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A novel alternative spliced variant of the brain natriuretic peptide gene is superior to BNP as a molecular marker of ventricular dysfunction

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Background and Aim: Two mRNA isoforms of BNP (i.e., immature and mature transcripts) were found to be expressed in both normal and diseased porcine heart. Given that there were indications suggesting that heart failure (HF) may be associated with production of various isoforms of BNP, we searched for novel variants of alternative splicing of BNP in the porcine model of HF.
Methods: HF was induced in 6-day-old neonatal piglets by a single i.v. injection of 2 mg/kg of Doxorubicin (Dox). To profile the cardiac gene expression changes associated with Dox-induced HF, a non-radioactive mRNA differential display (DDRT-PCR) was used. A novel alternative spliced variant of the BNP gene (designated ΔE2-BNP) was identified, characterized, cloned and expressed. ΔE2-BNP mRNA levels were determined by semi-quantitative and qRT-PCR.
Results: On day 24 after Dox-administration, experimented piglets developed the features of diastolic HF. Using DDRT-PCR, the band corresponding to the ΔE2-BNP was identified as being overexpressed in failing versus normal piglet myocardium. The sequence of this band displayed a 100% homology with exon 1 and exon 3 sequences of BNP. Further RT-PCR amplification of BNP (through exons 1-3) from the failing piglet myocardium cDNA produced three products, two of which were identical to the reported BNP immature and mature transcripts, whereas the third one was characterized by the precise deletion of the exon 2. Skipping of exon 2 (ΔE2) causes a frame-shift from the beginning of exon 3, generating a coding sequence for a new protein with no C-terminal homology to known natriuretic peptides. In newborn piglets (n=5), the ΔE2-BNP mRNA was 15 fold more abundant in the left ventricle (LV) than in the right ventricle (RV). During development, the ΔE2-BNP gene expression is rapidly downregulated in both ventricles being, at postnatal day 30, 10-20-fold lower as compared to newborn piglets. By contrast, in 30-day-old piglets with Dox-induced HF (n=12) the ΔE2-BNP mRNA levels were found to be consistently and significantly augmented in both ventricles being much more up-regulated in the RV (40±2.6 fold) than in the LV (10±1.6 fold) as compared with age-matched controls. The average fold-increase of the BNP mRNA content in the failing porcine RV and LV was only 6.3±0.5 and 5.1±0.3, respectively.
Conclusion: As such, variation in ΔE2-BNP mRNA transcript abundance within the ventricular wall can be used to diagnose heart conditions in large-animal models of HF. In addition, the ΔE2-BNP seems to be superior to BNP as a molecular marker for HF.

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Overexpression of the transcription factor Hand1 causes sudden cardiac death in mice

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The re-expression of fetal isoforms of contractile proteins, ion channels and transcription factors in failing cardiomyocytes is well recognised. The contributions of these molecules to the complex heart failure phenotype is less understood.
Hand1 is a bHLH transcription factor that has been implicated in control of cardiac morphogenesis. Hand1 RNA expression persists at low levels in the adult heart, and there is evidence that Hand1 transcription is upregulated towards fetal levels in some adult cardiac diseases. It is unclear whether this re-expression is adaptive or maladaptive with respect to cardiac function. We therefore sought to investigate the effects of overexpression of Hand1 in the adult mouse heart. To allow this we developed a novel cardiac-specific inducible transgenic system.
We show that overexpression of Hand1 in the adult mouse heart leads to mild cardiac hypertrophy and sudden death, accompanied by metabolic derangements characteristic of heart failure without large-scale changes in expression of cardiac markers. Hand1 overexpression leads to altered surface ECG morphology and cardiac electrophysiology, with a prolonged QTc and greatly reduced threshold for ventricular tachycardia compared with controls. Over the same time period of induction, Hand1 overexpression results in a dramatic loss of connexin43 from the cardiac intercalated discs, despite no change in connexin43 RNA levels, and upregulation of β-catenin at RNA and protein levels, both changes seen inheart failure/cardiomyopathy, and potentially the molecular substrate for ventricular arrhythmias in heart failure.
We believe that the Hand1 cardiac overexpression mouse will be useful in the study of heart-failure-associated arrhythmias. Furthermore, we hypothesise that HAND1 upregulation observed in human heart failure and some cardiomyopathies is directly maladaptive, driving remodelling of the cardiac intercalated disc and providing the substrate for cardiac arrhythmias. Consistent with this, elevated HAND1 levels are detected in biopsies from human patients in heart failure and their recovery after LVAD treatment is associated with a decline in HAND1 expression.

