In Vitro Activity of a Novel Antimicrobial Agent, TG44, for Treatment of Helicobacter pylori Infection

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Due to concerns about the current therapeutic modalities for Helicobacter pylori infection, e.g., the increased emergence of drug-resistant strains and the adverse reactions of drugs currently administered, there is a need to develop an anti-H. pylori agent with higher efficacy and less toxicity. The antibacterial activity of TG44, an anti-H. pylori agent with a novel structural formula, against 54 clinical isolates of H. pylori was examined and compared with those of amoxicillin (AMX), clarithromycin (CLR), and metronidazole (MNZ). Consequently, TG44 inhibited the growth of H. pylori in an MIC range of 0.0625 to 1 μg/ml. The MIC ranges of AMX, CLR, and MNZ were 0.0078 to 8 μg/ml, 0.0156 to 64 μg/ml, and 2 to 128 μg/ml, respectively. The antibacterial activity of TG44 against AMX-, CLR-, and MNZ-resistant strains was nearly comparable to that against drug-susceptible ones. In a pH range of 3 to 7, TG44 at 3.13 to 12.5 μg/ml exhibited potent bactericidal activity against H. pylori in the stationary phase of growth as early as 1 h after treatment began, in contrast to AMX, which showed no bactericidal activity at concentrations of up to 50 μg/ml at the same time point of treatment. TG44 at 25 μg/ml exhibited no antibacterial activity against 13 strains of aerobic bacteria, suggesting that its antibacterial activity against H. pylori is potent and highly specific. The present study indicated that TG44 possesses antibacterial activity which manifests quickly and is potentially useful for eradicating not only the antibiotic-susceptible but also the antibiotic-resistant strains of H. pylori by monotherapy.

In recent years, the association between Helicobacter pylori and gastritis, gastric ulcer, and duodenal ulceration (14, 21), as well as gastric cancer, has been clarified (7, 24, 28). The eradication of H. pylori drastically reduces the recurrence of ulceration and is therefore considered essential to treat ulceration. Currently, proton pump inhibitor-based triple therapy using a proton pump inhibitor and two antibiotics is frequently conducted to eradicate H. pylori in patients with gastric and/or duodenal ulcer. Among the antibiotics frequently used are amoxicillin (AMX), clarithromycin (CLR), and metronidazole (MNZ). Many clinical studies have reported eradication rates of 80 to ≥90%, attained by the relevant triple therapy (1, 2, 3, 8, 9, 15). However, many concerns remain to be addressed in the future, including the increased emergence of drug-resistant strains of H. pylori due to the overuse of antibiotics (4, 5, 6, 11, 12, 16, 17, 19, 23, 25, 26, 27, 29) and the indiscriminate use of eradication therapy for the bacterium, as well as the adverse reactions (e.g., diarrhea, dysgeusia, and eruption) to the drugs administered. Therefore, there is a strong need to develop an anti-H. pylori agent which is suitable for the next generation of eradication therapy. Ideally, such an agent is expected to satisfy the following requisites: (i) potent antibacterial activity against H. pylori (administrable by mono-therapy); (ii) high specificity for H. pylori (without efficacy for other intestinal bacteria); (iii) bactericidal activity against AMX-, CLR-, or MNZ-resistant strains of H. pylori; (iv) stability in the stomach (10); (v) possible synergism with other drugs; and (vi) less likelihood of generating drug-resistant strains of H. pylori.

TG44 (4-methylbenzyl 4-trans-4-(guanidinomethyl)cyclohexyl carbamoyloxyl[biphenyl-4-carboxylate monohydrochloride [CAS registry number 178748-55-3]], synthesized by Nagase Chemtex Corporation, is an antimicrobial agent with potent anti-H. pylori activity to which the bacterium exhibits high susceptibility.

In the present study, we used 56 strains of H. pylori, including two reference strains and 54 clinical isolates, to examine the antibacterial activity of TG44 in comparison with those of three antibiotics which are frequently used for the eradication of the bacterium, AMX, CLR, and MNZ.

