Membrane-lipids anti-bacterial and anti-tumoral therapies revisited: an example with *Helicobacter pylori* infection

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H. pylori infection is recognized as a major etiological factor in gastric cancer development. Eradication of *H. pylori* infection has not changed to a large extent in the last decades and can raise concerns due to recurrence of infection, and most importantly, acquired resistance to classically used antibiotics. In this context, the use of compounds other than antibiotics, that could impact *H. pylori* infection, might provide an alternative therapeutic tool to overcome this problem. Lipid-therapy-based approaches have been proposed in the treatment of various pathologies, such as cancer and infectious diseases. The latter rely on the ability to modulate lipid composition of cell membranes. Recently, it became unquestionable that *H. pylori* extracts cholesterol from host cell-membrane rafts, modifies it into α-glycosylated forms, and uses this mechanism to increase its survival. The main aim of this review is to explore the auxotrophy of *H. pylori* for cholesterol as a new therapeutic target for the management of this infection. In this context, some molecules such as the polyunsaturated fatty acids (PUFAs) and statins can alter key components of cell membrane, such as cholesterol availability/location within the cell membrane. Such ability makes these molecules possible therapeutic agents to inhibit *H. pylori* growth. Both statins and PUFAs are emphasized as harbouring a potential role in managing *H. pylori* infection. Indeed, they challenge *H. pylori*'s ability to uptake and use epithelial cholesterol, as well as favouring bacteria lysis. New targets for *H. pylori* eradication and infection management are of utmost importance, since resistance to antibiotics is increasing and new prophylactic/therapeutic strategies against *H. pylori* infection are needed in clinical settings.

Keywords *Helicobacter pylori*; Cholesterol; n-3 Polyunsaturated fatty acids; Docosahexaenoic acid; statins

1. Introduction

Studies have proposed lipid-therapies approaches in several pathologies, such as cancer, cardiovascular diseases, neurodegenerative processes, obesity, inflammation and infectious diseases. Both the type and relative amount of lipids in the membrane control numerous functions, such as the regulation of the activity and location of membrane proteins [1]. The existence of specific lipid regions and domains in the cell membrane supports the possibility to design therapies that act on the lipid composition of the cell membrane [1]. This is of special interest in the case of *H. pylori* infection due to constant increase of the incidence of antibiotic resistant strains.

2. Membrane lipid composition and structure in relation to pathology and therapy

2.1. Membrane lipid structure and composition: its importance to cell function

Biological membranes have lipid bilayers as their basic structural unit, which are sheet-like assemblies of thousands of amphiphilic lipid molecules held together by hydrophobic interactions between their acyl chains [2]. These bilayers form the boundaries between the intracellular cytoplasm and extracellular environment. The discovery of large membrane domains (basal, lateral and apical membrane regions of glandular, endothelial and epithelial cells) and lateral microdomains structures (lipid rafts, caveolae and coated pits) revealed the complex nature of the cell membrane structure [3]. Membrane lipids can be divided into three groups based on their chemical composition: glycerol-based lipids (phospholipids), ceramide-based sphingolipids, and cholesterol [4]. Membrane constituents are not homogeneously arranged in the bilayer membrane but organized in complex lateral microdomains. All eukaryotic cells are intimately involved in membrane budding, vesicle trafficking and sustaining lipid bilayers asymmetry, particularly through the sequestration of phospholipids, such as phosphotidylserine and cardiolipin, to the inner leaflet of the membrane [5]. Moreover, changes in the asymmetric distribution of phospholipids can lead to various biological consequences, as triggering apoptotic stimuli and immunogenic responses in the cell [6]. Sphingolipids have a sphingoid base as a backbone, which impacts greatly the hydrophobicity of the lipid bilayer [7]. Cholesterol, for instance, has an hydrophilic hydroxyl group that interacts with the phospholipids hydrophilic head groups, while the bulky steroid group interact with the hydrophobic acyl chains of lipids [4]. Overall, the polymorphic nature of lipids arrangement and their interaction with membrane proteins influence the fluidity and packing of the lipid membrane, regulate cell signalling, transport, adhesion and structure.

