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Exposure to Metronidazole In Vivo Readily Induces Resistance in *Helicobacter pylori* and Reduces the Efficacy of Eradication Therapy in Mice

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The *Helicobacter pylori* SS1 mouse model was used to characterize the development of resistance in *H. pylori* after treatment with metronidazole monotherapy and to examine the effect of prior exposure to metronidazole on the efficacy of a metronidazole-containing eradication regimen. Mice colonized with the metronidazole-sensitive *H. pylori* SS1 strain were treated for 7 days with either peptone trypsin broth or the mouse equivalent of 400 mg of metronidazole once a day or three times per day (TID). In a separate experiment, *H. pylori*-infected mice were administered either peptone trypsin broth or the mouse equivalent of 400 mg of metronidazole TID for 7 days, followed 1 month later by either peptone trypsin broth or the mouse equivalent of 20 mg of omeprazole, 250 mg of clarithromycin, and 400 mg of metronidazole twice a day for 7 days. At least 1 month after the completion of treatment, the mice were sacrificed and their stomachs were cultured for *H. pylori*. The susceptibilities of isolates to metronidazole were assessed by agar dilution determination of the MICs. Mixed populations of metronidazole-resistant and -sensitive strains were isolated from 70% of mice treated with 400 mg of metronidazole TID. The ratio of resistant to sensitive strains was 1:100, and the MICs for the resistant strains varied from 8 to 64 μg/ml. In the second experiment, *H. pylori* was eradicated from 70% of mice treated with eradication therapy alone, compared to 25% of mice pretreated with metronidazole ($P < 0.01$). Mice still infected after treatment with metronidazole and eradication therapy contained mixed populations of metronidazole-resistant and -sensitive isolates in a ratio of 1:25. These results demonstrate that *H. pylori* readily acquires resistance to metronidazole in vivo and that prior exposure of the organism to metronidazole is associated with failure of eradication therapy. *H. pylori*-infected mice provide a suitable model for the study of resistance mechanisms in *H. pylori* and will be useful in determining optimal regimens for the eradication of resistant strains.

Helicobacter pylori is a gram-negative, microaerobic, spiral bacterium that colonizes the stomachs of approximately half the world's population (44). Infection with *H. pylori* is associated with severe gastrointestinal disease, including peptic ulceration, and eradication of the organism from the stomach facilitates duodenal ulcer healing and reduces ulcer relapse (16, 28). Although the 5-nitroimidazole metronidazole is an important component of many currently used *H. pylori* eradication regimens, resistance to this class of antibiotics is relatively common. It has been estimated that 10 to 30% of clinical strains isolated in Western Europe and the United States are metronidazole resistant (9, 11, 13, 36), and this prevalence is far higher in developing countries and in certain immigrant populations (2, 9, 11, 13). Metronidazole is commonly used to treat anaerobic and parasitic infections, and there is epidemiological evidence that metronidazole resistance in *H. pylori* is associated with prior use of this antibiotic in certain patient groups (2, 4, 11, 13, 37). However, previous use of metronidazole is frequently not reported by patients (11), and consequently the development of resistance in *H. pylori* after metronidazole monotherapy is poorly characterized.

The relationship between previous exposure to metronidazole, the development of resistance to this antibiotic, and the eventual outcome of *H. pylori* eradication regimens remains unclear (30, 36). Many studies have demonstrated that infection with metronidazole-resistant strains is an important predictor of failure of metronidazole-containing eradication regimens, even when quadruple therapy is used (3, 5–7, 17, 19, 22, 31, 34, 37, 40–42, 47). However, there is also evidence that resistance to metronidazole can be partly overcome when antisecretory drug-based triple or quadruple therapies are used, and such regimens may achieve eradication in around 75% of patients infected with resistant strains (3, 6, 7, 17, 19, 22, 30, 32, 37, 40, 41, 48).

The aims of this study were to use a well-validated mouse model of *H. pylori* infection (24) to (i) characterize the development of resistance in *H. pylori* after treatment with metronidazole monotherapy at doses normally used to treat anaerobic and parasitic infections and (ii) examine the impact of prior exposure of *H. pylori* to metronidazole on the efficacy of a metronidazole-containing eradication regimen.

MATERIALS AND METHODS

Bacteria and growth conditions. *H. pylori* SS1 is a mouse-adapted strain originally isolated from a patient with peptic ulcer disease (24). *H. pylori* SS1 was routinely cultured on a blood agar medium (Blood Agar Base no. 2; Oxoid, Lyon, France) supplemented with 10% horse blood (bioMérieux, Marcy L'Etoile, France) and the following antibiotics: 10 μg of vancomycin (Dakota Pharmaceuticals, Creteil, France)/ml, 2.5 IU of polymyxin (Pfizer Laboratories, Orsay, France)/liter, 5 μg of trimethoprim (Sigma Chemicals, Saint-Quentin Fallavier, France)/ml, and 4 μg of amphotericin B (Bristol-Myers Squibb, Paris, France)/ml. The plates were incubated at 37°C under microaerobic conditions in an anaerobic jar (Oxoid) with a carbon dioxide generator (CampyGen; Oxoid) without a catalyst. For the selection of metronidazole-resistant colonies and their subsequent subculture, the medium was additionally supplemented with metronidazole at 8 μg/ml (Sigma).

