Open Journal of Medical Microbiology, 2013, 3, 70-79
http://dx.doi.org/10.4236/ojmm.2013.31011 Published Online March 2013 (http://www.scirp.org/journal/ojmm)

Interaction of *Helicobacter pylori* Cell Membrane with Non-Esterified Cholesterol and Other Steroids

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Received December 10, 2012; revised January 19, 2013; accepted February 2, 2013

ABSTRACT

Helicobacter pylori performs the unique action of assimilating exogenous non-esterified cholesterol into its cell membrane. This bacterium aggressively incorporates non-esterified cholesterol into the membrane, induces its glucosylation, and uses both non-esterified cholesterol and glucosylated cholesterols as membrane lipid compositions. The reason for this assimilation of non-esterified cholesterol into the cell membrane of *H. pylori* has eluded investigators for many years. Recent hypotheses posit that the sterol-uptake and sterol-glucosylation contribute to the survival of *H. pylori* cells in different ways. The incorporation of the non-esterified cholesterol into the cell membrane fortifies the resistance of *H. pylori* against the antibacterial actions of phosphatidylcholines, antibiotics, and bile salts. In parallel, the glucosylation of the non-esterified cholesterol incorporated into the cell membrane serves *H. pylori* in two ways. First, it helps the bacterium evade host immune responses, such as phagocytosis by macrophages and activation of antigen-specific T cells. Second, it detoxifies sterols fatal to the bacterium via a novel action of sterol glucosylation recently described in another report from our group. The reluctance of *H. pylori* to absorb esterified cholesterol remains unexplained. A recent study by our group has demonstrated that the phosphatidylethanolamine (PE) in the outer membrane of *H. pylori* serves as a steroid-binding lipid the incorporation of non-esterified cholesterol into the membrane. We have also discovered that the myristic acid ($C_{14:0}$) molecule attached to the PE of this bacterium plays an important role in the selective binding of non-esterified cholesterol but not esterified cholesterol.

Keywords: *Helicobacter pylori*; Cholesterol-*α*-Glucosyltransferase; Phosphatidylethanolamine

1. Introduction

Helicobacter pylori, a Gram-negative curved rod, is a pathogen responsible for chronic gastritis and peptic ulcers in humans [1,2]. In addition, the colonization of the stomach by this pathogen for many years contributes to the development of gastric cancer and marginal zone B-cell lymphoma [3-6]. Approximately half of population in the world is infected with *H. pylori*, and the majority of infected persons develop atrophic gastritis with or without symptoms. Among *H. pylori*-infected individuals, about 10% persons develop gastric and duodenal ulcers, 1% to 3% persons develop gastric adenocarcinoma, and 0.1% or less person develops gastric mucosa-associated lymphoid tissue (MALT) lymphoma [3,4, 7-9].

While sterols are generally unnecessary for the growth or viability of bacteria, a few bacterial species assimilate exogenous non-esterified cholesterol into their cell membranes and glucosylate it once assimilated. As of this writing, five unique bacterial genera are known to exhibit this cell membrane assimilation of non-esterified cholesterol: *Borrelia* spp., *Acholeplasma axanthum*, *Spiroplasma* spp., *Mycoplasma gallinarum*, and *Helicobacter* spp. [10-15]. Unlike plants and fungi, which produce glucosyl sterols such as glucosyl sitosterol and glucosyl ergosterol universally, bacterial species produce glucosyl sterols only in rare and special cases [16-21]. Plants and fungi biosynthesize the sterols by themselves and attach D-glucose molecules to the sterols via the catalytic action of sterol-*β*-glucosyltransferase. In contrast, *Helicobacter pylori* and the other bacteria lack the synthetic pathway for sterols. Thus, bacterial cells can only biosynthesize glucosyl cholesterol by ingesting non-esterified cholesterol from outside the cells.

While *H. pylori* attaches a D-glucose molecule to a cholesterol molecule absorbed into the cell membrane via *α*-glucosidic linkage, *Borrelia hermsii* attaches its sugar molecule to a cholesterol molecule via *β*-glucosidic linkage [22], as with the glucosyl sterols in plants and fungi. Recent studies by other groups have demonstrated that *Borrelia burgdorferi*, *B. garinii*, and *B. afzelii* attach a D-galactose molecule to a cholesterol molecule via *β*-galactosidic linkage in place of a D-glucose molecule

[22-24]. Put simply, the *O*-glycoside bond between sugar molecule and sterol molecule in *H. pylori* differs from the *O*-glycoside bond between the same two molecules in other organisms.

