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Review

4-hydroxynonenal in redox homeostasis of stomach in health and diseases

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Abstract:

The integrity, high functional activity and sufficient regeneration rate of gastric mucosa (GM) in harsh conditions is very challenging pathophysiological demand. The health of gastric epithelium highly depends of efficiency of redox balance maintenance, antioxidant defense and activity of detoxifying systems within the cells as well as robustness of blood supply. Bioactive products of lipid peroxidation, in particular second messengers of free radicals, the bellwether of which is 4-hydroxynonenal (HNE), are important mediators in (patho)physiological adaptive reactions, cells signaling, and are also implicated in pathogenesis of numerous gastric diseases. However, while mechanisms and consequences of HNE and its protein adducts production in response to strong stressors during acute and chronic gastric injury are well studied, many other important issues related to gastric carcinogenesis, tumor growth and progression, the condition of GM after eradication of *Helicobacter pylori*, the relevance of antioxidants for HNE-related redox homeostasis in GM and many other still need extensive studies and new comprehensive approaches. Therefore, in order to address existing issues preclinical studies and clinical intervention trials are required, which should include also determination of HNE preferably by immunohistological and specific HNE-His ELISA analyses.

Keywords: 4-Hydroxynonenal; lipid peroxidation; redox balance; oxidative stress; stomach; peptic ulcer; gastritis; *Helicobacter pylori;* gastric cancer; non-steroid anti-inflammatory drugs-induced gastropathy

1. Introduction

Gastrointestinal tract serves as a highly specialized interface between environment and organism's internal medium aimed primarily to digest food, absorb nutrients and water. In addition, it is responsible for a wide variety of other important functions including, but not limited to, immune defense, excretion of metabolic waste/detoxification, secretory/regulation, repository for gut bacteria, and physical barrier[1]. The gut it is exposed mechanically and chemically to food/chyme, digestive enzymes, different, often very aggressive pH conditions and numerous bacteria, therefore, high efficiency of protection and regeneration is required for its function. It is particularly important in case of stomach, where extremely aggressive acidic pH often reaching the values of 1-2 is accompanied with the action of proteolytic enzymes [2]. Numerous toxins that may be ingested together with food, as well as some drugs may contribute to the damage of gastric mucosa (GM) and expose it to additional risk. Furthermore, in more than a half of the population worldwide bacterial agent, known as *Helicobacter pylori* (*H. pylori*)[3], persists in GM and causes chronic gastritis, peptic ulcer and is major contributor to gastric adenocarcinoma and MALT-lymphoma [4].

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The redox balance is a major homeostatic parameter and regulatory factor for the metabolic functions of the organism and gut in particular[5]. Redox imbalance often referred to as "oxidative stress" may be caused either by exposure to excessive power of oxidants, or decreased activity of reducing systems and lack of antioxidants [6]. Certain degree of lipid peroxidation may take place in many cellular processes in physiological conditions, but redox imbalance that is typical for a wide range of diseases very often leads to excessive accumulation of oxidized lipids and their degradation products. Among such products of lipid peroxidation 4-hydroxy-2-nonenal (HNE), is known to be ubiquitous and one of the most studied also considered as "second messenger of free radicals" [7]. It is generated from of omega-6 fatty acids, along with its role in pathogenesis of multiple diseases has been shown to be involved in various signaling pathways. It contributes to the regulation of energy metabolism, detoxification, cell proliferation and differentiation, maintenance of cytoskeleton, metabolic adaptations to redox derangements as well as a number of other functions [8–10].

Considering sophisticated functions of mucous membrane and a wide variety of damaging exposures, the maintenance of redox balance in GM is particularly challenging [5]. On one hand, the cells of mucosal epithelium have high rate of proliferation and an exceptional regenerative potential, while on the other hand, gastritis, peptic ulcer and gastric cancer remain among the most common gastrointestinal diseases that cause severe health and overall socio-economic damage [3]. The general progress in understanding the roles of lipid peroxidation and HNE as its end-product in health and disease stimulated respective studies focused on specific diseases of stomach and gastrointestinal tract. Our mini-review is aimed to address the most important issues related to the role of HNE in normal functioning and development of the diseases of stomach.

