CARDIOPROTECTIVE PROPERTIES OF A PROMISING ISOTHIOCYANATE: FROM THE H_2S-RELEASING PROPERTIES TO THE *IN VIVO* CARDIOPROTECTIVE EFFECTS

Valentina Citi[1], Simone Brogi[1], Lorenzo Flori[1], Era Gorica[1], Eugenia Piragine[1], Beatrice Severino[2], Angela Corvino[2], Alma Martelli[1], Lara Testai[1], Giuseppe Caliendo[2], Vincenzo Calderone[1]

[1]Department of Pharmacy, University of Pisa, Pisa - Italy, [2]Department of Pharmacy, School of Medicine, University of Naples "Federico II", Naples - Italy

Introduction: The endogenous gasotransmitter hydrogen sulfide (H_2S) is an important regulator of the cardiovascular system, particularly of myocardial function. Moreover, H_2S exhibits cardioprotective activity against ischemia/reperfusion (I/R) or hypoxic injury and is considered an important mediator of "ischemic preconditioning". Accordingly, isothiocyanates molecules, that act as H_2S-donors, are viewed as promising therapeutic agents for a number of cardiovascular diseases. In this work, a small library of isothiocyanates has been synthetized and all the compounds were evaluated for their H_2S releasing properties; through a computational analysis of their physiochemical properties, the compound named ISOTHIA25has been selected to further investigation about its protective activity both in vitro and *in vivo* experimental procedures of I/R injury.

Material and methods: The H_2S releasing properties of the isothiocyanates were evaluated by an amperometric approach. Furthermore, the analysis of the physio-chemical properties has been performed to predict their pysico-chemical properties in order to prioritize the best predicted compounds for a comprehensive pharmacological characterization. After this first screening, the ISOTHIA25was selected to investigate its pharmacological effects: the H_2S release was measured into the H9c2cell line (rat cardiomyoblasts) by a specific dye (WSP-1). The protective effect of ISOTHIA25was first evaluated in vitro: H9c2were treated with the isothiocyanate for 1h, and then exposed to $H_2O_2$200μM for 2h; cell viability was measured with the WST-1method. The cardioprotective effect of ISOTHIA25was evaluated in an *ex vivo* rat model of myocardial I/R: male Wistar rats were treated i.p. with increasing concentrations of ISOTHIA25. After 2h, the animals were anaesthetized, sacrificed and the heart was subjected to an I/R injury. The ischemic area was detected by 1% aqueous solution of tetrazolium chloride and the LDH enzyme activity was measured in the perfusate. Furthermore, the ISOTHIA25cardioprotection has been also evaluated in an *in vivo* model of acute rat myocardial infarction.

Results: In the presence of L-Cysteine, all the isothiocyanates were able to release H_2S in a cell-free environment while in the absence the organic thiols, the release of H_2S was negligible. The computational analysis lead to the selection of the compound named ISOTHIA25, which was able to release about 60 μM of H_2S when incubated at the concentration of 1mM in the presence of L-Cysteine and which showed good solubility and a promising ADME profile. Furthermore, ISOTHIA25was able to enter into the H9c2cells and release H_2S in a concentration dependent manner without the addition of exogenous organic thiols. The protective effect was first evaluated using H9c2: the incubation of ISOTHIA25before the treatment with H_2O_2, lead to a significant recovery in cell viability in a concentration dependent manner, with an almost complete recovery of the viability when incubated at the concentration of 1μM.The H_2S-donor ISOTHIA25has been then tested in different experimental models of myocardial I/R: in Langendorff-perfused rat hearts subjected to I/R, ISOTHIA25significantly improved the post-ischemic damage, limiting the tissue injury in a concentration dependent manner. Accordingly, also the LDH biomarker was reduced. These effects were antagonized by 5-hydroxydecanoic acid (a blocker of mitoK_{ATP} channels). Finally, in an *in vivo* model of acute myocardial infarction in rats, ISOTHIA25significantly decreased I/R-induced tissue injury.

Discussion and conclusion: ISOTHIA25exhibits H_2S-releasing properties both in a cell-free and in a cell-based assay, and due to this property, has cardioprotective effects in vitro and *in vivo*. Thus, isothiocyanate-based H_2S-releasing drugs, like ISOTHIA25, can actually be considered a suitable pharmacological option in anti-ischemic therapy.