JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1999, 50, 1, 17—23

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ROLE OF MAST CELLS AS A TRIGGER OF INFLAMMATION IN *HELICOBACTER PYLORI* INFECTION

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Helicobacter pylori (*H. pylori*) induces severe inflammation and plays a key role in gastric mucosal diseases. In general, mast cells have been believed to play an important role in inflammation. Although mast cells were detected in the gastric mucosa, the role of mast cells in the gastric mucosal inflammation caused by *H. pylori* is still unclear. Therefore, we examined the effects of *H. pylori* water extract on the degranulation of mast cells to clarify the role of these cells in gastric mucosal inflammation induced by *H. pylori*. Mast cells prepared from rat abdominal cavity were incubated with *H. pylori* for 30 min. The protein concentrations of *H. pylori* water extract used in this study were 0.5—3 mg/ml. The degranulation of mast cells were monitored morphologically by phase contrast microscopy equipped with time-lapse video recording system and biochemically by measuring histamine and β-hexosaminidase. *H. pylori* water extract induced the degranulation of mast cells dose-dependently. The identical experiment was performed without extracellular calcium, and no significant degranulation was found. The data indicates that the degranulation of mast cells by *H. pylori* water extract depend on extracellular calcium. The present results indicate that *H. pylori* might be involved in the gastric mucosal inflammation as a trigger of mast cell degranulation for releasing chemical mediators.

Key words: *Helicobacter pylori, histamine, mast cells, gastric mucosa, degranulation*

INTRODUCTION

Helicobacter pylori (*H. pylori*) induces severe inflammation and plays a key role in gastric mucosal diseases (1—4). In general, mast cells have been considered to play an important role in inflammation through the secretion of a variety of chemical mediators and inflammatory cytokines. *H. pylori* has been demonstrated to potentiate the release of histamine from purified rat serosal mast cells induced by 48/80, calcium ionophore A23187 or bile acid (5).

This paper was presented at the International Symposium Growth Factors and Polyamines in Mucosal Protection and Repair, 12—13 September, 1998, Cracow, Poland.

However, the detailed mechanisms of mast cell degranulation induced by *H. pylori* are still unclear. Therefore, we examined the effects of *H. pylori* water extract on the degranulation of mast cells to clarify the role of mast cells in the gastric mucosal inflammation induced by *H. pylori.*

MATERIALS AND METHODS

H. pylori water extract

H. pylori used in this study was obtained from ATCC (strain ATCC 43504, cagA (+), vacA (+) (6), American Type Culture Collection, Rockville, MD). *H. pylori* water extract was prepared according to the method reported by Yoshida *et al.* (7). Briefly, *H. pylori* was grown in a CO_2 incubator for 48 h and the cells were harvested with sterile swabs into distilled water, using 1 ml per plate ($10^{11}-10^{12}$ *H. pylori*). The cell suspension was kept at room temperature for 20 min, following the centrifugation at 12000 rpm for 15 min. Then the supernatant was stored at −20°C. Before use, the supernatant was brought to room temperature and centrifuged at 18000 rpm for 20 min and applied to experiments at a final protein concentrations of 0.5—3.0 mg/ml.

Isolation of rat peritoneal mast cells

Rats (Wistar albino rat, usually 800—1500g, male) were anaesthetised with diethylether and exsanguinated by cutting cervical artery. Tyrode's buffer (137 mM NaCl, 5.6 mM glucose, 10 mM Hepes, 2.7 mM KCl, 0.4 mM NaH_2PO_4, PH 7.3) was injected (50 ml) into the peritoneal cavity. The abdominal cavity was then massaged for 200 s, thereafter the peritoneal fluid was collected using a plastic Pasteur pippette. Cells were recovered by centrifugation (1200 rpm, 2 min, 4°C) and washed twice in Tyrode's buffer, giving us preparations of approximately 5% mast cells. Further, rat peritoneal mast cells were purified by density gradient centrifugation with 100% and 75% Percoll (8—11), resulting in 95% purity (12) (mast cells 5×10^5/rat).

