

Highlights

- Proton pump inhibitors increase the risk of developing iron deficiency
- Omeprazole enhances the expression of hepcidin via aryl hydrocarbon receptor activation in HepG2 cells
- Levels of hepcidin increase in the blood and liver of mice treated with omeprazole
- Levels of duodenal and splenic ferroportin decrease in mice treated with omeprazole
- Omeprazole suppresses iron absorption through hepcidin-ferroportin-dependent axis in addition to elevated gastric pH levels, causing iron deficiency

1 **Proton pump inhibitors block iron absorption through direct**
2 **regulation of hepcidin via the aryl hydrocarbon**
3 **receptor-mediated pathway**

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1 27 **Abstract**

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4 28 Proton pump inhibitors (PPIs) have been used worldwide to treat gastrointestinal
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6 29 disorders. A recent study showed that long-term use of PPIs caused iron deficiency;
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9 30 however, it is unclear whether PPIs affect iron metabolism directly. We investigated
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12 31 the effect of PPIs on the peptide hepcidin, an important iron regulatory hormone. First,
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15 32 we used the FDA Adverse Event Reporting System database and analyzed the
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18 33 influence of PPIs. We found that PPIs, as well as H2 blockers, increased the odds ratio
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21 34 of iron-deficient anemia. Next, HepG2 cells were used to examine the action of PPIs
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24 35 and H2 blockers on hepcidin. PPIs augmented hepcidin expression, while H2 blockers
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27 36 did not. In fact, the PPI omeprazole increased hepcidin secretion, and
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30 37 omeprazole-induced hepcidin upregulation was inhibited by gene silencing or the
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33 38 pharmacological inhibition of the aryl hydrocarbon receptor. In mouse experiments,
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36 39 omeprazole also increased hepatic hepcidin mRNA expression and blood hepcidin
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39 40 levels. In mice treated with omeprazole, protein levels of duodenal and splenic
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41 41 ferroportin decreased. Taken together, PPIs directly affect iron metabolism by
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42 42 suppressing iron absorption through the inhibition of duodenal ferroportin via hepcidin
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43 43 upregulation. These findings provide a new insight into the molecular mechanism of
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44 44 PPI-induced iron deficiency.

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46 **Keywords:** proton pump inhibitor; hepcidin; iron deficiency

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- 47 **Abbreviations:** AhR, aryl hydrocarbon receptor; DMT1, divalent metal transporter 1;
- 48 FAERS, FDA Adverse Event Reporting System; FPN, ferroportin; FTH, ferritin heavy
- 49 chain; FTL, ferritin light chain; OME, omeprazole; PPIs, protein pump inhibitors;
- 50 ROR, reporting odds ratio; TfR1, transferrin receptor 1

1 51 **1. Introduction**

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4 52 Iron is an essential trace metal element in the body, and its deficiency causes
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7 53 anemia of microcytic and hypochromic corpuscles by interfering with hemoglobin
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10 54 synthesis. Almost all iron is recycled by the degradation of hemoglobin derived from
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13 55 old erythrocytes, however, it is also supplied by food. Iron has two forms, heme and
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16 56 non-heme. Whether non-heme iron is absorbed by the duodenum or the jejunum
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19 57 depends on a number of factors, including the presence of gastric acid (Zhang and Enns,
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22 58 2009). The acidic environment of the stomach promotes iron absorption by reducing
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25 59 insoluble iron (Fe^{3+}) to soluble iron (Fe^{2+}) ions and permitting the formation of soluble
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28 60 chelates (Jacobs and Miles, 1969).

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31 61 Worldwide, proton pump inhibitors (PPIs) and H2 blockers have been used
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34 62 for decades to treat gastric acid-related disorders (Scarpignato et al., 2006). Long-term
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37 63 use of PPIs or H2 blockers increases pH levels in the stomach by reducing the secretion
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40 64 of gastric acid, which decreases the digestion of proteins and the absorption of vitamins
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43 65 and minerals, including iron (Ito and Jensen, 2010). Several clinical studies have
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46 66 demonstrated that iron deficiency is induced in patients with long term use of PPIs and
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49 67 H2 blockers (Aymard et al., 1988; Lam et al., 2017). PPI- and H2 blocker-induced iron
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52 68 deficiency reduces iron absorption by altering the acidic environment of the
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55 69 gastrointestinal tract.

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57 70 Iron is normally absorbed from the small intestine via the action of
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60 71 ferroportin (FPN), a cellular iron exporter. Absorbed iron is delivered into the cytosol

1 72 and mitochondria of erythroblast, where it is used for heme synthesis, including the
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4 73 synthesis of hemoglobin. Hemoglobin is synthesized through an eight-step enzymatic
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6 74 cascade, and ferrous iron (Fe^{2+}) is inserted into protoporphyrin IX to form a heme
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9 75 group at the last step (Severance and Hamza, 2009). Heparin, plays a crucial role in
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12 76 the regulation of systemic iron metabolism (Park et al., 2001), regulates the efflux of
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15 77 intracellular iron by inducing the internalization and degradation of FPN (Nemeth et al.,
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18 78 2004). Therefore, iron absorption by the small intestine decreases as hepcidin levels
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21 79 increase. In fact, transgenic mice with hepatic hepcidin overexpression have severe
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24 80 iron deficiency anemia due to FPN downregulation, which reduces iron absorption
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27 81 (Nicolas et al., 2002). In humans, hepcidin is the key mediator of inflammation
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30 82 associated anemia. (Ganz, 2003).

