Prominent role of γ-glutamyl-transpeptidase on the growth of Helicobacter pylori

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Abstract

AIM: γ-glutamyl transpeptidase (GGT) has been reported as a virulence and colonizing factor of Helicobacter pylori (H pylori). This study examined the effect of GGT on the growth of H pylori.

METHODS: Standard H pylori strain NCTC 11637 and 4 clinical isolates with different levels of GGT activity as measured by an enzymatic assay were used in this study. Growth inhibition and stimulation studies were carried out by culturing H pylori in brain heart infusion broth supplemented with specific GGT inhibitor (L-serine sodium borate complex, SBC) or enhancer (glutathione together with glycyl-glycine), respectively. The growth profiles of H pylori were determined based on viable bacterial count at time interval.

RESULTS: Growth was more profuse for H pylori isolates with higher GGT activity than those present with lower GGT activity. However, in the presence of SBC, growth of H pylori was retarded in a dose dependent manner (P = 0.034). In contrast, higher growth rate was observed when GGT activity was enhanced in the presence of glutathione and glycyl-glycine.

CONCLUSION: Higher GGT activity provides an advantage to the growth of H pylori in vitro. Inhibition of GGT activity by SBC resulted in growth retardation. The study shows that GGT plays an important role on the growth of H pylori.

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INTRODUCTION

Helicobacter pylori (H pylori) is a gram-negative spiral bacterium that causes chronic infection of the human stomach in adults[1-3] as well as children[4]. The chronic H pylori infection may lead to peptic ulceration[5,6] and may be a potential risk factor for gastric carcinoma[7-9]. The exact mechanism on how H pylori promotes gastric neoplasia is not known but is hypothesized to have occurred through the production of reactive oxygen species leading to oxidative stress and DNA damage in gastric epithelial cells[10-12].

The level of reduced form of the tripeptide thiol, glutathione (GSH), one of the major endogenous defense mechanisms against oxidative stress, was shown to be decreased in gastric mucosal after H pylori infection[13,14]. Furthermore, it has been well established that GGT plays a major role in glutathione metabolism. This enzyme catalyses transpeptidation reaction in which a γ-glutamyl moiety is transferred from γ-glutamyl compounds, such as glutathione, a non-protein sulphydryl molecule, to amino acids. In addition, GGT can use γ-glutamyl peptides as substrates in the reciprocal hydrolysis reaction, thus playing a role in the synthesis of glutathione[15-19].

It has been reported that GGT activity could be inhibited by the presence of inhibitors like L-serine sodium borate complex (SBC)[20] and acivicin[21]. Although acivicin is a more effective GGT inhibitor, it is nonspecific and inhibits a number of glutamine amino-transferase[21]. In contrast, SBC is a highly specific GGT inhibitor but substantially higher concentration of SBC as compared to acivicin is needed for an effective inhibition of GGT activity[20].

GGT being a constitutive enzyme of H pylori was shown to participate in the colonization of H pylori in Swiss specific pathogen-free mice[22]. However, a later study using a different animal model demonstrated that GGT was not essential for colonization but acted as a virulence factor[23]. The present study examined the effect of GGT on H pylori growth in vitro in the presence of GGT inhibitor as well as enhancer.

MATERIALS AND METHODS

Bacterial strains and culture conditions

A standard H pylori strain NCTC 11 637 and 4 clinical isolates with different levels of GGT activity were used in this study. Strains 712 and 1 018 showed high GGT activity (>1 U/mg protein) while strains 1 082 and 888 had low GGT activity (<0.4 U/mg protein).

H pylori was grown for 3 d at 37 °C on chocolate blood agar containing 40 g/L blood agar base No. 2 (Oxoid) and 50 mL/L horse blood (Gibco) in a humidified incubator (Forma Scientific) supplied with 50 mL/L CO2[24]. The bacterial cells were harvested and washed with PBS buffer (pH 7.4) to give a suspension of ca. 5×107 CFU/mL (A600 = 0.2) in either PBS or brain heart infusion (BHI, Gibco) broth.

Inhibitory effect of SBC on H pylori GGT activity

A bacterial population of 3 d old 107 CFU/mL H pylori NCTC 11 637 was incubated in BHI broth containing various concentrations (2-10 mmol/L) of SBC (Sigma) at 37 °C for 30 min. GGT activity of H pylori cells was then measured by an enzymatic assay as described by Meister et al.[25].