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Chronic adenosine receptor blockade with BG9928 attenuates cardiac and renal histopathology and improves diabetes in animals with the metabolic syndrome

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Purpose: In animals and patients with left ventricular dysfunction, short-term A1 adenosine receptor blockade causes diuresis/natriuresis without altering potassium excretion and, in contrast to loop diuretics, improves renal function. Blockade of the A2b adenosine receptor has an anti-diabetic effect. The purpose of this study was to examine the long-term efficacy and safety of BG9928, a novel, orally active, and highly potent A1 adenosine receptor antagonist with moderate activity at the A2b receptor, in an animal system designed to model the complex pathology that characterizes, with increasing frequency, the modern cardiac patient.
Methods: The ZSF1 rat is a model of the metabolic syndrome that expresses obesity, hypertension, type 2 diabetes, dyslipidemia, dilated cardiomyopathy, and severe nephropathy. BG9928 (10 mg/kg/day), captopril (100 mg/kg/day, orally), and furosemide (50 mg/kg/day, orally), alone or in combination, were administered for 24 weeks to ZSF1 rats (9 or 10 per group). An untreated control group was included.
Results: BG9928 reduced urinary glucose excretion (from 823±179 to 196±80 mg/kg/day; P=0.004) and attenuated captopril-induced worsening of the oral glucose tolerance test as well as captopril-induced increases in fasting plasma glucose and insulin levels. Chronic captopril doubled the urinary excretion rate ($P<0.001$) and the renal interstitial levels (P=0.008) of adenosine. Compared with control animals, BG9928 blocked the age-related increase in plasma triglycerides and significantly reduced focal segmental glomerulosclerosis and cardiac vasculitis, degenerative ischemic changes, and necrosis ($P<0.05$). At 12 weeks of treatment, BG9928 increased captopril- and furosemide-induced renin release, but this effect was lost by 24 weeks into treatment.
Conclusion: In heart and renal disease complicated by the metabolic syndrome, chronic administration of BG9928 was found to be safe and may improve type 2 diabetes, lower plasma triglycerides, and attenuate

renal and cardiac histopathology by inhibition of both A1 and A2b adenosine receptors.

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Controlled reperfusion with adiponectin in porcine myocardial infarction model

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Background: Despite the recent progress in inteventional and pharmacological treatment of myocardial infarction, a reperfusion injury remains an important limitation of the therapy.
Aim: The aim of the study was to evaluate the influence of intracoronary injection of adiponectin on reperfusion injury and cardiomyocyte death in porcine myocardial infarction model.
Methods: The experiment was conducted with the consent of the Institutional Animal Review Committee of a Medical School. An acute infarction in 14 Polish domestic pigs was induced by inflation of a balloon catheter in medial Left Anterior Descending artery for 60 minutes. The animals were randomized into two groups. A control group consisted of 7 pigs, which was given intracoronary infusion of 5 ml saline (placebo) through "over the wire" catheter before and after reperfusion. The study group (n=7) was given 5 ml of adiponectin (10μg/1ml) in the same way. A control angiography and ventriculography was performed before and after ischaemia. Animals were sacrificed after two day follow-up. The infarct size area (ISA) was evaluated with 2,3,5-triphenyltetrazolium (TTC) staining and the area at risk (AAR) with Evans Blue staining. The size of infarct was presented as a percentage of a whole myocardium. Serial CPK-MB, Troponin I and biochemical inflamation markers measurements additionally were carried out.
Results: The pigs in both groups were comparable with regard to sex and weight. Hearts in each group had similar AAR (46,3±9,9% vs. 48,4±6,2% p=ns). The average infarct size was 20,5±5,6% vs. 11,7±4,9% in the control and study group respectively (p=0,01). The results of other biochemical data are pending and will be available during abstract presentation.
Conclusions: The administration of adiponectin into infarct related artery is safe and feasible. The treatment significantly reduced the infarct size.

