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*Basic Study*

**Mechanistic research: Selenium regulates virulence factors, reducing adhesion ability and inflammatory damage of *Helicobacter pylori***

Qin C *et al.* Selenium regulates virulence factors of *H. pylori*

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**Abstract**

**BACKGROUND**

The pathogenicity of *Helicobacter pylori* (*H. pylori*) is dependent on factors including the environment and the host. Although selenium is closely related to pathogenicity as an environmental factor, the specific correlation between them remains unclear.

**AIM**

To investigate how selenium acts on virulence factors and reduces their toxicity.

**METHODS**

*H. pylori* strains were induced by sodium selenite, The expression of cytotoxin-associated protein A (*CagA*) and vacuolating cytotoxin gene A (*VacA*) was determined by RT-qPCR and Western blotting, Transcriptomisc was used to analyze *CagA*, *CagM*, *CagE*, *Cag1*, *Cag3*, and *CagT*; C57BL/6A mice were infected with the attenuated strains subjected to sodium selenite induction; and *H. pylori* colonization, inflammatory reactions, and the cell adhesion ability of *H. pylori* were assessed.

## RESULTS

*CagA* and *VacA* expression was upregulated at first and then downregulated in the *H. pylori* strains after sodium selenite treatment; their expression was significantly and steadily downregulated after the 5<sup>th</sup> cycle (10 d). Transcriptome analysis revealed that sodium selenite altered the levels affect *H. pylori* virulence factors, such as *CagA*, *CagM*, *CagE*, *Cag1*, *Cag3*, and *CagT*. Of these factors, *CagM* and *CagE* expression was continuously downregulated and was further downregulated after 2 h of induction with sodium selenite. Moreover, *CagT* expression was upregulated before the 3<sup>rd</sup> cycle (6 d) and downregulated significantly after the 5<sup>th</sup> cycle. *Cag1* and *Cag3* expression was upregulated and downregulated, respectively, but no significant change was observed by the 5<sup>th</sup> cycle. C57BL/6A mice were infected with the attenuated strains subjected to sodium selenite induction; the extent of *H. pylori* colonization in the stomach increased. However, sodium selenite also induced a mild inflammatory reaction in the gastric mucosa of *H. pylori*-infected mice, and the cell adhesion ability of *H. pylori* was significantly weakened.

## CONCLUSION

These results demonstrated that *H. pylori* displayed virulence attenuation after the 10<sup>th</sup> day of sodium selenite treatment. Sodium selenite is a low toxicity compound with strong stability that can reduce the cell adhesion ability of *H. pylori*, thus mitigating the inflammatory damage to the gastric mucosa.

**Key Words:** *Helicobacter pylori*; Sodium selenite; Virulence factors; Adherence; Inflammation

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**Core Tip:** The situation caused by *Helicobacter pylori* (*H. pylori*) drug resistance is critical. Selenium is one of the trace elements in the human body, and its content in the stomach is related to the degree of *H. pylori* infection. Initial studies found that sodium selenite had an inhibitory effect on *H. pylori*, so we further investigated the mechanism of action of sodium selenite on *H. pylori*. This study provides an experimental basis for use of the trace element selenium in the treatment of *H. pylori* infection.

## INTRODUCTION

<sup>2</sup> *Helicobacter pylori* (*H. pylori*) is a common gram-negative bacterium that colonizes the stomach. Half of the global population is infected with this bacterium, which causes gastritis, stomach ulcers, and other diseases. In 1994, <sup>4</sup> the World Health Organization listed *H. pylori* as a Class I carcinogen. In 2022, the United States listed this bacterium on the list of microorganisms associated with cancer<sup>[1,2]</sup>.

The control and treatment of *H. pylori* infections can reduce gastric cancer incidence. However, because of the extensive use of antibiotics against *H. pylori*, the drug resistance rate has increased, and the eradication rate has significantly decreased<sup>[3]</sup>. Therefore, new antibacterial drugs or prevention and control programs need to be developed to alleviate the public health threat posed by *H. pylori*. Imitating live vaccines that lower *H. pylori* toxicity and reduce pathogenicity serves as better control methods.