Indeed, over the last three decades it has been shown that the plasma membrane lipid composition and structure play a pivotal role in cell signalling through several ways. The wide variety of functions displayed by the cell barrier includes the selectivity between hydrophobic hormones and hydrophilic signalling molecules; the control of the activity

of membrane signalling proteins by the membrane lipid composition and fluidity; the net negative charge surface at the inner leaflet of the eucaryotic cells plasma membrane,provided mainly by phosphotidylserine; the hydrolysis of phosphatidylinositol biphosphate into inositol triphosphate (IP3) and diacylglycerol, which are well-known second messengers among others [8, 9].

2.2. Modulation of membrane lipid structure and composition as a therapeutic approach in human pathologies

The type and relative abundance of lipids in the membrane control numerous functions and thus regulate the localization and activity of membrane proteins. Because most cellular functions occur in or in the vicinity of cell membranes and because alterations in the types and/or levels of membrane lipids have been described in many human pathologies, it is conceivable that specific therapies could be designed based on regulation of membrane lipids structure [10]. Membrane-lipid therapy is an innovative therapeutic approach aimed at developing drugs to regulate membrane-lipid composition and/or structure. Due to alteration of membrane lipids composition reported in various different pathologies, membrane-lipid therapy might have potential use for the treatment of several illnesses. Indeed, alterations in membrane-lipids composition and structure appear to be implicated in the development of various cardiovascular pathologies, such as hypertension, atherosclerosis, coronary heart disease, sudden cardiac death, blood vessels integrity, and thrombosis. In addition they also concern cancer and obesity. As regard to nutritional modulation it is well accepted that cellular membrane lipids can be remodelled by altering dietary fat intake, which consequently affects physical properties of membranes and membrane proteins functionality [11, 12]. Recent data have shown that specific classes of fatty acids can also remodel microdomains, such as rafts and caveolae microdomains [13, 14]. n-3 polyunsaturated fatty acids (PUFAs) and specially docosahexaenoic acid (DHA) are rapidly incorporated into membrane phospholipids, and alter membrane physical properties, including permeability, lateral diffusion, lipids packing and domains formation [15]. These changes affect the activation status of membrane proteins, such as GTPase, components of ion channels, tyrosine kinases and adenyl-cyclase, most of them having a central role in cell oncogenic pathways [16].

Eicosapentaenoic acid (EPA), another n-3 PUFA, and DHA when incorporated into the fatty acyl groups of caveolae phospholipids, decrease of 50% the caveolar content of cholesterol and caveolin-1, the major structural component of caveolae, unlike cyclodextrines that have been used to perturb microdomain integrity *in vitro* [12]. Concomitantly, alterations in the caveolar microenvironment by n-3 PUFA selectively inhibited the localization of caveolae-targeted proteins to caveolae, H-Ras and iNOS, whereas the localization of non-caveolae proteins, K-Ras and clathrin, are unchanged. Moreover, epidermal growth factor (EGF)-induced activation of H-Ras, but not of K-Ras, is significantly decreased following n-3 PUFA treatment [17].

Overall, reported studies provide evidence of n-3 PUFA ability to change the biochemical makeup of lipid rafts and caveolae membrane microdomains, thereby influencing cellular signalling. Therefore, cell microdomains are molecular targets for long-chain n-3 PUFA to modulate diverse biological systems leading to anti-inflammatory effects and inhibition of cancer development.

2.2.1. The specific case of lipid modulation as therapy to treat viral and bacterial infections

It has been proposed that certain PUFAs hold an inhibitory effect on bacterial growth [18-23]. Some mechanisms have been reported for PUFAs antibacterial action and gastric protective effect. These include the ability of PUFAs to disrupt bacteria membrane leading to lysis and also to modulate the synthesis of mucosal anti-inflammatory prostaglandins, such as Prostaglandin E_2 (PGE_2) [24].

The microbicidal activity of certain PUFA and their derivatives has been reported on various enveloped viruses, parasites and pathogenic bacteria such as *Pseudomonas aeroginosa*, *Staphylococcus aureus*, *Neisseria gonorrhoeae* and *Burkholderia cenocepacia* [25-28]. Although these lipids are found in certain food products no association between their intake and infection rates has been establish which clearly reflects the multifactorial and complexity of infection success.

2.2.2. Involvement of PUFAs in inflammation and in antitumor processes

Changes in the fatty acid content of typical human diets have occurred over time, and n-6 PUFAs are the most abundant form of fat in the diet, especially in the "*Western-style*" diet [29]. This imbalance between n-3 and n-6 PUFAs is associated with increased risks for both cardiovascular and some cancer diseases [30]. Because of this intricate metabolic and inflammation relation between n-6 and n-3 PUFAs it is recommended that the ratio between their intake is as low as possible, with a gold standard of 4:1 to 1:1 comparatively to what is actually observed in "*Western-style"* diets 20:1 to 16:1 [31].