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effect of pravastatin on left ventricular remodeling and endothelin-1 in pacing-induced canine of heart failure

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Purpose: To investigate the remodeling of left ventricular and ET-1 in serum and myocardium in the heart failure model of canine by high-speed pacing, and the effect of pravavstatin on left ventricular remodeling and endothelin.
Methods: 15 canines were divided into three groups. Group I comprised normal canines. Group II included canines with high-speed pacing for 4 weeks. Canines in group III accepted pravastatins (1.5mg/kg/d) as well as high-speed pacing for 4 weeks. UCG, serum and local ET-1 in myocardium were measured before and 4 weeks after the operation.
Results: Left ventricular end-diastolic diameter, end-systolic diameter, end-diastolic volume enlarged 4 weeks after pacing in control group (3.45±0.39cm vs. 2.76±0.24cm, p=0.006;2.21±0.30cm vs. 1.77±0.17cm, p=0.009;44.5±4.80ml vs. 29.00±7.09ml, p=0.003), left ventricular ejection fraction lowered greatly (0.38±0.24 vs. 0.67±0.08, p=0.011), cardiomyocytes enlarged with irregular range, lymphotytes and inflammatory cells and fibrosis intercellarly. In that with pravastatins, only left ventricular end-diastolic diameter enlarge little (3.02±0.10cm vs. 2.85±0.17cm, p=0.034),with little cardiomyocytes enlargment and irregular range, almost no lymphotytes and inflammatory cells and fibrosis intercellarly. ET-1 in both serum and myocardium enhanced significant in controls (6.54±0.95pg/ml vs 3.42±0.36 pg/ml, p=0.002; 324.75±9.86pg/ml vs. 136.14±7.53 pg/g, p=0.001), but only a little in those with pravastatins (4.02±0.54pg/ml vs. 3.54±0.76pg/ml, p=0.067; 198.17±5.34pg/ml vs. 142.95±8.46 pg/g, p=0.052).
Conclusion: Pravastatin can prevent left ventricular remodeling in heart failure model of canine by high-speed pacing. Pravastatin can decrease ET-1 in serum and myocardium of canine with heart failure.

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Effects of in vivo neuregulin-1/ErbB activation on diabetes-induced myocardial dysfunction in the rat

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Purpose: Neuregulin-1 (NRG-1) is a paracrine growth factor released by myocardial microvascular endothelial cells which activates ErbB4 and ErbB2 receptors on cardiomyocytes. Endothelium-derived NRG-1 promotes hypertrophy of cardiomyocytes, affects myocardial contractility through nitric oxide, and protects against stress-induced apoptosis in vitro. In vivo, therapy with recombinant NRG-1 (rNRG-1) improves ventricular function and survival of rats with ischemic and anthracycline-induced cardiomyopathy. In this study, we hypothesized that therapy with rNRG-1 would protect against diabetes-induced myocardial dysfunction.
Methods: Diabetes was induced in male SD rats (n=10) by injection of streptozotocin (45 mg/kg, i.v.). Five additional rats were injected with vehicle and served as non-diabetic control rats. A subgroup of diabetic rats (n=5) received NRG-1 daily (10 μg/kg, by intraperitoneal injection), starting 5 days before the induction of diabetes and then for an additional 2 weeks, until sacrifice. Global left ventricular (LV) function and LV mass were measured by 2D and M-mode transthoracic echocardiography. After sacrifice, myocardial mechanical twitch performance was studied in isolated right ventricular papillary muscles (n=29).
Results: The presence of diabetes did not affect LV fractional shortening, but significantly reduced LV mass (209±26 mg vs. 363±42 mg in non-diabetic rats, $p<0.01$, n=5). Diabetes induced a delayed onset of isometric twitch relaxation (101±2 ms vs. 84±3 ms, $p<0.001$) and a marked prolongation of time to 50% relaxation (75±5 ms vs. 51±5 ms, $p<0.001$) as compared to non-diabetic rats ($p<0.001$).
Intraperitoneal injection of rNRG-1 induced rapid and sustained phosphorylation of myocardial ErbB2 and ErbB4 receptors and led to activation of downstream signalling molecules Akt-1 and ERK1/2. However, this treatment did neither reverse diabetes-induced reduction in LV mass (204±18mg, $p>0.05$ vs. untreated diabetic, n=5) nor the delayed onset (100±3 ms) and prolongation (68±6 ms) of isometric twitch relaxation ($p>0.05$ vs. untreated diabetic).
Conclusions: Diabetes induces a marked dysfunction of myocardial twitch performance, and more specifically a delayed onset and slowing of isometric relaxation. Although rNRG-1, when administered intraperitoneally to diabetic rats, stimulates ErbB receptor signalling in the myocardium and has, in previous studies, protected against various forms of cardiomyopathy, this treatment does not prevent the development of diabetes-induced myocardial dysfunction.

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Bisoprolol in experimental cancer cachexia

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Background: Cachexia is a common co-morbidity in cancer patients (up to 80% depending on the tumour type), which drastically reduces quality of life and survival. In chronic heart failure, beta-blockers have been shown to reduce the onset of cachexia and induce weight gain (mainly fat mass).