FOLIA MEDICA CRACOVIENSIA
Vol. LVII, 1, 2017: 55–64
PL ISSN 0015-5616

Nicotine affects hydrogen sulfide concentrations in mouse kidney and heart but not in brain and liver tissues

Jerzy Wiliński[1], Bogdan Wiliński[2], Eugeniusz Somogyi[3], Joanna Piotrowska[3], Tomasz Kameczura[4], Małgorzata Zygmunt[5]

[1]1st Department of Internal Medicine with Cardiology Subdivision, Blessed Marta Wiecka District Hospital Bochnia, Poland
[2]Department of Human Developmental Biology, Institute of Nursing and Midwifery Faculty of Health Sciences, Jagiellonian University Medical College, Kraków, Poland
[3]Department of Inorganic and Analytical Chemistry, Jagiellonian University Medical College Kraków, Poland
[4]Department of Electroradiology, Institute of Nursing and Health Sciences, Faculty of Medicine University of Rzeszow, Rzeszów, Poland
[5]Department of Pharmacological Screening, Jagiellonian University Medical College, Kraków, Poland

Corresponding author: Jerzy Wiliński, MD, PhD
1st Department of Internal Medicine with Cardiology Subdivision, Blessed Marta Wiecka District Hospital
ul. Krakowska 31, 32-700 Bochnia, Poland
Phone: +48 14 615 33 17; Fax: +48 14 615 32 02; E-mail: putamen@interia.pl

Abstract: Nicotine, a potent parasympathomimetic alkaloid with stimulant effects, is contributing to addictive properties of tobacco smoking and is though used in the smoking cessation therapy. Hydrogen sulfide (H_2S) is involved in physiology and pathophysiology of various systems in mammals. The interactions between nicotine and H_2S are not fully recognized. The aim of the study is to assess the influence of nicotine on the H_2S tissue concentrations in different mouse organs. Adult CBA male mice were administered intraperitoneally 1.5 mg/kg b.w. per day of nicotine (group D1, n = 10) or 3 mg/kg b.w. per day of nicotine (group D2, n = 10). The control group (n = 10) received physiological saline. The measurements of the free and acid-labile H_2S tissue concentrations were performed with the Siegel spectrophotometric modified method. There was a significant increase in H_2S concentrations in both nicotine doses groups in the kidney (D1 by 54.2%, D2 by 40.0%). In the heart the higher nicotine dose caused a marked decrease in H_2S tissue level (by 65.4%), while the lower dose did not affect H_2S content. Nicotine administration had no effect on H_2S concentrations in the brain and liver.

In conclusion, nicotine affects H_2S tissue concentrations in kidney and heart but not in the liver and brain tissues.

Key words: nicotine, hydrogen sulfide, brain, heart, kidney, liver, mouse.

Introduction

Nicotine is a potent parasympathomimetic alkaloid found in the nightshade family of plants including *Nicotiana tabacum*. It serves as an agonist towards different subtypes of five-subunit nicotinic acetylocholine receptors (nAChR), members of the 'Cys-loop' superfamily, whereas in specific subtypes of them — nAChRα9 and nAChRα10 it acts as an antagonist [1]. Nicotinic receptors play diverse and often critical functions throughout the central and peripheral nervous systems and have emerging roles in non-neuronal systems. The nAChRs have been identified i.a. in bronchial epithelial cells, endothelial cells, vascular smooth muscle cells, mesangial cells, inflammatory cells and keratinocytes [2, 3].

Nicotine has stimulant effects and is contributing to addictive properties of tobacco use and is therefore widely applied in the smoking cessation therapy [4]. Cigarette smoking has great socioeconomic repercussions — it is the leading cause of preventable mortality and disability worldwide — the habit affects one billion people throughout the world and causes near six million deaths each year [5]. Furthermore, nicotine administration poses several health hazards. The alkaloid has been demonstrated to affect cell proliferation, oxidative stress, apoptosis, decrease immune response and contribute to DNA damage what altogether leads in effect to increased risk of cardiovascular events, respiratory, reproductive and gastrointestinal disorders and cancer development [6, 7].

