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In Vitro Activities of Linezolid Alone and in Combination with Amoxicillin, Clarithromycin, and Metronidazole against *Helicobacter pylori*

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Linezolid was tested against 70 strains of *Helicobacter pylori* by the agar dilution method. The MIC range and MICs at which 50 and 90% of strains were inhibited were 8 to 64, 16, and 32 μg/ml, respectively. With minimum and maximum fractional inhibitory concentration summation values of 0.31 and 2.50, respectively, the combination of linezolid with amoxicillin, clarithromycin, or metronidazole showed either partial synergy or indifference for the majority of strains.

Infection with *Helicobacter pylori* is one of the most frequent infections in humans. The established indications for eradication therapy primarily include chronic ulcer disease and mucosa-associated lymphoid tissue lymphoma (3). In addition, there has also been an obvious trend for prescribing eradication therapy in patients with dyspeptic disorders, although various studies have produced controversial results (1, 11). Simultaneously, a significant increase of *H. pylori* resistance to nitroimidazoles and macrolides as well as isolated cases of resistance to amoxicillin (AMX) have been observed, a development considerably reducing the available therapy options (5, 6). Hence, it appears meaningful to evaluate novel antimicrobial agents for their effectiveness against *H. pylori*.

Linezolid (LNZ), a novel oxazolidone primarily indicated for use in staphylococcal and streptococcal infections, has also shown certain activity against gram-negative bacteria and anaerobes (2, 4, 7, 8, 15, 16). We studied the susceptibility of various *H. pylori* strains to LNZ alone and in combination with AMX, clarithromycin (CLR), or metronidazole (MTZ).

The strains were isolated in 1998 and 1999 from gastric biopsy specimens and frozen in cryobank tubes (Mast Group Limited, Bootle, Merseyside, United Kingdom) at −70°C. Strains for which MICs were ≤0.25 μg/ml were classified as CLR susceptible; those for which MICs were ≥1.0 were classified as resistant (14). Based on experience in clinical trials (9), strains for which the MIC was >8 μg/ml were classified as MTZ resistant. MICs of AMX for all strains tested were in the range of ≤0.016 to 0.03 μg/ml; therefore, these strains were classified as susceptible.

Of the 70 strains, including the reference strains CCUG 38770 (MTZ resistant) and 38771 (MTZ susceptible), 30 were AMX, CRL, and MTZ susceptible, 20 were AMX as well as CRL susceptible but MTZ resistant, and 20 were AMX susceptible and CRL as well as MTZ resistant. *Staphylococcus aureus* ATCC 29213 was included as an additional control strain.

The MICs of LNZ and of the LNZ-AMX, LNZ-CLR, and LNZ-MTZ combinations were determined by using the agar dilution test or an agar dilution checkerboard method. Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, United Kingdom) with 5% sterile defibrinated horse blood was used to prepare the plates containing the antimicrobial agents.

LNZ was provided by Pharmacia Upjohn, Vienna, Austria; AMX and MTZ were provided by Biochemie, Kundl, Austria; and CRL was provided by Abbott, Vienna, Austria.

The concentration ranges of the substances tested (twofold dilutions) were as follows: LNZ, 1 to 64 μg/ml; AMX, 0.016 to 0.25 μg/ml; CLR, 0.004 to 0.1 μg/ml for CLR-susceptible and 1.0 to 512 μg/ml for CLR-resistant strains; and MTZ, 0.03 to 1.0 μg/ml for MTZ-susceptible and 2.0 to 512 μg/ml for MTZ-resistant strains.

To determine the interactions between LNZ and any of the other agents, all possible combinations were tested across the whole range of concentrations. The *H. pylori* strains to be studied were grown on Mueller-Hinton agar with 5% blood under microaerobic conditions for 48 h at 37°C. Then, the strains were suspended in physiological saline and adjusted to a McFarland standard of 3 to 4 (equivalent to 5×10^8 to 5×10^9 CFU/ml). The plates containing the antimicrobial agents were inoculated using a multipoint inoculator (approximately 2 μl/spot) and incubated for 72 h at 37°C under microaerobic conditions and a relative humidity of >95%.

In divergence from NCCLS guidelines (14), the strains to be tested were suspended after 48 h, instead of the 72 h recommended, and adjusted to a McFarland standard of 3 to 4. This well-proven procedure, having been used in numerous studies (e.g., 12), was chosen because 48-h cultures show significantly better viability than 72-h cultures and because the larger inoculum facilitates the identification of resistant mutants.

Reading of MICs and determination and interpretation of

TABLE 1. In vitro activity of LNZ against 70 strains of *H. pylori*

H. pylori susceptibility pattern[a] (no. of strains tested)	MIC (μg/ml)		
	Range	50%	90%
AMX +, CLR +, MTZ + (30)	8–32	16	32
AMX +, CLR +, MTZ − (20)	8–32	16	32
AMX +, CLR −, MTZ − (20)	16–64	16	32

[a] +, susceptible; −, resistant.