To determine viable counts of *H. pylori*, samples to be tested were serially diluted in sterile saline and then plated in duplicate onto blood agar plates

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TABLE 1. Development of metronidazole resistance after metronidazole monotherapy

Characteristic	Result of the following treatment[a] for mice in:					
	Group 1				Group 2	
	PTB OD (n = 5)	MTZ OD (n = 5)	PTB TID (n = 4)	MTZ TID (n = 5)	PTB TID (n = 10)	MTZ TID (n = 10)
Infection with *H. pylori*[b]	5/5	5/5	4/4	4/5	10/10	9/10
Infection with MTZ-resistant *H. pylori*[c]	0/5	1/5	0/4	2/5	0/10	9/10
Range of MICs for resistant isolates (μg/ml)		32		32		8–64

[a] PTB, peptone trypsin broth; MTZ, metronidazole; OD, once daily.
[b] Proportion of mice infected with *H. pylori* 1 month after the completion of therapy.
[c] Proportion of mice from which resistant strains were isolated. In all cases, these were mixed with sensitive strains.

supplemented with either 10% horse blood or fetal calf serum (Gibco BRL, Cergy Pontoise, France), 10 g of agar (Bacteriological Agar no. 1; Oxoid)/liter, 200 μg of bacitracin/ml, and 10 μg of nalidixic acid (Sigma)/ml. After 5 days of incubation, colonies with *H. pylori* morphology were identified according to standard criteria (morphology on Gram staining and the presence of catalase, oxidase, and urease enzyme activities) and enumerated (12).

Infection of mice with *H. pylori* SS1. Six-week-old specific-pathogen-free Swiss mice (Centre d'Elevage R. Janvier, Le-Genest-St-Isle, France) were housed in polycarbonate cages in isolators and fed a commercial pellet diet with water ad libitum. All animal experimentation was performed in accordance with institutional guidelines. Mice were inoculated intragastrically with a suspension of *H. pylori* SS1, which had been harvested directly from 48-h plate cultures into peptone trypsin broth (Organotéchnique, La Courneuve, France). Each animal was administered a single 100-μl aliquot of an inoculating suspension of 10^5 CFU/ml (equivalent to 100 times the 100% infectious dose [12]). This was administered with polyethylene catheters (Biotrol, Paris, France) attached to 1-ml disposable syringes. A control group of mice (n = 10) was given peptone trypsin broth alone.

Three groups of mice were used in the treatment study. The mice in group 1 were used in a pilot study to determine if metronidazole resistance was induced in *H. pylori* after treatment with two different doses of metronidazole monotherapy. Group 2 animals were used to further characterize the acquisition of resistance after metronidazole monotherapy. The mice in group 3 were used to examine the effect of prior exposure to metronidazole on the efficacy of a metronidazole-containing *H. pylori* eradication regimen.

Antimicrobial chemotherapy. All solutions were administered intragastrically in a final volume of 100 μl via polyethylene catheters as previously described. Seven weeks after infection, the *H. pylori*-colonized mice in group 1 were treated for 7 days either with peptone trypsin broth (n = 5) or 0.171 mg of metronidazole (Rhône-Poulenc Rorer, Vitry sur Seine, France) (n = 5) in a single daily dose or with peptone trypsin broth (n = 4) or 0.171 mg of metronidazole (n = 5) three times daily (TID) (see Table 1). The dose of 0.171 mg in a 30-g mouse is the body weight equivalent of a 400-mg dose in a 70-kg human. Fifteen weeks after infection, the *H. pylori*-colonized mice in group 2 were given either peptone trypsin broth (n = 10) or 0.171 mg of metronidazole TID for 7 days (n = 10) (see Table 1).

The *H. pylori*-colonized mice in group 3 were administered two treatment regimens (see Table 2). Treatment 1 was administered 15 weeks after infection and consisted of either peptone trypsin broth (n = 20) or 0.171 mg of metronidazole TID for 7 days (n = 17). Treatment 2 was administered 1 month after the completion of treatment 1 and consisted of either peptone trypsin broth (n = 19) or the mouse body weight equivalent of a recommended *H. pylori* eradication regimen of 20 mg of omeprazole (0.0086 mg; Astra Hassle AB, Molndal, Sweden), 250 mg of clarithromycin (0.107 mg; Abbott Laboratories, Saint-Rémy-sur-Avre, France), and 400 mg of metronidazole (0.171 mg) twice daily for 1 week (n = 18) (10).

