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Project title: Development of small molecular activators of SERCA2a: towards a new generation of therapeutics

Abstract

The sarcoplasmic/endoplasmic reticulum Ca2+-ATPase 2a (SERCA2a) performs active reuptake of cytoplasmic Ca2+ and is a major regulator of cardiac muscle contractility. Dysfunction or dysregulation of SERCA2a is associated with heart failure (HF), while restoring its function is considered as a therapeutic strategy to restore cardiac performance.

In last 5 years we developed innovative molecules derived from steroids that are active in selectively activating SERCA2a that might be new leads for drugs against cardiac diastolic dysfunction. The proposed project aims to exploit all the information on pharmacophore obtained from the first generation of molecules, to design, synthesize and test new SERCA2a-active compounds with different chemical structures. The new molecules will be tested in vitro and in vivo and their mechanism of action will be investigated.

Background, aims and significance of the proposed work

The cardiac contractility is tightly regulated by the activity of the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a), which is responsible for the reuptake of cytosolic Ca^{2+} into the sarcoplasmic reticulum (SR) of cardiomyocytes. SERCA2a enables cardiac muscle relaxation and determines how much Ca^{2+} can be released for contraction, which in turn controls contractile strength. In the heart, the activity of SERCA2a is regulated by several transmembrane (TM) micropeptides among these the most important being Phospholamban (PLB). A reduced SERCA2a activity, at least in part, contributes to the progressive deterioration of cardiac contractility in HF. There is an increasing pharmacological interest in finding molecules that stimulate selectively SERCA2a activity without interfering with the activity of other transporters, as novel drugs targeting HF. However, the structure-based design of molecules that are able to target this protein has so far been challenging because the SERCA2a structure was not known, and it has been determined very recently (1)(Fig. 1). Small molecule SERCA2a agonists have however been reported (2-4), but molecular details of their working mechanism are still lacking.

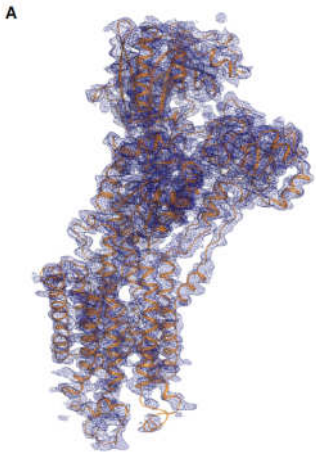

Fig. 1: The SERCA2a structure, determined in 2019

In last 5 years a collaboration among chemists, physiologists and biologists of the BTBS joint lab with the company CVie Therapeutics, allowed to develop new steroid derivatives that selectively activate SERCA2a without interfering with the activity of Na/K pump. These molecules are the first SERCA2a selective activators, are first-in-class drug leads for HF and their chemical structure has been protected by a family of patents jointly submitted by UNIMIB and the company (5). The aim of this 2-years is to project, synthesize and test on cells and animal models new generations of molecules obtained by ligand-based computer-assisted design. The design of new molecules will be based on the pharmacophore identified through structure-activity studies on the first generation of molecules.

Experimental plan

- **WP1** (months 1-6): Ligand-based computer-assisted rational design of potential SERCA2a activators based on the pharmacophore from QSAR studies on the first generation of compounds (in collaboration with Prof. Luca De Gioia, UNIMIB)

We have developed a panel of SERCA2a activators based on a steroid structure, that have been validated in vitro and in vivo and whose structure has been patented. The WP1 will be developed in collaboration with the computation unit of Prof. Luca De Gioia and will consist in the ligand-based design of new potential SERCA2a activators based on the pharmacophore and quantitative structure-activity data analysis on the first generation of compound. In particular, we will focus on compounds in which the steroid core is replaced by another chemical structure. The new SERCA2a activators will be patentable because the chemical structure will greatly differ from their precursors.

- **WP2** (months 3-24): Synthesis of new molecules projected in WP1

Aim of this task is the synthesis of the molecules designed in WP1. Multistep synthetic pathway will be designed and after pilot synthesis the single steps will be optimized. The upscale of the synthesis will be then envisaged in WP5 to provide the amount of compounds required by in vivo studies, only for those compounds that turned out to be active after in vitro testing (WP 3)

- **WP3** (months 3-18): *In vitro* studies (in collaboration with Prof. Marcella Rocchetti and Prof. Antonio Zaza, UNIMIB)

Two cell models will be used to assess the potential of newly synthesized molecules (WP2) on SERCA2a activity: myocytes from normal guinea-pig and from streptozotocin (STZ) diabetic rats. The STZ model will allow the analysis of molecules functional effects in a contest SERCA2a downregulation, the main cause of diastolic dysfunction in the diabetic cardiomyopathy.

The compounds synthesized in WP2 will be tested for their ability to stimulate SERCA2a activity in a primary screening assay by using SR microsomes derived from normal guinea pig heart tissues over a range of concentrations from 1 to 200 nM. The molecules will be also tested for their effects on SR Ca^{2+} uptake parameters in rat ventricular myocytes isolated from STZ rats. The SR Ca^{2+} uptake parameters will include: Ca^{2+} transient (CaT) amplitude, Ca^{2+}-induced Ca^{2+} release (CICR) gain and the time constant (τ) of CaT decay.

- **WP4** (months 12-18): Synthesis upscale

The synthesis of compounds that will be found to be active in in vitro experiments described in WP3, will be up-scaled to hundred milligrams-gram scale, to provide material for in vivo studies (WP5). Reaction conditions and yields will be optimized, as well as purification procedures by crystallization and chromatography.

- **WP5** (months 18-24): *In vivo* studies: pharmacokinetic, toxicity, activity on STZ rats (in collaboration with Prof. Marcella Rocchetti, Dr. Mara Ferrandi, Dr. Paolo Barassi)

Bioavailability and acute toxicity of formulated molecules will be determined in rats after an intravenous (iv) injection at 1 mg/kg and an oral administration at 10 mg/kg. Plasma concentrations of the synthetic compounds will be measured at intervals from time 0 to time 24h and detected by LC-MS method.

To determine the in vivo activity, the effects of compounds will be studied on echocardiographic parameters in STZ rats after an intravenous infusion in comparison to the reference drug Digoxin.

Feasibility and financial support

The research collaboration contract with Windtree and other pharmaceutical companies will provide financial support to this project

Literature references

1) A. Sitsel et al. The EMBO J. , 2019, DOI 10.15252/embj.2018100020

2) S. De Munari, et al., J. Med. Chem. 2003, 64, 3644-3654

3) M. Rocchetti et al. J Pharmacol Exp Ther.2005, 313, 207-215

4) Kaneko M. et al. Eur J Pharmacol 2017; 814, 1-7

5) PCT application n° "New androstane derivatives with activity as pure or predominantly pure stimulators of SERCA2a for the treatment of heart failure" 2019

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