2. Approaches to determine HNE in case of diseases of stomach

Along with conventional approach to measure the concentrations of substances of interest in biological liquids like blood (serum, plasma, whole blood), urine, cerebrospinal fluid etc., in case of diseases of stomach, several other options are available. First, stomach is available for endoscopy which is a routine clinical intervention. During endoscopy it is possible to obtain biopsies of mucous membrane from different parts of stomach for further morphological studies. Second, gastric juice can be obtained for chemical analysis. Third, a number of "breath-tests" (determination of metabolites of ingested reagents in exhaled air) are available for gastroenterological diagnostics. And finally, some tests can be performed with the use of feces as a material, for example, to test *H. pylori* bacterial contamination [3]. The researchers have to keep in mind that the blood from stomach flows through portal vein to the liver and many substances, for example xenobiotics, lipid peroxidation products, some hormones and cytokines may be cleaved there and thus, may appear in peripheral blood within normal concentration range despite the clear evidence of toxicity/inflammation etc. [11].

HNE and other lipid peroxidation products, such as for example acrolein, malonic dialdehyde and many others can be used as biomarkers of redox imbalance [7]. However, their high reactivity and capacity for interactions with multiple functional groups of macromolecules significantly reduces concentrations of free lipid peroxidation products and their transfer to blood and/or urine from other compartments of the organism. Therefore, the most of the detectable HNE is found to be conjugated with proteins or glutathione. Through a Michael-type reaction of nucleophilic addition HNE binds covalently with cysteine, lysine and histidine residues of proteins [12]. Development of specific antibodies against HNE-histidine adducts facilitated further research and enabled implementation of respective analytical methods [13,14].

In this regard, widely used methods of HNE determination in human samples include HNE-immunohistochemistry (qualitative/semiquantitative evaluation) that is used in order to map tissue or intracellular distribution of respective HNE-conjugates in the samples, obtained by the means of gastric biopsy [12]. A variety of HNE-ELISA (quantitative methods) have been proposed that are applicable also for evaluation of the levels of HNE-adducts in biological fluids like blood serum, urine or gastric juice [15]. Other antibody-based methods including immune fluorescence, immune-gold electron microscopy, Western-blotting, immunoblotting etc. that are also often successfully used [16]. In turn, free HNE can be accurately determined by high performance liquid chromatography and a

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number of modifications of mass spectroscopy-based methods [17]. However, due to high reactivity of free HNE appropriate handling of the samples can be very problematic and its levels in many cases are very low, therefore the determination of respective conjugates reveals more biologically/clinically relevant information and may have substantial advantages [12].

3. HNE in stomach under physiological conditions

In physiological conditions ingested food is exposed to low pH (hydrochloric acid) and proteolytic enzymes in order to provide degradation of proteins to peptides and amino-acids. However, a highly-acidic medium facilitates also a variety of chemical reactions between different food components [1,2]. A modelling of chemical processes taking place during gastric digestion reveals the possibility of iron- or metmyoglobin-catalyzed generation of substantial amounts of hydroperoxides and other lipid peroxidation products from components of common diets containing meat and unsaturated fats at low pH in presence of water-dissolved oxygen. Notably, addition of food rich on polyphenols dramatically reduces generation of hydroperoxides and may be at least in part responsible for preventive effects of fruits and vegetables [2].

