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In Vitro Activities of Rabeprazole, a Novel Proton Pump Inhibitor, and Its Thioether Derivative Alone and in Combination with Other Antimicrobials against Recent Clinical Isolates of *Helicobacter pylori*

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The MICs of rabeprazole sodium (RPZ), a newly developed benzimidazole proton pump inhibitor (PPI), against 133 clinical *Helicobacter pylori* strains revealed a higher degree of activity than the another two PPIs, lansoprazole and omeprazole. Time-kill curve assays of RPZ, when combined with amoxicillin, clarithromycin, or metronidazole, disclosed that synergistic effects were demonstrated in combination with each antibiotic examined. Moreover, no apparent antagonistic effect appeared among all of the strains tested.

It is well known that *Helicobacter pylori* is associated with gastric disorders, such as gastritis, and the gastric or duodenal ulcer (2, 5, 8, 15, 21). The combination chemotherapy, i.e., amoxicillin (AMC) plus clarithromycin (CAM) or metronidazole (MNZ) with a proton pump inhibitor (PPI) is now widely recommended for eradication chemotherapy (1, 3, 4, 6, 9, 13, 14, 16). Rabeprazole sodium (RPZ), a benzimidazole PPI, is a new substituted benzimidazole H^+, K^+ ATPase inhibitor. It acts as an irreversible, noncompetitive inhibitor of the H^+, K^+ ATPase, and preliminary studies demonstrate that RPZ produces a potent and long-lasting inhibition of gastric acid secretion and a low level of hypergastrinemia (3, 17, 20). A novel RPZ demonstrating a chemical structure of $C_{18}H_{20}N_3SNa$ with a molecular weight of 381.43, as shown in Fig. 1, was developed in 1997 and has been proven to be effective against *H. pylori* strains, like other PPIs, such as lansoprazole (LPZ) and omeprazole (OPZ) (11, 19). It has been demonstrated to act as an irreversible noncompetitive inhibitor of the enzyme urease that is an important virulence factor of pathogenicity of gastric *H. pylori* (17, 20). The in vivo evaluation study of RPZ has recently been reported (18). However, no in vitro data has been available to date concerning the interaction studies of RPZ in combination with some kinds of antibiotics. Therefore, we tried to evaluate its bactericidal activity when combined with an antibiotic compound against *H. pylori* strains by the time-kill curve assay (10).

We at first determined the in vitro activities of RPZ and its thioether (TH) derivative, RPZ-TH, together with OPZ and LPZ.

The 133 *H. pylori* strains tested were recent clinical isolates from different patients with chronic gastritis and gastric and/or duodenal ulcer during the 2 years between April 1996 and March 1998, at the Central Clinical Laboratories, Shinshu University Hospital, Matsumoto, Japan. In addition, two reference strains, *H. pylori* NCTC 11637 and NCTC 11916, were also included in this study. All of the strains examined were preserved in Microbank (Pro-Lab Diagnostic, Richmond Hill, Ontario, Canada) vials in a deep freezer at −83°C.

The antimicrobials and PPIs used were as follows: AMC from Meiji Seika Kaisha, Ltd., Tokyo, Japan; CAM from Taisho Pharmaceuticals, Co., Ltd., Tokyo, Japan; MNZ from Shionogi & Co., Ltd., Tokyo, Japan; RPZ and its derivative, RPZ-TH, from Eisai Pharmaceuticals Co., Ltd, Tokyo, Japan; LPZ from Takeda Chemical Industries, Ltd., Osaka, Japan; and OPZ from Astra Japan Ltd., Tokyo, Japan.

In determining the MICs, twofold serial dilutions of each drug were made in 50 μl of brucella broth (BBL Microbiology Systems Inc., Cockeysville, Md.) supplemented with 5% horse serum (Irvin Scientific, Santa Ana, Calif.) in microplates with 96 wells (Eiken Chemical Co., Ltd., Tokyo, Japan). The broth dilutions in the wells containing the agent at each concentration were prepared on the day of use.

For a preparation of inocula of *H. pylori* strains, the cells of *H. pylori* strains were scraped from 72-h culture lawns on the blood agar plates (Columbia agar base; BBL) containing 5% defibrinated sheep blood) to adjust the density of MacFarland no. 3 standard. Fifty microliters from each broth prepared was applied within 30 min to the wells with 50 μl of drug diluent containing the test compound to make the final concentration approximately 10^6 CFU/ml. Inoculation into each well was made with a multipoint inoculating device.

The final concentrations prepared in the wells of the microplates were from 0.0078 to 32 μg/ml for AMC, from 0.0078 to 128 μg/ml for CAM and MNZ, and from 0.031 to 64 μg/ml for RPZ, RPZ-TH, LPZ, and OPZ. The results were read after

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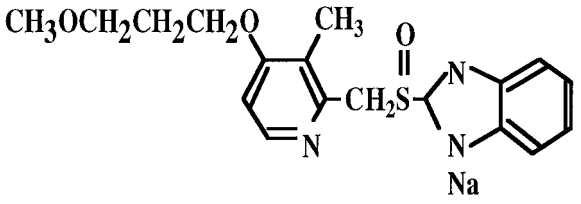

FIG. 1. Chemical structure of RPZ ($C_{18}H_{20}N_3SNa$; molecular weight, 381.43).

incubation at 35°C for 72 h in the CO_2 incubator (Sanyo Co., Ltd., Tokyo, Japan) with 15% CO_2 in an atmosphere with high humidity.

