The Use of Hydrogen Sulfide Donor in Cardiac Control During Low Doses of Ionizing Radiation (Experimental Study)

Iryna Kovalchuk[1], Mechyslav Gzhegotskyi[1], Svitlana Kovalchuk[1], Vasyl Dukach[1]

[1] Department of Physiology, Danylo Halytsky Lviv National Medical University, Ukraine

Corresponding author: Iryna Kovalchuk, tarakanchikova@gmail.com

Abstract

Aim: To analyze the influence of the hydrogen sulfide donor NaHS on the cardiac control in rats during low doses of ionizing radiation and characterize the predictors of imbalance in autonomic nervous system activities.

Methods: Adult male rats were treated once with NaHS 7.4 mg/kg, i.p. or vehicle (saline), without and with total body irradiation (TBI). The irradiation of animals in experimental groups was single-fractional, total, with total absorbed dose – 2 Gy. Heart rate variability (HRV) was recorded in rats after 30 minutes with NaHS pre-treatment, 24 h of TBI exposure and NaHS and TBI combination.

Results: HRV analysis revealed that low doses of radiation cause cardiac autonomic dysfunction. TBI rats exhibited signs of activation of sympathetic nervous system, while NaHS-treated rats showed mobilization of all parts of regulatory systems with predominant activation of the parasympathetic nervous system (PSNS). Overall, these results indicate that NaHS is involved in cardiac control and decreases sensitivity to low doses of radiation.

Conclusion: The gaseous messenger H_2S under conditions of radiation modulates adaptive processes, acting as a regulator for imbalance of autonomic nervous system and may serve as a novel cardioprotective agent for ANS imbalance.

(Kovalchuk I, Gzhegotskyi M, Kovalchuk S, Dukach V. The Use of Hydrogen Sulfide Donor in Cardiac Control During Low Doses of Ionizing Radiation (Experimental Study). SEEMEDJ 2018; 2(1); 36-43)

Received: Dec 22, 2017; revised version accepted: Nov 30, 2018; published: November 27. 2018

KEYWORDS: hydrogen sulfide H_2S, sodium bisulfide, radiation effects, autonomic nervous system (ANS), cardiovascular system (CVS), heart rate control

Introduction

Recent data on extracellular and intracellular control of biological activities of gas transmitter Hydrogen Sulfide (H_2S) has shown that it plays multiple roles in several signaling pathways of physiological functions in almost all systems of the body [4, 12, 22]. According to the latest data, H_2S is an important key regulator in the cardiovascular system [1, 17], neural signaling [27], gastrointestinal tract [13, 25], anti-inflammatory activity [28], as well as metabolic disorders [29]. Moreover, it has been established that the cardio-protective effect of hydrogen sulfide manifests in the reduction of myocardial damage during ischemia-reperfusion injury, oxidative stress, etc.[8, 20, 21]. H_2S in physiological endogenous concentrations shows local [24, 25] and central effects (control of blood pressure and cardiac activity) [4, 12]. According to the other reports, H_2S has been registered to have dose-dependent negative chronic and inotropic effects on the heart, which obviously depends on the initial state of the myocardium [23]. It is known that H_2S can act as a free radical scavenger, reacting with radical oxygen species (ROS) and radical nitrogen species (RNS), including superoxide anion radical, peroxide, peroxynitrite and hypochlorite [5, 30, 14]. Several studies have also been conducted to show mechanisms of regulation of the physiological functions involving higher levels of control with the participation of H_2S. Thus, the authors of the present study tried to analyze the impact of hydrogen sulfide on peripheral and central mechanisms involved in control of cardiac activity. One of the integrative tests for assessing the functional activity of different levels of regulatory systems was the monitoring of heart rate variability (HRV) [3, 7, 11, 16]. This non-invasive method enables not only high accuracy in assessing the activity of the autonomic nervous system (ANS) in experimental conditions, but also reflects the physiological and biochemical regulatory mechanisms, the capacity of aerobic metabolism, as well as the physiological reserve capabilities of the physiological system [15, 18, 19].

