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Alpha-ketoglutarate oxidoreductase, an essential salvage enzyme of energy metabolism, in coccoid form of *Helicobacter pylori*

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ABSTRACT

In the Krebs cycle of *Helicobacter pylori*, the absence of alpha-ketoglutarate dehydrogenase and succinyl CoA synthetase are shown. Instead, alpha-ketoglutarate is converted to succinyl CoA and succinate by alpha-ketoglutarate oxidoreductase (KOR) and CoA transferase (CoAT). In the present study, when *H. pylori* transformed to the coccoid form, a viable but non-culturable form of *H. pylori* with reduced metabolic activity, the KOR activity was enhanced while the CoAT activity was reduced. Direct inactivation of KOR could potently kill the bacteria without allowing conversion to the coccoid form, suggesting a novel treatment strategy for the eradication of *H. pylori*, especially in cases infected with multiple antibiotic-resistant strains.

Helicobacter pylori (H. pylori), a gram-negative spiral bacterium, colonizes the mucosal layer of the gastric epithelium and is recognized as a major cause of gastritis and peptic ulcer disease in humans [1]. Eradication of *H. pylori* by drugs has been found to drastically reduce the recurrence rates of ulcers, and is therefore considered to be essential in the treatment of peptic ulcer disease. In recent years, however, the increase in the number of patients with failure of *H. pylori* eradication therapy has become a serious problem [2–4]. The most common cause of failure of *H. pylori* eradication therapy is the emergence of drug-resistant strains. Therefore, it is important to explore new and effective strategies for the Eradication of *H. pylori*. In this context, some researchers have shown the possible involvement of the coccoid form of the bacterium in the development of relapses following antimicrobial therapy [5,6]. Therefore, for successful therapy, it may be essential to not only to eliminate the spiral form, but also to rapidly suppress and/or destroy the coccoid forms of *H. pylori*.

H. pylori obtained from fresh cultures assumes the spiral form rods, however, under various environmental conditions, such as aerobiosis, extended incubation periods, and antibiotic treatment, the organisms are transformed to a coccoid form. Many studies have shown that the coccoid form is one of the stages of the putative *H. pylori* biological life cycle, because it retains cellular structures compatible with viability and polyphosphate energy reserves [7,8]. On the other hand, the DNA and RNA contents and ATP production in the coccoid form of *H. pylori* are lower than those in the spiral form of the bacterium [8–10]. The metabolic activity of the coccoid form, however, has not yet been fully characterized. Several studies have indicated that the coccoid form of *H. pylori* might be involved in the pathogenicity of this organism, including interleukin-8 induction, adhesion to MKN45 cells [11] and *ureA*, *cagA*, and *vacA* expression [12], and also in the transmission of this infection [13,14].

H. pylori is deficient in enzymes of the Krebs cycle such as alpha-ketoglutarate dehydrogenase, which converts alpha-ketoglutarate to succinyl CoA, and succinyl CoA synthetase, which converts succinyl CoA to succinate [15]. Instead, alpha-ketoglutarate is converted to succinyl CoA and succinate by alpha-ketoglutarate oxidoreductase (KOR) and CoA transferase (CoAT) [16,17]. Furthermore, it has been shown that alpha-ketoglutarate is directly oxidized to succinate by KOR [18]. However, the relationship between these two enzymes have not yet been clarified in detail.

The aim of the present study was to examine the changes in the activities of these enzymes involved in energy metabolism during conversion of *H. pylori* from the spiral to the coccoid form, and to develop a potent bactericidal strategy to prevent conversion to the coccoid form.

Abbreviations: CoAT, CoA transferase; KOR, alpha-ketoglutarate oxidoreductase; Tet, tetracycline hydrochloride; SEM, scanning electron microscopy.

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Materials and methods

Bacterial strains and culture conditions. H. pylori strain ATCC 43504 was used throughout this study. The strain was maintained at −80 °C in Brucella broth (Becton–Dickinson, Cockeysville, MD) containing 25% (vol/vol) glycerol. The bacterium was cultured on Columbia HP agar (Becton–Dickinson) for 2 days at 37 °C under microaerobic conditions maintained with AnaeroPack MicroAero (MITSUBISHI GAS, Tokyo Japan), and then harvested and inoculated into Brucella broth supplemented with 7% fetal bovine serum (FBS). Cultures were incubated at 37 °C under microaerobic conditions maintained with a AnaeroPack MicroAero (MITSUBISHI GAS).