The MIC was defined as the lowest concentration of the agent that completely inhibited the growth of the strain examined as detected by the unaided eye.

The time-kill curve assay (10) was performed to determine the behavior of drug interaction of RPZ with another antimicrobial(s), using four representative *H. pylori* strains, i.e., two reference strains, NCTC 11637 and NCTC 11916, and two clinical isolates, SHP 107 and SHP 133. The medium used was brucella broth (BBL) supplemented with 5% horse serum (Irvin Scientific). The strains tested were inoculated to a final concentration of 5×10^6 CFU/ml and cultured in 10 ml of the medium containing a single agent or a combination of two agents with gentle shaking at 35°C for 24 h. Agent concentrations were based on half the MICs, once the MICs, and twice the MICs of the agent.

The numbers of viable *H. pylori* cells were calculated as CFU per milliliter. At 0, 3, 6, 12, and 24 h, the samples (100 μl) were removed and 10-fold serial dilutions were performed. An aliquot (100 μl) of each dilution was spotted in duplicate onto blood agar plates (Columbia agar base; BBL) containing 5% defibrinated sheep blood for colony counts. The inoculated plates were grown at 35°C for 7 days. Data obtained were analyzed by determining the number of strains that yielded a $\Delta\log_{10}$ CFU per milliliter reduction after 24 h of incubation, compared with the counts at time zero. Synergism was defined as a $\geq$2-$\log_{10}$ CFU/ml decrease after 24 h of incubation with the drug combination, in comparison with the most active agent alone. Antagonism was defined as a $\geq$2-$\log_{10}$ CFU/ml increase after 24 h of incubation with the drug combination, compared with the most active agent alone (12).

The MIC distributions of all the agents, including RPZ and RPZ-TH, are shown in Table 1.

Among the three PPIs, RPZ and RPZ-TH are the most active, with almost the same MICs. On the other hand, OPZ is the least active compound among the PPIs.

TABLE 1. MICs[a] (μg/ml) of anti-*H. pylori* compounds against 133 clinical *H. pylori* isolates by the microdilution method

Drug	MIC range	MIC_{50}	MIC_{90}
AMC	0.015–0.5	0.031	0.031
CAM	≤0.007–64	0.007	1
MNZ	1–128	4	16
RPZ	0.063–1	0.25	0.5
RPZ-TH	0.031–4	0.25	0.25
LPZ	0.25–2	1	1
OPZ	0.5–16	8	16

[a] MIC_{50} and MIC_{90}, MICs at which 50 and 90% of the isolates tested are inhibited, respectively.

TABLE 2. MICs (μg/ml) of respective agent to the selective strains tested for the time-kill curve assay

Antibiotic or PPI	MIC against strain[a]			
	NCTC 11916	NCTC 11637	SHP 107	SHP 133
AMC	0.016	0.016	0.016	0.016
CAM	0.5	0.5	0.007	8
MNZ	64	0.25	4	2
RPZ	0.016	0.25	0.25	0.5
RPZ-TH	0.031	0.13	0.25	0.25
LPZ	0.25	0.25	1	1
OPZ	2	8	16	2

[a] SHP, clinical *H. pylori* strains isolated in Shinshu University Hospital.

MICs required by the four *H. pylori* strains used for the evaluation of the combination effect by the time-kill assay are tabulated in Table 2.

Time-kill curves obtained were almost the same as those obtained with half, once, and twice the MICs. Therefore, among the various plot curves, only the ones representative of once the MIC, demonstrating synergy and indifference, were shown in Fig. 2A and B. Figure 2A demonstrates a representation of indifference in combination with CAM. Figure 2B shows an example of synergy when combined with MNZ. Synergistic effects when combined with CAM were also observed in the NCTC 11637 and NCTC 11916 strains. In combination with MNZ, only the clinical SHP 107 strain exhibited synergy. No apparent antagonistic effect was observed with any combination of a drug.

The emergence of synergistic effects (two out of four strains tested for CAM plus RPZ and one out of four strains examined for MNZ plus RPZ) was almost the same as those obtained when combined with LPZ, as previously described (7). Moreover, we observed that RPZ caused no apparent decrease in CFU even after incubation for 24 h. These findings may suggest that the activities of RPZ and RPZ-TH were not bactericidal but bacteriostatic.

We previously demonstrated that synergistic effects were observed only when the strains were sensitive to both of the combined antibiotics investigated, such as AMC and CAM or AMC and MNZ (7). In this study, the NCTC 11916 strain with resistance to MNZ revealed no synergistic effect when determined by applying twice the MIC, thus confirming that synergism did not occur when the strains were resistant to both the agents tested. These findings, thereby, led us to consider that the strains tested revealed different drug interactions.