It has long been unclear why *H. pylori* needs to assimilate non-esterified cholesterol into its cell membrane. Recent investigations by our group and others have helped us more fully understand the purposes of this cell membrane assimilation of non-esterified cholesterol in the bacterium. This review article describes the biological significance of the cholesterol uptake and glucosylation for the survival of *H. pylori*, and the interaction of the *H. pylori* cell membrane with other steroid compounds.

2. Biosynthesis and Membrane Localization of Glucosyl Cholesterols

H. pylori like most other bacteria, is capable of growing *in vitro* without help from non-esterified cholesterol. Yet, when non-esterified cholesterol is added into a culture medium, *H. pylori* aggressively absorbs the cholesterol into the cell membrane, glucosylates the cholesterol, and uses both non-esterified cholesterol and glucosylated cholesterol as cell membrane lipid components. Lebrun *et al.* (2006) identified the enzyme involved in the biosynthesis of glucosyl cholesterol in *H. pylori* as a cholesterol-*α*-glucosyltransferase (CGT) encoded by *hp*0421 gene on chromosomal DNA. CGT uses the uridine diphosphate glucose (UDP-Glc) as a sugar source, catalyzes the dehydration reaction between the 1*α*-hydroxyl (OH) group in a D-glucose molecule and the 3*β*-OH group in a cholesterol molecule, and synthesizes cholesteryl-*α*-D-glucopyranoside (CGL) [25].

H. pylori is known to produce at least three types of glucosyl cholesterols, namely CGL, cholesteryl-6-*O*-tetradecanoyl-*α*-D-glucopyranoside (CAG), and cholesteryl-6-*O*-phosphatidyl-*α*-D-glucopyranoside (CPG), but the enzymes catalyzing the acylation and phosphatidylation of the CGL molecule involved in the biosynthesis of CAG and CPG have yet to be identified [26]. A study by our group in 2009 revealed that glucosyl cholesterols are more potent lipid constituents in the outer membrane than in the inner membrane, but that the non-esterified cholesterol absorbed into the *H. pylori* cell is present in both the inner membrane and the outer membrane (**Figure 1**) [27].

3. A Role of Cholesterol Glucosylation in the Survival of *H. pylori*

Wunder *et al.* (2006) were the first to identify a role of cholesterol glucosylation in *H. pylori*. Specifically, they demonstrated that *H. pylori* cells glucosylate the non-esterified cholesterol extracted from the lipid raft of

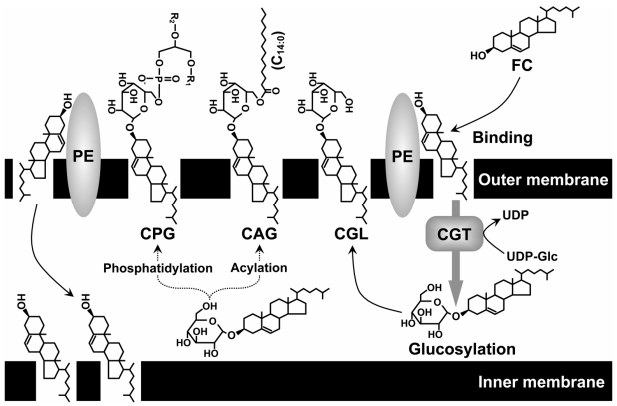

Figure 1. Localization of non-esterified cholesterol and glucosylated cholesterols in *H. pylori* cell membranes. FC, non-esterified cholesterol; CGL, cholesteryl-*α*-D-glucopyranoside; CAG, cholesteryl-6-*O*-tetradecanoyl-*α*-D-glucopyranoside; CPG, cholesteryl-6-*O*-phosphatidyl-*α*-D-glucopyranoside; PE, phosphatidylethanolamine; UDP-Glc, uridine diphosphate glucose; CGT, cholesterol-*α*-glucosyltransferase