*CagA* and *VacA*, the main *H. pylori* virulence factors, are associated with inflammation, apoptosis, autophagy, and the epithelial-mesenchymal transformation<sup>[4]</sup>. *CagA*, a bacterial oncoprotein<sup>[5]</sup>, and *VacA*, which promotes the survival of *H. pylori* in gastric epithelial cells, are associated with phenomena such as mitochondrial damage<sup>[4]</sup>. The *cag* pathogenicity island (CagPAI) contains secretory protein-encoding *CagA* genes and type IV secretion system (T4SS) genes, including *CagE*, *CagM*, *Cag3*, *CagT*, *CagX*,

and *CagY*. These genes are involved in the T4SS activity<sup>[6]</sup>. However, the mutual regulation of these genes is not well understood.

The incidence of *H. pylori* infection is 10%-15%, and most of the population is infected with *H. pylori* but does not develop disease. However, the reason for this remains unclear. The mechanism underlying the interaction between *H. pylori* and the environment and body needs to be further explored. Selenium (Se), a trace element in the human body<sup>[7,8]</sup>, plays a major role in immunoregulation, antioxidation, and antitumor and antibacterial functions<sup>[9-12]</sup>. As an environmental factor in the stomach, Se is closely associated with *H. pylori* pathogenesis. Ustündağ *et al*<sup>[13]</sup> found that the stomach Se level was higher in the early stage of *H. pylori* infection but significantly decreased when *H. pylori* induced precancerous lesions. Burguera *et al*<sup>[14]</sup> found that the stomach Se content was significantly lower in patients with gastric ulcers and gastric cancer than in those with gastritis. Liu<sup>[15]</sup> found that sodium selenite can promote gastric ulcer healing<sup>[16,17]</sup>, but why Se content in the patient's stomach decreases and how Se promotes ulcer healing remain unknown. Therefore, in this study, we explored how the Se-rich environment acts on *H. pylori* virulence factors by inducing *H. pylori* with sodium selenite and investigated the interaction between Se and *H. pylori* in light of the toxicity and inflammatory injury mediated by strains in the stomach. These findings provide an experimental basis for Se application for the prevention and treatment of *H. pylori* infection-related diseases.

## **MATERIALS AND METHODS**

### ***The cells and bacteria***

The human gastric mucosal epithelial cell line GES-1 (Guangzhou Cellcook Biotech Co., Ltd., article number: CC4026) and human gastric cancer cell line BGC823 (Nanjing Kaijian Biotech Co., Ltd.) were purchased. *H. pylori* strains (159, 26695, G27, and NSH57) were all provided by Professor Bi Hongkai, Laboratory of Pathogen Biology, Nanjing Medical University.

### ***Induction and culture of *H. pylori* cells with sodium selenite***

Hp G27 and NSH57 bacterial solutions were prepared at an initial concentration of  $1 \times 10^5$  CFU/mL. The sodium selenite concentration for induction was determined according to the MIC results, and the induction time was determined according to the growth curve. Sequential induction was performed using culture medium containing the same sodium selenite concentration for continuous passages.

### ***RT-qPCRs targeting virulence factors in *H. pylori* strains to determine mRNA expression after sodium selenite induction***

RNA was extracted using a FastPure Cell/Tissue Total RNA Isolation Kit V2 (Vazyme Biotech Co., Ltd, item number: RC112-01). cDNA was obtained through RNA reverse transcription by using the dsDNase-containing reverse transcription premix (Monad Biotech Co., Ltd, item number: MR05101M). MonAmp SYBR Green qPCR Mix (Monad Biotech Co., Ltd, MQ10301S) was used to configure an RT-qPCR system, and Light Cycler 96 was used for amplification, with the procedures adjusted as follows: An initial denaturation was performed at 95°C for 30 s, denaturation at 95°C for 10 s, annealing at 60°C for 10 s, and extension at 72°C for 30 s; all of these steps were performed for 40 cycles. The corresponding calculations were made according to the equation " $2^{-\Delta CT(\text{test}) - \Delta CT(\text{calibrator})} = \text{relative expression}$ ". The mRNA expression of *CagA*, *VacA*, 16s RNA, IL-6, IL-8, TNF- $\alpha$ , glyceraldehyde-3-phosphate dehydrogenase (GAPDH), *Cag1*, *Cag3*, *CagT*, *CagM*, and *CagE* was determined. Table 1 lists the design and synthesis of the primers.

