Праці НТШ
Мед. науки 2015. Т. XLIII. С. 63–74
Оригінальні дослідження:
Фундаментальні науки

Proc. Shevchenko Sci. Soc.
Medicine 2015. Vol. XLIII. P. 63–74
Research:
Fundamental sciences

УДК: 616.392/.33–018.73: 615.212

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INFLUENCE OF HYDROGEN SULFIDE-RELEASING ASPIRIN ON MUCOSAL INTEGRITY OF ESOPHAGEAL AND GASTRIC MUCOSA

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Introduction. *Aspirin is one of the widely prescribed antiplatelet and anti-inflammatory drugs and last data expanded its role into complex biological processes such as cancerogenesis, despite the fact that its effects for peptic ulcer or gastrointestinal (GI) bleeding, which can develop even in an achlorhydric environment, are well known. Recent extensive research of activities of hydrogen sulfide (H_2S) has proven that the addition of H_2S-releasing moiety to the classical nonstreroid anti-inflammatory drugs (NSAID) results in GI cytoprotective activity.*

The aim *of this study was to evaluate dose-dependent effects of a novel NSAID-H2S compound - ATB 340 (Antibe Therapeutics Inc), hydrogen sulfide-releasing derivative of aspirin, 4-(5-thioxo-5H-dithiol-3-yl) phenyl 2-acetoxybenzoate (H_2S-aspirin) vs classical aspirin (ASA) on esophageal and gastric mucosal integrity.*

Methods: *A blind, randomized study on rats treated with single administration of the vehicle (control), ASA (in the doses 3.0; 10.0; 30.0; 100.0 mg/kg) and ATB-340 (in the doses 5.25; 17.5; 52.5; 175.0 mg/kg) with a vehicle (1.0 ml of 1% carboxymethylcellulose) and 7-days administration of ASA (3.0 and 10.0 mg/kg) and ATB-340 (5.25 and 17.5 mg/kg) was done. The damage of the esophageal mucosa (EM) and gastric mucosa (GM) was estimated by macro- and microhistological analyses using damage scores; endothelial leukocyte adherence was examined using intravital microscope before and after ASA and ATB-340 administration.*

Results: *Treatment by ATB-340 resulted in cyprotective effect and lower grade of EM and GM lesions with damage score being twice less than ASA. Effective cytoprotective and an anti-inflammatory dose of ATB-340 was 17.5 mg/kg during single or 7-day administration. Treatment by ATB-340 (in the dose of 17.5 mg/kg) vs ASA exerted a strong anti-inflammatory effect by decreasing leukocyte adherence 4 times vs ASA and showing vasoprotective effect.*

Conclusion: *H_2S-aspirin exerts strong cytoprotective and anti-inflammatory dose-depended effects on EM and GM. An effective anti-inflammatory and vasoprotective dose of H_2S-aspirin with low risk of damage of GI mucosal barrier is 17.5 mg/kg.*

Key words: *Aspirin, H_2S, mucosal barrier, leukocyte adherence, esophagus, stomach.*

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ВПЛИВ ГІДРОГЕН СУЛЬФІД-ВИВІЛЬНЮВАЛЬНОГО АСПІРИНУ НА ЦІЛІСНІСТЬ СЛИЗОВОЇ ОБОЛОНКИ СТРАВОХОДУ ТА ШЛУНКУ

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***Вступ**: Аспірин є одним з препаратів, який часто призначають як дезагрегант і протизапальний засіб, але останні дані свідчать про його лікувальний вплив для комплексних біологічних процесів – канцерогенез тощо, незважаючи на добре знані побічні ефекти (утворення пептичних виразкок або шлунково-кишкових кровотеч), що можуть розвинутися навіть за умов ахлоргідрії. Останні інтенсивні дослідження активності гідроген-сульфіду (H_2S) довели, що додавання H_2S-вивільнювальної частки до класичних нестероїдних протизапальних препаратів (НПЗП) дає змогу змогу активувати природні цитопротекторні механізми в шлунково-кишковому тракті (Wallace, 2007–2015).*

