

Featured research

Abstracts and commentaries

Reduction of hyperacute rejection and protection of metabolism and function in hearts of human decay accelerating factor (h-DAF)-expressing pigs

Smolenski RT, Forni M, Maccherini M, et al. *Cardiovasc Res.* 2007;73:143–152.

Heart failure is currently one of the major causes of mortality and factors affecting the quality of life in humans. Despite advances in pharmacological treatment and recent developments in mechanical circulatory support devices, cardiac transplantation remains the most effective treatment of endstage heart failure. However, the availability of human donor hearts is limited, and only a small percentage of those who would benefit from this treatment could actually receive a transplant. It is expected that the disparity between organ availability and demand will increase even further in the future. One possible solution of the donor organ shortage is the use of animal organs. To date, pigs seem to be the best source of organs for xenotransplantation because of their physiological compatibility and ethical acceptability. However, after transplantation into primates, pig hearts undergo a rapid and vigorous reaction termed hyperacute rejection, causing total dysfunction within minutes. An important challenge is therefore the development of effective and inexpensive procedures for the generation of transgenic animals. Sperm-mediated gene transfer (SMGT), developed by Smolenski and colleagues several years ago [1], appears to be an efficient and cost-effective procedure for that purpose. Smolenski's group successfully established a pig strain expressing human decay-accelerating factor (hDAF) [2]. They evaluated in the present paper whether hearts of hDAF transgenic pigs generated using SMGT were protected from structural damage, metabolic changes, and mechanical dysfunction during perfusion with human blood.

Commentary

Hearts from control or transgenic pigs were perfused ex vivo for 4 h with fresh human blood using an ex-vivo working model system allowing monitoring of function, metabolism, and structure.

Cardiac output remained constant in transgenic animals throughout the experiment, whereas it decreased in control pigs after 30 min of perfusion ($P < 0.01$ compared with transgenic animals). The maximum increase in coronary perfusion pressure was reduced to $154 \pm 16\%$ in transgenic animals, and to $237 \pm 10\%$ in control pigs ($P < 0.001$). After 4 h, myocardial ATP was 21.1 ± 1.1 nmol/mg dry weight (similar to the baseline value) in transgenic pigs, whereas it decreased to 17.2 ± 1.4 nmol/mg dry weight in control animals ($P < 0.05$).

Deposition of complement factors C3 and C5b9 was present in control but not transgenic animals after perfusion. Attenuation of hyperacute rejection was further confirmed by microscopic analysis of cardiac specimens: there was no structural damage in transgenic hearts.

There are several limitations to the system used in this study. First, it allows for only several hours of perfusion, essentially limiting its application to the study of hyperacute rejection. Secondly, not all mechanisms of rejection could be reproduced; for example, platelet-mediated rejection that was blunted by high-dose heparin. The ex-vivo system also neglects the effect of other organs on blood homeostasis. However, this study has shown that hearts from transgenic pigs produced by SMGT were protected from hyperacute rejection after exposure to human blood, and that those hearts, in a human blood environment, were relatively metabolically stable and maintained mechanical function above the threshold for life support. Further application of this method for the generation of multigene transgenic pigs, and in

particular its combination with knockout techniques, could be a significant step towards clinical xenotransplantation.

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Free fatty acid depletion acutely decreases cardiac work and efficiency in cardiomyopathic heart failure

Tuunanen H, Engblom E, Naum A, et al. *Circulation*. 2006;114:2130–2137

Metabolic modulators that enhance myocardial glucose metabolism by inhibiting free fatty acid (FFA) metabolism may improve cardiac function in patients with heart failure. The effect of acute FFA withdrawal on cardiac function was studied in 18 fasting non diabetic patients with heart failure caused by idiopathic dilated cardiomyopathy (IDCM) (14 men, four women, ages 58.8 ± 8.0 years, ejection fraction $33 \pm 8.8\%$) and eight matched healthy controls. They underwent examination of myocardial perfusion and oxidative and FFA metabolism before and after acute reduction of serum FFA concentrations by acipimox, an inhibitor of lipolysis. Metabolism was monitored by positron emission tomography (PET) and [^{15}O]H $_2$ O, [^{11}C]acetate, and [^{11}C]palmitate. Left ventricular function and myocardial work were measured by echocardiography and the efficiency of forward work was calculated. Acipimox decreased myocardial FFA uptake by $>80\%$ in both groups. The rate–pressure product and myocardial perfusion remained unchanged, but stroke volume decreased similarly in both groups. In the healthy controls, reduced cardiac work was accompanied by a decrease in oxidative metabolism (from 0.071 ± 0.019 l/min to 0.055 ± 0.016 l/min; $P < 0.01$). In patients with IDCM, cardiac work decreased, whereas oxidative metabolism remained unchanged and efficiency decreased (from 35.4 ± 12.6 mm Hg/ μL per μg to 31.6 ± 13.3 mm Hg/ μL per μg ; $P < 0.05$). It was concluded that acutely decreased serum FFA depresses cardiac work. In healthy hearts, this was accompanied by a parallel decrease in oxidative metabolism, and