Incubation of mast cells with H. pylori water extract

Mast cells (1×10^5/200 µl) were incubated at 30°C in Tyrode's buffer with *H.pylori* water extract (0.5, 1, 2, and 3 mg/ml) for 30 min with or without 1 mM Ca^{2+}. Mast cells were stimulated by compound 48/80 and the degranulation of mast cells were monitered morphologically by phase contrast microscopy equipped with time-lapse video recording system (CRV disc LVR-3000N, Sony, Tokyo, Japan) and by measuring histamine and β-hexosaminidase biochemically.

Histamine assay

The supernatants of the samples were placed into designated acylation tubes, and 50 µl of acylation buffer was added to each tube. Then 500 µl iodinated (^{125}I) acylated histamine was added and the materials were mixed well by vortex. 50 µl aliquots of these test solutions were incubated in the anti-histamine at 4°C for 18 hours. Solutions were aspirated and discarded, then anti-body-coated tubes were counted in a Beckman 5500 gamma counter (Beckman Instruments, Irvine, CA) for 1 min. Histamine standard solutions in dialyzed plasma (0.2—50.0 ng/ml Pharmacia RIA calc.) were used to construct standard curves (13).

Values were compared with that of basal histamine secretion which was obtained by the stimulation using 0.05 µg/ml compound 48/80 (Sigma-Aldrich Fine Chemicals, St. Louis, MO) and histamine release was expressed as follows.

Histamine release ratio = histamine release by *H.pylori* water extract/ histamine release by compound 48/80

Determination of β-hexosaminidase activity

50 μl of the supernatants of samples were placed into 1% BSA coated tubes, and to which 50 μl of 1.3 mg/ml N-acetyl glucosamine was added. After the incubation at 37°C for 90 min, 0.2 M glycine buffer was added to each tube to stop the reaction. Then the samples were applied to spectrometer (OD 405) (Beckman DU-64 Spectrophotometer, Irvine, CA). Values were compared with that of basal β-hexosaminidase activity stimulated by compound 48/80 and β-hexosaminidase release was expressed as follows.

β-hexosaminidase activity ratio =
β-hexosaminidase release stimulated by *H. pylori*/β-hexosaminidase release stimulated by compound 48/80

In additional experiments, β-hexosaminidase activity was measured *H. pylori* water extract without Ca^{2+} to investigate the role of extracellular Ca^{2+} in the degranulation process.

Statistical analysis

The results were expressed as mean ± standard deviation of 3—6 independent experiments and statistical analysis was performed using analysis of variance and non paired Student's T test. A p value < 0.05 was regarded as significant.

RESULTS

Degranulation of mast cells after the incubation with *H. pylori* water extract was monitored morphologically by phase contrast microscopy with time-lapse video recording and then the number of degranulated cells was counted. *Fig. 1*

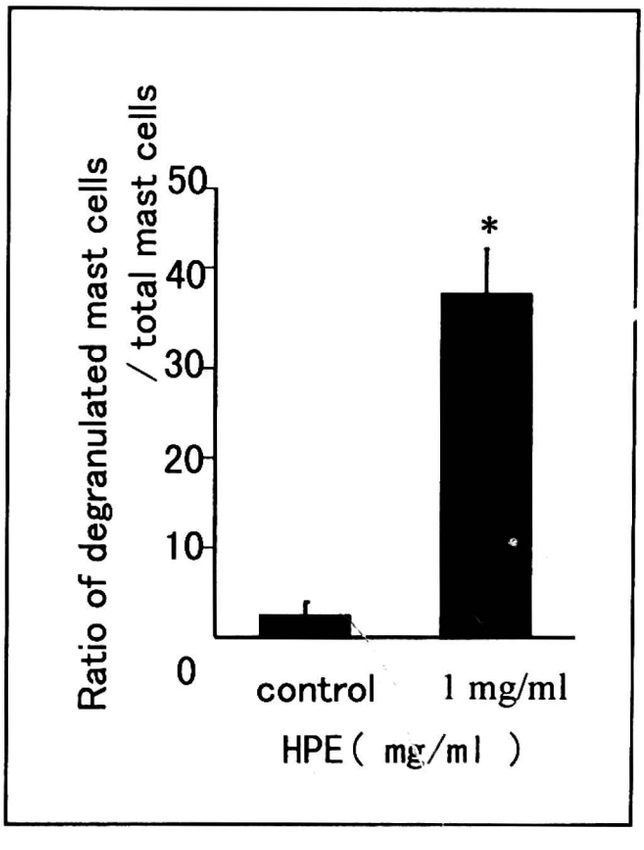

Fig. 1. Degranulation of mast cells stimulated with *H. pylori* water extract (HPE). Mast cells were incubated with 1 mg/ml of HPE and the degranulation of mast cells were monitered morphologically by phase contrast microscopy. Values represent degranulated mast cells/total mast cells and are expressed as the mean ± SD for 4 experiments. * P < 0.05 as compared with Tyrode's buffer alone.