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TABLE 2. ΣFIC values and number of *H. pylori* strains against which synergy, partial synergy, indifference, or antagonism was shown by the combination of LNZ with AMX, CLR, or MTZ

Drug with which LNZ was combined	*H. pylori* susceptibility pattern[a] (no. of strains tested)	ΣFIC		No. of strains showing:			
		Minimum	Maximum	Synergy	Partial synergy	Indifference	Antagonism
AMX	AMX +, CLR +, MTZ + (29)	0.31	2.50	1	11	29	0
CLR	AMX +, CLR +, MTZ + (15)	0.38	2.06	1	14	14	0
	AMX +, CLR −, MTZ − (15)	0.56	2.50	0	14	15	0
MTZ	AMX +, CLR +, MTZ + (15)	0.50	2.12	2	12	15	0
	AMX +, CLR +, MTZ − (15)	0.50	2.12	1	13	15	0

[a] +, susceptible; −, resistant.

fractional inhibitory concentrations (FICs) and FIC indices were done as described elsewhere (13).

Table 1 shows the results of the MIC determination for LNZ against 70 *H. pylori* strains. With regard to susceptibility to LNZ, no significant differences between CLR- and MTZ-susceptible, CRL-susceptible and MTZ-resistant, and CRL- and MTZ-resistant *H. pylori* strains were found. MICs at which 50 and 90% of strains were inhibited (MIC_{50} and MIC_{90}, respectively) were 16 and 32 μg/ml, respectively, for all three groups of strains. Identical results were obtained with a smaller (10^7 to 10^8 CFU/ml), NCCLS-consistent inoculum.

The MICs determined for the strains studied were distributed across a rather small concentration range, with the lowest MIC determined being 8 μg/ml and the highest (measured twice) being 64 μg/ml. Hence, the majority of MICs measured were beyond the breakpoint of ≤8 μg/ml derived from pharmacokinetic studies (7) and recommended by the manufacturer of LNZ.

The in vitro interactions between LNZ and AMX, CLR, or MTZ are summarized in Table 2. The minimum and maximum fractional inhibitory concentration summation (ΣFIC) values calculated for all strains tested with a given combination of agents are shown. If different types of interactions occurred for one organism within the same checkerboard, all interpretations were reported. The presence of an interaction was reported even if this interaction was observed only once within the checkerboard. The results show that the combinations tested most often exhibited different types of interactions against one and the same strain, predominantly partial synergy ($0.5 < \Sigma FIC < 1.0$) and indifference ($1.0 < \Sigma FIC \leq 4.0$). While actual synergy ($\Sigma FIC \leq 0.5$) was found in some isolated instances, no case of antagonism ($\Sigma FIC > 4$) could be identified.

The extent and the type of interactions were virtually identical when CLR- and/or MTZ-susceptible and -resistant strains were tested.

In none of the cases was the observed increase in activity sufficient to classify CLR- or MTZ-resistant strains as CLR or MTZ susceptible when tested with a combination with LNZ. However, it must be emphasized that for the majority of the *H. pylori* strains, the combination of AMX and LNZ caused a reduction of the MIC of LNZ by one to two dilutions for at least one of the concentrations tested, thus resulting in MICs of ≤8 μg/ml.

LNZ is an antimicrobial agent with a spectrum of activity primarily covering gram-positive bacteria, including multiresistant strains of staphylococci and streptococci. In addition, it has also shown in vitro activity against certain gram-negative species, such as *Fusobacterium*, *Prevotella*, and *Porphyromonas* spp. and *Pasteurella multocida* (4).

While the MICs of LNZ determined in this study for *H. pylori* were highly uniform, they were significantly higher than those for the above bacteria and comparable to those reported for *Neisseria*, *Moraxella*, and *Eikenella* (4).

Whether such MICs are to be interpreted as susceptible or as resistant remains to be clarified. The currently recommended limit of 8 μg/ml is based on attainable serum drug levels, whose relevance for superficial infections of the gastric mucosa is still unknown.

As yet, there has been no pharmacological approach providing satisfactory means for assessing the effectiveness of existing treatment regimens for *H. pylori* infections or for evaluating new treatment modalities. This situation is primarily due to the fact that there is no simple and generally accepted model allowing for pharmacokinetic studies of the microenvironment of the infected gastrum. Furthermore, the physicochemical conditions prevailing in this microenvironment are only partly known (10). For the reasons outlined, it would be interesting to determine the *H. pylori* status before and after LNZ treatment in the course of ongoing clinical studies by using noninvasive methods, such as the ^{13}C-urea breath test or, possibly, antigen or DNA detection in stool samples. The above tests allow for a sufficiently accurate quantification of *H. pylori* so that not only genuine eradication—being rather unlikely with LNZ monotherapy—but also temporary suppression—to be interpreted as evidence for a certain activity—can be demonstrated. Such in vivo susceptibility tests should generally be considered for use in clinical studies with new antimicrobial agents.

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