MATERIALS AND METHODS

Test compound and antibiotics. TG44 (Fig. 1) was synthesized by Nagase Chemtex Corporation (Osaka, Japan). AMX, CLR, and MNZ were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Reagents. Brain heart infusion broth (BHIB) and brain heart infusion agar (BHIA) were purchased from Difco Laboratories, Inc. (Detroit, MI), β-Cyclo-dextrin (β-CD) was purchased from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Calf serum was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Strains. The fifty-six strains of H. pylori used were supplied by the following sources: the American Type Culture Collection provided 2 reference strains...
(ATCC 43504 and ATCC 43629), S. Kamiya (Department of Microbiology, Kyorin University School of Medicine, Tokyo, Japan) provided 12 clinical isolates (KR 2098, TK 1003, TK 1025, TK 1027, TK 1030, TK 1042, TK 1047, TK 1126, TK 1147, TK 1307, TK 1308, and TK 1310), and M. Sasatsu (Department of Microbiology, School of Pharmacy, Tokyo University of Pharmacy and Life Science, Tokyo, Japan) provided 42 clinical isolates (TH 517, TH 555, TH 582, TH 607, TH 627, TH 1818, TH 2095, TH 3391, TH 3892, TH 4165, TH 1126, TH 1609, TH 1611, TH 1614, TH 1663, TH 1711, TH 1723, TH 1729, TH 1735, TH 1775, TH 1826, TH 1831, TH 1832, TH 1876, TH 1887, TH 1889, TH 1890, TH 1892, TH 1893, and TH 1899).

Helicobacter mastilae ATCC 45772, Helicobacter pullorum ATCC 51864, Helicobacter bilis ATCC 51630, Arcobacter cryaerophilus ATCC 49615, Campylobacter helveticus ATCC 51209, Campylobacter jejuni ATCC 700819, and Campylobacter jejuni ATCC 29428 were purchased from the American Type Culture Collection.

Stock cultures were stored in a freezer at −85°C in BHIB supplemented with 5% heat-inactivated calf serum and 15% glycerol. Thirty reference strains of common aerobic bacteria, preserved at the Department of Microbiology at Kyoto Pharmaceutical University (Kyoto, Japan), were used in the present study. Gram-positive strains included Staphylococcus aureus (ATCC 29212), the stock cultures of 13 reference strains of aerobic bacteria were grown at 35°C for 3 days. The MIC was defined as the lowest concentration at which the compound inhibited visible bacterial growth.

Bacterial suspension (20 µl) was seeded into 4 ml of BHIB supplemented with 0.1% β-CyD and containing two-fold serial dilutions of the compound. The agar plate was inverted and incubated at 35°C for 20 h. The MIC was defined as the lowest concentration at which the compound inhibited visible bacterial growth.

Determination of the MICs for H. pylori. The MICs were determined by the agar dilution method using BHIA. Under microaerobic conditions (5% O2, 10% as an alternative to bovine serum in an attempt to avoid the enzymatic inactivation of the culture was incubated, and aliquots were collected at various time points. Each sample was serially diluted 10-fold with saline, and 10 µl of the diluted sample was plated on BHIA supplemented with 7% horse blood (TG44-treated sample) or on BHIA supplemented with 7% horse blood and 5% pen-

FIG. 1. Structural formula of TG44.

