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Original article

Functional brown adipose tissue limits cardiomyocyte injury and adverse remodeling in catecholamine-induced cardiomyopathy

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ABSTRACT

Brown adipose tissue (BAT) has well recognized thermogenic properties mediated by the uncoupling protein 1 (UCP1); more recently, BAT has been demonstrated to modulate cardiovascular risk factors. To investigate whether BAT also affects myocardial injury and remodeling, UCP1-deficient ($UCP1^{-/-}$) mice, which have dysfunctional BAT, were subjected to catecholamine-induced cardiomyopathy. At baseline, there were no differences in echocardiographic parameters, plasma cardiac troponin I (cTnI) or myocardial fibrosis between wild-type (WT) and $UCP1^{-/-}$ mice. Isoproterenol infusion increased cTnI and myocardial fibrosis and induced left ventricular (LV) hypertrophy in both WT and $UCP1^{-/-}$ mice. $UCP1^{-/-}$ mice demonstrated exaggerated myocardial injury, fibrosis, and adverse remodeling, as well as decreased survival. Transplantation of WT BAT to $UCP1^{-/-}$ mice prevented the isoproterenol-induced cTnI increase and improved survival, whereas $UCP1^{-/-}$ BAT transplanted to either $UCP1^{-/-}$ or WT mice had no effect on cTnI release. After 3 days of isoproterenol treatment, phosphorylated AKT and ERK were lower in the LV of $UCP1^{-/-}$ mice than in that of WT mice. Activation of BAT was also noted in a model of chronic ischemic cardiomyopathy, and was correlated to LV dysfunction. Deficiency in UCP1, and accompanying BAT dysfunction, increases cardiomyocyte injury and adverse LV remodeling, and decreases survival in a mouse model of catecholamine-induced cardiomyopathy. Myocardial injury and decreased survival are rescued by transplantation of functional BAT to $UCP1^{-/-}$ mice, suggesting a systemic cardioprotective role of functional BAT. BAT is also activated in chronic ischemic cardiomyopathy.

1. Introduction

Cardiomyocyte injury leading to cardiac remodeling and subsequent progression to heart failure represents a major cause of human morbidity and mortality [1]. To maintain adequate cardiac output in the face of decreased ventricular pump function, reflex pathways including the sympathetic nervous system are activated, in turn leading to increased catecholamine release from the heart and endocrine tissues. This exposure to excess catecholamines increases cardiomyocyte death and augments myocardial adverse remodeling [2].

Sympathetic nervous system activation and norepinephrine release [3], concomitant with the release of natriuretic peptides [4] occur after myocardial injury and during the development of adverse left ventricular (LV) remodeling. Interestingly, these molecules are also major contributors to the growth and stimulation of brown adipose tissue (BAT) [5,6]. Brown adipose tissue, a relatively sparse brownish-colored adipose tissue well recognized in rodents and children, has recently been detected in adult humans [7–9]. Upon activation of BAT, the brown adipocytes consume glucose and lipids and convert the energy from free fatty acids and glucose oxidation

Abbreviations: BAT, brown adipose tissue; UCP1, uncoupling protein 1; $UCP1^{-/-}$, UCP1-deficient; LV, left ventricle; WT, wild-type; H/R, wall thickness/LV radius; LVMI, LV mass index; cTnI, cardiac troponin I; MI, myocardial infarct; LVEDD, LV end-diastolic diameter; LVESD, LV end-systolic diameter; PWT, posterior wall thickness; IVS, interventricular septal thickness; AWT, anterior wall thickness; LVEF, LV ejection fraction; SBP, systolic blood pressure; DBP, diastolic blood pressure; GTT, glucose tolerance test; ITT, insulin tolerance test; FABP3, fatty acid binding protein 3; MYL3, myosin light chain 3.

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into heat (thermogenesis) [10]. The unique metabolic and thermogenic properties of BAT have generated substantial research interest in exploring its potential therapeutic applications for obesity and type II diabetes [6,11,12].

The thermogenic capacity of BAT is mediated by the mitochondrial proton transporter uncoupling protein 1 (UCP1), which disperses the proton motive force generated by oxidative phosphorylation, generating heat as a by-product of this futile cycle [13]. Mice deficient in UCP1 (UCP1$^{-/-}$ mice) display impaired activation of BAT in response to cold and beta-adrenergic agonists, characterized by a mildly decreased thermogenic capacity [14,15], and reduced local blood flow [16].

