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ORIGINAL ARTICLE

Suppression of vacuolar-type ATPase and induction of endoplasmic reticulum stress by proton pump inhibitors

Wei-Ping Lee[a,b,*]

[a]*Department of Medical Research, Taipei Veterans General Hospital, Taipei, Taiwan, ROC;* [b]*Institute of Biochemistry and Molecular Biology, College of Life Sciences, National Yang Ming Chiao Tung University, Taipei, Taiwan, ROC*

Abstract

Background: Proton pump inhibitors (PPIs), such as esomeprazole, pantoprazole, dexlansoprazole, and rabeprazole, are one of the most commonly prescribed medications. Several studies have linked the long-term use of PPIs to a potentially increased risk of gastric cancer. Therefore, this study aimed to determine the underlying mechanism of PPI-mediated gastric cancer.

Methods: Lysosomes were isolated using immunoprecipitation. The inhibition of vacuolar-type ATPase (V-ATPase) by PPIs was assayed using a PiColorLock Gold Phosphate Detection System. PPI-induced lysosomal stress was analyzed using transcription factor EB (TFEB) nuclear translocation. PPI-induced endoplasmic reticulum (ER) stress was analyzed using the expression of protein kinase RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6). Finally, reactive oxygen species (ROS) removal was determined using the activity of superoxide dismutase (SOD).

Results: PPIs caused a 70% inhibition of V-ATPase activity at 20 μM, leading to lysosomal stress through TFEB nuclear translocation; ER stress by inducing the expression of PERK, IRE1, and ATF6; and enhanced SOD activity for ROS removal.

Conclusion: The long-term use of PPIs inhibits lysosomal V-ATPase, leading to ER stress and ROS accumulation, which may result in an increased risk of gastric cancer. Because lysosomes and the ER are common organelles in cells, physicians prescribing PPIs for gastroesophageal reflux and peptic ulcer diseases should pay more attention to the general effects of these agents on the human body.

Keywords: ER stress; Lysosome; Proton pump inhibitor; ROS; V-ATPase

1. INTRODUCTION

Proton pump inhibitors (PPIs), such as esomeprazole, pantoprazole, dexlansoprazole, and rabeprazole, are one of the most commonly prescribed medications for gastroesophageal reflux disease (GERD) and peptic ulcers by inhibiting the secretion of gastric acid from parietal cells.[1] The first PPI, omeprazole, was introduced in 1989. PPIs are also used in stress ulcer prophylaxis, *Helicobacter pylori* eradication, and gastric protection against mucosal damage induced by nonsteroidal anti-inflammatory drugs (NSAIDs) and in the treatment of Zollinger-Ellison syndrome, upper gastrointestinal bleeding, chronic esophagitis, and dyspepsia.[2,3] Similar to various medications prescribed daily in hospitals and clinics, PPIs induce common minor adverse effects, including headache, nausea, diarrhea, abdominal pain, fatigue, and dizziness, which can be resolved by switching to a different PPI. Some infrequent adverse effects of PPIs are rashes, itching, flatulence, constipation, anxiety, and depression. PPI use may also be associated with myopathies, including rhabdomyolysis, which is considered a serious condition.[4]

Various studies have linked the long-term use of PPIs to some systemic severe adverse effects, such as an increased risk of osteoporosis-related fractures, infection with *Clostridium difficile*, malabsorption of vitamins and minerals (e.g., vitamin B12, calcium, and iron), dementia, pneumonia, kidney disease, and stroke.[5–7] In addition, some local effects induced by long-term PPI use are atrophic gastritis due to prolonged acid suppression, hypergastrinemia, chronic infection with *H. pylori*, and the development of gastric polyps.[8] Despite the established effectiveness of PPIs, several concerns have been raised regarding the safety of administering PPIs for extended periods and the serious adverse effects that PPIs may cause. For example, some studies have indicated that long-term use of PPIs is associated with an increased risk of gastric cancer, which ranks third among cancer-related mortalities worldwide.[9]

In 2006, García Rodríguez et al reported that PPI use for esophageal purposes to achieve long-term acid suppression (i.e., to ameliorate the symptoms of reflux and treat cases of esophagitis, Barrett's esophagus, or hiatal hernia) resulted in a fivefold increased risk of esophageal adenocarcinoma (odds ratio [OR] = 5.42, 95% confidence interval [CI] = 3.13-9.39). They also reported that PPI use for peptic ulcer purposes (i.e., for treating gastric, duodenal, and unspecified peptic ulcers) resulted in a greater-than-fourfold increased risk of gastric noncardia adenocarcinoma among long-term users (OR = 4.66, 95% CI = 2.42-8.97).[10] In a more recent study involving 63,397

*Address correspondence: Dr Wei-Ping Lee, Department of Medical Research, Taipei Veterans General Hospital, 201, Section 2 Shi-Pai Road, Taipei 112, Taiwan, ROC. E-mail address: wplee@vghtpe.gov.tw (W.-P. Lee).