### ***Western blotting of *H. pylori* virulence factor proteins after sodium selenite induction***

In total, 3-7 mL *H. pylori* bacterial solution was centrifuged at 12000 rpm for 2 min. The supernatant was removed and dosed with approximately 130 mL RIPA lysate (Shanghai Beyotime Biotech Co., Ltd., article number: P0013B). Then, a protease inhibitor and 0.1% EDTA were added at a 100: 1 ratio. Thereafter, the solution was mixed on ice for cracking and ultrasonicated for approximately 5 min until the solution became clear. The solution was centrifuged at 13000 rpm for 5 min (4°C) to obtain the

supernatant protein solution. The protein concentration of the obtained solution was determined and the sample was divided to ensure 100 mg per tube; then, the samples were mixed with SDS-PAGE loading buffer, denatured in boiling water at 100°C for 5–10 min, and stored at –80°C.

A PAGE gel rapid preparation kit (10%) (Shanghai Epizyme Biomedicine Technology Co., Ltd., item number: PG112) was used for colloid preparation. Electrophoresis was performed using the loading protein and marker at 80 V for 30 min and 120 V for 1 h, respectively; the transmembrane was help at a constant flow of 300 mA for 3 h. The protein gel was placed in a PVDF membrane for 1 h for blocking; transferred into the primary antibody dilution buffer containing *CagA* (A-10), *VacA*, and GAPDH antibodies at dilution ratios of 1: 500, 1: 500, and 1: 6000, respectively; and incubated at 4°C overnight.

Thereafter, the PVDF membrane was washed three times with TBST (1 ×) on a shaker (10 min each time). The secondary antibody dilution buffer containing the m-IgGk BP-HRP antibody and the goat anti-rabbit IgG (H + L) HRP antibody (both diluted at 1: 6000) was added. After incubation for 2 h, the aforementioned washing steps were repeated.

Using an ECL luminescence detection kit, the image was developed in the automatic chemiluminescence image analyzer. The gray values of the bands were quantified by using Image J. The expression of all proteins was compared with that of GAPDH. Table 2 presents the antibody information.

*Differentially expressed genes of H. pylori after sodium selenite induction were determined by transcriptome sequencing*

To explore the effect of the half inhibitory concentration of sodium selenite on the Hp G27 strain, the bacterial solution was prepared ( $1 \times 10^8$  CFU/mL); treated with sodium selenite at concentrations of 1/8, 1/5, 1/4, 1/2, and 1 times minimal inhibitory concentration (MIC); and cultured for 0, 2, and 8 h. Thereafter, 1 mL of solution was coated over the plate and the plate was cultured for 3 d. The colonies were counted to

determine the concentration of the original bacterial solution. The concentration at which the colony count remained relatively stable for 0, 2, and 8 h after sodium selenite treatment was considered the half inhibitory concentration and used as the transcriptome induction concentration.

The transcriptome analysis samples were treated with sodium selenite at a concentration of 1/5 the MIC. The intervention experiment was repeated three times to obtain three sample groups ( $n = 9$  samples). The markers S\_0, S\_2, and S\_8 represent the treatment groups at 0, 2, and 8 h, respectively. Sequencing and analysis were performed by Nanjing Fengzi Biomedical Technology Co., Ltd.

#### ***Detection of the cell adhesion ability of *H. pylori* strains after induction with sodium selenite***

The PBS, *H. pylori*, induction, and *H. pylori* + 4  $\mu\text{mol/L}$  sodium selenite groups were used for the Alma blue experiments. GES-1 cells were cultured in a 96-well plate for 18~24 h ( $1 \times 10^4$  cells/well) and infected with *H. pylori* for 1 h (MOI = 300: 1). Then, the cells that did not adhere to the GES-1 cells were slowly removed, and the adhering cells were treated with 50 mL of 0.4% saponin solution for 5 min. Thereafter, the cells with the spent solution were removed, added to the culture medium containing 100 mL *H. pylori*, treated with 10 mL Alma blue, inoculated in a three-gas incubator for 4-6 h, and analyzed using a multimode reader.

