Cardiovascular characterization of p-Carboxyphenyl-Isothiocyanate as a novel H_2S-donor

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Hydrogen sulfide (H_2S), is a relevant mediator in cardiovascular physiology. Indeed, impaired production of H_2S contributes to the pathogenesis of important cardiovascular disorders [1].
Accordingly, exogenous compounds, acting as H_2S-releasing agents, are viewed as promising therapeutic agents for cardiovascular diseases. Thus, this work aimed at evaluating the H_2S-releasing properties of the p-Carboxyphenyl-Isothiocyanate (PhNCS-COOH) derivative and its cardiovascular effects.
H_2S release was determined by the amperometric approach. Unlike NaHS, a fast H_2S-donor widely used in the laboratory but unsuitable for clinical use, PhNCS-COOH exhibited a slow H_2S-releasing profile, similar to the slow-releasing reference drugs diallyldisulfide (DADS) and GYY4137. H_2S release from PhNCS-COOH occurred only in the presence of an excess of L-Cysteine: this thiol-dependency has been viewed as a particularly advantageous property, because it allows this compound to release H_2S only in a biological environment.
The vascular activity of PhNCS-COOH was tested in rat aorta and coronary arteries. Like NaHS, PhNCS-COOH displayed concentration-dependent vasorelaxing effects on endothelium-denuded rat aortic rings. PhNCS-COOH also inhibited the vasoconstricting effect of noradrenaline (NA), with greater potency than NaHS.
In addition, the isothiocyanate derivative increased basal coronary flow similarly to NaHS. Furthermore PhNCS-COOH was more effective than NaHS in counteracting the coronary vasoconstriction induced by angiotensin II.
Since H_2S is known to hyperpolarize vascular smooth muscle by activating K_{ATP} and Kv7 channels [1,2], we evaluated its effects on the membrane potential of human aortic smooth muscle cells (HASMC) using a membrane potential sensitive fluorescent dye. In this experimental protocol, PhNCS-COOH 100mM evoked a marked hyperpolarization when compared with levocromakalim, a K_{ATP}-opener used as reference drug. In contrast, NaHS caused weaker membrane hyperpolarization [3].
We next evaluated the cardioprotective activity of PhNCS-COOH in ex-vivo models of ischemia-reperfusion (IR), such as Langendorff-perfused hearts from Wistar rats and C57BL/6J mice. IR caused marked damage to isolated rat hearts, as shown by a 50% impairment of myocardial contractility, associated with a high degree of tissue injury, measured by morphometric analysis. PhNCS-COOH significantly improved functional parameters and reduced the ischemic area. This improvement was completely abolished by a selective blocker of mitochondrial K_{ATP} channels (5-hydroxydecanoic acid). The involvement of this channel was confirmed in isolated rat cardiac mitochondria in which PhNCS-COOH caused depolarization and inhibition of Ca^{2+} uptake. In agreement with these results, PhNCS-COOH showed protective effects also in mouse hearts, attenuating IR-induced arrhythmias and decreasing NA release.
In conclusion, PhNCS-COOH can be viewed as a suitable slow H_2S-releasing drug, endowed with vasorelaxing and cardioprotective effects, typical of the endogenous gasotransmitter. PhNCS-COOH might be employed as a novel chemical tool in basic studies and in the development of novel protective drugs in cardiovascular diseases.

[1] Martelli et al. (2012) *Med Res Rev.* 32(6), 1093-1130.
[2] Martelli et al. (2013) *Pharmacol Res.* 70(1), 27-34.
[3] Martelli et al. (2014) *Vasc Pharmacol.* 60(1), 32-41.