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individuals, Cheung et al indicated that PPI use was associated with an increased risk of gastric cancer (hazard ratio [HR] = 2.44, 95% CI = 1.42-4.20) whereas H_2 blocker use was not (HR = 0.72, 95% CI = 0.48-1.07).[11] In a study involving 797,067 individuals, Brusselaers et al reported that the standardized incidence ratio of gastric cancer among PPI users was 3.38 (95% CI = 3.23-3.53) and that the risk of cancer increased with the duration of PPI use.[12]

Gastric carcinogenesis involves multiple gene mutations. In a whole-genome study on 153 patients with gastric cancer, Cai et al identified 35 substantially mutated genes, including *TP53*, *AKAP9*, *DRD2*, *PTEN*, *CDH1*, and *LRP2*. When the findings were referenced against the Cancer Genome Atlas, 29 of these genes were found to be novel substantially mutated genes.[13] Generally, *TP53* is the most frequent driver mutation, with a high mutation burden in *TP53* wild-type benign lesions. In addition, excessive reactive oxygen species (ROS) accumulation within cells is the main factor in progressive mutations during the course of carcinogenesis.[14] Here, we investigated the mechanism underlying PPI-mediated gastric cancer. The results indicated that PPIs also inhibited vacuolar-type ATPase (V-ATPase), leading to increased lysosomal and endoplasmic reticulum (ER) stress and ROS accumulation in gastric epithelial cells.

2. METHODS

2.1. Chemicals and reagents

Four PPIs (rabeprazole, dexlansoprazole, esomeprazole, and pantoprazole) were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) and dissolved in dimethyl sulfoxide, and a pcDNA3.1-Tmem192-3xHA plasmid was purchased from Addgene.

2.2. Cell culture

GES-1 cells (a normal human gastric epithelial cell line transformed by simian virus 40) were purchased from the American Type Culture Collection (CVCL EQ22) and grown in Dulbecco's modified Eagle's medium/F-12 medium (Thermo Fisher Scientific Inc, Waltham MA, USA) supplemented with 100 U/mL penicillin G, 100 μg/mL streptomycin, and 20% fetal bovine serum (Cytiva, Logan UT, USA) at 37°C with 5% CO_2. HEK-293T cells were grown under the same conditions used for the GES-1 cells.

2.3. Isolation of lysosomes by antihemagglutinin magnetic bead immunoprecipitation

Tmem192 is a transmembrane protein of lysosomes involved in lysosomal autophagy and apoptosis. Here, the pcDNA3.1-Tmem192-3xHA plasmid was first transfected into HEK-293T cells, and stable cells were selected with G418 (Merck KGaA, Darmstadt, Germany). Subsequently, the Tmem192-3xHA (3xHA-tagged Tmem192) protein was translocated to the lysosome membrane. To isolate the lysosomes, antihemagglutinin (anti-HA) magnetic beads were used to pull down the Tmem192-3xHA–lysosome cells (HA-Lyso cells), as described by Abu-Remaileh et al.[15] Briefly, 1×10^7 HA-Lyso HEK-293T cells cultured on 15-cm plates (with or without PPI treatment) were used for each lysosomal immunoprecipitation (LysoIP) assay. The cells were then quickly rinsed twice with phosphate-buffered saline (PBS), scraped in 1 mL of KTris buffer (136 mM KCl, 10 mM Tris, pH 7.25), and centrifuged at 1000*g* for 2 minutes at 4°C. Next, pelleted cells were resuspended in 1 mL of KTris buffer and gently homogenized with 20 strokes in a 2-mL homogenizer. Subsequently, the homogenate was centrifuged at 1000*g* for 2 minutes at 4°C, and the supernatant containing the cellular organelles (including the lysosomes) was incubated with 150 μL of KTris prewashed anti-HA magnetic beads (Thermo Fisher Scientific Inc, Waltham MA, USA) on a gentle rotary shaker for 3 minutes. The immunoprecipitates were then gently washed three times with KTris on a DynaMag-Spin magnet (Thermo Fisher Scientific Inc, Waltham MA, USA). Finally, the anti-HA lysosomes were eluted from the magnetic beads in a buffer containing 40 mM Tris, 80 mM NaCl, 8 mM $MgCl_2$, and 1 mM EDTA (pH 7.5).

2.4. Lysosomal vacuolar-type ATPase assay

Protein from HA-LysoIP (i.e., lysosomes) was quantitated using the Bio-Rad Protein Assay. The V-ATPase activity of the lysosomes (2 μg) was assayed in a buffer containing 40 mM Tris, 80 mM NaCl, 8 mM $MgCl_2$, and 1 mM EDTA (pH 7.5), with a total volume of 45 μL. The reaction mixture was then completed to 50 μL by adding 5 μL of ATP (100 mM), followed by incubation at room temperature for 10 minutes. ATPase activity was calculated as units per milligram of protein, with one unit of ATPase activity defined as the hydrolysis of 1 μmol ATP per minute. The PO_4^{3-} released was assayed using a PiColorLock Gold Phosphate Detection System (Abcam, Cambridge, United Kingdom). After the incubation of the mixture for 10 minutes at room temperature, 2 μL of 0.5 M EDTA was added to the aforementioned 50 μL reaction mixture to stop the reaction, followed by the addition of 15 μL of an accelerator agent (diluted at 1:100, available in the kit) and 7 μL of a stabilizer agent (available in the kit). Finally, color development was assessed at 635 nm (OD_{635}) on a 96-well plate.