Induction of the coccoid forms. Coccoid forms were induced by two different methods: (i) the bacteria were transferred after overnight culture into an aerobic atmosphere (incubation in air) and cultured for a further 3 days. (ii) 15 μg/mL tetracycline hydrochloride (Tet) (Sigma, St. Louis, MO, USA) was added to the Brucella broth after overnight culture, followed by cultivation for a further 4 days [19]. The spiral form was sedimented by centrifugation at 600*g* for 5 min, and the supernatant was centrifuged at 11,000*g* for 5 min; the resultant pellet contained the induced coccoid forms of *H. pylori*.

Scanning electron microscopy (SEM) of the coccoid forms. After removal of the residual spiral bacteria by centrifugation at 600*g* for 5 min, the induced coccoid forms were sedimented by centrifugation at 11,000*g* for 5 min. Samples were washed three times with PBS and fixed in 1% (vol/vol) glutaraldehyde in 60 mM Hepes overnight at 4 °C. The samples were then dehydrated for 10 min in a graded series of ethyl alcohol (50%, 70%, 80%, 90%, and 100% (vol/vol)) and isoamyl acetate, subjected to critical-point drying and gold–palladium coating, and then examined by scanning electron microscopy (SEM) (S-4000 model electron microscope; Hitachi, Tokyo, Japan).

Measurement of the enzymatic activities. After sonication (7-min cycles of a 5-s pulse at 25% power and a 3-s pause) of the bacteria, the resultant bacterial lysates were centrifuged and the enzymatic activities were measured in the supernatant as follows; CoAT activity was measured by monitoring the increase in absorbance at 310 nm, corresponding to the formation of acetoacetyl CoA. CoAT reaction was started by the addition to the supernatant of the bacterial lysate (10 μg protein) of 50 mM Tris–HCl (pH 9.1) containing 67 mM lithium acetoacetate (Sigma), 300 μM succinyl CoA (Sigma), and 15 mM $MgCl_2$ [16]. KOR activity was measured by monitoring the increase in absorbance at 550 nm, caused by the ferredoxin-dependent reduction of a horse heart cytochrome *c* in the presence of 2-oxoglutarate as the substrate. KOR reaction was started by the addition to the supernatant of the bacterial lysate (10 μg protein) of 100 mM phosphate buffer (pH 7.0) containing 50 μM horse heart cytochrome *c* (Sigma), 100 μM coenzyme A (Sigma), and 4 mM 2-oxoglutarate (Sigma) [20,21].

Inhibition of the enzyme activities. The activity of CoAT obtained from the pig heart was shown to be inhibited by NaI [22], and that of 2-ketoglutarate ferredoxin oxidoreductase of *Thermococcus litoralis* was reported to be inhibited by $NaNO_2$ [23]. NaI, an inhibitor of CoAT, and $NaNO_2$, an inhibitor of KOR of *H. pylori*, were added to the supernatant of the bacterial lysate, in a way as to ensure that these agents were present throughout the enzymatic activity assay period.

Bacterial cell counts. In order to examine the effects of NaI (CoAT inhibitor) and $NaNO_2$ (KOR inhibitor), the number of viable bacteria (cfu per milliliter) was counted as follows: after overnight culture, NaI or $NaNO_2$ was added to the culture and incubated for a further 12 h. The cultures were then suspended in sterile saline and 100-μL aliquots were plated onto Nissui plate *Helicobacter* Agar (Nissui, Tokyo, Japan). The plates were incubated at 37 °C under microaerobic conditions. After 4 days' incubation, the colonies were counted and the results were expressed as cfu per milliliter.

Bacterial growth, viability, and morphology. The effects of $NaNO_2$ (KOR inhibitor) and Tet on the bacterial growth, viability and morphology was examined. A bacterial culture was grown in Brucella broth at 37 °C under microaerobic conditions to an OD of 0.1 at 600 nm, and then 50 mM $NaNO_2$, 15 μg/mL Tet, or both, were added to the culture, followed by incubation for a further 4 days. The bacterial viability was measured by the 25× Alamar Blue dye (Biosource, Carlsbad, CA, USA) staining method [24,25], as follows: the bacterial cells were added to the Alamar Blue plate, and the plate was then incubated overnight at 37 °C. The absorbance of the plate was read at 570 and 595 nm. The relative absorbances of the samples were calculated as the percent absorbance relative to the absorbance of the non-treated control sample. The bacterial cultures were centrifuged at 11,000*g* for 5 min, and the resultant bacterial pellet was processed for and examined by SEM, as described above.

Results and discussion

Alteration of enzymatic activities of the Krebs cycle in *H. pylori* from the spiral to the coccoid form.

