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Effect of Metronidazole Resistance on Bacterial Eradication of *Helicobacter pylori* in Infected Children

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A prospective study was performed with 23 *Helicobacter pylori*-infected children (mean age, 9.5 ± 4.4 years) with clinical symptoms of gastritis and positive results of culture and histologic examination of gastric biopsy specimens to evaluate the influence of antibiotic resistance on eradication. Positive children were treated for 4 weeks with lansoprazole and for 2 weeks with either amoxicillin-metronidazole or spiramycin (a macrolide)-metronidazole. At endoscopy 1 month after the discontinuation of therapy, the eradication rate and improvement of histologically related gastritis were significantly dependent on the susceptibility or the resistance of the infecting organism to metronidazole (83 versus 17% and 88 versus 16.6%, respectively). Pretreatment determination of the susceptibility is appropriate in any anti-*H. pylori* regimen, including one with metronidazole.

The role of *Helicobacter pylori* in colonizing the stomach in adults with chronic gastritis, gastric ulceration, peptic ulcer disease, and possibly, gastric carcinoma is now firmly established. Several studies have also shown that *H. pylori* may be involved in children with abdominal pain. Eradication of the organism has a great effect on preventing the development of peptic ulcer relapses in both adults (12) and children (23). Triple therapy is the most successful, as first reported by Borody et al. (3), with a regimen of three antimicrobial drugs, which was efficient in most patients. Bazzoli et al. (1) reported a high eradication rate (93%) with 1 week of triple therapy combining a proton pump inhibitor, metronidazole, and amoxicillin. We previously reported on a study of triple therapy in children with a proton pump inhibitor, metronidazole, and either amoxicillin or spiramycin, with eradication rates of 85.7 and 72.7%, respectively (19).

Eradication rates are lower for adults infected or colonized with a metronidazole-resistant strain than for those infected or colonized with a susceptible one (2). Furthermore, the presence of CagA-positive strains leads to bacterial eradication difficulties, as reported previously (17).

The aim of this study was to determine in children the effect of metronidazole resistance on bacteriological eradication and the improvement of histological gastritis as determined by endoscopic and histological examinations of biopsy samples. The CagA antibody status of all children was checked in order to avoid bias in the interpretation of the results.

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MATERIALS AND METHODS

A prospective study was performed over 1 year with 23 children (10 girls and 13 boys) with a mean age of 9.5 ± 4 years. All children underwent exploratory upper gastrointestinal endoscopy as part of an examination of the following symptoms of clinical gastritis: recurrent abdominal pain for at least 3 months, nausea, vomiting, and weight loss. Endoscopy, performed with an Olympus x P

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20 GIF 100 device, detected abnormalities in 17 children (74%). The most common feature was antral nodularity, which was found in 14 of the 23 children (60%); 2 children had antral erythema and 1 had petechial gastritis.

Three gastric biopsy specimens were obtained from the antral region (20). Susceptibility to antimicrobial compounds was tested under microaerobic conditions over 3 days by using a turbidity approximating that of a McFarland no. 3 standard. The MICs of amoxicillin and metronidazole were determined by an E-test (MIC range, 256 to 0.01 mg/liter), and the MICs of the other antibiotics (cephalosporins, macrolides, quinolones, and co-trimoxazole) were determined by the disk diffusion susceptibility test (Kirby-Bauer). The E-test has been described as a reliable method for determination of the MICs of antibiotics for *H. pylori* (7) and likely detects antibiotic-resistant subpopulations. The inoculum size does not alter MIC results (5). The strains were considered metronidazole resistant when the MIC was greater than 8 mg/liter (15, 16). CagA antibody status was assessed in all children by using Western blots (Helicoblot 2.0; Genelabs, Singapore).

Treatment. All children included in the study had tested positive for *H. pylori* by both culture and histology. They were alternately enrolled in one of two groups and treated for 4 weeks with lansoprazole at 30 mg/day and metronidazole at 30 mg/kg of body weight/day for 2 weeks plus either amoxicillin at 100 mg/kg/day (AML) for 12 children or spiramycin at 300,000 IU/kg/day (SML) for 11 children. Spiramycin, a macrolide, was chosen because of its extensive use without side effects in children (22). Compliance with the study drug regimen was monitored through analysis of the drug containers, which were checked at the end of the treatment. Noncompliance was defined as taking less than 75% of the prescribed course of medication.

Follow-up. One month after the end of treatment, clinical symptoms were evaluated and another endoscopy was performed, with biopsy samples taken for bacteriological and histological examination.

Informed consent was obtained from the parents of the children. Patients living in an institution were excluded because of high rates of infection and the high probability of reinfections. Statistical analysis was performed by the chi-square test.

RESULTS AND DISCUSSION

All children exhibited good compliance with the study drug regimen.

