Zinc Salts Provide a Novel, Prolonged and Rapid Inhibition of Gastric Acid Secretion

Philipp Kirchhoff, MD[1,2], Thenral Socrates, MD[1], Shafik Sidani, MD[1], Andrew Duffy, MD[1], Tobias Breidthardt, MD[3], Christian Grob, MD[2], Carsten T. Viehl, MD[2], Christoph Beglinger, MD[3], Daniel Oertli, MD[2] and John P. Geibel, MD[1,4]

OBJECTIVES: **The overproduction of acid and the associated illnesses linked to hypersecretion have a lifetime prevalence of 25–35% in the United States. Although a variety of pharmaceutical agents have been used to reduce the production of acid, alarming new evidence questions the long-term efficacy and safety of the agents. These issues coupled with the delayed onset of action and the return of symptoms in over 60% of the patients is less than satisfactory. The purpose of this study was to determine whether administration of a zinc salt could lead to a rapid and sustained increase in gastric pH in both animals and in humans and provide a new rapid acid suppression therapy.**

METHODS: **Intracellular pH was measured with 2′,7′-bis-(2-carboxyethyl)-5-and-6-carboxy-fluorescin in both human and rat gastric glands following an acid load±a secretagogue. In a separate series of studies, whole stomach acid secretion was monitored in rats. A final study used healthy human volunteers while monitoring with a gastric pH measurement received placebo, zinc salt, or a zinc salt and proton pump inhibitor (PPI).**

RESULTS: **We demonstrate that exposure to $ZnCl_2$ immediately abolished secretagogue-induced acid secretion in isolated human and rat gastric glands, and in intact rat stomachs. Chronic low-dose zinc exposure effectively inhibited acid secretion in whole stomachs and isolated glands. In a randomized cross-over study in 12 volunteers, exposure to a single dose of $ZnCl_2$ raised intragastric pH for over 3 h, including a fast onset of effect.**

CONCLUSIONS: **Our findings demonstrate that zinc offers a novel rapid and prolonged therapy to inhibit gastric acid secretion in human and rat models.**

Am J Gastroenterol 2011; 106:62–70; doi:10.1038/ajg.2010.327; published online 24 August 2010

INTRODUCTION

The generation of concentrated 0.16 N hydrochloric acid by the mammalian parietal cell involves a complex combination of neuronal and hormonal regulatory feedback loops (1). A disruption in any of these regulatory components can lead to unregulated acid secretion. Hypersecretion and acid-related diseases are common and have a lifetime prevalence of 25–35% in the United States (2–6). Clinically, the continued hypersecretion of acid can lead to changes in the gastric epithelium, but can in more serious cases lead to erosions of the esophagus that can result in metaplasia and death (7).

In an attempt to design the therapies to prevent hyperacid secretion, a variety of approaches have been used in recent years with two of the most successful being: (i) inhibition of the histamine receptor on the basolateral membrane (H2-blockers) and (ii) proton pump specific drugs targeted against the H^+,K^+-ATPase (the so-called proton pump inhibitors (PPIs)) (8,9). Both of these therapies have greatly improved the quality of life for patients suffering from this disease; however, there is an ever increasing number of patients that have experienced recurrent disease while still taking the drugs (10). It has been estimated that about 30% of gastroesophageal reflux disease patients remain symptomatic on a standard dose of PPI (11). One explanation relates to the fact that typically PPIs have a short plasma half life, which often leads to nocturnal acid breakthrough. This increased and recurrent failure in the treatment of acid-related diseases has lead to the

[1]Department of Surgery, Yale University School of Medicine, New Haven, Connecticut, USA; [2]Department of Surgery, University of Basel, Basel, Switzerland; [3]Division of Gastroenterology, Department of Internal Medicine, University of Basel, Basel, Switzerland; [4]Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut, USA. **Correspondence:** Philipp Kirchhoff, MD, Department of Surgery, University of Basel, Spitalstrasse 21, 4031 Basel, Switzerland. E-mail: pkirchhoff@hotmail.com
Received 28 April 2010; accepted 20 July 2010

continued development of new agents focused on faster speed of onset, while maintaining symptom relief that remains the top priority for patients suffering from these disorders (12). Currently, administration of therapeutic oral doses of PPIs reach steady state and thus achieve maximal effective levels after 4–5 days with typical dosing regimens (13). Even after the administration of a PPI, there is a return of acid secretion that is partly due to *de novo* synthesis of the enzyme (14).

In this study, we investigate zinc as a novel agent for the rapid inhibition of acid secretion targeted directly to the parietal cell. Zinc is an essential dietary element that is required to maintain membrane integrity and function. Deficiency in intracellular zinc leads to apoptotic events and eventual cell death (15–19). Previous studies have investigated the potential role of zinc in the proliferation and generation of the protective barrier, namely the mucous gel layer at the surface of the stomach (20–22). These studies concluded that the reduction in acid secretion observed over time was related to an increase in the thickness of the gel layer (23).

METHODS

Animals

Male Sprague-Dawley rats 150–250 g (Charles River Laboratory, MA) were housed in climate- and humidity-controlled, light-cycled rooms, fed standard chow with free access to water. Prior to experiments, animals were fasted for 18–24 h with free access to water. The use of rats, as well as the protocol for isolating colon tissue, was approved by the Institutional Animal Care and Use Committee (IACUC No. 2000-10253) at Yale University.

Isolation of rat and human gastric glands

Rat studies. Following removal of the stomach, the corpus was isolated and sliced into 0.5 cm square sections, and washed with cold 4 °C Ringer solution to remove residual food particles.

Human studies. Surgical specimens were obtained from obese patients who underwent gastric reduction surgery. Single pieces of stomach ~0.2 cm square were resected laparoscopically and transferred to a cold 4 °C Ringer solution. The tissues were transferred to the stage of a dissecting microscope. Individual glands were isolated using a hand-dissection technique as described previously (24). Individual isolated glands were allowed to adhere to cover slips that had been pre-coated with Cell-Tak (Collaborative Research, Bedford, MA) and were transferred to the stage of an inverted microscope. The human tissue was transferred from the operating room in a HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl] ethanesulfonic acid)-buffered Ringer solution.

