THE NOVEL H2S-DONOR ISOTHIA25PROMOTES CARDIOPROTECTIVE EFFECT AGAINST MYOCARDIAL ISCHEMIA/REPERFUSION INJURY IN RAT HEARTS AND REDUCTION OF OXIDATIVE STRESS IN H9C2CELLS

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Introduction: Hydrogen sulfide (H_2S) is an endogenous gasotransmitter, involved in the regulation of several biological functions. Even though it has been considered a well-known toxic gas, recent studies have described its important role in the regulation of cardiovascular homeostasis, and its deficiency is etiologically associated with several cardiovascular diseases. Therefore, the research of original moieties able to release H_2S represents a timely issue for drug discovery. In this work, the H_2S-releasing properties of the isothiocyanate ISOTHIA25were evaluated and its protective effects in different models of myocardial injury were further investigated using H9c2cell line (rat cardiomyoblasts) and a model of rat myocardial ischemia/reperfusion (I/R) injury.

Material and methods: The ISOTHIA25H_2S releasing properties have been detected both in a cell-free environment, using an amperometric approach, through an Apollo-4000 Free Radical Analyzer (WPI) detector, and in cell-based assays. In particular, the H_2S release after ISOTHIA25incubation in H9c2cells was detected by a spectrofluorometric method, using the Washington state probe -1(WSP-1) dye. The protective effect of ISOTHIA25was first evaluated in vitro: H9c2were treated with the isothiocyanate for 1h, and then exposed to the oxidative stress using $H_2O_2$200μM for 2h. Cell viability was then measured with the WST-1method. Finally, the cardioprotective effect of this isothiocyanate was 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 with sodium pentobarbital, sacrificed and the hearts were subjected to an I/R injury using the Langendorff apparatus. The ischemic area was detected by 1% aqueous solution of tetrazolium chloride.

Results: In the presence of organic thiols (L-Cysteine 4mM) ISOTHIA25at the concentration of 1mM was able to release about 60 μM of H_2S. In the absence of organic thiols, the H_2S release was negligible. Furthermore, the incubation of Isothia-25with the H9c2cells led to a significant increase of the H_2S release into the cells in a concentration-dependent manner.
ISOTHIA25showed a significant and concentration-dependent protection effect against the oxidative stress, resulting in an almost total recovery of the H9c2cell viability at the concentration of 1μM. The cardioprotective effect was also confirmed in the *ex vivo* model: the treatment with ISOTHIA25led to a decrease of the ischemic area of the left ventricle after the I/R injury. Accordingly, the lactate dehydrogenase LDH enzyme activity measured in the perfused Krebs solution resulted to be was reduced.

Discussion and conclusions: ISOTHIA25exhibits H_2S-releasing properties showed cardioprotective effects both in vitro and in *ex vivo* models: it increased the cell viability in cells exposed to oxidative stress and reduced the ischemic area in isolated rat hearts against the I/R injury. Thus, this compound can be viewed as a promising pharmacological tool for the treatment of such disease.