Helicobacter pylori associated phospholipase A₂ activity: a factor in peptic ulcer production?

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Abstract

Aims: To examine the potential role of the lipolytic enzyme phospholipase A₂, produced by *Helicobacter pylori* in ulcer formation.

Methods: Phospholipase A₂ activity in *H pylori* was compared with that in 10 commonly occurring pathogenic bacteria. Phospholipase A₂ activity and its cytotoxic metabolite, lysolecithin, in the basal gastric aspirates of 12 patients infected with *H pylori* were compared with those in 12 subjects not infected with *H pylori*.

Results: The phospholipase A₂ activity in *H pylori* was substantially higher than that in most of the other bacteria tested, and the activities of phospholipase A₂ and lysolecithin in the basal gastric aspirates of those infected with *H pylori* were significantly higher than the activities found in the basal gastric aspirates of subjects who were not infected. The lysolecithin proportion of total phospholipids in the gastric aspirates was also much higher in the infected than the non-infected group and a weak positive correlation (0.415) was found between phospholipase A₂ and lysolecithin in the infected group.

Conclusions: *H pylori* has clinically important concentrations of phospholipase A₂, an enzyme capable of hydrolysing gastric mucosal phospholipids. The high values of phospholipase A₂ and lysolecithin in gastric fluid from subjects with *H pylori* infections supports the notion that phospholipase A₂ is involved in the inflammation and mucosal damage associated with peptic ulcer formation.

The pathogenesis of ulcer formation has still not been satisfactorily elucidated despite the increasing acceptance of the role of *Helicobacter pylori* in duodenal and, to a lesser extent, gastric ulcers.¹ Hypotheses include impaired mucosal defence by *H pylori* toxins,^{2,3} ammonia,⁴ inflammation⁵ or hydrogen ion back-diffusion.⁶

We suggest another hypothesis that involves the action or metabolites of phospholipase A₂ (PLA₂) [E.C.3.1.1.4], an enzyme capable of hydrolysing membrane phospholipids, which in the presence of strong gastric acidity, leads to mucosal damage. This study was designed to examine the possible clinical importance of PLA₂ activity in gastritis and peptic ulceration.

H pylori, a Gram negative bacterium, has been reported to have PLA₂ activity,⁷ and this has been shown to exist in several other pathogenic bacteria, particularly in the context of a bacterial cause of premature labour.⁸

The magnitude of PLA₂ activity in the *H pylori* bacterium was important to this study and the activity in two strains was compared with PLA₂ activity in other bacteria to provide a frame of reference. Furthermore, to determine if PLA₂ activities were higher in the stomachs of *H pylori* positive patients than *H pylori* negative patients, the basal gastric aspirates from both types of patients attending a gastroscopy clinic were analysed for PLA₂, total choline phospholipids, and lysolecithin, and the results compared.

Lysolecithin, a cytotoxic, amphipathic lipid produced by the action of PLA₂ on phospholipids,⁹ was measured as an indirect indicator of PLA₂ activity. It is itself capable of directly causing tissue damage, but is also a precursor for platelet activating factor, a known powerful pathogen in the development of ulcers.¹⁰

Methods

The 10 commonly occurring pathogenic bacteria tested, grown from stock cultures, were *Bacteroides fragilis*, *Campylobacter jejuni*, *Fusobacterium nucleatum*, *Staphylococcus epidermis*, *Enterococcus* (*Streptococcus faecalis*, *Escherichia coli*, haemolytic group A and group B streptococci, *Clostridium perfringens*, *Lactobacillus* and two strains of *H pylori* (NCTC 11637 and a patient's strain, PSR). All cultures were collected from overnight growth, except *H pylori* which is a slow growing microaerophilic bacterium usually obtained after five to six days growth for best yield. It was collected on day 3 to ensure that all colonies were live. Each bacterial type was harvested from up to 10 blood agar culture plates, by scraping with a sterile loop, into a small plastic centrifuge tube. They were ultrasonicated in an ice bath with a Sonic Dismembrator (Dynatech; Billingshurst, Kent) using a microprobe, for a total of 3 × 15 seconds at 4°C. After sonication the bacteria were centrifuged at 12 000 rpm for five minutes to remove cell debris. The supernatant cytoplasm or "sonicates" were analysed for protein and PLA₂ analysis. Saline washings were prepared by gently washing the *H pylori* culture plates with a few millilitres of 0.9% physiological saline. The remaining bacteria were removed by centrifugation and the washings assayed as for the sonicates. Analyses were

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performed in duplicate in three separate analyses.