gastric epithelial cells to evade the host immune responses [28]. The glucosylation of the non-esterified cholesterol absorbed into the cell membrane allows the bacterium to colonize the gastric mucosa tissues of hosts for long periods by conferring resistance against the phagocytosis of macrophages and regulating the activation of antigen-specific T cells. Yet an *hp*0421 gene-disrupted *H. pylori* mutant that regained only the non-esterified cholesterol without glucosylation was strong activator of macrophages and antigen-specific T cells and was promptly banished from the gastric mucosa tissues of hosts. The same pattern was observed in an abnormal *H. pylori* strain bioengineered to artificially incorporate excessive amounts of non-esterified cholesterol into the cell membrane, a strain that turned out to be incapable of biosynthesizing sufficient amounts of glucosyl cholesterol. We can thus infer that non-esterified cholesterol itself poses a threat to the survival of any *H. pylori* cells that fail to glucosylate it. If *H. pylori* behaved like other general bacterial species and had a cell membrane impervious to exogenous non-esterified cholesterol, its cells would have no need to glucosylate the non-esterified cholesterol. In sum, no one has yet unraveled why the cell membrane of *H. pylori* actively assimilates non-esterified cholesterol.

4. Biological Significance of Cholesterol Uptake by *H. pylori*

Our study in 2009 revealed that *H. pylori* cells without non-esterified cholesterol or other cholesterol analogues succumb to the bacteriolytic action of phosphatidylcholines (PCs), while the *H. pylori* cells with absorbed non-esterified cholesterol or the cholesterol analogue estrone

acquire resistance against the bactericidal action of PCs (**Figure 2**) [27]. *H. pylori* was incapable of glucosylating estrone absorbed into the cell membrane. Hence, its resistance against the bacteriolytic action of the PCs was acquired independently of the glucosylation of the steroid compounds. PC is the most prevalent glycerophospholipid in mammals and exists in sufficient abundance to kill *H. pylori* in gastric mucosa and gastric juice of humans [29,30]. In sum, our study demonstrated that *H. pylori* aggressively incorporates exogenous non-esterified cholesterol into the cell membrane, in order to survive in the presence of PCs.

In separate experiments, McGee *et al.* (2011) and Trainor *et al.* (2011) inactivated the CGT of an *H. pylori* strain by inserting a chloramphenicol-resistant (*cat*) gene cassette into the *hp*0421 gene. With this strain, they found that non-glucosylated non-esterified cholesterol absorbed into the cell membrane fortified this bacterium's resistance to antibacterial agents such as ciprofloxacin, tetracycline, and clarithromycin, and to bile salts and ceragenins [31,32]. The studies by our group and others indicate that non-esterified cholesterol strengthens the cell membrane lipid barrier of *H. pylori*. Incidentally, McGee et al. have also proposed that the glucosylation of the non-esterified cholesterol absorbed by *H. pylori* confers further resistance against the bactericidal action of antibacterial peptides such as polymixin B and colistin, at least in part [31].

5. Response of *H. pylori* Cell Membrane to Other Steroid Compounds

It has been unclear whether the *H. pylori* cell glucosylates only the non-esterified cholesterol. Steroid hormones such as sex hormones (steroid compounds) and corticoids, typical sterol analogues in mammals, are derived from non-esterified cholesterol. Earlier investigations by other groups have shown that the enzymes involved in the biosynthesis and activation of sex hormones are also expressed in human gastric tissues [33-36]. Still other studies have revealed the expression of sex hormone receptors in gastric cancer [37-39]. These studies, taken together, indirectly demonstrate that sex hormones exist in the human stomach, an environment that *H. pylori* colonizes and inhabits for many years. Our group has looked closer at this issue by investigating how the cell membrane of *H. pylori* interacts with sex hormones. In 2009 we found that *H. pylori* glucosylates not only non-esterified cholesterol, but also various steroid compounds with a 3*β*-OH group, such as pregnenolone, dehydroepiandrosterone, and epiandrosterone [40]. Yet again, as mentioned earlier, estrone with a 3-OH group was incorporated into the cell membrane of *H. pylori* but not glucosylated once absorbed [27]. These results indicate that the 3*β*-OH group in a steroid molecule is a crucial conformation for glucosylation by the enzymatic action of CGT regardless of the differences of other structures among the steroid compounds.

Our group also found that some steroid compounds impair the viability of *H. pylori*. In particular, progesterone with an oxo group at the carbon 3-position of the steroid framework destabilized the cell membrane structure of *H. pylori* and ultimately destroyed the cells [41]. The cell membrane of *H. pylori* apparently interacted with a number of steroid compounds, some of which were even toxic to this bacterium (**Figure 3**). Intriguingly, a synthetic progesterone derivative acylated by a caproic acid ($C_{6:0}$) at the carbon 17-position in a progesterone molecule had conspicuously stronger anti-*H. pylori* activity than the progesterone, whereas a natural progesterone derivative hydroxylated at the same carbon position of a progesterone molecule lacked this activity. This may open the way to the development of a new class of steroidal medicines that lack the hormonal activity but maintain the anti-*H. pylori* activity, using the progesterone molecule as a basic structure (**Figure 4**).