Digital imaging for intracellular pH

Isolated gastric glands were incubated in a HEPES-buffered Ringer's solution containing either 10 µmol of the pH-sensitive dye 2′,7′-bis-(2-carboxyethyl)-5-and-6-carboxy-fluorescin, aceto-methyl ester (Molecular Probes, Eugene, OR) for 10 min as described previously (25–27). Following dye loading, the chamber was flushed with a HEPES solution to remove all non-de-esterified dye. The perfusion chamber was mounted on the stage of an inverted microscope (Olympus IX50, Boston, MA), which was used in the epifluorescence mode with a ×40 objective. 2′,7′-bis-(2-carboxyethyl)-5-and-6-carboxy-fluorescin was successively excited at 440 and 490 nm from a monochromatic light source, and the resultant fluorescent signal was monitored at 535 nm using an intensified charge-coupled device camera. Individual parietal cells were identified due to its unique conical shape and regions of interest were outlined and simultaneously monitored every 15 s during the course of the experiment. A minimum of eight cells or regions was selected per gland.

Proton extrusion by individual parietal cells was monitored by observing recovery of pH_i after acid loading the cells with Na^+-free HEPES solution containing 20 mM NH_4Cl. Parietal cells were subsequently superfused with Na^+-free HEPES, which abolished all Na^+/H^+-exchanger activity, trapping H^+ within the cytosol and initiating an immediate drop in pH_i. Under these conditions, the only potential H^+ extrusion pathway is via the H^+,K^+-ATPase activation.

The intensity ratio data (490/440) were converted to pH values using the high-K+/nigericin calibration technique (28). Intracellular pH recovery rates were calculated from the same initial starting pH to eliminate the potential variation in the individual intracellular buffering power of the cells under the different experimental conditions. All data including the individual images for all wavelengths were recorded to the hard disk, which allowed us to return to the individual images after the experiment for further analysis. The recovery rates are expressed as the ΔpH/min, and were calculated over the pH range of 6.5–6.8. All chemicals were obtained from Sigma (St Louis, MO) and Molecular Probes. All data were summarized as means±s.e. and were analyzed by grouping measurements at baseline values.

Oral zinc supplementation in rats

These studies were designed to modulate acid secretion by increasing dietary zinc. In these studies, we used an oral $ZnCl_2$ solution (zinc chloride in tap water). The animals had free access to food and the zinc containing water for the duration of the study. In all, 150 or 0.5 mg/kg/day $ZnCl_2$ was added to the drinking water for 5 days. Animals had free access to water prior to the experiment and were fed with standard chow until 24 h before the experiment, at which point they had free access to $ZnCl_2$ containing water only. Following 5 days *ad lib* exposure, and the 24-h fast, a total gastrectomy was performed on the animals. Individual gastric glands were isolated with the hand-dissection technique as described above.

Digital imaging for intracellular chloride

For intracellular chloride (Cl^-_i) measurements, Cl^- influx and efflux from individual parietal cells was measured by exciting *N*-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide at 340±10 nm, with emission monitored at 460±10 nm, with an intensified charge-coupled device camera and recorded as arbitrary fluorescence units. The data are expressed as the change in arbitrary fluorescence unit per minute. A decrease in fluorescence intensity represents a rise in Cl^-_i concentration (influx), whereas an increase signifies a reduction in Cl^-_i concentration (efflux).

Digital imaging for intracellular zinc

To measure intracellular zinc, gastric glands were loaded with 5 µmol of the zinc-sensing dye Fluo-Zin-2-AM (Molecular Probes) in the perfusion chamber for 20 min at 37 °C. To eliminate residual non-de-esterified dye from the bath, the glands were superfused with standard HEPES-buffered Ringer solution. Fluo-Zin-3-AM was excited with light of 490 nm wavelength, and the change of the intracellular zinc concentration was expressed as fluorescence-Units/min.

Whole stomach pH measurements

Before the experiments, all animals were fasted for 24 h to reduce basal acid secretion to a consistent minimum. Animals were euthanized with an overdose of isoflurane, and the stomach was ligated at the duodenal and esophageal junction and excised. Then 1 ml of non-buffered, isotonic saline (140 mM) was infused into the lumen of the stomach using a small guage needle. The stomachs were then placed in oxygenated 50 ml HEPES-buffered Ringer solution, or in the same solution containing 100 µM histamine alone or additionally 300 µM $ZnCl_2$ (pH 7.4) and maintained at 37 °C. After 1 h, the stomach contents were aspirated (1 ml), and the pH was measured with a micro pH meter.

pH measurements after zinc administration in 12 healthy volunteers

The study was conducted as an open, randomized, cross-over study. In all, 12 healthy *Helicobacter pylori*-negative adult subjects were included and each underwent four different treatments after an overnight fast of 10 h. On 4 different days and in random order after a 30-min basal intragastric pH measurement period, each participant received 200 ml water, 20 mg omeprazole (Astra-Zeneca, Mölndal, Sweden), 200 mg zinc sulfate (Aliud-Pharma, Laichingen, Germany), or a combination of 200 mg zinc sulfate plus 20 mg omeprazole orally. The treatments were separated by a washout phase of at least 3 days. For the intragastric pH monitoring, a glass electrode (Medical Instruments Corporation, Solothurn, Switzerland) was used, which was attached to a Digitrapper Mark III Gastrograph (Medical Instruments Corporation, Herford, Germany). The electrode was calibrated in buffer solution before and after recording and inserted transnasally into the gastric body. The position of the pH electrode in the stomach was 8–10 cm below the gastro-esophageal junction, which was recognized by an abrupt drop in pH from neutral to acid (intragastric pH < 2). Measurements of the intragastric pH began with a 30-min basal period. Then, one of the four treatments was given according to the randomization scheme, and pH monitoring was continued for the following 3 hours. The primary end point was the median pH during the 180 min after drug administration. The study was approved by the local ethical committee (University of Basel, Switzerland) and subjects gave written informed consent.

Statistical analysis

Data are presented as means±s.d. Groups were compared with Student's *t* test. Comparisons of the four different treatments in the intragastric pH study were done by Kruskall–Wallis test. A two-sided *P* value of < 0.05 indicated statistical significance.