H. pylori, lacks alpha-ketoglutarate dehydrogenase, which converts alpha-ketoglutarate to succinyl CoA, and of succinyl CoA synthetase which converts succinyl CoA to succinate in the Krebs cycle [15]. Instead, CoAT catalyzes the conversion of succinyl CoA to succinate [16], and KOR catalyzes the conversion of alpha-ketoglutarate to succinyl CoA [17]. Furthermore, it has been shown that alpha-ketoglutarate is directly oxidized to succinate by KOR [18] (Fig. 1). *H. pylori* incubated overnight under standard culture conditions (37 °C, microaerobic atmosphere) assumed the spiral form, as observed by SEM (Fig. 2A). Morphological change from the spiral to the coccoid form occurred under aerobic incubation for 3 days (Fig. 2B) or exposure to 15 μg/mL Tet for 4 days (Fig. 2C). The coccid forms were obtained without residual spiral forms by centrifugation at 600*g* for 5 min (Fig. 2B and C). Reduction in the activity of CoAT was observed during morphological change of the bacterium from the spiral to the coccoid form (Fig. 2D). On the other hand, the KOR activity was increased by about 1.7-fold ($p < 0.01$) and 1.4-fold ($p < 0.05$), respectively, in the coccoid form induced by aerobiosis and exposure to 15 μg/mL Tet (Fig. 2E). Fig. 2F and G indicates

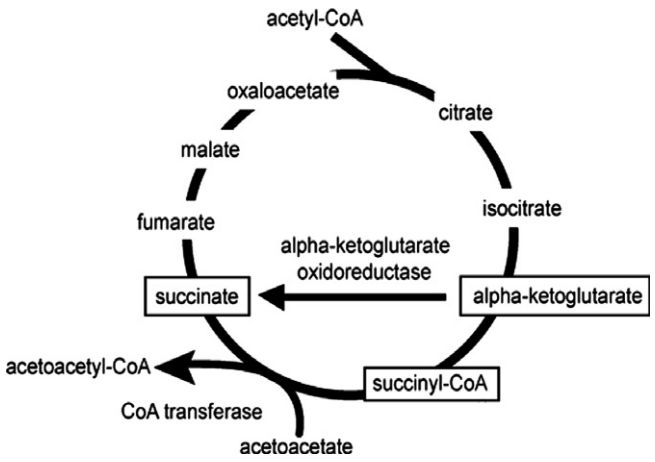

Fig. 1. The Krebs cycle of *H. pylori*. The existence of unusual enzymes, namely, KOR, that converts alpha-ketoglutarate to succinate, and CoAT, that converts succinyl CoA to succinate.