***Мета** дослідження – оцінити дозо-залежні ефекти новітньої сполуки H_2S-НПЗП – АТВ 340 (Antibe Therapeutics Inc.), похідної аспірину – гідроген-сульфід вивільнювального аспірину, 4-(5-тіоксо-5Н-дітіол-3-ил) феніл 2-ацетоксибензоату (H_2S-аспірину) vs класичного аспірину (ASA) на цілісність слизової оболонки стравоходу та шлунка.*

***Методи**: Подвійні сліпі рандомізовані дослідження проведено на щурах, які отримували разово плацебо (контроль), ASA (у дозах 3.0; 10.0; 30.0; 100.0 мг/кг) або АТВ-340 (у дозах 5.25; 17.5; 52.5; 175.0 мг/кг); та впродовж 7-денного введення ASA (у дозах 3.0 та 10.0 мг/кг) і АТВ-340 (5.25 та 17.5 мг/кг); препарати змішували з плацебо; як плацебо використовували 1.0 мл 1% карбоксиметилцелюлози. Дослідження стану слизової оболонки стравоходу (СОС) та слизової оболонки шлунка (СОШ) було проведено за допомогою аналізу макро- і мікроскопічних змін; пошкодження СОС і СОШ ранжовано за індексом ураження; адгезію лейкоцитів до ендотелію судин – за допомогою інтравітальної мікроскопії до та після введення аспірину та АТВ-340.*

***Результати**: Виявлено, що АТВ-340 володіє захисною дією на СОС та СОШ, індекс ураження вдвічі нижчий vs ASA. Ефективною цитопротекторною та протизапальною дозою АТВ-340 є 17.5 мг/кг за умов разового та 7-денного застосування. Продемонстровано, що АТВ-340 у дозі 17.5 мг/кг ефективно знижувало лейкоцитарну адгезію у 4 рази порівняно до дії ASA, підтверджуючи протизапальну дію і здатність змінювати адгезивні властивості ендотелію.*

***Висновки**: H_2S-аспірин має виразну протизапальну дозо-залежну дію та цитопротектними властивостями на СОС та СОШ. Ефективна протизапальна та вазопротекторна доза з малим ризиком ураження слизової шлунково-кишкового тракту H_2S-аспірину – 17.5 мг/кг.*

***Ключові слова**: Аспірин, H_2S, слизовий бар'єр, адгезія лейкоцитів, стравохід,*

шлунок.

INTRODUCTION

The therapy with nonsteroidal anti-inflammatory drugs (NSAIDs) has become widespread nowadays (Schrör K., 2015). Aspirin (acetylsalicylic acid), one of the common NSAIDs, has been broadly used in medical practice for 130 years. Experimental and clinical studies have shown that acetylsalicylic acid can be used for treatment purpose as an anti-fever or strong anti-aggregatory compound, anti-inflammatory painkiller, or for both treatment and prophylactic purposes for endothelial dysfunction in cardiovascular diseases, as well as breast, neck, esophageal, colon, prostate, and lung cancer chemoprevention (Sadeghi, 2008; Chan and Ladabaum, 2015; Macfarlane et al., 2015). Many enzymes are acetylated and modified by aspirin. Thus, this process is irreversible; the enzymatic properties of the targeted enzyme are changed. Aspirin's acetylating property is not intrinsic to other NSAIDs. Since the time of discovery of the 1982 Nobel Prize winner Sir John R. Vane establishing that major aspirin's targets are cyclooxygenases (COXs) at low antiplatelet doses (75 to 325 mg/day), which prevents the production of prostaglandins (PGs) and thromboxane A2 from arachidonic acid (Higgs et al., 1987; Schrör, 2015), it has been well-known that aspirin and other nonsteroidal anti-inflammatory preparations cause a number of side effects. Most dangerous ones are related to the cytolytic effect on mucosal barrier in gastrointestinal (GI) organs in people with a changed pattern of arachidonic acid metabolism, inducing hemorrhage and ulceration (Vane, 1971-2002; Wallace, 1990-2015;). At the same time, according to scientific data, a combination of aspirin with acid suppression therapy using proton pump inhibitors (PPI therapy), which has become widespread in prevention of GI mucosal injury, is controversial (Dang et al, 2010; Kuramoto et al, 2013; Goldstein and Cryer, 2015). Moreover, latest data attest that this kind of treatment is not very effective in NSAIDs-associated oesophageal injury because it has no effect on postprandial acid reflux (proximal "acid pocket"), causes dysmotility of the stomach and the esophagus, changes the anti-reflux barrier, does not eliminate the effects of bile, a permanent component of the refluxate, and specifically bile acids and trypsin, and causes a change of the natural microflora, which, according to numerous reports, is the main pathogenic agent for mutagenesis and the onset of oncological diseases (Saunders, 2006; Sheen and Triadafilopoulos, 2011; Freedberg et al., 2014; Hvid-Jensen et al, 2014; Nasser et al., 2015; Saunders and Frellick, 2015). Also, an induction of hypergastrinemia causes oxyntic cell hyperplasia, increased parietal cell mass, glandular dilatations and stimulation of enterochromaffin-like cells to release chromogranin and histamine, raising their concentrations in serum, which are links of the oncogenesis chain (Dang et al., 2010) or induced mucosal injury in upper part of GI tract (Zayachkivska O, 2014). Moreover,