Assessment of *H. pylori* infection in mice. Colonization with *H. pylori* was assessed at least 1 month after the completion of each treatment regimen as recommended by recent guidelines (46). The animals were sacrificed, the stomach of each mouse was removed, and serum was recovered in microtubes (Sarstedt France, Orsay, France). The presence of *H. pylori* infection was determined by biopsy urease, quantitative culture, and serology. Stomachs were washed in physiological buffered saline and divided longitudinally into tissue fragments so that each fragment contained cardia, body, and antrum. For each stomach, one fragment was immediately placed in urea-indole medium and another was placed in peptone trypsin broth. The presence of urease activity in tissue fragments was detected in urea-indole medium incubated for 24 h at room temperature (12). For the performance of quantitative bacterial cultures on stomach samples, tissue fragments were homogenized in peptone trypsin broth by using disposable plastic grinders and tubes (PolyLabo, Strasbourg, France). The homogenates were serially diluted in sterile saline and plated directly onto blood and serum plates for enumeration and onto a selective plate containing 8 μg of metronidazole/ml. To increase the sensitivity of detection of metronidazole-resistant strains, all colonies that grew on the two enumeration plates were pooled and subcultured onto plates containing 8 μg of metronidazole/ml. *H. pylori* colonies were identified according to standard criteria and enumerated as described above.

Serum samples were tested for *H. pylori* antigen-specific immunoglobulin G antibody by a previously described enzyme-linked immunosorbent assay technique (12). Briefly, 96-well Maxisorb plates (Nunc, Kamstrup, Denmark) were coated with 25 μg of a sonicated whole-cell extract of *H. pylori* SS1. Serum samples were diluted 1:100 and were added in 100-μl aliquots to coated microtiter wells. To allow for nonspecific antibody binding, samples were also added to uncoated wells. Bound *H. pylori*-specific antibodies were detected by using biotinylated goat anti-mouse immunoglobulin and streptavidin-peroxidase conjugate (Amersham, Les Ulis, France).

The readings for uncoated wells were subtracted from those of the respective test samples. A cutoff value was determined from the mean optical density value ± 2 standard deviations for the corresponding samples from naive uninfected mice. Samples with optical density readings greater than this cutoff value were considered positive for *H. pylori*-specific antibodies.

Analysis of isolates. Five colonies identified as metronidazole-resistant *H. pylori* were selected from each mouse for further analysis. The susceptibility to metronidazole of each colony was assessed by agar dilution determination of the MIC. Inoculates yielding 10^4 CFU/spot were inoculated onto plates of IsoSensitest agar (Oxoid) enriched with 10% horse blood containing doubling dilutions of metronidazole. The MIC was defined as the lowest concentration of metronidazole inhibiting growth when the plates were read after 72 h of incubation under microaerobic conditions (generated as described above) at 37°C. Isolates were considered resistant to metronidazole if the MIC was ≥8 μg/ml (46). To assess if resistance to metronidazole was stable in vitro, resistant strains were subcultured three times on nonselective media before redetermination of the MIC.

To quantify the proportion of metronidazole-resistant and -sensitive colonies isolated from mice that received each of the different combinations of treatments in group 3, 200 colonies isolated on nonselective blood agar plates were subcultured in parallel onto media with and without metronidazole (8 μg/ml). The ratio of metronidazole-resistant to metronidazole-sensitive colonies was assessed after 72 h of incubation under microaerobic conditions at 37°C.

Intracage transmission of *H. pylori*. Two groups of animals were used in order to exclude the possibility of transmission of *H. pylori* between mice housed in the same cage. To study short-term transmission, two mice infected with *H. pylori* were kept in the same cage as six uninoculated mice for 9 weeks. In a further experiment, two mice infected with *H. pylori* were kept in the same cage as eight uninoculated mice for the period covered by the treatment studies (7 months).

Statistical analysis. Differences in the eradication rates between the groups of mice were determined by the chi-square test. A P value of <0.05 was considered significant.

RESULTS

Development of metronidazole resistance after metronidazole monotherapy. In group 1, quantitative culture of gastric tissue samples 1 month after completion of treatment was positive for *H. pylori* in 18 of 19 mice (Table 1). The bacterial counts obtained ranged from 2.0×10^3 to 1.8×10^6 CFU/g of tissue and were similar in mice treated with peptone trypsin broth and those treated with metronidazole. *H. pylori* was not cultured from one mouse treated with metronidazole TID. The *H. pylori*-specific humoral response in this mouse was of a magnitude similar to that in other infected mice, suggesting that infection had been established and subsequently eradicated by metronidazole in this animal. A mixed population of metronidazole-resistant and metronidazole-sensitive *H. pylori*

TABLE 2. Effect of prior exposure to metronidazole on the efficacy of *H. pylori* eradication therapy

Characteristic	Infection, treatments,[a] and results for group 3 mice, by subgroups				
	Control	Expt 1	Expt 2	Expt 3	Expt 4
No. of mice	10	10	9	10	8
Inoculating suspension	PTB	*H. pylori* SS1	*H. pylori* SS1	*H. pylori* SS1	*H. pylori* SS1
Treatment 1	PTB	PTB	MTZ	PTB	MTZ
Treatment 2	PTB	PTB	PTB	OCM	OCM
Infection with *H. pylori*[b]	0/10	10/10	7/9	3/10[d]	6/8[d]
Infection with MTZ-resistant *H. pylori*[c]	0/10	0/10	6/9	2/10[d]	6/8[d]
Ratio of resistant to sensitive isolates			1:100	<1:200	1:25
Range of MICs for resistant isolates (μg/ml)			16–32	16	16–32

[a] PTB, peptone trypsin broth; MTZ, metronidazole; OCM, omeprazole, clarithromycin, and metronidazole.
[b] Proportion of mice infected with *H. pylori* 2 months after the completion of treatment.
[c] Proportion of mice from which resistant strains were isolated. In all cases, these were mixed with sensitive strains.
[d] Statistically significant difference ($P < 0.01$).

strains was isolated from one mouse treated with 0.171 mg of metronidazole once daily and from two mice treated with 0.171 mg TID (Table 1). The MIC for all the resistant strains tested from these three mice ($n = 15$) was 32 μg/ml (compared to the MIC for the parental SS1 strain, which was 0.064 μg/ml).

