon J., Nihoyannopoulos P., Perez Isla L., Schwehr U., Torraca L., Vachiery J.L. and Task Force for the Diagnosis and Management of Acute Pulmonary Embolism of the European Society of Cardiology (2008). Guidelines on the diagnosis and management of acute pulmonary embolism: the Task Force for the Diagnosis and Management of Acute Pulmonary Embolism of the European Society of Cardiology (ESC). Eur. Heart J. 29, 2276-2315.

Varagic J., Trask A.J., Jessup J.A., Chappell M.C. and Ferrario C.M. (2008). New angiotensins. J. Mol. Med. 86, 663-671.

Watts J.A., Zagorski J., Gellar M.A., Stevinson B.G. and Kline J.A. (2006). Cardiac inflammation contributes to right ventricular dysfunction following experimental pulmonary embolism in rats. J. Mol. Cell. Cardiol. 41, 296-307.

Watts J.A., Gellar M.A., Obraztsova M., Kline J.A. and Zagorski J. (2008). Role of inflammation in right ventricular damage and repair following experimental pulmonary embolism in rats. Int. J. Exp. Pathol. 89, 389-399.

Watts J.A., Gellar M.A., Stuart L., Obraztsova M. and Kline J.A. (2009). Proinflammatory events in right ventricular damage during pulmonary embolism: Effects of treatment with Ketorolac in rats. J. Cardiovasc. Pharmacol. 54, 252.

Watts J.A., Marchick M.R. and Kline J.A. (2010). Right ventricular heart failure from pulmonary embolism: key distinctions from chronic pulmonary hypertension. J. Card. Fail. 16, 250-259.

Zagorski J., Debelak J., Gellar M., Watts J.A. and Kline J.A. (2003). Chemokines accumulate in the lungs of rats with severe pulmonary embolism induced by polystyrene microspheres. J. Immunol. 171, 5529-5536.

Zagorski J., Gellar M.A., Obraztsova M., Kline J.A. and Watts J.A. (2007). Inhibition of CINC-1 decreases right ventricular damage caused by experimental pulmonary embolism in rats. J. Immunol. 179, 7820-7826.

Zagorski J., Obraztsova M., Gellar M.A., Kline J.A. and Watts J.A. (2009). Transcriptional changes in right ventricular tissues are enriched in the outflow tract compared with the apex during chronic pulmonary embolism in rats. Physiol. Genomics 39, 61-71.

Zagorski J., Sanapareddy N., Gellar M.A., Kline J.A. and Watts J.A. (2008). Transcriptional profile of right ventricular tissue during acute pulmonary embolism in rats. Physiol. Genomics 34, 101-111.

Zhang R., Wu Y., Zhao M., Liu C., Zhou L., Shen S., Liao S., Yang K., Li Q. and Wan H. (2009). Role of HIF-1alpha in the regulation ACE and ACE2 expression in hypoxic human pulmonary artery smooth muscle cells. Am. J. Physiol. Lung Cell. Mol. Physiol. 297, L631-L640.

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