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Preclinical evaluation of a novel dual acting V1a/V2 vasopressin receptor antagonist by using a noninvasive cardiac output monitor

T Thomas Mondritzki[1]; P Boehme[2]; P Sandner[1]; W Dinh[2]; J Hueser[1]; H Truebel[2]; P Kolkhof[1]
[1]Bayer AG, Cardiovascular Research, Wuppertal, Germany; [2]Bayer AG, Experimental Medicine, Wuppertal, Germany

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Background/Introduction: Elevated levels of arginine-vasopressin (AVP) may mediate deleterious effects via renal V2 and vascular V1a AVP receptors. While the only outcome trial in HF with a selective V2 receptor antagonist (EVEREST-trial) showed no mortality benefit, a respective trial in HF patients with a dual V1a/V2 receptor antagonist has not been conducted so far. Preclinical studies suggest beneficial aquaretic and hemodynamic effects of a dual acting V1a/V2 antagonist. However, there is a lack of predictive diagnostic readouts used in experimental models in view of transferability in a clinical scenario.
Purpose: Noninvasive cardiac output monitoring with bioreactance can be used to identify and differentiate the hemodynamic effects of a dual acting V1a/V2 antagonist compared to a selective V2 antagonism avoiding hemodynamic changes induced by anesthesia which would be required for invasive assessment.
Methods: Healthy beagle dogs (n = 9) were instrumented with telemetric sensors for hemodynamics assessment. Additional surface sensors were attached for noninvasive cardiac output monitoring (CHEETAH NICOM™, USA) and bladder catheter were placed for investigations diuretic effects. We compared the hemodynamic effects of a novel, dual acting V1a/V2 vasopressin receptor antagonist, BR-6819 with the selective V2 antagonist tolvaptan during intravenous AVP challenge in conscious animals.
Results: BR-6819 (3 mg/kg) induced a significant increase of 0.26 l/min ($p = 0.029$ vs. placebo) in cardiac output (CO) and 0.58 (l/min)/m2 ($p = 0.018$ vs. placebo) in cardiac index (CI) during vasopressin challenge, whereas tolvaptan was without any significant effect, respectively. In addition, BR-6819 treatment significantly reduced (-36.4%, $p = 0.015$) total peripheral resistance vs. placebo. BR-6819 and tolvaptan significantly ($p < 0.05$) increased urinary volume.
Conclusion: This is the first study demonstrating the feasibility of noninvasive cardiac output monitoring to differentiate between V1a/V2 and selective V2 antagonism. Use of the dual V1a/V2 antagonist BR-6819 significantly improved CO and CI while tolvaptan was without any effect here. Noninvasive cardiac output monitoring has the potential to improve the transferability of preclinical findings to the clinics.

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Enzyme activity evaluation and tissue distribution of Neprilysin in heart failure

MC Asensio Lopez[1]; MC Sanchez Perez[1]; A Hernandez Vicente[1]; E Cabrero Romero[2]; D Fernandez Vazquez[2]; DJ Vazquez Andres[2]; MT Perez Martinez[1]; E Fernandez[1]; DA Pascual Figal[2]; AM Lax[1]
[1]Biosanitary Research Institute of Murcia (IMIB-Arrixaca)., Cardiology, Murcia, Spain; [2]University Hospital Virgen de la Arrixaca, Department of Cardiology, Murcia, Spain

Background: Neprilysin is a ubiquitous enzyme involved in degradation of numerous vasoactive peptides, notably including natriuretic peptides (NP). Despite recent data indicate a substantial mortality reduction associated with use of neprilysin inhibition in patients with reduced ejection fraction, had no data about enzymatic activity and tissue distribution.
Purpose: To evaluated the enzymatic activity and tissue distribution of neprilysin in presence of myocardial infarction and heart failure.
Methods. Wistar rats underwent MI by left coronary artery ligation were sacrificed at 4 weeks after surgery (n = 5 per group). Sham-operated animals were also included. Frozen tissue (100 mg) was homogenized using N2 liquid in adequate buffer. Neprilysin enzyme activity was measured with a fluorometric assay for the generation of free dansyl-D-Ala-Gly (DAG) from N-dansyl-Ala-Gly-D-nitro-Phe-Gly (DAGNPG)., using 562 and 342 nm as wavelengths of emission and excitation, respectively. Unpaired t-test was used to compare neprisilin activity respect sham in each tissue and, for multiple comparisons between tissues, paired t-test with Holm-Bonferroni correction was used.
Results: Neprylisin activity exhibited a gradient between tissues, with the highest level in lungs ($p < 0.01$ for all comparisons) followed by liver ($p < 0.01$) compared with kidney and heart); whereas, kidney and myocardial tissues showed similar levels. This gradient was observed in both sham animals and infarcted animals after 4 weeks (Figure 1). Of interest, animals with myocardial infarction and heart failure showed higher levels in all non-cardiac tissues as compared with sham animals: lung (1375.8 ± 166.0 vs. 152.8 ± 15.6, $p = 0.0017$), liver (68.55 ± 2.49 vs. 12.66 ± 0.76, $p < 0.0001$), and kidney (16.23 ± 3.96 vs. 2.29 ± 0.79, $p = 0.023$). In myocardium, neprylisin activity was observed at higher levels than sham (1.61 ± 0.22) in both infarcted (29.85 ± 1.84, $p < 0.0001$) and ischemic border of left ventricle (29.0 ± 4.73, $p = 0.0044$); however no differences were observed in remote myocardium compared with sham (2.59 ± 0.76, $p = 0.28$) (Figure 1).
Conclusion: Neprilysin activity has a gradient of levels from lung, to liver and kidney/heart. In the presence of myocardial infarction and heart failure, an increase of neprylisin activity was observed in non-cardiac tissues, as well as infarcted and ischemic myocardium, but not in remote non-ischemic myocardium.