In group 2, 19 of 20 mice were infected with *H. pylori* 1 month after the completion of treatment (Table 1). The *H. pylori* bacterial loads were similar in mice treated with peptone trypsin broth and those treated with metronidazole and ranged from 4.0×10^3 to 3.1×10^6 CFU/g of tissue. Infection was eradicated from one mouse treated with metronidazole TID (Table 1). A mixture of metronidazole-resistant and metronidazole-sensitive colonies were isolated from the other nine mice treated with 0.171 mg of metronidazole TID. The MICs for the resistant strains isolated from these nine mice ($n = 45$) ranged from 8 to 64 μg/ml (Table 1). Strains with varying degrees of resistance to metronidazole were isolated from seven of these nine mice. The MIC for each resistant isolate was unchanged after three consecutive subcultures on nonselective medium. No resistant bacteria were isolated from the mice treated with peptone trypsin broth.

Prior exposure to metronidazole and the efficacy of eradication therapy. In group 3, none of the 10 mice inoculated with peptone trypsin broth were infected with *H. pylori* 2 months after the completion of treatment 2 (Table 2). In contrast, quantitative culture of gastric tissue samples was positive for *H. pylori* in all 10 SS1-inoculated mice that were treated twice with peptone trypsin broth (Table 2), with bacterial counts of 3.1×10^4 to 1.0×10^7 CFU/g of tissue. No metronidazole-resistant strains were isolated from these mice. *H. pylori* was cultured from seven of nine SS1-infected mice that received metronidazole monotherapy followed by peptone trypsin broth (Table 2). Mixed populations of metronidazole-resistant and metronidazole-sensitive *H. pylori* was isolated from six of these mice. The range of MICs for the resistant strains tested ($n = 30$) was 16 to 32 μg/ml, and the ratio of metronidazole-resistant to -sensitive isolates was 1:100 (Table 2).

H. pylori was eradicated by a recommended triple-therapy regimen (omeprazole, clarithromycin, and metronidazole) from 25% of mice pretreated with metronidazole compared to 70% of mice not pretreated with this antibiotic ($P < 0.01$) (Table 2). The bacterial counts in the three mice still infected after treatment with peptone trypsin broth and eradication therapy were similar to those observed in nontreated mice (between 6.5×10^4 and 5.3×10^6 CFU/g of tissue). Two of these mice were infected by mixed populations of metronidazole-resistant and -sensitive isolates. The MIC for all the resistant isolates tested ($n = 10$) was 16 μg/ml, and the ratio of metronidazole-resistant to -sensitive isolates was <1:200 (Table 2). The stomachs of the six mice still infected with *H. pylori* after receiving both metronidazole monotherapy and eradication treatment contained 1.9×10^5 to 1.0×10^7 CFU/g of tissue. All six of these mice harbored mixed populations of metronidazole-resistant and -sensitive isolates. The range of the MICs for the resistant isolates tested ($n = 30$) was 16 to 32 μg/ml, and the ratio of metronidazole-resistant to -sensitive isolates was 1:25 (Table 2). All MICs were unchanged after three consecutive subcultures on nonselective medium. At the time of sacrifice (2 months), serological testing was not predictive of successful eradication of *H. pylori*.

Intracage transmission of *H. pylori*. Transmission from *H. pylori*-colonized mice to uninoculated mice was not observed. At 9 weeks and 7 months, the two infected mice were confirmed as colonized with *H. pylori* SS1 (by culture, biopsy urease, and serology), while the uninoculated mice remained uncolonized.

DISCUSSION

Acquired resistance of *H. pylori* to metronidazole and other 5-nitroimidazole drugs has been reported worldwide. There is epidemiological evidence suggesting that variation in the use of metronidazole is responsible for the uneven distribution of resistance among different populations. It has been proposed that the high level of resistance in developing countries is associated with the prior use of metronidazole to treat parasitic infections (2, 4, 11, 13). It has also been suggested that the higher prevalence of primary metronidazole resistance found in *H. pylori* isolated from female patients correlates with the use of metronidazole to treat bacterial vaginosis and other infections of the female genital tract (4, 11, 13, 37). However, many of these studies have been unable to demonstrate a direct association between prior use of metronidazole and the presence of resistant strains, presumably because such use often goes unrecognized by the patient (11, 13, 37). Although resistance to metronidazole in *H. pylori* has been shown to arise readily in vitro (18, 43), the development of resistance after metronidazole monotherapy in vivo remains poorly characterized. Given the potential implications of metronidazole resistance for the selection of optimal eradication regimens, this is an important phenomenon calling for further study.