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33 83 Here, we evaluated the effect of PPIs on iron absorption via hepcidin
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36 84 regulation.

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40 41 42 86 **2. Materials and Methods**

43 44 45 87 *2.1. Large-scale database analysis*

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48 88 We used the World Medical & Drug Information Service to define drug
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51 89 names and the Medical Dictionary for Regulatory Activities (MedDRA/J) version 18D
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54 90 to define “iron deficiency anaemia”. We analyzed data recorded from January 2007 to
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57 91 January 2017 (among a total of 6,992,882 reports) in the FDA Adverse Event

1 92 Reporting System (FAERS) database, using reporting odds ratio (ROR), a signal
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4 93 detection method. The reports were divided into the following four groups: (a)
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6 94 individuals who received PPIs and exhibited iron deficiency anemia, (b) individuals
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9 95 who received PPIs but did not exhibit iron deficiency anemia, (c) individuals who did
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12 96 not receive PPIs and exhibited iron deficiency anemia, and (d) individuals who did not
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15 97 receive PPIs and did not exhibit iron deficiency anemia. ROR was calculated for each
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18 98 group using the following equation: $ROR = (a/b)/(c/d)$, 95% CI = $\exp [\log (ROR) \pm$
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21 99 $1.96 \sqrt{(1/a+1/b+1/c+1/d)}]$. We assumed that there was a signal when the calculated
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24 100 lower limit value of the 95% confidence interval of ROR was > 1 (van Puijenbroek et
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27 101 al., 2002).

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32 33 34 103 *2.2 Chemicals and reagents*

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37 104 Omeprazole (OME) was purchased from the Fujifilm Wako Pure Chemical
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40 105 Corporation, (Osaka, Japan); lansoprazole, rabeprazole, pantoprazole, and famotidine
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43 106 were purchased from the Tokyo Chemical Industry (Tokyo, Japan); and CH-223191,
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46 107 an aryl hydrocarbon receptor (AhR) inhibitor, was obtained from Sigma-Aldrich (St.
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49 108 Louis, MO, USA). The following commercially available antibodies were used in this
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52 109 study: anti-NRAMP2/divalent metal transporter 1 (DMT1), anti-ferritin heavy chain
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55 110 (FTH), anti-ferritin light chain (FTL), and anti-AhR antibodies were purchased from
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58 111 Santa Cruz Biotechnology (Santa Cruz, CA, USA); anti-transferrin receptor 1 (TfR1)
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61 112 antibody (Zymed Technologies; Carlsbad, CA, USA); anti-FPN antibody (Alpha

1 113 Diagnostics; San Antonio, TX, USA); anti- α -tubulin (Merck KGaA, Darmstadt,
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4 114 Germany) was used as protein loading control; and anti-histone H3 antibody (Abcam,
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6 115 Cambridge, UK) was used as a loading control for nuclear proteins.
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10 117 *2.3. Cell culture*

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15 118 HepG2, a human hepatoma cell line, was purchased from the Japanese
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18 119 Collection of Research Bioresources (Osaka, Japan). The methods of cell culture have
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21 120 been described previously (Hamano et al., 2017). In brief, when the cells reached
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24 121 sub-confluency, the cells were placed in serum-free media overnight. Subsequently, the
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27 122 cells were treated with PPIs and an H2 blocker for 24 hours. In another experiment,
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30 123 cells were pre-treated for 1 hour with CH-223191 (AhR inhibitor) before stimulation
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33 124 with OME.
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37 38 39 126 *2.4. Small interfering RNA experiments*

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42 127 Small interfering RNA (siRNA) targeting human AhR, and a non-targeting
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45 128 siRNA control sequence, were obtained from Sigma Aldrich (Mission siRNA; Tokyo,
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48 129 Japan), and used as previously described (Hamano et al., 2017).
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53 54 55 131 *2.5. Experimental animals and treatment*