Due to its high reducing properties, H_2S participates in the regulation of changes in oxidative and nitrosative stress [11, 14, 17]. One of the factors that causes oxidative stress is the effect of ionizing radiation. Natural and man-made sources of ionizing radiation contribute to human exposure and, accordingly, increase potential risk to human health. Much radiation is unavoidable, e.g., natural radiation, and represents a potential health risk [6]. As known, the human body responds to various physical, chemical and biological stimuli by developing a potent inflammatory response that triggers defense mechanisms crucial for maintaining tissue integrity and restoring tissue homeostasis and functions. Ionizing radiation-induced systemic effects usually arise from local exposure of an organ or part of the body [10]. The target for low doses of radiation are the membranes of subcellular and cellular structures, in which free radical processes are induced. For experimental rats, who are highly radioresistant animals (LD_{50} 7 – 7.5 Gy), the dose of 2 Gy is considered low [2]. Relatively little work has been done on this topic associated with the effects of H_2S and its main mechanisms involved in the systemic actions of ionizing radiation on the level of the whole body or on cellular level. Recently, it has been shown that low-dose radiation has a harmful systemic effect on the human body [6, 10, 26], but the influence of NaHS pretreatment during LDR injury is still not clear.

Therefore, the aim of this study was to analyze the effects of exogenous administration of hydrogen sulfide donor NaHS on cardiac control in rats under LDR and implement a comprehensive analysis of these changes in the parameters of HRV.

Materials and methods

Twenty-five male rats with an average weight 180-200 g that were used in our study were kept in conditions that were in accordance with university policies, and all experiments were approved by the Ethics Committee of Lviv National Medical University (20.01.2015; №1). The animals were selected into experimental groups (n=5): 1) control group – rats with single administration of 0.9% solution of NaCl at a dose 0.5 ml, i.p.; 2) group with single administration of NaHS at a dose of 7.4 mg/kg (Sigma Aldrich, USA), i.p. and registration of HRV values in 30 minutes; 3) group with administration of NaHS at the same dose and determination of HRV in 1 day; 4) group that underwent irradiation in the total absorbed dose of 2 Gy; 5) group with introduction of NaHS + irradiation (2 Gy). Animals of the experimental group were irradiated with a single-fractional teletherapy machine device "Teragam" (source 60Co) at a dose rate of 0.0393 R/s and a "source-surface" of 0.8 m. The total absorbed dose was 2 Gy. During irradiation, the animals were placed in individual cages-fixators.

We recorded a peripheral pulse non-invasively in non-anaesthetized animals for the purpose of HRV analysis (Hzhehotskyi MR, Patent UA, 2008) [9]. To record the parameters, 5 minutes after the rats stabilized, they were placed in a Plexiglas chamber and a photoplethysmographic transducer was attached to the base of each animal's tail [9]. Measuring of HRV parameters was carried out before the administration of NaHS, after 30 minutes and one day after the procedure, as well as one day after irradiation. Changes in HRV parameters were compared to their baseline values (before manipulations), which served as a control.

The duration of cardiointervals (AverVal Interv) was determined using software and a special fast-acting recording device [9]. During recording, dynamic series of cardiointervals were presented in the form of a cardiointervalograms. There was simultaneous analysis of dynamic series of cardiointervals and interpretation of HRV analysis data. The activity of the system-regulatory mechanisms of experimental animals was evaluated on the basis of spectral statistical methods, as well as variation pulsometry. Among the statistical parameters of the dynamic series of cardiointervals, the following indexes were determined: SDNN – the mean square deviation of the mean cardiointerval duration, CV – the coefficient of variation of the full array of cardiointervals, which characterizes the total effect of autonomous blood flow regulation; RMSSD – the square root of the sum of the differences in the series of cardiointervals, which was an indicator of activity of the parasympathetic link of autonomous regulation [3, 18].

Among the parameters of the variational pulsometry, the following ones were determined: the difference between the maximum and minimum values of cardiointervals (MxDMn) – the maximum amplitude of regulatory influences; mode (Mo) – the most likely level of functioning of the sinus node of the heart; mode amplitude (AMo) was a conditional indicator of the activity of the sympathetic link of regulation [3, 18]. With the help of spectral analysis, it was possible to characterize such parameters as the total power of the HRV spectrum (TP, 0.015 – 3.0 Hz) – the total absolute level of activity of regulatory systems [3, 18]. For the correct reproduction of the results of studies conducted in laboratory animals, the analysis of HRV was carried out using spectral components in the following frequency bands: Low Frequency – LF (0.015-0.25) Hz, which was associated with the level of activity of ergotropic slow influences, Mid Frequency – MF – (0.25-0.75) Hz, caused by sympathetic modulating effect on the cardiovascular system, High Frequency – HF – (0.75-3.0) Hz, which was characteristic of the activity level of the parasympathetic link of regulation [20, 21]. The digital results of all measurements were subjected to statistical variation analysis. The results were processed using Microsoft Excel spreadsheets and statistical software Statistica for Windows.