Phospholipase A₂, total phospholipid, and lysolecithin activities were assayed in fasting gastric fluid aspirated from the body of the stomach at the beginning of endoscopy, from 12 patients subsequently confirmed by biopsy, culture, and histology to be *H pylori* positive and 12 patients who were attending a gastroscopy clinic after an overnight fast and no medication on the day of the test, as described previously.¹¹

Total protein, used to assess the concentrations of the bacterial supernatants, was assayed by the bicinchoninic acid assay.¹² This has proved more tolerant than the Lowry method to interference, in particular detergents. Phospholipase A₂ was measured using the fluorometric method of Thuren,¹³ with a specific pyrene labelled substrate and phospholipase A₂ standard obtained from KSV Chemicals (Helsinki, Finland). A unit is defined as 1 nmol of substrate converted per minute. All PLA₂ values reported in this study were assayed at pH 7.4, although assays at pH 4.0 and 2.0 were performed to determine the enzyme's activity in an acid environment. Total choline phospholipids were measured by the enzymatic-colorimetric method of Takayama¹⁴ and lysolecithin by a semiautomated method utilising the phospholipid assay. Samples were incubated with phospholipase B to hydrolyse completely the lysolecithin; the reduction in phospholipid activity equalled the lysolecithin in the original sample.¹⁵

The differences in gastric fluid concentrations of PLA₂, total phospholipids, and lysolecithin between *H pylori* positive and *H pylori* negative patients were tested for significance by Student's unrelated *t* test. Pearson's correlations were calculated between PLA₂ activity, lysolecithin, and phospholipids, in *H pylori* positive and *H pylori* negative patients.

Results

Phospholipase A₂ activities in the bacterial sonicates are shown in the table. The PLA₂ activities of the two strains of *H pylori*, freshly harvested, sonicated, and centrifuged were mean 66.9 (SD 4.5) and 63.3 (4.0) Units/gram total protein, or about 1020 pmol/min/ml of supernatant, over six replicate assays. The activity found in the saline washings of *H pylori*

Table Mean (SD) phospholipase A₂ activity in pathogenic bacteria

	Mean (SD) phospholipase A ₂ activity U/g protein
1 <i>Bacteroides fragilis</i>	22.5 (6.5)
2 <i>Campylobacter jejuni</i>	31.9 (6.7)
3 <i>Lactobacillus</i>	34.7 (7.3)
4 <i>Fusobacterium nucleatum</i>	35.4 (3.9)
5 <i>Staphylococcus epidermis</i>	46.5 (5.4)
6 <i>Enterococcus faecalis</i>	53.9 (7.0)
7 <i>Escherichia coli</i>	55.5 (6.6)
8 Haemolytic group A <i>Streptococcus</i>	68.0 (4.3)
9 <i>Helicobacter pylori</i> (PSR)	63.3 (4.0)
10 <i>Helicobacter pylori</i> NCTC11637	66.9 (4.5)
11 <i>Clostridia perfringens</i>	70.0 (3.8)
12 Haemolytic group B <i>Streptococcus</i>	90.5 (8.1)

Helicobacter pylori showed relatively high activity.

Figure 1 Basal gastric fluid phospholipase A₂ activity (pmol/min/ml) in *H pylori* positive and in *H pylori* negative patients. The positive group level was significantly higher ($p < 0.001$).

was 36 (7) Units/gram total protein, which was considerably lower than that found in the total bacterial sonicate.

PLA₂ activity in the gastric aspirates of the positive patients, 86.7 (45) pmol/min/ml, was significantly higher than in the aspirates of the negative patients, 21.5 (19) pmol/min/ml (fig 1), using Student's *t* test for unrelated data ($p < 0.001$). The optimal activity of PLA₂ in both the gastric fluids and *H pylori* sonicates was at pH 7.4. Activity was noticeably reduced by about 60% at pH 4.0 and virtually non-existent at pH 2.0.

The gastric aspirate concentrations of lysolecithin and phospholipids are shown in fig 2. The mean total phospholipid and lysolecithin concentrations in the positive group (1.19 (0.30) mmol/l and 0.56 (0.36) mmol/l, respectively) were both significantly higher in the *H pylori* positive group than in the *H pylori* negative group (0.77 (0.34) mmol/l and 0.15 (0.08) mmol/l respectively), tested by Student's *t* test ($p < 0.001$). Furthermore, the average lysolecithin proportion of the total choline phospholipids in the *H pylori* positive group was 49%, which was significantly higher than the 19.5% found in the *H pylori* negative

Figure 2 Basal gastric fluid lysolecithin and total phospholipids (mmol/l) in *H pylori* positive patients and *H pylori* negative patients. The positive group level was significantly higher for both parameters ($p < 0.001$).

group. There was a small overlap between groups on all the parameters measured, reflecting the wide range of values that may be found in gastric aspirates, as noted by Begemann and Schumpelick in assessing gastroduodenal reflux.¹⁶

The correlations between PLA₂ activity and the other parameters, total phospholipids and lysolecithin, were close to zero in all cases, except for a positive correlation of 0.415 between PLA₂ and lysolecithin in *H. pylori* positive patients.