In the last years special attention has been given to the relation between fat provided by the diet and the inflammation-mediated pathologies. PUFAs have been extensively studied in the context of diseases for which inflammation plays an essential role [31]. Arachidonic acid (AA; n-6 PUFA), after being metabolized, is a crucial substrate for the synthesis of eicosanoids holding pro-inflammatory properties, such as series 2- and series 4- of prostaglandins (PGs) and leukotrienes (LTs), respectively. These classes of eicosanoids lead to pro-inflammatory effects that include fever, increased vascular permeability, generation of reactive oxygen species and finally pro-

inflammatory cytokines production, like IL-6, IL-1β and TNFα [29]. In contrast to n-6 PUFAs, n-3 PUFAs (EPA, DHA) once incorporated into cell phospholipids, are metabolized to PG and LTs of series 3- and series 5-, with an anti-inflammatory action. These n-3 PUFAs decrease the expression of several pro-inflammatory genes such as TNFα, IL-1 and IL-6 [32, 33].

EPA and DHA supplementation replaces n-6 PUFAs (such as AA) in cell membranes phospholipids, particularly platelets, erythrocytes, neutrophils, monocytes and liver cells [30, 34]. Ultimately, it will lead to *1*) decreased production of prostaglandin E_2 metabolites; *2*) decreased concentration of Thromboxane A2 (TXA_2), a potent platelet aggregator and vasoconstrictor; *3*) decreased formation of LTB_4, an inducer of inflammation and a powerful inducer of leukocyte chemotaxis and adherence; *4*) increased concentration of TXA_3, a weak platelet aggregator and vasoconstrictor; *5*) increased concentration of prostacyclin PGI_3 leading to an overall increase in total prostacyclins with no decrease of PGI_2 (both PGI_2 and PGI_3 are active vasodilators and inhibitors of platelet aggregation); and *6*) increased concentration of LTB_5, a weak inducer of inflammation and chemotactic agent [35, 36]. Additionally, DHA competitively inhibits eicosanoid biosynthesis from AA [37]. As already addressed, n-6/n-3 PUFA ratio rather than the absolute levels of the two classes of unsaturated fatty acids, is crucial to regulate AA eicosanoid biosynthesis. Indeed, the ability of n-3 PUFA to suppress the conversion of LA to AA is dependent, not only on its amount in diet, but also on n-6 PUFA [37].

Cancer preventive studies using EPA and DHA supplementation have shown the n-3 PUFA ability to inhibit the formation of papilloma, of mammary tumours and carcinogenesis of the large and small intestine [38]. DHA-enriched diets have also been shown to reduce formation of aberrant crypt foci, metastatic colon cancer carcinoma, sarcoma, prostate cancer and lung cancer [39-42]. *Fat*-1 gene encodes the desaturase responsible for the conversion of n-6 PUFAs into n-3 PUFAs is lacking in humans. The *Fat*-1 transgenic mouse model provides strong evidence for EPA and DHA involvement in cancer development. Indeed, in this mouse model both formation and growth of melanoma and colon cancer were significantly reduced when compared with non-transgenic mice [43-45]. Furthermore, an epidemiologic study reported a significant decrease in cancer incidence amongst Alaska's native Inuit childhood population comparatively to North America's. Noteworthy, the Inuit population which has dietary habits that include seal and fat fish, presents in their diets significant higher DHA levels than Caucasian population [46, 47].

There are several proposed mechanisms that might explain the anti-tumour properties of n-3 PUFAs. The n-3 PUFA tumour-suppressive effects might be at least in part, though to activate apoptotic pathways since they have been implicated in the regulation of the expression of components of Bcl-2 family. In fact, Serini *et al*. have reported that DHA increases levels of the pro-apoptotic proteins Bak and Bcl-xs [48]. On the other hand, reduced levels of anti-apoptotic proteins, Bcl-2 and Bcl-xl, were observed [49]. Another possible mechanism by which n-3 PUFAs induce apoptosis is via the induction of cytochrome c from mitochondria and depolarization of mitochondrial membrane [50, 51]. Finally, adhesion and angiogenesis, which are essential for solid tumour establishment and growth, are promoted by a variety of factors, such as Ras homolog gene (Rho)GTPase, intercellular Adhesion Molecule(ICAM)-1, vascular cell adhesion molecule (VCAM)-1, VEFG and platelet-derived growth factor, which are downregulated by n-3 PUFA [15, 37, 52-54].

