Upon reperfusion of occluded arteries, deleterious cellular mediators particularly located at the mitochondria level can be activated thus limiting the outcome in patients. This may lead to so-called ischemia/reperfusion (I/R) injury. Calpains are cysteine proteases and mediators of caspase-independent cell death. They emerge as central transmitters of cellular injury in several cardiac pathologies e.g. hypertrophy and acute I/R injury. Here we investigated the role of cardiac calpains in acute I/R in relation to mitochondrial integrity and whether calpains can be effectively inhibited by posttranslational modification by S-nitrosation.
Purpose: To investigate the involvement of calpains in myocardial I/R injury.
Methods and results: Following in vivo myocardial I/R injury, we isolated cardiac mitochondria and assessed integrity using electron microscopy. We could show that mitochondrial swelling and, mitochondrial membrane disruption occurred within minutes after reperfusion. We next assessed the levels of calpains 1, 2 and 10 as specific myocardial calpain subtypes in mitochondria following I/R. These studies using electronmicroscopy in combination with immuno-gold labelling showed that calpain 10 increased, while calpain 1 and 2 decreased in the course of I/R. Taking advantage of the cardiomyocyte HL1 cell line, we next determined cell viability, intracellular calcium concentration and pH after simulated ischemia and reoxygenation. We exemplary measured calpain 1 activity as being the calpain with comparably high concentrations in many tissues. Cell viability was lower compared to control and calpain 1 activity was higher in hypoxia/reoxygenation samples. Nitrate is known to protect from I/R injury by yet incompletely resolved downstream mechanisms. Following an oral nitrate treatment to induces nitric oxide (NO) formation in the same murine I/R model, we discovered a significant increase in S-nitrosation of calpains. We could reproduce calpain S-nitrosation in HL1 cells in vitro after hypoxia and reoxygenation. Fittingly, cell viability improved and calpain 1 activity decreased compared to PBS-treated samples in this in vitro approach.
Conclusion: Mitochondrial-mediated cell death remains an important research field. We show that calpains, as key players in caspase-independent apoptosis, increasingly locate at mitochondria following I/R, which is accompanied with the swelling of the mitochondria. We also demonstrate that inhibitory post-translational modification by S-nitrosation of calpains reduces deleterious calpain activity in murine cardiomyocytes. Further studies will need to show if the consequences of calpain activity can also be prevented in vivo after post-translational S-nitrosation.

3247 | BENCH
Experimental cerebral ischemia in rats increases myocardial vulnerability to ischemia-reperfusion injury ex vivo

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For years, the relationship between cardiac and neurological ischemic events has been mainly attributed to overlapping pathophysiological mechanisms and common risk factors. However, acute stroke may induce dramatic alterations of cardiovascular function. The aim of this work was to evaluate how prior cerebrovascular lesions affect myocardial function in vivo and ex vivo, as well as myocardial vulnerability to ischemic injury.
Cerebral embolization was performed in adult Wistar male rats by the injection of microspheres into the left internal carotid artery. Left ventricular function, investigated in vivo using echocardiography (1 hour, 24 hours and 7 days after the embolization), was not significantly impaired; however, the heart rate was significantly increased in the stroke group (+7.2%). Epinephrine (E) and norepinephrine (NE) plasma levels increased in rats from the stroke group (E: 47.3±2.1 vs. 24.3±8.7 and NE: 22.7±4.2 vs. 10.9±3.7). One hour after stroke or sham embolization, hearts were isolated and perfused ex vivo in the Langendorff mode. In hearts from the stroke group, the baseline left ventricular developed pressure was diminished (-11%); moreover, a greater myocardial vulnerability to ischemic injury was observed, with impaired coronary flow recovery after 40 minutes of total global normothermic ischemia.
Our study provides original exciting data indicating that myocardial vulnerability to ischemia can be worsened by prior ischemic stroke, a situation that does not agree with the concept of remote preconditioning. The underlying molecular mechanisms of the stroke-induced myocardial alterations after cerebral embolization remain to be established, insofar as they may involve the sympathetic nervous system.

3248 | BENCH
Inhibition of the S1P lyase improves cardiac remodeling after acute myocardial infarction in mice