Results

As a result of the conducted analyses, it was found that the total power of the spectrum has increased to 78% in comparison to the basal level (control group) due to the increase of spectral power in all frequency bands, significantly 30 minutes after the administration of the H2S donor (Figure 1). Analysis of the internal structure of the spectrum has shown increased proportion of HF (24% ($p<0.05$)) and tendency of decreasing proportion of MF and LF. Correspondingly, the sympathovagal index (MF/HF) decreased to 26% (<0.05) vs the control group (Figure 1).

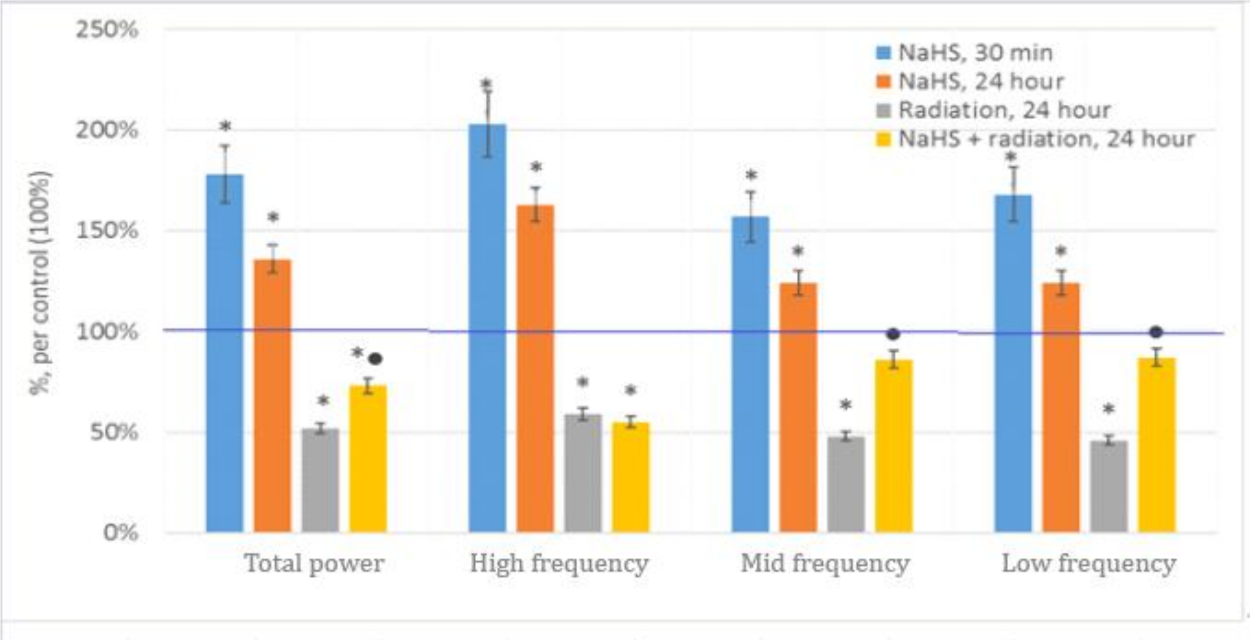

Figure 1. Changes in HRV spectrum indexes under the radiation exposure and previous administration of NaHS vs control group, which is marked as 100 % (blue line). Note: *– p<0.05 significance in relation to the control group; • – p<0.05 significance in relation to the irradiation group.

Also, the increased spectral power in the range of high frequency oscillations was detected, which correlates with rise of CV to 75% ($p<0.05$), SDNN to 32%, RMSSD to 41% ($p<0.05$), and MxDMn to 53% ($p<0.05$)). In general, this testifies to the activation of the autonomous circuit of regulation of cardiac activity (Table 1).

Decreased AM o (18% ($p<0.05$)), as well as MF/HF in comparison to control group indicates inhibition of sympathetic nervous system (SNS) activity. At the same time, a positive chronotropic effect was recorded, which may result in an increase in cardiac output (Table 1).