1 132 All experimental animal procedures were performed in accordance with the
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4 133 guidelines of the Animal Research Committee of Tokushima University Graduate
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6 134 School (Permit Number: T30-125). Eight-week-old male C57BL6/J mice were
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9 135 purchased from Nippon CLEA (Tokyo, Japan). The mice were maintained under
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12 136 conventional conditions, with a regular 12-hour light/dark cycle. They were given free
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15 137 access to food (Type NMF; Oriental Yeast, Tokyo, Japan) and water during the study.
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18 138 OME (20 mg/kg/day) was orally administered to mice (Hess et al., 2015; Wang et al.,
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21 139 2015) for 1 or 2 weeks. OME was suspended in 0.5 % carboxymethylcellulose. Control
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24 140 mice received orally-administered carboxymethylcellulose alone. The mice were
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27 141 euthanized by intraperitoneal over-dose injection of anesthetic. Tissues and blood
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30 142 samples were collected and stored at -80 °C until use.
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36 144 *2.6. Measurement of plasma iron levels, plasma ferritin levels, tissue iron*
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39 145 *concentration, and peripheral blood*
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42 146 Serum iron levels and tissue iron concentrations were measured using an iron
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45 147 assay kit (Metallo Assay) according to the manufacturer's instructions (Metallogenics
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48 148 Co. Ltd., Chiba, Japan), and plasma ferritin levels were determined using a Mouse
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51 149 Ferritin ELISA Kit (Immunology Consultants Laboratory; Newberg, OR, USA)
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54 150 according to the manufacturer's instructions (Hamano et al., 2017). Complete blood
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57 151 count was evaluated using Microsemi LC-662 (HORIBA, Ltd., Kyoto, Japan).
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1 153 2.7. RNA extraction and evaluation of mRNA expression levels

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4 154 The methods of RNA extraction, cDNA synthesis, and quantitative RT-PCR
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6 155 have been described previously (Ikeda et al., 2016). The primer sets used were as
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9 156 follows: 5'-CTGCCTGTCTCCTGCTTCTC-3' and 5'-
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11 AGATGCAGATGGGGAAGTTG-3' for mouse *hepcidin-1*, and
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13 158 5'-GCTCCAAGCAGATGCAGCA-3' and 5'-CCGGATGTGAGGCAGCAG-3' for
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15 159 *36B4* (an internal control). The expression levels of all target genes were normalized to
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17 160 *36B4*. Values were compared to the control group, and expressed as relative fold
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33 164 The methods of protein preparation and western blotting have been
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36 165 previously described (Ikeda et al., 2016). In brief, prepared protein samples were
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39 166 separated using SDS-PAGE and transferred onto a PVDF membrane. A
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42 167 chemiluminescent reagent was used to detect immunoreactive bands. Immunoblot
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45 168 bands were visualized by exposure to X-ray film or by a C-DiGit chemiluminescent
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48 169 scanner (LI-COR C-DiGit Blot Scanner, Lincoln, Nebraska, USA). Densitometry of
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51 170 the visualized bands was quantified using Image J 1.38x software (U. S. National
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54 171 Institutes of Health, Bethesda, Maryland, USA, <http://imagej.nih.gov/ij/>, 1997-2014).

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60 173 2.9. Measurement of hepcidin concentrations

1 174 Hepcidin concentrations in mouse plasma and in cell culture media were
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4 175 measured using surface-enhanced laser desorption ionization time of flight mass
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6 176 spectrometry as described previously (Tomosugi et al., 2006). The assays were
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9 177 performed by the Medical Care Proteomics Biotechnology Co. Ltd. (Kanazawa,
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17 180 *2.10. Statistical analysis*

18 181 Data are presented as the mean \pm standard deviation (SD). An unpaired,
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21 182 2-tailed, Student's *t*-test was used to compare two groups. To compare among more
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24 183 than two groups, the statistical significance of each difference was evaluated using a
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27 184 post-hoc test (either Dunnett's method or Tukey-Kramer's method). P values < 0.05
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30 185 indicated statistical significance.

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38 187 **3. Results**

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41 188 *3.1. Analysis of the effects of PPIs and H2 blocker on iron deficiency anemia using the*
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44 189 *FAERS database*

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49 190 A larger analysis to evaluate the influence of PPIs in iron deficiency anemia
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52 191 using data from many hospitals was conducted using the FAERS database (Table 1).
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55 192 Treatment with PPIs was positively associated with the incidence of iron deficiency
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1 193 anemia. Increasing RORs were observed in combination therapy that included OME
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4 194 (ROR = 3.90, 95% CI = 3.43–4.43), lansoprazole (ROR = 5.02, 95% CI = 4.27–5.89),
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6 195 rabeprazole (ROR = 7.29, 95% CI = 5.77–9.21), and pantoprazole (ROR = 4.75, 95%
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9 196 CI = 4.15–5.45). Similarly, the increase was observed for famotidine (ROR = 5.06,
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12 197 95% CI = 4.01–6.38). Consistent with a previous report (Lam et al., 2017), PPIs, as
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15 198 well as the H2 blocker, were significantly associated with the incidence of iron
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18 199 deficiency anemia by database analysis.
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23 24 25 201 *3.2. Effect of PPIs and H2 blocker on hepcidin mRNA and secretion in HepG2 cells*

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29 202 We checked the action of PPIs and the H2 blocker on hepcidin expression in
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32 203 HepG2 cells. All PPIs, including OME, lansoprazole, rabeprazole, and pantoprazole
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35 204 enhanced hepcidin mRNA expression, but the H2 blocker, famotidine, did not (Fig.
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38 205 1A). In addition, secreted hepcidin-25 protein levels also increased in the culture
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41 206 medium with OME treatment (Fig. 1B).
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46 47 48 49 208 *3.3. Effect of OME on hepcidin expression via an AhR-mediated pathway*