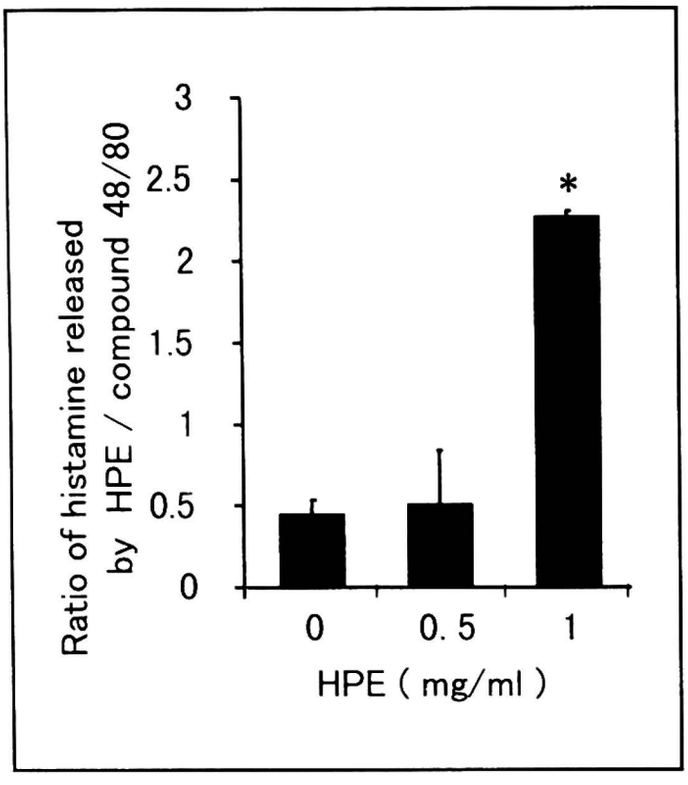

Fig. 2. Histamine released from mast cells stimulated with HPE. Mast cells were incubated with 0.5 and 1 mg/ml of HPE, and the supernatants were evaluated by radioimmunoassay for histamine measuremment. Values represent histamine released from mast cells with HPE/histamine released by compound 48/80 and are expressed as the mean ± SD for 3 experiments. * $P < 0.05$ as compared with Tyrode's buffer alone.

shows the ratio of degranulated mast cells/total mast cells. The addition of *H. pylori* water extract (1 mg/ml) caused significant degranulation of 38% of mast cells ($p < 0.05$). Without *H. pylori* water extract, only 3% of mast cells showed degranulation. Biochemical evaluation was also performed for histamine and β-hexosaminidase which were released from mast cells upon degranulation. Addition of *H. pylori* water extract caused significant histamine release from peritoneal mast cells, and the ratio of histamine release jumped up to 2.4 from 0.5 without *H. pylori* ($p<0.05$) (*Fig. 2*).

