Effect of Drug Combination on Omeprazole Metabolism by Cytochrome P450 2C19 in Helicobacter pylori Eradication Therapy

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Received January 30, 2019; accepted May 7, 2019

Helicobacter pylori (H. pylori) infection is common and can result in gastric and duodenal ulcers, and in some cases, gastric lymphoma and cancer. Omeprazole (OMP)—in combination with clarithromycin (CLR), amoxicillin (AMX), tinidazole (TND), or metronidazole (MET)—is used in double or triple combination therapy for eradication of H. pylori. However, the roles of the drugs other than OMP are not clearly understood. Therefore, in the present study, we aimed to investigate any effects of these drugs on OMP metabolism by wild-type CYP2C19 using spectroscopy and enzyme kinetics. The dissociation constants (Kd) for CYP2C19 with OMP, CLR, AMX, TND, and MET were 8.6, 126, 156, 174, and 249 µM, respectively. The intrinsic clearance of OMP was determined to be 355 mL/min/µmol of CYP2C19. Metabolism of OMP was significantly inhibited by 69, 66, 28, and 40% in the presence of CLR, TND, AMX, and MET, respectively. Moreover, the combination of CLR and TND resulted in 76% inhibition of OMP metabolism, while the combination of AMX and MET resulted in 48% inhibition of OMP metabolism. Both combinations of drugs not only have antibacterial effects, but also enhance the effect of OMP by inhibiting its metabolism by CYP2C19. These results indicate that drug–drug interactions of co-administered drugs can cause complex effects, providing a basis for OMP dose adjustment when used in combination therapy for H. pylori eradication.

Key words  omeprazole; CYP2C19; Helicobacter pylori; drug–drug interaction

Introduction

Helicobacter pylori (H. pylori) is a Gram-negative bacterium that enters the body through food, drink, or direct person-to-person contact (saliva and vomiting), and can survive in the human digestive tract. More than 50% of people worldwide have an H. pylori infection,1 and many individuals are infected during childhood. H. pylori attacks the lining of the stomach and upper part of the small intestine, damaging the protective layer of the stomach and small intestine and thereby contributing to the development of gastric and duodenal ulcers. H. pylori infection is considered a major risk factor for development of gastric mucosa-associated lymphoid tissue lymphoma and stomach cancer.2–7 Effective eradication of H. pylori is required for preventing the recurrence of such diseases.8,9 Omeprazole (OMP), a common proton pump inhibitor, is used in combination with antibiotics or antiparasitics for eradication of H. pylori.10 There are several drugs and lines for eradication treatment; clarithromycin (CLR), amoxicillin (AMX), tinidazole (TND), and metronidazole (MET) are commonly used in double and triple combination therapy with OMP for treatment of H. pylori infection.11–14 The chemical structures of OMP, CLR, AMX, TND, and MET are shown in Fig. 1.

Drug–drug interactions (DDIs) are major factors that can affect the pharmacological activities, therapeutic efficacies, and adverse effects of the most commonly used drugs. They can lead to changes in absorption, metabolism, and excretion of most drugs. CYP enzymes comprise a class of monooxygenases that are responsible for oxidative metabolism of most (>90%) drugs currently available for use in humans.15 CYPs catalyze drug detoxification by epoxidation, hydroxylation, desulfuration, dealkylation, oxidation, or sulfoxidation.16 CYPs can be induced or inhibited by several drugs and are therefore sensitive to clinically important DDIs that can lead to either drug toxicity or reduction of pharmacological activity.17 Consequently, identification of drugs that act as enhancers or inhibitors might prevent the occurrence of clinically significant DDIs.