Overall, reported studies provide evidence of n-3 PUFA ability to change the biochemical makeup of lipid rafts and caveolae membrane microdomains, thereby influencing cellular signalling. Therefore, cell microdomains are molecular targets for long-chain n-3 PUFA to modulate diverse biological systems leading to anti-inflammatory effects and inhibition of cancer development.

3. Management of *H. pylori* infection: is there a space for a new therapy?

H. pylori is a gram-negative bacterium that colonizes half of the world's human population and is recognized as a major etiological factor in chronic active gastritis, gastric duodenal ulcers and gastric cancer (GC). The unifying mechanism by which *H. pylori* infection leads to disease is the establishment of a chronic inflammation that will increase GC risk [55]. Additionally, as a result of *H. pylori* infection host's sustained inflammation and hypochlorhydria enable other bacteria to grow and permit the latter to sustain a pro-neoplastic drive [56, 57]. Players in *H. pylori*-mediated chronic inflammation are mainly cytokines, bacterial by-products and the bacteria itself. Altogether, they interact with receptors at the surface of epithelial cells and activate signal transduction pathways often inducing transformation of the phenotype. The clinical outcome of *H. pylori* infection is determined by multiple factors, such as environmental factors, bacterial strain heterogeneity and host genetic predisposition [58, 59]. Additionally, *H. pylori* variants of adhesion molecules at the surface of the bacteria, known as outer membrane proteins (OMPs), composition of peptidoglycans, and the presence of virulence factors among them the pro-apoptotic cytotoxin VacA and a component of the cag pathogenicity island CagA, are crucial for the outcome of the infection [55, 60-62]. An intriguing fact is that most of *H. pylori* carriers will remain asymptomatic, and it is only a small proportion of individuals will develop clinical disease manifestations [63]. Recent reports suggest that the risk of developing clinical outcomes is related with immunological response during childhood [64]. Hence, immunological active tolerance towards *H. pylori* infection would protect individuals from gastric severe diseases.

3.1. Standard clarithromycin-based triple therapy: current therapeutic recommendations

The triple therapy was proposed in 1997 as the recommended treatment for the eradication of *H. pylori* infection and since then, it has become universal. It includes a proton pump inhibitor (PPI)-clarithromycin and amoxicillin or metronidazole administered for 7-days [65-67]. However, recent multicentric and meta-analysis studies show failure of eradication with this combination, with eradication rates below 80%, that is far from what should be expected for an infectious disease [68]. This scenario has raised a growing interest for new therapeutic approaches [69-74]. Moreover, prolonging exposure to antibiotics through increased duration of triple therapy has generated neither consensual results, nor remarkable benefits among patients [75-77].

Gisbert *et al*. proposed a different scheme of administration and sequence of antibiotics for *H. pylori* eradication, the so called sequential treatment [78]. It consists of a dual regimen of a 5-day period with PPI-amoxicillin, followed by 5-day period with PPI-clarithromycin-tinidazole (or metronidazole) [78-80]. Although sequential regimen has achieved nearly 90% *H. pylori* eradication rates, recent results led to debate the need to take all components concomitantly [81-86]. Indeed, concomitant administration of PPI-clarithromycin-amoxicillin-nitroimidazole, originally proposed by Treibner *et al.,* had previously been proved to be efficacious [87, 88], most probably because of the larger number of antibiotics included [79]. However, and although results were satisfactory, ecological impact of this regimen can be expected to be relatively negative with the selection of multi-resistant strains [10]. Indeed, randomized controlled trials demonstrate that administering all components (3 antibiotics plus PPI) at the same time is more efficient in *H. pylori* eradication than the sequential treatment, which is especially true for longer periods of administration (7-10 days *vs.* 5 days) [89].