Progesterone non-reversibly bound to the cell membrane of *H. pylori* and inhibited the interaction of non-

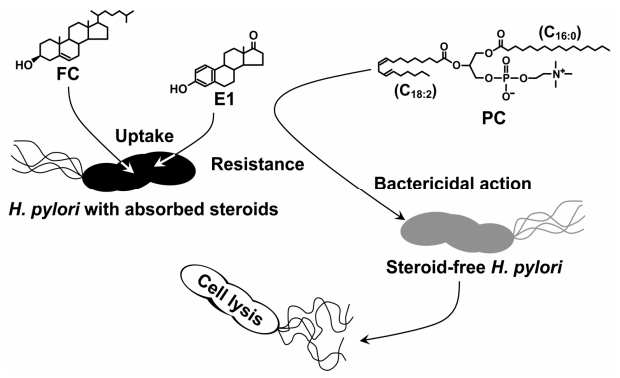

Figure 2. How *H. pylori* acquires resistance to the bacteriolytic action of phosphatidylcholine by incorporating steroid compounds into the cell membrane. FC, non-esterified cholesterol; E1, estrone; PC, phosphatidylcholine.

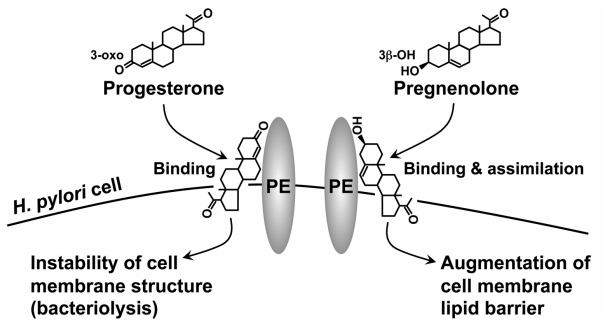

Figure 3. Cell membrane of *H. pylori* interacting with steroid compounds regardless of toxicity. PE, phosphatidylethanolamine.

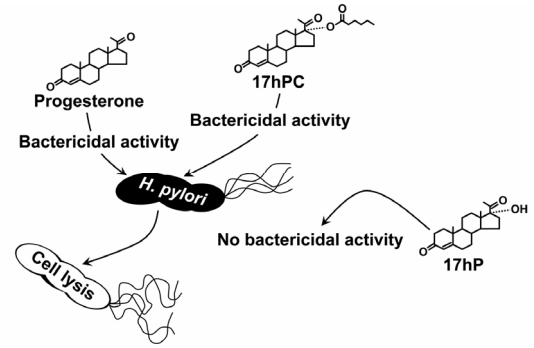

Figure 4. Anti-*H. pylori* activity of progesterone and its derivatives. 17hPC, 17*α*-hydroxyprogesterone caproate; 17hP, 17*α*-hydroxyprogesterone.

esterified cholesterol to the bacterial cell membrane. Conversely, non-esterified cholesterol protected the *H. pylori* cells from the bacteriolytic action of progesterone to some degree. This suggested that non-esterified cholesterol and progesterone bind to the identical component on the cell membrane of *H. pylori* and thereby obstruct each other's effects on the bacterial cell. We were unable, however, to identify which of the cell membrane components of *H. pylori* took part in the binding of the steroid compounds in that series of experiments.