TABLE 1. Antibacterial activities of TG44, AMX, CLR, and MNZ against H. pylori

| H. pylori strain | TG44 | AMX | CLR | MNZ |
|------------------|------|-----|-----|-----|
| Reference strains | ATCC 43504 | 0.5 | 0.0313 | 0.0156 | 64 |
|                  | ATCC 43629 | 0.5 | 0.0313 | 0.0156 | 2 |
| Clinical isolates |                 |     |     |     |
| KL 2098          | 0.5 | 0.0313 | 0.0313 | 2 |
| TK 1003          | 1   | 0.0313 | 0.0625 | 64 |
| TK 1025          | 0.25 | 0.0313 | 0.0156 | 16 |
| TK 1027          | 0.125 | 0.0313 | 0.0313 | 8 |
| TK 1030          | 0.5  | 0.0156 | 0.0313 | 8 |
| TK 1042          | 0.5  | 0.125 | 0.0625 | 8 |
| TK 1047          | 0.125 | 0.0156 | 2  | 4 |
| TK 1126          | 0.25 | 0.0313 | 0.0313 | 8 |
| TK 1147          | 0.25 | 0.0156 | 4  | 4 |
| TK 1307          | 0.125 | 0.0156 | 0.0313 | 32 |
| TK 1308          | 1   | 0.0625 | 0.0625 | 4 |
| TK 1310          | 1   | 0.0156 | 0.0156 | 8 |
| TK 517           | 1   | 8   | 8   | 4 |
| TK 555           | 0.25 | 0.0313 | 0.0313 | 64 |
| TK 582           | 0.25 | 0.0156 | 0.0156 | 32 |
| TK 607           | 0.125 | 0.0156 | 0.0625 | 8 |
| TK 627           | 0.25 | 0.0313 | 0.0625 | 32 |
| TK 1818          | 0.5  | 4   | 8   | 4 |
| TK 2095          | 0.125 | 0.0156 | 0.0625 | 128 |
| TK 3391          | 0.25 | 0.0156 | 0.0313 | 128 |
| TK 3392          | 0.25 | 0.0156 | 0.0313 | 128 |
| TK 4165          | 0.125 | 0.0156 | 0.0313 | 32 |
| TS 119           | 0.25 | 0.0156 | 0.0156 | 128 |
| TS 120           | 0.25 | 0.0156 | 0.0156 | 32 |
| TS 251           | 0.25 | 0.0156 | 0.0313 | 16 |
| TS 279           | 0.25 | 0.0156 | 0.0156 | 128 |
| TS 1131          | 0.25 | 0.5  | 4   | 4 |
| TS 1367          | 0.25 | 0.0156 | 16  | 2 |
| TS 1407          | 0.25 | 0.0078 | 32  | 8 |
| TS 1419          | 0.25 | 0.0156 | 32  | 8 |
| TS 1445          | 0.5  | 0.0156 | 32  | 4 |
| TS 1459          | 0.25 | 0.0078 | 32  | 4 |
| TS 1556          | 0.25 | 0.0156 | 32  | 2 |
| TS 1609          | 0.125 | 0.0313 | 4  | 2 |
| TS 1611          | 0.25 | 0.0625 | 8  | 2 |
| TS 1614          | 0.125 | 0.0625 | 4  | 2 |
| TS 1664          | 0.125 | 0.0625 | 8  | 8 |
| TS 1683          | 0.25 | 0.0078 | 8  | 2 |
| TS 1711          | 0.25 | 0.0313 | 16 | 32 |
| TS 1723          | 0.5  | 0.0156 | 16  | 2 |
| TS 1729          | 0.25 | 0.0313 | 8  | 2 |
| TS 1735          | 0.25 | 0.0156 | 0.0156 | 16 |
| TS 1775          | 0.5  | 0.0313 | 1  | 32 |
| TS 1826          | 0.25 | 0.0078 | 8  | 8 |
| TS 1831          | 0.5  | 0.5  | 2   | 64 |
| TS 1832          | 0.5  | 0.5  | 4   | 64 |
| TS 1876          | 0.125 | 0.0156 | 4  | 4 |
| TS 1887          | 0.125 | 0.0156 | 4  | 4 |
| TS 1888          | 0.0625 | 0.0313 | 8  | 4 |
| TS 1889          | 0.25 | 0.0313 | 0.0156 | 2 |
| TS 1890          | 0.125 | 0.0078 | 4  | 2 |
| TS 1892          | 0.5  | 0.0156 | 64  | 4 |
| TS 1893          | 0.5  | 0.0156 | 32  | 2 |
| TS 1899          | 0.125 | 0.0156 | 0.0313 | 2 |

* MICs were determined by the agar dilution method on brain heart infusion agar supplemented with 0.1% β-cyclodextrin which was seeded with a bacterial suspension of 10^7 CFU/ml.

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of penicillinase (AMX-treated sample). The plate was incubated at 37°C under microaerobic conditions for 3 days, and the colonies of H. pylori were then counted. **Effects of pH on bacterialidal activity.** At pH 7, 6, 5, and 3, H. pylori ATCC 43504 was used to examine the bactericidal activity of TG44 at 1×, 2×, 4×, 8×, and 16× the MIC of the compound. The bacterial suspension (20 μl at 2×10⁶ CFU/ml) was seeded into 4 ml of BHIB supplemented with 0.1% β-Cyd and containing TG44 at each concentration. The culture medium suspensions were adjusted to pH 7, 6, 5, and 5 with 1 N HCl and to pH 3 with 1 N HCl plus urea (1.4 mmol/liter). Urea was added to the medium because of H. pylori lethality under acidic pH conditions. With urea, a substrate of urease, H. pylori can produce ammonia to survive at pH 3. Cultures were incubated, and aliquots were collected at various time points. Each sample was serially diluted 10-fold with saline, and 10 μl of the diluted sample was plated on BHIA supplemented with 7% horse blood (TG44-treated sample) or on BHIA supplemented with 7% horse blood and 5% penicillin (AMX-treated sample). The plate was incubated at 37°C under microaerobic conditions for 3 days, and the number of colonies of H. pylori was counted.