Discussion

Significant PLA₂ activities were found in the cell sonicates of all the pathogenic bacteria examined in this study. The concentrations of PLA₂ in the sonicates of the two strains of *H. pylori* were among the highest values found in the other bacteria. This study confirms a previous finding of PLA₂ activity in many pathogenic bacteria,⁸ although Lumb *et al* found significant PLA₂ activity only in *E. coli* and not in *Lactobacillus* or group B streptococci.¹⁷ Also confirmed is a report indicating high PLA₂ activity in eight strains of *H. pylori*.⁷

The protein adjusted PLA₂ activity in the saline washings of *H. pylori* was less than half that of the sonicates, but because PLA₂s are small molecular weight proteins of 12–18 kilodaltons,¹⁸ this suggests it can diffuse, or be secreted, to the outside of the bacterium in sufficient concentration to hydrolyse membrane phospholipids and initiate the inflammatory process seen in gastritis. The likelihood of PLA₂ coming from lysed non-viable *H. pylori* was reduced by collecting the bacteria at three days of culture and not more. The PLA₂ activity outside the cells contradicts the finding of Lumb *et al*, who tested some pathogenic bacteria (but not *H. pylori*), and found no spontaneous release of PLA₂ into culture media from them.¹⁷

A small significant increase in PLA₂ activity was found in the gastric fluid from the *H. pylori* positive group compared with the negative group, but the values were much lower than those obtained in the *H. pylori* saline washings. The origin of the gastric fluid PLA₂, which is optimised at pH 7.4, is under investigation. It may be released from elsewhere other than *H. pylori*—that is, from the gastric mucosa, or infiltrating white blood cells in the inflamed regions of the stomach. The activity of the PLA₂ was found to be very low at the acidic pH expected in the lumen of the stomach, but *H. pylori*, while motile, resides in the proximity of the gastric epithelial surface protected by a thick mucous layer,¹⁹ where the environmental pH is physiological.

The phospholipid and lysolecithin concentrations of the gastric aspirates were also significantly higher in the *H. pylori* positive group than the negative group. This confirms an earlier study in which lysolecithin was suggested as being a factor in gastric ulceration, long before *H. pylori* was identified as a gastric infection.²⁰ Lysolecithin is normally relatively high in gastric fluid, but in the *H. pylori* positive

patients it represented an average 49% of total phospholipids, while in the *H. pylori* negative patients it was only 19.5% of the total, indicating that significantly more phospholipid hydrolysis had occurred in the *H. pylori* positive patients.

Increased duodenal reflux of biliary fluid rich in lysolecithin in gastric inflammation has been reported,²¹ but Schumpelick *et al* found that such reflux was very moderate in ulcer disease.²² Lysolecithin concentrations in the basal gastric secretion from patients with gastric ulcer, however, have been found to be higher than in patients with duodenal ulcer,²³ which militates against gastroduodenal reflux being a major factor causing increased gastric lysolecithin concentrations.

Since 1982, when Warren and Marshall first identified *H. pylori* on gastric epithelium²⁴ and made the connection between the bacterium, gastritis, and ulceration,²⁵ its role has become widely accepted in these conditions. Despite a large amount of information being generated about this organism, as recently reviewed by Buck,²⁶ it is still not known how *H. pylori* is involved in gastric tissue damage.

This study has shown that *H. pylori* has significant PLA₂ activity, which acts by hydrolysing the fatty acid from the second carbon of membrane phospholipids to release highly cytotoxic lysophospholipids, such as lysolecithin, and fatty acids.²⁷ Lysolecithin is metabolised more slowly than fatty acids, persists in tissues longer, and because of its amphipathic properties is worthy of attention as an ulcer producing agent. It is also a precursor to platelet activating factor^{28,29} which in studies on rats has been acknowledged as the most powerful ulcerogenic agent so far encountered.¹⁰ The simple approach is that the inflammation and impaired mucosal defence caused by the action of the bacterium creates an environment which, in the presence of strong gastric acidity, leads to ulceration. A more complex mechanism may be involved, however. For example, one of the fatty acids released by PLA₂ activity is arachidonic acid from which powerful lipid mediators such as prostaglandins, leucotrienes, and thromboxanes of the "arachidonic acid cascade" are derived,³⁰ and these may contribute directly to tissue damage.

Significant PLA₂ activities in *H. pylori* and significantly higher concentrations of both PLA₂ and lysolecithin in the gastric fluids of *H. pylori* positive compared with *H. pylori* negative patients suggest that the organism is capable of initiating the inflammatory processes found in gastritis and ulceration, through mechanisms involving lysolecithin and possibly platelet activating factor.

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