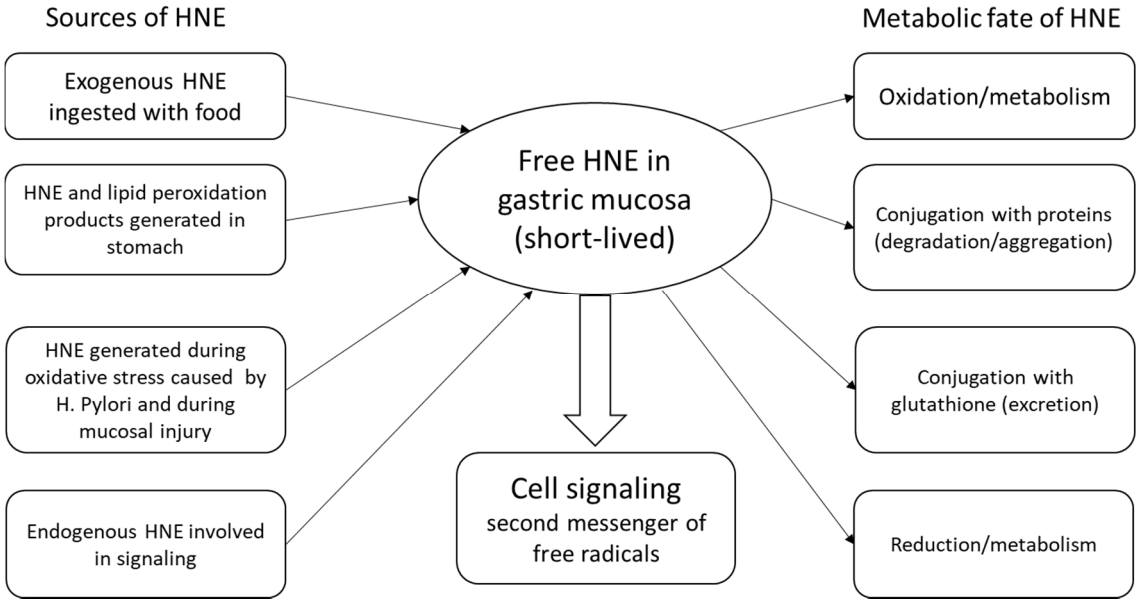

Figure 1. Schematic presentation of major sources of HNE in gastric mucosa and the ways of its further transformations. Free HNE is a highly reactive molecule and is capable to react with numerous targets within the cells interfering with redox-sensitive pathways. Metabolites of HNE are less important for signaling, however, HNE-protein/peptide adducts could develop on numerous enzymes, cytokine and receptors so they have important regulatory roles, in particular since HNE-binding if often reversible. Hence, such aldehydic adducts can represent source of HNE and secondary oxidative stress, while they can also be used for immunochemical HNE detection in the cells and tissues as advanced lipoxidation end products (ALEs).

However, accumulation of exogenous lipid peroxidation products in GM may be enhanced by consumption of large amounts of unsaturated fats that may be a part of many "healthy" diets or popular supplements containing polyunsaturated fatty acids (PUFAs) [18]. Therefore, the products containing significant quantities of PUFAs should be carefully processed and properly stored in order to prevent their oxidation and possible toxic impact on GM. Actual levels of HNE in GM are the function of the rates of their generation/absorption and utilization [19]. The formation of protein conjugates is proportional to the mean levels of free HNE in the cells, therefore antibody-based methods of staining and quantitative determination of HNE are considered to be quite accurate and reliable, especially if HNE-histidine adducts are monitored [12].

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Certain degree of accumulation of HNE-histidine adducts in mucosa of gastric corpus and antrum was demonstrated in majority of healthy volunteers [20]. Notably, almost all the samples, obtained from asymptomatic apparently healthy subjects regardless of whether the patients have been *H. pylori*-positive or not, have had mild to moderate HNE-immunopositivity in cytoplasm of gastric glandular epithelium, with only a few cases of HNE-negative samples [20]. The reasonable explanation of these findings suggests that HNE plays a role in normal signalling and regulation of cellular functions in GM under physiological conditions and its levels are strictly maintained within reasonable range providing adaptations to adverse factors like metabolic or emotional stress, exogenous toxins that are occasionally ingested with food or latent *H. pylori* infection. The impacts with excessive power may lead to distress and cause GM injury and inflammation that is discussed below.

Interestingly, the most of *H. pylori*-positive subjects never experience clinically overt forms of gastritis, peptic ulcer or gastric cancer [21]. This observation lays in line with the findings, that apparently healthy *H. pylori*-positive subjects have no difference in HNE-histidine conjugates in GM compared to controls, despite occasional presence of inflammatory cells in the samples [20]. It is likely, that asymptomatic subjects have sufficient compensatory power to cope with the negative influence of the pathogen and only excessive virulence of certain *H. pylori* strains or reduced due to different reasons resistance of host organism leads to clinically significant manifestations. It is known that, for example, sedentary lifestyle is leading to deleterious metabolic changes that are associated with activation of symapthethic tone (with subsequent parasympathetic impairment) [22]. Genetic defects, psychoemotional stress and a number of other factors may also contribute to autonomic imbalance that may contribute to increased vulnerability of GM [23–25].