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53 209 We previously reported that uremic toxin indoxyl sulfate-induced hepcidin
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56 210 upregulation is mediated through activation of the AhR (Diaz et al., 1990), and OME
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59 211 can activate AhR in human and rat hepatocytes (Diaz et al., 1990; Kashfi et al., 1995).
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1 212 Consistent with these data, we found that OME promoted translocation of AhR from
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4 213 the cytosol to the nucleus in HepG2 cells (Fig. 1C). To examine whether OME-induced
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6 214 hepcidin upregulation was mediated through AhR, we used AhR-specific siRNA and
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9 215 the AhR inhibitor CH-223191. Both AhR silencing and CH-223191 reduced
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12 216 OME-induced hepcidin expression (Fig. 1D and E). These findings suggest that OME
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15 217 induces hepcidin upregulation via an AhR-mediated pathway.
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23 219 *3.4. Changes of hepatic hepcidin mRNA and plasma hepcidin levels in OME-treated*
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26 220 *mice*

29 221 To assess the effect of OME on iron metabolism *in vivo*, we administered
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32 222 OME orally to mice. Similar to the findings in HepG2 cells, hepatic hepcidin
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35 223 expression significantly increased in mice after both 1 and 2 weeks of OME treatment
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38 224 (Fig. 2A). Additionally, plasma hepcidin concentration increased after 1 week of OME
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41 225 treatment (Fig. 2B).
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49 227 *3.5. Changes in ferroportin expression in OME-treated mice*

52 228 As expected, FPN expression decreased in the duodenum and the spleen of
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55 229 mice treated with OME (Fig. 2C and D); however, 1 week of OME treatment did not
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59 230 change TfR1 and DMT1 expression in the duodenum and spleen (Fig. 3). These
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1 231 findings suggest that in mice treated with OME, reduced FPN expression is due to
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4 232 increased hepcidin production.
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11 234 *3.6. Alteration of tissue and plasma iron content by OME administration*
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15 235 We examined the iron content of the liver and iron concentration in the
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18 236 plasma of mice treated with OME. As shown in Table 2, OME administration for 1 or
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21 237 2 weeks did not change the iron content of the liver. Meanwhile, the incidence of
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24 238 anemia increased **with reduced** plasma iron levels. There were no differences in the
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27 239 **protein** expression of liver and spleen ferritin (Fig. 4), or in plasma ferritin levels **at 2**
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30 240 **weeks when** comparing vehicle-treated and OME-treated mice (OME 399.9 ± 48.1
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33 241 ng/mL , vehicle $389.8 \pm 51.3 \text{ ng/mL}$).
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1 242 **4. Discussion**

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4 243 Using the FAERS database, we demonstrated that there is an increased risk
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7 244 of iron deficiency anemia in patients treated with PPIs or an H2 blocker. *In vitro*, we
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10 245 found that PPIs, but not the H2 blocker, upregulated hepcidin through the AhR
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13 246 pathway. Mice treated with OME had increased expression of hepatic hepcidin mRNA
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16 247 and higher plasma hepcidin levels, leading to a decrease in FPN expression in the
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19 248 duodenum and spleen. These findings indicate that PPI-induced iron deficiency
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22 249 involves a hepcidin-FPN dependent pathway in addition to elevated gastric pH levels.
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26 250 The FAERS is a database of self-reported drug-related adverse events from
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29 251 multiple treatment centers that reflects the realities of clinical practice. Patients treated
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32 252 with PPIs or an H2 blocker had a significantly higher rate of iron deficiency anemia
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35 253 compared to patients who had not taken PPIs or an H2 blocker. These data are
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38 254 consistent with (Lam et al., 2017) who found that patients who had taken PPIs
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41 255 long-term (i.e. for more than two years) were at increased risk of iron deficiency. **This**
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44 256 **case-control study also showed that the risk of iron deficiency was higher in the**
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47 257 **patients using PPIs (adjusted odds ratio, 2.49) than in patients using H2 blockers (odds**
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50 258 **ratio, 1.58) (Lam et al., 2017). The subsequent risk for iron deficiency due to PPI use**
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53 259 **may be attributed to elevation of pH levels in the stomach and other factors.**
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56 260 Hepcidin is an important iron regulator that controls cellular iron efflux via
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59 261 FPN (Nemeth et al., 2004). We found that hepcidin mRNA expression increased with
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1 262 PPI treatment; however, famotidine, a H2 blocker, did not augment hepcidin
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4 263 expression in HepG2 cells. Hepcidin expression is regulated by many factors including
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6 264 iron, anemia, and inflammation (Ganz, 2011). In addition, indoxyl sulfate regulates
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9 265 hepcidin expression through an AhR-mediated pathway (Hamano et al., 2017),
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12 266 suggesting the involvement of AhR in hepcidin regulation. Previous studies have
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15 267 shown that OME activates AhR in hepatocytes (Diaz et al., 1990; Kashfi et al., 1995).
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18 268 Consistent with these data, we confirmed that OME promoted AhR translocation from
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21 269 the cytosol to the nucleus, suggesting that OME may be an activating ligand for AhR.
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24 270 Silencing or inhibiting AhR suppressed OME-induced hepcidin upregulation. Taken
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27 271 together, these data indicate that OME upregulates hepcidin through an AhR-mediated
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30 272 pathway.