2.5. Lysosomal stress assay

GES-1 cells were treated with 5 μM PPIs (rabeprazole, pantoprazole, esomeprazole, or dexlansoprazole) for 1 week and then subjected to nuclear fractionation. Briefly, 5×10^6 cells were collected in PBS by centrifugation and washed twice with cold PBS. Subsequently, the cells were gently resuspended in 500 μL of a hypotonic buffer (20 mM Tris HCl [pH 7.4], 10 mM NaCl, and 3 mM $MgCl_2$) by pipetting up and down several times and incubated on ice for 15 minutes. Next, 25 μL of 10% NP-40 was added, followed by vortexing for 10 seconds. The homogenate was then centrifuged for 10 minutes at 3000 rpm at 4°C. The supernatant obtained contained the cytoplasmic fraction, and the pellet obtained was the nuclear fraction. Thereafter, the nuclear fraction (pellet) was resuspended in 50 μL of a nuclear extraction buffer (10 mM Tris [pH 7.4], 2 mM Na_3VO_4, 100 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10% glycerol, 1 mM EGTA, 0.1% sodium dodecyl sulfate, 1 mM NaF, 0.5% deoxycholate, and 20 mM $Na_4P_2O_7$) for 30 minutes on ice along with vortexing at 10-minute intervals. Subsequently, the sample was centrifuged for 30 minutes at 14,000*g* at 4°C. Finally, the supernatant (nuclear fraction) was collected and subjected to Western blotting by using anti–transcription factor EB (anti-TFEB) and anti-histone H3B (Abcam, Cambridge, United Kingdom) antibodies.

2.6. Endoplasmic reticulum stress assay

GES-1 cells were treated with 5 μM esomeprazole, rabeprazole, dexlansoprazole, or pantoprazole for 4 weeks. At the end of each week, the cells were harvested, saved, and subjected to Western blotting by using anti–protein kinase RNA-like endoplasmic reticulum kinase (anti-PERK; Abcam, Cambridge, United Kingdom), anti–inositol-requiring enzyme 1 (anti-IRE1; Abcam, Cambridge, United Kingdom), anti–activating transcription factor 6 (anti-ATF6; Abcam, Cambridge, United Kingdom), and anti-β-actin (Santa Cruz) antibodies. Briefly, the cells were lysed in a buffer containing 50 mM Tris HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 5 mM EDTA, and SIGMAFAST Protease Inhibitor Cocktail. The cell lysates were then resolved using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane. Finally, the membrane was treated with

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a primary antibody, followed by incubation with a peroxidase-conjugated secondary antibody and detection with an enhanced chemiluminescence method.

2.7. Superoxide dismutase assay

In total, 2×10^5 GES-1 cells in 10-cm tissue culture dishes were treated with 5 μM esomeprazole, rabeprazole, dexlansoprazole, or pantoprazole for 1 week and then subjected to a superoxide dismutase (SOD) assay. The SOD activity was then analyzed using a SOD assay kit acquired from Cayman Chemical Company (Ann Arbor, MI, USA). Briefly, cells were harvested using trypsin digestion followed by washing with PBS and centrifugation at 1000*g*. Subsequently, cell pellets were resuspended and homogenized in a 20 mM cold HEPES buffer (pH 7.2) containing 1 mM EGTA, 210 mM mannitol, and 70 mM sucrose in a 2-mL homogenizer. Finally, the lysate was centrifuged at 1500*g* for 5 minutes at 4°C, and the supernatant was collected to perform the assay exactly per the supplier's protocol.

3. RESULTS

3.1. Inhibition of vacuolar-type ATPase by PPIs

After the lysosomes were prepared, they were subjected to Western blotting with anti-HA, anti–lysosomal-associated membrane protein 1 (anti-LAMP1; Abcam, Cambridge, United Kingdom), and anti–voltage-dependent anion channel (anti-VDAC, a mitochondrial marker; Abcam, Cambridge, United Kingdom) antibodies to verify the successful isolation of the lysosomes (Fig. 1A). Tmem192-3xHA-expressing HEK-293T cells were then treated with PPIs (esomeprazole, rabeprazole, lansoprazole, or dexlansoprazole) at concentrations of 0, 1, 5, 10, and 20 μM for 24 hours and subjected to lysosome preparation. Generally, V-ATPase is the major ATPase present in the lysosomes, pumping protons into the lysosomes to maintain an acidic pH (approximately 4.5-5.5). As inhibitors of H^+-K^+-ATPase in gastric parietal cells, PPIs also inhibited lysosomal V-ATPase in a dose-dependent manner (Fig. 1B). Both esomeprazole and rabeprazole exhibited similar inhibitory activity (approximately 75% reduction) greater than that of pantoprazole and dexlansoprazole (approximately 65% reduction) at 20 μM.