The fluorescence experiential groups were divided into the same categories as those in the Alma blue experiment, as previously described. After culturing BGC823 cells in a 24-well plate for 18-24 h ( $5 \times 10^4$  cells/well), the cells were fluorescently stained with SYTO9 reagent, infected with *H. pylori* strains (MOI = 100: 1), and cultured for another 3 h. The cells adhering to *H. pylori* strains were observed under an inverted fluorescence microscope.

#### ***Determination of inflammatory factor levels in *H. pylori*-infected cells induced with sodium selenite***



The GES-1, GES-1 + G27, GES-1 + G27 induced, GES-1 + G27 + Se, GES-1 + Se, and Se groups were included in this experiment, and all groups were treated with 4  $\mu\text{mol/L}$  sodium selenite. GES-1 cells were cultured in a 6-well plate for 18-24 h ( $3 \times 10^5$  cells/well). After 24 h of infection with *H. pylori* strains (MOI = 100: 1), the levels of inflammatory factors, such as IL-6, were determined.

***Determination of the pathogenicity of H. pylori after sodium selenite induction in mice***

Thirty SPF C57BL/6J mice (age: 6-7 wk) were purchased from Changsha Tianqin Biological Co., Ltd (The number of animals: NO. 430726210100134118; SPF animal use license number: SYXK Gui 2017-0004; animal experiment ethics number: NO. 2019112501). The mice were randomly divided into the following five groups: The PBS, Se, NSH57, NSH57-induced, and NSH57 + Se groups. Mice in the Se group were intragastrically administered sodium selenite (4  $\mu\text{mol/L}$ ), and those in the induction group were administered sodium selenite (4  $\mu\text{mol/L}$ ) for 6 cycles. The corresponding bacterial solutions were centrifuged at 12000 rpm for 2 min, and the supernatant was removed to obtain the precipitate. Thereafter, the solutions were resuspended in fresh medium or medium containing 4 sodium selenite, with the concentration adjusted to  $1 \times 10^9$  CFU/mL.

The mice were fasted and deprived of water for 12 h before intragastric administration. Thereafter, they were intragastrically administered the solutions (0.5 mL/mouse), once every alternate day five consecutive times, and the diets were resumed diet 4 h after intragastric administration. After all rounds of intragastric administration, the mice were kept for 3 wk and dissected following the aseptic operation procedure. The tissues collected from stomachs, livers, spleens, and kidneys were fixed with 10% formalin and stained with hematoxylin and eosin (HE). The whole stomach was divided into two parts along its major and minor curves, with the stomach contents gently scraped away. One part was fixed with HE staining, and a fluorescence immunoassay was conducted to measure the levels of the inflammatory factors IL-1 $\beta$ , IL-6, and TNF- $\alpha$ . The other part was placed in a sterile Eppendorf tube containing

magnetic beads, and the medium was weighed and recorded before and after induction, and crushed well using a multi-sample tissue grinder at 50 Hz three times (3 min each time). Then, 100 mL abrasive solution was diluted 10-fold, 100-fold, and 1000-fold. Thereafter, 100 mL of each of the three diluted concentrations was coated over a solid plate containing selective antibiotics and cultured for 3-4 d. Then, the number of *H. pylori* colonies in the gastric tissues per gram (CFU/g) or the extent of *H. pylori* colonization in the stomach was calculated.

#### *Statistical processing and analysis*

Statistical analysis and mapping were performed using GraphPad Prism 8.0. The continuous data were expressed as the mean  $\pm$  standard deviation. Differences in means between the groups were analyzed using one-way ANOVA.  $P < 0.05$  was considered statistically significant.