Growth inhibition and stimulation studies

H pylori NCTC 11 637 was suspended in fresh BHI broth at a final cell concentration of approximately 5×106 CFU per ml. For growth inhibition study, appropriate volumes of filter-sterilized (pore size, 0.2 µm; Nalgene sterile syringe filter) SBC stock solution (100 mmol/L) were added to sterile BHI broth to provide final concentrations of SBC in BHI in the range of 2-10 mmol/L. For growth stimulation study, filter-sterilized glycyl-glycine
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(Sigma) and GSH (Sigma) were added to BHI broth at a final concentration of 1 mmol/L and 0.1 mmol/L, respectively. Growth inhibition and stimulation curves were constructed based on viable bacterial count at different time interval.

**Growth of various H pylori strains that expressed different levels of GGT activity**

Two strains each with high GGT activity (strains 1 018 and 712) and low GGT activity (strains 1 082 and 888) were grown in BHI broth at 37 °C over a period of 3 wk. The bacterial populations of the various strains were enumerated at time interval.

**Statistical analysis**

Data was analyzed using one-way ANOVA test (SPSS). A value of \( P \leq 0.05 \) was considered statistically significant.

**RESULTS**

**SBC inhibits GGT activity of H pylori**

Figure 1 shows that H pylori strain NCTC 11 637 GGT activity was inhibited in a dose dependent manner upon exposure to a range of SBC concentrations (2-10 mmol/L) for 30 min at 37 °C, where >99% GGT activity was inhibited by ≥4 mmol/L SBC. The maximum inhibitory effect of 96% of GGT activity was achieved at the concentration of 10 mmol/L SBC.

**Effect of GGT on H pylori growth**

Since SBC has inhibitory effect on H pylori GGT activity, H pylori NCTC 11 637 was cultured in BHI broth supplemented with different concentrations of SBC (2-10 mmol/L). Figure 2 shows that the H pylori cultured in the presence of various concentrations of SBC (2-10 mmol/L) over 3 wk displayed marked inhibition on the growth of H pylori in a dose dependent manner (ANOVA, \( P = 0.034 \), \( \Delta \geq 99\% \) decrease in viability of the bacterial population was observed within 72 h after culturing H pylori in the presence of 10 mmol/L SBC. In contrast, H pylori proliferated at twice the normal growth rate in the presence of GSH and glycyl-glycine with increased GGT activity as compared to the control. And this acceleration in growth lasted over a period of more than two weeks. Similar growth profile was observed with another H pylori strain SS1 (data not shown).

**Growth of different H pylori strains**

Growth of the four H pylori strains with disparate GGT activities was followed over a period of 3 wk. Figure 3 shows that H pylori isolates with higher GGT activity (strains 1 018 and 712) grew better and more abundant than those with lower GGT activity (strains 1 082 and 888) by 10-100 folds depending on the age of culture. However, all 4 cultures showed similar growth rate at 3 wk-old.

**DISCUSSION**

Inhibition of GGT activity by SBC was caused by competition with respect to γ-glutamyl substrate, and it was suggested that a serine-borate complex is formed which may bind to the active site of the enzyme by interacting with a carbohydrate residue of the enzyme [20]. Although acivicin is 20 times more effective in inhibiting GGT activity as compared to SBC (data not shown), it was not used for the growth inhibition study because it is a non-specific GGT inhibitor [21]. In this study, more than 90% of GGT activity was suppressed by 4 mmol/L SBC. Inhibition of...
GGT activity by SBC resulted in retarding the growth of *H. pylori* in a dose dependent manner indicating that GGT is an essential enzyme in the growth of *H. pylori*. In contrast, when physiological concentration of GSH and glycyl-glycine[26,27] were supplemented into the culture medium, growth of *H. pylori* was accelerated by more than two folds. The results show that the growth of *H. pylori* was enhanced under GGT stimulation condition demonstrating the possibility of such event in *in vivo* situation.

In this study, it is interesting to note that *H. pylori* with higher GGT activity (>1 U/mg protein) grows better than those with lower GGT activity (<0.4 U/mg protein). However, in the presence of SBC, a specific GGT inhibitor, the growth of *H. pylori* was shown to be retarded. This finding that GGT is vital for the growth of *H. pylori* could possibly explain why there was a reduction in the recovery of GGT isogenic mutant in the postinfected mice in the *in vivo* study carried out by McGovern et al.[23], while no ggt mutant *H. pylori* were recovered in the animal model of Chevalier et al.[22]. The explanation supports the role of GGT activity in the ability of *H. pylori* to proliferate in *in vivo* condition. However, the variation could also be due to the difference in animal model as suggested by McGovern et al.[23], or owing to strain difference in terms of the level of GGT activity expressed as demonstrated in this study.

It has been reported that GGT plays a significant role in *H. pylori*-mediated apoptosis[23]. It was therefore suggested that *H. pylori* induces apoptosis by GGT and that the bacteria gain essential nutrients from the apoptotic cells, thus to play on the growth of *H. pylori*.

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