Preclinical Development of Gene Therapy

SUMMARY

Heart failure (HF) incidence is increasing and the current therapeutic options have remained the same over the past years, without resplendent improvements in the disease's outcomes. In addition to this, there are no efficacious treatments for specific subgroups of heart failure patients, such as those with heart failure with preserved ejection fraction (HFpEF). Early disease detection can offer the opportunity of lifestyle changes and pharmacologic interventions that can effectively manage the disease, although the complexity and heterogeneity of HF makes early diagnosis difficult (1). Furthermore, the management of some risk factors of HF, like diabetes, is still challenging with restricted number and efficacy of the available treatments (2-4). Therefore, new therapies for HF and its risk factors are warranted.

Among a plethora of agents that could benefit HF patients are the corticotropin releasing factor (CRF) family peptides. The four know members of this family are CRF, which binds predominantly to CRF receptor 1 (CRFR1), Ucn1, which binds to both CRFR1 and CRFR2, and Ucn2 and Ucn3, which bind exclusively to CRFR2. In the last two decades, promising findings on the beneficial effects of Ucns IV infusions on healthy or failing animal and human hearts, have demonstrated the potency of those peptides to be used as therapeutic agents for heart failure (5-18). Ucns' regulatory role in glucose metabolism also suggests a potential use in diabetes management. However, the exact molecular mechanisms that are activated by Ucns and underlie the therapeutic effects in diabetes and HF are still unclear. The use of Ucn2 and Ucn3 for chronic treatment is restricted by peptides' short half-life (about 10 minutes) (19); therefore, gene transfer of those peptides has been proposed.

The main goal of this thesis was to evaluate the effects Ucn2 and Ucn3 gene transfer on heart function and glucose metabolism, as well as to unravel the molecular mechanisms contributing in these effects. The major findings of this work are:

- Ucn2 and Ucn3 gene transfer increased systolic and diastolic function in healthy young mice. (Chapter 2)
- Ucn2, but not Ucn3, gene transfer improved glucose clearance in healthy young mice. (Chapter 2)
- Ucn2 and Ucn3 gene transfer resulted in increased Ca^{2+} transients and decreased time constant of relaxation (Tau) of isolated cardiac myocytes from young healthy mice. (Chapter 2)
- Ucn2 and Ucn3 gene transfer increased the left ventricular (LV) levels of sarco/endoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a). (Chapter 2)
- Ucn3 gene transfer increased the function of the failing heart in a murine model of HF with reduced ejection fraction. (Chapter 3)
- Ucn3 gene transfer increased Ca^{2+} transients and decreased Tau of isolated cardiac myocytes from failing murine hearts. (Chapter 3)

- Ucn3 gene transfer in a murine model of HFrEF increased the LV content of SERCA2a and decreased the phosphorylation of Ca^{2+}/calmodulin mediated kinase II (CaMKII). (Chapter 3)
- Ucn2 gene transfer improved glucose clearance in two murine models of type 2 diabetes mellitus (T2DM). (Chapter 4)
- Ucn2 gene transfer in a T2DM murine model increased adenosine monophosphate-activated protein kinase (AMPK) activation and induced translocation of glucose transporter 4 (Glut4). (Chapter 4)
- Ucn2 gene transfer reversed, and also prevented, age-related diastolic dysfunction in aged mice. (Chapter 5)
- Ucn2 gene transfer increased the levels of SERCA2a and decreased the phosphorylation of CaMKII in the LVs of 2-year-old mice. (Chapter 5)
- There was no observation of adverse effects related to UCn2 and Ucn3 gene transfer. (Chapters 2, 3, 4, 5)