## **RESULTS**

### *Induction with sodium selenite in vitro downregulated the expression of the H. pylori virulence factors CagA and VacA*

According to the MIC and cytotoxic effects of sodium selenite on *H. pylori* observed in the Supplementary materials (Supplementary Table 1 and Supplementary Figure 1), 0, 4, 8, and 16  $\mu\text{mol/L}$  of sodium selenite was used to induce Hp G27 (48 h as a cycle), and sequential induction was performed after the medium was changed.

RT-qPCR results revealed that after Hp G27 was sequentially induced with sodium selenite for 1-6 cycles, the mRNA expression of *CagA* was slightly downregulated in the 1<sup>st</sup> cycle, upregulated in the 2<sup>nd</sup> cycle, significantly upregulated in the 3<sup>rd</sup> cycle, downregulated again in the 4<sup>th</sup> cycle, and downregulated steadily and significantly throughout the 5<sup>th</sup> and 6<sup>th</sup> cycles (Figure 1A).

*VacA* mRNA expression was slightly upregulated in the 1<sup>st</sup> cycle, significantly upregulated in the 2<sup>nd</sup> cycle, downregulated again throughout the 3<sup>rd</sup> and 4<sup>th</sup> cycles, and downregulated steadily and significantly throughout the 5<sup>th</sup> and 6<sup>th</sup> cycles (Figure 1B).

Western blotting results revealed that *CagA* protein expression changed in a manner consistent with that of mRNA in the 1<sup>st</sup> cycle. In the 3<sup>rd</sup> cycle, the amount of mRNA at 4 and 8  $\mu\text{mol/L}$  increased significantly, whereas the protein expression basically remained unchanged. In the 6<sup>th</sup> cycle, the protein expression was downregulated in a manner consistent with the decreased mRNA expression.

*VacA* mRNA expression increased in the 1<sup>st</sup> and 3<sup>rd</sup> cycles, whereas its protein expression remained unchanged. In the 6<sup>th</sup> cycle, the protein expression was downregulated in a manner consistent with the decreased mRNA expression (Figure 1C-E). The mRNA expression of *CagA* and *VacA* was upregulated, whereas their protein expression remained unchanged. These results indicated that sodium selenite was possibly acting on biological processes posttranscription or posttranslation. The same results were obtained after the sequential induction of NSH57 strains for 6 cycles, and the protein expression was downregulated in a manner consistent with the decreased mRNA expression (Figure 1F-H). However, the aforementioned results suggested that the doses of 4, 8, and 16  $\mu\text{mol/L}$  could induce virus attenuation, with 16  $\mu\text{mol/L}$  being the best concentration.

#### *Changes in the H. pylori transcriptome after sodium selenite induction*

*H. pylori* induced with sodium selenite at a concentration of 1/5 the MIC exhibited only slight growth. This result suggested that this concentration could be used to achieve a half inhibitory effect (Figure 2A). Therefore, this concentration was selected for induction. The RNA-seq correlation test of the transcriptional group revealed that  $R^2$  values in the repeated intervention group were all  $> 0.9$ , thereby exhibiting good repeatability among the samples. The differences in  $R^2$  values were noted among the different intervention groups (Figure 2B). Principal component analysis revealed significant differences between the repeated and non-repeated samples (Figure 2C). The transcriptome analysis results are as follows: (1) In total, 329 differentially expressed genes (DEGs) were detected between S\_2 and S\_0; (2) A total of 466 DEGs were detected between S\_8 and S\_0; and (3) In total, 85 DEGs were detected between S\_8 and

S\_2. Overall, 247, 25, and 54 identical genes were detected between (1) and (2), (1) and (2), and (2) and (3), respectively, and 13 identical DEGs were detected between (1), (2), and (3) (Figure 2D and E). Among the top 20 pathways where the DEGs of (1), (2), and (3) were enriched, signal transduction pathways of epithelial cells associated with *H. pylori* infection were found to be associated with virulence factors (as shown in Figure 2F-H). The “*CagPAI-CagA*” pathway related to *CagA* virulence was further identified (Figure 2I). Thereafter, follow-up verification experiments were conducted on the genes with upregulated expression, *Cag1*, *Cag3* and *CagT*, and the genes with downregulated expression, *CagM* and *CagE*, that had been identified based on significant differences, repeatability of differential expression between (1) and (2), and whether they encoded unknown proteins, as shown in Tables 3 and 4. Therefore, the transcriptional analysis suggested that virulence factors of *H. pylori* after sodium selenite induction were mainly enriched in *H. pylori* infection-associated epithelial cell signal transduction pathways. The main virulence factors of *CagPAI* included *CagA*, *CagM*, *CagE*, *Cag1*, *Cag3*, and *CagT*, with the urease gene (*Ure*) and *VacA* also being key genes.

