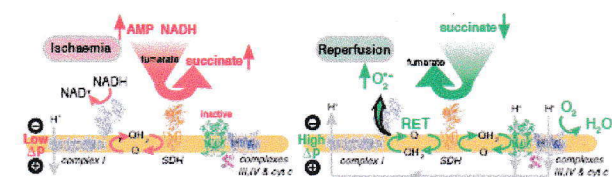


Methods & Results: We applied a comparative metabolomics method in five in vivo tissue models to identify metabolic signatures that were both distinct to ischaemia and common across many tissue types. We unexpectedly identified selective accumulation of the citric acid cycle intermediate succinate as a universal metabolic signature of ischaemia in a range of tissues which is responsible for mtROS production during reperfusion. We show ischaemic succinate accumulation arises from reversal of succinate dehydrogenase (SDH). Upon reperfusion, the accumulated succinate is rapidly re-oxidised by SDH, driving extensive ROS generation by reverse electron transport at mitochondrial complex I. Decreasing ischaemic succinate accumulation by pharmacological inhibition is sufficient to ameliorate in vivo IR injury in murine models of heart attack and stroke.

Conclusions: We have identified a conserved metabolic response of tissues to ischaemia and reperfusion that reveals a novel pathway for metabolic control of ROS production in vivo, while demonstrating that inhibition of ischaemic succinate accumulation and its oxidation upon subsequent reperfusion is a potential therapeutic target to decrease IR injury in a range of pathologies.



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The soluble guanylate cyclase activator cinaciguat inhibits pressure overload-induced cardiac hypertrophy

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Purpose: Pathological cardiac hypertrophy is observed in pressure overload of the left ventricle. Elevated intracellular cGMP-levels have been reported to prevent the development of pathological myocardial hypertrophy. We investigated the effects of chronic activation of the cGMP producing enzyme, soluble guanylate cyclase (sGC) by cinaciguat in a rat model of pressure overload-induced cardiac hypertrophy.

Methods: We performed aortic banding (AB) to evoke pressure overload-induced cardiac hypertrophy in our rats. Sham operated animals served as controls. Experimental and control groups were treated with 10 mg/kg/day cinaciguat (Cin) or placebo (Co) p.o., respectively. Development of cardiac hypertrophy was investigated by echocardiography. We performed left ventricular (LV) pressure-volume analysis with a pressure-conductance microcatheter to assess cardiac function. In addition to our functional experiments, histological and molecular biological measurements were carried out.

Results: Echocardiography showed marked myocardial hypertrophy in the AB-Co group following 6 weeks of pressure overload of the heart (left ventricular mass index [LVMI]: 3.15 ± 0.09 AB-Co vs. 2.09 ± 0.05 g/bwkg Sham-Co) which was verified by post mortem investigation of the hearts (heart weight/tibial length ratio [HW/TL]: 38.4 ± 1.5 AB-Co vs. 29.3 ± 0.8 mg/mm Sham-Co) and by histology (cardiomyocyte diameter [CD]: 17.37 ± 0.04 AB-Co vs. 14.55 ± 0.12 μm Sham-Co). Increased left ventricular dimensions (left ventricular end-diastolic volume: 414 ± 19 AB-Co vs. 341 ± 19 μl Sham-Co) were observed while ejection fraction and fractional shortening remained unchanged. Cinaciguat did not alter blood pressure (183 ± 7 AB-Co vs. 177 ± 5 mmHg AB-Cin, $p = n.s.$) but effectively attenuated left ventricular hypertrophy (LVMI: 2.57 ± 0.06 g/bwkg, HW/TL: 33.5 ± 0.9 mg/mm, CD: 15.08 ± 0.10 μm, $p < 0.05$ vs. AB-Co), and normalized the maladaptive increase in contractility (end-systolic pressure-volume relationship [ESPVR]: 1.000 ± 0.057 Sham-Co, 1.232 ± 0.049 AB-Co vs. 0.969 ± 0.073 mmHg/ml AB-Cin, $p < 0.05$) following AB.

Conclusions: Our results demonstrate that chronic stimulation of the NO-cGMP signaling pathway by pharmacological activation of the soluble guanylate cyclase might be a novel therapeutic approach in the prevention of pathological myocardial hypertrophy.

