The Turkish Journal of Pediatrics 1999; 41: 335-339 Original

INCREASED GASTRIC JUICE LEUKOTRIENE B_4, C_4 AND E_4 CONCENTRATIONS IN CHILDREN WITH HELICOBACTER PYLORI COLONIZATION*

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SUMMARY: Kasırga E, Çoker I, Aydoğdu S, Yağcı RV, Taneli B, Gousseinov A. (Division of Gastroenterology, Department of Pediatrics, Ege University Faculty of Medicine, İzmir, Turkey). Increased gastric juice leukotriene B_4, C_4 and E_4 concentrations in children with Helicobacter pylori colonization. Turk J Pediatr 1999; 41: 335-339.

During recent years, the role of inflammatory lipid mediators in the pathophysiology of Helicobacter pylori (H. pylori) infections has been investigated in several studies. The concentrations of leukotrienes (LTs) in gastric juice from H. pylori positive (n = 13) and negative (n = 18) children with recurrent abdominal pain were studies in order to determine whether these lipid inflammatory mediators are involved in local and systemic biological actions. Gastric juice samples and biopsy specimens of mucosa were obtained endoscopically from 31 patients with recurrent abdominal pain for assessment of LTs and histopathological examination. In this study, all children with recurrent abdominal pain were investigated by rapid urease test and histological assessment for H. pylori colonization. Leukotriene levels were measured by high performence liquid chromatography (HPLC) and raioimmunoassay (RIA) in gastric juice samples. Gastric juice LTB_4, LTC_4, and LT_4 levels were significantly higher in patients with H. pylori colonization than in children without H. pylori colonization. These results indicate that increased gastric content of proinflammatory mediators (LTB_4, LTC_4, and LT_4) may be related to the pathogenesis of H. pylori-associated gastritis. *Key words: Helicobacter pylori, antral gastritis, leukotrienes, lipid mediators, gastric juice.*

Helicobacter pylori (H. pylori) is the most prevalent agent of chronic gastritis and duodenal ulcer disease in adults as well as in children[1,2]. It is known that H. pylori initates an inflammatory cascade that leads to self injury[3]. During recent years, the role of inflammatory lipid mediators in the pathophysiology of H. pylori infections has been investigated in several studies[4-6]. Leukotrienes (LTs) are synthesized from arachidonic acid which derived from lipid layers of the cell membrane by phospholipase A_2 through the lipoxygenase pathway by immunologic and nonimmunologic stimulation[7]. LTs are well known proinflammatory mediators[8]. LTB_4 is a potent chemoattractant of polymorphonuclear cells and causes degranulation

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and a relase of lysosomal enzymes. Peptide LTs (LTC_4, LTD_4 and LTE_4) could mediate gastric mucosal damage both by their vasoconstrictive actions and effects on vascular permeability, promoting vascular stasis and subsequent reduction in tissue perfusion[9,10]. Increases of both LTB_4 and LTC_4 have also been reported with gastritis associated with H. pylori[11-13].

The aim of this study was therefore to investigate the relation between H. pylori colonization and synthesis of eicosanoids by gastric mucosa, as detected by LT concentrations in gastric juice from H. pylori positive and negative children with recurrent abdominal pain (RAP).

Material and Methods

Thirty-one patients (16 girls, 15 boys; mean age ± SD: 12.7 ± 3.6 years) with RAP underwent diagnostic upper gastrointestinal endoscopy. All patients had normal laboratory values and radiological findings. At endoscopy, three biopsies were taken from the antrum. One sample was placed in a quick urease test for assessment of H. pylori colonization. Two samples were fixed in 10 percent formalin for histopathological examination. Gastric juice was obtained endoscopically. Gastric juice sample from all children with RAP was placed in a filtered tube containing a 5 ml mixture of methanol: water: acetic acid (70:30:0.01 v/v) for LTs analysis and was stored at -70 °Ç until assayed. Before assessment, cold PBS was added to the contents of the tube and this mixture was centrifuged at 6,000 rpm for 10 min for precipitating proteins and other deposits. The supernatant was filled into Sep. Pak C18 cartridges which were activated previously by 10 ml each of methanol and water both at the flow rate of 1.5 ml/min. The flow rate of the supernatant was 2.5 ml/min. These cartridges were rinsed twice with 5 ml of water and then with 5 ml of 20 percent methanol; the rinse solution was discarded. The LTs which were separated from other small molecule-sized, nonpolar lipids were extracted from the columns by 3 ml of methanol and then collected into a tube by passing through a 0.45 µ pore membrane filter and evaporated to dryness by speed vacuum concentrator. Dried samples were dissolved in 20 µl of solvent and kept at -70 °C.