#### ***Verification of and changes in DEGs following induction***

The main virulence genes of *CagPAI* were verified through RT-qPCR (Figure 3). After the induction, *CagM* expression was significantly downregulated at 2 and 8 h and from the 1<sup>st</sup> to the 3<sup>rd</sup> cycles, upregulated again in the 4<sup>th</sup> cycle, and significantly downregulated in the 5<sup>th</sup> cycle (4–10 d). After the induction, *CagE* expression was significantly downregulated at 2 and 8 h with no significant change noted in the 1<sup>st</sup> cycle (2 d) and significantly downregulation from the 2<sup>nd</sup> to 5<sup>th</sup> cycles (4–10 d). The expression of these two genes was upregulated at some stages, but was significantly and continuously downregulated overall. *CagM* expression was upregulated at some stages but significantly and continuously downregulated overall; *CagM* and *CagE* expression was continuously and significantly downregulated at the fastest rate. *Cag1* expression was significantly upregulated at 2 and 8 h and in the 3<sup>rd</sup> cycle after induction, but no significant change was observed at the other time points. Although

*Cag3* expression was significantly upregulated in the 3<sup>rd</sup> cycle after induction, no significant change was observed at other time points. Because no uniform trend of change was observed in the two genes, they might not be considered key genes in attenuation. *CagT* expression was significantly upregulated at 2 and 8 h and in the 2<sup>nd</sup> and 3<sup>rd</sup> cycles after induction, downregulated in the 4<sup>th</sup> cycle, and significantly downregulated in the 5<sup>th</sup> cycle. However, the expression at most of the detection sites was consistent with the result of the transcriptome analysis; the expression was upregulated, but whether the expression would continue to be downregulated after the 5<sup>th</sup> week remains to be determined.

#### ***Induction with sodium selenite in vitro reduced the cell adhesion ability of H. pylori***

The cell adhesion ability of *H. pylori*, an important prerequisite for bacterial colonization and pathogenicity, was evaluated by inducing *H. pylori* with 4 µmol/L sodium selenite for 6 cycles. The Alamar Blue assay revealed that the number of Hp G27 cells adhering to GES-1 cells after induction significantly decreased ( $P < 0.05$ ) and that of Hp G27 cells adhering to cells after induction with 4 µmol/L sodium selenite exhibited a decreasing trend ( $P > 0.05$ ) (Figure 4A). The fluorescence experiment demonstrated that the number of Hp G27 cells that adhered to cells in the induction group was significantly reduced (the red fluorescence represents BGC823 cells, and the green fluorescence represents *H. pylori* strains). The cell adhesion ability of NSH57 strains induced under the same conditions was the same as that of Hp G27 strains (Figure 4B). In conclusion, 6 induction cycles of different *H. pylori* strains with sodium selenite significantly reduced the cell adhesion ability of these strains.

#### ***Induction with sodium selenite reduced the inflammatory response triggered by H. pylori***

After sodium selenite induction, GES-1 cells and mice were infected with *H. pylori* strains. The results of the cell infection experiment suggested that the relative mRNA expression of the inflammatory factors IL-6, IL-8, and TNF-α was significantly

downregulated (Figure 5A-C). In the experiment including infected mice, the extent of *H. pylori* colonization in the *H. pylori*-induced groups was significantly increased ( $P < 0.0001$ ), and inflammation was significantly reduced. However, inflammation was reduced in the mice in the noninduced *H. pylori* groups that had received sodium selenite intragastrically compared with that of those that had not in the same group. Furthermore, the degree of inflammation reduction in the noninduced group was not as good as that in the induced group. The gastric mucosal expression of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  was reduced, similar to the degree of inflammation (Figure 5D and E).