**Electron microscopy.** Under microaerobic conditions (5% O₂, 10% CO₂, and 85% N₂) in an AnaeroPack Campylo jar (Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan), the stock culture of H. pylori ATCC 43504 was grown at 37°C for 24 h in BHIB supplemented with 0.1% β-Cyd by shaking the bacteria on a shaker at 125 rpm. The bacterial suspension (10 ml) in the exponential phase of growth was seeded into BHIB (990 ml) supplemented with 0.1% β-Cyd and containing TG44 (at concentrations of 0.20, 0.39, 1.56, and 25 μg/ml). After incubation at 37°C under microaerobic conditions for 3 days and 6 h with shaking at 125 rpm, the sample was collected.

After prefixation with an aqueous solution of 1.5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 30 min at 5°C, the sample was washed twice with 0.05 M phosphate buffer (pH 7.2) and then fixed with 1% OsO₄ in Veronal-acetate buffer (pH 6.1) for 1 h at room temperature by the method of Kellenberger et al. (13). After treatment with 0.5% uranyl acetate, the sample was subsequently dehydrated in alcohol solutions at serial concentrations. For scanning electron microscopic observation, alcohol from serial concentrations was replaced with isooctyl acetate for further dehydration, and samples were then dried by the critical-point drying method and further evaporated with carbon and gold. The surface structures of bacterial cells were then observed with a transmission electron microscope (model 1200EX; Japan Electron Optics Laboratory, Tokyo, Japan).

**RESULTS**

**Table 2. Summary of the antibacterial activities of TG44, AMX, CLR, and MNZ against 54 clinical isolates of H. pylori**

| Compound | MIC (μg/ml) | 50% | 90% |
|----------|-------------|-----|-----|
| TG44 | 0.0625–1 | 0.25 | 0.5 |
| AMX | 0.0078–8 | 0.0156 | 0.125 |
| CLR | 0.0156–64 | 2 | 32 |
| MNZ | 2–128 | 8 | 64 |

**Table 3. Antibacterial activities of TG44 against Helicobacter species and common bacteria**

| Microorganism | MIC (μg/ml) | AMX | CLR | MNZ |
|---------------|-------------|-----|-----|-----|
| Helicobacter pylori ATCC 43504 | 0.5 | 0.0313 | 0.0078 | 64 |
| Helicobacter mustelae ATCC 43772 | 8 | 4 | 1 | 4 |
| Helicobacter pullorum ATCC 51864 | >16 | 4 | 2 | 16 |
| Helicobacter bilis ATCC 51630 | >16 | 4 | 8 | 2 |
| Aerococcus pyraerophilus ATCC 49615 | >16 | 16 | 4 | 8 |
| Campylobacter helveticus ATCC 51209 | >16 | 16 | 4 | 16 |
| Campylobacter jejuni ATCC 700819 | >16 | 16 | 2 | 1 | >64 |
| Campylobacter jejuni ATCC 29428 | >16 | 0.5 | 0.25 | 32 |

*MICs were determined from the agar dilution method on brain heart infusion agar supplemented with 0.1% β-cyclodextrin, which was seeded with a bacterial suspension of 10⁵ CFU/ml.

**TABLE 4. Antibacterial activities of TG44, AMX, CLR, and MNZ against common aerobic bacteria**

| Microorganism | MIC (μg/ml) | AMX | CLR | MNZ |
|---------------|-------------|-----|-----|-----|
| Gram positive | | | | |
| Staphylococcus aureus | 209P JC | >25 | 0.20 | 0.10 | >200 |
| Staphylococcus aureus Smith | | >25 | 0.20 | 0.20 | >200 |
| Staphylococcus epidermidis ATCC 12228 | | >25 | 0.78 | 0.20 | >200 |
| Enterococcus faecalis ATCC 29212 | | >25 | 0.78 | 1.56 | >200 |
| Bacillus subtilis PCI 219 | | >25 | 0.05 | 0.10 | 200 |

