

## SERCA2 stimulation by Istaroxime follow-on compounds in a rat model of diabetic cardiomyopathy

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Diabetic cardiomyopathy is a complex and multifactorial disease characterized by an early onset of diastolic dysfunction (DD), which precedes the development of systolic impairment. The streptozotocin (STZ)-induced type 1 diabetes in rat is characterized by an impaired diastolic function attributable to a reduced protein expression and activity of cardiac SERCA2a (see poster P48, E. Torre *et al*). Istaroxime is a compound endowed with a dual mechanism of action that consists in the inhibition of the Na<sup>+</sup>/K<sup>+</sup>-ATPase (NKA) and stimulation of SERCA2a, resulting in a combined luso-inotropic effect. New “SERCA2 pure” Istaroxime derivatives have been developed in the BTBS Department thanks to a multi-disciplinary collaboration with the research groups of prof. Peri and prof. Zaza.

Overall, the aim of the project is to verify whether SERCA2 stimulation by Istaroxime derivatives can improve DD in streptozotocin (STZ)-treated rats thanks to a better control of intracellular Ca<sup>2+</sup> handling. To this end, in this study we firstly evaluated effects of Istaroxime follow-on Compounds (Cpds A, B, C) on both Istaroxime targets (NKA and SERCA2) in CTR and STZ rats 8 weeks following STZ injection. Measurements in cell free system and in isolated myocytes were performed.

Heart homogenates from CTR and STZ rats were utilized to measure SERCA2a activity as <sup>32</sup>P-ATP hydrolysis assay by using increasing Ca<sup>2+</sup> concentrations from 100-4000 nM; maximal velocity (V<sub>max</sub>) and Ca<sup>2+</sup> affinity (K<sub>d</sub>) were estimated through a logistic function. Effects on NKA were evaluated by using kidney homogenates and by measuring I<sub>NKA</sub> in isolated rat ventricular myocytes. Moreover, evaluation of sarcoplasmic reticulum (SR) Ca<sup>2+</sup> uptake function in CTR and STZ myocytes was assessed by dedicate voltage clamp protocols in FLUO4-loaded myocytes.

In comparison to the lead compound Istaroxime, its derivatives showed low affinity for NKA (EC<sub>50</sub>>100μM). They stimulated SERCA2 activity at submicromolar concentration in heart homogenates from STZ rats but they did not display any effect in CTR rats. Moreover, SR Ca<sup>2+</sup> uptake following SR depletion became steeper in the presence of each Cpd in STZ myocytes, thus confirming their stimulating effect on SERCA2.

Overall, these data suggest that the novel Cpds are able to restore the impaired SERCA2 activity in a pathological model of STZ-induced diabetic cardiomyopathy. Their potential role in ameliorating DD will be verified by their *in vivo* administration in STZ-treated animals.

