Metalloproteinase Inhibition Prevents Diastolic Stiffening, AMP-Breakdown, and Oxypurine Accumulation in Accelerated Heart Failure

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Background: Recent studies indicated increased plasma oxypurine (hypoxanthine/xanthine oxidase) levels as an independent risk factor for worsened heart failure, suggesting oxypurine output may be important for diastolic dysfunction.

Methods: We used a canine model of enhanced diastolic failure (1 week AII infusion + subacute tachypacing; 250 bpm for 48 hrs; All-P), displaying pronounced diastolic stiffening and marked increase in median plasma oxypurine (AMP) accumulation. MMP inhibition (MMPI) prevents diastolic stiffening, without influencing collagen content/subtype or cross-linking. In this study we tested whether All-P (n=7) enhances AMP catabolism to increase cardiac nucleoside/oxypurine and diastolic stiffening, assessing the ability of MMPI to prevent these changes.

Results: All-P raised diastolic chamber stiffness and end-diastolic pressure ~100% and markedly activated gelatinases MMP-9 and MMP-2 (abundance and in situ assays). With All-P, ATP declined while AMP catabolites increased: nucleotides (inosine, adenosine) raised from 254±84 to 1700±363 nmol/g (p<0.005), and oxypurines from 100±20 to 66±7 nmol/g (p<0.01). MMP inhibition (PD-166793; 5 mg/kg/day; n=9) prevented diastolic stiffening as well as MMP-9 and -2 activation, and countered the rise in both nucleotides (804±35 nmol/g; p<0.02 vs All-P) and oxypurines (397±17; p<0.01 vs All-P). MMPI directly inhibited in vitro AMP-deaminase activity in a dose-dependent manner but did not affect other steps of the purine catabolism cascade. In All-P hearts hallmark of oxidative stress were evident: malondialdehyde (lipid peroxidation index) raised from 0.31±0.1 (controls) to 6.7±2.3 mmol/g (p<0.01) while antioxidant levels (ascorbate and reduced glutathione) significantly declined. However, MMPI did not mitigate All-P-induced oxidative stress (MDA = 6.1±1.3).

Conclusions: Diastolic stiffening is associated with a rise in myocardial content of AMP-breakdown byproducts (i.e. nucleotides and oxypurines). MMP inhibition prevents diastolic stiffening by limiting this accumulation, directly interfering with AMP catabolic activities (i.e.AMP deaminase), independently from the extent of cardiac oxidative stress.

Survival, Differentiation, and Contractility of Immature Cardiac Cells Implanted Into the Outer Wall of Aorta in Rats as a Step in the Development of an Auxiliary Circulatory Pump

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Purpose: We proposed to build an auxiliary circulatory pump by implanting neonatal cardiac cells into the wall of aorta in rats. As a first step, we investigated the survival, differentiation, and contractility of immature cardiac cells implanted into the wall of the abdominal aorta.

Methods: Cardiomyocytes from neonatal Fischer rats (both sexes) were injected into the outer wall of the abdominal aorta at a site 3 mm above the take-off of the renal arteries in female Fischer rats. Rats were divided into two groups: (1) medium only (n=2); (2) neonatal cardiomyocytes (n=22), 5×10⁶ cells each. At 2 or 6 weeks, the graft site on the aorta was exposed for histological and immunohistochemical analysis.

Results: At 2 weeks after transplantation, 7 out of 10 aortas in the cell group, but none of the aortas in the control group, were positive for c-Kit, vimentin, and CD45. At 6 weeks, 7 out of 10 aortas in the cell group, but none of the aortas in the control group, were positive for muscle specific actin, cardiac troponin-I, and CD45. In the cell group, the graft site on the aorta was organized in a compact, longitudinally oriented cardiac muscle layer and stained positive for c-Kit and vimentin.

Conclusion: We have successfully implanted neonatal cardiac cells in the abdominal aorta of rats. The transplanted cells survived and differentiated into mature cardiomyocytes, indicating the feasibility of this technique for the development of a new type of auxiliary pump.