Five CYP isoforms (1A2, 2C19, 2C9, 2D6, and 3A4) are the predominant metabolizing enzymes for xenobiotics.18 One...
of the CYP isoforms, CYP3A4, is well-known for possessing a wide catalytic cavity that allows for several DDIs.\textsuperscript{19,20} For instance, rifampin enhances the metabolism of warfarin \textit{in vivo} by increasing the metabolic activity of CYP3A4.\textsuperscript{21} On the other hand, itraconazole, ketoconazole, erythromycin, and clarithromycin each inhibit the metabolism of imidafacin by decreasing the metabolic activity of CYP3A4.\textsuperscript{22} Although CYP3A4 could reasonably enable DDIs owing to its wide substrate cavity, a recent study showed that etizolam metabolism by another isoform, CYP2C19, is affected by co-administration of itraconazole.\textsuperscript{23}

CYP2C19 is one of the major CYP isoforms\textsuperscript{7} and is responsible for metabolism of at least 10% of commonly used clinical drugs. CYP2C19 is responsible for metabolism of proton pump inhibitors, such as OMP.\textsuperscript{24-26} Several \textit{in vivo} studies clarified the effects of CYP2C19 genotype on OMP combination therapy used for eradication of \textit{H. pylori}.\textsuperscript{27-34} Interestingly, higher OMP plasma concentrations were detected following co-administration of OMP and CLR in healthy volunteers,\textsuperscript{35} implying that OMP and CLR cause a DDI and affect OMP metabolism by CYP2C19. This work aimed to investigate the effects of CLR, AMX, TND, and MET in double and triple combination therapies on OMP metabolism by CYP2C19 in order to detect and clarify any DDIs—which could help in optimizing the therapeutic dose regimen for eradication of \textit{H. pylori}. The binding affinity of each investigated drug for CYP2C19 was measured spectrophotometrically. In addition, the metabolic activity of CYP2C19 on OMP was investigated in the absence and presence of co-existing drugs (CLR, AMX, TND, and MET) using ultra performance liquid chromatography (UPLC).

**Experimental**

**Chemicals and Reagents**

TND was purchased from Tokyo Chemical Industry Development Co., Ltd. (Tokyo, Japan) and cytochrome \textit{b}, was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). OMP, CLR, AMX, MET, and all other chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). CYP2C19 was expressed and purified as previously reported.\textsuperscript{36} Recombinant human CYP reductase was prepared and its purity and activity were checked in accordance with a previously reported method.\textsuperscript{37} Reduced nicotinamide adenine dinucleotide phosphate (NADPH) generating solution—containing 1 mM of \textit{β}-nicotinamide-adenine dinucleotide phosphate (\textit{β}-NAD\textsuperscript{P}\textsuperscript{+}), 2.5 mM of glucose-6-phosphate (G6P), and 2 U/mL of glucose-6-phosphate dehydrogenase (G6PDH) purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan)—was prepared in 100 mM potassium phosphate buffer (KPi buffer, pH 7.4) with 2.5 mM MgCl\textsubscript{2}.

**Drug Binding Affinity Measurements**

CYP2C19 binding affinities for OMP, CLR, AMX, TND, and MET were investigated by spectral titration using an Ultraviolet/Visible DU\textsuperscript{®} 800 spectrophotometer from Beckman Coulter Inc. (Brea, CA, U.S.A.). Each drug was studied in triplicate. CYP2C19 was diluted and prepared to 8 µM in 100 mM KPi buffer. The studied drugs were prepared as high concentration stock solutions (500, 5.01, 6.22, 6.98, and 9.97 mM for OMP, CLR, AMX, TND, and MET; respectively), and the concentrations were determined by calibrated curves with peak areas in HPLC from precisely weighed drug solutions. Spectral titration was carried out according to a previous work.\textsuperscript{38} Briefly, an aliquot of drug stock solution was added gradually to the CYP2C19 solution, and spectral changes of CYP2C19 were monitored in the Soret band region. The absorbance changes between approx. 390 nm and approx. 420 nm were monitored and used to determine the molar fraction (\textit{α}) of drug-bound CYP2C19. The molar fractions were plotted against the concentrations of the titrated drugs and a theoretical curve was fit to the data using the following Eq. 1:

\[
\alpha = 1/(1+K_d/[\text{drug}])
\]