Metronidazole resistance was detected in 26% (6 of 23) of the patients: 2 in the AML group and 4 in the SML group. Macrolide resistance was detected in 4.3% (1 of 23) of the children. We did not observe secondary resistance.

Considering global treatment results independently from the results for those in the AML or SML arm and independently from metronidazole resistance, bacteriological eradication occurred in 74% of the patients and histological gastritis cleared from 84.6% of the patients. The bacterial eradication rate in the AML group (83.3%) was not significantly different from that in the SML group (63.6%) ($P = 0.3$).

When excluding children infected or colonized with metronidazole-resistant strains, eradication occurred in seven of nine (77.7%) children in the AML group and in seven of eight (87.5%) children in the SML group ($P = 0.1$).

When the results were analyzed according to metronidazole resistance, the bacterial eradication rate was significantly higher: 83% (14 of 17) in the case of susceptibility versus 17% (1 of 6) in the case of resistance ($P < 0.05$). The improvement in histological gastritis was also significantly increased ($P < 0.05$) in the case of susceptibility: 88% (15 of 17) versus 16.6% (1 of 6) in the case of resistance. The only child infected or colonized with a strain resistant to macrolides was treated with the AML combination.

Overall, 10 of the 23 (43%) children were CagA positive. Bacteriological eradication occurred in 7 of the 10 (70%) CagA-positive children and in 10 of the 13 (77%) CagA-negative children ($P = 0.1$).

The most effective eradication of *H. pylori* was achieved with triple therapy that included metronidazole as a major component, with eradication rates of more than 90% (1). Eradication is not always as successful and resistance to metronidazole has been incriminated (8), with some controversies existing since in some studies metronidazole resistance is associated with only a minor decrease in effectiveness (11). Actually, eradication rates differ from 93% (13), 96% (10), 91% (18), and 90% (2) in adult patients infected or colonized with metronidazole-susceptible strains to 19% (13), 71% (10), 63% (18), and 31% (2) in those infected or colonized with metronidazole-resistant strains.

In addition, the cutoff values used for metronidazole resistance vary among investigators. The work of Bouchard et al. (4) states that a breakpoint of 8 mg/liter is valid and in accordance with those used in other studies (14). Our results with the same cutoff values suggest that it is clinically relevant in the case of a triple therapy that includes metronidazole.

Furthermore, we report here the same metronidazole resistance rate reported in the work of Rautelin et al. (18) and in a European study carried out in 1991 (9), which showed a mean resistance rate of 26%, with values ranging from 7% in Spain to 49% in Greece. The rate of resistance among strains from adults in France is between 7.9% (6) and 18.9% (4). In tropical regions, especially South America, the rate is higher (80 to 90%) and could be attributed to previous treatment for parasitic diseases.

Although our study included only 23 children, we report a significant difference in the bacteriological eradication rate between children infected or colonized with metronidazole-susceptible strains and children infected or colonized with metronidazole-resistant strains. These results do not appear to be biased by any differences in pathogenicity (17) because of the similar CagA statuses of the subjects in both groups.

The significant improvement in histological gastritis indicates that it is correlated with the presence of bacteria and that eradication rapidly leads to normalization of the mucosa, as reported by Valle et al. (21).

Briefly, in children, as in adults, *H. pylori* eradication seems to depend on the susceptibility of the strain to metronidazole. Pretreatment determination of metronidazole susceptibility might be important in deciding any anti-*H. pylori* therapeutic regimen. In view of geographic discrepancies in levels of metronidazole resistance, local routine surveys would probably provide adequate information to help physicians determine which triple therapy should be used.