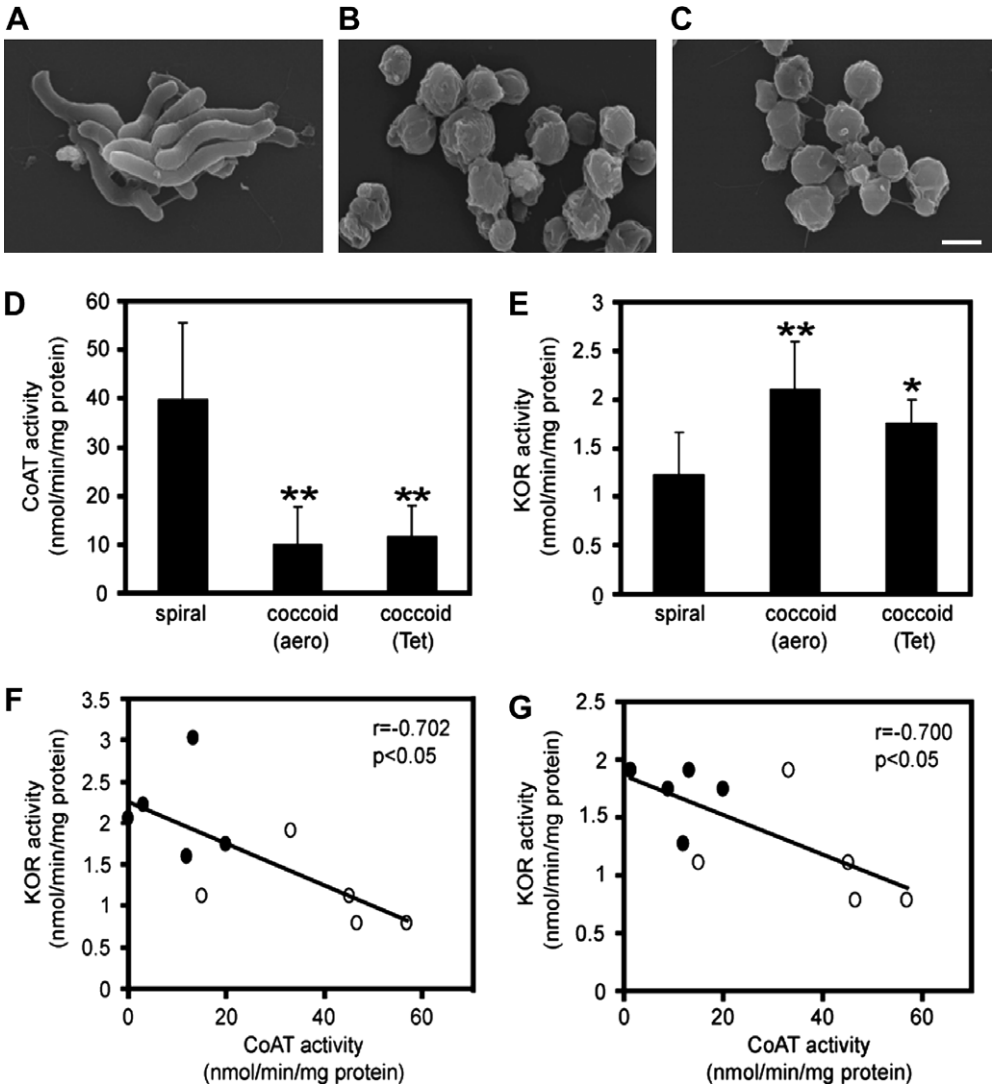

Fig. 2. Effect of morphological change of the bacterium on the activities of CoAT and KOR. Morphology of the spiral forms produced by overnight microaerobic culture (A), coccoid forms induced by 3 days' aerobic culture (B) and coccoid forms induced by 4 days' exposure to 15 μg/mL Tet (C) of *H. pylori* ATCC 43504. White scale bar represents 1 μm. The enzymatic activities were measured in a bacterial lysate (D,E). Spiral forms produced by overnight microaerobic culture. Coccoid forms induced by 3 days' aerobic culture (coccoid (aero)) or 4 days' exposure to 15 μg/mL Tet (coccoid (Tet)) (D,E). Results are means ± SD of three independent assays. Asterisks indicate statistical significance as determined by Student's *t* test ($^{*}p < 0.05$, $^{**}p < 0.01$). Relationship between the CoAT activity (nmol/min/mg protein) and KOR activity (nmol/min/mg protein). Spiral form produced by overnight microaerobic culture, clear circles; coccoid form induced by 3 days' aerobic culture (F) or 4 days' exposure to 15 μg/mL Tet (G), filled circles. Spearman's rank order correlation analysis revealed a significant linear inverse correlation between the CoAT activity (nmol/min/mg protein) and KOR activity (nmol/min/mg protein).

the relationship between the CoAT activity and the KOR activity; a significant linear inverse correlation was found between the CoAT activity and the KOR activity ($r = -0.702$, $p < 0.05$ (Fig. 2F) $r = -0.700$, $p < 0.05$ (Fig. 2G)). These results suggest that succinyl CoA was not required for the energy metabolism in the coccoid form of *H. pylori*. Additionally, since Pitson et al. reported that no formation of succinyl CoA was observed in the oxidative reaction of alpha-ketoglutarate to succinate [18], it was thought that *H. pylori* could survive in the coccoid form because of the KOR activity which directly oxidize of alpha-ketoglutarate to succinate.

Effect of NaI on the CoAT activity and bacterial growth

In this study, exposure to 10 mM NaI inhibited the CoAT activity of *H. pylori* by 41% (Fig. 3A). Although CoAT activity was inhibited by applying NaI to exponentially growing *H. pylori* cell cultures, the colony-forming ability (cfu per milliliter) of the organisms was not influenced by this procedure (Fig. 3B). As shown in Fig. 3C and D, while the CoAT activity was inhibited during the growth of the spiral form, the KOR activity increased by 2.3-fold ($p < 0.01$). Therefore, KOR was thought to be involved not only the viability of the coccoid form, but also in energy metabolism in the spiral form of *H. pylori*. The catalytic pathway involving KOR which directs oxidation of alpha-ketoglutarate to succinate as a salvage pathway is an essential pathway of energy metabolism in *H. pylori* when the CoAT activity is suppressed.

The existence of an alternative pathway for the conversion of alpha-ketoglutarate to succinate has been reported in *Mycobacterium tuberculosis*, which is dependent for its viability on alpha-ketoglutarate decarboxylase (kgd) [26]. Since this kgd is lacking in humans, its activity in *M. tuberculosis* could be a potential therapeutic target. In a similar manner, as KOR is also lacking in humans, its activity in *H. pylori* could be a potential target in cases with *H. pylori* infection.