Notwithstanding the emergence of indifferent and synergistic plot curves observed, it is notably favorable that no antagonistic effect was observed among the four *H. pylori* strains examined in any combination of RPZ with any of the three antibiotics. These findings were just the same as the previously reported LPZ results (7).

Because of its strong in vitro activity against *H. pylori* strains, RPZ should be regarded as an additional novel PPI to be administered in combination regimens against *H. pylori* infections. The fact that clinical *H. pylori* strains were inhibited in their growth at the lowest MICs of the three PPIs tested is noteworthy and potentially useful information.

Further testing should be done to confirm the activity of RPZ when combined with certain antibiotics using larger numbers of strains. In addition, the accumulation of data concerning clinical efficacy of RPZ appears warranted. Indeed, the highest growth inhibitory activity among the three PPIs tested

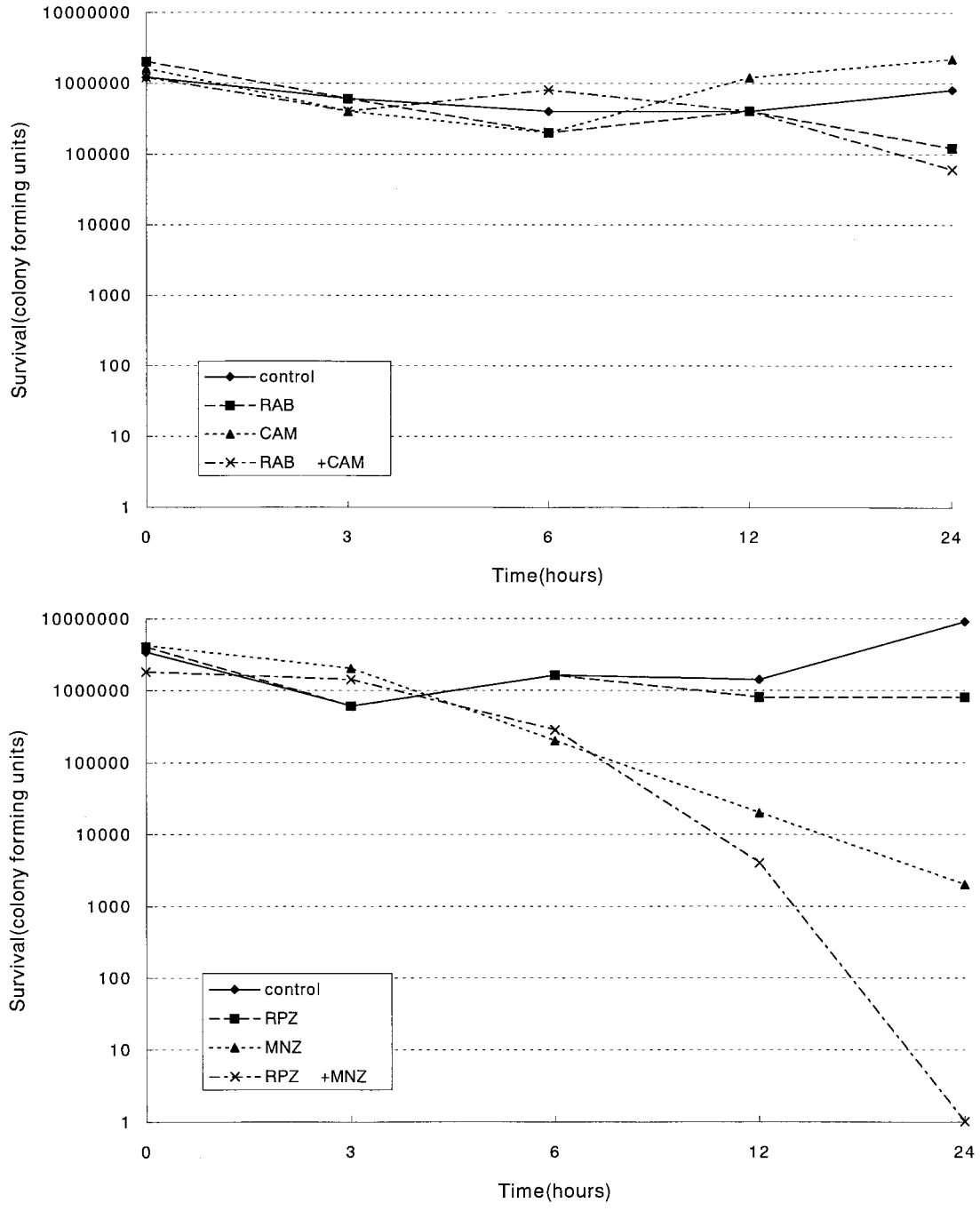

FIG. 2. Time-kill curves of *H. pylori* strains in the presence of the drug(s) at their MICs. The numbers of viable cells per milliliter were plotted versus incubation time. (A) A time-kill curve showing indifference of a clinical strain, *H. pylori* SHP 133. (B) A time-kill curve showing synergism of a clinical strain, *H. pylori* SHP 107.

against *H. pylori* strains is noteworthy; however, development of novel antimicrobials with specific activity against *H. pylori* strains is urgently desired.

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