The studies of the recent decade have uncovered the biological significance of hydrogen sulfide (H_2S) and subsequently changed its perception from an odorous by-product of putrescence processes to a crucial co-modulator in physiology and pathophysiology of various systems in mammals [8]. H_2S is formed in a series of enzymatic reactions in which the substrates are sulfur-containing amino acids such as L-cysteine, L-homocysteine and D-cysteine. The key enzymes are cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS), cysteine aminotransferase (CAT), 3-mercaptopyruvate sulfur transferase (3-MST) and D-amino acid oxidase (DAO). H_2S is also a product in a few other enzymatic reactions of the complex sulfur metabolism and in non-enzymatic reactions [9]. It is recognized that H_2S acts mainly in a paracrine manner but it is also transported to other tissues as a component of the serum and inside erythrocytes. This gaseous messenger exerts a broad array of biological actions. No specific receptor to H_2S has been identified. The molecular

targets of H_2S are varied and comprise many proteins which participate in the regulation of crucial physiological processes. The most important mechanisms of action involve sulfhydration of proteins and their redox regulation, modification of prosthetic groups, interaction with nitric oxide (NO) and carbon monoxide (CO), participation in S-nitrozylation of proteins, interaction with reactive oxygen species (ROS) and electrophile lipid derivatives [10]. The biological effects of H_2S cover changes in the activity of ion channels, N-methyl-D-aspartate (NMDA) receptors, various signaling proteins, transcription factors, enzymes, structural and transport proteins [11].

Data on the share of H_2S in the development of complications of smoking are scarce. H_2S alleviated airway reactivity induced by acetylocholine (ACh) or potassium chloride and reduced inflammatory cytokines interleukin 8 and tumor necrosis factor α (TNF-α) in a rat model of chronic exposure to cigarette smoke [12]. The mediator ameliorated tobacco smoke-induced oxidative stress and emphysema in mice and pulmonary fibrosis in smoking rats via attenuation of oxidative stress and inflammation [13, 14]. H_2S improved left ventricular function in smoking rats via regulation of apoptosis and autophagy [15]. Additionally, mounting evidence shows pivotal role of H_2S in the function of the brain and changes of H_2S generation in cases of exposure to different compounds and drugs [9, 16, 17].

The interactions between nicotine, cigarette toxins and H_2S are not fully recognized [18]. The indication of the influence of nicotine on sulfur metabolism might help to evolve more efficient smoking cessation and addiction therapies. Moreover, the elaboration of compounds correcting sulfur biology disturbances observed in various smoking-related diseases might hamper their progression and improve mortality and morbidity outcomes.

The aim of the study is to assess the influence of nicotine on the endogenous H_2S tissue concentrations in different mouse organs.

Material and methods

Animals

The study has been approved by the II Local Ethics Committee for Animal Experimentation in Krakow at the Polish Academy of Sciences (Krakow, Poland) with the permission no. 24/2016 issued on 22nd March 2016.

Thirty CBA male mice (15-week-old individuals) of approximate 20 g weight were involved in the study. The animals were housed under standard laboratory conditions and had free access to water and food. They were kept at temperature of 22–24°C with a light/dark cycle of 12 h (8 am — 8 pm and 8 pm — 8 am, respectively).

Study design

With the median lethal dose estimated at around 4 kg per kg body weight in mice, the study protocol comprised intraperitoneal injections of nicotine dissolved in a saline solution in doses of 1.5 mg per kg of body weight daily (group D1, n = 10) or 3 mg per kg of body weight daily (group D2, n = 10) for 5 consecutive days at the same time of the day (9:00 am) — each administration of 0.2 ml [19]. The control group (n = 10) received intraperitoneally physiological saline in portions of the same volume. The individuals were randomly assigned to each group. The animals tolerated the applied doses of nicotine well and remained in good condition till the end of the experiment. Measurements of the free and acid-labile tissue H_2S concentrations were performed by the use of the modified method of Siegel [20, 21]. A standard curve was prepared with sodium sulfide solutions simultaneously analyzed by the iodometric titration.