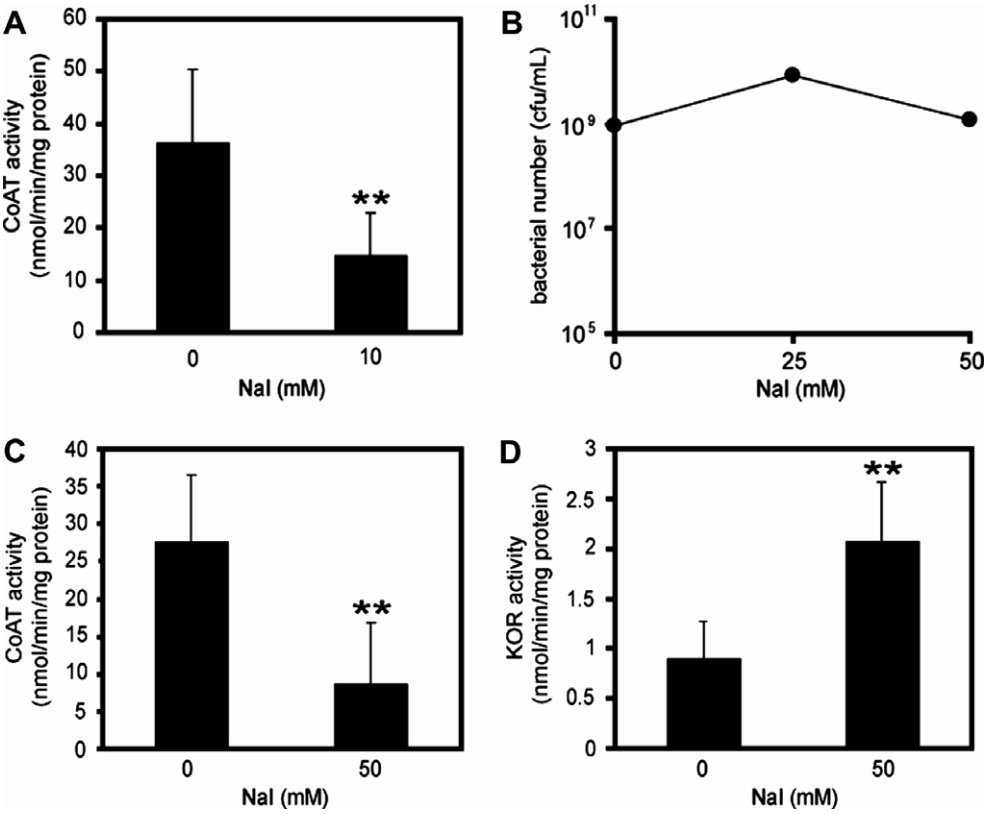

Fig. 3. Effect of NaI on the CoAT and KOR activity and growth of *H. pylori*. CoAT activity was measured in a cell lysate in the presence or absence (control) of 10 mM NaI (A). *H. pylori* was cultured in the presence of NaI for 12 h, and the number of viable bacteria (cfu per milliliter) was counted (B). The enzymatic activities were measured in a cell lysate of spiral form which was cultured in the presence or absence (control) of 50 mM NaI for 12 h (C,D). Results are means ± SD of three independent assays. Asterisks indicate statistical significance as determined by Student's *t* test ($^{**}p < 0.01$).

Effect of $NaNO_2$ on the KOR activity and bacterial viability

The KOR activity of *H. pylori* was inhibited in a dose-dependent manner by the $NaNO_2$ concentration (Fig. 4A). Along with the inhibition of the KOR activity by the addition of $NaNO_2$ to an exponentially growing culture of *H. pylori*, the colony-forming ability (cfu per milliliter) of the organisms was also reduced in a dose-dependent fashion, which was different from the case for inactivation of CoAT by the addition of NaI (Fig. 3B). Although a residual bacterial cell viability of 15% following the Tet treatment was kept, a significant decrease of the cell viability to less than 5% was shown following treatment with $NaNO_2$ ($p < 0.05$) (Fig. 4C). Complete conversion of *H. pylori* to the coccoid form was observed following incubation with Tet for 96 h (Fig. 4D). On the other hand, following incubation with 50 mM $NaNO_2$ for 96 h, most of the bacteria were transformed to the degraded coccoid form, while a few intact coccoid forms remained (Fig. 4E). After 96-h incubation with both 15 μg/mL Tet and 50 mM $NaNO_2$, most organisms exhibited the degraded coccoid form, while some exhibited a shrunken form (a small subset maintained the spherical form) (Fig. 4F).