Table 1. Changes of HRV in rats

Parameters	Control	NaHS 30 min	NaHS 1st day	Irradiation 1st day	NaHS + irradiation 1st day
HR (heart rate), bpm	431.4±27.3	539.3±39.7*	444.7±29.7	433.1±31.2	406.1±24.7
SDNN (mean square deviation of the mean cardiointerval duration), s	0.030±0.003	0.039±0.003*	0.037±0.002*	0.024±0.002	0.029±0.002
CV (coefficient of variation), %	22.1±19	38.6±2.8*	27.9±2.3 *	17.7±1.3*	19.0±1.5
RMSSD (square root of the sum of the differences in series of cardiointervals), s	0.031±0,003	0.043±0.004*	0.038±0.003	0.025±0.002	0.026±0.002
Mo (mode), s	0.151±0.012	0.151±0.014	0.151±0.013	0.147±0.012	0.151±0.013
AMo (mode amplitude), %	37.1±3.1	30.4±2.7	25.4±2.1 *	36.3±3.2	37.2±3.4
MxDMn (maximum and minimum values of cardiointervals), s	0.128±0.011	0.196±0.017*	0.148±0.013	0.138±0.012	0.122±0.014

Note: * – $p<0.05$ significance in relation to the control group.

The results of HRV 24 h after administration of NaHS have shown noted maintenance of a high level of TP which exceeded the control value on 37% ($p<0.05$), however, it decreased on about 23% ($p<0.05$) after the influence of hydrogen sulfide on the 30th minute (Figure 1). As in the previous term, the spectral power increased the most in the range of high-frequency oscillations, both in comparison with the initial level and the level measured 30 minutes after the influence of NaHS. The increase of statistical parameters of HRV as CV, SDNN, RMSSD, as well as MxDMn (on average 25% ($p<0.05$) vs control data) was also noted, which indicates an increase in the tonus of the PSNS, but it was of a lesser degree of severity than data recorded at the 30th minute (Table 1).

One day after TBI at a dose of 2 Gy, the total power of spectrum (TP) decreased twofold compared to the control group (Figure 1). The decrease in power in all spectrum ranges was recorded: LF and MF to 54% and 52% ($p<0.05$) respectively, HF – to 40% ($p<0.05$). This data suggests a significant inhibition of the activity of all parts of cardiac regulatory systems. In that case, in the internal structure of the spectrum, the proportion of LF decreased to 16%, and MF practically did not change. The reduction of HRV time parameters (SDNN, CV, RMSSD) was marked (20% on average ($p<0.05$)), which indicates inhibition of PSNS activity (Table 1). In terms of the influence of irradiation accompanied by administration of NaHS, it was found that the values of time indexes have increased significantly relative to irradiation and almost reached the baseline (Table 1). Thus, the total power of the spectrum 1 day after irradiation accompanied by administration of a donor hydrogen sulfide was reduced to 27% relative to the results of control, but it was significantly higher than at single irradiation (Figure 1). At the same time, the spectral power in the range of LF and MF, which was 86% vs control, was significantly increased compared to the rats with TBI effect.

Discussion

The obtained data are consistent with the results of other researchers, which have established the cardioprotective effect of H_2S, manifested by an increase in the strength of cardiac contractions, as well as vasodilator effect in the period after the administration of a donor hydrogen sulfide [12, 13]. The increase in TP, as well as the spectral power in the HF and MF, RMSSD and other HRV statistical indexes, the decrease in MF/HF can be regarded as an increase in functional and metabolic reserves under the influence of H_2S. Consequently, the revealed complex array of changes in the parameters of HRV indicates the mobilization of all regulatory systems with the predominant activation of the parasympathetic nervous system (PSNS) after the administration of NaHS [8, 11].

An elevated level of balanced autonomous components (HF + MF) and decrease of MF/HF index was a prominent marker of influence of peripheral regulatory system on central control. The obtained results of the study indicated a protective effect of NaHS under influence of LDR, which indirectly indicates improvement of processes of adaptation to the action of radiation under the influence of hydrogen sulfide biosynthesis modification [19], which consisted of activation of various links of regulatory systems with prevailing mobilization of the parasympathetic nervous system and moderate activation of peripheral control system regulated by H_2S. On the 1st day after administration of NaHS, there was a prolonged effect in comparison to that found in the initial (30 min.) period of exposure to the hydrogen sulfide donor, NaHS, which ensures the maintenance of increased activity of regulatory processes. Thus, the reduction of the total effect of autonomic regulation by SDNN, RMSSD and CV correlated with the reduced total level of activity of regulatory processes by the spectral index of TP and indicated the activation of central regulatory systems. The previous administration of NaHS before irradiation led to a less pronounced reduction in TP than with irradiation alone, indicating the maintaining of a much higher level of regulatory processes in these conditions than with irradiation alone.