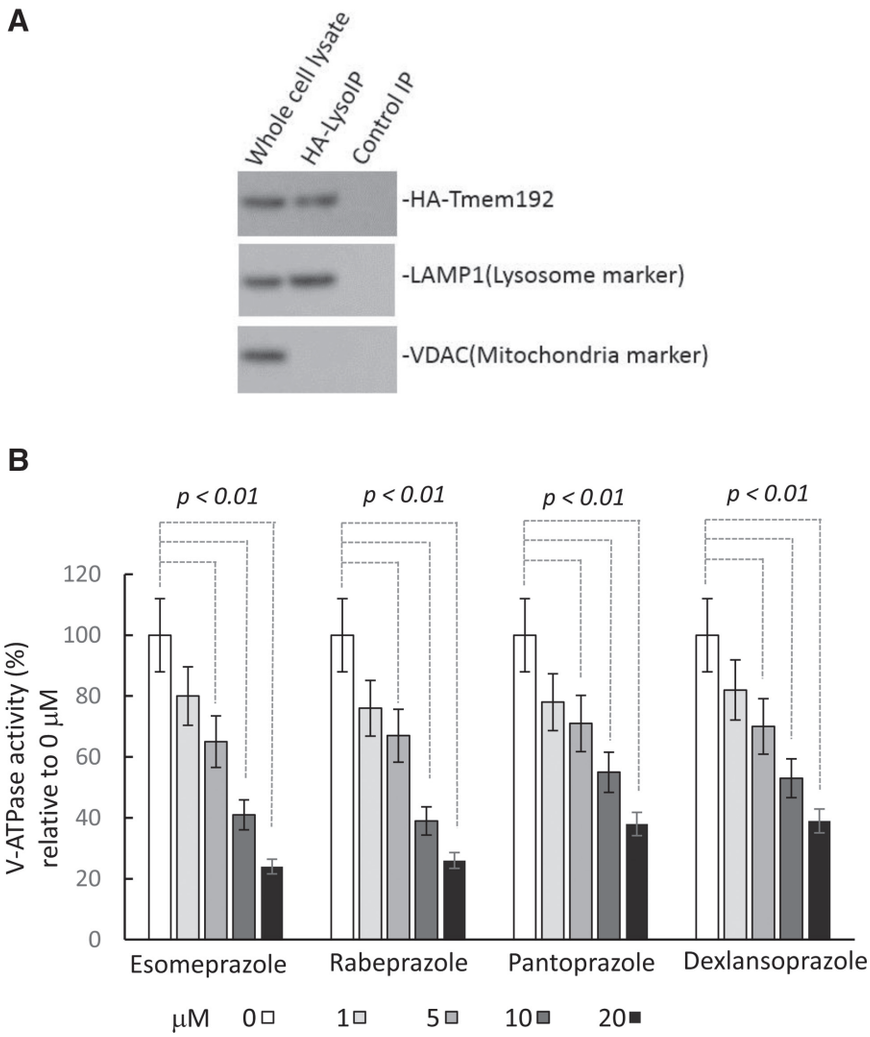

Fig. 1 Lysosome preparation and vacuolar-type ATPase (V-ATPase) assay. A, Western blot showing that hemagglutinin-lysosomal immunoprecipitation (HA-LysoIP) contains the lysosome marker lysosomal-associated membrane protein 1 (LAMP1) but not the mitochondrial marker voltage-dependent anion channel (VDAC). HA-LysoIP, immunoprecipitation using anti–HA-antibody-conjugated magnetic beads. Control IP, immunoprecipitation using anti–FLAG-antibody-conjugated magnetic beads. B, ATPase assay showing the inhibitory effect of the four proton pump inhibitors on the V-ATPase activity of the lysosomes. Each bar is for an average of triplicate samples. All *p* values were determined using Student's *t* test.

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3.2. Induction of lysosomal stress by PPIs

As shown in Fig. 2, the inhibitory effect of PPIs on lysosomal V-ATPase suggests that PPIs interfere with lysosomal acidification and thereby trigger lysosomal stress.[16] In this scenario, phosphorylated TFEB (a master regulator of lysosomal gene transcription) becomes dephosphorylated and translocates into the nucleus to transcribe genes for lysosomal biogenesis. To confirm this process, GES-1 cells were treated with 5 μM PPIs for 1 week. Subsequently, the cells were harvested on days 1, 2, 4, and 7 for nuclear fractionation and subjected to Western blotting by using anti-TFEB and anti-histone H3B (Abcam, Cambridge, United Kingdom) antibodies (Fig. 3). As shown in Fig. 3, the four PPIs used in this study increased the translocation of TFEB into the nucleus: 2.81-fold increase with esomeprazole, 3.94-fold increase with rabeprazole, 2.14-fold increase with pantoprazole, and 3.51-fold increase with dexlansoprazole on day 7 compared with the respective densities of bands on day 0.