last data has shown that co-therapy of aspirin and PPI can be implicated in vitamin B12 deficiency (Lam et al, 2013), osteopenia (Ngamruengphong et al, 2011), or pneumonia (Eom et al 2011).

Former and recent data have demonstrated that non-organic Sulfhydryl compounds or synthesis of the natural gasotransmitter hydrogen sulfide (H_2S) plays a major role in several physiological processes, including cytoprotection, local blood flow, inflammation and changes of adhesive properties of endothelium and platelet aggregation (Satoh et al., 1990; Wallace J, 1990-2014; Gao et al. 2015), and can decrease GI complications related to NSAISs (Goldstein and Cryer, 2015; Herrera et al., 2015; Kodela et al, 2015). H_2S is the key to controlling vascular permeability; it participates in the formation of the so-called "hemodilution barrier" of GI mucosal integrity (Zanardo et al., 2006). Vasoprotective effects of H_2S can prevent the side effects of NSAIDs causing erosive lesions or ulcer of GI mucosa, as demonstrated by the effects of using H_2S-enriched drugs, for instance, S-diclofenac or ATB 337 (Wallace JL, 2007–2015; Chan and Wallace, 2013); H_2S-releasing naproxen derivative, ATB-346 (Fomenko et al, 2014; Blackler et al, 2014; Wallace, 2015; Zayachkivska et al, 2015). Additionally, several studies of different substances named H_2S-realizing aspirin in vitro and in vivo have shown the ability to decrease oxidative stress (Rossoni et al., 2010; Liu et al., 2012; Huang et al., 2014), as well as strong antithrombotic effects (Pircher et al., 2012). Therefore, the first aim of our research was to compare the effects of novel H_2S-enriched NSAIDs derivative – ATB-340 (H_2S-aspirin) (Fig.1) with effects of aspirin in regard to the integrity of the epithelial barrier of the stomach; the second aim was to determine an effective and safe dose of ATB-340 for mucosal integrity in upper part of GI tract by its single or 1 week treatment.

MATERIALS AND METHODS

Male rats weighing 180-220 g were used in our study in accordance with the norms of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, as well as of the University Committee on Bioethics (protocol No 5 of 17.05.2014).

Rats were maintained under a constant 12 h light/dark cycle and an ambient temperature of 21-23° C, and fed according to a standard diet. Animals were deprived of food for 18 h before the experiment but had free access to water. The rats were anesthetized with sodium pentobarbital (BioLab, Brazil) in the dose of 60 mg/kg intraperitoneally (ip). Six rats were used in each group. The aspirin-related lesions of esophageal and gastric mucosa were induced via single oral administration of aspirin (Sigma-Aldrich, USA) in a vehicle (1.0 ml) of 1% carboxymethylcellulose in the doses of 3.0, 10.0, 30.0, and 100.0 mg/kg. ATB-340

(4-(5-thioxo-5H-dithiol-3-yl) phenyl 2-acetoxybenzoate; H_2S-aspirin), a novel hydrogen sulfide-releasing derivative of aspirin (Antibe Therapeutics Inc; Fig.1), was tested in the doses of 5.25, 17.5, 52.5, and 175.0 mg/kg per day. In the next series of experiments, the dose-dependent effect on gastric mucosal integrity was estimated according to the damage scores after 7 days of aspirin (3.0 and 10.0 mg/kg) and ATB-340 (5.25; 17.5) treatment.