The effect of donor hydrogen sulfide NaHS against low-dose radiation injury, which was reflected in the modulation of adaptive processes, acting as a regulator of imbalance of autonomic nervous system, may serve as a novel cardioprotective agent increasing the capacity of restorative processes.

Conclusion

In conclusion, the data of the present study support that exogenous stimulation of peripheral H_2S-dependent signaling processes by administration of NaHS significantly affected the systemic regulatory mechanisms of cardiac activity on different levels. After 30 minutes and 1 day following the administration of the donor hydrogen sulfide, activation of all the links of regulatory systems with the predominant mobilization of PSNS was marked. One day after irradiation at a dose of 2 Gy, the TP decrease was twofold, with decreasing power in all ranges, indicating significant inhibition of the activity of all regulatory systems. Finally, irradiation accompanied by previous administration of NaHS leads to a less pronounced reduction in TP than with irradiation alone, indicating the maintaining of a higher level of regulatory processes.

Acknowledgement. None.

Disclosure
Funding. No specific funding was received for this study.
Competing interests. None to declare

References:

1. Apaijai N, Pintana H, Chattipakorn SC et al. Effects of vildagliptin versus sitagliptin, on cardiac function, heart rate variability and mitochondrial function in obese insulin-resistant rats. Br J Pharmacol 2013;169:1048–1057.

2. Asrar M. Havas. The biosensitivity of certain organs in rats exposed to low doses of γ -radiation. 2013;6:56-62.

3. Baevsky RM, Ivanov GG, Chirejkin LV et al. The analysis of Heart Rate Variability with use various electrocardiographical systems. The Bulletin of arrhythmology 2001;24:65-87.

4. Berezovsky VJ, Plotnikova LM. Hydrogen sulfide and its role in the vascular tone regulation. Med hydrol and rehabilit 2012;10:4–10.

5. Cipak Gasparovic A, Zarkovic N, Zarkovich K, Semen K, Kaminskyy D, Yelisyeyeva O, Bottari SP. Biomarkers of oxidative and nitro-oxidative stress: conventional and novel approaches. Br J Pharmacol 2017;174(12):1771–1783. doi: 10.1111/bph.13673

6. Tang FR, Loke WK. Molecular mechanisms of low dose ionizing radiation-induced hormesis, adaptive responses, radioresistance, bystander effects, and genomic instability Int J Radiat Biol 2015;91(1):13-27. doi: 10.3109/09553002.2014.937510.

7. Hillebrand S1, Gast KB, de Mutsert R, Swenne CA, Jukema JW, Middeldorp S, Rosendaal FR, Dekkers OM. Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose-response meta-regression. Europace 2013;15(5):742-9. doi: 10.1093/europace/eus341.

8. Hoshovs'ka JV, Shymans'ka TV, Semenychina OM, Sagach VF. Cardioprotective effects of hydrogen sulfide donor. Fiziol Zh 2012;58(6):3-15.

9. Hzhehotskyi MR, Storchun YV, Panina LV, Kovalchuk SM et al. Method for evaluation of experimental animal functional state based on heart rate variability test. Patent 29596 UA, 2008. http://uapatents.com/5-29596-sposib-ocinki-funkcionalnogo-stanu-eksperimentalnikh-tvarin-na-osnovi-analizu-variabelnosti-sercevogo-ritmu.html

10. Mavragani IV, Laskaratou DA, Frey B, Candéias SM, Gaipl US, Lumniczky K, Georgakilas AG. Key mechanisms involved in ionizing radiation-induced systemic effects. A current review. Toxicology Research 2016;5:12-33.

11. Jeppesen J, Fuglsang-Frederiksen A, Brugada R, Pedersen B, Rubboli G, Johansen P, Beniczky S. Heart rate variability analysis indicates preictal parasympathetic overdrive preceding seizure-induced cardiac dysrhythmias leading to sudden unexpected death in a patient with epilepsy. Epilepsia 2014;55(7):e67-71. doi: 10.1111/epi.12614.

12. Kimura H. Hydrogen polysulfide signaling along with hydrogen sulfide (H2S) and nitric oxide (NO). J Neural Transm 2016;123(11):1235-45.