Separation of LTs was performed by high performance liquid chromatography (HPLC) (Waters 625 LC System). The system was composed of a multisolvent delivery pump system, powerline system controller, water 486 tunable absorbance UV detector, Rheodyne 7012 injector, column areas and Baseline 810 HPLC software program. Separon SGX C18 super (250 x 2.0 mm 1.0) analytical column and precolumn (100 x 2-0.10) containing the same filling material were used with methanol: water:acetic acid gradient for separations of LTs. LTs were sequenced at 280 mm and 235 mm wavelengths and 1.0 ml/min and 1.5 ml/min flow rates, respectively. The LTs were collected from HPLC UV detector output according

to their retention times and were dried again in a vacuum speed evaporator. The retention time for LTE_4 was 7 min, LTB_4 min, 11 LTD_4 13 min, and LTC_4 15 min. The amounts of LTB_4, LTC_4 and LTE_4 were quantitatively measured using LTB_4 (^3H), LTC_4 (^3H) and LTE_4 (^3H) assay systems (Amersham Life Science, UK). The procedure was performed according to kit instructions, LTB_4, LTC_4 and LTE_4 concentrations were measured by Beta-liquid scintillation counter (TRI-CRAB-1600 TR, LSA-Packard, Canberra Company).

Results of LTs were expressed as ng/ml in gastric juice samples.

Statistics: Data on leukotriene concentrations are expressed as mean and standard error. Student's t test was used for comparisons between H. pylori positive and negative groups. A p value of less than 0.05 was considered significant.

Results

In gastric juice, LTB_4 concentrations in patilents with H. pylori colonization (mean ± SEM: 1.6 ± 0.29, range: 0.36-3.2) were significantly higher than in children without H. pylori colonization (mean ± SEM: 0.28 ± 0.09, range: 0-1.2) ($p < 0.001$). The values for LTC_4 in those with colonized H. pylori (mean ± SEM: 0.80 ± 0.16, range: 0.3-2.1) were higher than in those not colonized (mean ± SEM: non-detectable). LT_4 levels in children with H. pylori colonization (mean ± SEM: 2.75 ± 0.67, range: 0-0.67) were found to be significantly elevated when compared to H. pylori negative patients (mean ± SEM: 0.88 ± 0.31, range: 0-5.2) ($p < 0.05$).

In gastric juice, LTB_4, LTC_4 and LTE_4 were non-detectable in 55.5 percent (10/18), 100 percent (18/18) and 55.5 percent (10/18) respectively, of children without H. pylori colonization. On the other hand, LTE_4 was also non-detectable in 7.6 percent (1/13) of patients with H. pylori colonization.

Discussion

In this study we have shown that H. pylori colonization in children is associated with an increased gastric juice LTB_4 level as compared with children not colonized. Our study also shows that H. pylori colonization of gastric mucosa results in increased concentrations of gastric juice peptide LTs (LTC_4 and LTE_4). It has been reported that patients have higher LT concentrations in gastric mucosa if they are colonized by H. pylori than if they are not[4,11-13]. On the other hand, in the gastric juice of children without H. pylori colonization, LTs (LTB_4 and LTE_4) were also assessed in detectable concentrations but significantly less than the levels measured in children with H. pylori colonization. In H. pylori negative children, the source of these LTs are gastric epithelial cells and resident peripheral blood leukocytes in normal gastric mucosa. Increased concentration of these mediators in children with H. pylori colonization may be explained by increased mononuclear

cells and neutrophil infiltration in the gastric mucosa. LTB_4 is a potent chemotactic facor, as it attracts neutrophils, monocytes and lymphocytes to the inflammation area[4,8]. LTC4 causes local ischemia by vasoconstriction in gastric mucosa, and infiltration of inflammatory cells to this ischemic area leads to a release of secondary mediators[13]. Previous studies in humans have shown that synthesis of LTs correlates with mucosal histological injury[11-13]. However, the mechanisms of inflammation reaction in H. pylori infections are often complex and multiple[14,15]. The ammonia produced by H. pylori has been reported to have a cytotoxic effect on the gastric mucosa[16-19]. The pathogenesis of H. pylori-associated gastritis is also related to other factors such as free oxygen radicals, cytotoxins and cytokines[19-21]. Basso et al's[22] results supported the role of increased synthesis of several cytokines in the pathogenesis of H. pylori-associated gastritis. In addition, phospholipases produced by H. pylori induce the synthesis and relase of LTs from cytoplasmic membrane phospholids[23]. In conclusion, we investigated the influence of H. pylori colonizatilon on gastric juice LT concentrations in children with recurrent abdominal pain, and showed that H. pylori seemed to enhance the synthesis of LTs by promoting mucosal inflammatory cell infiltration. These results suggest that antileukotrienes will probably become effective agents in H. pylori-associated antral gastritis treatment.