Esophageal and gastric mucosal integrity was graded via damage scores by an observer unaware of the treatment using the system described previously that takes into consideration both the number and size of the lesions (Wallace, et al., 1990; Zayachkivska, et al., 2015).

The previous data has shown leucocyte adherence within the gastric microcirculation after the administration of NSAID. The adherence within mesenteric venules was examined after the administration of aspirin in the presented study. Examination of the mentioned vessels does not require an extensive surgery. They were examined using intravital videomicroscopy (Leitz Wetzlar L25/0.35). Rats were anesthetized with sodium pentobarbital (60 mg/kg ip), their jugular veins were cannulated for the administration. The rats were kept in the supine position. The mesentery was carefully placed over an optically clear viewing pedestal that allowed transillumination of a $2m^2$ of tissue. The mesentery was superfused with bicarbonate-buffered saline (pH 7.4). Leukocyte adherence was quantified from the images of the vessels made over 5-minute periods prior to and then 30 and 60 min after intragastric administration of aspirin. Leucocytes were adherent while staying stationary during 30 s. Rolling leucocytes were defined as white blood cells that moved slower than erythrocytes in the same vessel. Flux refers to the number of rolling leucocytes that moved past the reference point in a given period of time (McCafferty, et al., 1995).

Statistical analysis of the results is presented as the mean values (M) ± standard errors (SE). Statistica 10 software and Microsoft Excel Analysis Tool Pak in Microsoft Office Excel 2010 incorporating aposterior test with the comparison of middle indexes after Newman-Keuls criteria for homogeneity the variance were used to analyze the data.

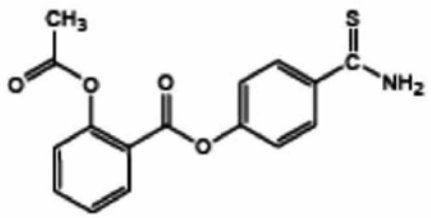

Fig. 1. Chemical structure of 4-(5-thioxo-5H-dithiol-3-yl) phenyl 2-acetoxybenzoate (1) — Antibe's H_2S-releasing aspirin (ATB-340)

Differences with $p < 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

In the first series of investigations, we investigated the dose-dependent effect of ATB-

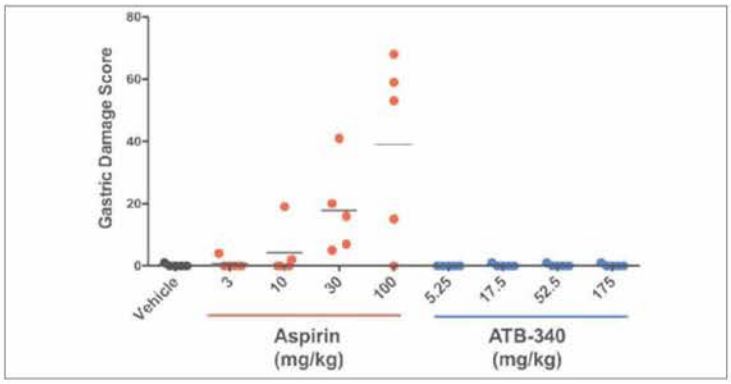

Fig. 2. Dose-dependent effects on gastric mucosal integrity estimated by damage scores after single administration of aspirin (10.0 mg/kg) and ATB-340 (17.5 mg/kg).

340 in comparison with the influence of classical aspirin and vehicle (control group) on esophageal and gastric mucosa (Fig.2). Macroscopic and histological features of damage of the esophageal and gastric mucosa were absent in rats of the control group. The degree of damage of esophagus mucosa in rats that received ASA macroscopically manifested with hyperemia of mucosa during administration of the dose of 10 mg/kg, as well as histologically manifested with ASA-induced esophagitis (shelling of the corneal layer, loss of keratin, epithelial stratification, thickening of the basal membrane, and formation