myocardial efficiency was preserved. In failing hearts, FFA depletion did not downregulate oxidative metabolism, and myocardial efficiency deteriorated. Thus failing hearts are, unexpectedly, more dependent than healthy hearts on the availability of FFA. It is proposed that both glucose and fatty acid oxidation are required for optimal function of the failing heart.

Commentary

In this interesting study, both patients with IDCM and healthy volunteers underwent [^{11}C]acetate PET imaging for measurement of oxidative metabolism, [^{11}C]palmitate PET imaging for measurement of the free fatty acid uptake and the rate of β -oxidation, [^{15}O]H $_2$ O PET imaging for myocardial perfusion, and echocardiography to determine left ventricular stroke volume and left ventricular mass. The same measurements were repeated after administration of acipimox, a drug that decreases free fatty acid concentrations in blood.

The most significant part of the study concerned the measurement of cardiac efficiency in patients and volunteers. This parameter is determined by combining the echocardiographic data with the [^{11}C]acetate data:

$$\text{efficiency} = (\text{LV work power}/\text{LV mass})/\text{LV } K_{\text{mono}}$$

where LV (left ventricular) work power is ‘systolic blood pressure \times stroke volume \times heart rate’ and K_{mono} is a value derived from [^{11}C]acetate in which the decline in tracer activity of the heart is fitted with a monoexponential curve. (For an overview of cardiac efficiency, see [1].)

As expected, acipimox decreased fatty acid concentrations in both patients and volunteers. Also expected was that left ventricular work power was decreased in patients with IDCM compared with that in healthy volunteers, both before and after the administration of acipimox. In both groups, administration of acipimox led to a small but non significant reduction of left ventricular work power. Oxidative metabolism (K_{mono} value) was similar between patients and healthy volunteers at baseline but, after the administration of acipimox, K_{mono} values decreased significantly in the healthy volunteers alone. By combining these work power data and K_{mono} values in the calculation of efficiency, the authors showed that decreasing the concentration of fatty acids by the use of acipimox resulted in a small but significant decrease (of 11%) in cardiac efficiency in patients with IDCM and an increase (of 18%) in the healthy volunteers.

These findings are quite surprising, and in contrast with what was to be expected. A wealth of experimental and clinical data have shown that a metabolic intervention that increases glucose metabolism and

AQ1

AQ2

inhibits fatty acid metabolism in heart failure (eg, trimetazidine or glucose–insulin–potassium) improves cardiac function.

In the editorial accompanying the paper by Tuunanen and colleagues, Taegtmeyer and Ballal [2] offered some explanations for the observed phenomena. First, recent data in lipase-deficient mice have shown that fatty acids are needed for normal cardiac contraction. This is in line with the findings discussed here. Secondly, acipimox itself may have direct hemodynamic effects. This was not measured in the study, and may have altered left ventricular work power. Thirdly, acipimox itself may decrease insulin secretion. As the patients were insulin-resistant, the intervention to decrease fatty acids, combined with the insulin resistance, may have depleted the heart of the required energy nutrients.

Another point worthy of consideration is that, in this study by Tuunanen et al, K_{mono} values before the acipimox intervention were similar between IDCM patients and volunteers. Other studies [3] have shown that K_{mono} values were lower in patients with cardiomyopathy, suggesting a patient bias in the present study. Finally, earlier experimental studies have clearly shown that the clearance of radioactivity from the heart is biexponential: the fast part of the time–activity curve is related to turnover of [^{11}C]acetate in the Krebs' cycle, and the slower part is related to clearance of the radioactivity from Krebs' cycle intermediates such as

glutamate [4]. As is usual in human studies, in the present study oxidative metabolism was measured by fitting the time–activity curve solely with a monoexponential curve. It is therefore unknown to what extent the second, slow, component of the tracer influenced the values of the first. This may be different in healthy volunteers and in patients.

Despite some points of criticism, the findings of Tuunanen and colleagues show that the relationship between cardiac function and metabolism is complex, and that there remain many details that need to be studied. In conclusion, I will quote a statement by Taegtmeyer and Ballal: 'When it comes to energy substrate metabolism of the heart, extremes are never good'.

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