On the other hand, numerous epidemiological observations associate *H. pylori*-positivity with so called extra-gastric manifestations that include, but are not limited to atherosclerosis, insulin resistance/diabetes type 2, diseases of liver and pancreas etc. [26–30]. Proposed pathogenesis include an initial damage of GM caused by *H. pylori* and its virulence factors, oxidative stress and lipid peroxidation, local inflammation, release of pro-inflammatory cytokines and other bioactive mediators to the blood circulation thus causing systemic effects and metabolic derangements that result in respective extra-gastric disease [4,31–34]. Indeed, in *H. pylori*-positive healthy male subjects with sedentary lifestyle higher levels of fasting insulin and elevated homeostatic model assessment index (HOMA-index) were observed compared to *H. pylori*-negative matches [28]. The other study showed significantly increased heart rate and sympathetic tone in H. pylori positive asymptomatic volunteers. However, the levels of water-soluble HNE derivative 1,4-dihydroxynonane mercapturic acid (DHN-MA), iso-PGF2, pro and anti-inflammatory cytokines, C-reactive protein, and a number of selected hormones were not different between the groups, indicating that either the degree of local mucosal damage is not strong enough to cause marked elevation of studied factors or their mild/moderate elevation is cleaved by the passage of blood through the liver [33].

4. The HNE presence in patients with *H. pylori*-associated gastritis and peptic ulcer

Despite of its recent decline, the prevalence of *H. pylori* infection it is still extremely high world-wide reaching the rates between 20-40% in Western countries to over 90% in many developing countries [3]. There is a clear evidence that this microorganism is causative factor for chronic gastritis type B and peptic ulcer. However, as it was mentioned above, most of *H. pylori*-positive subjects are clinically healthy and never develop gastritis or ulcer. This suggests that along with *H. pylori* and its virulence factors, conditions of host organism play a crucial role in the results of this complex host-microbe interaction [4,35]. This fits well into the framework of the classical concept of balance of factors of “aggression” and “cytoprotection” in GM. On the cellular level this paradigm is consistent with current understanding of the principles of redox balance maintenance under stress conditions[6]. Namely, GM injury and subsequent inflammation takes place when the capacity of antioxidant mechanisms is not sufficient to protect the cells from the damaging factors and related oxidative stress [5].

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Peptic ulcer and gastritis are for a long time known to be associated with redox imbalance and excessive lipid peroxidation [36], as was confirmed in numerous studies and with different study models [37]. Clinical studies are less abundant and only a few of them are addressing the issue of oxidative stress and lipid peroxidation in GM. The use of gastric endoscopy enables the obtaining of the mucosal tissue samples for further histological examination. In the group of *H. pylori*-positive peptic ulcer patients significantly higher accumulation of HNE-histidine adducts in GM compared to control group was clearly demonstrated [20]. Notably, in some cases severe immunopositivity of nuclei and perinuclear spaces along with diffuse accumulation of HNE-histidine conjugates in cytoplasm of the cells was observed, confirming the evidence of decompensated redox imbalance in GM of these patients [20,38].