Mizoguchi et al. is reported that the coccoid forms of *H. pylori* obtained from a 7-day aerobic culture exhibited a degraded forms and roughed surfaces as compared with the coccoid forms obtained from a 3-day aerobic culture [27]. From these findings, we concluded that there are at least two different coccoid forms of *H. pylori*, one that exists in a viable dormant stage, that is, the coccoid form induced in the 3-day aerobic culture, and the other that exists under the condition of impending cell death, that is, the degraded coccoid form induced in the 7-day aerobic culture. Our present study results indicate that the inactivation of KOR by $NaNO_2$ also resulted in the accumulation of the degraded coccoid form, destined for cell death.

Recently, some studies have reported that the coccoid forms of *H. pylori* might be involved in relapses of *H. pylori* infection following antimicrobial therapy, and that to ensure complete eradication, it may be essential to not only eliminate the spiral form, but also to rapidly suppress the coccoid form of *H. pylori* [5,6]. The results of our present study indicated that inactivation of KOR activity produced a potent bactericidal effect against *H. pylori*, without induction of the coccoid form. Furthermore, antimicrobial agents, such as amoxicillin, kanamycin and Tet, have been shown to induce the conversion to the coccoid form [5,19,28]. In this study, after treatment with both Tet and $NaNO_2$, most of the micro-organisms exhibited a degraded coccoid form, and a minor proportion showed a shrunken form (Fig. 4F). Therefore, $NaNO_2$ induced degradation of the coccoid forms more strongly when it was administered in combination with the antimicrobial agent, which could induce conversion to the coccoid form.

In conclusion, KOR is an essential survival enzyme for energy metabolism in the coccoid form of *H. pylori*, and inactivation of the KOR activity exerted a potent bactericidal action against *H. pylori* by preventing induction of the coccoid form.

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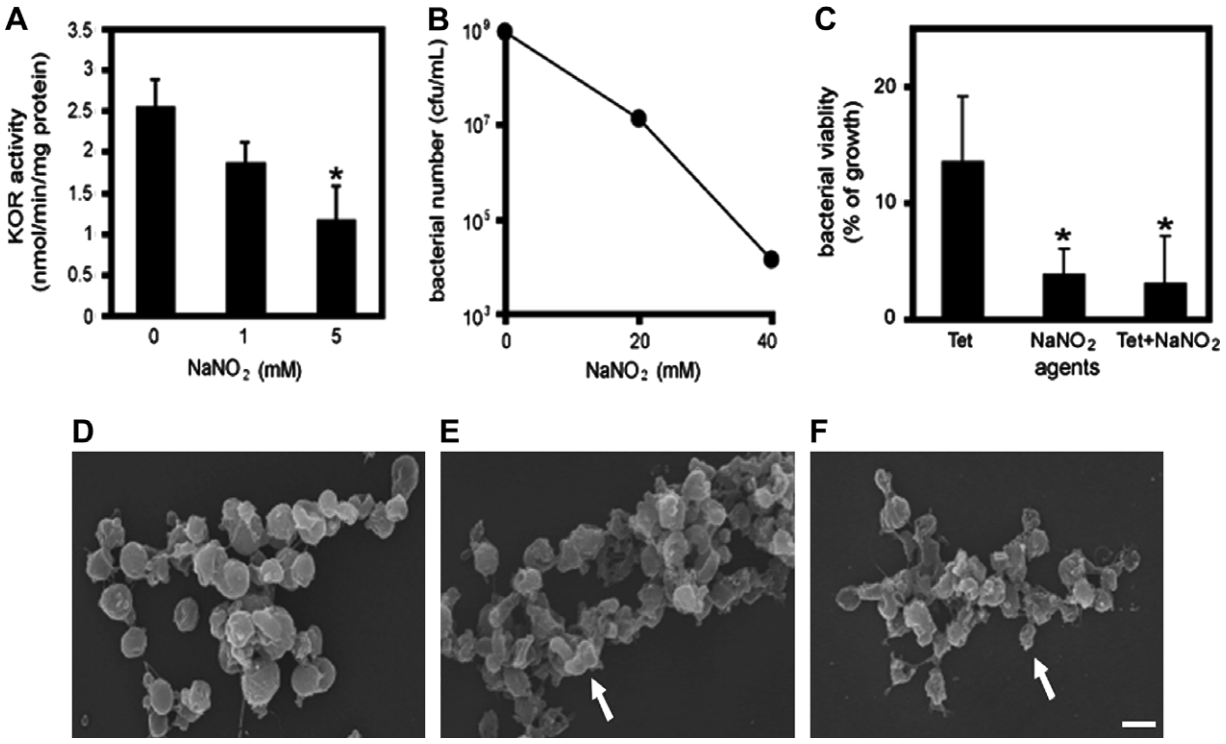