6. Uptake Mechanism of Non-Esterified Cholesterol by *H. pylori* Cell Membrane

When *H. pylori* was incubated in a serum-supplemented culture medium, the cell membrane of this bacterium incorporated only the non-esterified cholesterol, even though esterified cholesterol was more abundant. Year after year we were unable to explain why the cell membrane of *H. pylori* absorbs non-esterified cholesterol but not esterified cholesterol. In an earlier investigation we found that the components of glucosyl cholesterols hardly changed in *H. pylori* cells undergoing growth phase changes from the logarithmic phase to the decline phase in a serum-supplemented culture medium. The level of CGL, a basic structure of glucosyl cholesterols, decreased in close correlation with the increases in CAG and CPG [42]. This meant that the active uptake of non-esterified cholesterol by the *H. pylori* cells was only observable in the logarithmic growth phase. Hence, the decrease in the CGL reflected a reduction of non-esterified cholesterol uptake by the *H. pylori* cell. Intriguingly, the amount of phosphatidylethanolamine (PE) in the membrane lipid composition decreased remarkably, from about 66% to 29%, in *H. pylori* cells undergoing growth phase changes. Further, the decline curve of PE along the time axis of the growth phase in *H. pylori* cells matched the decline curve of the CGL almost exactly. In contrast to the PE, the amounts of phosphatidylglycerol-cardiolipin (PG-CL), other glycerophospholipids of *H. pylori*, negligibly altered in its bacterial cells undergoing growth phase changes from the logarithmic phase to the decline phase. We therefore assumed that the PE of *H. pylori* regulated the uptake of non-esterified cholesterol by the cells. PE is the most prevalent glycerophospholipid in *H. pylori*, as in many other commonplace bacteria, yet the function of PE is still incompletely understood.

In a more recent study in 2012 we found that PE took part in the incorporation of non-esterified cholesterol into the cell membrane of *H. pylori* [43]. *H. pylori* PE clearly bound to non-esterified cholesterol more potently than PG-CL of this bacterium, or a reference *Escherichia coli* PE. Surprisingly, *E. coli* PE bound to both esterified cholesterol and non-esterified cholesterol at similar levels, whereas *H. pylori* PE bound to far less esterified cholesterol than to non-esterified cholesterol. In sum, *H. pylori* PE bound non-esterified cholesterol selectively and left esterified cholesterol alone. *H. pylori* PE also turned out to be involved in the incorporation of non-esterified steroids, that is, pregnenolone and dehydroepiandrosterone, other steroid compounds with a 3*β*-OH group, into the cell membrane.

When the heat-killed cells of *H. pylori* and *E. coli* were treated with 2,6-di-*O*-methyl-*β*-cyclodextrin (dM*β*CD), a lipid solubilizer, the conspicuous elution of PE from the cells was observed in *H. pylori*. Meanwhile, the elution of PE from the heat-killed *E. coli* cells was negligible levels, whereas the elution of PG-CL from the same cells was conspicuous. Given that dM*β*CD makes its first contact with the bacterial cell in the outermost layer of the outer membrane, and given that the conspicuous elution of PE from the *H. pylori* cells is induced via the action of dM*β*CD, we can assume that the outermost layer of the outer membrane of *H. pylori* contained more PE than PG-CL. Conversely, the outermost layer of the outer membrane of *E. coli* was seemed to contain more PG-CL than PE. *E. coli* cells show no signs of interaction with either esterified cholesterol or non-esterified cholesterol. Two of our results may partly explain why no cholesterol is absorbed into the cell membrane of *E. coli*: first, relatively less PE is expressed on the cell surface of *E. coli* than on the cell surface of *H. pylori*; second, *H. pylori* PE clearly binds to non-esterified cholesterol more efficiently than *E. coli* PE binds to non-esterified cholesterol or esterified cholesterol.

In one set of experiments we analyzed the fatty acid compositions of *H. pylori* PE to identify which structure is most closely involved in the selective binding of non-esterified cholesterol. The predominant saturated fatty acid component of *H. pylori* PE was a myristic acid ($C_{14:0}$) molecule, whereas that of *E. coli* PE turned out to be a palmitic acid ($C_{16:0}$) molecule. Several studies by others have identified a palmitic acid ($C_{16:0}$) as the predominant saturated fatty acid component of PE in typical

Gram-negative bacteria such as *E. coli*, *Klebsiella pneumoniae*, *Salmonella enterica* serovar Typhimurium, and *Pseudomonas aeruginosa* [44-47]. These findings, taken in sum, proved that the saturated fatty acid composition of *H. pylori* PE was quite distinct from that of other bacterial species PEs.

Noting this, we decided to examine the importance of the myristic acid ($C_{14:0}$) molecule attached to PE in the selective binding of non-esterified cholesterol by dimyristoyl PE. As expected, dimyristoyl PE bound to non-esterified cholesterol selectively and bound to esterified cholesterol to only a negligible degree. In contrast, dipalmitoyl PE bound to both esterified cholesterol and non-esterified cholesterol. Hence, the myristic acid ($C_{14:0}$) molecule in *H. pylori* PE played an important role in the binding of non-esterified cholesterol. These results demonstrate how *H. pylori* PE contributes as a steroid-binding lipid functionally important to the assimilation of non-esterified cholesterol and 3*β*-OH steroids (**Figure 5**).