3.3. Induction of endoplasmic reticulum stress by PPIs

As shown in Figs. 1 and 3, PPIs inhibited V-ATPase and induced the translocation of TFEB into the nucleus. In other words, PPIs caused lysosomal stress, leading to inefficient digestion of erroneous or unfolded proteins produced in the ER. To determine whether ER stress occurred in PPI-treated GES-1 cells, the cells were treated with 5 μM PPIs for 4 weeks. At the end of each week, the cells were harvested and subjected to Western blotting by using anti-PERK (Abcam, Cambridge, United Kingdom), anti-IRE1 (Abcam, Cambridge, United Kingdom), anti-ATF6 (Abcam, Cambridge, United Kingdom), and anti-β-actin (Santa Cruz) antibodies (Fig. 4). PERK, IRE1, and ATF6 triggered three pathways of ER stress.[17] In addition, the densitometric data obtained revealed progressively enhanced expression of PERK, IRE1, and ATF6 compared with their levels at week 0 (Fig. 5). These results suggest that PPIs induced ER stress in GES-1 cells.

3.4. Induction of superoxide dismutase by PPIs

During ER stress, misfolded proteins are transferred to the lysosome for degradation. Because the formation of disulfide bonds by protein disulfide isomerase and endoplasmic reticulum oxidoreductin 1 (ERO1) may be the source of ROS, the burden of protein folding increases in the ER.[19] SOD converts superoxides into hydrogen peroxide, which is then eliminated by glutathione