RESULTS

pH measurements in isolated gastric glands

To examine the rapid cellular effect of zinc on acid secretion, we conducted studies using the isolated gastric gland preparation. In the absence of any stimulation, a low-basal rate of pH_i recovery was observed (0.011±0.002 ΔpH_i/min). Following exposure of the glands to the secretagogue histamine (100 µM), the intracellular alkalinization rate increased to 0.051±0.004 ΔpH_i/min (**Figure 1a**). Adding 300 µM $ZnCl_2$ to the superfusion bath in the presence of histamine (100 µM) prevented the stimulatory effect of histamine on the pH_i recovery rate (0.0012±0.004 ΔpH_i/min) and reduced it to the same level as seen in the control glands not exposed to

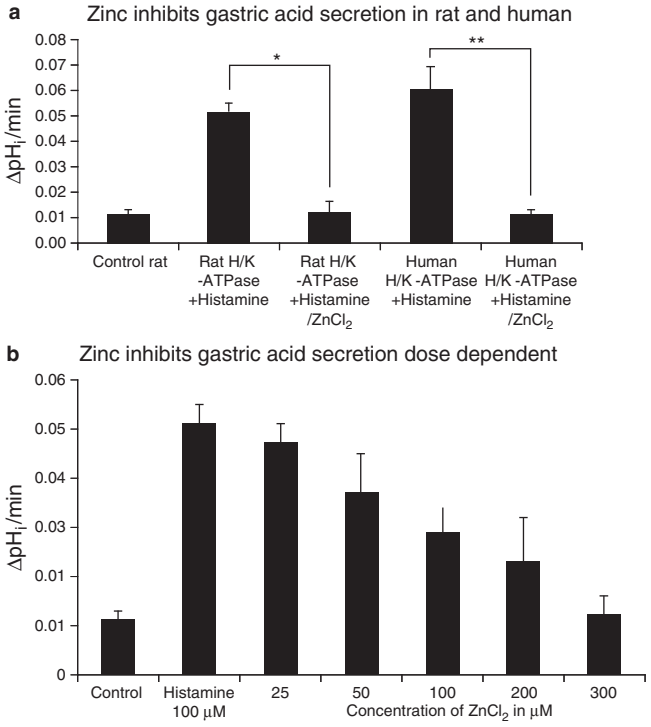

Figure 1. Histamine-induced acid secretion and inhibition by $ZnCl_2$ in isolated human and rat gastric glands. Single human and rat gastric glands were isolated, loaded with the pH-sensitive dye 2′,7′-bis-(2-carboxyethyl)-5-and-6-carboxy-fluorescin (BCECF) to measure intracellular pH over single parietal cells and the pH_i recovery rate. (**a**) Intracellular alkalinization in resting cells and stimulated by histamine (100 µM) in the absence of extracellular Na^+ as a function of H^+/K^+-ATPase in rat and human gastric glands. The histamine-induced proton efflux can be blocked by 300 µmol $ZnCl_2$ in rat and human gastric glands. Bar graph summarizing data as means s.e. (control: $n=32$ cells, 3 gland, 3 animals; histamine: $n=120$ cells, 15 glands, 8 animals; histamine+$ZnCl_2$: $n=60$ cells, 6 gland, 4 animals, human glands ($n=26$ cells, 3 glands in each protocol) (*$P<0.0001$, **$P<0.0001$). Histamine-induced acid secretion and dose-dependent inhibition by $ZnCl_2$ in isolated rat gastric glands. (**b**) $ZnCl_2$ concentration dependence of H^+/K^+-ATPase activity (intracellular alkalinization expressed as ΔpH/min) in the presence of 100 µM histamine in comparison to basal and histamine-induced acid secretion ($n=40$ cells, 3–4 glands, 3–4 animals for each $ZnCl_2$ concentration).

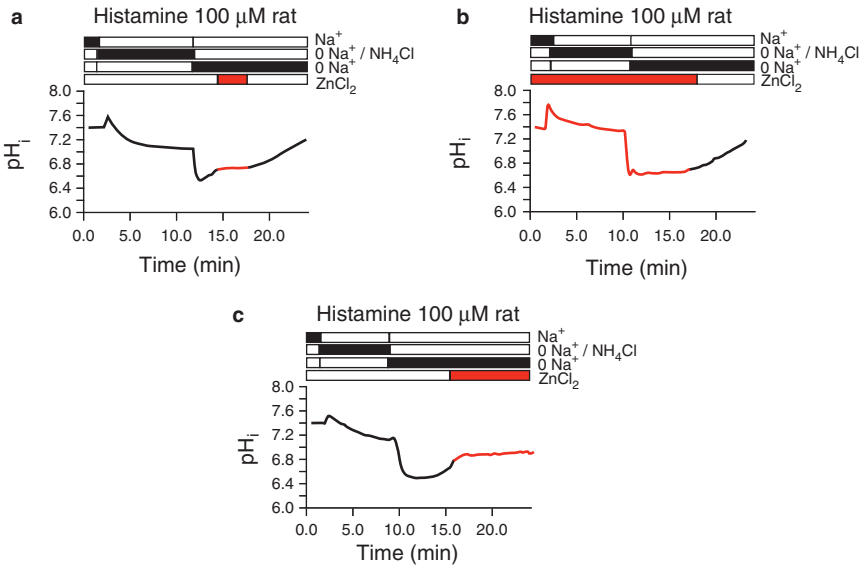

Figure 2. Fast onset inhibitory effect and reversibility $ZnCl_2$. Histamine (100 µM) was added through the whole experiment. (**a**) When the intracellular alkalinization (proton efflux) was observed, $ZnCl_2$ (300 µM) was added to the superfusion bath (red boxes). The acid secretion was abolished after a few seconds (flat red part). After the removal of $ZnCl_2$ out of the perfusion bath, the drug was washed out and the increase of the intracellular pH continued. (**b**) Cells were incubated and superfused over 20 min with $ZnCl_2$ (300 µM) and histamine (100 µM) (red box and line). After removal of $ZnCl_2$ out of the superfusion bath, the intracellular alkalinization (proton extrusion) occurs. (**c**) The rapid inhibitory effect on acid secretion. Alkalinization (proton efflux) was abolished after a few seconds after adding 300 µM $ZnCl_2$ to the superfusion bath (red box and red tracing line).

histamine. Freshly isolated human gastric glands also showed a robust proton efflux under histamine stimulation. This effect was abolished by 300 µM $ZnCl_2$ (**Figure 1a**). $ZnCl_2$ inhibited H^+ extrusion in a dose-dependent manner (**Figure 1b**). In this protocol, acid secretion was stimulated by histamine and expressed as ΔpH_i/min. To investigate the inhibitory potency of $ZnCl_2$ we used different concentrations (25–300 µM). In all, 300 µM $ZnCl_2$ showed a 98% inhibition of proton extrusion compared with the histamine-induced rate and the control (**Figure 1b**).