Tissue sample preparation

An hour after the last nicotine solution or physiological saline injections the animals were killed by cervical dislocation. Brain, heart, kidney and liver tissues of each animal were quickly removed, homogenized with 0.01 M sodium hydroxide (NaOH) and frozen. Each tissue was combined with NaOH in different proportions (brain: 1 to 4, kidney and liver: 1 to 5 and heart: 1 to 10). Then, 50% trichloroacetic acid (TCA) was added to the samples. The TCA solution (0.5 ml) was added to 2 g of brain or liver samples in tight 3 ml capsules, and 0.25 ml was added to 1 g of heart or kidney sample in tight 2 ml capsules. These suspensions were shaken, and the resultant mixture was centrifuged. Subsequently, 1.5 ml brain or liver and 0.75 ml heart or kidney supernatant samples were moved to 2 ml tight capsules with 0.15 ml or 0.075 ml of 0.02 M N,N-dimethyl-p-phenylenediamine sulfate in 7.2 M hydrochloric acid (HCl), and 0.15 ml or 0.075 ml of 0.03 M iron (III) chloride ($FeCl_3$), respectively, was then added in 1.2 M HCl portions. After 20 min in the dark, the contents were shaken for 1 min with 1 ml of chloroform.

H_2S tissue concentration measurements

The absorbance was measured at 650 nm with a Varian Cary 100 spectrophotometer. A standard curve was prepared with an iodometrically determined 0.0001 M sodium sulfide (Na_2S) solution. Four concurrent analyses of every analyzed tissue type were performed for each group of animals.

Statistical analysis

The statistical analysis was performed with the Statistica 10 PL version (Statsoft, Tulsa, USA). Normal distribution of variables was disproved using the Shapiro-Wilk test. Subsequently, the existence of differences in H_2S tissue concentrations among groups within one organ were assessed with Kruskal–Wallis one-way analysis of variance. The Mann-Whitney U test was used in the post hoc analysis for comparisons between selected pairs of groups (control group vs D1 group and control group vs D2 group). Statistical significance was considered when $p < 0.05$.

Results

There was a significant increase in H_2S concentrations in both nicotine doses groups in the kidney (D1 by 54.2%, D2 by 40.0%). In the heart the higher nicotine dose caused a marked decrease in H_2S tissue level (by 65.4%), whereas the lower one did not affect H_2S content (control group vs D2 group: 8.45 ± 0.07 μg/g vs 8.15 ± 0.13 μg/g, $p = 0.1143$). Nicotine administration had no effect on H_2S concentrations in the brain and liver. H_2S tissue levels' results are presented in the Table 1.

Table 1. Hydrogen sulfide (H_2S) tissue concentration in mouse brain, heart, liver and kidney following the administration of 1.5 mg/kg b.w. per day or 3 mg/kg b.w. per day of nicotine (groups D1 and D2, respectively).

H_2S tissue concentration [μg/g]	Control group (n = 10)	D1 (n = 10)	D2 (n = 10)	Kruskal-Wallis one-way analysis of variance	Control vs D1 — Mann-Whitney U test	Control vs D2 — Mann-Whitney U test
Brain	1.70 ± 0.06	1.74 ± 0.03	1.81 ± 0.07	NS	NS	NS
Heart	8.45 ± 0.07	8.15 ± 0.13	2.92 ± 0.12	H(2, 12) = 8.80 p = 0.0123	NS	p = 0.0294
Liver	2.43 ± 0.06	2.73 ± 0.10	2.46 ± 0.08	NS	NS	NS
Kidney	4.48 ± 0.17	6.91 ± 0.21	6.27 ± 0.16	H(2, 12) = 9.85 p = 0.0073	p = 0.0286	p = 0.0303