The emergence of a single metronidazole-resistant *H. pylori* strain after exposure to metronidazole was recently reported in a euthymic mouse model (29). However, the relevance of this model to human infection can be questioned, particularly because only low levels of colonization occur (24). In another study, metronidazole-resistant bacteria were isolated after treat-

ment of *H. pylori*-infected gnotobiotic piglets (23). Although this model mimics many aspects of human *H. pylori* disease, it is necessary, for practical reasons, to use short study periods (3 weeks). In addition, the parental *H. pylori* strain used to colonize piglets (26695) already possesses intermediate resistance to metronidazole (MIC, 6.25 μg/ml; intermediate resistance has been defined as a MIC of 4 to 8 μg/ml [47]). It has been demonstrated that immunocompetent mice inoculated with *H. pylori* SS1 develop chronic infection (≥16 months), with gastric bacterial loads and host inflammatory responses similar to those found in humans (12, 24). We therefore selected the SS1 *H. pylori* mouse model for the study of the emergence of acquired resistance to metronidazole in this bacterium in vivo and for the examination of the effect of this resistance on the efficacy of a metronidazole-containing *H. pylori* eradication regimen. All experimentation was performed on long-term-colonized mice and, as far as was possible, in accordance with recent guidelines for clinical trials in *H. pylori* infection (46).

Using the *H. pylori* SS1 mouse model, we have demonstrated that *H. pylori* readily develops stable resistance to metronidazole in vivo after treatment with metronidazole monotherapy. In total, 73% of mice in groups 1 and 2 harbored resistant strains after receiving the TID treatment regimen. In a separate experiment, 67% of mice in group 3 that were treated with metronidazole TID followed by peptone trypsin broth developed resistant strains. The high numbers of mice colonized with resistant isolates cannot be explained by transmission of metronidazole-resistant strains between mice. We demonstrated that transmission of *H. pylori* SS1 does not occur between mice housed in the same cage, and similar results have recently been presented by other workers (26). These results provide direct evidence that exposure of a clonal population of *H. pylori* to doses of metronidazole normally used to treat parasitic and anaerobic bacterial infections does result in the emergence of resistance to this antibiotic. Moreover, the development of resistance appears to occur at a high frequency at the individual level. The fact that *H. pylori* survives beneath the mucous layer and in the gastric pits in both the human and murine stomachs may explain its propensity to develop resistance to metronidazole (21, 24). The antibiotic penetrates such sites poorly, exposing the bacterium to sublethal doses and encouraging the development of resistance.

The phenomenon of heteroresistance, in which susceptible and resistant bacteria may be isolated from the same patient, has previously been described for *H. pylori* (8, 21, 39, 45). Our study clearly demonstrates that a mixed population of sensitive and resistant bacteria may arise after exposure of a clonal, metronidazole-susceptible strain of *H. pylori* to either metronidazole monotherapy or a metronidazole-containing eradication regimen. Accurate determination of the proportion of resistant strains demonstrated that after metronidazole monotherapy, the ratio of metronidazole-resistant to metronidazole-sensitive isolates was 1 in 100. This ratio rose to 1 in 25 in mice treated with both metronidazole monotherapy and eradication treatment, indicating that repeated exposure to metronidazole in vivo selects for a resistant population of *H. pylori* in the stomach. It is recognized that current methods of susceptibility testing may underestimate the frequency of metronidazole resistance in patients coinfected with metronidazole-resistant and -sensitive *H. pylori* strains (8, 45). If heteroresistance after treatment with metronidazole is confirmed to occur frequently in *H. pylori*-infected patients, this may have important implications for the susceptibility testing of this organism.

We also observed that, despite the fact that they originated from the same metronidazole-sensitive parental strain, the degree of susceptibility to metronidazole of the emergent resistant isolates varied, with MICs ranging from 8 to 64 μg/ml. Recently, resistance to metronidazole in *H. pylori* was demonstrated to be associated with mutational inactivation of the *rdxA* gene, which encodes an oxygen-insensitive NADPH nitroreductase (14). This model will allow us to evaluate the contribution of *rdxA* to the development of resistance in these strains and to evaluate if other potential resistance mechanisms might exist in *H. pylori*.

Clinical trials of the efficacy of omeprazole, clarithromycin, and metronidazole have yielded conflicting results for the successful eradication of metronidazole-sensitive and -resistant strains. While a number of studies have demonstrated that eradication is significantly reduced for metronidazole-resistant strains (3, 19, 22, 27, 33, 35, 47), others have shown that this regimen is equally effective for both sensitive and resistant isolates (1, 25). In this study, prior exposure of *H. pylori* to metronidazole had a considerable negative influence on eradication; this is the first time that a direct link between prior exposure to metronidazole and the outcome of eradication therapy has been clearly demonstrated. The magnitude of this effect is likely to reflect the high frequency of resistance induced by the pretreatment of mice with metronidazole monotherapy and is consistent with the observation that the effectiveness of metronidazole-containing eradication regimens is dependent on the prevalence of metronidazole-resistant *H. pylori* in the population (17). In addition, secondary resistance to metronidazole, which is recognized to arise during the course of *H. pylori* eradication therapy (15, 20, 37, 38), was observed to arise in strains isolated from 20% of mice that received eradication therapy only.