13. Magierowski M, Magierowska K Hubalewska-Mazgaj M, Adamski J, Bakalarz D, Sliwowski Z, Pajdo R, Kwiecien S, Brzozowski T. Interaction between endogenous carbon monoxide and hydrogen sulfide in the mechanism of gastroprotection against acute aspirin-induced gastric damage. Pharmacol. Res 2016;114:235-250. doi: 10.1016/j.phrs.2016.11.001

14. Mancardi D, Penna C, Merlino A, Del Soldato P, Wink DA, Pagliaro P Physiological and pharmacological features of the novel gasotransmitter: Hydrogen sulfide. Biochimica et

Biophysica Acta (BBA)-Bioenergetics 2009;1787:864-72.

15. McCarthy R, Shaffer F. Heart rate variability: new perspectives on physiological mechanisms, assessment of self-regulatory capacity and health risk. Global advances in health and medicine 2015;1:46-61.

16. Monfredi O, Lyashkov AE, Johnsen AB, Inada S, Schneider H, Wang R, Nirmalan M, Wisloff U, Maltsev VA, Lakatta EG, Zhang H, Boyett MR. Biophysical characterization of the underappreciated and important relationship between heart rate variability and heart rate. Hypertension. 2014;64:1334-43.

17. Mys LA, Budko AYu, Strutynska N.A, Sagach VF. Pyridoxal-5-phosphate restores hydrogen sulfide synthes and redox state of heart and blood vessels tissue in old animals. Fiziol Zh 2017;63(1):3-9.

18. Reyes del Paso GA, Langewitz W, Mulder LJ, van Roon A, Duschek S. The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies. Psychophysiology 2013;50:477-87. doi: 10.1111/psyp.12027

19. Sacha J. Interaction between heart rate and heart rate variability. Ann Noninvasive Electrocardiol 2014;19:207–216.

20. Sgoifo A, Carnevali L, Alfonso Mde L, Amore M. Autonomic dysfunction and heart rate variability in depression. Stress 2015;18:343-52. doi: 10.3109/10253890.2015.1045868.

21. Shaffer F, Ginsberg JP. An overview of heart rate variability. Metrics and norms. Front Public Health 2017;5:258.

22. Shao M, Zhuo C, Jiang R, Chen G, Shan J, Ping J, Tian H, Wang L, Lin C, Hu L. Protective effect of hydrogen sulphide against myocardial hypertrophy in mice. Oncotarget. 2017;8(14):22344-22352. doi: 10.18632/oncotarget.15765.

23. Strutyns'ka NA, Semenykhina OM, Chorna SV, Vavilova HL, Sahach VF. Hydrogen sulfide inhibits calcium–induced mitochondrial pore opening in the heart of adult and old rats. Fiziol Zh 2011;57(6):3-15.

24. Wallace JL, Blackler RW, Chan MV, Da Silva GJ, Elsheikh W, Flannigan KL, Gamaniek I, Manko A, Wang L, Motta JP, Buret AG. Anti-inflammatory and cytoprotective actions of hydrogen sulfide: translation to therapeutics. Antioxid Redox Signal 2015;22:398-410.

25. Wallace JL, Muscara MN. Hydrogen sulfide: an endogenous mediator of resolution of inflammation and injury. Antioxid Redox Signal 2012;17:58-67.

26. William FM, William JB. Issues in low dose radiation biology: the controversy continues. A perspective. Radiation research 2013;179: 501-510.

27. Zang X, Bian JS. Hydrogen sulfide: a neuromodulator and neuroprotectant in the central nervous system. ACS Chem Neurosci 2014;5:876-83.

28. Zayachkivska O, Bula N, Khyrivska D, Gavrilyuk E, Wallace JL. Exposure to non-steroid anti-

inflammatory drugs (NSAIDs) and suppressing hydrogen sulfide synthesis leads to altered structure and impaired function of the oesophagus and oesophagogastric junction. Inflammopharmacology 2015;23:91-9.

29. Zayachkivska O, Havryluk O, Hrycevych N, Bula N, Grushka O, Wallace JL. Cytoprotective effects of hydrogen sulfide in novel rat models of non-erosive esophagitis. PloS one. 2014;9(10):e110688.

Zhao Y, Biggs TD, Xian M. Hydrogen sulfide (H_2S) releasing agent: chemistry and biological application. Chem Commun (Camb) 2014;50(80):11788-805. doi: 10.1039/c4cc00968a.