As described above, progesterone non-reversibly binds to the *H. pylori* cells and inhibits the absorption of non-esterified cholesterol into its cell membrane. On this basis, *H. pylori* PE is also thought to be involved in the binding of progesterone to *H. pylori* cells.

7. A Novel Role of Cholesterol Glucosylation in the Survival of *H. pylori*

Several studies have clarified the respective roles of cholesterol-uptake and cholesterol-glucosylation in *H. pylori* cells, as mentioned earlier. The uptake of non-esterified cholesterol into the cell membrane is important to *H. pylori*'s resistance to the antibacterial activity of phosphatidylcholines (PCs), antibiotics, and bile salts. Likewise, the glucosylation of the non-esterified cholesterol absorbed into the cell membrane is important to the regulation of host immune responses against *H. pylori*.

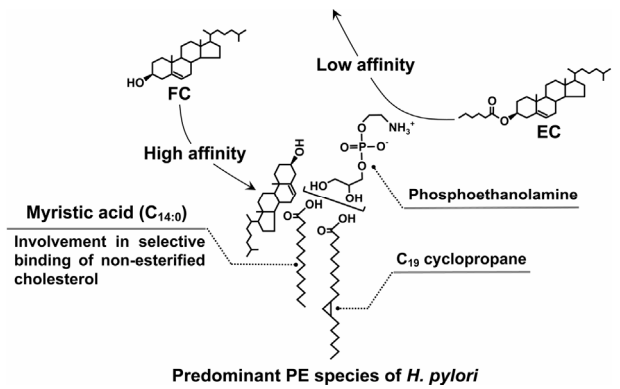

Figure 5. Selective binding of non-esterified cholesterol to *H. pylori* phosphatidylethanolamine. FC, non-esterified cholesterol; EC, esterified cholesterol; PE, phosphatidylethanolamine.

The most recent study by our group revealed a novel role of cholesterol glucosylation in the survival of *H. pylori* cells, a role distinct from host immune system evasion [48].

The sterol 7-dehydrocholesterol is the direct precursor of non-esterified cholesterol in the cholesterol biosynthetic pathway in mammalian tissues. The non-esterified cholesterol and 7-dehydrocholesterol differ in the number of hydrogen atoms in the steroid framework. The precursor lacks the two hydrogen atoms at carbon positions 7 and 8 of the non-esterified cholesterol molecule. Yet no studies have shown whether 7-dehydrocholesterol is useful or harmful for the growth and/or survival of the *H. pylori* cell.

When *H. pylori* at a low cell density ($10^{6.5}$ CFU/ml) was incubated in the presence of 7-dehydrocholesterol at a 100 μM concentration, we were intrigued to find that the non-esterified cholesterol precursor induced the bacteriolysis of this bacterium. Put simply, 7-dehydrocholesterol proved to be fatally toxic to *H. pylori*. Incidentally, non-esterified cholesterol at the same concentration had no influence on the viability or growth of this bacterium. In contrast, when *H. pylori* at a high cell density (10^9 CFU/ml) was incubated in the presence of 7-dehydrocholesterol (100 μM), we were surprised to find that the *H. pylori* cells lasted somehow to the bacteriolytic action of 7-dehydrocholesterol, glucosylated its toxic sterol, and admitted the glucosylated 7-dehydrocholesterols as membrane lipid components. Observing this, we assumed that the *H. pylori* detoxified the 7-dehydrocholesterol via the glucosylation. In our next experiment we examined the glucosylation-induced abolishment of the anti-*H. pylori* activity of 7-dehydrocholesterol by incubating the *H. pylori* at a low cell density ($10^{6.5}$ CFU/ml) in the presence of glucosyl 7-dehydrocholesterol (100 μM). As expected, the glucosylated 7-dehydrocholesterol had no effect on the viability or growth of *H. pylori* cells. In addition, the *hp*0421 gene-disrupted *H. pylori* mutants with inactivated CGT and no ability to glucosylate sterols were clearly more susceptible to the bactericidal action of 7-dehydrocholesterol than the wild type *H. pylori* cells. These results indicate that the cholesterol-*α*-glucosyltransferase (CGT) encoded by the *hp*0421 gene plays an important role in the inactivation of sterols toxic to *H. pylori* (**Figure 6**).