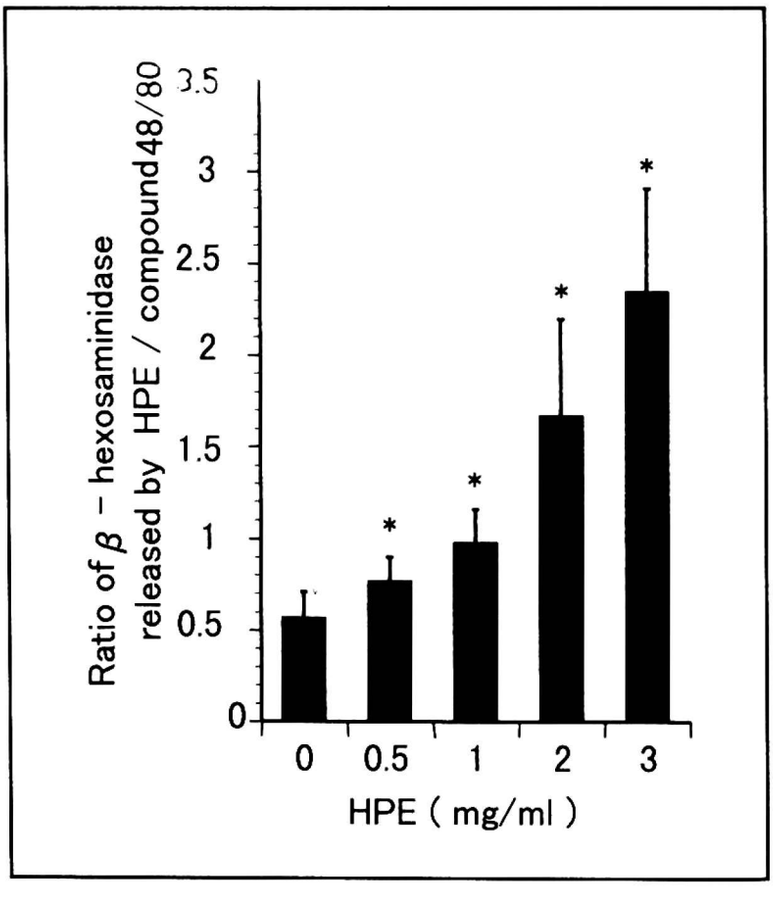

Fig. 3. β-hexosaminidase released from mast cells stimulated with HPE. Mast cells were incubated with 0.5—3 mg/ml of HPE for 30 min, and the degranulation was evaluated by β-hexosaminidase. Values represent β-hexosaminidase released from mast cells with HPE/β-hexosaminidase released by compound 48/80 and are expressed as the mean ± SD for 4 experiments. * $P < 0.05$ as compared with Tyrode's buffer alone.

β-hexosaminidase activity was determined as another marker of mast cell degranulation. *H. pylori* water extract increased β-hexosaminidaseactivity in a dose-dependent manner. Ratio of β-hexosaminidase release after the stimulation with *H. pylori* water extract was 0.8 with 0.5 mg/ml of *H. pylori* water extract ($p < 0.05$), 1.0 with 1mg/ml *H. pylori* water extract ($p<0.05$), 1.6 with 2mg/ml *H.pylori* water extract ($p<0.05$) and 2.4 with 3mg/ml *H. pylori* water extract ($p<0.05$) (*Fig. 3*).

In another series of experiments, we clarified the role of Ca^{2+} in the process of degranulation of mast cells upon the stimulation with water extract of *H. pylori.* Ratio of β-hexosaminidase release was significantly reduced in Ca^{2+} omitted medium (ratio; 0.65) as compared with Ca^{2+} added medium (ratio; 1.2, $p<0.05$) (*Fig. 4*).