**Gram negative**

| Microorganism | MIC (μg/ml) | AMX | CLR | MNZ |
|---------------|-------------|-----|-----|-----|
| Escherichia coli K-12 | >25 | 3.13 | 6.25 | >200 |
| Klebsiella pneumoniae | >25 | 50 | 12.5 | >200 |
| NCTC 9632 | | | | |
| Serratia marcescens IFO 3736 | >25 | 100 | >100 | >200 |
| Proteus vulgaris OX-19 | >25 | >100 | >100 | >200 |
| Proteus mirabilis 1287 | >25 | 0.39 | >100 | >200 |
| Morganella morganii KONO | >25 | >100 | >100 | >200 |
| Providencia rettgeri NIH 96 | >25 | 3.13 | >100 | >200 |
| Pseudomonas aeruginosa PAO-1 | >25 | >100 | >100 | >200 |

*MICs were determined from the agar dilution method on Mueller-Hinton S agar which was seeded with a bacterial suspension of 10⁶ CFU/ml.
Antibacterial activities of TG44, AMX, CLR, and MNZ against common aerobic bacteria. The MICs of TG44, AMX, CLR, and MNZ for 13 reference strains of aerobic bacteria were determined by the agar dilution method. The MICs thereof are shown in Table 4. At a concentration of 25 μg/ml, TG44 showed no antibacterial activity against gram-positive aerobic bacteria (i.e., S. aureus 209P JC, S. aureus Smith, S. epidermidis ATCC 12228, E. faecalis ATCC 29212, and B. subtilis PCI 219) or gram-negative aerobic bacteria (i.e., E. coli K-12, K. pneumoniae NCTC 9632, S. marcescens IFO 3736, P. vulgaris OX-19, P. mirabilis 1287, M. morganii KONO, P. rettgeri NIH 96, and P. aeruginosa PAO-1). The MICs of AMX and CLR for these aerobic bacteria ranged from 0.05 to >100 μg/ml and from 0.10 to >100 μg/ml, respectively.

Bactericidal activity of TG44 against H. pylori. The bactericidal activity of TG44 against reference strains of H. pylori was compared with that of AMX by using a short-term assay. Time-kill studies were conducted using the microbes at a concentration of approximately 10^5 CFU/ml for the initial seeding.

Figure 2a shows the effects of TG44 on the viability of H. pylori ATCC 43629. H. pylori ATCC 43629 was incubated in BHIB supplemented with 0.1% β-CyD containing various concentrations of TG44. Aliquots were removed at various time points, and the numbers of colonies of H. pylori were counted. (b) Bactericidal activity of AMX against H. pylori ATCC 43629. H. pylori ATCC 43629 was incubated in BHIB supplemented with 0.1% β-CyD containing various concentrations of AMX. Aliquots were removed at various time points, and the numbers of colonies of H. pylori were counted.

Bactericidal activity of TG44 against H. pylori ATCC 43504. H. pylori ATCC 43504 was incubated in BHIB supplemented with 0.1% β-CyD containing various concentrations of TG44. Aliquots were removed at various time points, and the numbers of colonies of H. pylori were counted. (b) Bactericidal activity of AMX against H. pylori ATCC 43504. H. pylori ATCC 43504 was incubated in BHIB supplemented with 0.1% β-CyD containing various concentrations of AMX. Aliquots were removed at various time points, and the numbers of colonies of H. pylori were counted.

Antibacterial activities of TG44, AMX, CLR, and MNZ against common aerobic bacteria. The MICs of TG44, AMX, CLR, and MNZ for 13 reference strains of aerobic bacteria were determined by the agar dilution method. The MICs thereof are shown in Table 4. At a concentration of 25 μg/ml, TG44 showed no antibacterial activity against gram-positive aerobic bacteria (i.e., S. aureus 209P JC, S. aureus Smith, S. epidermidis ATCC 12228, E. faecalis ATCC 29212, and B. subtilis PCI 219) or gram-negative aerobic bacteria (i.e., E. coli K-12, K. pneumoniae NCTC 9632, S. marcescens IFO 3736, P. vulgaris OX-19, P. mirabilis 1287, M. morganii KONO, P. rettgeri NIH 96, and P. aeruginosa PAO-1). The MICs of AMX and CLR for these aerobic bacteria ranged from 0.05 to >100 μg/ml and from 0.10 to >100 μg/ml, respectively.