Non-esterified cholesterol is biosynthesized from 7-dehydrocholesterol via the catalytic action of 7-dehydrocholesterol reductase encoded by a gene on chromosome 11 in humans [49]. A mutation of the gene for 7-dehydrocholesterol reductase leads to an accumulation of the sterol in plasma and tissues and has a role in the development of Smith-Lemli-Opitz (SLO) syndrome [49,50]. The plasma concentration of 7-dehydrocholesterol is vastly higher in SLO patients (from about 100 μM to 400

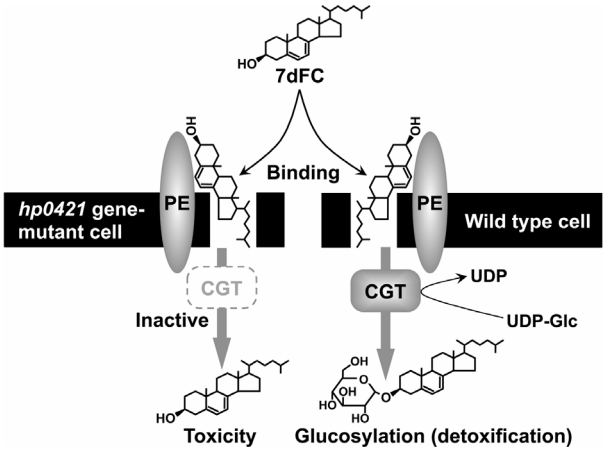

Figure 6. Detoxification of 7-dehydrocholesterol via the enzymatic action of *H. pylori* cholesterol-*α*-glucosyltransferase. 7dFC, 7-dehydrocholesterol; UDP-Glc, uridine diphosphate glucose; PE, phosphatidylethanolamine; CGT, cholesterol-*α*-glucosyltransferase.

μM), than in normal subjects (10 μM) [50-53].

As described above, we have found that phosphatidylcholines (PCs) induce the cell lysis of *H. pylori* without retained steroid compounds. Given that the SLO patients have a mutation only in the 7-dehydrocholesterol reductase gene, we can assume that PC is present at a normal concentration even in the gastric mucosa tissues of SLO patients. If *H. pylori* colonizes the gastric mucosa tissues of SLO patients, the cell membrane of this bacterium may ingest the 7-dehydrocholesterol from the gastric mucosa tissues of the hosts in order to survive around the PCs, even though the sterol is toxic to the bacterium. The benefit in this case, survival around the PCs, may outweigh the damage incurred by the toxicity to the bacterium. Hence, *H. pylori* is thought to be capable of glucosylating, and thereby detoxifying, the 7-dehydrocholesterol. Moreover, given the common occurrence of 3*β*-OH sterols such as ergosterol, sitosterol, campesterol, and stigmasterol in diverse forms of life, from fungi to mammals and plants, we can safely assume that some exert toxicity against *H. pylori* much in the same way as 7-dehydrocholesterol. *H. pylori* cells thus need to inactivate toxic 3*β*-OH sterols in order to survive *in vivo* or *in vitro*. The glucosylation of 3*β*-OH steroid compounds seems to be an essential mechanism for accomplishing this in *H. pylori*.

8. Conclusions

This review article has described the interaction of the *H. pylori* cell membrane with non-esterified cholesterols (including 7-dehydrocholesterol) and other steroid compounds. As described earlier, the inactivation of cholesterol-*α*-glucosyltransferase (CGT) via the insertion of the chloramphenicol-resistant (*cat*) gene cassette into the *hp*0421 gene prevents *hp*0421 mutant *H. pylori* cells from glucosylating non-esterified cholesterol. Even so, the cell membrane of the mutant cells absorbed non-esterified cholesterol from serum-supplemented culture medium and incorporated it as a lipid component [48]. This indicates that the *hp*0421 gene-mutant cells bind to non-esterified cholesterol via the mediation of phosphatidylethanolamine (PE) in the outermost layer of the outer membrane. In sum, the cell membrane of *H. pylori* glucosylates non-esterified cholesterol and other 3*β*-OH steroid compounds via at least two pathways: first, via the direct binding of 3*β*-OH steroid compounds to the CGT protein followed by glucosylation; second, via the transport of 3*β*-OH steroid compounds to the CGT protein after the binding of those compounds to the PE for glucosylation. Yet it remains unclear whether *H. pylori* cells possess a transport molecule that carries the 3*β*-OH steroid compounds from the PE molecules to the CGT proteins. Another unsolved question is the localization of the CGT protein in the outer membrane and inner membrane of the *H. pylori* cell. Earlier we established that the glucosylated cholesterols (CGL, CAG, and CPG) in *H. pylori* cells are more predominantly localized in the outer membrane than in the inner membrane, while non-esterified cholesterol in bacterial cells is localized in both the inner and outer membranes. This prompted us to consider the localization of the CGT protein in the inner membrane of *H. pylori* cell.

