Recombinant hSERCA2a in yeast: a new tool for mechanistic studies on drug-induced SERCA2a stimulation

M. Arici[1], **C. Aspetti[1]**, F. Testa[1], A. Metallo[1], L. Fiandra[1], M.E. Regonesi[1], C. Airoldi[1], M. Rocchetti[1]

E-mail: c.aspetti@campus.unimib.it

[1] Dept of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy

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Abstract:

Istaroxime is a promising agent combining Na^+/K^+ pump inhibition and SERCA2a stimulation, showing a safety profile for acute HF treatment. Recently, new istaroxime follow-on compounds, able to improve cardiac performance in diseased animals by selective SERCA2a stimulation, were developed and characterized. Despite evidence of SERCA2a/phospholamban (PLN) interaction inhibition, the molecular mechanism of action of these compounds is still unknown.

The aim of the project was to investigate the molecular mechanism of action of these new molecules, analysing their interaction with SERCA2a and/or PLN through NMR studies.

Preliminary NMR data showed that one of the new follow-on compounds, Cpd 8, was able to bind the synthetic full-length $hPLN_{1-52}$, but not the citosolic $hPLN_{1-32}$ fragment, and it was able to interact with a mammalian membrane mimetic, like its parent compound istaroxime.

Concerning SERCA2a, we engineered S. cerevisiae to product hSERCA2a with pRS426-Gal1-hSERCA2a-GFP-His8 plasmid. After 22h of induction, the yeast effectively expressed SERCA2a and it mirrored the ER- and the plasmalemma-protein localization pattern. Transformants were used to obtain vesicles containing the protein of interest, ranging from 50 to 400 nm diameter. Vesicles were solubilized and hSERCA2a was purified through an affinity chromatography. Despite the procedure has to be optimized, a small quantity of protein was purified. This small fraction will be used to verify its activity and to perform preliminary NMR experiments after reconstitution in vesicles.

Overall, these encouraging preliminary results are fundamental for a complete ligand-based NMR analysis of SERCA2a/PLN complex inhibition by istaroxime and its follow-on compounds.