Rapid onset and reversal of acid secretion

We investigate the reversibility and onset of the inhibitory effect of $ZnCl_2$ in our *in vitro* setting. Individual glands were stimulated with histamine (100 µM) during the entire experiment to promote acid secretion. Following the NH_4Cl acid challenge and during the subsequent recovery indicative of acid secretion (intracellular alkalinization), $ZnCl_2$ (300 µM) was added to the superfusion bath. The acid secretion was rapidly abolished after a few seconds in both rat and human gastric glands (**Figure 2a, c**). The subsequent removal of $ZnCl_2$ in the same experiment allowed acid secretion to return to normal secretagogue-induced levels (**Figure 2a**). We were also able to demonstrate reversibility following incubation and superfusion of parietal cells over 20 min with $ZnCl_2$ (300 µM) and histamine (100 µM). After removal of $ZnCl_2$ from the superfusion bath, the intracellular alkalinization (proton extrusion) proceeded at normal rate (**Figure 2b**).

Zinc prevents acid secretion in whole animal studies

In the next series of studies, we added 150 or 0.5 mg/kg/day $ZnCl_2$ to the drinking water of rats for 5 days, while allowing *ad lib* access to food. Following this 5-day period, glands were isolated, and the secretagogue histamine was added to induce acid secretion. **Figure 3a** shows that $ZnCl_2$ (150 or 0.5 mg/kg/day) in the drinking water decreased the histamine-induced acid secretion significantly in isolated gastric glands in comparison to the control group (no zinc in water) with histamine alone—150 mg/kg/day: 0.022±0.0045 ΔpH_i/min, 0.5 mg/kg/day: 0.034±0.0036 ΔpH_i/min. Histamine-stimulated parietal cells showed a robust recovery rate (proton extrusion) of 0.051±0.004 ΔpH_i/min.

To determine whether $ZnCl_2$ could inhibit gastric acid secretion in the whole organ, we examined gastric luminal pH in freshly isolated rat stomachs after incubation in HEPES or in the presence of 100 µM histamine, 100 µM histamine, and 300 µM $ZnCl_2$. As illustrated in **Figure 3b**, in the presence of histamine mean luminal pH was lower than in control stomach preparations incubated in HEPES alone (3.15 pH±0.27 vs. 4.59 pH±0.48). In the presence of histamine and $ZnCl_2$, the luminal pH was similar to the control group without secretagogue stimulation (4.54 pH±0.065 vs. 4.59 pH±0.48).

Zinc permeability in parietal cells

To determine whether the effect of Zinc was via a membrane receptor or through modulation of the intracellular compartment, we measured intracellular zinc concentration using a fluorescent recorder dye. Individual gastric glands were incubated for 15 min with 5 µM Zin-Fluo-3, an intracellular zinc dye. $ZnCl_2$ (100–600 µM) was added to the bath to determine an intracellular concentration profile for zinc transport. Addition of different concentrations of $ZnCl_2$ to the bath solution led to a sustained concentration-dependent increase of intracellular Zinc (**Figure 4a**).

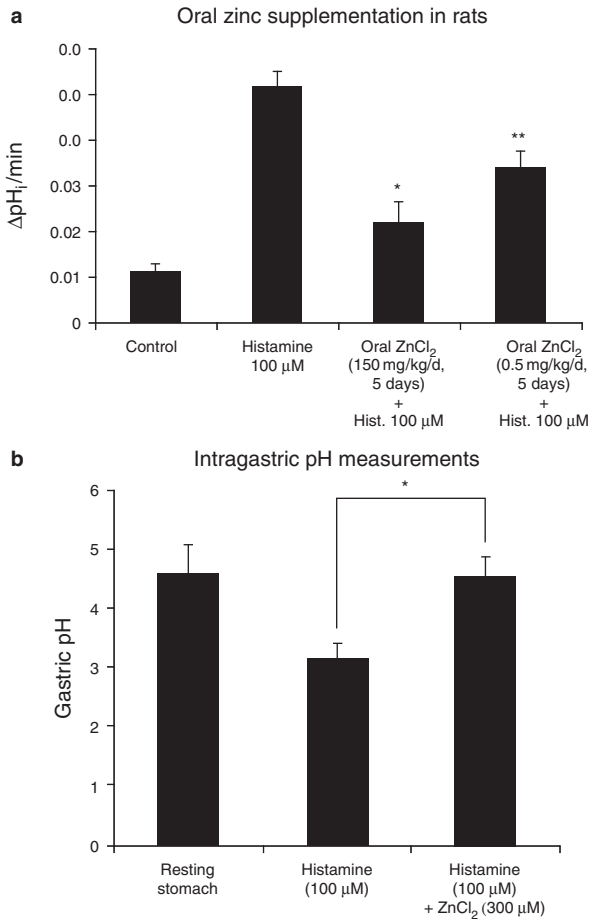

Figure 3. Acid secretion after oral $ZnCl_2$ application. (**a**) $ZnCl_2$ was added to the drinking water. Animals ate and drank as much as control animals. The histamine-induced acid secretion was measured as described before. The cells of the $ZnCl_2$-treated animals showed a lower rate of proton efflux—150 mg/kg/day: 0.022±0.0045 ΔpH/min (*P<0.001 compared with histamine alone) (n=60 cells, 10 glands, 3 animals), 0.5 mg/kg/day: 0.034±0.0036 ΔpH/min (**P<0.008 compared with histamine alone) (n=60 cells, 6 glands, 4 animals). (**b**) *Ex vivo* rat whole stomach preparations were incubated in HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid)-buffered Ringer solution (control: n=9), HEPES-buffered Ringer solution plus 100 μM histamine (n=8), or HEPES-buffered Ringer solution plus 100 μM histamine and 300 μM $ZnCl_2$ (n=8). Stomach preparations incubated with histamine and $ZnCl_2$ had a higher pH than those in HEPES-buffered Ringer solution with histamine and their pH was similar to the pH of the control stomach (*P<0.006).

Zinc entry via Ca^{2+} and Cl^- channels

Figure 4b shows zinc entry into the parietal cell using the intracellular zinc dye Fluo-Zin-2. The control tracing illustrates a robust entry of zinc into the parietal cell with an extracellular zinc concentration of 200 μM. Addition of 200 μM Nifedipine, a specific Ca^{2+}-channel blocker to the superfusion bath significantly reduced zinc uptake. Application of the stilbene derivative DIDS (4,4′-diisothiocyano-2,2′-stilbene disulphonic acid) (500 μM), who at high concentrations is a well-known inhibitor of Cl^- channels, had a more pronounced inhibition of zinc uptake than Nifedipine. Addition of both, DIDS (500 μM) and Nifedipine (200 μM), produced an additive effect suggesting that zinc entry occurs via both Ca^{2+} and Cl^- channels.