NS — statistically not significant

Discussion

In our experiment nicotine administration caused H_2S tissue content elevation in the kidneys. Nicotine has worsened kidney function in different studies on animal and human acute and chronic renal failure. The alkaloid was shown to be a powerful stimulus for human mesangial cell proliferation and fibronectin production. These effects were accompanied by the increased activity of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase, leading to ROS and nitrotyrosine production, mitogen-activated protein kinases (MAPKs) activation, and were prevented by protein kinase C (PKC) inhibition [2, 22, 23]. Glomerular injury induced by nicotine was also shown to be mediated by augmented cyclooxygenase-2 (COX-2) expression and activity what was partially triggered by ROS [24]. Interestingly, NADPH oxidase, MAPKs, PKC are COX-2 are among molecular targets of H_2S and H_2S exerts a broad array of protective actions in kidney failure conditions [10, 25]. H_2S was demonstrated to inhibit nicotine and lipopolysaccharide-induced osteoclastic differentiation by blocking the activation of MAPKs, phosphoinositide 3-kinase (PI3K), PKC and nuclear factor-κB (NF-κB) [26]. Plasma concentration of H_2S correlated negatively with the levels of conventional PKC βII activation and cardiovascular mortality index in chronic hemodialysis patients [27]. H_2S administration in uranium-intoxicated rats inhibited uranium-induced nuclear translocation and phosphorylation of NF-κB-p65, which decreased protein expression of target-p65 inflammatory genes of TNF-α, inducible nitric oxide synthase (iNOS) and COX-2 [28]. H_2S quenched angiotensin II-induced NADPH expression in cardiac fibroblasts what was associated with decreased ROS production, reduced MAPKs (Erk 1/2) phosphorylation and connective tissue growth factor (CTGF) expression [29].

In contrast, there are studies demonstrating anti-inflammatory effects of nicotine occurring under specific circumstances. Single injections of the alkaloid attenuated the severity of renal injury in rat models of ischemia-reperfusion and in a model of sepsis which was associated with reductions in inflammatory cytokines and NF-κB, what is observed among numerous biological actions of H_2S [30–32]. Additionally, long-term administration of nicotine in the drinking water resulted in improvements in proteinuria and renal function in a rat model of proteinuria-induced renal inflammation with unclear mechanisms [33]. These protective effects of nicotine might be partially mediated by H_2S.

As we have shown, nicotine caused decrease in H_2S levels in the heart. Nicotine was proven to dysregulate sympathovagal balance in the heart, affecting heart rate, blood pressure, myocardial contractility, increasing myocardial demand for oxygen and nutrients, causing vasoconstriction and promoting tissue remodeling and fibrosis [3, 34]. In the study by JI and colleagues nicotine administration to isolated perfused rat hearts caused a brief decrease followed by a much larger increase in the heart

rate [35]. The effects were attributed by the authors to different nAChRs subtypes share but interactions with mediators in autonomic nervous system including H_2S should strongly be considered. H_2S was shown to modulate fast cholinergic synaptic input acting on presynaptic terminals in peripheral ganglia and neuromuscular junction activity what involved presynaptic ryanodine receptor [36, 37]. Furthermore, H_2S was considered as an endothelium-derived hyperpolarizing factor mediated by Ach in rat cerebral vessels [38].

It should be emphasized that nicotine is only one of more than 9000 compounds found in cigarette smoke. This smoke contains many oxidizing chemicals, CO, volatile organic compounds, particulates, heavy metals, all with more than 60 identified carcinogens [39, 40]. The effects of nicotine are accompanied by many other actions of cigarette smoke contents.

Growing evidence has revealed that H_2S improves heart function and reduces cardiac complications in different pathogenic conditions. H_2S exerts antioxidative actions, preserves mitochondrial function, has anti-inflammatory and antiapoptotic properties, angiogenic effects on the ischemic area, participates in the regulation of ion channels and microRNAs (miRNAs) expression, and interferes with NO and CO [41]. Thus, our findings of nicotine reducing H_2S content in the heart stresses the need for new directions of study on possible exogenous H_2S supplementation in smoking and nicotine replacement therapy.

We have not observed any effect of nicotine administration on H_2S tissue levels in the brain and liver. In tobacco smoking the addictive nature of nicotine involves psychoactive effects, drug-reinforced behavior, compulsive use, relapse after abstinence, physical dependence and tolerance [42]. These complex actions are mediated by close interactions of the glutamatergic, dopaminergic and γ-aminobutyric acidergic systems mainly in the mesocorticolimbic system [5]. H_2S is perceived as a potent neuroprotector and neuromodulator [9].