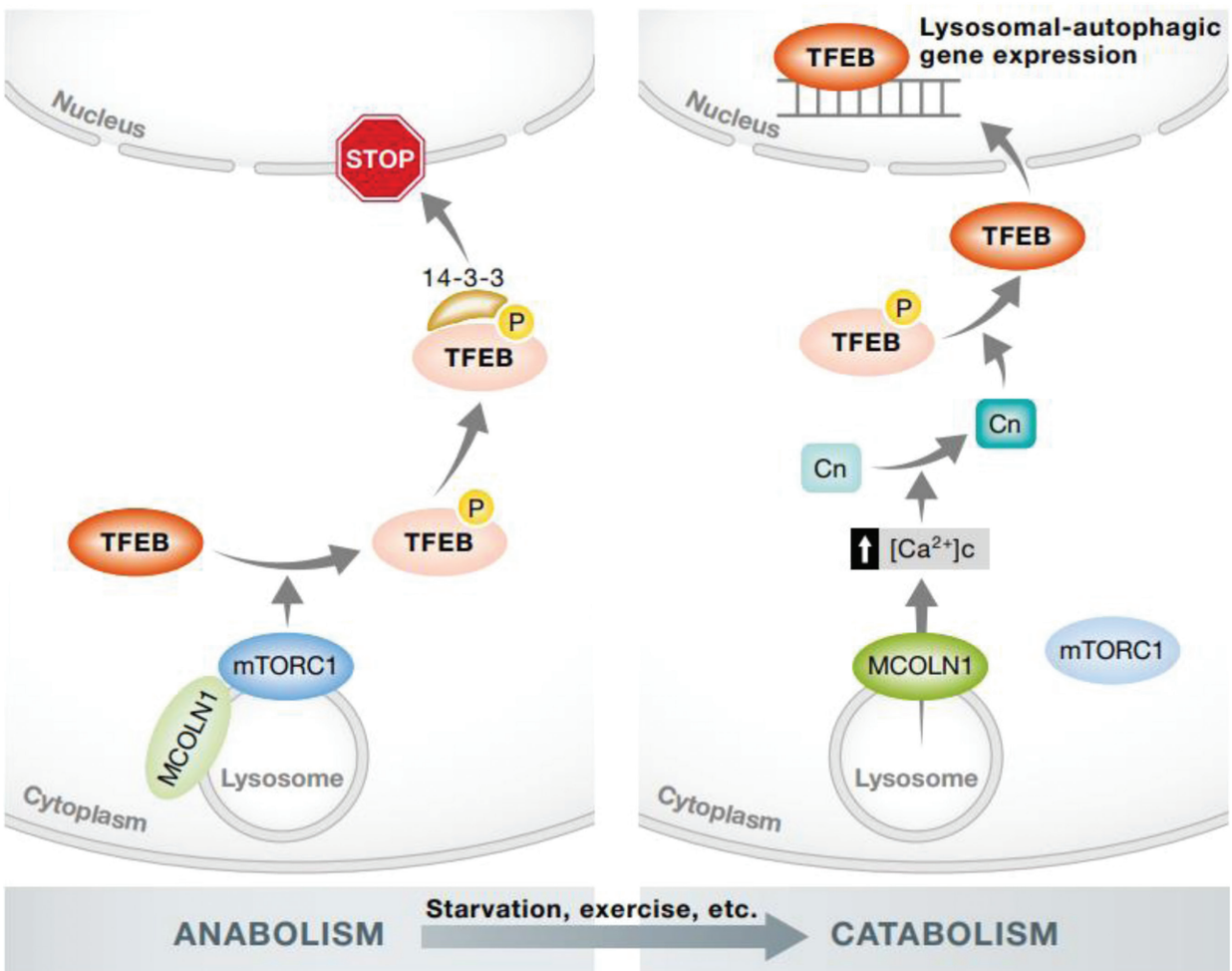

Fig. 2 Transcription factor EB (TFEB) nuclear translocation during lysosomal stress. Under normal conditions, TFEB is phosphorylated by mTORC1 on the lysosomal surface and sequestered in the cytoplasm by 14-3-3 proteins. During lysosomal stress, mTORC1 is inactivated and Ca^{2+} is released from the lysosome through MCOLN1, leading to local calcineurin (Cn) activation and TFEB dephosphorylation. The dephosphorylated TFEB can no longer bind 14-3-3 proteins and can freely translocate to the nucleus, where it transcriptionally activates the autophagy lysosomal pathway. This figure and its legend were adapted from Ballabio's review article with some modifications.[17]

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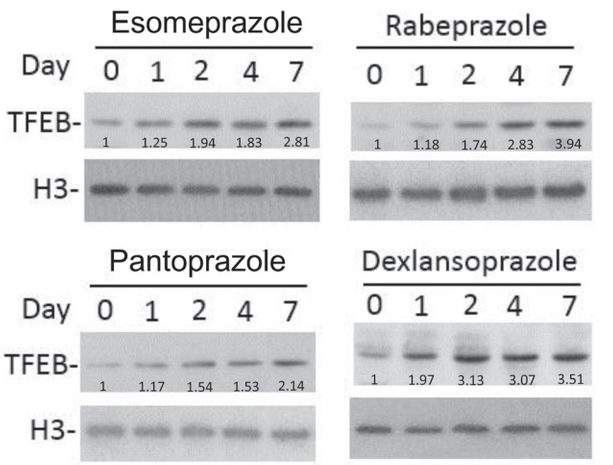

Fig. 3 Western blot showing increased transcription factor EB (TFEB) in the nuclear fraction of GES-1 cells treated with 5 μM PPIs (rabeprazole, pantoprazole, esomeprazole, or dexlansoprazole) for 1 wk. Cells were collected on days 0, 1, 2, 4, and 7. H3: histone H3B, a marker protein of the cell nucleus as a loading control. Relative band intensity was quantified using densitometric analysis, and relative protein expression levels were normalized to their respective histone H3B levels.

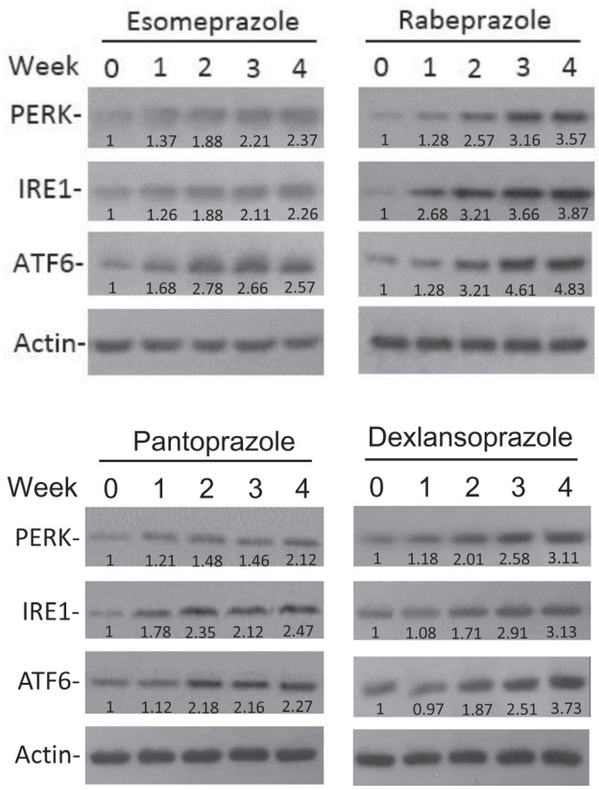

Fig. 4 Western blot showing increased expression of the three major ER stress proteins (i.e., protein kinase RNA-like endoplasmic reticulum kinase [PERK], inositol-requiring enzyme 1 [IRE1], and activating transcription factor 6 [ATF6]) in GES-1 cells treated with 5 μM proton pump inhibitors for 4 wk. Cells were collected at the end of each week. Here, β-actin is used as a loading control. Relative band intensity was quantified using densitometric analysis, and relative protein expression levels were normalized to their respective β-actin levels.

peroxidase or catalase. Here, we examined cellular ROS removal by analyzing the activity of SOD. GES-1 cells were treated with 5 μM PPIs for 1 week and then subjected to a SOD assay. As shown in Fig. 6, PPIs resulted in the induction of SOD (2.2-fold increase with esomeprazole, 1.7-fold increase with rabeprazole, 1.6-fold increase with pantoprazole, and 1.9-fold increase with dexlansoprazole compared with their respective controls), which helped decrease the amount of ROS within the cells, suggesting that PPIs induced ROS production.