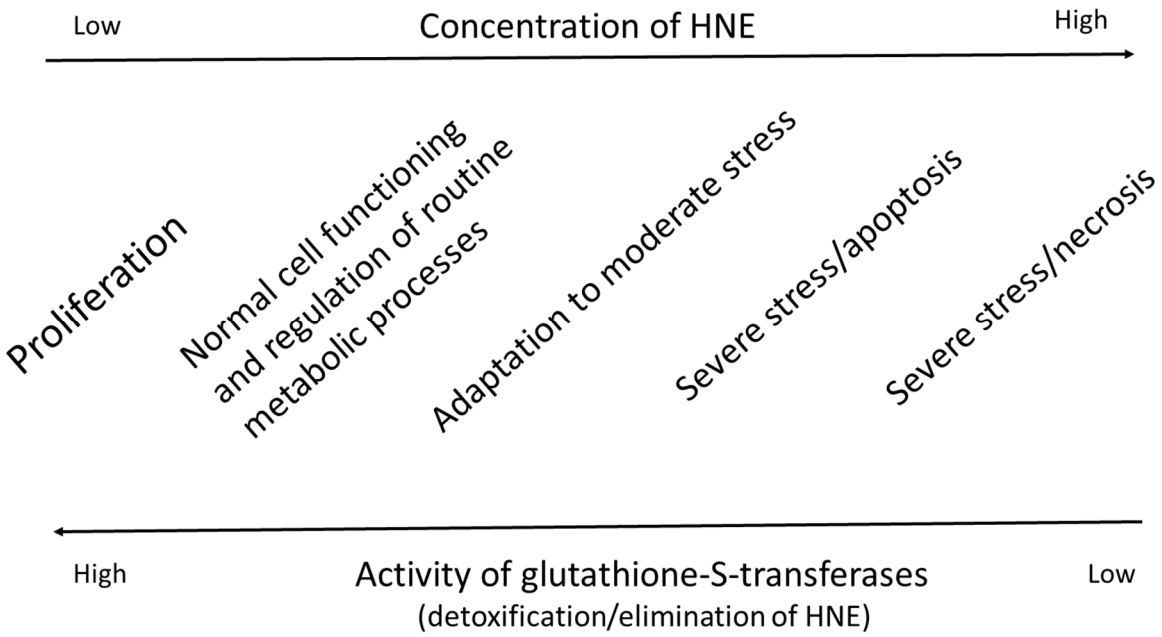

Figure 2. Dose-dependent effects of HNE levels on regulation of cellular functions in gastric mucosa. Actual concentration of HNE inversely correlates with the redox status of the cell and is a function of the rate of its generation and utilization. HNE content is regulated by the activities of alcohol and aldehydes dehydrogenases and of glutathione S-transferases, depending mostly on the level of reduced glutathione and affinity for the other cellular proteins [39]. The overall pathophysiological consequences reflect the tissue/cellular redox (un)balance, the type of cells and the reaction of neighboring cells to the onset of lipid peroxidation and HNE generation in the cells studied, which often behave as individuals, not as constituents of any tissue, which is relevant for carcinogenic effects of HNE and for its involvement in host defense against cancer [40–43].

Pharmacological approach to treat chronic gastritis and peptic ulcer via eradication of *H. pylori* proved to be very successful from the clinical point of view and provided great improvement of treatment efficiency and allows to cure these diseases in most of the patients [44]. In addition, there are reasons to expect that eradication of this microorganism may be useful for prevention and/or treatment of other diseases, associated with *H. pylori*, including metabolic syndrome, type 2 diabetes, non-alcoholic fatty liver disease, atherosclerosis and possibly several others [45–47]. However, precise mechanisms of how the infection leads to systemic pathological effects as well as biochemical mechanisms that may contribute to metabolic deteriorations in *H. pylori* positive patients need to be further elucidated.

Despite obvious clinical efficiency, there are reports indicating persistence of HNE-histidine adducts hyper-accumulation in peptic ulcer patients even after successful eradication of *H. pylori* at least in the period of 4 weeks after completing anti-microbial treatment [38]. This is consistent with clinical observations that some patients still have symptoms (epigastric pain, nausea, reduced

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appetite etc.) for several months after treatment [48]. It might be possible that metabolic dysfunction in these patients, as integral part of ulcer disease, contributes to pathogenesis of gastric injury independently to *H. pylori*. Combination of these two factors and additionally other factors is known to increase the risk of ulcerations, like smoking, psycho-emotional stress, unhealthy lifestyle and suboptimal nutrition may be crucial for the outcome of host-microbial interaction [24,49]. Thus, it depends on the power of intrinsic cytoprotective mechanisms (genetics, sufficient blood microcirculation in stomach, effective autonomic regulation) and exogenous factors (*H. pylori*, ingestion of toxins and products of PUFAs peroxidation) and may vary from long-term asymptomatic carrying to chronic gastritis type B with the periods of exacerbation and remission, peptic ulcer of stomach and/or duodenum or tumorous transformations in the form of mucosa-associated lymphoid tissue lymphoma (MALT-lymphoma) or gastric adenocarcinoma.