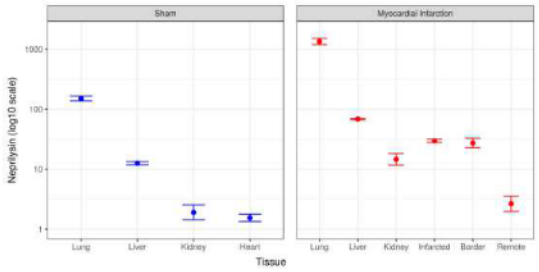

Figure 1

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Different pathophysiological mechanisms for heart failure progressions in male and female mRen2 rats

N Nikolett Olah[1]; T Csipo[1]; A Kovacs[1]; GA Fulop[1]; I Edes[1]; Z Papp[1]; A Toth[1]
[1]University of Debrecen, Division of Clinical Physiology, Institute of Cardiology, Debrecen, Hungary

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Introduction: In view of the effectiveness of renin-angiotensin-aldosteron system (RAAS) inhibitors in heart failure (HF) therapy RAAS appears to play a crucial role in the pathomechanism of HF. Transgenic rats (mREN2) harboring an extra copy of renin gene develop fulminant hypertension at an early age which progresses into HF.
Purpose: Here we studied hypothetical gender dependent differences in the pathomechanisms in the mREN2 model of HF.
Methods: Mean arterial pressure (MAP) of transgenic renin overexpressing rats (mRen2) were high in both females (138.5 ± 11.7 mmHg) and males (168 ± 5.8). Internal non-transgenic wild type rats (WT) served as controls. To reveal the mechanisms contributing to HF progression, levels of RAAS activity in isolated tissues were studied in vitro.
Results: Male rats had higher mortality till 1 year of age (survival rate: males: 23% versus females: 75%). At 1 year rats exhibited signs of mixed systolic and diastolic cardiac dysfunctions, indicating the progression of hypertension to HF (EF in females: WT: 68.28 ± 2.1 versus mRen2: 68.26 ± 2.3; males: WT: 74.7 ± 6.1 versus mRen2: 60.2 ± 4.9; E/A: in females: WT: 1.74 ± 0.03 versus mRen2 1.47 ± 0.052; males: WT: 1.56 ± 0.01 versus mRen2: 1.06 ± 0.038). In parallel, a dysregulation of the tissue RAAS was observed. In particular angiotensin converting enzyme (ACE) activity was higher in male mREN2 left ventricles (9.5 ± 0.8 U/mg) than in those of their WT littermates (5.5 ± 0.2 U/mg), while no similar differences were observed in the lungs (71 ± 21 versus 76 ± 9 U/mg) and in any of the above parameters in females. Activities of angiotensin 2 eliminating ACE2 enzymes were similar in the left ventricles, lung, kidney of WT and mRen2 animals irrespectively of gender.
Conclusions: Our work illuminated important gender differences in the progression of hypertension to HF. In particular, our data implicate that left ventricular ACE activities increase in males more than in females. This is in accordance with the higher clinical effectiveness of ACE inhibitors and the higher HF risks in males than those in females.

