TOPIC 22 – Heart failure, cardiomyopathies – D

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Urban carbon monoxide pollution aggravates heart failure through oxidative stress
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People with heart failure (HF) are particularly sensitive to outdoor air quality as assessed by the increased risk of hospital cardiac readmission and mortality during peak pollution. Here, we explored the mechanisms underlying the increased cardiac sensitivity of HF patients following chronic exposure to carbon monoxide (CO), an ubiquitous environmental pollutant (second-hand smoke, vehicular exhaust, industrial emissions...).

Seven weeks after coronary artery ligation, rats were exposed to CO in an airtight exposure container for 4 weeks to reproduce air quality variations environmentally relevant.

We found that exposure of myocardial infarcted (MI) rats to CO exacerbated the MI-associated cardiac remodelling, resulting in increased occurrence of ventricular tachycardia and in vivo contractile dysfunction. The cellular origin for the aggravation of HF phenotype involved a remodeling of both Ca^2+ handling (Ca^2+transient) and contractile machinery properties. The β-adrenergic reserve has been investigated both in vivo and in vitro. It was altered in MI animals, as expected, but was not worsened by chronic CO exposure.

In addition the altered mitochondrial metabolism and the pre-existent oxidative stress in MI rats were more pronounced after CO exposure. Acute antioxidant treatment with N-acetylcysteine reversed some of the MI-induced and CO-induced alterations at the cellular level, therefore suggesting potential beneficial effects of antioxidant strategies. Moderate exercise training performed in standard filtered air attenuated cardiac remodelling and prevented the additional lesions induced by chronic CO exposure.

The present study shows that chronic exposure to low CO levels mimicking urban atmospheric pollution potentiates and worsens HF-associated cardiac remodelling, thus providing an explanation for epidemiological reports. Since oxidative stress is involved in the deleterious effects of CO pollution, antioxidant therapy or mild exercise could provide cellular protection against pollution effects.

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Endocardial endothelium dysfunction leads to an increased β1-adrenergic cardiac contractility through the cyclooxygenases pathway at the early endotoxic shock
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The endothelial dysfunction plays an important role in the cardiovascular alterations during the septic shock. In addition the impaired sympathetic regulation of the cardiovascular system appears early in the septic shock. The aim of this project was to determine the role of the endocardial endothelium (EE) in the cardiac β-adrenergic (β-AR) dysfunction at the early phase of septic shock.

Twelve-weeks-old Sprague Dawley rats received either 5 mg.kg⁻¹ of lipopolysaccharide (LPS) or saline (C) intravenously. 3h later, β-AR cardiac contractility was evaluated on papillary muscle with or without a functional EE. EE removal was performed with immersion in Triton X-100 at 0.5% during 3s and was validated by electronic microscopy and functional studies.

Surprisingly, isoproterenol (non-selective β-AR agonist) induced contractility was strongly increased in papillary muscle from LPS rat (+102±19% vs C; p<0.05). A similar increase was observed with a β₁-AR stimulation (+90±25% vs C; p<0.05) whereas β₂-AR and β₁-AR produced similar contractility both in C and LPS muscles. The EE removal did not modified β₁-AR-induced contractility in C whereas it abolished the increased β₁-AR response in LPS. In LPS papillary muscle, the increased β₁-AR-induced contractility was not modified by pretreatment with 1 µM L-NMMA, a NO synthase inhibitor, or 10 µM bosentan, an endothelin receptor antagonist. Conversely, the increased β₁-AR-induced contractility was abolished by 10 µM indomethacin, a non selective cyclooxygenase (COX) inhibitor, as well as by selective inhibitors of COX1 (SC560, 3 µM) and COX2 (NS398, 3 µM).

Our results demonstrate the EE involvement in the increased cardiac β₁-AR contractility at the early phase of endotoxic shock. This effect is mediated through the activation of both COX1 and COX2 which appear as new putative therapeutic targets at the early phase of septic shock.
Deletion of tenasin C decreases inflammation and attenuates pressure overload induced cardiac dysfunction

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Introduction: The extracellular matrix protein tenasin C (TnC) is highly expressed during embryogenesis. In adult tissues, TnC is not expressed under physiologic conditions but strongly induced in inflamed and remodeled tissues. Growing evidence linked TnC with development and complications of cardiovascular disease. We hypothesized that the absence of TnC would protect the heart from pressure overload induced cardiac dysfunction.

Methods: Transverse aortic constriction (TAC) or Sham operations were performed in male C57BL/6N wild type (WT) and TnC knockout (KO) mice. After 1 or 6 weeks, echocardiographic measurements of left ventricular (LV) parameters and standard methods for cardiac gene expression, collagen coloration and immunolocalization were employed.