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Background: Shingosine-1-Phosphate (S1P) is a bioactive sphingolipid with important roles in cardiac protection against ischemia/reperfusion injury as well as pre- and post-conditioning. Exogenous S1P administration as well as the inhibition of its degradation by genetic or pharmacological means has been shown to decrease infarct size in mice with acute myocardial infarction (AMI). The impact of high endogenous S1P in cardiac remodeling and function after AMI over time has not been investigated. We hypothesized that inhibition of the S1P degrading enzyme S1P lyase improves cardiac remodeling after AMI independently of its effects on infarct size.
Purpose: To investigate the effect of pharmacological S1P lyase inhibition on cardiac remodeling after AMI in terms of ventricular geometry and left ventricular function by magnet resonance imaging (MRI).
Methods: 12±2 week old C57BL/6 mice on standard rodent chow underwent AMI by ligation of the left anterior descending artery. The S1P lyase inhibitor 4-deoxypyridoxine (DOP) was administered orally beginning 7 days prior to AMI. Effectiveness was monitored by determining peripheral CD4+ and CD8+ lymphocyte counts. Infarct size was measured 24 hours post AMI by late gaudolinum enhancement, and cardiac remodeling and function evaluated at day 1 and 21 by MRI. Data are mean ± standard deviation. P below 0.05 was considered significant.
Results: Infarct size did not differ between DOP-treated and control mice (27±9mg vs. 31±8mg, n.s.). In contrast, remodeling after AMI was improved by DOP. Stroke volume index improved in DOP treated mice (0.5±1.4 μl/beat/body surface area [BSA] vs. 0.8±0.2 μl/beat/BSA; p=0.01, while it did not improve in control mice (0.5±0.1 μl/beat/BSA vs. 0.6±0.1 μl/beat/BSA; n.s.). Stroke volume index at day 21 was higher in DOP treated mice as compared to control mice (0.8±0.2 μl/beat/BSA vs. 0.6±0.1 μl/beat/BSA; p=0.009).
Myocardial mass increased between day 1 and 21 only in control but not DOP-treated mice (control: 54±1mg versus 68±9mg, P=0.03; DOP 70±10mg versus 60±10mg, n.s.). Heart rate was decreased in DOP-treated mice 21 days after AMI. In comparison, heart rate in control mice increased between day 1 and day 21 (DOP day 1: 608±47 beats per minute [bpm], day 21: 527±39bpm, 0.03 vs. control day 1, 540±28bpm, day 21, 618±52bpm, P=0.003).
Conclusion: Pharmacological inhibition of the S1P lyase improves cardiac remodeling after AMI in mice without affecting infarct size. This may be a promising approach to improve cardiac performance after AMI.

GENETIC RISK SCORE IN CARDIOVASCULAR PREVENTION: DAWN OF A NEW ERA

3267 | BEDSIDE
Do metabolic risk factors mediate the genetic risk for coronary heart disease?

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Background: Family history of coronary heart disease (CHD) as well as directly assessed genetic predisposition to CHD (genetic risk score, GRS) are strong predictors of CHD risk. It is, however, uncertain to what extent these risk predictors are mediated through the pathways of major metabolic risk factors. Some studies have shown that GRS and family history predict cardiovascular risk independently of established cardiovascular risk factors.
Purpose: We quantitatively assessed the fraction of family history and GRS mediated through established cardio-metabolic risk pathways.
Methods: Statistical mediation analysis was used to estimate the total effects of self-reported family history and a 50-variant genetic risk score (GRS50), as well as the effects mediated by apolipoprotein B (apoB), apolipoprotein A-I (apoA-I), blood pressure, and diabetes mellitus (DM) on the incidence of CHD. Analyses were done in the Malmö Diet and Cancer study, a prospective, population-based study of 23,595 men and women aged 45–73 years recruited between 1991 and 1996. During a median follow-up of 14.4 years, 2,213 participants experienced a first CHD event.
Results: Family history and GRS50 (highest vs. lowest quintile) were associated with incident CHD, with hazard ratios of 1.52 (95% CI (confidence interval): 1.39 to 1.65) and 2.01 (95% CI: 1.76 to 2.30), respectively, after adjusting for age, sex, and smoking status. Small proportions of the family history effect were mediated by traditional risk factors: 8.3% (95% CI: 5.8% to 11.7%) through the apoB pathway, 1.7% (95% CI: 0.2% to 3.4%) through apoA-I, 8.5% (95% CI: 5.9% to 12.0%) through blood pressure, and 1.5% (95% CI: -0.8 to 3.8%) through DM. Similarly, small proportions of GRS50 were mediated by traditional risk factor pathways: 6.0% (95% CI: 3.7% to 8.6%) of the effect was mediated through apoB, 1.1% (95% CI: -0.2% to 2.6%) through apoA-I, 3.5% (95% CI: 1.0% to 5.9%) through blood pressure, and 0.2% (95% CI: -1.6% to 2.7%) through DM. In total, 20.0% (95% CI: 14.8% to 26.4%) of the family history effect and 10.7% (95% CI: 5.8% to 16.0%) of GRS50 effect were mediated by these metabolic risk factors.
Conclusions: A fraction of the CHD risk associated with family history or with GRS50 is mediated through dyslipidemia and hypertension, but not through diabetes. However, a major part (≥80%) of the genetic effect operates independently of established metabolic risk factor pathways. Therefore, family history and genetic disposition might warrant assessment in addition to established metabolic risk factors.