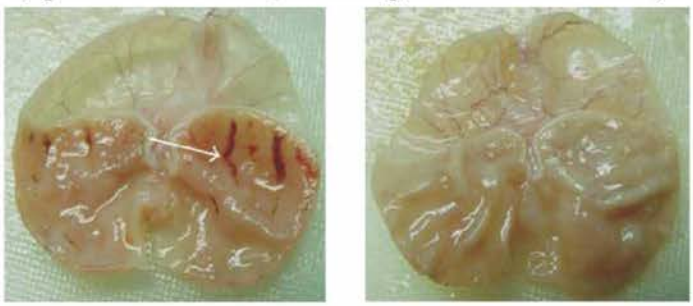

Fig. 3. The ulceration on gastric mucosa caused by aspirin (on the left) and the abcense of ulceration after ATB-340 administration (on the right) after single administration of doses 10 mg/kg and 17.5 mg/kg, respectively.

of an irregular subepithelial edema); in case of ASA application in the doses of 30 and 100 mg/kg, the manifestations included engorgement and perivascular diapedesis in subepithelial stromal structures. During investigations of aspirin associated changes in gastric mucosa, the appearance of solitary erosions in case of administration of 3 mg/kg, and the appearance of ulcerogenic changes in case of administration of 10 mg/kg (Fig. 3), which were characterized by invariably linear erosions confined to the corpus region of stomach, were revealed. They were frequently located on the crests of gastric rugal folds. The influence of ASA in the dose of 30 mg/kg caused multiple ulcers on gastric mucosa, which were characterized by the damage score of around 28, whereas the dose of 100 mg/kg caused its two-fold increase in comparison to the previous one. The application of ATB-40 in the doses of 5.25, 17.5, 52.5, and 175.0 mg/kg per day did not cause any

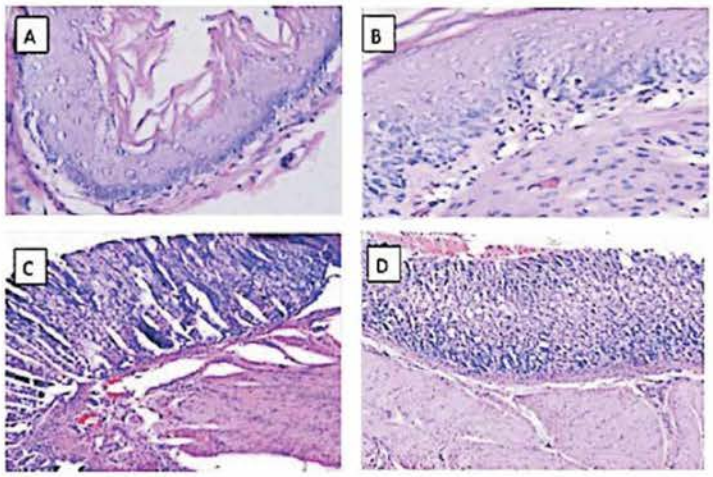

Fig. 4. Histological appearance of esophageal and gastric mucosa in rats during administration of aspirin (microphoto A and C, respectively) and ATB-340, the H_2S-releasing aspirin (microphoto B and D, respectively)..

damages in the esophageal and gastric mucosa. Histological examination showed local slimming and epithelial desquamation of the surface layer into the lumen (Fig. 4). The described reactions of esophageal and gastric mucosa in case of ATB-340 administration in the of dose 17.5 mg/kg could be attributed to triggering natural defense reactions, such as vasodilatative effect of H_2S (Wallace, 2010-2015), decreasing of thrombocyte aggregation (Pircher et al, 2012; Gao L et al, 2014), antiradical activity (Huang et al, 2013), and anti-inflammatory action (Zanardo et al, 2006), which facilitates the barrier function of stomach and esophagus providing mucosal integrity of upper part of gastrointestinal tract.

It was established during the course of investigation of leukocyte adhesion by

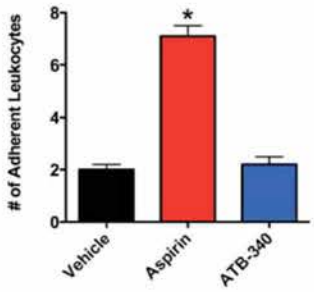

Fig. 5. The number of leukocytes that adhere along a 100 um length of mesenteric venule (counted by an observer unaware of the treatment) during single administration of aspirin (10 mg/kg and 17.5 mg/kg) (*p<0.05 vs control).