Here, we found that the 3*β*-OH steroid compounds binding to the outer membrane of bacterial cells via the mediation of PE seemed to somehow shift to the inner membrane, and that later, the 3*β*-OH steroid compounds glucosylated by the catalytic action of CGT in the inner membrane seemed to be carried outward to the outer membrane (**Figure 7**). Investigations into the carrier molecules involved in the transport of 3*β*-OH steroid

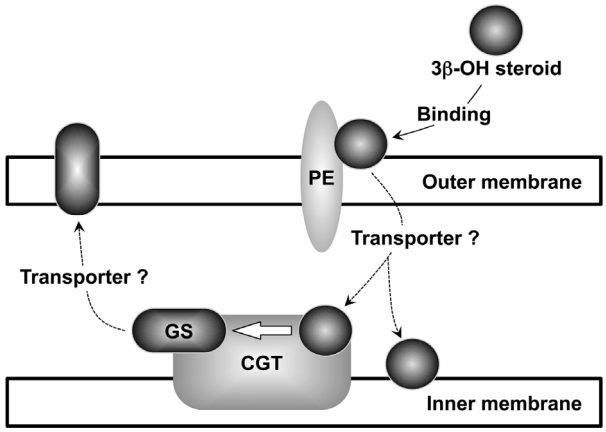

Figure 7. Proposed membrane transport of 3*β*-OH steroid compounds in *H. pylori*. PE, phosphatidylethanolamine; CGT, cholesterol-α-glucosyltransferase; GS, glucosyl steroid.

compounds and glucosylated steroid compounds may elucidate the precise mechanisms of the transport systems in *H. pylori*.

Lipopolysaccharide (LPS) is generally the main membrane lipid component of the outermost layer of the outer membrane of Gram-negative bacteria. LPS is a glycolipid consisting of a long polysaccharide chain and fatty acid molecules. It comes into direct contact with the outsides of the bacterial cells via part of the polysaccharide chain and regulates the permeability of the cell membrane to various lipophilic compounds [54]. Gram-negative bacteria are therefore intrinsically resistant to phosphatidylcholines (PCs) and steroid compounds. Though *H. pylori* is Gram-negative and naturally possesses LPS, the bacterium succumbs to the bacteriolytic activity of PCs if the cell membrane of the bacterium fails to incorporate the beneficial steroid compounds. The cell membrane of *H. pylori* also easily ingests a number of steroid compounds, even though some are fatally toxic to the bacterium. As described earlier, the predominant saturated fatty acid molecule attached to the PE of *H. pylori* is a myristic acid that is composed of 14 carbon atoms, whereas the predominant saturated fatty acid molecule attached to the other Gram-negative bacterial PEs is a palmitic acid that is composed of 16 carbon atoms. Although it is unclear what this means, intriguingly, noticeable acyl groups in *H. pylori* LPS molecule are fatty acid molecules that are composed of 16 carbon atoms and 18 carbon atoms, while noticeable acyl groups in typical bacterial LPS molecules, such as *E. coli* and *Salmonella enterica* serovar Typhimurium, are a fatty acid molecule that is composed of 14 carbon atoms [55-57]. These suggest that the membrane lipid conformation of *H. pylori* may be quite distinct from those of other Gram-negative bacteria, or that the LPS contents of the outer membrane of *H. pylori* are conspicuously lower than those of the outer membranes of other Gram-negative bacteria. On account of the unique membrane lipid conformation differing from those of other Gram-negative bacteria, the outer membrane of *H. pylori* cells without assimilated sterols or steroids is considered to cause the rise of membrane permeability to hydrophobic compounds, and therefore the bacterial cells succumb even to the bacteriolytic action of PCs. Hence, to overcome the membrane collapse induced by the invasion of such glycerophospholipids, *H. pylori* cells are suggested to allow the contact with various steroid compounds via the mediation of PE in the outer membrane. To clarify whether the steroid compound-free membrane of *H. pylori* cell is structurally vulnerable to exogenous lipophilic compounds compared with those of other Gram-negative bacteria, it will be necessary to carry out the further analyses for the total lipid compositions of both the outer membrane and inner membrane of the *H. pylori* cell.

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