4. DISCUSSION

Since their introduction to the market in 1989, PPIs have been considered a safe medication, whose minor side effects do not impede their use. In some clinical situations, a 4-month course of PPIs is considered routine therapy for GERD and peptic ulcer diseases. However, in *H. pylori*-negative individuals, the long-term use of PPIs is associated with an increased risk of gastric cancer,[11] although the underlying mechanism of this process remains unclear. Here, we reported that PPIs also inhibited lysosomal V-ATPase and suppressed H^+-K^+-ATPase in gastric parietal cells, which resulted in lysosomal and ER stress, leading to increased ROS accumulation within the cells. Notably, ROS are a major cause of genomic mutations and cancer.

Various studies have indicated that PPIs are overprescribed and even inappropriately prescribed in clinical practice. For example, because of the aging population, aspirin has been increasingly prescribed for cardiovascular and cerebrovascular diseases. According to the guidelines set by medical societies and scientific reports, PPIs should be used as a main option to prevent gastrointestinal complications in high-risk patients treated with aspirin. These PPI prevention therapies should also be used with patients taking NSAIDs for pain control, for example, to alleviate lower-back pain and arthritis. However, several studies have reported increased prescription of PPI co-therapy for irrelevant cases.[18–20] A similar trend of overprescription has also been observed in other clinical situations, such as in cases of dyspepsia, which can be treated with probiotics or lifestyle modifications. Overall, the lack of patent protection and the availability of most PPIs as generic drugs have substantially lowered their price and contributed to their increased use.

In 2016, PPI use was reported to be associated with an increased risk of senile dementia and chronic kidney disease, prompting clinicians to be more cautious when prescribing medications. In a study involving 73,679 participants aged 75 and older with no dementia at baseline, Gomm et al reported that patients who regularly received PPIs were at a significantly increased risk of incident dementia compared with those who did not receive PPI medications (HR = 1.44, 95% CI = 1.36-1.52, $p < 0.001$).[21] However, although Wijarnpreecha et al observed the same risk of dementia (44% increase),[22] subsequent studies in Germany and the United Kingdom have revealed that PPIs are associated with a decreased risk of dementia.[23,24] For example, Torres-Bondia et al indicated that PPI use was not associated with a risk of Alzheimer's disease (AD) (adjusted OR = 1.06, 95% CI = 0.93-1.21, $p = 0.408$), although a weakly but significantly increased risk of non-AD dementia was observed among PPI users (adjusted OR = 1.20, 95% CI = 1.05–1.37, $p = 0.007$).[25] These results suggest that PPI use is somehow associated with incident senile dementia.

In a study on the association between PPI use and renal disease, Lazarus et al indicated that twice-daily PPI use (adjusted HR = 1.46, 95% CI = 1.28-1.67, $p < 0.001$) was associated with an increased risk of renal disease compared with once-daily PPI use (adjusted HR = 1.15, 95% CI = 1.09-1.21, $p < 0.001$).[26] In a retrospective cohort study, Hart et al reported that PPI exposure was associated with an increased risk of

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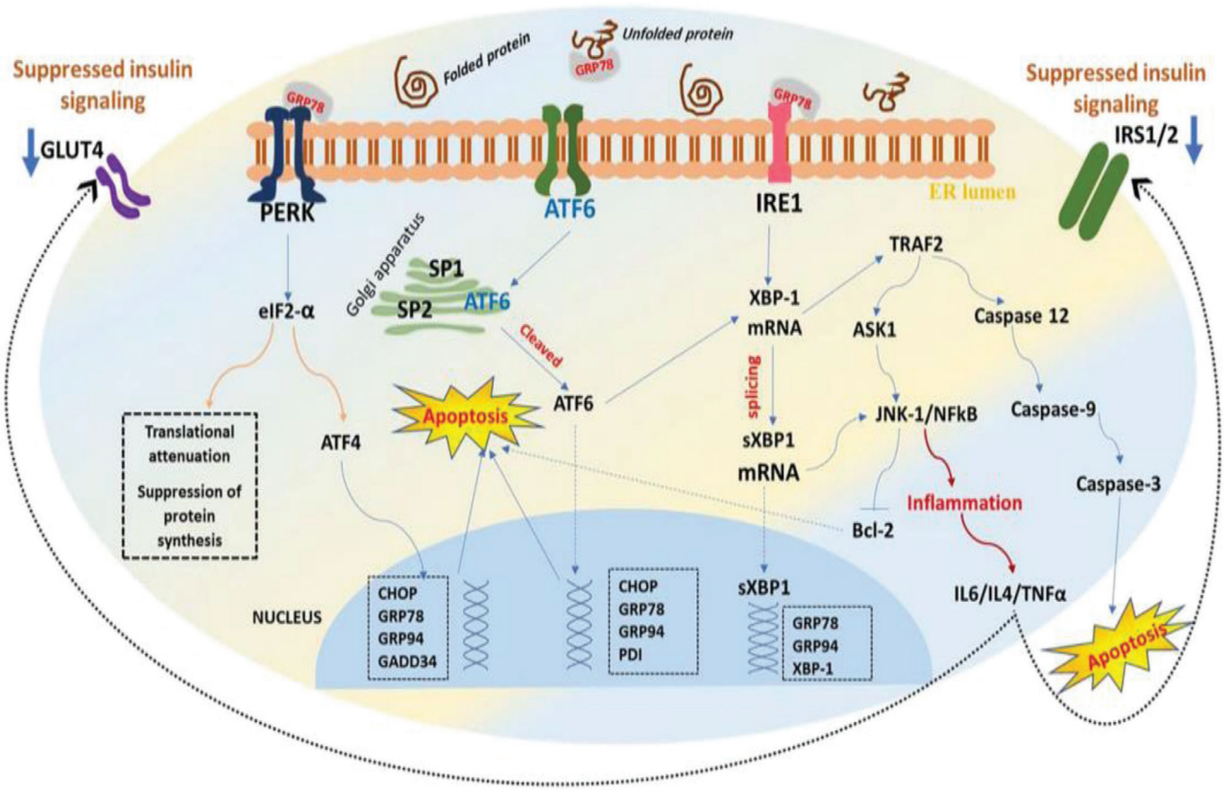