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34 273 Similar to the effect of PPIs on hepcidin *in vitro*, mice treated with OME for
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37 274 1 week showed increased hepatic hepcidin mRNA expression and increased plasma
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40 275 hepcidin levels. The mice also displayed reduced FPN expression in the duodenum and
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43 276 spleen. Moreover, the increase in hepatic hepcidin mRNA lasted for 2 weeks, and the
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46 277 mice developed anemia, suggesting that iron absorption and utilization were impaired.
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49 278 Therefore, in addition to increasing gastrointestinal pH levels, PPIs may also inhibit
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52 279 iron absorption but regulating the hepcidin-FPN pathway. We did not find a decrease
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55 280 in hepatic iron content or ferritin expression, therefore, we propose that OME-induced
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58 281 hepcidin upregulation by was not due to iron accumulation. Further studies are
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1 282 necessary to further clarify the effect of PPIs on hepcidin, and on the induction of iron
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7 284 In conclusion, PPIs upregulate hepcidin expression by activating AhR.
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10 285 Increased hepcidin production by PPIs leads to a reduction in FPN expression, which
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13 286 inhibits iron absorption and utilization, and promotes the development of iron
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16 287 deficiency anemia. The effect of PPIs on iron metabolism suggests that the risk of iron
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19 288 deficiency anemia should be carefully **monitorized in** patients who receive long-term
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22 289 PPI treatment. **Although future clinical study is needed, our study would caution**
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25 290 **healthcare providers to consider PPIs' potential effects on body iron dysmetabolism.**

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17 356 **Figure legends**

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21 357 **Fig. 1.** (A) Effect of proton pump inhibitors and a histamine H2-receptor antagonist on

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23 358 hepcidin mRNA expression in HepG2 cells. Cells were treated with 200 μ M

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25 359 omeprazole (OME), 50 μ M lansoprazole, 100 μ M pantoprazole, 12.5 μ M rabeprazole,

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27 360 200 μ M famotidine, or vehicle. Values are expressed as the mean \pm SD. *P < 0.05, **P

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29 361 < 0.01; n = 5–10 in each group. (B) Concentration of hepcidin, secreted by HepG2

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31 362 cells, in the culture media. Cells were treated with either 200 μ M OME or vehicle.

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33 363 Values are expressed as the mean \pm SD. *P < 0.05 (vs. vehicle treatment); n = 3 in each

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35 364 group. (C) OME action on AhR translocation from the cytoplasm to the nucleus of

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37 365 HepG2 cells. Cells were treated with either 200 μ M OME or vehicle. Values are

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39 366 expressed as the mean \pm SD. *P < 0.05 (vs. vehicle treatment); n = 3 in each group. (D)

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41 367 Treatment with AhR siRNA inhibits OME-induced hepcidin upregulation in HepG2

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43 368 cells. Forty-eight hours after siRNA transfection, cells were treated with either 200 μ M

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45 369 OME or vehicle. Values are expressed as the mean \pm SD. **P < 0.01; n = 6–9 in each

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47 370 group. (E) Treatment with CH-223191 inhibits OME-induced hepcidin upregulation in

1 371 HepG2 cells. Cells were treated with either 200 μ M OME or vehicle 1 hour after 10
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4 372 μ M CH-223191 pretreatment. Values are expressed as the mean \pm SD. **P < 0.01; n =
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7 373 5–10 in each group.

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10 374 **Fig. 2.** (A) Hepcidin mRNA expression in the liver of mice treated for 1 and 2 weeks
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13 375 with vehicle or OME. Values are expressed as the mean \pm SD. **P < 0.01 (vs. vehicle
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16 376 treatment); n = 9–13 in each group. (B) Plasma hepcidin concentration. Values are
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19 377 expressed as the mean \pm SD. *P < 0.05 (vs. vehicle treatment); n = 3–6 in each group.
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22 378 Effects of 1 and 2 weeks of treatment with vehicle or OME on the expression of FPN
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25 379 and tubulin in murine (C) duodenum and (D) spleen. Upper panels: Representative
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28 380 immunoblots. Lower panels: Semi-quantitative densitometric analyses of FPN protein
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31 381 levels normalized to tubulin. Values are expressed as the mean \pm SD. *P < 0.05; n =
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34 382 6–16 in each group.

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38 383 **Fig. 3.** Effects of 1 week of treatment with vehicle or OME on protein expression in
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41 384 murine duodenum and spleen. Upper panels: Representative immunoblots for tubulin,
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44 385 (A) duodenal DMT1, (B) splenic DMT1, and (C) splenic TfR1. Lower panels:
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47 386 Semi-quantitative densitometric analyses of DMT1 and TfR1 protein levels normalized
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50 387 to tubulin. Values are expressed as means \pm SD. n = 7–9 in each group.