Fig. 4. Cell viability and SEM observation of the morphology of *H. pylori* treated with Tet and $NaNO_2$. KOR activity was measured in a cell lysate in the presence or absence (control) of 1 or 5 mM $NaNO_2$ (A). *H. pylori* was cultured in the presence of $NaNO_2$ for 12 h, and the number of viable bacteria (cfu per milliliter) was counted (B). Results are means ± SD of three independent assays. Cell viabilities following exposure to Tet, $NaNO_2$ and combined exposure to both were determined by an assay method using Alamar Blue staining (C). Results are means ± SD of two independent assays. Morphology of the coccoid form induced by 96-h exposure to Tet (D), the coccoid form + degraded forms (arrow) induced by 96-h exposure to $NaNO_2$ (E). In the culture exposed to both Tet and $NaNO_2$, most organisms exhibited the degraded coccoid form, with some exhibiting a shrunken form (arrow) (a small subset maintained their spherical form) (F). White scale bar presents 1 μm. Asterisks indicate statistical significance as determined by Student's *t* test ($^{*}p < 0.05$).

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References

[1] H. Suzuki, T. Hibi, B.J. Marshall, *Helicobacter pylori*: present status and future prospects in Japan, J. Gastroenterol. 42 (2007) 1–15.
[2] T. Shimoyama, S. Fukuda, T. Mikami, M. Fukushi, A. Munakata, Efficacy of metronidazole for the treatment of clarithromycin-resistant *Helicobacter pylori* infection in a Japanese population, J. Gastroenterol. 39 (2004) 927–930.
[3] F. Kaneko, H. Suzuki, N. Hasegawa, K. Kurabayshi, H. Saito, S. Otani, H. Nakamizo, K. Kawata, M. Miyairi, K. Ishii, H. Ishii, High prevalence rate of *Helicobacter pylori* resistance to clarithromycin during long-term multiple antibiotic therapy for chronic respiratory disease caused by non-tuberculous mycobacteria, Aliment. Pharmacol. Ther. 20 (Suppl. 1) (2004) 62–67.
[4] T. Matsuhisa, T. Kawai, T. Masaoka, H. Suzuki, M. Ito, Y. Kawamura, K. Tokunaga, M. Suzuki, T. Mine, S. Takahashi, N. Sakaki, Efficacy of metronidazole as second-line drug for the treatment of *Helicobacter pylori* infection in the Japanese population: a multicenter study in the Tokyo metropolitan area, Helicobacter 11 (2006) 152–158.
[5] V. Berry, K. Jennings, G. Woodnutt, Bactericidal and morphological effects of amoxicillin on *Helicobacter pylori*, Antimicrob. Agents Chemother. 39 (1995) 1859–1861.
[6] M.I. Brenciaglia, A.M. Fornara, M.M. Scaltrito, F. Dubini, *Helicobacter pylori*: cultivability and antibiotic susceptibility of coccoid forms, Int. J. Antimicrob. Agents 13 (2000) 237–241.
[7] M. Benaissa, P. Babin, N. Quellard, L. Pezennec, Y. Cenatiempo, J.L. Fauchere, Changes in *Helicobacter pylori* ultrastructure and antigens during conversion from the bacillary to the coccoid form, Infect. Immun. 64 (1996) 2331–2335.
[8] M. Sorberg, M. Nilsson, H. Hanberger, L.E. Nilsson, Morphologic conversion of *Helicobacter pylori* from bacillary to coccoid form, Eur. J. Clin. Microbiol. Infect. Dis. 15 (1996) 216–219.
[9] S. Narikawa, S. Kawai, H. Aoshima, O. Kawamata, R. Kawaguchi, K. Hikiji, M. Kato, S. Iino, Y. Mizushima, Comparison of the nucleic acids of helical and coccoid forms of *Helicobacter pylori*, Clin. Diagn. Lab. Immunol. 4 (1997) 285–290.
[10] H.O. Nilsson, J. Blom, W. Abu-Al-Soud, A.A. Ljungh, L.P. Andersen, T. Wadstrom, Effect of cold starvation, acid stress, and nutrients on metabolic activity of *Helicobacter pylori*, Appl. Environ. Microbiol. 68 (2002) 11–19.
[11] T. Osaki, H. Yamaguchi, H. Taguchi, M. Fukada, H. Kawakami, H. Hirano, S. Kamiya, Interleukin-8 induction and adhesion of the coccoid form of *Helicobacter pylori*, J. Med. Microbiol. 51 (2002) 295–299.
[12] F. Sisto, M.I. Brenciaglia, M.M. Scaltrito, F. Dubini, *Helicobacter pylori*: *ureA*, *cagA* and *vacA* expression during conversion to the coccoid form, Int. J. Antimicrob. Agents 15 (2000) 277–282.
[13] J. Parsonnet, H. Shmuely, T. Haggerty, Fecal and oral shedding of *Helicobacter pylori* from healthy infected adults, JAMA 282 (1999) 2240–2245.
[14] J.G. Fox, B.J. Paster, F.E. Dewhirst, N.S. Taylor, L.L. Yan, P.J. Macuch, L.M. Chmura, *Helicobacter mustelae* isolation from feces of ferrets: evidence to support fecal-oral transmission of a gastric *Helicobacter*, Infect. Immun. 60 (1992) 606–611.
[15] B. Kather, K. Stingl, M.E. Rest, K. Altendorf, D. Molenaar, Another unusual type of citric acid cycle enzyme in *Helicobacter pylori*: the malate:quinone oxidoreductase, J. Bacteriol. 182 (2000) 3204–3209.
[16] I.E. Corthesy-Theulaz, G.E. Bergonzelli, H. Henry, D. Bachmann, D.F. Schorderet, A.L. Blum, L.N. Ornston, Cloning and characterization of *Helicobacter pylori* succinyl CoA:acetoacetate CoA-transferase, a novel prokaryotic member of the CoA-transferase family, J. Biol. Chem. 272 (1997) 25659–25667.
[17] N.J. Hughes, C.L. Clayton, P.A. Chalk, D.J. Kelly, *Helicobacter pylori porCDAB* and *oorDABC* genes encode distinct pyruvate:flavodoxin and 2-oxoglutarate:acceptor oxidoreductases which mediate electron transport to NADP, J. Bacteriol. 180 (1998) 1119–1128.
[18] S.M. Pitson, G.L. Mendz, S. Srinivasan, S.L. Hazell, The tricarboxylic acid cycle of *Helicobacter pylori*, Eur. J. Biochem. 260 (1999) 258–267.
[19] J.G. Kusters, M.M. Gerrits, J.A. Van Strijp, C.M. Vandenbroucke-Grauls, Coccoid forms of *Helicobacter pylori* are the morphologic manifestation of cell death, Infect. Immun. 65 (1997) 3672–3679.
[20] T. Iwasaki, T. Oshima, Ferredoxin and related enzymes from *Sulfolobus*, Methods Enzymol. 334 (2001) 3–22.
[21] Y.J. Park, C.B. Yoo, S.Y. Choi, H.B. Lee, Purifications and characterizations of a ferredoxin and its related 2-oxoacid:ferredoxin oxidoreductase from the hyperthermophilic archaeon, *Sulfolobus solfataricus* P1, J. Biochem. Mol. Biol. 39 (2006) 46–54.
[22] L.B. Hersh, W.P. Jencks, Coenzyme A transferase. Kinetics and exchange reactions, J. Biol. Chem. 242 (1967) 3468–3480.
[23] X. Mai, M.W. Adams, Characterization of a fourth type of 2-keto acid-oxidizing enzyme from a hyperthermophilic archaeon: 2-ketoglutarate ferredoxin oxidoreductase from *Thermococcus litoralis*, J. Bacteriol. 178 (1996) 5890–5896.