Zinc reduced Cl entry and efflux into isolated gastric glands

Figure 4c summarizes the effects of addition of $ZnCl_2$ to the basolateral perfusate to prevent either Cl^- entry or Cl^- exit from the cells as measured by the Cl^--sensitive dye *N*-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide. These data show that transcellular Cl^- transport in the parietal cell is greatly reduced in the presence of $ZnCl_2$.

Zinc inhibits gastric acid secretion in 12 healthy volunteers

The bar graph shown in **Figure 5a** illustrates how 200 mg zinc increases intragastric pH in healthy subjects in comparison to water (200 ml) and 20 mg omeprazole. The effect of the zinc is apparent within seconds after drug intake. The median curves in **Figure 5b** show that immediately after zinc administration, the pH rises >2 Units. This effect continues for about 3 h until the pH decreases to baseline values. Water and omeprazole show only a small peak after oral intake. The combination of zinc and omeprazole does not potentiate the effect of zinc and shows similar action as zinc alone.

DISCUSSION

Acid secretion is an essential component of the digestive process and provides both concentrated acid and enzymes to aid in the breakdown of foodstuffs into components that can be absorbed in the intestine (29). Unfortunately, for ~25–50 million patients in the United States, this process does not work properly leading to the hypersecretion of acid causing symptomatic pain and discomfort (29,30). The risk for erosions of the epithelia and internal bleeding climb dramatically when this hypersecretion occurs for long periods of time. In an effort to combat the effects of this hypersecretion, a wide variety of agents have been proposed that either block the histamine receptors, or target the acid extrusion pump (31). Of these various agents, the members of the omeprazole class of compounds targeting the pump have been highly successful in providing inhibition of acid secretion (29). However, recent data now demonstrates new potential side effects of these agents resulting in everything from zinc depletion in parietal cells to reduction in efficacy of statins (32). Furthermore, recent surveys have shown that in ~60% of the patients chronically taking these drugs, they are experiencing "breakthrough" or recurrence of symptoms while still taking the medication (33,34).

With this overlying dissatisfaction of therapy for acid secretory disease, we chose to investigate the potential of using Zinc to suppress acid secretion.

Zinc is an important element for cell growth differentiation and viability, a decrease in cellular zinc levels can result in neuronal damage, digestive tract issues, and developmental issues in infants (35). As zinc has such a key role in cellular viability and levels of zinc appear to fall in patients receiving PPI therapy, we hoped that by increasing the concentration of intracellular zinc we would not only retain cellular viability but could potentially inhibit acid secretion directly in the parietal cells (36).

Previous studies showing a positive effect of zinc on ulcers and gastric erosions focused on the barrier and the positive effect zinc

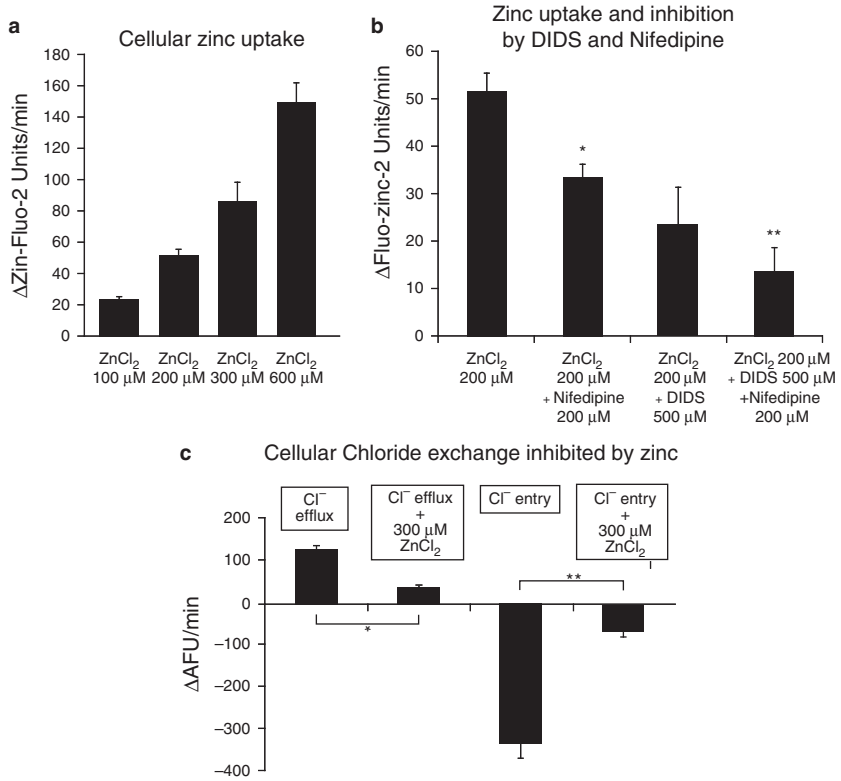

Figure 4. Zinc uptake by the parietal cell. (**a**) The zinc uptake by the parietal cell is proportional to the extracellular $ZnCl_2$ concentration (30 cells, 4 glands, 3 animals for each $Zncl_2$ concentration). Zinc uptake and inhibition by DIDS and Nifedipine. The control in (**b**) shows a robust entry of zinc in the parietal cell with an extracellular zinc concentration of 200 μM (51.71±3.68 ΔUnits/min). Adding 200 μM Nifedipine to the superfusion bath, the zinc uptake was significantly reduced (33.41±2.79 ΔUnits/min). In all, 500 μM DIDS (4,4′-diisothiocyano-2,2′-stilbene disulphonic acid) decreased the zinc uptake into the cell even more than Nifedipine (23.47±7.8 ΔUnits/min). Adding both, DIDS and Nifedipine, the inhibitory effect on the zinc entry was increased in comparison to the drug alone (13.58±5.26 ΔUnits/min) (*$P<0.0001$ compared with $ZnCl_2$ alone, **$P<0.004$ compared with $ZnCl_2$ and Nifedipine). Cellular chloride exchange inhibited by Zinc. (**c**) Cl^- influx and efflux from individual parietal cells measured by using the intracellular Cl^- dye *N*-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide (MQAE). A decrease in fluorescence intensity represents a rise in Cl^-_i concentration (influx), whereas an increase signifies a reduction in Cl^-_i concentration (efflux). As shown in the bar graph, 300 μM $ZnCl_2$ reduced the Cl^- entry and the Cl^- efflux of the parietal cell significantly in comparison to the control experiments (Cl^- efflux 126.74±7.77 ΔAFU/min reduced by $ZnCl_2$ to 36.07±.7.7 ΔAFU/min) (Cl^- entry −325.83±31.52 ΔAFU/min reduced by $ZnCl_2$ to −62.60±5.51 ΔAFU/min) (*$P<0.0001$, **$P<0.0001$). AFU, arbitrary fluorescence unit.