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53 388 **Fig. 4.** (A, B) Effects of 1 week of treatment with vehicle or OME on protein
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56 389 expression in murine liver. Upper panels: Representative immunoblots for tubulin, (A)
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58
59 390 FTH, and (B) FTL. Lower panels: Semi-quantitative densitometric analyses of FTH

1 391 and FTL protein levels normalized to tubulin. Values are expressed as the mean \pm SD.
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4 392 *P < 0.05; n = 4–6 in each group. (C, D) Effects of 1 week of treatment with vehicle or
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6 393 OME on protein expression in murine spleen. Upper panels: Representative
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9 394 immunoblots for tubulin, (C) FTH, and (D) FTL. Lower panels: Semi-quantitative
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12 395 densitometric analyses of FTH and FTL protein levels normalized to tubulin. Values
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15 396 are expressed as the mean \pm SD. n = 4–9 in each group.
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Table 1. Number of reported cases and reporting odds ratio of iron deficiency anemia in patients who took proton pump inhibitors and an H2 blocker in FAERS analysis

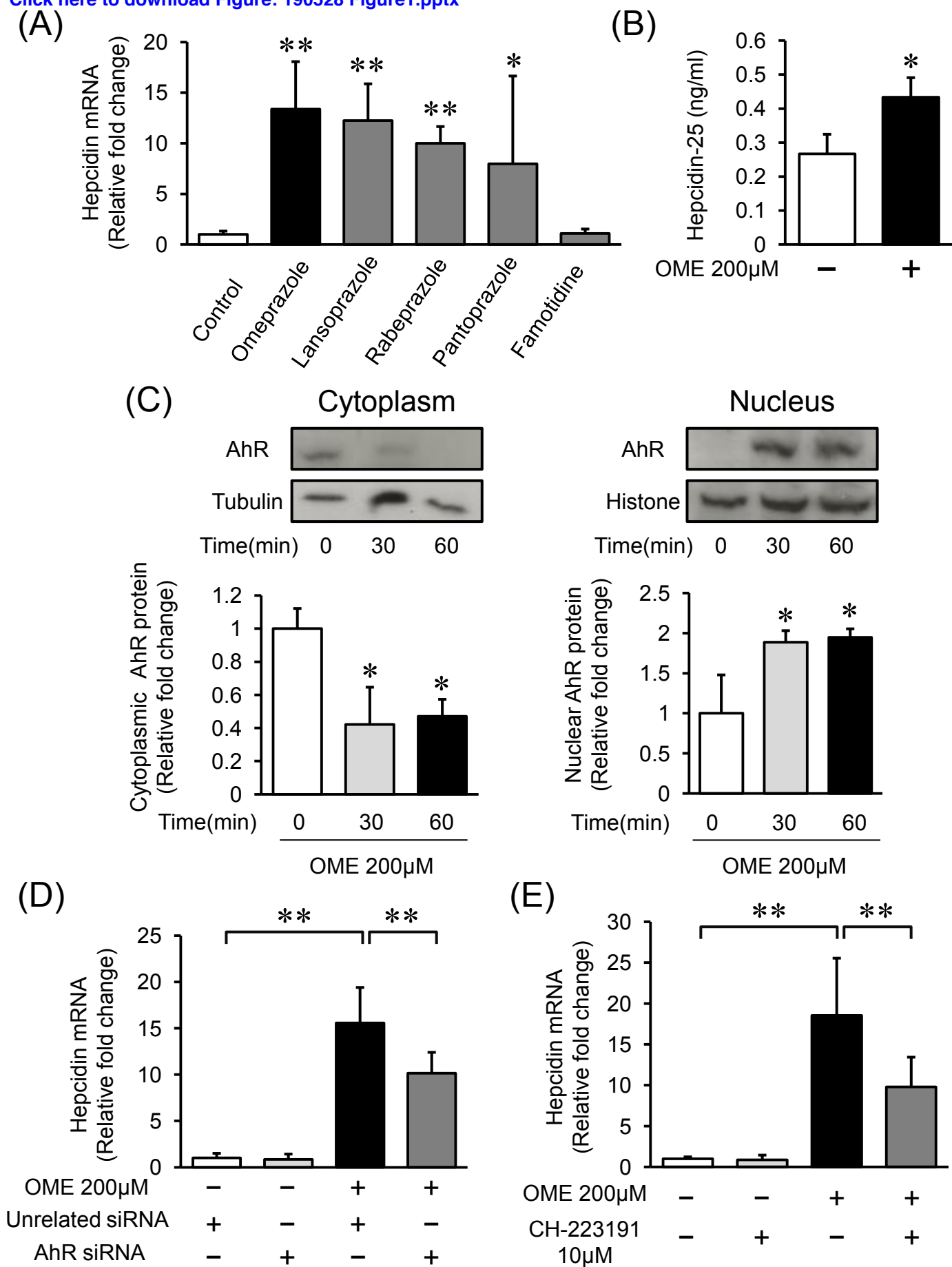
Drug name	Iron deficiency anemia without drug	Iron deficiency anemia with drug	Reporting odds ratio (95% CI)
Omeprazole	3275/6856188	254/136695	3.90 (3.43–4.43)
Lansoprazole	3372/6925076	157/64277	5.02 (4.27–5.89)
Rabeprazole	3457/6972908	72/19975	7.29 (5.77–9.21)
Pantoprazole	3309/6892944	220/96409	4.75 (4.15–5.45)
Famotidine	3456/6960307	73/29046	5.06 (4.01–6.38)