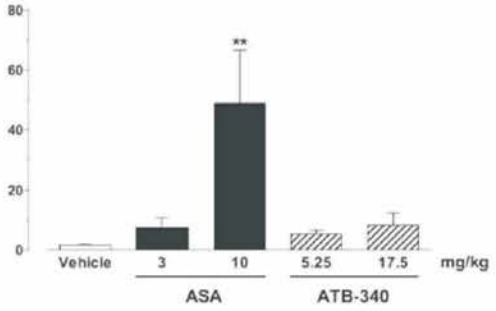

Fig. 6. Dose-dependent effects on gastric mucosal integrity estimated by damage scores after daily administration of aspirin (3 and 10.0 mg/kg) and ATB-340 (5.25 and 17.5 mg/kg) per 7 days; (**p<0.01 vs control).

means of intravital microscopy that during the application of placebo, the number of leukocytes stood at 2, whereas ATB in the dose of 17.5 mg/kg reduced the number of adherent leukocytes 3.5-fold as compared to aspirin in the dose of 10.0 mg/kg – 7 ($p<0.05$ as compared to control group) (Fig. 5). In the next series of investigation, during which preparations were administered for seven days, it was established (Fig. 6) that placebo did not cause changes of the gastric mucosa, whereas application of aspirin in the dose of 10 mg/kg caused a three-fold increase in the injury index as compared to aspirin in the dose of 3 mg/kg ($p<0.01$ as compared to the control group). Meanwhile, application of ATB in the dose of 5.25 mg/kg facilitated the appearance of gastric mucosa irritations, which corresponded to the injury index of 5, whereas the dose of 17.5 mg/kg to the injury index of 8. The data received allow concluding that 17.5 mg/kg is a safe dose of ATB-340, which, according to scientific publications - due to the release of hydrogen sulfide – triggers endogenous multimodal signal systems for resistance to ulcerotrophic factors for the healing of ulcers (Blackler et al, 2012; Herrera et al, 2015).

CONCLUSION

New compound H_2S-releasing aspirin, modification of classical aspirin with enrichment of H_2S, provides a novel approach to the development of "smart drugs" that help activate mucosal defense mechanisms in order to maintain the mucosal integrity and vascular physiological responses. H_2S-aspirin exerts strong cytoprotective and anti-inflammatory dose-dependent effects on the esophageal and gastric mucosa. An effective anti-inflammatory and vasoprotective dose of H_2S-aspirin with low risk of damage to

barrier function in upper part of the gastro-intestinal tract is 17.5 mg/kg.

REFERENCES

1. Blackler R, Syer S, Bolla M, Ongini E, Wallace JL. Gastrointestinal-sparing effects of novel NSAIDs in rats with compromised mucosal defence. PloS One. 2012; 7. e35196.
2. Blackler RW, Gemic B, Manko A, Wallace JL. NSAID-gastroenteropathy: new aspects of pathogenesis and prevention. Curr Opin Gastroenterol. 2014; 19C: 11-16.
3. Chan AT, Ladabaum U. Where do we stand with aspirin for the prevention of colorectal cancer? The USPTF recommendations. Gastroenterol 2015. pii: S0016-5085(15)01665-0. doi: 10.1053/j.gastro.2015.11.018.
4. Chan MV, Wallace JL. Hydrogen sulfide-based therapeutics and gastrointestinal diseases: translating physiology to treatments. American J Physiol Gastrointest Liver Physiol. 2013; 305(7), G467-G473.
5. Dang MN, Richardson P, El–Serag HB. Medications (NSAIDs, statins, proton pump inhibitors) and the risk of oesophageal adenocarcinoma in patients with Barrett's oesophagus. Gastroenterol. 2010; 138(Suppl.7): 2260-2266.
6. Eom CS, Jeon CY, Lim JW, Cho EG, Park SM, Lee KS. Use of acid-suppressive drugs and risk of pneumonia: a systematic review and meta-analysis. Can Med Assoc J 2011; 183: 310–9.
7. Fomenko I, Sklyarov A, Bondarchuk T, Biletska L, Panasyuk N, Wallace JL. Effects of conventional and hydrogen sulfide-releasing non-steroidal anti-inflammatory drugs in rats with stress-induced and epinephrine-induced gastric damage. Stress. 2014 Dec 1;17(6):528-37.
8. Freedberg DE, Lebwohl B, Abrams JA. The impact of proton pump inhibitors on the human gastrointestinal microbiome. Clin Lab Med. 2014; 34(Suppl.4): 771–785.
9. Gao L, Cheng C, Sparatore A, Zhang H, et al. Hydrogen Sulfide inhibits human platelet aggregation in vitro in part by interfering gap junction channels: effects of acs14, a hydrogen sulfide-releasing aspirin. Heart, Lung and Circulation. 2015; 24(1): 77 – 85.
10. Goldstein JL, Cryer B. Gastrointestinal injury associated with NSAID use: a case study and review of risk factors and preventative strategies. Drug Healthc Patient Saf. 2015; 22(Suppl.7): 31-41. doi: 10.2147/DHPS.S71976.
11. Herrera BS, Coimbra LS, da Silva AR, Teixeira SA, et al. H2S-releasing drugs: anti-inflammatory, cytoprotective and chemopreventative potential. Br J Pharmacol. 2015; 159: 1236-1246, 201. doi: 10.1186/s13618-015-0025-3. eCollection 2015.46.