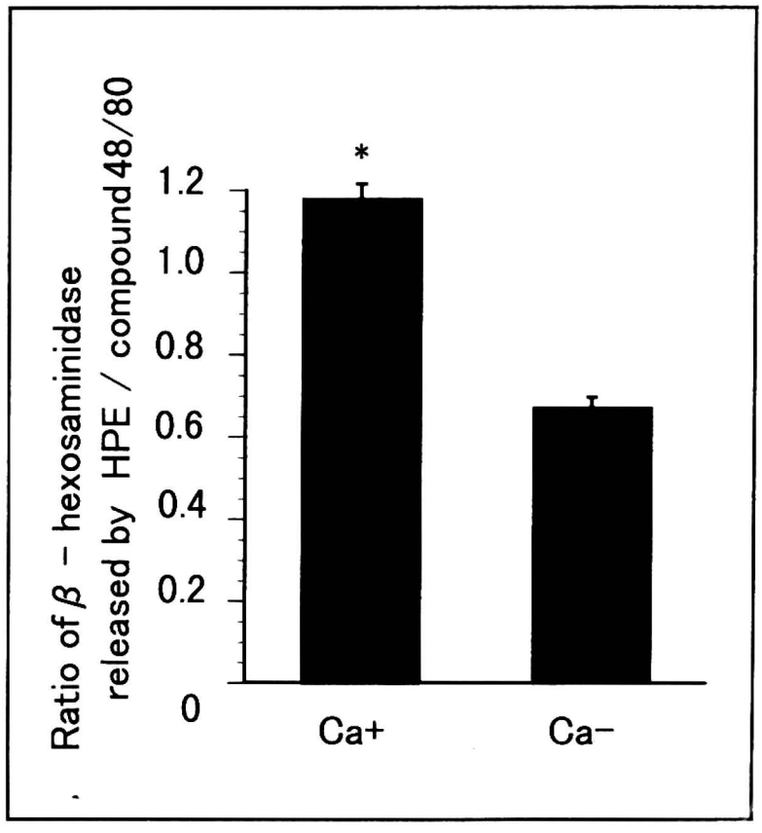

Fig. 4. β-hexosaminidase released from mast cells stimulated with HPE with or without Ca^{2+}. Mast cells were incubated with 1 mg/ml of HPE with or without Ca^{2+} for 30 min, and the degranulation was evaluated by β-hexosaminidase. Values represent β-hexosaminidase released from the cells with HPE/β-hexosaminidase induced by compound 48/80 and are expressed as the mean ± SD for 6 experiments. (* $p < 0.05$).

DISCUSSION

Many reports indicate that *H. pylori* plays an important role in the pathogenesis of active and chronic gastritis, duodenitis and gastric duodenal ulcers (1—4). Indeed, Hirayama *et al.* (14) reported that after the infection of *H. pylori* in mongolian gerbil, severe gastric mucosal inflammation occurred, resulting in the deep gastric ulcer formation. *H. pylori* has been reported to contain substances that increase the adhesion of glycoproteins on neutrophils, increase neutrophil chemotactic activity, elicit an oxidative burst response from the venules and activated macrophage/monocytes (6). Although the biochemical characteristics of these substances are unclear, water extract used in this study

has been believed to contain similar components and has been widely used as a tool for the investigation of *H. pylori*-induced gastric mucosal lesions. In this study, we clearly demonstrated that water extract of *H. pylori* induced degranulation of mast cells in a dose-dependent manner. This mast cell degranulation was confirmed by measuring released-histamine and β-hexosaminidase activity in the supernatants. As expected, histamine and β-hexosaminidase were increased with *H. pylori* water extract. and this degranulaton process was inhibited by omitting extracellular Ca^{2+}. Present data suggest that probably mast cell degranulation with the release of inflammatory cytokines might trigger gastric mucosal inflammation in *H. pylori* infection.

It is well documented that *H. pylori* infection (cagA positive strains) results in increased mucosal immune responses and more intense gastritis (15—17), and is highly associated with peptic ulcer (18—19), atrophic gastritis (20—21), and gastric cancer (22—24). ATCC 43504 used in this study contains the cagA and vacA gene with a vacuolating cytotoxin (23). Although we had no chance to use cagA gene and vacuolating cytotoxin negative strain, Yamaoka *et al.* (23) indicated that the main proinflammatory factor in water extract was different from bacterial urease, cytotoxin and lipopolysaccharide. Further studies are necessary to determine the biochemical character of this factor, leading to the resolution of the strain diversity of *H. pylori* infection.

Acknowledgenents: The autors thank Mr Atsusi and Ms Miki Aihara (Otsuka Pharmaceutical Co.) for his valuable technical assistance.

REFERENCES

1. Nakajima S, Krishnan B, Ota H *et al.* Mast cell involvement in gastritis with or without *Helicobacter pylori* infection. *Gastroenterology* 1997; 113: 746—754.
2. Wallace JL, Keenan CM, Mugridge KG *et al.* Possible mechanisms and mediators of gastritis associated with *Helicobacter pylori* infection. *Scand J Gastroenterol* 1991; 26 (Suppl 187): 65—70.
3. Bertram TA, Krakowka S, Morgan DR. Gastritis associated with infection by *Helicobacter pylori* : comparative pathology in humans and swine. *Rev Infect Dis* 1991; 13 (suppl 18): S714-S722.
4. Megraud F, Lamouliatte H. *Helicobacter pylori* and duodenal ulcer: Evidence suggesting causation. *Dig Dis Sci* 1992; 37: 769—772.
5. Bechi P, Dei R, Bello D. *H. pylori* potentiates histamine release from serosal rat mast cells in vitro. *Dig Dis Sci* 1993; 38: 944—949.
6. Tiveljung A, Borch K, Jonasson J *et al.* Identification of *Helicobacter* in gastric biopsies by PCR based on 16S rDNA sequences: a matter of little significance for the production of H. pylori-associated gastritis? *J Med Microbiol* 1998; Aug 47(8): 695—704.
7. Yoshida N, Granger DN, Evans DJ, Jr *et al.* Mechanisms involved in *Helicobacter pylori* — induced inflammation. *Gastroenterology*. 1993; 105: 1431—1440.
8. Johnson AR, Moran NC. Comparison of several methods for isolation of rat peritoneal mast cells. *Proc Soc Exp Biol Med* 1966; 123: 886—888.