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Development of therapeutic and preventative strategies for takotsubo syndrome

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Takotsubo syndrome (TTS) is an acute heart failure characterised by a hypokinetic apical myocardium coupled to a hyperkinetic basal myocardium. First reported in 1990, little is known about its pathophysiology, and even less about possible treatments. We investigated possible mitigating and exacerbating factors for TTS, either as pre-treatments or post-treatments. Those found to be useful as a pre-treatment could guide research into preventing recurrent TTS; therapies that reduce the heart failure once established can guide research into alternatives to the current treatment for TTS that causes cardiogenic shock (i.e. ECMO). Previously we have reported that levosimendan can reduce apical dysfunction, as can PTX pre-treatment.

We used two rat models of TTS: epinephrine (4.3×10^{-7} mol/kg) IV bolus (isoflurane anaesthesia) to produce apical hypokinesia within 20 minutes that resolves within 60 minutes; and isoprenaline (50mg/kg) IP injection (ketamine/midazolam anaesthesia) that produces apical hypokinesia within 90 minutes and resolves over a matter of days. We have investigated the effects of sex, ovariectomy (along with hormone replacement) and anaesthesia on TTS.

Epinephrine model: compared to males, females had a significantly reduced mortality rate (6.25% vs. 46%, $p < 0.01$) and did not show apical dysfunction; ovariectomised rats had a very high mortality rate (75%), both through acute VF and cardiac failure. 2-week estradiol therapy (238ug/day) significantly reduced mortality (10%, $p < 0.001$). Isoprenaline model: we found that anaesthesia with isoflurane instead of ket/mid completely inhibited apical dysfunction, which was ordinarily very reproducible under ket/mid anaesthesia. Treatment with isoflurane after induction of TTS resulted in some mortality through acute cardiac decompensation. A subset of rats were injected with isoprenaline while conscious and imaged 3 hours later: these showed a reduced level of apical dysfunction.

Our data agrees with published data that isoflurane pretreatment can prevent apical dysfunction: this did not translate into isoflurane being a potential therapy as it worsened the heart failure. Further research is needed to establish why this occurs and we caution the use of isoflurane in acute TTS patients. Ketamine/midazolam anaesthesia appears to potentiate TTS induction, which may allude to the induction pathway: We hypothesise that ketamine could potentiate the effect of the exogenous catecholamine. Estrogen, lost in post-menopausal women (the primary TTS patient), and isoflurane may play a critical role in dampening the initial response to catecholamines.

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Modulation of survivin reverses cardiac and pulmonary remodeling in monocrotaline-induced pulmonary arterial hypertension

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Purpose: Cardiac and lung expression of the inhibitor of apoptosis survivin and its inhibitor smac was characterized in the progression of monocrotaline(MCT)-induced pulmonary arterial hypertension(PAH). The effects of survivin antagonist terameprocol(TMP) were assessed, both in vitro and in vivo.

Methods I: adult male Wistar rats received a subcutaneous injection of MCT(60mg/Kg) or equal volume of vehicle. On days 1,3,7,14 and 21 after injection ($n = 7-12$), right ventricular(RV) pressures were measured, heart and lungs were weighted and collected for histological analysis, immunohistochemistry and western blot. II: adult male Wistar rats injected with MCT were treated with TMP (166 mg/Kg,ip;MCT-TMP, $n = 7$) or vehicle (MCT-V, $n = 9$) on days 7, 12 and 17 after injection and compared with a SHAM group ($n = 7$). On day 21, RV pressure and cardiac output were measured and heart and lungs were weighted and collected for histology. III: Primary cultures of pulmonary artery smooth muscle cells(PASMC) were obtained from sham and MCT rats (day 21) in order to assess the effects of TMP in proliferation and apoptosis.

Results: Survivin upregulation and smac downregulation were present since day 7 after MCT injection and preceded the hemodynamic manifestations of PAH. Increases in RV systolic pressure, dP/dtmax and dP/dtmin were only present since day 14. In vivo, TMP treatment reduced RV hypertrophy and pulmonary arterial wall thickness, decreased RV systolic pressure, dP/dtmax and dP/dtmin and normalized cardiac output. TMP inhibited proliferation and induced apoptosis of PASMC in a dose-dependent manner (Fig 1).

Conclusions: Our findings suggest that survivin has a role in PAH. TMP could be an effective and selective therapeutic strategy for PAH by reversing cardiac and pulmonary remodeling and improving hemodynamic features.