These data establish the *H. pylori* SS1 mouse model as a suitable system for the study of resistance mechanisms in this organism. It will also be useful for examining factors that influence the efficacy of *H. pylori* eradication and for determining the optimal eradication regimens for resistant strains.

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REFERENCES

1. **Adamek, R. J., S. Suerbaum, B. Pfaffenbach, and W. Opferkuch.** 1998. Primary and acquired *Helicobacter pylori* resistance to clarithromycin, metronidazole and amoxycillin—influence on treatment outcome. Am. J. Gastroenterol. **93:**386–389.
2. **Banatvala, N., G. R. Davies, Y. Abdi, L. Clements, D. S. Rampton, J. M. Hardie, and R. A. Feldman.** 1994. High prevalence of *Helicobacter pylori* metronidazole resistance in migrants to east London: relationship with previous nitroimidazole exposure and gastrointestinal disease. Gut **35:**1562–1566.
3. **Bazzoli, F., M. Zagari, P. Pozzato, O. Varoli, S. Fossi, L. Ricciardiello, G. Alampi, G. Nicolini, S. Sottili, P. Simoni, A. Roda, and E. Roda.** 1998. Evaluation of short-term low-dose triple therapy for the eradication of *Helicobacter pylori* by factorial design in a randomized, double-blind, controlled study. Aliment. Pharmacol. Ther. **12:**439–445.
4. **Becx, M. C., A. J. H. M. Janssen, H. A. L. Clasener, and R. W. de Koning.** 1990. Metronidazole-resistant *Helicobacter pylori*. Lancet **335:**539–540.
5. **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 pylori* eradication therapy: side effects and the importance of testing the pre-treatment bacterial isolate for metronidazole resistance. Aliment. Pharmacol. Ther. **6:**427–435.
6. **Bell, G. D., K. Powell, S. M. Burridge, A. F. Bowden, W. Atoyebi,**