The aforementioned results indicated that the inflammatory effect and virulence of *H. pylori* on cells and mice were significantly weakened after sodium selenite induction. However, the increased colonization might be a result of the weakened immune reaction in mice due to the reduced virulence of *H. pylori*.

## **DISCUSSION**

Whether *H. pylori* infection causes disease depends on the bacteria, the environment, and the infected organism. Sodium selenite, a common inorganic Se, is also a common form of Se used for supplementation. Se has antibacterial effects<sup>[18,19]</sup>. The MIC of Se against *H. pylori* is 185  $\mu\text{mol/L}$ , which is not strong enough to inhibit growth and makes it difficult to achieve a therapeutic effect, and high doses of Se can damage gastric mucosal cells. In addition, some infected people with high Se content in the body do not show any symptoms, although they carried *H. pylori* for a long time. This also reveals that the antibacterial effect of Se in the body is not the main factor and there are other factors affecting the development of *H. pylori* infection. In this study, after creating a Se-enriched environment and inducing *H. pylori* cells with sodium selenite, the expression of the *H. pylori* virulence factors *CagA* and *VacA* was significantly downregulated, and the downregulation of *VacA* expression was basically in line with the reports of Duan *et al*<sup>[20]</sup> and Shao *et al*<sup>[21]</sup>. However, as toxicity was not rapidly reduced after induction with sodium selenite, the reason and potential mechanism for the reduction in *H. pylori* virulence by sodium selenite is not clear.

In *H. pylori*, the *CagPAI*-encoded T4SS has 15–16 components. These components are mainly involved in *CagA* protein transport and play a certain role in immunogenicity<sup>[22]</sup>. T4SS can be divided into the core complex, inner membrane component protein, and outer membrane component protein. The core complex includes *CagT*, *CagX*, and *CagY*, the inner membrane component proteins include *CagE*, *CagW*, and *CagV*, and the outer membrane component proteins encompass *CagM* and *Cag3*<sup>[23]</sup>. Regarding the T4SS of *H. pylori*, studies have been conducted to determine changes in *CagPAI* expression to understand the relationship between this gene and diseases and the integrity of virulence genes to study the size of virulence factors<sup>[24,25]</sup>. *CagPAI* deletion caused changes in the T4SS, thereby weakening *H. pylori* toxicity; however, the bacterium was not completely detoxified<sup>[26,27]</sup>. Kumari *et al*<sup>[28]</sup> found for the first time that *CagW* could interact with *CagA* and play a crucial role in secretion while affecting the expression of flagellar components such as *CagL*. Similarly, *Cag1* deletion could lead to a decrease in *CagA* heterotopia function<sup>[29]</sup>.

The DEGs of epithelial cell pathways were all screened from *CagPAI*. Meanwhile, *VacA*, which is outside *CagPAI*, was analyzed. *CagA* and *VacA* expression first increased and then decreased after sodium selenite induction and significantly decreased after 10 d. After sodium selenite induction for 2 h, *CagM* and *CagE* expression was continuously downregulated, whereas *CagT* expression was upregulated before the 6th day and downregulated significantly after the 10<sup>th</sup> day; while *Cag1* and *Cag3* expression was upregulated and downregulated, with no significant change noted on the 10<sup>th</sup> day. The above results suggest that these five genes may be key genes in the reduction of *H. pylori* virulence by sodium selenite in a Se-enriched environment and that the time needed to induce a reduction in *H. pylori* virulence is at least 10 d.

After induction of *H. pylori* by sodium selenite, its adhesion ability was tested *in vitro*, and its colonization and inflammatory damage to the gastric mucosa of mice was tested *in vivo*, both results demonstrated that sodium selenite could significantly reduce the adhesion ability and toxicity of inflammatory damage, and *H. pylori* stability was

better in a Se-enriched environment. The application of sodium selenite to reduce the toxicity of *H. pylori* is an ideal method for the development of attenuated vaccines<sup>[30]</sup>.

