Helicobacter pylori associated phospholipase A_2 activity: a factor in peptic ulcer production?

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Abstract

Aims: **To examine the potential role of the lipolytic enzyme phospholipase A_2, produced by *Helicobacter pylori* in ulcer formation.**

Methods: **Phospholipase A_2 activity in *H pylori* was compared with that in 10 commonly occurring pathogenic bacteria. Phospholipase A_2 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 A_2 activity in *H pylori* was substantially higher than that in most of the other bacteria tested, and the activities of phospholipase A_2 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 A_2 and lysolecithin in the infected group.**

Conclusions: ***H pylori*** **has clinically important concentrations of phospholipase A_2, an enzyme capable of hydrolysing gastric mucosal phospholipids. The high values of phospholipase A_2 and lysolecithin in gastric fluid from subjects with *H pylori* infections supports the notion that phospholipase A_2 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] inflammation[5] or hydrogen ion back-diffusion.[6]

We suggest another hypothesis that involves the action or metabolites of phospholipase A_2 (PLA_2) [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 PLA_2 activity in gastritis and peptic ulceration. *H pylori*, a Gram negative bacterium, has been reported to have PLA_2 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 PLA_2 activity in the *H pylori* bacterium was important to this study and the activity in two strains was compared with PLA_2 activity in other bacteria to provide a frame of reference. Furthermore, to determine if PLA_2 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 PLA_2, total choline phospholipids, and lysolecithin, and the results compared.

Lysolecithin, a cytotoxic, amphipathic lipid produced by the action of PLA_2 on phospholipids,[9] was measured as an indirect indicator of PLA_2 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 fragillis, 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 Dysmembrator (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 cytoplasms or "sonicates" were analysed for protein and PLA_2 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 A_2, 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 A_2 was measured using the fluorometric method of Thuren,[13] with a specific pyrene labelled substrate and phospholipase A_2 standard obtained from KSV Chemicals (Helsinki, Finland). A unit is defined as 1 nmol of substrate converted per minute. All PLA_2 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 Takayama[14] 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 PLA_2, 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 PLA_2 activity, lysolecithin, and phospholipids, in *H pylori* positive and *H pylori* negative patients.

Results

Phospholipase A_2 activities in the bacterial sonicates are shown in the table. The PLA_2 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* was 36 (7) Units/gram total protein, which was considerably lower than that found in the total bacterial sonicate.

Table Mean (SD) phospholipase A_2 activity in pathogenic bacteria

	Mean (SD) phospholipase A_2 activity U/g protein
1 *Bacteroides fragillis*	22·5 (6·5)
2 *Campylobacter jejuni*	31·9 (6·7)
3 *Lactobaccillus*	34·7 (7·3)
4 *Fusobacterium nucleatum*	35·4 (3·9)
5 *Staphylococcus epidermis*	46·5 (5·4)
6 *Enterococcus faecalis*	53·9 (7·0)
7 *Escherichia coli*	55·5 (6·6)
8 Haemolytic group A *Streptococcus*	68·0 (4·3)
9 *Helicobacter pylori* (PSR)	63·3 (4·0)
10 *Helicobacter pylori* NCTC11637	66·9 (4·5)
11 *Clostridia perfringens*	70·0 (3·8)
12 Haemolytic group B *Streptococcus*	90·5 (8·1)

Helicobacter pylori showed relatively high activity.

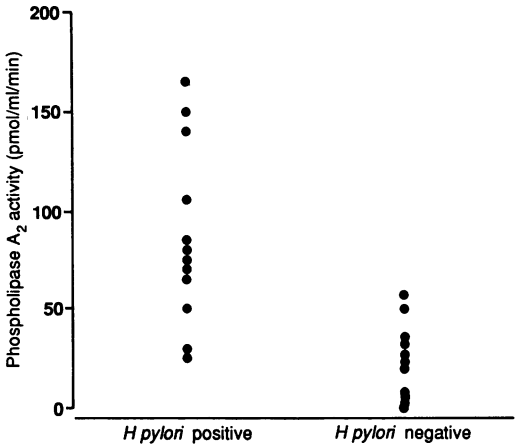

Figure 1 Basal gastric fluid phospholipase A_2 activity (pmol/min/ml) in H pylori positive and in H pylori negative patients. The positive group level was significantly higher ($p < 0·001$).