Results: In WT mice, TAC led to re-expression and protein deposition of TnC with a peak after 1 week (6.5% of heart surface vs 0% in Sham). Echocardiographic measurements after 6 weeks of TAC exhibited significantly increased of ventricular hypertrophy in both WT+TAC and KO+TAC compared to Sham (LV posterior wall dimension WT+TAC: 0.102±0.006 vs Sham: 0.078±0.003 cm, p<0.01; KO+TAC: 0.092±0.002 vs Sham: 0.072±0.005 cm, p<0.5). However, cardiac dilation induced by TAC was observed in WT but not in KO (LV internal diastolic dimension; WT+TAC: 0.487±0.012 vs Sham: 0.375±0.009 cm, p<0.001; KO+TAC 0.430±0.011 vs Sham 0.407±0.011). Myocardial contractility was also less reduced in KO+TAC than in WT+TAC (fractional shortening 23.4±1.5 vs 13.9±2. %, p<0.001). In parallel, collagen deposition was lower in KO+TAC than in WT+TAC. Strikingly, up regulation of inflammatory cytokines genes induced by TAC in WT was completely abolished in KO. Heart deposition of TnC was clearly colocalized with area enriched in leukocyte cell marker and the deletion of TnC prevented macrophage recruitment induced by TAC.

Conclusion: The absence of TnC attenuates the development of heart failure through possibly down regulation of inflammatory response.

Role of Neutrophil Gelatinase Associated Lipocalin (NGAL) in cardiovascular remodeling induced by aldosterone

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Background: Neutrophil Gelatinase Associated Lipocalin (NGAL) is a circulating protein, member of the lipocalin family, which binds MMP9 and modulates its stability and activity. We have recently shown that NGAL is a primary target of aldosterone/mineralocorticoid receptor (MR) in endothelial cells, vascular smooth muscle cells and cardiomyocytes.

Objective: We hypothesized that NGAL could be a mediator of aldosterone/MR profibrotic and proinflammatory effects in the cardiovascular system.

Methods: Wild type (WT) and NGAL Knock Out (KO) mice were subjected to an uni-nephrectomy aldosterone salt challenge (NAS, 200 μg/kg/day of ald, 1% NaCl in tap water) for 4 weeks. Blood pressure (SBP) was measured by tail cuff method. Vascular reactivity was assessed by wire myograph. Cardiovascular fibrosis and inflammation were analyzed by RT-PCR, western blot, immunohistochemistry and ELISA.

Results: There was no difference in SBP between transgenic mice compared to WT mice in basal condition. With NAS challenge, SBP was increased only in WT. Ex vivo aortic contraction induced by phenylephrine (Phe) and potassium chloride (KCl) were increased by NAS challenge in WT and KO. Responses to vasodilators (acetylcholine and sodium nitroprussiate) were altered in NAS condition in both groups. Quantification of pro collagen I-terminal peptide (PINP) in plasma showed an increase of PINP due to NAS treatment in WT that was prevented by NGAL inactivation. In myocardium, NAS treatment increased collagen type I and perivascular fibrosis in WT whereas KO were resistant to fibrosis. In aorta, collagen type I, vascular fibrosis and osteopontin were also increased by NAS in WT. These increases were prevented by NGAL inactivation.

Conclusion: Our results show that NGAL plays a role in aldosterone/MR-mediated vascular fibrosis and inflammation, but not in cardiac interstitial fibrosis and vascular dysfunction. We suggest that NGAL could be a new biotarget in cardiovascular fibrosis.
High risk of severe cardiac adverse events in patients with mitochondrial m.3243A>G MELAS mutation

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Background: To determine the long-term incidence of cardiac life-threatening complications and death in patients with the m.3243A>G mutation, and to identify cardiac prognostic factors.

Methods: We retrospectively included patients carrying the m.3243A>G mutation who were admitted to the Neuromuscular Disease Clinic of Pitié Salpêtrière Hospital between January, 1992 and December, 2010. We collected information relative to their yearly neurological and cardiac investigations, their mutation load in blood, urine and muscle at initial admission, and the occurrence of cardiac life-threatening adverse events and death during follow-up.

Results: Forty-one patients (median age=47 years [36-55], men=13) were included, of whom 38 had clinical manifestations of MELAS and 3 were asymptomatic. One patient had a personal history of cardiac transplantation. Cardiac investigations displayed left ventricular hypertrophy, left ventricular dysfunction, or both abnormalities in 18 patients, along with Wolff Parkinson White syndrome in 7, conduction system disease in 4, and atrial fibrillation in 1. Over a median 5-year [3-9] follow-up period, 11 patients died, including 3 due to heart failure; 7 had life-threatening adverse events, including 6 hospitalizations for severe heart failure and 1 resuscitated cardiac arrest. By multivariate analysis, left ventricular hypertrophy was the only parameter independently associated with occurrence of cardiac adverse events.

Conclusion: Patients with the m.3243A>G mutation have a high incidence of cardiac death and life-threatening adverse events. Left ventricular hypertrophy was the only parameter independently associated with occurrence of these events.

Deletion of the mineralocorticoid receptor in vascular smooth muscle cells improves coronary and cardiac function in mice with heart failure

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MR deletion in cardiomyocytes improves cardiac remodeling and function in CHF, however the specific role of vascular MR in CHF remains unknown. Thus, the vascular and cardiac consequences of MR deletion specifically in vascular smooth muscle cells (VS MCMR/-/-) were studied in mice with CHF (2months coronary artery ligation).