G. H. Bolton, P. H. Jones, and C. Browns. 1995. Rapid eradication of *Helicobacter pylori* infection. Aliment. Pharmacol. Ther. **9:**41–46.
7. **Buckley, M. J. M., H. X. Xia, D. M. Hyde, C. T. Keane, and C. A. O'Morain.** 1997. Metronidazole resistance reduces efficacy of triple therapy and leads to secondary clarithromycin resistance. Dig. Dis. Sci. **42:**2111–2115.
8. **Dore, M. P., M. S. Osato, D. H. Kwon, D. Y. Graham, and F. A. K. El-Zaatari.** 1998. Demonstration of unexpected antibiotic resistance of genotypically identical *Helicobacter pylori* isolates. Clin. Infect. Dis. **27:**84–89.
9. **Dunn, B. E., H. Cohen, and M. J. Blaser.** 1997. *Helicobacter pylori*. Clin. Microbiol. Rev. **10:**720–741.
10. **European *Helicobacter pylori* Study Group.** 1997. Current European concepts in the management of *Helicobacter pylori* infection. The Maastricht Consensus Report. Gut **41:**8–13.
11. **European Study Group on Antibiotic Susceptibility of *Helicobacter pylori*.** 1992. Results of a multicentre European survey in 1991 of metronidazole resistance in *Helicobacter pylori*. Eur. J. Clin. Microbiol. Infect. Dis. **11:**777–781.
12. **Ferrero, R. L., J. M. Thiberge, M. Huerre, and A. Labigne.** 1998. Immune responses of specific-pathogen-free mice to chronic *Helicobacter pylori* (strain SS1) infection. Infect. Immun. **66:**1349–1355.
13. **Glupczynski, Y., A. Burette, E. De Koster, J. F. Nyst, M. Deltenre, S. Cadranel, L. Bourdeaux, and D. De Vos.** 1990. Metronidazole resistance in *Helicobacter pylori*. Lancet **335:**976–977.
14. **Goodwin, A., D. Kersulyte, G. Sisson, S. J. O. Veldhuyzen van Zanten, D. E. Berg, and P. S. Hoffman.** 1998. Metronidazole resistance in *Helicobacter pylori* is due to null mutations in a gene (*rdxA*) that encodes an oxygen-insensitive NADPH nitroreductase. Mol. Microbiol. **28:**383–393.
15. **Goodwin, C. S., B. J. Marshall, E. D. Blincow, D. H. Wilson, S. Blackbourn, and M. Phillips.** 1988. Prevention of nitroimidazole resistance in *Campylobacter pylori* by coadministration of colloidal bismuth subcitrate: clinical and in vitro studies. J. Clin. Pathol. **41:**207–210.
16. **Graham, D. Y., G. M. Lew, P. D. Klein, D. G. Evans, D. J. Evans, Z. A. Saeed, and H. M. Malaty.** 1992. Effect of treatment of *Helicobacter pylori* infection on the long-term recurrence of gastric or duodenal ulcer. A randomized, controlled study. Ann. Intern. Med. **116:**705–708.
17. **Graham, D. Y., W. A. de Boer, and G. N. Tytgat.** 1996. Choosing the best anti-*Helicobacter pylori* therapy: effect of antimicrobial resistance. Am. J. Gastroenterol. **91:**1072–1076.
18. **Haas, C. E., D. E. Nix, and J. J. Schentag.** 1990. In vitro selection of resistant *Helicobacter pylori*. Antimicrob. Agents Chemother. **34:**1637–1641.
19. **Harris, A. W., D. I. Pryce, S. M. Gabe, Q. N. Karim, M. M. Walker, H. Langworthy, J. H. Baron, and J. J. Misiewicz.** 1996. Lansoprazole, clarithromycin and metronidazole for seven days in *Helicobacter pylori* infection. Aliment. Pharmacol. Ther. **10:**1005–1008.
20. **Hirschl, A. M., E. Hentschel, K. Schutze, H. Nemec, R. Potzi, A. Gangl, W. Weiss, M. Pletschette, G. Stanek, and M. L. Rotter.** 1988. The efficacy of antimicrobial treatment in *Campylobacter pylori*-associated gastritis and duodenal ulcer. Scand. J. Gastroenterol. **23**(Suppl.)**:**S76–S81.
21. **Jorgensen, M., G. Daskalopoulos, V. Warburton, H. M. Mitchell, and S. L. Hazell.** 1996. Multiple strain colonization and metronidazole resistance in *Helicobacter pylori*-infected patients: identification from sequential and multiple biopsy specimens. J. Infect. Dis. **174:**631–635.
22. **Kist, M., S. Strobel, U. R. Folsch, T. Kirchner, E. G. Hahn, D. H. Kleist, H. U. Klor, and H. G. Dammann.** 1997. Prospective assessment of the impact of primary antimicrobial resistances on cure rates of *Helicobacter pylori* infections, abstr. 09/328. *In* Abstracts of the Xth International Workshop on Gastroduodenal Pathology and *Helicobacter pylori*. Gut **41**(Suppl. 1)**:**A90.
23. **Krakowka, S., K. A. Eaton, and R. D. Leunk.** 1998. Antimicrobial therapies for *Helicobacter pylori* infection in gnotobiotic piglets. Antimicrob. Agents Chemother. **42:**1549–1554.
24. **Lee, A., J. O'Rourke, M. Corazon de Ungria, B. Robertson, G. Daskalopoulos, and M. F. Dixon.** 1997. A standardized mouse model of *Helicobacter pylori* infection: introducing the Sydney strain. Gastroenterology **112:**1386–1397.
25. **Lerang, F., B. Moum, J. B. Haug, P. Tolas, O. Breder, E. Aubert, O. Hoie, T. Soberg, B. Flaaten, P. Farup, and T. Berge.** 1997. Highly effective twice-daily triple therapies for *Helicobacter pylori* infection and peptic ulcer disease: does *in vitro* metronidazole resistance have any clinical relevance? Am. J. Gastroenterol. **92:**248–253.
26. **Leung, V., J. O'Rourke, and A. Lee.** 1998. Transmission of *Helicobacter pylori* between C57/BL6 mice, abstr. 05/146. *In* Abstracts of the XIth International Workshop on Gastroduodenal Pathology and *Helicobacter pylori*. Gut **43** (Suppl. 2)**:**A44.
27. **Lind, T., F. Mégraud, K. D. Bardhan, E. Bayerdorffer, M. Hellblom, C. O'Morain, R. C. Spiller, P. Unge, S. J. O. Veldhuyzen van Zanten, M. Wrangstadh, L. Zeijlon, and C. Cederberg.** 1997. The MACH2 study: antimicrobial resistance in *Helicobacter pylori* therapy—the impact of omeprazole, abstr. 09/324. *In* Abstracts of the Xth International Workshop on Gastroduodenal Pathology and *Helicobacter pylori*. Gut **41**(Suppl. 1)**:**A89.