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Vanhoutte and Bowditch phenomena in heart failure: their relation to ischemia-reperfusion impact

L Tacu[1]; M Ivanov[2]; E Cobet[3]; A Rotaru[4]; L Ciobanu[2]; V Rotaru[1]; V Lutan[1]; IU Feghiu[1]; V Cobet[1]; M Popovici[2]
[1]State University of Medicine and Pharmacy, Chisinau, Moldova Republic of; [2]Institute of cardiology , Chisinau, Moldova Republic of; [3]State University of Moldova , Biopharmaceutical chemistry , Chisinau, Moldova Republic of; [4]Harvard Medical School, Cambridge, United States of America

Aim: Evaluation of both 15,16-epoxyeicosatrien (15,16-EET) induced coronarodilation and Bowditch's staircase in experimental heart failure (HF) as well as their

influence on ischemic contracture and functional recovery of isolated heart during repefusion.
Material and methods: HF was reproduced by doxorubicin administration in rat (16 mg/kg in 2 weeks). Vanhoutte's phenomenon was estimated by coronary flow raising rate in izovolumic isolated heart perfused by Langendorff method during action of 15,16-EET (10-4 M). Bowditch's staircase was assayed by electrically induced heart rate (HR) rise till maximal level suitable to left ventricle (LV) systolic pressure elevation (LVSP). Ischemic contracture was appreciated by LV end-diastolic pressure (LVEDP) at the end of global 20 min ischemia period followed by 30 min period of reperfusion when LVSP dynamics has been recorded. Likewise, ischemia-reperfusion impact was attested in condition of 15,16-EET action during pre-ischemia (20 min) and reperfusion as well as after maximal HR reaching.
Results: Coronarodilation effect of 15,16-EET has not been compromised in HF, because the coronary flow increased like in control comparatively to basal value (13,2 ± 1,2% vs 13,9 ± 1,1%). However, Bowditch's staircase was earlier interrupted in comparison to control according to maximal HR matching to positive slope of LVSD: 285 ± 22,6 vs 372 ± 29,4 1/min ($p < 0,05$). Maximal ischemic LVEDP was significantly higher in HR: 47,6 ± 3,3 vs 24,9 ± 1,8 mmHg ($p < 0,001$). On the other hand LVSP was at the end of reperfusion lower than control: 72,4 ± 6,5 vs 112,3 ± 7,5 mmHg ($p < 0,01$). Remarkably, 15,16-EET action before ischemia and during reperfusion notably improved dynamics of LVEDP and LVEDP in both control and HF (in the last even more evidently). Relative diminution of LVEDP measured 14,3 ± 1,4% in HF and 12,5 ± 1,2% in control, and LVESP increment: 15,1 ± 1,5% vs 13,7 ± 1,3%. If the ischemia-reperfusion onset started after frequency pacing ischemic contracture and functional LV recovery worsened similarly in both control and HF series: LVEDP increased by 19-20% while LVSP decreased by 17-18%.
Conclusions: 1. Derived (in cytochrome P450 biochemical way) from arachidonic acid 15,16-EET increases coronary flow in HF similarly to control and could be an important factor of coronary regulation by hyperpolarization mechanism in cases of endothelium dependent compromised coronary reactivity.
2. 15,16-EET mitigates ischemia-reperfusion impact in HF while HR elevation worsens ischemic contracture and LV contraction recovery in reperfusion.

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A link between oxidative stress and CaMKII activity in post-ischemic failing hearts: a subcellular analysis with pathophysiological implications

T Tomas Rajtik[1]; A Szobi[1]; M Lichy[1]; J Hrdlicka[2]; P Galis[1]; J Neckar[2]; F Kolar[2]; A Adameova[1]
[1]Comenius University, Faculty of Pharmacy, Department of Pharmacology and Toxicology, Bratislava, Slovak Republic; [2]Academy of Sciences of the Czech Republic, Institute of Physiology, Prague, Czech Republic

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Background: Heart failure (HF) is generally associated with increased oxidative stress (OS) which has been suggested to be linked with detrimental activity of Ca2+/calmodulin-dependent protein kinase II (CaMKII) and thereby dysregulation of Ca2+-homeostasis. As CaMKII is widely localized in various cellular compartments throughout the cell, oxidative damage of these particular cellular fractions can underlie the increase in kinase activity observed in HF and thereby its influence on cellular function such as excitation-contraction coupling.
Purpose: To test this hypothesis, we measured the activity of CaMKII and various forms of oxidative stress in whole cell lysates, and subcellular fractions, such as cytoplasmic (Cy), membrane (Me) and mitochondrial (Mi) in left ventricles of failing hearts and sham operated hearts.
Methods: In male Wistar rats, a ligation of left descending coronary artery was performed to induce post-ischemic HF. Immunoblot analysis was used to assess the levels and activity of CaMKII (evidenced as p-Thr286-CaMKII). Lipoprotein oxidation, protein S-glutathionylation (PSSG) and protein carbonylation (PC) were evaluated by measuring the levels of TBARS, formation of glutathionylated proteins in non-reduced samples and carbonylated proteins detected in DNPH-derivatized lysates, respectively.
Results: In the whole cell lysates of failing hearts, oxidative stress evidenced itself as higher levels of PSSG and slightly increased carbonylated proteins. In the Me fraction of HF, only TBARS were increased. Despite the unchanged levels of PSSG in Me fraction in HF, there was a positive correlation with p-Thr286-CaMKII indicating a novel link between glutathione homeostasis and the kinase activity. In addition, a negative correlation of p-Thr286-CaMKII with PC was found in this fraction suggesting the abolishment of its activity under such conditions. In the mitochondria of diseased hearts, TBARS and PC were higher and were not in line with the active form of CaMKII.
Conclusions: This study has shown that the various types of OS are selectively upregulated in particular cellular compartments of failing hearts. Moreover, we have indicated for the first time that contractile dysfunction of post-ischemic HF might be, at least in part, linked with the activity of the membrane-bound CaMKII being dependent on protein S-glutationylation and carbonylation.