Table 2. Body weight, liver iron content, plasma iron levels and hematological data in mice treated with vehicle or omeprazole

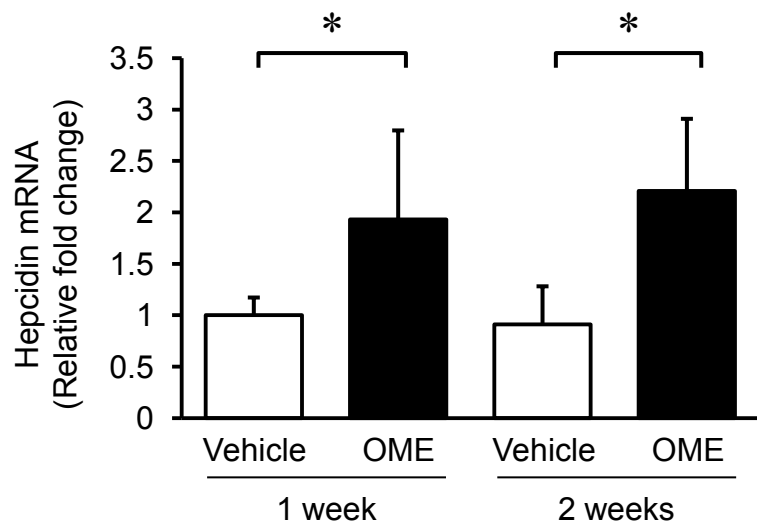
	1 wk		2 wk	
	Vehicle	OME	Vehicle	OME
Body weight (g)	23.2 ± 0.2	22.9 ± 0.8	23.9 ± 0.9	23.5 ± 0.5
Liver iron (µg/g protein)	125.2 ± 32.0	117.9 ± 20.7	107.2 ± 44.5	120.1 ± 47.4
Plasma iron (µg/dL)	114.6 ± 21.1	80.8 ± 31.7*	115.7 ± 31.4	78.6 ± 15.3*
RBC (× 10⁴/µl)	821 ± 31	773 ± 27	867 ± 51	777 ± 18*
Hb (g/dL)	12.0 ± 0.2	11.1 ± 0.3**	12.8 ± 0.7	11.5 ± 0.4*
Ht (%)	37.2 ± 1.5	35.0 ± 1.2	39.1 ± 2.4	35.1 ± 0.9*

Data are means ± SD; *n* = 4-10, respectively. **P* < 0.05, ***P* < 0.01 vs. vehicle mice at the same week.

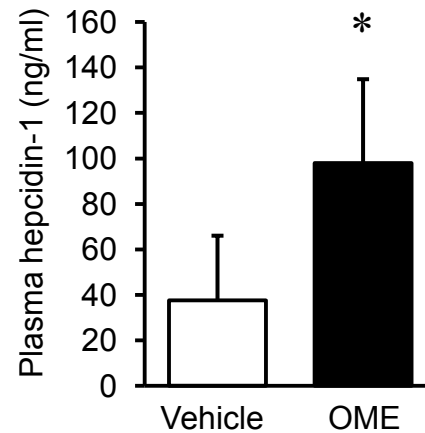
RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit



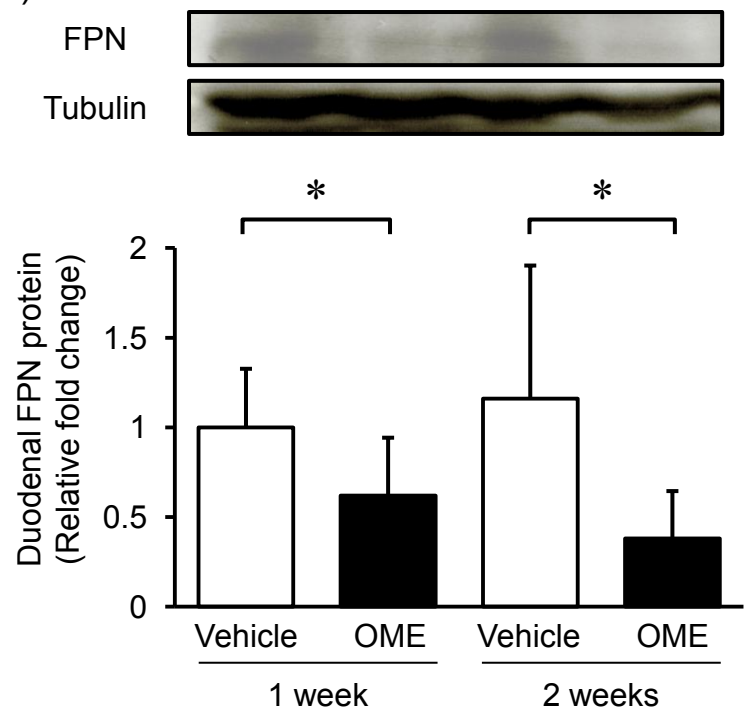
(A)