5. HNE in gastric carcinogenesis

GM is heavily exposed to different types of exogenous chemical agents and many other reactive species are generated directly in the stomach during process of digestion. Some of them may be toxic and cause the damage to gastric epithelium, and some may also be carcinogenic [2]. Chronic inflammation and oxidative stress caused by *H. pylori* infection are also major contributors to malignant transformation of the cells of GM [31,50]. The idea to eradicate *H. pylori* in all carriers, even asymptomatic, is growing in popularity and there some recently published results of respective trials confirming this reasoning [51]. Moreover, eradication of H. pylori seems to be reasonable also in patients with early stages of gastric cancer undergoing endoscopic resection since it decreases the rates of metachronous cancers comparing to control group [52]. In this context, genotoxicity of supraphysiological levels of HNE and other lipid peroxidation products may be important for carcinogenesis as well [53].

The role of HNE in malignant transformation and growth is ambiguous. On one hand, HNE can diffuse from the site of generation into the nucleus and bind covalently to the molecule of DNA causing mutations and supporting carcinogenesis, while on the other hand, it is influencing pathways regulating proliferation, differentiation and apoptosis of transformed cells. Depending on the concentration and activity of detoxifying systems of cancer cells the effects of HNE may be toxic to them or can stimulate their growth and enforce resistance to cytostatic drugs [54].

While in case of acute and chronic GM injury caused by *H. pylori* and gastrotoxic agents oxidative stress and increased lipid peroxidation is well documented, in case of gastric cancer it is not the case. As it was shown by Ma Y., et al. 2013, serum levels of major lipid peroxidation products such as HNE, malonic dialdehyde, conjugated dienes and 8-iso-prostaglandin F2α all were decreased in cancer patients compared to control group [55]. Hence not statistically significant, also lower levels of HNE were observed in *H. pylori*-positive vs *H. pylori*-negative patients that may support the idea that moderate (or local) activation of lipid peroxidation may stimulate systemic activation of detoxification mechanisms through, for example, Nrf2-dependent mechanisms [54].

6. HNE in alcohol and non-steroid anti-inflammatory drugs-induced gastropathy

Alcohol and a rapidly growing use of non-steroid anti-inflammatory drugs (NSAIDs) jointly are the second most important cause of gastric injury after *H. pylori* [49]. The evidence from well-established animal models of GM injury suggests two principal mechanisms responsible for tissue damage. The first, direct toxic effect on GM and the second, limitation of gastric microcirculatory blood flow that is essential for proper rate of proliferation, mucus secretion etc., through decreased levels of gastroprotective prostaglandin E_2 with subsequent endothelial dysfunction and autonomic dysregulation that may cause oxidative stress [56,57]. Both mechanisms contribute to the development of severe local oxidative stress, excessive lipid peroxidation and accumulation of its products, including HNE, mostly covalently bounded to proteins [36].

Important role of autonomic dysregulation is often ignored in case of diseases of stomach. It is known that increased sympathetic tone limits blood flow in the organs of gastrointestinal tract and

caused endothelial dysfunction which is crucial for gastroprotection, therefore autonomic imbalance may significantly potentiate damaging effects of alcohol and NSAIDs [58,59].

7. Pharmacological and non-pharmacological approaches to reduce redox imbalance in GM

Considering multiple etiologic and pathogenic factors that may interact with each other and contribute to GM damage, there is a number of different approaches used in order to prevent or treat gastric injuries (Table 1).

Hence, eradication of *H. pylori* with a complex of two antibiotics and proton pump inhibitors is proved to be effective in most of the cases of *H. pylori* positive gastritis and peptic ulcer [44]. However, in some of these patients elimination of the microbial factor is not sufficient and symptoms as well as redox imbalance may persist long after completion of the treatment [38,48]. Moreover, eradication of *H. pylori* does not reduce significantly risk of gastric cancer at least within a few years after eradication and statistical difference becomes significant only after 8-10 years [60]. Therefore, other approaches are also needed in order to overcome these limitations and to address other aspects of GM injury pathogenesis.