12. Higgs GA, Salmon JA, Henderson B, Vane JR. Pharmacokinetics of aspirin and salicylate in relation to inhibition of arachidonate cyclooxygenase and antiinflammatory activity. Proceedings of the National Academy of Sciences 1987 Mar 1;84(5):1417-20.
13. Huang Q, Sparatore A, Del Soldato P, Wu L, et al. Hydrogen sulfide releasing aspirin, acs14, attenuates high glucose-induced increased methylglyoxal and oxidative stress in cultured vascular smooth muscle cells. PLoS ONE. 2014; 9(6): e97315. doi:10.1371/journal.pone.0097315.
14. Hvid-Jensen F, Pedersen L, Funch-Jensen P, Drewes AM. Proton pump inhibitor use may not prevent high-grade dysplasia and ooesophageal adenocarcinoma in Barrett's ooesophagus: a nationwide study of 9883 patients. Alimentary Pharmacol Therapeutics. 2014; 39: 984–991. doi: 10.1111/apt.12693.
15. Kodela R, Chattopadhyay M, Velázquez-Martínez CA, Kashfi K. NOSH-aspirin (NBS-1120), a novel nitric oxide- and hydrogen sulfide-releasing hybrid has enhanced chemo-preventive properties compared to aspirin, is gastrointestinal safe with all the classic therapeutic indications./ Biochem Pharmacol. 2015; 98(Suppl.4): 564-72. doi: 10.1016/j.bcp.2015.09.014.
16. Kuramoto T, Umegaki E, Nouda S, Narabayashi K, et al. Preventive effect of irsogladine or omeprazole on non-steroidal anti-inflammatory drug-induced oesophagitis, peptic ulcers, and small intestinal lesions in humans, a prospective randomized controlled study. BMC Gastroenterology. 2013.; 13(Suppl. 1): 85.
17. Lam JR, Schneider JL, Zhao W, Corley DA. Proton pump inhibitor and histamine 2 receptor antagonist use and vitamin B12 deficiency. JAMA 2013; 310: 2435–42.
18. Liu L., Cui J, Song CJ, Bian JS, et al. H(2)S-Releasing aspirin protects against aspirin-induced gastric injury via reducing oxidative stress. PLoS ONE. 2012; 7(9), e46301. /doi.org/10.1371/journal.pone.0046301.
19. Macfarlane TV, Murchie P, Watson MC. Aspirin and other non-steroidal anti-inflammatory drug prescriptions and survival after the diagnosis of head and neck and oesophageal cancer. Cancer Epidemiol. 2015; 39(Suppl. 6): 1015-1022. doi: 10.3748/wjg.v21.i15.4599.
20. McCafferty DM, Granger DN, Wallace JL. Indomethacin-induced gastric injury and leukocyte adherence in arthritic versus healthy rats. Gastroenterology 109: 1173-1180, 1995.
21. Nasser SC, Slim M, Nassif JG, Nasser SM. Influence of proton pump inhibitors on gastritis diagnosis and pathologic gastric changes. World J Gastroenterol. 2015; 21(Suppl. 15): 4599-606. doi: 10.3748/wjg.v21.i15.4599.
22. Ngamruengphong S, Leontiadis GI, Radhi S, Dentino A, Nugent K. Proton pump

inhibitors and risk of fracture: a systematic review and meta-analysis of observational studies. Am J Gastroenterol 2011; 106: 1209–18.

