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Short-term zinc supplementation attenuates *Helicobacter felis*-induced gastritis in the mouse

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Abstract *Background*. Mucosal damage by *H. pylori* infection is mainly caused by neutrophils producing large quantities of reactive oxygen species (ROS). Metallothionein (MT) an intracellular, low-molecular, cysteine-rich protein, which is inducible by dietary zinc (Zn), has been implicated in sequestering ROS. This study examines the effects of Zn supplementation on *Helicobacter* colonisation and associated gastritis and the relationship with gastric MT levels.

Methods. C57Bl/6 mice were inoculated with either 10^8 *H. pylori* or *H. felis* and were infected for 4 weeks or 6 and 12 weeks, respectively. Mice infected with *H. pylori* (4 weeks) or *H. felis* (6 weeks) were treated with either Zn acetate (ZnA; 1 mg/ml), or Zn sulphate ($ZnSO_4$; 5 mg/ml) for 2 weeks with 0.1 ml oro-gastric gavage twice daily. *H. pylori* load and *H. felis* colonisation density were determined by culture and microscopy, respectively. MT levels and *H. felis*-induced gastritis were also determined.

Results. Zn treatment showed no significant difference in *Helicobacter* load and gastric MT, however, $ZnSO_4$ treatment showed a significant ($p<0.05$) increased in gastric MT in *H. felis* infected mice. Both Zn-treated groups showed a significant ($p<0.05$) difference in gastritis score in the antrum of the stomach within the basal and submucosal compartments compared to *H. felis*-infected controls.

Conclusions. We found that *H. felis*-induced gastritis can be attenuated by short-term treatment of Zn. This observation suggests that Zn alone may be effective for the suppression of gastric mucosal inflammation induced by *Helicobacter*.

Introduction

Helicobacter pylori (*H. pylori*) infection has been associated with gastritis and peptic ulcer disease[1,2] and is recognised as a risk factor in gastric carcinogenesis.[2] Mucosal damage by

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H. pylori infection is mainly caused by activation and infiltration of neutrophils and monocytes producing large quantities of reactive oxygen species (ROS).[3] Excessive production of ROS can contribute to various diseases of the gastrointestinal tract as shown in animal models[4,5] and human studies.[6,7] The host possesses enzymatic as well as non-enzymatic defence mechanisms against ROS.[8] The gastric manganese-superoxide dismutase scavenger enzyme has been demonstrated to increase after *H. pylori* infection[9,10] and these levels reversed towards normal after successful treatment.[8] Whereas, glutathione concentrations were lower in gastric biopsies from *H. pylori*-infected patients compared to controls.[11]

Metallothionein (MT) is an intracellular, low molecular weight, cysteine-rich protein with a high binding affinity for heavy metal ions. MT is induced in many tissues by a variety of factors including inflammatory mediators and zinc (Zn) and it has putative roles in regulating Zn homeostatsis,[12,13] detoxifying heavy metals,[14] providing Zn for metalloenzymes[15] and sequestering ROS.[16] In regard to the latter, MT has been found to decrease oxidative damage as a result of host-mediated inflammatory responses to microbial infection.[17] MTs are also antioxidant agents because the Zn-sulphur clusters are sensitive to changes to cellular redox state and the oxidising sites induce the transfer of Zn from its binding sites of MTs to those of lower affinity in other proteins.[18] MT may also be important in providing Zn for gastric repair mechanisms. However, the implication of this scavenging enzyme in the defence against ROS during *H. pylori* infection has yet to be fully defined.

Studies regarding the effect of Zn compounds on the eradication of *H. pylori* infection have been limited. However, Sunairi et al.[19] reported that a combination of aluminium chloride, sofalcone [2′-carboxymethyl 4, 4′-bis (3-methyl-2-butenyloxy) chalcone] and Zn chloride inhibited the growth of *H. pylori* in vitro and suggested that the effectiveness may be attributable to the Zn ion. Another insoluble chelate compound consisting of Zn ion and L-carnosine, polaprezinc, has been shown to inhibit *H. pylori*-associated gastric mucosal oxidative inflammation and may be effective for the treatment of gastric inflammation induced by *H. pylori*.[20] Furthermore, in a randomised clinical trial, polaprezinc in combination with the triple therapy (lansoprazole, amoxicillin, clarithromycin) significantly improved the cure rate of *H. pylori* infection with no increase in side effects.[21] The aim of the present study was to evaluate the effect of zinc supplementation on *Helicobacter* colonisation and associated gastritis in the mouse, in addition, to determine the relationship between gastric MT and *Helicobacter* infection after zinc supplementation.

Materials and methods

Culture of *H. pylori* and *H. felis*

The *Helicobacter* strains, *H. pylori*, Sydney strain 1 (SS1) and *H. felis*, were obtained from Professor Adrian Lee (University of NSW, Sydney, Australia).[22] The *H. pylori* strain was grown on *Helicobacter* selective agar (HSA) supplemented with 5% lysed horse blood and Dent's selective supplement (Oxoid Australia Pty Ltd, Victoria, Australia) at 37 °C for 2 days. The bacteria were inoculated into a brain heart infusion (BHI) broth with 5% horse serum, agitated for 24 h in a microaerophilic environment (Oxoid Australia Pty Ltd, Victoria, Australia). The broth containing *H. pylori* was centrifuged and resuspended in sterile BHI at a concentration of 10^9 bacteria/ml. *H. felis* was grown on Campylobacter selective agar (CSA) containing 5% lysed horse blood and Skirrows selective supplement for 4 days in a microaerophilic environment (Oxoid Australia Pty Ltd, Victoria, Australia) and resuspended in sterile BHI at a concentration of 10^9 bacteria/ml. The suspensions were then tested for urease, oxidase and catalase activities. Morphology and motility of the bacteria were assessed with a wet-preparation to ensure bacterial viability.

In vitro studies

The *H. pylori*, SS1 strain was grown on HSA at 37 °C for two days. The bacteria were collected and subcultured in a BHI broth with 5% horse serum and then agitated for 24 h in a microaerophilic environment. Various concentrations of Zn acetate (ZnA; 0, 5, 15 and 30 mg/ml) and Zn sulphate ($ZnSO_4$; 0, 5, 15 and 30 mg/ml) were incubated in the presence of *H. pylori* for 0, 2, 4, 6, 8 and 24 h in a microaerophilic environment while being agitated. After each time point the suspension containing *H. pylori* and various Zn concentrations was serially diluted and plated out on HSA (*H. pylori*). The plates were then incubated in a 10% CO_2 environment at 37 °C for 7 days. *H. pylori* colonies were identified by positive urease, counted and results