REFERENCES

1. **Bazzoli, F., R. C. Zagari, S. Fossi, P. Pozzato, G. Alampi, P. Simoni, S. Sottili, A. Roda, and E. Roda.** 1994. Short-term low dose triple therapy for the eradication of *Helicobacter pylori*. Eur. J. Gastroenterol. Hepatol. **6:**773–777.
2. **Bell, G. D., K. Powell, S. M. Burridge, A. Pallecaros, P. H. Jones, P. W. Gant, G. Harrison, and J. E. Trowell.** 1992. Experience with "triple" anti-Helicobacter eradication therapy: side effects and the importance of testing the pretreatment bacterial isolate for metronidazole resistance. Aliment. Pharmacol. Ther. **6:**427–435.
3. **Borody, T. J., P. Cole, S. Noonan, A. Morgan, G. Ossip, J. Maysey, and S. Brandl.** 1989. Recurrence of duodenal ulcer and *Campylobacter pylori* infection after eradication. Med. J. Aust. **151:**431–435.
4. **Bouchard, S., C. Birac, H. Lamouliatte, S. Forestier, and F. Megraud.** 1996. Correlation between the MICS of metronidazole and *Helicobacter pylori* strains and the outcome of a lansoprazole-amoxicillin-metronidazole therapy. Gut **39**(Suppl. 1A)**:**5. (Abstract.)
5. **Cederbrant, G., G. Kahlmeter, and A. Ljungh.** 1993. The E-test for antimicrobial susceptibility testing of Helicobacter pylori. J. Antimicrob. Chemother. **31:**65–71.
6. **Conray, M. C. H., J. D. De Korwin, A. Loznieswski, and M. Weber.** 1996. Resistance of *Helicobacter pylori* to clarithromycin and metronidazole in Eastern France. Gut **39**(Suppl. 1A)**:**8. (Abstract.)
7. **Glupczynski, Y., M. Labbe, F. Crokaert, and E. Yourassowsky.** 1991. Evaluation of the E test for quantitative antimicrobial susceptibility testing of *Helicobacter pylori*. J. Clin. Microbiol. **29:**2072–2075.
8. **Glupczynski, Y., A. Burette, E. De Koster, J. F. Nyst, M. Deltenre, S. Cadranel, L. Bordeaux, and D. De Vos.** 1990. Metronidazole resistance in *Helicobacter pylori*. Lancet **335:**976–977. (Letter.)
9. **Glupczynski, Y., and the European Multicentre Study Group on Antibiotics.** 1992. Susceptibility of *Helicobacter pylori*: results of a multicentre European survey in 1991 of metronidazole resistance in *Helicobacter pylori*. Eur. J. Clin. Microbiol. Infect. Dis. **11:**777–781.
10. **Glupczynski, Y., and A. Burette.** 1992. Eradication of *Helicobacter pylori*. Lancet **339:**54–55. (Letter.)
11. **Graham, D. Y., W. A. De Boer, and G. N. J. Tytgat.** 1996. Choosing the best anti-*Helicobacter pylori* therapy: effect of antimicrobial resistance. Am. J. Gastroenterol. **91:**1072–1076.
12. **Hentschel, E., G. Brandstatter, B. Dragosics, A. B. Hirsch, H. Nemec, K. Schutze, M. Tauffer, and H. Wurzer.** 1993. Effects of ranitidine and amoxicillin plus metronidazole on the eradication of *Helicobacter pylori* and the recurrence of duodenal ulcer. N. Engl. J. Med. **328:**308–312.
13. **Logan, R. P. H., P. A. Gummett, J. J. Misiewicz, Q. N. Karim, M. M. Walker, and J. H. Baron.** 1991. One week eradication regimen for *Helicobacter pylori*. Lancet **338:**249–252.
14. **Midolo, P. D., J. R. Lambert, and I. Turnidge.** 1996. Metronidazole resistance: a predictor of failure of *Helicobacter pylori* eradication by triple therapy? J. Gastroenterol. Hepatol. **11:**290–292.
15. **Midolo, P. D., J. Turnidge, and J. R. Lambert.** 1994. Validation of a modified Kirby-Bauer disk diffusion method for metronidazole susceptibility testing of *Helicobacter pylori*. Am. J. Gastroenterol. **89:**1383. (Abstract.)
16. **National Committee for Clinical Laboratory Standards.** 1990. Standard MF-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
17. **Oderda, G., P. Ravanini, M. C. Malosso, F. Altare, M. Forni, P. Cerro, P. Caristo, and G. Fortina.** 1996. Detection and quantification of serum antibodies to *H. pylori* specific antigens of childhood Hp-gastritis. Gut **39**(Suppl. A)**:**51. (Abstract.)
18. **Rautelin, H., T. U. Kosunen, and K. Seppälä.** 1992. Eradicating *Helicobacter pylori*. Lancet **339:**54–55. (Letter.)
19. **Raymond, J., N. Kalach, M. Bergeret, P. H. Benhamou, F. Briet, B. Flourie, L. Senouci, D. Gendrel, and C. Dupont.** 1996. A controlled study of the efficacy of two different dual antibiotic associations in combination with lanzoprazole during *Helicobacter pylori* gastric infection in children. *In* Proceedings of the Third ICMAS. (Abstract.)
20. **Raymond, J., M. Bergeret, P. H. Benhamou, K. Mensah, and C. Dupont.** 1994. A 2-year study of *Helicobacter pylori* in children. J. Clin. Microbiol. **32:**461–463.
21. **Valle, J., K. Seppälä, P. Sipponen, and T. U. Kosunen.** 1991. Disappearance of gastritis after eradication of *Helicobacter pylori*. Scand. J. Gastroenterol. **26:**1057–1065.
22. **Wilson, C. B., and J. S. Remington.** 1987. Toxoplasmosis, p. 2067–2078. *In* R. D. Feigin and J. D. Cherry (ed.), Textbook of pediatric infectious diseases. The W. B. Saunders Co., New York, N.Y.
23. **Yeung, C. K., K. H. Fu, K. Y. Yuen, W. F. Ng, T. M. Tsang, F. J. Branicki, and H. Saing.** 1990. *Helicobacter pylori* and associated duodenal ulcer. Arch. Dis. Child. **65:**1212–1216.