**Assessment of Metabolic Activities**

The enzyme kinetic parameters (\textit{K}_m, \textit{V}_\text{max}, and \textit{CL}_{int}) for OMP metabolism by CYP2C19 were measured by substrate depletion, as reported previously.\textsuperscript{50} In addition, the effect of co-existing drugs on OMP metabolism by CYP2C19 was investigated by measuring the enzyme kinetic parameters in the presence of 629 µM CLR, 778 µM AMX, 872 µM TND, or 1250 µM MET. The concentrations were set to be 5-times higher than the evaluated \textit{K}_d values. Stock solutions of investigated drugs were prepared in 100 mM KPi buffer—except CLR, which was dissolved in 100 mM KPi buffer with 1.75% (v/v) of acetone. The decrease in OMP concentration was estimated by measuring the peak area of the UPLC chromatograms. The initial reaction velocities (\textit{V}) were estimated from the dataset of OMP depletion and times in each concentration, and then were plotted against OMP concentrations. Finally, metabolic activity of CYP2C19 on OMP was determined using the Michaelis–Menten Eq. 2, and kinetic parameters (\textit{K}_m and \textit{V}_\text{max}) were determined.

\[
V = V_{\text{max}} \times ([\text{OMP}]/(K_m + [\text{S}])
\]

\textit{V} is the reaction velocity, \textit{V}_\text{max} is the maximal product formation rate, and \textit{K}_m is the Michaelis constant—the concentration of OMP at the rate of half-maximal product formation. In addition, the value of intrinsic clearance (\textit{CL}_{int})—an index of metabolic efficiency—was also calculated as the ratio of \textit{V}_\text{max} to \textit{K}_m. These enzymatic parameters were evaluated in triplicate for each drug. Finally, the values were analyzed and assessed statistically using Student’s \textit{t}-test.

**Chromatographic Conditions**

An analytical column (C18 ACQUITY UPLC\textsuperscript{®} CSH\textsuperscript{TM}, 50 × 2.1 mm, 1.7 µm particle size) was purchased from Nihon Waters K. K., Tokyo, Japan. Isoacetic elution was carried out on the column using 0.1% (v/v) acetic acid and acetonitrile at a ratio of 70:30 as the mobile phase. The flow rate was set to 0.5 mL/min. The sample storage compartment was kept at 10°C, while the column was maintained at 40°C. The injection volume for metabolized solutions ranged from 2 to 10 µL. Detection of OMP was carried out by measuring the absorbance at 300 nm.

**Results**

**Binding Affinity and Metabolic Activity of CYP2C19 for OMP**

The binding affinity of OMP for CYP2C19 was estimated using spectrophotometry. Titration of OMP into a solution containing CYP2C19 resulted in a decrease in absorbance at the Soret band (423.5 nm) with a subsequent increase at 387 nm (Fig. 2A). Molar fractions (\textit{α}) of OMP-bound CYP2C19 were calculated and plotted against concentrations of titrated OMP (Fig. 2B). The plots were fit to a theoretical curve Eq. 1, and the \textit{K}_d of CYP2C19 for OMP was determined.
to be 8.6 µM (Table 1). Subsequently, the metabolic activity of CYP2C19 for OMP was evaluated by measuring the enzymatic parameters. The $K_m$ and $V_{max}$ were determined to be 20 µM and 7.1 µM/min/µM of CYP2C19, respectively. The value of $CL_{int}$ was calculated from the $V_{max}$ to $K_m$ ratio and was determined to be 355 mL/min/µmol of CYP2C19. These parameters are summarized in Table 2.

**CLR, AMX, TND, and MET Binding Affinities for CYP2C19**

As performed for the binding affinity of OMP for CYP2C19, the binding affinities of CLR, AMX, TND, and MET for CYP2C19 were also determined by following the spectral change of CYP2C19 upon titration with each drug. Upon addition of CLR, a decrease around the Soret band of CYP2C19 could be observed without any subsequent increase at any other wavelength (Fig. 3A, inset). The molar fractions of drug-bound forms were plotted against drug concentration (Fig. 3A). The $K_m$ of CYP2C19 for CLR was 126 µM. During measurement of the binding affinity of CYP2C19 for AMX, TND, and MET, only a decrease around the Soret band of CYP2C19 could be observed, similarly to what was observed with CYP2C19 and CLR. The $K_m$ of CYP2C19 for each of AMX, TND, and MET was calculated by plotting the molar fraction of the drug-bound form against the drug concentration, as before (Figs. 3B–D). The $K_m$ values were estimated to be 156, 174, and 249 µM for AMX, TND, and MET, respectively. The values for all drugs tested are summarized in Table 1.