9. Purcell WM, Cohen DL, Hanahoe TH. Contribution of post-secretory mechanisms to the observed pattern of histamine and 5-hydroxytryptamine secretion from peritoneal rat mast cells in response to compound 48/80. *Int Archs Allergy Appl Immunol* 1989; 90: 387—394.
10. Sullivan TJ, Parker KL, Stenson W *et al.* Modulation of cyclic AMP in purified rat mast cells. I. Responses to pharmacologic, metabolic and physical stimuli. *J Immunol* 1975; 114: 1473—1479.
11. Enerback L, Svensson I. Isolation of rat peritoneal mast cells by centrifugation on density gradients of Percoll. *J Immunol Methods* 1980; 39: 135—145.
12. Mackay GA A comparison of rat peritoneal mast cells purified using Percoll and Path-O-Cyte 4. *Agents Actions* 1983; 13: 122—126.
13. McBride P, Bradley BS, Kaliner M. Evaluation of a radioimmunoassay for histamine measurement in biologic fluids. *J Allergy Clin. Immunol* 1988; 82; 4: 638—646
14. Hirayama F, Takagi S, Yokoyama Y *et al.* Establishment of gastric *Helicobacter pylori* infection in Mongolian gerbils. *J Gastroenterol* 1996; 31 (suppl 9): 24—8.
15. Yamaoka Y, Kita M, Kodama T *et al. Helicobacter pylori* cagA gene and expression of cytokine messenger RNA in gastric mucosa. *Gastroenterology* 1996; 110: 1744—52.
16. Peek RM, Miller GG, Tham KT *et al.* Heighteened inflmmatory response and cytokine expression in vivo to cagA+ *Helicobacter pylori* strains. *Lab Invest* 1995; 73: 760—70.
17. Crabtree JE, Tayler JD, Wyatt JI *et al.* Mucosal IgA recognition of *Helicobacter pylori* 120 kDa protein, peptic ulceration and gastric pathology. *Lancet* 1991; 338: 332—5.
18. Weel JFL, Hulst RW, Gerrits Y *et al.* The interrelationship between cytotoxin-associated gene A, vacuolating cytotoxin, and *Helicobacter pylori-* related disease. *J Infect Dis* 1996; 173: 1171—5.
19. Kuipers EJ, Perez GI, Meuwissen SG *et al. Helicobacter pylori* and atrophic gastritis: importance of the cagA status. *J Natl Cancer Inst* 1995; 87: 1777—80.
20. Parsonnet J, Friedman GD, Orentreich N *et al.* Risk for gastric cancer in people with CagA positive or cagA negative *Helicobacter pylori* infection. *Gut* 1997; 40: 297—301.
21. Blaser MJ, Perez GI, Kleanthous H *et al.* Infection of strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 1995; 55: 2111—15.
22. Crabtree JE,Wyatt JI, Sobala GM *et al.* Systemic and mucosal humoral responses to *Helicobacter pylori* in gastric cancer. *Gut* 1993; 34: 1339—43.
23. Sato N, Watanabe S, Wang XE *et al.* Inhibition of rabbit gastric epithelial restoration by a water extract of *Helicobacter pylori : Inflammopharmacology* 1996; 4: 341—349.
24. Yamaoka Y, Kodama T, David Y *et al.* Search for putative virulence factors of *Helicobacter pylori* : the low-molecular-weight (33—35 K) antigen. *Dig Dis Sci* 1998; 43 (7): 1482—7.

Received: October 26, 1998
Accepted: January 8, 1999

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