Furthermore, recently the addition of bismuth to a PPI(omeprazole)-metronidazole-tetracycline regimen (known as OBMT) originally proposed by de Boer *et al*, as a "rescue treatment" to a clarithromycin-based treatment failure, presents good eradications rates. Besides, this OBMT treatment overcomes *H. pylori* clarithromycin and levofloxacin (which is a fluoroquinolone antibiotic, more recently prescribed to circumvent clarithromycin resistance) resistant-strain concerns [90-92]. However, bismuth salts raise toxicity concerns with documented cases of encephalopathy among patients presenting plasma levels higher than 50 mg/L. Nevertheless, new formulations of OBMT administered for shorter periods, seem to guarantee maintenance of bismuth plasma levels within the threshold of potential toxicity [56, 91, 93]. Furthermore, in a multicentric randomized open-label phase 3-trial that compared *H. pylori* eradication success between a capsule containing OBMT, and clarithromycin-based triple therapy, eradication rates were 80% versus 55% in the standard therapy group. Hence, due to the constant increase of the prevalence of *H. pylori* clarithromycin-resistant strains, quadruple therapy should be considered for the first-line treatment since it provides a better eradication with similar safety and tolerability to standard therapy [91, 94].

Overall, the main reason for the decreased efficacy of standard triple therapy is the increased resistance of *H. pylori* from 9% to almost 20% during the last decade [95]. Furthermore, in certain parts of Central, Western and Southern Europe resistance rates exceed 20% in comparison with Northern parts of Europe (<10%). Hence, International Guidelines have recommended taking into account geographical differences in resistance rates for *H. pylori* strains and suggests a threshold of 15-20% to separate the regions of high and low clarithromycin resistance. Therefore, different therapeutic options are proposed according to clarithromycin resistance: low rate areas should use clarithromycin-containing treatments; whereas areas presenting high rates of resistance should prescribe bismuth-containing quadruple therapies [94]. In conclusion, standard clarithromycin triple-based therapy is still the most widely used treatment in clinical practice. However, in areas where clarithromycin resistance is high, other options, such as non-bismuth or bismuth quadruple concomitant therapy, should be warranted since they yield better eradication rates, besides bypassing clarithromycin high resistance rates [85, 94-96].

In spite of *H. pylori* infecting 50% of the world's population, only a very small percentage of these individuals will eventually develop gastric pathology. Therefore, the question of which patients should be enrolled in *H. pylori* infection eradication programs is still pertinent. There is strong evidence that *H. pylori* eradication reduces both gastric atrophy and gastric cancer development [97]. In the absence of preneoplastic gastric lesions, a successful *H. pylori* eradication restores the inflamed gastric mucosa back to normal. Indeed, the active inflammatory process characterized by infiltration with polymorphonuclear cells is usually abolished within 4 weeks [98]. However, intestinal metaplasia is irreversible even in successful *H. pylori* eradication [22]. Hence, a population-based screening is probably the best option for primary prevention of gastric cancer, especially in high-risk areas of gastric cancer, such as some parts of Asia and of Europe [99]. At risk patients considered for *H. pylori* eradication should include first-degree relatives of family members with a diagnosis of gastric cancer; patients with previous gastric neoplasia or with a risk of gastritis; patients with chronic gastric acid inhibition for more than 1 year; or with strong environmental factors for gastric cancer (high exposure to dust, coal, quartz, cement, heavy smoking, among others) [94]. Screen-and-treat strategy of *H. pylori* infection should be explored in communities with high burden of gastric cancer and eradication therapy prescribed [22, 99, 100].

3.2. Revisiting PUFAs as an approach for *H. pylori* infection treatment: direct effect of PUFAs on *H. pylori* infection