Recently, several lines of evidence indicate that BAT and related tissues are capable of modulating several endocrine and cardiovascular risk factors. BAT activation or transplantation normalized both glucose tolerance and insulin resistance in obese or old mice [17,18]. Recently, this beneficial effect on glucose metabolism was also extended to humans: in healthy volunteers Chondronikola *et al.* demonstrated that activated BAT increased insulin sensitivity [19]. In addition. perivascular and epicardial adipose tissue depots, which share characteristics of BAT [20], are implicated in the modulation of atherosclerosis and blood pressure [21].

Although BAT may be activated after myocardial injury and during ventricular remodeling, whether this activation has an effect on these processes is unknown. The objectives of the present study were to study whether BAT was activated after myocardial injury and whether this activation was cardioprotective. To approach this question we first used a model of cardiac injury in which the activation of BAT is well recognized. The model we chose, chronic catecholamine (isoproterenol) exposure, leads to cardiac injury and cardiomyopathy [22]. Using this model, we compared the cardiac response of wild-type (WT) and UCP1$^{-/-}$ mice (with functional and dysfunctional BAT respectively). In a series of separate experiments, we then investigated whether BAT was also activated in an easily clinically translatable model of ischemic cardiomyopathy.

2. Material and methods

Additional material and methods are detailed in the Supplemental Data.

2.1. Experimental animals

All animal procedures were conducted in accordance with guidelines published in the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, Washington, DC, 1996) and were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care. C57Bl6/J WT mice were obtained from Jackson Laboratory (Bar Harbor, ME). Whole body UCP1 knockout mice (UCP1$^{-/-}$) mice, as described in Enerbäck *et al.* [15], were obtained courtesy of Dr. Randall Mynatt (Pennington Biomedical Research Center, Baton Rouge, LA) and were bred at Massachusetts General Hospital. All mice were housed at thermoneutrality (31 °C) at all times and were studied at the age of 12–14 weeks.

2.2. Experimental protocol

All experimental groups are summarized in Fig. 1. Alzet osmotic minipumps (no. 1002; Alza Durect Corp., Mountain View, CA, USA) containing isoproterenol (60 mg/kg/d) [23] or saline were subcutaneously and dorsally inserted in mice under isoflurane anesthesia. Cardiomyocyte injury, gene expression levels and immunoblots were measured 3 days after the start of the infusion, and myocardial fibrosis after 14 days. Echocardiographic measurements and blood pressure were measured at baseline and after 14 days of saline or isoproterenol infusion. Metabolic parameters were obtained at baseline after 16 h of fasting.

Brown adipose tissue transplantation was performed as described previously [18]. Mice were allowed to recover for 8 weeks before saline or isoproterenol infusion was started.

Myocardial infarction (MI) was studied in WT mice only and was produced by ligation of the left anterior descending coronary

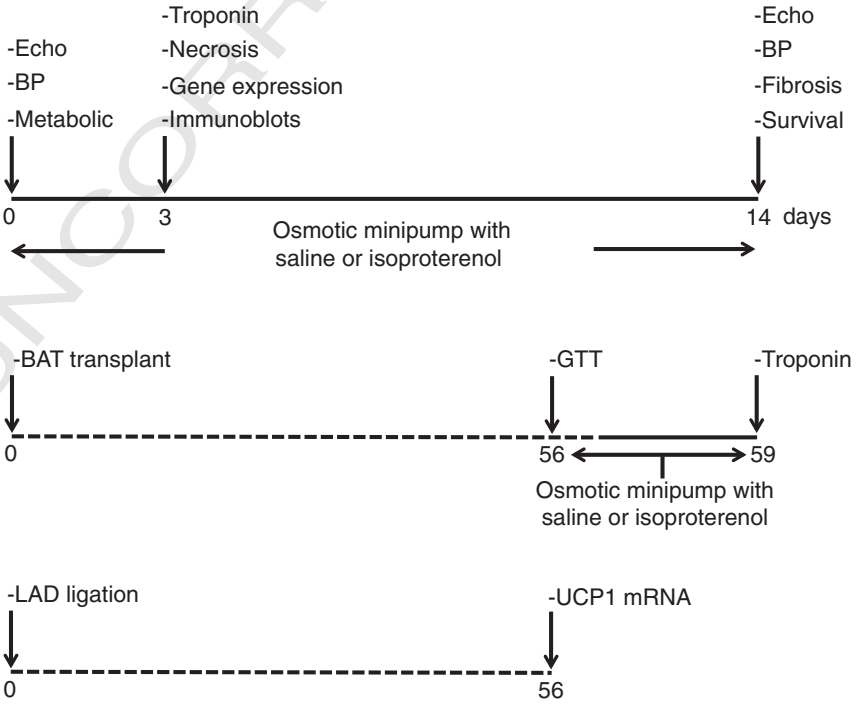

Fig. 1. Flow chart of experimental procedures.

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