REFERENCES

1. Lamireau T, Rigot A, Mégraud F, de Mascarel A. Helicobacter pylori gastritis in children. Arch Pediatr 1995; 2: 310-316.
2. Saita H, Murakami M, Yoo JK, et al. Link between Helicobacter pylori-associated gastritis and duodenal ulcer. Dig Dis Sci 1993; 38: 117-122.
3. Miegraud F. Duodenal ulcer disease: a new infectious disease. Eur J Gastroenterol Hepatol 1993; 5: 17-22.
4. Hudson N, Balsitis M, Everitt S, Hawkey CJ. Enhanced gastric mucosal leukotriene B_4 synthesis in patients taking non-steroidal anti-inflammatory drugs. Gut 1993; 34: 742-747.
5. Hudson , Balsitis sM, Filipowiez F, Hawkey CJ. Effect of Helicabacter pylori colonisation on gastric mucosal eicosanoid synthesis in patients taking non-steroidal anti-inflammatory drugs. Gut 1993; 34: 748-751.
6. Martin A, Struniolo GC, Fochesato M, et al. LTB_4 in gastritis and peptic ulcer. Minerva Gastroenterol Dietol 1991; 37: 163-167.
7. Lewis RA, Austen KF, Soberman RJ. Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. N Engl J Med 1990; 323: 645-655.
8. Lewis RA, Austen KF. The biologically active leukotrienes. Biosynthesis, metabolism, receptors, functions, and pharmacology. J Clin Invest 1984; 73: 889-897.
9. Bisgaard H. Sulphidoleukotrienes in human allergic diseases. Dan Med Bull 1989; 36: 142-159.
10. Henderson WR Jr. The role of leukotrienes in inflammation. Ann Intern Med 1994; 121: 684-697.
11. Fukuda T, Kimura S, Arakawa T, Kobayashi K. Possible role o fleukotrienes in gastritis associated with Campylobacter pylori. J Clin Gastroenterol 1990; 1: 5131-5134.

12. Ahmed A, Vaira D, Cairns SR, et al. Increased formation of leukotriene C4 in Campylobacter pyloritis. Gut 1987; 28: 1405.

13. Ahmed A, Holton J, Vaira D, Smith SK, Hoult JR. Eicosanoid synthesis and Helicobacter pylori associated gastritis: increase in leukotriene C4 generation associated with H. pylori colonization. Prostaglandins 1992; 44: 75-86.

14. Malfertheiner P, Bode G. Helicobacter pylori and the pathogenesis of duodenal ulcer disease. Eur J Gastroenterol Hepatol 1993; 5: 1-8.

15. Terada S, Negayama K, Kawanishi K. Neutrophil migration into the mucous layer in Helicobacter pylori-associated gastritis. Eur J Gastroenterol Hepatol 1993; 5: 45-49.

16. Lee A, Fox J, Hazell S. Pathogenicity of Helicobacter pylori: a perspective. Infect Immun 1993; 61: 1601-1610.

17. Dekigai H, Murakami M, Saita H, et al. Effect of monochloramine on neutrophil-mediated gastric vascular damage in cultured endothelial cells. Eur J Gastroenterol Hepatol 1993; 5: 41-44.

18. Suzuki M, Miura S, Suematsu M, et al. Helicobacter pylori elicits gastric mucosal cell damage associated with neutrophil-derived toxic oxidants. Eur J Gastroenterol Hepatol 1993; 5: 35-39.

19. Fujioka T. [Virulence and pathogenesis of Helicobacter pylori infection]. Rinsho Byori 1995; 43: 557-561.

20. Tamura K, Yamamur aM, Yamamoto I, et al. Ammonia produced by Helicobacter pylori, related to superoxide generation in situ, as a major factor in acute gastritis. Eur J Gastroenterol Hepatol 1993; 5: 51-56.

21. Broide E, Klinowski E, Varsano R, Eshchar J, Herbert M, Scapa E. Superoxide dismutase activity in Helicobacter pylori-positive antral gastritis in children. J Pediatr Gastroenterol Nutr 1996; 23: 609-613.

22. Basso D, Scrignerr M, Toma A, et al. Helicobacter pylori infection enhances mucosal interleukin-1 beta, interleukin-6, and the soluble receptor of interleukin-2. Int J Clin Lab Res 1996; 26: 207-210.

23. Zhou HL, Chabot-Fletcher M, Foley JJ, et al. Association between leukotriene B_4-induced phospholipase D activation and degranulation of human neutrophils. Biochem Pharmacol 1993; 46: 139-148.