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Lung diffusion capacity is positively correlated to pulmonary capillary wedge pressure in heart failure: a capillary volume effect?

T Tania Deis[1]; L Balling[1]; K Rossing[1]; E Wolsk[1]; F Gustafsson[1]
[1]Rigshospitalet - Copenhagen University Hospital, Copenhagen, Denmark

Background: Patients with heart failure (HF) are known to have a reduced pulmonary diffusion capacity for carbon monoxide (DLCO) but little is known about how hemodynamics relate to lung function. This study tested the hypothesis that pulmonary capillary wedge pressure (PCWP) is associated with alveolar volume adjusted pulmonary diffusion capacity (DLCO/VA) in patients with advanced chronic HF.
Methods: We retrospectively studied the data on 262 HF patients (mean age 51 ± 13) with a LVEF < 45 % referred non-urgently for evaluation for heart transplantation or LVAD, who underwent right heart catheterization and lung function testing. Univariate and multivariate linear regression models were constructed to examine the associations between DLCO/VA, FVC, FEV1 and hemodynamic parameters (PCWP, central venous pressure (CVP), cardiac index (CI), mean pulmonary artery pressure (MPAP) and mean arterial pressure (MAP)) as well as other factors known to affect lung function in HF.
Results: There was no significant correlation between hemodynamics and FVC or FEV1. DLCO/VA correlated positively with PCWP in both univariate ($p < 0,001$) and multivariate ($p = 0,012$) regression analysis but did not correlate to other hemodynamic factors. DLCO/VA was not a significant predictor of mortality. FVC and FEV1 were found to be significant predictors of mortality (HR for lower versus highest FEV1 and FVC tertiles 2,0 (1,4-2,9) and 1,9 (1,3-2,6)).
Conclusions: Surprisingly, PCWP was positively associated with DLCO/VA. This relation might be driven by increased lung capillary volume (Vc) in patients with lung congestion. Unlike dynamic lung parameters, DLCO did not predict mortality.

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Quantitative estimation of extravascular lung water volume and preload by dynamic 15O-H2O positron emission tomography

R Roni Nielsen[1]; J Sorensen[2]; L Tolbod[2]; AKO Alstrup[2]; P Iversen[2]; CA Fredriksen[1]; J Frokiaer[2]; HJ Harms[2]
[1]Aarhus University Hospital, Skejby, Department of Cardiology, Aarhus, Denmark; [2]Aarhus University Hospital, Department of Nuclear Medicine & PET center, Aarhus, Denmark

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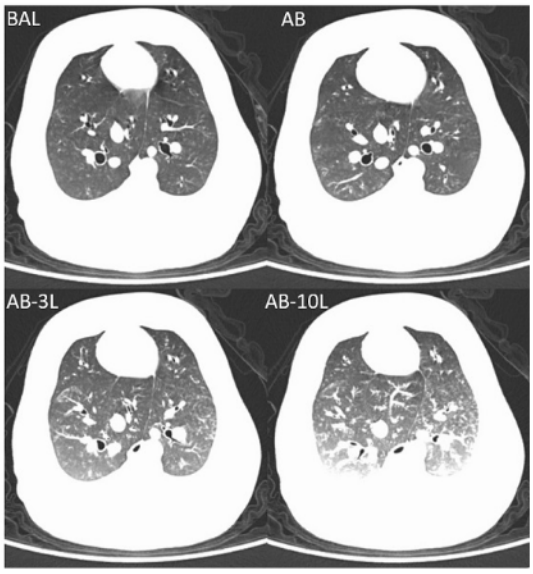
Background: Pulmonary congestion is an important finding in heart failure. It can be assessed invasively by the PICCO method using transpulmonary thermodilution to measure extravascular lung water content (EVLW) and preload by global



CT during the study of a pig