Table 1. Selected pharmacological and non-pharmacological interventions and their effects on HNE production/utilization in gastric mucosa.

Intervention	Target process/ pharmacological effect	References
Proton pump inhibitors, H_2 histamine receptor inhibitors	Reduction of acidity, decreased proteolytic activity of gastric juice/ decreased gastric injury (production of HNE)	[44,49]
Antibiotics	*H. pylori* eradication/ decreased gastric injury (production of HNE)	[44,49]
NO,CO, H_2S-releasing NSAIDs	Release of CO, NO and/or H_2S modulates redox signaling, improves endothelial function and improves microcirculation/ reduced production and improved utilization of HNE	[57,58]
Antioxidants/polyphenols present in food	Reduced lipid peroxidation of PUFAs in stomach/ reduced absorption of exogenous HNE	[2,61]
Phytochemical and phytotoxins with moderate prooxidant action	Nrf-2 activators induce expression of antioxidant genes and increase detoxification of HNE	[62,63]
Interval hypoxic training	Improvement of autonomic control of microcirculation and function of internal organs	[64,65]
Exercise, intermittent fasting, caloric restriction	Activation of autophagy, reduction of systemic inflammatory response, improvement of protein quality control and autonomic regulation	[66]
Ulcer-healing drugs (Actovegin, Solcoseryl etc.)	Mechanism unknown, suggested influence on microcirculation and/or endothelial function	[67,68]

Since substantial amounts of gastrotoxic substances may be ingested with food or generated during digestion, the idea to use drugs, supplements or certain types of food able to neutralize toxins or reduce the rate of lipid oxidation was actively explored. Indeed, subjects consuming more fruits and vegetables show lower incidence of gastric diseases, especially gastric cancer [69]. Studies show also that polyphenols reduce formation of hydroperoxides in stomach and *in vitro* models of gastric digestion [2,61]. Use of pre- and pro-biotics [70] as well as a number of plant derived traditional medicines or extracts was also shown to be protective against gastric and intestinal mucosal damage and may improve redox balance in mucous membranes in different parts of gastrointestinal tract [63]. Exact mechanisms of their effects are often not clear, but at least some of them may act via hormetic

effect, when moderate prooxidant action is leading to activation of defense mechanisms (for example by stimulation of Nrf-2 transcription factor) [54]. Alternatively, they may contribute to increased mucosal microcirculation through improvement of endothelial function or parasympathetic tone as it is in case of Actvegin, which is used as antiulcer drug for several decades [68]. Among non-pharmacological interventions that showed some efficiency in case of peptic ulcer disease is also interval hypoxic training [64]. Exact gastroprotective mechanisms in this case are not clear as well, but it is likely that the mechanism includes improvements of autonomic balance and enhanced microcirculation [65].

Therapeutic use of NSAIDs is overwhelming and in order to reduce their gastrotoxicity a wide range of new formulations are introduced or are under development [71]. For example, a number of nitric oxide (NO), carbon monoxide (CO) or hydrogen sulfide (H_2S) releasing derivatives of acetylsalicylic acid and other NSAIDs were shown to be pharmacologically as effective as traditional drugs, but have preventive effects against NSAID-induced gastrotoxicity via improvement of endothelial function, anti-inflammatory and cytoprotective effects [57,58]. Protective mechanisms of action of these drugs are closely related to HNE signaling pathways and maintenance of redox balance in GM.

8. Conclusions

The integrity, high functional activity and sufficient regeneration rate of GM in harsh conditions is very challenging. The health of gastric epithelium highly depends of efficiency of redox balance maintenance, antioxidant defense and activity of detoxifying systems within the cells as well as robustness of blood supply. The products of lipid peroxidation, in particular of HNE and its protein/histidine adducts, are important mediators in physiological adaptive reactions, cells signaling, and are also implicated in pathogenesis of numerous gastric diseases. Hence, while the mechanisms and consequences of HNE generation in response to strong stressors during acute and chronic gastric injury are well studied, many other important issues related to gastric carcinogenesis, tumor growth and progression, the condition of GM after eradication of *H. pylori*, and many others still need extensive studies and new comprehensive approaches.

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