In the liver nicotine evoked organ damage inducing oxidative stress, biochemical changes and histopathological injuries [43–45]. To the contrary, H_2S displayed a crucial role in physiology and protective actions in liver pathology [46].

In conclusion, the influence of nicotine on H_2S tissue concentrations in the kidney and heart tissues proves that the biological actions of nicotine involve impact on sulfur metabolism of undetermined mechanisms. Interestingly, the interactions between H_2S and nicotine seem to begin long before nicotine is inhaled with cigarette smoke or administered as replacement therapy in smoking cessation: H_2S has been recently identified as a crucial modulator of nicotine biosynthesis in *Nicotiana tabacum* [47]. Our results are highly indicative but less conclusive. They open new fields of research for the use of H_2S donors and HS-releasing agents in the prevention of the complications of smoking and nicotine replacement therapy. Neither have we examined the influence of long-term exposure to nicotine, nor the effects of other

cigarette smoke constituents, what poses our experiment's limitations. No effect of nicotine on H_2S levels in the liver and brain absolutely does not preclude their interactions, especially in chronic exposure to nicotine.

Acknowledgments, funding, and disclosures

This study has not been funded.

Conflict of interest

None declared.

References

1. *Millar N.S., Gotti C., Marks M.J., Wonnacott S.*: Nicotinic acetylcholine receptors, introduction. IUPHAR Database. International Union of Basic and Clinical Pharmacology. Last modified on 15/10/2014. www.guidetopharmacology.org/GRAC/FamilyIntroductionForward?familyId=76.
2. *Jain G., Jaimes E.A.*: Nicotine signaling and progression of chronic kidney disease in smokers. Biochem Pharmacol. 2013; 86: 1215–1223.
3. *Benowitz N.L., Burbank A.D.*: Cardiovascular toxicity of nicotine: Implications for electronic cigarette use. Trends Cardiovasc Med. 2016; 26: 515–523.
4. *Jackson K.J., Muldoon P.P., De Biasi M., Damaj M.I.*: New mechanisms and perspectives in nicotine withdrawal. Neuropharmacology. 2015; 96: 223–234.
5. *Pistillo F., Clementi F., Zoli M., Gotti C.*: Nicotinic, glutamatergic and dopaminergic synaptic transmission and plasticity in the mesocorticolimbic system: focus on nicotine effects. Prog Neurobiol. 2015; 124: 1–27.
6. *Grando S.A.*: Connections of nicotine to cancer. Nat Rev Cancer. 2014; 14: 419–429.
7. *Mishra A., Chaturvedi P., Datta S., Sinukumar S., Joshi P., Garg A.*: Harmful effects of nicotine. Indian J Med Paediatr Oncol. 2015; 36: 24–31.
8. *Kimura H.*: Physiological roles of hydrogen sulfide and polysulfides. Handb Exp Pharmacol. 2015; 230: 61–81.
9. *Panthi S., Chung H.J., Jung J., Jeong N.Y.*: Physiological importance of hydrogen sulfide: emerging potent neuroprotector and neuromodulator. Oxid Med Cell Longev. 2016; 2016/9049782: 1–11.
10. *Zaichko N.V., Melnik A.V., Yoltukhivskyy M.M., Olhovskiy A.S., Palamarchuk I.V.*: Hydrogen sulfide: metabolism, biological and medical role. Ukr Biochem J. 2014; 86: 5–25.
11. *Olson K.R., Straub K.D.*: The role of hydrogen sulfide in evolution and the evolution of hydrogen sulfide in metabolism and signaling. Physiology (Bethesda). 2016; 31: 60–72.
12. *Chen Y.H., Wang P.P., Wang X.M., He Y.J., Yao W. Z., Qi Y.F., Tang C.S.*: Involvement of endogenous hydrogen sulfide in cigarette smoke-induced changes in airway responsiveness and inflammation of rat lung. Cytokine. 2011; 53: 334–341.
13. *Han W., Dong Z., Dimitropoulou C., Su Y.*: Hydrogen sulfide ameliorates tobacco smoke-induced oxidative stress and emphysema in mice. Antioxid Redox Signal. 2011; 15: 2121–2134.
14. *Zhou X., An G., Chen J.*: Inhibitory effects of hydrogen sulphide on pulmonary fibrosis in smoking rats via attenuation of oxidative stress and inflammation. J Cell Mol Med. 2014; 18: 1098–1103.
15. *Zhou X., An G., Chen J.*: Hydrogen sulfide improves left ventricular function in smoking rats via regulation of apoptosis and autophagy. Apoptosis. 2014; 19: 998–1005.