had on healing and on thickening of the mucous gel layer over time, which would buffer excess acid. In those previous studies, no attention was given to immediate actions of zinc, rather only on the replacement of the barrier and accelerated healing of the associated acid-induced leasons (37–39). Other studies described the inhibitory effect of zinc on gastric acid secretion also in animal and in a few human trials. These studies could not demonstrate a cellular mechanism for the inhibitory effect of zinc. In addition, most of the studies used very high doses of zinc (40–42). We are the first group to demonstrate a direct effect of zinc at the parietal cell level and its ability to inhibit acid secretion.

One key question for us was could we obtain a sufficiently high intracellular concentration of zinc to induce suppression of secretagogue-induced acid secretion. Previous studies in neurons demonstrated that zinc can enter cells either through the Cl^- cotransporters, or via Ca^{2+} channels (43).

In this study, we elucidate a role for zinc as a direct cellular suppressor of gastric acid secretion in animals and humans. Acid secretion was induced by the classically known secretagogue histamine, which resulted in a robust proton extrusion via the H^+,K^+-ATPase in comparison to basal acid secretion in the resting, non-stimulated rat and human gastric gland (**Figure 2**). In subsequent studies, we examined the inhibitory effects of $ZnCl_2$ on secretagogue-sensitive gastric acid secretion. We demonstrated the inhibitory potency of $ZnCl_2$ on histamine-induced acid secretion (**Figure 1**). $ZnCl_2$ abolished proton extrusion to a level comparable to that of the control experiments in both, human and rat gastric glands (**Figure 1a**).

The dose applied would be equivalent to 40 mg supplementation per day in humans. In the literature, the amount considered to be toxic is 10 times higher (44,45). With the daily recommended amount of zinc intake to maintain cell viability and membrane integrity being ~10–15 mg, a 40 mg $ZnCl_2$ oral acid blocker would be significantly lower than reported toxic doses and only slightly higher than the daily recommended amount (46). At the present time, it is not known if $ZnCl_2$ is working directly on inhibiting the H^+,K^+-ATPase of the parietal cell, or that the increased intracellular concentration of zinc leads

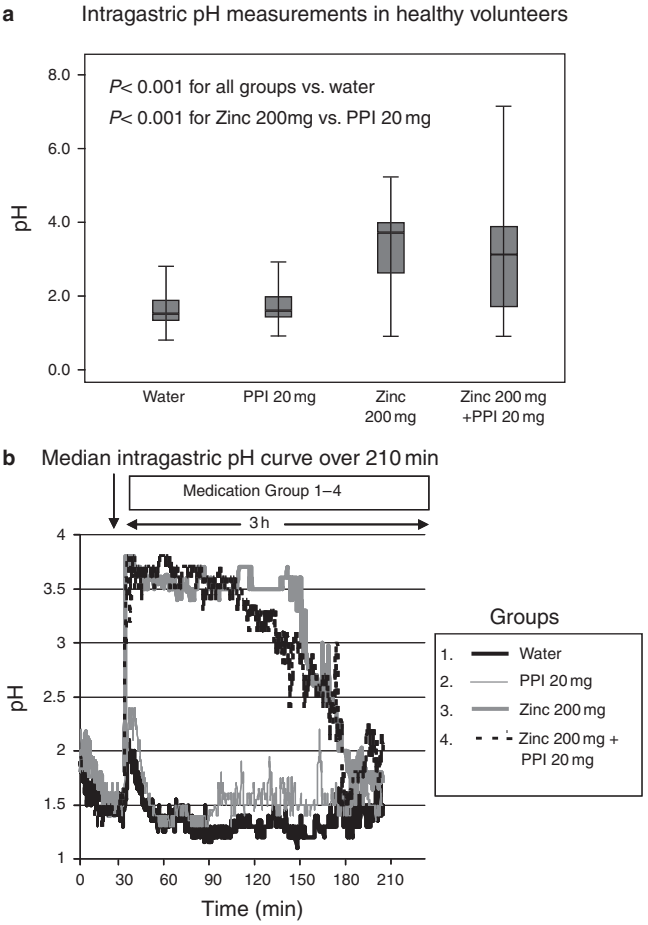

Figure 5. Zinc increased intragastric pH in healthy adult subjects. Box and Whisker Plots of the median pH of four groups (200 ml water, 20 mg proton pump inhibitor (PPI), 200 mg zinc sulfate, and 200 mg zinc sulfate + 20 mg omeprazole) over 180 min after the medication was given. In the zinc group, the pH raised >2 Units and was statistically significant in comparison to the PPI and water group. The combination of zinc and PPI also increased the pH significantly in comparison to water but did not show a stronger effect than zinc alone (**a**). Zinc increased intragastric pH in healthy adult subjects. (**b**) The intragastric pH measurements of the four groups shown as median curves of each group. After the first 30 min of basal pH, the randomized medication was given. Although the water and PPI group only show a small peak after drug administration, the zinc containing groups illustrate a rapid pH elevation that prolonged over 180 min.

to inhibition of Cl^- efflux and entry (47), or inhibits Ca^{2+} by competing with calcium, which therefore prevents the formation of acid. Furthermore, zinc has been shown to rapidly and reversibly effect Aquaporin 4, which has also been identified in the stomach (48). As shown in **Figure 4c**, addition of $ZnCl_2$ can significantly affect both the efflux of Cl^- from the apical surface or the influx of Cl^- from the basolateral membrane. Previous studies in a wide variety of cells described zinc entry into the cell through voltage-dependent Ca^{2+} channels and/or the Cl^-/HCO_3 exchanger on the basolateral membrane (49). This was confirmed by our observation that zinc entry can be blocked by Nifedipine and DIDS (**Figure 4b**). To determine whether our cellular data was relevant to what occurs in the intact stomach, we fed a series of rats two different doses of $ZnCl_2$ in their drinking water for 5 days prior to harvesting the gastric glands for study, paired control animals were run to compare. Proton extrusion in $ZnCl_2$-treated rats was significantly lower than acid secretion by our control group (**Figure 3**). These data suggest that following a long-term constant dosing of zinc, a sustained suppression of acid secretion occurred.