H. pylori is a gastric pathogen. This bacterium is responsible for the most common infection among the world population. Regarding *H. pylori* infection the decline in duodenal ulcer incidence associated with the rise in dietary intake of PUFAs, independently of *H. pylori* treatment, led to a growing interest on the antibacterial role of PUFAs [101]. Furthermore, it has been demonstrated that concentration of $2.5x10^{-4}$M of Linoleic acid (LA) an n-6 PUFA, could inhibit the growth of *H. pylori in vitro*. This inhibitory effect is thought to be related to the degree of unsaturation within PUFA, meaning the number of double bounds in the fatty acid molecule [18]. As previously addressed, the hypothesis suggested by Tarnawski *et al.* in 1986 that the continuous decline in peptic ulcer morbidity and mortality, regardless *H. pylori* treatment, was associated with a rising in PUFAs intake, is supported by several studies demonstrating PUFAs ability to inhibit gastric acid secretion while increasing cytoprotective prostaglandins [101]. These data are at the origin of two clinical trials in which PUFAs have been given to patients. In the first, forty *H. pylori* infected patients with past or present duodenal ulcer disease were randomized to receive either placebo capsules and low-PUFA margarine or a high-PUFA regimen and high-PUFA margarine for 35-days of treatment. No significant changes were observed in *H. pylori* infection colonization and related-inflammation among the different groups of patients [102]. In another study, the efficacy of fish oil was investigated as an antibiotic-sparing component of a triple *H. pylori* eradication regimen in non-ulcer dyspepsia (NUD) patients in a randomized, double-blind trial. Patients with a normal upper endoscopy and a positive C^{13}-urea breath test were randomly assigned to pantoprazole, clarithromycin and metronidazole or to pantoprazole, clarithromycin and fish oil for 7 days. In spite of *in vitro* evidence of the inhibitory effect of fatty acids on *H. pylori* growth, both clinical trials fail to inhibit gastric colonization and inflammation. However, when the authors provided a mixture of n-3 and n-6 PUFAs to the enrolled patients, while n-3 PUFAs are known for their anti-inflammatory role, n-6 PUFAs metabolization and incorporation into cell membranes generate potent inflammatory mediators. Therefore, this mixture of PUFAs may provide antagonistic effects regarding inflammation of the gastric mucosa or serum composition as regard to inflammation. However, Correia *et al* have demonstrated that n-3 PUFAs ability to inhibit *H. pylori* growth and mice gastric colonization is exclusive for DHA, [103, 104]. It has been shown that concentrations of 100 µM of DHA decreased *H. pylori* growth, whereas concentrations higher than 250 µM irreversibly inhibited bacteria survival. DHA reduced ATP production, adhesion to AGS cells and gastric inflammation in a mouse model. In addition, 2D-electrophoresis analysis revealed that DHA changed the expression of *H. pylori* outer membrane proteins associated with stress response and metabolism and modified bacterial lipopolysaccharide (LPS) phenotype [103, 104]. Moreover, DHA efficiency in eradicating *H. pylori* from mice gastric mucosa was significantly lower compared to the standard antibiotherapy. However, if DHA was added to the standard clarithromycin based treatment (ST), this combination yielded better results than ST alone, regarding *H. pylori* eradication. None of the DHA/ST treated mice presented gastric colonization by *H. pylori* two months after the end of the treatment [103].

3.2.1. Role of other fatty acids on *H. pylori* growth

It has been shown that certain short-chain fatty acids and medium chain fatty acids hold *in vitro* anti-*H. pylori* growth effect. However, this inhibition would be dependent on the presence of micelle-forming agents, that would foster the contact between fatty acids and bacterium [105]. Additionally, fatty acids do not seem to possess concern regarding *H. pylori* resistant strains, since low frequency of spontaneous resistance to their bactericidal activity and to their corresponding monoacylglycerol esters has been reported [21].

Regardless the evidence of PUFAs inhibitory effect on *H. pylori* growth, the responsible mechanisms remain unknown. The most efficient fatty acids in inhibiting *H. pylori* growth are still subject of debate. Additionally, their degree of unsaturation and local pH seem to influence their inhibitory effect. Moreover, not all bacteria are sensitive to bactericidal effect of fatty acids. In fact, difference in the susceptibilities of Gram-negative bacteria to lipids bactericide effect is notable and is probably due to differences in the outer membrane or the cell wall composition. The external leaflet of *Enterobacteriaceae*, such as *E. coli*, but also *Salmonella spp*., known to be insensitive to fatty acids treatment, is almost entirely composed by LPS, which due to its O side chain leads to a low fluidity and a great difficulty for fatty acids to enter [18]. In contrast, Gram-negative bacteria as *N. gonorrhoeae* are susceptible to fatty acids bactericide action. They do not possess LPS and are mainly composed by other polysaccharides lacking the O side chain of LPS [106].

4. Cholesterol as a new target for *H. pylori* eradication

4.1. *H. pylori's* auxotrophy for cholesterol from host epithelial cell membranes