23. Pircher J, Fochler F, Czermak T, Mannell H, et al. Integrative Physiology/Experimental Medicine: Hydrogen Sulfide–Releasing Aspirin Derivative ACS14 Exerts Strong Antithrombotic Effects In Vitro and In Vivo. Arterioscler Thromb Vasc Biol. 2012; 32: 2884-2891.
24. Rossoni G, Manfredi B, Tazzar V, Sparatore A, et al. Activity of a new hydrogen sulfide-releasing aspirin (ACS14) on pathological cardiovascular alterations induced by glutathione depletion in rats. Eur J Pharmacol. 2010; 648: 139–145.
25. Sadeghi S, Bain C J, Pandeya N, Webb PM, et al. Aspirin, nonsteroidal anti-inflammatory drugs, and the risks of cancers of the oesophagus. Cancer Epidemiol Biomarkers Prevention 2008; 17(Suppl. 5), 1169-1178.
26. Satoh H, Sato F, Takami K, Szabo S. New ulcerative colitis model induced by sulfhydryl blockers in rats and the effects of antiinflammatory drugs on the colitis. Jpn J Pharmacol. 1997; 73: 299-309.
27. Saunders WB, Anderson LA, Johnston BT, Watson RP, et all. Nonsteroidal anti-inflammatory drugs and the oesophageal inflammation-metaplasia-adenocarcinoma sequence. Cancer research. 2006; 66 (Suppl. 9): 4975-4982.
28. Saunders WB, Frellick M. Proton-pump inhibitors may boost death risk in inpatients. J Gen Intern Med 2015. http://www.medscape.com/viewarticle/854454nlid=915882163&src=wnl_edit_medp_gast&uac=94607HV&spon=20&impID=888097&faf=1.
29. Schrör K. Aspirin modes of action and clinical benefits: what we know today CME. 2015; http://rubensriotorto.blogspot.com/2015/09/aspirin-modes-of-action-and-clinical.html.
30. Sheen E, Triadafilopoulos G. Adverse effects of long-term proton pump inhibitor therapy. Dig Dis Sci 2011; 56 (Suppl. 4): 931–950.
31. Wallace JL, Caliendo G, Santagada V, Cirino G. Markedly reduced toxicity of a hydrogen sulfide-releasing derivative of naproxen (ATB-3 The H2S-releasing naproxen derivative, ATB-346, inhibits alveolar bone loss and inflammation in rats with ligature-induced periodontitis. Med Gas Res. 2015; 5: 4. doi: 10.1186/s13618-015-0025-3.
32. Wallace JL, Keenan CM, Granger DN. Gastric ulceration induced by non-steroidal anti-inflammatory drugs is a neutrophil-dependent process. Am J Physiol. 1990; 259: G462-G467.
33. Zanardo RC, Brancaleone V, Distrutti E, Fiorucci S, et al. Hydrogen sulfide is an

endogenous modulator of leukocyte-mediated inflammation. FASEB J. 2006; 20: 2118-2120.

34. Zayachkivska O, Bula N, Khyrivska D, Wallace JL et al. 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(2-3): 91.
35. Zayachkivska O, Havryluk O, Bula N, Wallace JL et al. Cytoprotective Effects of Hydrogen Sulfide in Novel Rat Models of Non-Erosive Esophagitis. PLoS ONE, 2014; 9(10).
36. Zayachkivska O, Pshyk-Titko I, Hrycevych N, Savytska M. New insight into oseophageal injury and protection in physiologically relevant animal models. J Physiol Pharmacol. 2014; 65(Suppl. 2): 295-307.
37. 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: 91-99.

Стаття надійшла 15. 10. 2015
Після доопрацювання 02. 12. 2015
Прийнята до друку 15. 12. 2015