(B)



(C)



(D)

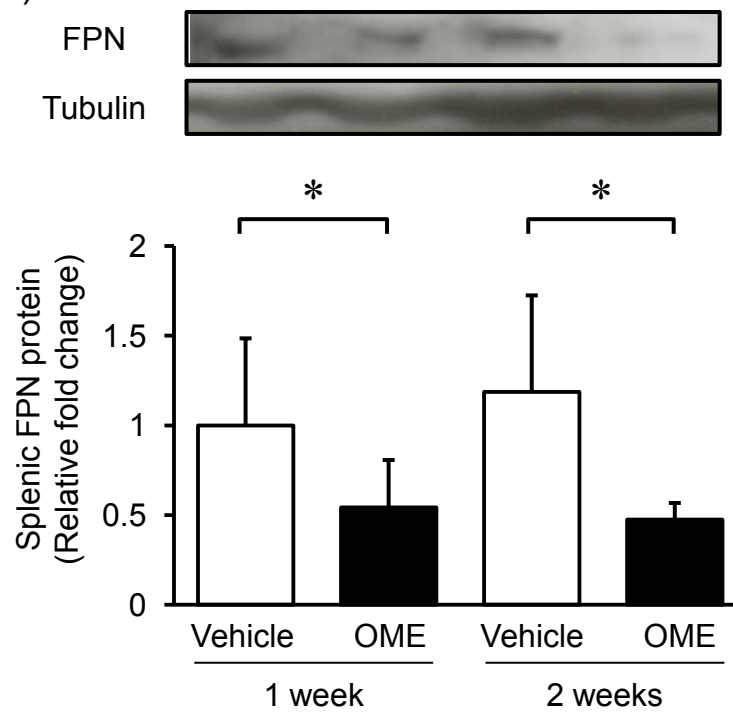


Figure 3 Hamano et al.

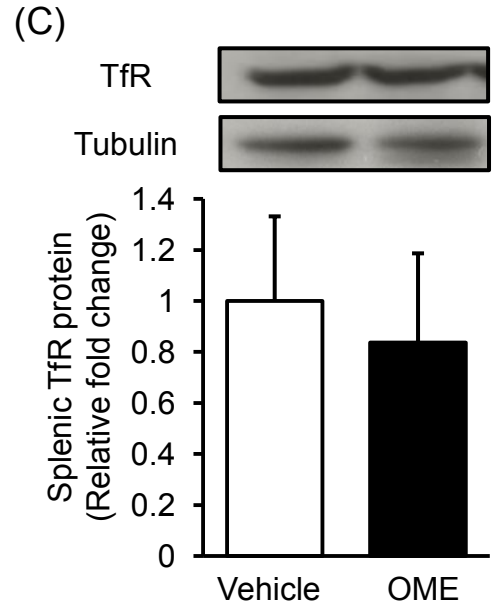
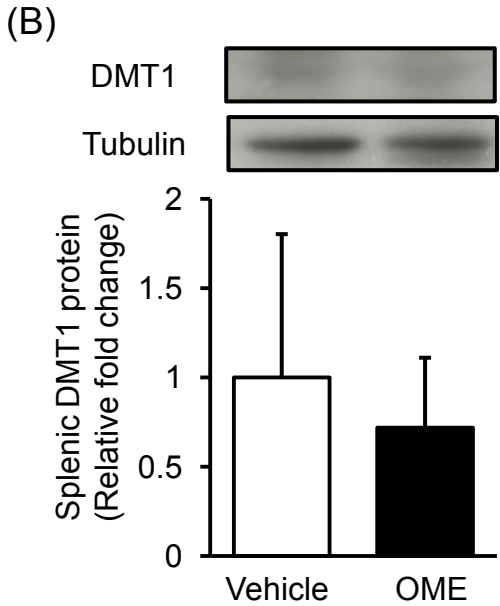
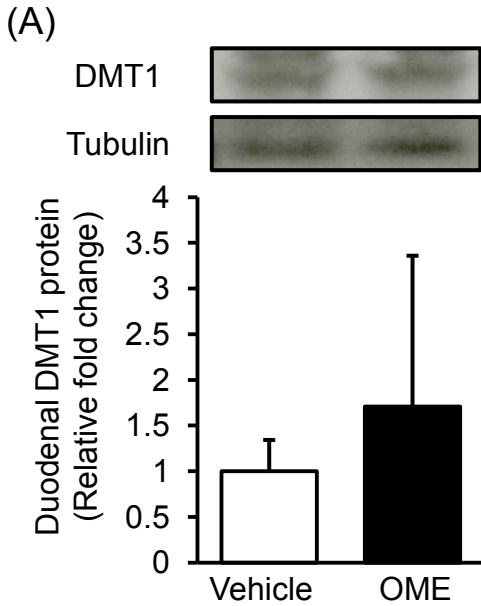


Figure 4 Hamano et al.