[24] G.X. Wei, A.N. Campagna, L.A. Bobek, Effect of MUC7 peptides on the growth of bacteria and on *Streptococcus mutans* biofilm, J. Antimicrob. Chemother. 57 (2006) 1100–1109.
[25] R.K. Pettit, C.A. Weber, M.J. Kean, H. Hoffmann, G.R. Pettit, R. Tan, K.S. Franks, M.L. Horton, Microplate Alamar blue assay for *Staphylococcus epidermidis* biofilm susceptibility testing, Antimicrob. Agents Chemother. 49 (2005) 2612–2617.
[26] J. Tian, R. Bryk, M. Itoh, M. Suematsu, C. Nathan, Variant tricarboxylic acid cycle in *Mycobacterium tuberculosis*: identification of alpha-ketoglutarate decarboxylase, Proc. Natl. Acad. Sci. USA 102 (2005) 10670–10675.
[27] H. Mizoguchi, T. Fujioka, K. Kishi, A. Nishizono, R. Kodama, M. Nasu, Diversity in protein synthesis and viability of *Helicobacter pylori* coccoid forms in response to various stimuli, Infect. Immun. 66 (1998) 5555–5560.
[28] G. Bode, F. Mauch, P. Malfertheiner, The coccoid forms of *Helicobacter pylori*. Criteria for their viability, Epidemiol. Infect. 111 (1993) 483–490.