In healthy human volunteers, we could demonstrate the rapid increase of gastric pH after oral zinc administration (**Figure 5a, b**), which was significantly higher than receiving either water or a PPI. The effect of 200 mg oral zinc continued for ~3 h. During this time, the pH in the PPI and water group remained low. As mentioned in the introduction, PPIs have a delayed onset of acute action and the full inhibitory effect is slow requiring several dose cycles. For example, omeprazole reaches only 30% inhibition of acid secretion on the first day of treatment (50).

In summary, our findings indicate that zinc offers a rapid and prolonged inhibition of gastric acid secretion. It is a reversible and fast acting inhibitor of acid secretion in single rat and human gastric glands and also in whole rat stomach preparations. In addition, we were able to confirm these effects in a randomized cross-over trial in 12 healthy subjects. This rapid onset of action and reversibility may be due to its rapid entry and blockade of a chloride efflux pathway, which is necessary for maintaining the negative charge into the gastric lumen necessary to allow for gastric acid formation and secretion. Furthermore, we demonstrate that increasing the concentration of the essential element zinc can provide an alternative or supplemental treatment strategy for patients with gastroesophageal reflux disease, and breakthrough gastroesophageal reflux disease by providing a rapid inhibition and protection from the hypersecretion of acid.

This new therapeutic can target patients that are unhappy with their present acid suppression therapy as well as providing an essential element for cell function that is potentially lost on conventional PPI therapy.

CONFLICT OF INTEREST

Guarantor of the article: Philipp Kirchhoff, MD.

Specific author contributions: Designed the study, analyzed the data, performed statistical analysis, drafted the manuscript, had full access to all of the data in the study, and took responsibility for the integrity of the data and the accuracy of the data analysis: Philipp Kirchhoff, John P. Geibel; participated in the design and coordinated the study: Thenral Socrates, Shafik Sidani, Tobias Breidthardt; helped to draft the manuscript: Christoph Beglinger and Daniel Oertli; helped to draft the manuscript and study design: Carsten T. Viehl; performed the intragastric pH measurements: Christian Grob. All authors read and approved the final manuscript.

Financial support: This study was supported by a grant from the Swiss National Science Foundation to P.K. (PBZHB-110427), the Nachwuchsförderung of the University of Basel to P.K., and by an Oshe Grant to J.P.G.

Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Zinc is known to promote wound healing, including gastric ulcers.
- ✓ Zinc-deficient patients are at risk to develop gastric lesions.
- ✓ Effect of proton pump inhibitors taken orally shows a late onset of effect, with no immediate symptom relief.

WHAT IS NEW HERE

- ✓ Zinc inhibits gastric acid secretion within seconds and the effect is prolonged.
- ✓ Gastric acid secretion is inhibited by zinc taken orally; intragastric pH remained elevated over 3 h.
- ✓ The inhibitory effect of zinc occurred within minutes in comparison to the proton pump inhibitor (Omeprazole).

How might it impact on clinical practice in the foreseeable future

- ✓ There are an increasing number of patients that are insensitive to proton pump inhibitor (PPI) treatment and have recurrent symptoms of acid reflux disease.
- ✓ Zinc can be taken orally and seems to have a potent effect. As it is an essential component to cell function, it might have fewer side effects than other commonly used acid inhibitory drugs.
- ✓ Zinc has a fast onset of efficacy. This is an important characteristic in the treatment of reflux disease. Symptom relief might be faster in comparison to other antacid preparations.
- ✓ Under long-term PPI treatment intestinal zinc absorption is decreased. This side effect would be avoided by a combination of PPI and zinc application in long-term treatment providing both short- and long-term efficacy.