Rafts are cholesterol-rich areas within the cell membrane that are responsible for clustering important signalling proteins platforms [107]. These raft-platforms are cell entry gates for bacteria, viruses, protozoans and prions (*Escherichia coli, Chlamydia spp, Mycobacterium spp, Shigella spp, Salmonella enteretica, Pseudomonas aeroginosa, Brucella spp, Legionella pneumophila and Coxiella burnetii*, Vaccinia, Adenovirus type 2 and poliovirus) [108-112]. Additionally, some bacteria are auxotrophic for cholesterol and need to incorporate it to survive [113]. Interestingly, *H.*

pylori is one of them; it extracts free-cholesterol (non-esterified) from host epithelial gastric cell membranes, modifies it by α-glycosylation and incorporates the modified cholesteryl glucosides into its membrane [63, 64]. The α-glycosylation of cholesterol is an enzymatic reaction performed by enzymes encoded by *HP0421* gene. The resultant cholesteryl glucosides become a component of *H. pylori* cell wall, without which bacteria would probably not survive [63, 65, 66]. Recently, Correa *et al* demonstrated that DHA also have indirect effect to inhibit *H. pylori* survival by depleting cholesterol in gastric epithelial cell membranes *(submitted)*. Moreover, Wunder *et al.* demonstrated that *H. pylori* successfully evades host immune system by cholesterol glycosylation, hence escaping phagocytosis, T-cell activation and bacteria clearance, all mechanisms that increase bacterial survival [63]. Additionally, cholesterol has been suggested to be involved in *H. pylori* host cell adhesion, due to proximity of bacteria to intercellular junctions, particularly tight junctions which present higher concentrations of cholesterol [114, 115]. *H. pylori* virulence factors VacA and CagA entry in cell is dependent on cell membrane cholesterol levels. In fact, it is demonstrated that vacuole biogenesis in the case of VacA, CagA translocation through the type 4 secretion system (T4SS) and its tyrosine phosphorylation in *H. pylori* infected cells is abrogated by cholesterol depletion carried out by β-cyclodextrines [116-118]. Nevertheless, the role of cholesterol incorporation and its derivatives into bacteria cell wall is not fully understood [67].

4.2. Inhibitors of 3-hydroxy-3-methylglutaryl(HMG)-CoA reductase as bactericidal agents

Statins, also known as 3-hydroxy-3-methylglutaryl(HMG)-CoA reductase inhibitors are potent anti-hyperlipidemic drug group that is widely used for the treatment of hyperlipidemia. The HMG-CoA reductase is responsible for the rate-limiting step in the cholesterol synthesis mevalonate pathway [119]. Additionally, HMG-CoA reductase inhibitors are known to have effects beyond their lipid depleting action, collectively known as pleiotropic effects [120]. Indeed statins have been proposed to treat viruses and bacterial infections [121-123]. Statins have been shown to hold immunomodulatory action, regulate serum cytokine concentration within a community with acquired pneumonia and associated with a better prognosis in severe bacterial infections [124-126]. Individuals treated with statins are less prone to bacterial infection and present better outcomes especially in the case of respiratory tract infections [126, 127]. In addition, statins have been reported to inhibit host cell invasion by *Staphylococcus aureus,* enhance its clearance, protect against pneumococcal infection in a mouse model and repress translocation of *Pseudomonas aeroginosa* across Madin-Darby canine kidney cell monolayers [128-130]. Although under debate, statins bactericidal concentrations might be close to concentrations frequently prescribed for hyperlipidemia treatment [131, 132].

4.2.1. Evidence of anti-*H. pylori* action of statins

Statins have been shown to reduce inflammation in gastrointestinal tract in both dextran-sulphate colitis mice model and in *H. pylori* infected mice [133, 134]. Although the mechanisms underlying this anti-inflammatory effects are not completely understood, statins do not affect *H. pylori* viability, indicating that they act in epithelial cells. Indeed, statins inhibited mRNA expression of eNOS and TNF-alpha in *H. pylori* infected mice [133]. Moreover, patients with chronic gastritis in long-standing statin therapy had their gastric inflammation severity reduced compared to patients with no statins therapy [135]. Interestingly, in a population-based case control study, the constant use of any statin was associated with a significant decrease in gastric cancer risk [136]. Nevertheless, in an *H. pylori* infected gerbil model pitavastatin fails to decrease gastric inflammation, suggesting that statins inhibition of *H. pylori*-mediated inflammation is dependent on the type of statin and on yet unknown factors [137].

Nseir *et al*., in a very interesting prospective, randomised clinical trial double-blind placebo-controlled study, tested the hypothesis that statin as adjuvant to standard triple therapy might improve *H. pylori* eradication [138]. Indeed, they concluded that simvastatin in combination with triple standard treatment regimen (two antibiotics plus a PPI) significantly improved *H. pylori* eradication.