16. *Wiliński B., Wiliński J., Somogyi E., Piotrowska J., Góralska M.*: Amlodipine affects endogenous hydrogen sulfide tissue concentrations in different mouse organs. Folia Med Cracov. 2011; 51: 29–35.
17. *Wiliński J., Somogyi E., Góralska M., Wiliński B., Czarnecka D.*: Ramipril enhances the endogenous hydrogen sulfide tissue concentration in mouse heart and brain. Folia Med Cracov. 2008; 49: 123–130.
18. *Cakmak Y.O.*: Coffee consumption, smoking, and Parkinson's disease? The beneficial role of hydrogen sulfide. Mov Disord. 2016; 31: 429.
19. *Bontempi B., Whelan K.T., Risbrough V.B., Lloyd G.K., Menzaghi F.*: Cognitive enhancing properties and tolerability of cholinergic agents in mice: a comparative study of nicotine, donepezil, and SIB-1553A, a subtype-selective ligand for nicotinic acetylcholine receptors. Neuropsychopharmacology. 2003; 28: 1235–1246.
20. *Siegel L.M.*: A direct microdetermination for sulfide. Anal Biochem. 1965; 11: 126–132.
21. *Somogyi E., Piotrowska J., Rzeszutko W.*: An effect of some parameters of the determination of the hydrogen sulfide in pig liver and brain on the obtained results. Farm Prz Nauk. 2008; 5: 33–36. (In Polish with English summary).
22. *Hua P., Feng W., Ji S., Raij L., Jaimes E.A.*: Nicotine worsens the severity of nephropathy in diabetic mice: implications for the progression of kidney disease in smokers. Am J Physiol Renal Physiol. 2010; 299: F732–739.
23. *Jaimes E.A., Tian R.X., Raij L.*: Nicotine: the link between cigarette smoking and the progression of renal injury? Am J Physiol Heart Circ Physiol. 2007; 292: H76–82.
24. *Jaimes E.A., Tian R.X., Joshi M.S., Raij L.*: Nicotine augments glomerular injury in a rat model of acute nephritis. Am J Nephrol. 2009; 29: 319–326.
25. *Koning A.M., Frenay A.R., Leuvenink H.G., Van Goor H.*: Hydrogen sulfide in renal physiology, disease and transplantation — the smell of renal protection. Nitric Oxide. 2015; 46: 37–49.
26. *Lee S.K., Chung J.H., Choi S.C., et al.*: Sodium hydrogen sulfide inhibits nicotine and lipopolysaccharide-induced osteoclastic differentiation and reversed osteoblastic differentiation in human periodontal ligament cells. J Cell Biochem. 2013; 114: 1183–1193.
27. *Feng S.J., Li H., Wang S.X.*: Lower hydrogen sulfide is associated with cardiovascular mortality, which involves cPKCβII/Akt pathway in chronic hemodialysis patients. Blood Purif. 2015; 40: 260–269.
28. *Zheng J., Zhao T., Yuan Y., Hu N., Tang X.*: Hydrogen sulfide (H2S) attenuates uranium-induced acute nephrotoxicity through oxidative stress and inflammatory response via Nrf2-NF-κB pathways. Chem Biol Interact. 2015; 242: 353–362.
29. *Pan L.L., Liu X.H., Shen Y.Q., et al.*: Inhibition of NADPH oxidase 4-related signaling by sodium hydrosulfide attenuates myocardial fibrotic response. Int J Cardiol. 2013; 168: 3770–3778.
30. *Azizi F., Seifi B., Kadkhodaee M., Ahghari P.*: Administration of hydrogen sulfide protects ischemia reperfusion-induced acute kidney injury by reducing the oxidative stress. Ir J Med Sci. 2016; 185: 649–654.
31. *Yeboah M.M., Xue X., Duan B., et al.*: Cholinergic agonists attenuate renal ischemia-reperfusion injury in rats. Kidney Int. 2008; 74: 62–69.
32. *Yeboah M.M., Xue X., Javdan M., Susin M., Metz C.N.*: Nicotinic acetylcholine receptor expression and regulation in the rat kidney after ischemia-reperfusion injury. Am J Physiol Renal Physiol. 2008: 295: F654–661.
33. *Agarwal P.K., Van Den Born J., Van Goor H., Navis G., Gans R.O., Bakker S.J.*: Renoprotective effects of long-term oral nicotine in a rat model of spontaneous proteinuria. Am J Physiol Renal Physiol. 2012; 302: F895–904.
34. *Harte C.B.*: Concurrent relations among cigarette smoking status, resting heart rate variability, and erectile response. J Sex Med. 2014; 11: 1230–1239.
35. *Ji S., Tosaka T., Whitfield B.H., et al.*: Differential rate responses to nicotine in rat heart: evidence for two classes of nicotinic receptors. J Pharmacol Exp Ther. 2002; 301: 893–899.