## **CONCLUSION**

In this study, we investigated how a sodium-rich environment acts on *H. pylori* virulence factors, verified that sodium selenite could reduce *H. pylori* toxicity, and confirmed that a Se-rich environment could significantly reduce *H. pylori* pathogenicity. We also indicated that further studies are needed to elucidate the underlying molecular mechanisms. This study offers an experimental basis for the use of Se, a trace element, in *H. pylori* infection treatment in the future and a reference for ensuring that humans coexist with bacteria without developing diseases. Sodium selenite treatment is a potential method that will allow attenuated vaccines to alleviate severe drug resistance in *H. pylori*.

## **ARTICLE HIGHLIGHTS**

### ***Research background***

*Helicobacter pylori* (*H.pylori*) is a common gram-negative bacterium that colonizes the stomach and is currently recognized as a class I carcinogen. The *H. pylori* infection rate is as high as approximately 60%, and the *H. pylori* eradication rate decreased markedly with increasing rate of drug resistance. Finding new antimicrobial drugs or control regimens to mitigate the threat of *H. pylori* to human health and mimicking live vaccines is crucial. The preparation of live vaccines with reduced virulence and reduced pathogenicity is a promising control method. Selenium (Se) is one of the essential trace elements in the human body. Se has been proven to have an attenuating effect, but whether it has the same effect on *H. pylori* is not known. The interaction between *H. pylori*, Se and its mechanism in the stomach has rarely been studied. *H. pylori* has coexisted with humans for 100000 years, and eliminating this coexisting relationship is extremely difficult, this makes studying the effect of Se on *H. pylori* difficult. The



influence of Se on the interaction between *H.pylori* and the organism may provide new ideas and <sup>1</sup> an experimental basis for the prevention and treatment of *H.pylori*.

### **Research motivation**

Currently, *H.pylori* is a persistent threat to humans, and the increase in drug resistance makes it increasingly difficult to eliminate this threat. Studying the mechanism of the attenuating effect of the trace element Se on *H. pylori* and its interaction with the organism after attenuation will help to better apply Se to the development of *H. pylori* attenuated vaccines and alleviate the problem of *H. pylori* drug resistance.

### **Research objectives**

<sup>5</sup> The aim of this study was to investigate the effect and mechanism of action of the trace element Se on the virulence factors of *H. pylori* and to provide an experimental basis for the use of the trace element Se in the prevention and treatment of *H. pylori*.

### **Research methods**

A Se-enriched environment was created with sodium selenite to induce *H. pylori*, and the effect of the Se-enriched environment on the virulence of *H. pylori* and its potential mechanisms were evaluated by real-time fluorescence quantitative polymerase chain reaction, protein immunoblotting, transcriptome gene sequencing, Alma blue assay, and cell adhesion assay. A mouse gastritis model was established to understand the attenuation of *H. pylori* in terms of the changes in virulence *in vitro* and *in vivo*.

### **Research results**

Se-enriched environments may lead to a reduction in the virulence factors CagA and VacA and significantly attenuate the pathogenicity of *H. pylori* by affecting the CagPAI-encoded type IV secretion systems of *H. pylori*.

### **Research conclusions**

The mechanism of action of sodium selenite leading to the reduction of *H. pylori* virulence was shown to be through the downregulation of virulence factor expression, which led to a significant reduction in adhesion capacity, as well as inflammatory damage, thus inhibiting the growth of *H. pylori* and presenting better stability under a Se-rich environment.

### ***Research perspectives***

This study demonstrated the antibacterial mechanism of sodium selenite against *H. pylori* in a Se-enriched environment through *in vivo* and *in vitro* experiments; these results provide theoretical support for further research and development of sodium selenite in the preparation of attenuated vaccines and contributes to the alleviation of drug resistance in *H. pylori*.

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Crossref

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Siqi Shen, FeiFei Ren, Haiming Qin, Ihtisham Bukhari et al. "Lactobacillus acidophilus NCFM and Lactiplantibacillus plantarum Lp-115 inhibit Helicobacter pylori colonization and gastric inflammation in a murine model", Frontiers in Cellular and Infection Microbiology, 2023  
Crossref

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