PLA_2 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 PLA_2 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

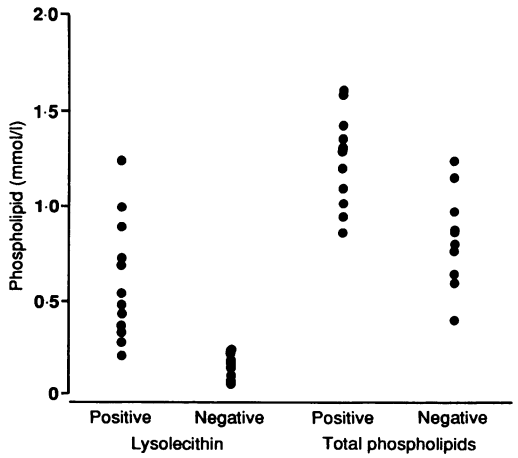

Figure 2 Basal gastric fluid lysolecithin and total phospholipids (mmol/l) in H pylori positive patients and H pylori negative patients. The positive group level was significantly higher for both parameters ($p < 0·001$).

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 PLA_2 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 PLA_2 and lysolecithin in *H pylori* positive patients.

Discussion

Significant PLA_2 activities were found in the cell sonicates of all the pathogenic bacteria examined in this study. The concentrations of PLA_2 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 PLA_2 activity in many pathogenic bacteria,[8] although Lumb *et al* found significant PLA_2 activity only in *E coli* and not in *Lactobacillus* or group B streptococci.[17] Also confirmed is a report indicating high PLA_2 activity in eight strains of *H pylori*.[7]

The protein adjusted PLA_2 activity in the saline washings of *H pylori* was less than half that of the sonicates, but because PLA_2s 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 PLA_2 coming from lysed non-viable *H pylori* was reduced by collecting the bacteria at three days of culture and not more. The PLA_2 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 PLA_2 into culture media from them.[17]

A small significant increase in PLA_2 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 PLA_2, 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 PLA_2 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 epithelium[24] 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 PLA_2 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 PLA_2 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 PLA_2 activities in *H pylori* and significantly higher concentrations of both PLA_2 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.

1 Blaser MJ. Gastric Campylobacter-like organisms, gastritis, and peptic ulcer disease. *Gastroenterology* 1987;93: 371–83.
2 Leunk RD, Johnson PT, David BC, Kraft WG, Morgan DR. Cytotoxic activity in broth-culture filtrates of Campylobacter pylori. *J Med Microbiol* 1988;26:93–9.
3 Figura N, Guglielmetti P, Rossolini A, *et al*. Cytotoxin production by *Campylobacter pylori* strains isolated from patients with peptic ulcers and from patients with chronic gastritis only. *J Clin Microbiol* 1989;27:225–6.