5. Conclusions

H. pylori colonizes half of the world population and if early eradicated it prevents the development of gastric atrophy and gastric precancerous lesions [100, 139], [97, 140]. The standard recommended treatment for eradicating *H. pylori* infection still resides in the combination of two antibiotics and a PPI [141]. The efficiency of this prescription has decreased over time, and currently holds less than 80% of success, mainly because of the high incidence of antibiotic resistant strains. Alternative therapeutic approaches and treatment strategies that could overcome *H. pylori* resistance strains are, therefore, needed. The antimicrobial activity of certain non-antibiotic compounds has been tested and deserves further attention. Among molecules known for inhibiting *H. pylori* are certain oils and compounds extracted from plants. The bactericidal activity of these molecules is most likely attributable to their lipid composition, specifically their fatty acid and lipid derivative profile [18, 142-144]. Although, the mechanisms by which these compounds inhibit *H. pylori* are still not clarified, increasing evidence suggests that changes in the cell wall structure and composition play a key role.

In this context, certain fatty acids inhibit up to 50% of *H. pylori* growth *in vitro* within concentrations of 1 mM, although the mechanisms underlying the inhibitory effect of lipids on *H. pylori* growth are unknown [20, 21, 23].

Supporting the cell membrane disruption hypothesis, Thompson *et al*. demonstrated that radiolabeled PUFAs, added to *H. pylori* liquid cultures, are incorporated into the *H. pylori* plasma membrane [23], as well Correia *et al* work [104]. Indeed, the latter have proposed DHA as a novel antibacterial agent that inhibits *H. pylori* growth *in vitro*, and in a murine model and also attenuates inflammation resulting from the infection. DHA has also been implicated to significantly change *H. pylori* protein outer membrane composition and ability to adhere to epithelial gastric cells, which overall might explain DHA effect in decreasing mice gastric colonization and inflammation [103, 104]. Despite these promising results, DHA use in eradication therapy should not be regarded as a replacement of conventional antibiotic treatment, but on contrary an adjuvant to optimize the efficiency of antibiotherapy and to limit the recurrence of the infection.

DHA is also known for inducing changes in host epithelial cells, such as lateral diffusion, lipid packing, domain formation and cell lipid profile [13, 107, 145]. It is suggested that the unique hairpin structure of DHA is incompatible with cholesterol and cholesterol efflux from cells will increase [13, 146]. The ability of DHA to affect membrane composition and structure raises the hypothesis that DHA might have an indirect effect on the survival of *H. pylori,* by modulating the availability of cholesterol to *H. pylori* in epithelial gastric cells. This is of utmost importance as *H. pylori* is auxotrophic for cholesterol; it extracts free-cholesterol from host epithelial gastric cells, modifies it by α-glycosylation and incorporates the modified cholesteryl glucosides into its membrane [116, 147-151]. This dependence on cholesterol is very well illustrated by the fact that the capacity of *cgt* knockout mutants (*H. pylori* strain that is unable to α-glycosylate cholesterol) to colonize gerbils is severely attenuated [152].

Although the physiologic significance of incorporation of cholesterol and its derivatives into the *H. pylori* membrane is still not well established, it seems plausible the hypothesis that cholesterol assimilation is important for *H. pylori'*s survival and growth [149, 153]. In fact, it has been shown that *H. pylori* grown with cholesterol is up to thousand-fold more resistant to amoxicillin treatment than *H. pylori* grown in free cholesterol medium [152]. *H. pylori* cholesterol-dependent resistance to some anti-*Helicobacter* molecules, such as bile salts, that inhibit *H. pylori* growth, is also documented [153]. This concept may be very important for the management of *H. pylori* infection, since it supports the idea that cholesterol enhances the resistance of *H. pylori* to antimicrobial compounds, some of which are, or could be used clinically to treat *H. pylori* infection [153, 154].

Statins, which directly inhibit endogenous cholesterol synthesis, have also been shown to decrease inflammation induced by *H. pylori* infection in both animal model and patients. Most importantly, when given as an adjuvant to triple regimen in patients with *H. pylori* infection eradication is more effective [138]. These results clearly suggest that alternative therapies, such as DHA and statins, which alters cholesterol levels and epithelial cell membranes rearrangements, might be identified as promising adjuvant therapies in the management of *H. pylori* infection.

Importantly, both DHA and statins are safe molecules, with a wide-ranging use in the clinical setting. Its action has been shown to yield significant results in the prevention and management of progression of certain inflammatory diseases, which can support their further testing in the context of *H. pylori* infection management.

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