Bactericidal activity of TG44 against H. pylori. The bactericidal activity of TG44 against reference strains of H. pylori was compared with that of AMX by using a short-term assay. Time-kill studies were conducted using the microbes at a concentration of approximately 10^5 CFU/ml for the initial seeding.

Figure 2a shows the effects of TG44 on the viability of H. pylori ATCC 43629 at 1/2 ×, 1 ×, 2 ×, 4 ×, and 8 × the MIC for up to 24 h. TG44 showed potent bactericidal activity at a concentration of 3.13 μg/ml for 1 to 24 h of treatment.

Figure 2b shows the effects of AMX on the viability of H. pylori ATCC 43629 at concentrations of 1/2 ×, 1 ×, 2 ×, 4 ×, and 8 × the MIC for up to 24 h of treatment. AMX had almost no bactericidal activity at 8 × the MIC for up to 6 h. At 24 h of treatment, when the number of cells in the control group
increased, AMX showed a slight inhibition of the viability of *H. pylori*.

Figure 3a shows the effects of TG44 on the viability of *H. pylori* ATCC 43504 at concentrations of 3.13 to 50 µg/ml for up to 1 h of treatment. TG44 showed potent bactericidal activity at 1 h of treatment. No visible microorganisms were detected after treatment with TG44 at 12.5 µg/ml or higher concentrations for 1 h of treatment.

Figure 3b shows the effects of AMX on the viability of *H. pylori* ATCC 43504 at concentrations of 3.13 to 50 µg/ml for up to 1 h of treatment. AMX had no bactericidal activity at a concentration of 50 µg/ml for 1 h of treatment.

Figure 4 shows the bactericidal activity of TG44 against *H. pylori* ATCC 43504 under various pH conditions. TG44 at 3.13 µg/ml showed bactericidal activity at pH 7 and 6 within 3 h of treatment. At pH 5, TG44 at 1.56 and 3.13 µg/ml also reduced the viable numbers of *H. pylori* cells at 24 and 6 h of treatment, respectively. At an acidic pH of 3, *H. pylori* survived for 2 h in the presence of urea. TG44 exerted a predominant bactericidal effect at a concentration of 0.78 µg/ml or higher for 2 h of treatment.

**DISCUSSION**

*H. pylori* is well recognized as a major etiologic factor for gastritis and peptic ulceration (14, 21) and has also been...
Implicated as a risk factor for gastric lymphoma and carcinoma (7, 24, 28). Considerable progress in the therapeutic modalities for \textit{H. pylori} infection has been achieved in recent years. However, the emergence of resistant bacteria has elicited a major clinical concern. The relevant resistance seems to be attributable to the types of antibiotics prescribed in eradication therapy, especially MNZ and CLR (16, 17, 23). Furthermore, the emergence of AMX-resistant strains has also been reported (4, 5, 6, 11, 19, 25). Hence, there are great medical needs for the eradication of \textit{H. pylori} by monotherapy if possible.

In the present study, in which the antibacterial activity of TG44 against 56 strains of \textit{H. pylori} (including 2 reference strains and 54 clinical isolates) was examined in comparison with those of AMX, CLR, and MNZ, TG44 exhibited equivalent antibacterial activities against both susceptible bacterial strains and highly resistant clinical isolates.

TG44 was found to have a high specificity because it clearly exhibited antibacterial activity against \textit{H. pylori}, a slight activity against \textit{H. mustelae}, and no activity against other bacterial species examined.

Transmission electron microscopy showed the detachment
of outer membranes of *H. pylori*, which might be the mechanism responsible for the rapid bactericidal activity of TG44, which is as short as 1 h of treatment. The mechanism of detachment is under investigation.

*H. pylori* is known to be transformed to the coccoid form after treatment with AMX or CLR. The coccoid form is hypersensitive to these antibiotics. In the present study, however, we observed no transformation of *H. pylori* to the coccoid form after treatment with TG44, implying its possible clinical relevance.

In conclusion, the present study revealed that (i) TG44 has equivalent antibacterial activities against both antibiotic-susceptible and -resistant strains of *H. pylori* and that (ii) TG44 exhibits bactericidal activity against *H. pylori* at a pH range of 3 to 7, confirming its high stability in the pH range as demonstrated in physicochemical studies (data not shown), in a short time of treatment. These facts suggest that TG44 is a promising chemotherapeutic agent which allows monotherapy against *H. pylori* infection, unlike conventional therapy which requires drug combinations and systemic circulation.
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