Invasive left ventricular pressure-volume curves showed that VS MCMR/-/- CHF mice had increased LV end-systolic pressure-volume relationships (elastance; $\text{ctrl } 11.9\pm0.5 \text{ mNmm}^{-1} \text{L}^{-1}; \text{VS MCMR}/-/- 19.9\pm2.5 \text{ mNmm}^{-1} \text{L}^{-1}, p<0.05$) and decreased LV end-diastolic pressure-volume relationships (compliance; $\text{ctrl } 4.08\pm0.45; \text{VS MCMR}/-/- 2.16\pm0.33, p<0.01$), in the absence of changes in cardiac fibrosis (% collagen density ctrl $1.23\pm0.33$, n=5; VS MCMR/-/- 1.40\pm0.31, n=4, ns).

Coronary endothelial dysfunction was assessed ex vivo testing the NO-mediated response to acetylcholine. In sham-operated animals, no difference between VS MCMR/-/- mice and littermate controls were observed (maximal relaxation $\text{ctrl } 91\pm1, n=5; \text{VS MCMR}/-/- 83\pm2, n=4, ns$). CHF was associated with reduced response to Ach, indicating endothelial dysfunction, which was less severe in VS MCMR/-/- mice ($\text{ctrl } 30\pm3, n=4; \text{VS MCMR}/-/- 54\pm2, n=4, p<0.01$).

Finally, coronary reserve was measured by MRI as the difference between basal and maximal LV perfusion obtained after stimulation by the A2a adenosinergic agonist ATL307. In sham-operated mice, MR deletion in VS MC resulted in a higher coronary reserve, ($\text{ctrl } 3.4\pm0.9, n=4; \text{VS MCMR}/-/- 6.7\pm1.4 \text{ mLmin}^{-1}\text{g}^{-1}$, n=4, $p=0.10$). In CHF mice, VS MC MR deficiency was associated with an increased basal coronary perfusion ($\text{ctrl } 10.5\pm0.41; \text{VS MCMR}/-/- 12.0\pm0.5 \text{ mLmin}^{-1}\text{g}^{-1}$, p<0.05), probably responsible for the observed decrease in coronary reserve ($\text{ctrl } 4.5\pm0.7, n=12; \text{VS MCMR}/-/- 1.5\pm0.6 \text{ mLmin}^{-1}\text{g}^{-1}$, n=9, p<0.01).

These results suggest that MR deletion in VS MC improves coronary endothelial function and cardiac perfusion, and this is associated with reduced cardiac dysfunction in this model of CHF.

Endothelial Protein Tyrosine Phosphatase 1B deficiency reduces both endothelial and cardiac dysfunction in a mouse model of heart failure

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Chronic heart failure (CHF) induces endothelial dysfunction, however the direct link between this dysfunction and aggravation of CHF is not demonstrated. We previously described a new treatment of CHF, based on inhibition of protein tyrosine phosphatase 1B (PTP1B), which both increases NO production (via restored PI3K/Akt/ENOS signaling) and reduces adverse Left Venricular (LV) remodeling and LV dysfunction. To address the direct link between endothelial protection and reduction of CHF, we evaluated in mouse CHF (left coronary ligation) the effects of endothelial PTP1B deficiency (endoPTP1B/-/- mice, obtained by crossing LOX-P PTP1B mice with mice expressing CRE under the control of the endothelial promoter Tie2).

Wild type (WT) CHF mice showed markedly impaired flow-mediated, NO-dependent dilatation of isolated mesenteric arteries (sham: $40\pm4\%$; CHF: $5\pm5\%; p<0.01$), which was improved in endoPTP1B/-/- mice (30\%; p<0.01 vs WT CHF).

WT CHF mice had increased LV diameters and decreased LV fractional shortening (at 3 months: sham: 49\%; CHF: 12\%; p<0.01), which were improved in endoPTP1B/-/- mice (20\%; p<0.01). LV pressure-volume curves showed that endoPTP1B/-/- mice with CHF had reduced LV end-systolic (sham: 22.5±1.2%; CHF WT: 14.9±1.9%, p<0.05) and increased end-diastolic pressure-volume relationships (sham: 2.1±0.9%; CHF WT: 4.9±0.7%, p<0.05) demonstrating impaired systolic and diastolic dysfunction, respectively, which were both improved in endoPTP1B/-/- mice (end-systolic: 20.6±2.3%; p<0.05; end-diastolic: 2.3±0.9%, p<0.05). Histological analysis showed cardiomyocyte hypertrophy and increased collagen density in WT CHF, which were reduced in endoPTP1B/-/- mice, at identical infarct size.

Thus, endoPTP1B deficiency not only induced an improvement of endothelial function, but also improved cardiac function and reduced adverse LV remodeling. These results provide a direct demonstration of the beneficial effect of endothelial protection in the treatment of CHF.