Fig. 5 Schematic of the downstream effectors and target genes in ER stress (unfolded protein response [UPR]), focusing on protein kinase RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6). Under ER stress, GRP78 dissociates from its ER luminal domain, leading to the oligomerization and autophosphorylation of PERK with subsequent activation of PERK with both kinase and endoribonuclease. Therefore, the α-subunit of eukaryotic initiation factor 2 (eIF2) undergoes phosphorylation, resulting in translational attenuation characterized by a reduction in protein biosynthesis. Simultaneously, this downstream phosphorylation of eIF2 leads to increased ATF4 expression and translocation into the nucleus, where it binds to the UPR element. This results in the transcriptional modification of UPR target genes, including proapoptotic transcription factors, C homologous protein (CHOP), GRP78, GRP94, and GADD34, and attenuation of the global translational process. However, PERK phosphorylation also inhibits the transcription of I kappa B alpha (IκBα), leading to NF-κB hyperactivation and increased production of inflammatory cytokines. Furthermore, ER stress leads to the autophosphorylation of IRE-1, resulting in the excision and splicing of its substrate, XBP-1 mRNA. This process, in turn, results in a spliced XBP-1 protein (sXBP1), which translocates into the nucleus, causing the upregulation of genes responsible for protein folding enzyme secretion and ER-associated protein degradation. Notably, ATF6 is activated after PERK and before IRE1. GRP78 dissociates from ATF6 and is recruited to luminal protein aggregates, resulting in the translocation of the cytosolic fraction of ATF6 to the Golgi apparatus, where it is spliced and proteolyzed by site-1 and site-2 proteases. Eventually, this process leads to the release of the cytosolic domain of ATF6 and its entry into the nucleus, where it is sequestered with the ER stress response element, resulting in the activation of UPR target genes (XBP1 and CHOP), GRP78/BiP, GRP94, and PDI. This figure and its legend were adapted from Amen et al.s' review article with some modifications.[18]

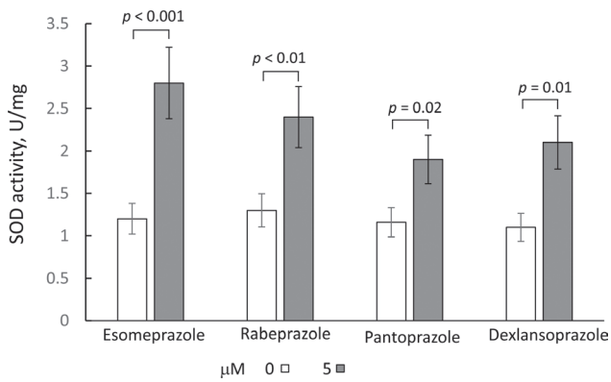

Fig. 6 Superoxide dismutase (SOD) activity in GES-1 cells treated with 5 μM proton pump inhibitors for 1 wk. Each bar is an average of triplicate samples. All *p* values were determined using Student's *t* test.

acute kidney injury (adjusted OR = 4.35, 95% CI = 3.14-6.04, $p < 0.0001$) and chronic kidney disease (adjusted OR = 1.20, 95% CI = 1.12-1.28, $p < 0.0001$).[27] Finally, a study employing the adverse event reporting system of the U.S. Food and Drug Administration indicated a strong association between PPI use and chronic kidney disease (relative OR = 8.80, 95% CI = 8.49-9.13) and acute kidney injury (relative OR = 3.95, 95% CI = 3.81-4.10).[28]

Overall, the mechanism underlying the associations between PPI use and gastric cancer, dementia, and renal disease remains unknown. The general inhibition of cellular lysosomal V-ATPase may be a major mechanism underlying PPI-induced ROS production within cells. Lysosomal acidification is required for the degradation of aged molecules and misfolded proteins. In this study, we reported that PPIs induced lysosomal and ER stress, leading to the accumulation of ROS, which may account for the clinical correlation between PPI use and an increased risk of gastric cancer, dementia, and renal disease.

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