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Slow Releasing H_2S Donor-Thioglycine Exerts Cardioprotective Effects in Myocardial Ischemia/ Reperfusion in Vivo

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Background: H_2S is produced continuously at low levels in biological systems playing an important role in the regulation of cardiovascular function[1]. The diffusion ability of this gaseous molecule turns it into an attractive pharmacological agent for cardioprotection. Exogenous administration of rapid releasing H_2S donors affords cardioprotection during ischemia/ reperfusion injury[2]. **Purpose:** Pharmacological characterization of newly synthetized thioaminoacids (thioglycine, L-thiovaline and L-thiolysine) as slow releasing H_2S donors. Further investigation of the cardioprotective role of thioglycine administrated in a rabbit model of ischemia/reperfusion injury and study of the underlying molecular mechanism(s) involved. **Methods:** H_2S release was determined through methylene blue method and a fluorescence- based assay[3]. Changes in cGMP levels in smooth muscle cells were measured by EIA in the absence of a phosphodiesterase inhibitor. *In vivo* infarct size was determined in 4 groups of anesthetized rabbits subjected to 30 minutes ischemia and 3 hours reperfusion: 1) *Control* group, no further intervention, 2) *Thioglycine* group, thioglycine was administrated at a dose of 16.26 $\mu g*kg^{-1}$ bolus on the 20^{th} min of ischemia followed by infusion of 0.16226 $mg*kg^{-1} *h^{-1}$ for the next 120 min, 3) *NaHS* group, NaHS was administrated at a dose of 100 $\mu g*kg^{-1}$ bolus on the 20^{th} min of ischemia followed by infusion of 1 $mg*kg^{-1} *h^{-1}$ for the next 120 min and 4) *PostC* group, animals were subjected to 8 cycles of 30sec ischemia/30sec reperfusion immediately after sustained ischemia. Drugs were administrated in saline. Dose of thioglycine was estimated as 1/10 equimolar of cardioprotective dose of NaHS[4]. The ratio of the infarct size (I) and the corresponding area at risk (R) was expressed as % I/R. Additional rabbits were subjected to the previous interventions up to 10^{th} min of reperfusion for Akt, eNOS and GSK3β assessment. **Results:** Thioglycine released more H_2S than L-thiolysine and L-thiovaline reaching a plateau after 60 min, in contrast to the rapid rate observed with NaHS. Exposure of cultured rat aortic smooth muscle to thioaminoacids led to a concentration-dependent increase in cGMP levels. Exposure to L-thiolysine and thioglycine had a much more robust effect on cGMP levels than NaHS. Glycine, valine and lysine failed to increase cGMP levels. In the *in vivo* model of ischemia/reperfusion the following results were obtained:

Groups	**Infarct/Area at**	**Phosphorylation state**

	Risk (±1SEM) %	*Akt*	*eNOS*	*GSK3β*
Control	45,30 ± 2,3	-	-	-
Thioglycine	17,70 ± 2,0 *	+ *	-	-
NaHS	12,30 ± 3,3 *	+ *	-	-
PostC	26,00 ± 2,3 *	+ *	+ *	+ *
	*p<0,05 vs Control group		*p<0,05 vs all other groups	

Conclusion: Thioaminoacids liberate H_2S at a slow rate versus inorganic salts and enhanced cGMP formation. Thioglycine triggered pharmacological postconditioning in rabbits during myocardial ischemia/reperfusion. The cytoprotective mechanism may involve activation of Akt and occurs independently of GSK3β and eNOS.

References: (1) Wang R, *Physiol Rev* 92: 791, 2012 (2) Lefer DJ et al, *Clin Sci* 120:219, 2011 (3) Zhou Z et al, *Bioorg Med Chem* 20:2675, 2012 (4) Bibli et al, ESC 2013, Amsterdam

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