4 Sidebottom RL, Baron JH. Hypothesis: Helicobacter pylori, urease, mucus, and gastric ulcer. *Lancet* 1990;**335**:193–5.
5 Wyatt JI, Rathbone BJ, Dixon MF, Heatley RV. Campylobacter pyloridis and acid induced gastric metaplasia in the pathogenesis of duodenitis. *J Clin Pathol* 1987;**40**: 814–8.
6 Hazell SL, Lee A. Campylobacter pyloridis urease, hydrogen back-diffusion and gastric ulcers. *Lancet* 1986;ii:15–7.
7 Raedsch R, Stiehl A, Pohl S, Plachky J. Quantification of phospholipase A_2 activity of Campylobacter pylori. *Gastroenterology* 1988;**95**:A404.
8 Bejar R, Curbelo V, Davis C, Gluck L. Premature labor. II. Bacterial sources of phospholipase. *Obstet Gynecol* 1981; 57:479–82.
9 Giessler AJ, Bekemeier H, Hirschelmann R, Bakathir HA. Some effects of lysolecithin in vivo and in vitro and their possible relations to the inflammatory process. *Agents Actions* 1982;**10 (suppl)**:195–211.
10 Rosam A-C, Wallace JL, Whittle BJR. Potent ulcerogenic actions of platelet-activating factor on the stomach. *Nature* 1986;**319**:54–6.
11 Marshall BJ, Langton SR. Urea hydrolysis in patients with Campylobacter pyloridis infection. *Lancet* 1986;i:965–6.
12 Smith PK, Krohn RI, Hermanson GT, *et al.* Measurement of protein using bicinchoninic acid. *Anal Biochem* 1985; **150**:76–85.
13 Thuren T, Virtanen JA, Lalla M, Kinnunen PKJ. Fluorometric assay for phospholipase A_2 in serum. *Clin Chem* 1985;**31**:714–7.
14 Takayama M, Itoh S, Nagasaki T, Tanimizu I. A new enzymatic method for determination of serum choline containing phospholipids. *Clin Chim Acta* 1977;**79**:93–8.
15 Langton SR. A rapid sensitive method for serum lysolecithin and its measurement in early acute pancreatitis. *Med Sci Res* 1991;**19**:203–4.
16 Begemann F, Schumpelick V. Problems in duodenogastric reflux assessment, using bile acid and lysolecithin measurement. *Scand J Gastroenterol* 1981;**67 (suppl)**: 51–3.
17 Lumb MR, Roseblade CK, Helmig R, Uldbjerg N, Sullivan MHF, Elder MG. Use of a new simplified assay for phospholipase A_2 to measure bacterial enzyme levels. *Clin Chim Acta* 1990;**189**:39–46.
18 Waite M. *The phospholipases*. New York: Plenum Press, 1987.
19 Slomiany BL, Nishikawa J, Piotrowski K, Okazaki K, Slomiany A. Lipolytic activity of Campylobacter pylori: Effect of sofalcone. *Digestion* 1989;**43**:33–40.
20 Johnson AG, McDermott SJ. Lysolecithin: A factor in the pathogenesis of gastric ulceration? *Gut* 1974;**15**:710–3.
21 Pozdnyakov VS, Kuzin NM, Lyadov KV. A procedure for measuring the gastric juice lysophosphatidylcholine (lysolecithin). *Lab Delo* 1987;**11**:839–41.
22 Schumpelick V, Stemme D, Begemann F. Ulcer disease and duodenal reflux. *Z Gastroenterol* 1983;**21**:11–20.
23 Sarosiek J, Slomiany BL, Gabryelewicz A. Lysolecithin and glyceroglucolipids in gastric secretion of patients with gastric and duodenal ulcers. *Scand J Gastroenterol* 1983;**18**:935–8.
24 Warren JR, Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983;i:1273–5.
25 Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984;i:1311–4.
26 Buck GE. Campylobacter pylori and gastroduodenal disease. *Clin Microbiol Rev* 1990;**3**:1–12.
27 Dennis EA. Phospholipase A_2 mechanism: Inhibition and role in arachidonic acid release. *Drug Development Res* 1987;**10**:205–20.
28 Chilton FH, Ellis JM, Olson SC, Wykle RL. 1-0-alkyl-sn-glycero-3-phosphocholine. A common source of platelet-activating factor and arachidonate in human polymorphonuclear leucocytes. *J Biol Chem* 1984;**259**:12014–9.
29 Stenson WF. Platelet-activating factor in inflammatory bowel disease. *Gastroenterology* 1988;**95**:1416–21.
30 Das UN. Biological significance of arachidonic acid. *Med Sci Res* 1987;**15**:1485–90.