28. **Marshall, B. J., C. S. Goodwin, J. R. Warren, R. Murray, E. D. Blincow, S. J. Blackbourn, M. Philips, T. E. Waters, and C. R. Sanderson.** 1988. Prospective double-blind trial of duodenal ulcer relapse after eradication of *Campylobacter pylori*. Lancet **ii:**1437–1442.
29. **Matsumoto, S., Y. Washizuka, Y. Matsumoto, S. Tawara, F. Ikeda, Y. Yokota, and M. Karita.** 1997. Appearance of a metronidazole-resistant *Helicobacter pylori* strain in an infected-ICR-mouse model and difference in eradication of metronidazole-resistant and -sensitive strains. Antimicrob. Agents Chemother. **41:**2602–2605.
30. **Mégraud, F., and H. P. Doermann.** 1998. Clinical relevance of resistant strains of *Helicobacter pylori*: a review of current data. Gut **43**(Suppl. 1)**:**S61–S65.
31. **Misiewicz, J. J., A. W. Harris, K. D. Bardhan, S. Levi, C. A. O'Morain, B. T. Cooper, G. D. Kerr, M. F. Dixon, H. Langworthy, D. Piper, and the Lansoprazole Helicobacter Study Group.** 1997. One week triple therapy for *Helicobacter pylori*: a multicentre comparative study. Gut **41:**735–739.
32. **Moayyedi, P., P. Sahay, D. S. Tompkins, and A. T. Axon.** 1995. Efficacy and optimum dose of omeprazole in a new 1-week triple therapy regimen to eradicate *Helicobacter pylori*. Eur. J. Gastroenterol. Hepatol. **7:**835–840.
33. **Moayyedi, P., P. L. Ragunathan, N. Mapstone, A. T. Axon, and D. S. Tompkins.** 1998. Relevance of antibiotic sensitivities in predicting failure of omeprazole, clarithromycin, and tinidazole to eradicate *Helicobacter pylori*. J. Gastroenterol. **33:**160–163.
34. **Noach, L. A., W. L. Langenberg, M. A. Bertola, J. Dankert, and G. N. J. Tytgat.** 1994. Impact of metronidazole resistance on the eradication of *Helicobacter pylori*. Scand. J. Infect. Dis. **26:**321–327.
35. **Peitz, U., A. Nusch, B. Tillenburg, M. Stolte, G. Borsch, and J. Labenz.** 1996. High cure rate of *H. pylori* (HP) infection by one-week therapy with omeprazole (OME), metronidazole (MET) and clarithromycin (CLA) despite a negative impact by MET resistance, abstr. 1A:03. *In* Abstracts of the IXth International Workshop on Gastroduodenal Pathology and *Helicobacter pylori*. Gut **39**(Suppl. 2)**:**A5.
36. **Peitz, U., M. Menegatti, D. Vaira, and P. Malfertheiner.** 1998. The European meeting on *Helicobacter pylori*: therapeutic news from Lisbon. Gut **43**(Suppl. 1)**:**S66–S69.
37. **Rautelin, H., K. Seppala, O. V. Renkonen, U. Vainio, and T. U. Kosunen.** 1992. Role of metronidazole in therapy of *Helicobacter pylori* infections. Antimicrob. Agents Chemother. **36:**163–166.
38. **Rautelin, H., W. Tee, K. Seppala, and T. U. Kosunen.** 1994. Ribotyping patterns and emergence of metronidazole resistance in paired clinical samples of *Helicobacter pylori*. J. Clin. Microbiol. **32:**1079–1082.
39. **Taylor, N. S., J. G. Fox, N. S. Akopyants, D. E. Berg, N. Thompson, B. Shames, L. Yan, E. Fontham, F. Janney, F. M. Hunter, and P. Correa.** 1995. Long-term colonization with single and multiple strains of *Helicobacter pylori* assessed by DNA fingerprinting. J. Clin. Microbiol. **33:**918–923.
40. **Thijs, J. C., A. A. Van Zwet, W. J. Thijs, E. J. Van der Wouden, and A. Kooy.** 1997. One-week triple therapy with omeprazole, amoxycillin and tinidazole for *Helicobacter pylori* infection: the significance of imidazole resistance. Aliment. Pharmacol. Ther. **11:**305–309.
41. **van de Hulst, R. W. M., A. van der Ende, A. Homan, P. Roorda, J. Dankert, and G. N. J. Tytgat.** 1998. Influence of metronidazole resistance on efficacy of quadruple therapy for *Helicobacter pylori* eradication. Gut **42:**166–169.
42. **Vanzanten, S. V., R. H. Hunt, A. Cockeram, G. Schep, D. Malatjalian, J. Sidorov, A. Matisko, and D. Jewell.** 1998. Adding once-daily omeprazole 20 mg to metronidazole/amoxicillin treatment for *Helicobacter pylori* gastritis—a randomized, double-blind trial showing the importance of metronidazole resistance. Am. J. Gastroenterol. **93:**5–10.
43. **van Zwet, A. A., J. C. Thijs, W. S. Vries, J. Schiphuis, and J. A. M. Snijder.** 1994. In vitro studies on stability and development of metronidazole resistance in *Helicobacter pylori*. Antimicrob. Agents Chemother. **38:**360–362.
44. **Warren, J. R., and B. Marshall.** 1983. Unidentified curved bacilli on gastric epithelium in chronic active gastritis. Lancet **i:**1273–1275.
45. **Weel, J. F. L., R. W. M. van der Hulst, Y. Gerrits, G. N. J. Tytgat, A. van der Ende, and J. Dankert.** 1996. Heterogeneity in susceptibility to metronidazole among *Helicobacter pylori* isolates from patients with gastritis or peptic ulcer disease. J. Clin. Microbiol. **34:**2158–2162.
46. **Working Party of the European *Helicobacter pylori* Study Group.** 1997. Guidelines for clinical trials in *Helicobacter pylori* infection. Gut **41**(Suppl. 2)**:** S1–S23.
47. **Xia, H. X., C. T. Keane, S. Beattie, and C. A. O'Morain.** 1994. Standardization of disk diffusion test and its clinical significance for susceptibility testing of metronidazole against *Helicobacter pylori*. Antimicrob. Agents Chemother. **38:**2357–2361.
48. **Yousfi, M. M., H. M. el-Zimaity, M. T. al-Assi, R. A. Cole, R. M. Genta, and D. Y. Graham.** 1995. Metronidazole, omeprazole and clarithromycin: an effective combination therapy for *Helicobacter pylori* infection. Aliment. Pharmacol. Ther. **9:**209–212.