36. *Sha L., Linden D.R., Farrugia G., Szurszewski J.H.*: Hydrogen sulfide selectively potentiates central preganglionic fast nicotinic synaptic input in mouse superior mesenteric ganglion. J Neurosci. 2013; 33: 12638–12646.
37. *Gerasimova E., Lebedeva J., Yakovlev A., Zefirov A., Giniatullin R., Sitdikova G.*: Mechanisms of hydrogen sulfide (H2S) action on synaptic transmission at the mouse neuromuscular junction. Neuroscience. 2015; 303: 577–585.
38. *Han J., Chen Z.W., He G.W.*: Acetylcholine- and sodium hydrosulfide-induced endothelium-dependent relaxation and hyperpolarization in cerebral vessels of global cerebral ischemia-reperfusion rat. J Pharmacol Sci. 2013; 121: 318–326.
39. *Huang M.F., Lin W.L., Ma Y.C.*: A study of reactive oxygen species in mainstream of cigarette. Indoor Air. 2015; 15: 135–140.
40. *Lau W.K., Cui L.Y., Chan S.C., Ip M.S. Mak J.C.*: The presence of serotonin in cigarette smoke — a possible mechanistic link to 5-HT-induced airway inflammation. Free Radic Res. 2016; 50: 495–502.
41. *Shen Y., Shen Z., Luo S., Guo W., Zhu Y.Z.*: The cardioprotective effects of hydrogen sulfide in heart diseases: from molecular mechanisms to therapeutic potential. Oxid Med Cell Longev. 2015; 2015/925167: 1–13.
42. *Caponnetto P., Campagna D., Papale G., Russo C., Polosa R.*: The emerging phenomenon of electronic cigarettes. Expert Rev Respir Med. 2012; 6: 63–74.
43. *Moździerz A., Rzepecka-Stojko A., Juszko-Piekut M., et al.*: The role of nicotine as a modulator of the activity of acid phosphotase and cathepsin D and L in the liver and kidney of mice. Przegl Lek. 2012; 69: 833–836. (In Polish with English summary).
44. *Salahshoor M., Mohamadian S., Kakabaraei S., Roshankhah S., Jalili C.*: Curcumin improves liver damage in male mice exposed to nicotine. J Tradit Complement Med. 2015; 6: 176–183.
45. *El Golli N., Jrad-Lamine A., Neffati H., et al.*: Impact of e-cigarette refill liquid with or without nicotine on liver function in adult rats. Toxicol Mech Methods. 2016; 26: 419–426.
46. *Mani S., Cao W., Wu L., Wang R.*: Hydrogen sulfide and the liver. Nitric Oxide. 2014; 41: 62–71.
47. *Chen X., Chen Q., Zhang X., et al.*: Hydrogen sulfide mediates nicotine biosynthesis in tobacco (Nicotiana tabacum) under high temperature conditions. Plant Physiol Biochem. 2016; 104: 174–179.