REFERENCES

1. Hersey SJ, Sachs G. Gastric acid secretion. Physiol Rev 1995;75:155–89.
2. Aihara T, Nakamura E, Amagase K *et al.* Pharmacological control of gastric acid secretion for the treatment of acid-related peptic disease: past, present, and future. Pharmacol Ther 2003;98:109–27.
3. Gardner JD, Sloan S, Miner PB *et al.* Meal-stimulated gastric acid secretion and integrated gastric acidity in gastro-oesophageal reflux disease. Aliment Pharmacol Ther 2003;17:945–53.
4. Williams JL. Gastroesophageal reflux disease: clinical manifestations. Gastroenterol Nurs 2003;26:195–200.
5. Lehmann F, Hildebrand P, Beglinger C. New molecular targets for treatment of peptic ulcer disease. Drugs 2003;63:1785–97.
6. Eisen GM, Sandler RS, Murray S *et al.* The relationship between gastroesophageal reflux disease and its complications with Barrett's esophagus. Am J Gastroenterol 1997;92:27–31.
7. Turcotte S, Duranceau A. Gastroesophageal reflux and cancer. Thorac Surg Clin 2005;15:341–52.
8. Robinson M. Proton pump inhibitors: update on their role in acid-related gastrointestinal diseases. Int J Clin Pract 2005;59:709–15.
9. Garnett WR. Lansoprazole: a proton pump inhibitor. Ann Pharmacother 1996;30:1425–36.
10. Katz PO, Hatlebakk JG, Castell DO. Gastric acidity and acid breakthrough with twice-daily omeprazole or lansoprazole. Aliment Pharmacol Ther 2000;14:709–14.
11. Carlsson R, Galmiche JP, Dent J *et al.* Prognostic factors influencing relapse of oesophagitis during maintenance therapy with antisecretory drugs: a meta-analysis of long-term omeprazole trials. Aliment Pharmacol Ther 1997;11:473–82.
12. Kleinman L, McIntosh E, Ryan M *et al.* Willingness to pay for complete symptom relief of gastroesophageal reflux disease. Arch Intern Med 2002;162:1361–6.
13. Tytgat GN. Shortcomings of the first-generation proton pump inhibitors. Eur J Gastroenterol Hepatol 2001;13 (Suppl 1): S29–33.
14. Gedda K, Scott D, Besancon M *et al.* Turnover of the gastric H+,K(+)-adenosine triphosphatase alpha subunit and its effect on inhibition of rat gastric acid secretion. Gastroenterology 1995;109:1134–41.
15. Diamond I, Hurley LS. Histopathology of zinc-deficient fetal rats. J Nutr 1970;100:325–9.
16. Elmes ME, Jones JG. Ultrastructural studies on Paneth cell apoptosis in zinc deficient rats. Cell Tissue Res 1980;208:57–63.
17. Fong LY, Lee JS, Chan WC *et al.* Zinc deficiency and the development of esophageal and forestomach tumors in Sprague-Dawley rats fed precursors of N-nitroso-N-benzylmethylamine. J Natl Cancer Inst 1984;72:419–25.
18. Ng WL, Fong LY, Ma L *et al.* Dietary zinc deficiency and tumorigenesis: a scanning electron microscope study. J Electron Microsc (Tokyo) 1984;33:344–8.
19. Sunderman FW Jr. The influence of zinc on apoptosis. Ann Clin Lab Sci 1995;25:134–42.
20. Cho CH, Fong LY, Ma PC *et al.* Zinc deficiency: its role in gastric secretion and stress-induced gastric ulceration in rats. Pharmacol Biochem Behav 1987;26:293–7.
21. Frommer DJ. The healing of gastric ulcers by zinc sulphate. Med J Aust 1975;2:793–6.
22. Watanabe T, Arakawa T, Fukuda T *et al.* Zinc deficiency delays gastric ulcer healing in rats. Dig Dis Sci 1995;40:1340–4.
23. Naess K. Zinc in the treatment of stomach ulcer. Tidsskr Nor Laegeforen 1976;96:1334.
24. Schettino T, Kohler M, Fromter E. Membrane potentials of individual cells of isolated gastric glands of rabbit. Pflugers Arch 1985;405:58–65.
25. McDaniel N, Lytle C. Parietal cells express high levels of Na-K-2Cl cotransporter on migrating into the gastric gland neck. Am J Physiol 1999;276 (5 Part 1): G1273–8.
26. Geibel JP, Wagner CA, Caroppo R *et al.* The stomach divalent ion-sensing receptor scar is a modulator of gastric acid secretion. J Biol Chem 2001;276:39549–52.
27. Dufner MM, Kirchhoff P, Remy C *et al.* the calcium-sensing receptor (CaSR) acts as a modulator of gastric acid secretion in freshly isolated human gastric glands. Am J Physiol Gastrointest Liver Physiol 2005;289:G1084–90.
28. Paradiso AM, Negulescu PA, Machen TE. Na+-H+ and Cl(-)-OH-(HCO3-) exchange in gastric glands. Am J Physiol 1986;250 (4 Part 1): G524–34.
29. Geibel JP, Wagner C. An update on acid secretion. Rev Physiol Biochem Pharmacol 2006;156:45–60.
30. Hersey SJ, Sachs G. Gastric acid secretion. Physiol Rev 1995;75:155–89.
31. Sachs G, Munson K, Hall K *et al.* Gastric H+,K(+)-ATPase as a therapeutic target in peptic ulcer disease. Dig Dis Sci 1990;35:1537–44.
32. Ma TK, Lam YY, Tan VP *et al.* Impact of genetic and acquired alteration in cytochrome P450 system on pharmacologic and clinical response to clopidogrel. Pharmacol Ther 2010;125:249–59.
33. Grigolon A, Cantu P, Savojardo D *et al.* Esophageal acid exposure on proton pump inhibitors in unselected asymptomatic gastroesophageal reflux disease patients. J Clin Gastroenterol 2008;42:969–73.
34. Chey WD, Mody RR, Izat E. Patient and physician satisfaction with proton pump inhibitors (PPIs): are there opportunities for improvement? Dig Dis Sci 2010; DOI: 10.1007/s10620-010-1209-2.
35. Song Y, Leonard SW, Traber MG *et al.* Zinc deficiency affects DNA damage, oxidative stress, antioxidant defenses, and DNA repair in rats. J Nutr 2009;139:1626–31.
36. Ali T, Roberts DN, Tierney WM. Long-term safety concerns with proton pump inhibitors. Am J Med 2009;122:896–903.
37. Cho CH, Ogle CW, Dai S. Effects of zinc sulphate pretreatment on gastric acid secretion and lesion formation in rats infused intravenously with graded doses of methacholine. Pharmacology 1978;17:32–8.
38. Cho CH, Ogle CW, Dai S. Effects of zinc chloride on gastric secretion and ulcer formation in pylorus-occluded rats. Eur J Pharmacol 1976;38:337–41.
39. Escolar G, Bulbena O. Zinc compounds, a new treatment in peptic ulcer. Drugs Exp Clin Res 1989;15:83–9.
40. Bulbena O, Esplugues JV, Escolar G *et al.* Zinc acexamate inhibits gastric acid and pepsinogen secretion in the rat. J Pharm Pharmacol 1990;42:252–6.
41. McLeay LM, Smith BL. Effects of intraruminal administration of zinc on gastric acid secretion in sheep. Res Vet Sci 1977;23:243–5.

42. Puscas I, Sturzu L, Buzas G. Effect of ZnSO4 upon gastric acid secretion and carbonic anhydrase. Int J Clin Pharmacol Ther Toxicol 1985;23:609–12.
43. Hershfinkel M, Moran A, Grossman N *et al.* A zinc-sensing receptor triggers the release of intracellular Ca2+ and regulates ion transport. Proc Natl Acad Sci USA 2001;98:11749–54.
44. Inoue K, Branigan D, Xiong ZG. Zinc-induced neurotoxicity mediated by transient receptor potential melastatin 7 channels. J Biol Chem 2010;285:7430–9.
45. Lewis MR, Kokan L. Zinc gluconate: acute ingestion. J Toxicol Clin Toxicol 1998;36:99–101.
46. Inoue K, Branigan D, Xiong ZG. Zinc-induced neurotoxicity mediated by transient receptor potential melastatin 7 channels. J Biol Chem 2010;285:7430–9.
47. Hoque KM, Rajendran VM, Binder HJ. Zinc inhibits cAMP-stimulated Cl secretion via basolateral K-channel blockade in rat ileum. Am J Physiol Gastrointest Liver Physiol 2005;288:G956–63.
48. Yukutake Y, Hirano Y, Suematsu M *et al.* Rapid and reversible inhibition of aquaporin-4 by zinc. Biochemistry 2009;48:12059–61.
49. Beharier O, Etzion Y, Katz A *et al.* Crosstalk between L-type calcium channels and ZnT-1, a new player in rate-dependent cardiac electrical remodeling. Cell Calcium 2007;42:71–82.
50. Dammann HG, Burkhardt F. Pantoprazole versus omeprazole: influence on meal-stimulated gastric acid secretion. Eur J Gastroenterol Hepatol 1999;11:1277–82.