**Effect of Double Combination Therapy on OMP Metabolism by CYP2C19**

OMP is commonly administered with CLR, AMX, MET, or TND as a double combination therapy for treatment of *H. pylori*. Therefore, the metabolic activity of CYP2C19 for OMP was investigated in the presence of each of CLR, AMX, TND, and MET (Fig. 4). First, the effect of CLR on OMP metabolism was studied. The examined concentration of CLR was 629 µM—five times higher than the $K_m$. CLR increased by 1.5-fold compared to that of OMP alone. As a consequence, the value of $CL_{int}$ was calculated as 110 mL/min/µmol of CYP2C19, which is almost one-third of that of OMP alone. Subsequently, the degree of inhibition by CLR (69%) was calculated from the absorbance changes are plotted against OMP concentration for determination of the dissociation constant ($K_d$).
the CLR-TND combination with OMP, the interactions: OMP-CLR-TND and OMP-AMX-MET. In the case of metabolism was measured for two sets of combinations. OMP metabolism in the presence of AMX (open circles), MET (filled triangles), TND (open triangles), or CLR (open square) was determined to evaluate the potential efficacy of double combination therapy on *H. pylori* treatment. AMX: amoxicillin, CLR: clarithromycin, MET: metronidazole, OMP: omeprazole, TND: tinidazole, $V$: initial reaction velocity.

was calculated by subtracting one from the ratio of the $CL_{int}$ of OMP in the presence of CLR to the $CL_{int}$ of OMP alone. Next, the effects of the other drugs—AMX, TND, and MET—on OMP-metabolism were also investigated, and enzymatic parameters were measured similarly to those for CLR. Finally, the degrees of inhibition were determined to be 28, 66, and 40%, for AMX, TND, and MET, respectively. The obtained enzymatic parameters are summarized in Table 2.

**Effect of Triple Combination Therapy on Metabolism of OMP by CYP2C19** The effect of triple combination therapy on OMP metabolism was measured for two sets of combinations: OMP-CLR-TND and OMP-AMX-MET. In the case of the CLR-TND combination with OMP, the $V_{max}$ decreased to 3.1 $\mu$M/min/$\mu$M of CYP2C19 and the $K_{m}$ increased to 36 $\mu$M (Fig. 5). These values resulted in a $CL_{int}$ of 86 mL/min/$\mu$mol of CYP2C19 and suggested that the combination resulted in 76% inhibition of OMP metabolism. Similarly, the AMX-MET combination resulted in $K_{m}$ and $V_{max}$ values of 24 $\mu$M and 4.4 $\mu$M/min/$\mu$M of CYP2C19, respectively (Table 2). These values indicated that the combination inhibited OMP metabolism by 48%. A comparison of double and triple combination therapies on OMP metabolism by CYP2C19 is represented in Fig. 6.

**Discussion**

*In vitro* studies can be used to screen possible DDIs that may occur during treatment of certain diseases. These DDIs, which can result from enzyme inhibition or induction, are of great importance to clinicians and patients. CYP2C19 is an adaptive or inducible enzyme with reversible or irreversible induction and inhibition. For this reason, a CYP2C19 substrate may inhibit its own metabolism or inhibit the metabolism of other substrates by competing for the enzyme active site. Thus, CYP2C19 enzyme induction and inhibition are of concern and may alter therapeutic outcomes of treatment.

*H. pylori* is a Gram-negative bacterium that can be treated with OMP in combination with CLR, AMX, TND, or MET. Several previous studies focused on the effects of CYP2C19 genotype on OMP metabolism to improve the therapeutic efficacy of drug combinations in *H. pylori* eradication. The roles and mechanisms of these co-administered drugs on OMP metabolism by CYP2C19 are not fully understood. Therefore, in this study, the effects of CLR, AMX, TND, and MET on OMP metabolism by CYP2C19 were thoroughly investigated by measuring their binding affinities using spectrophotometry and their metabolic activities using substrate depletion and UPLC.

**Binding Affinity and Metabolic Activity of CYP2C19 for OMP** In the spectral titration of CYP2C19 with OMP, a peak and trough were observed around 390 nm and 420 nm, respectively. This type of difference spectrum is known as a “type I” spectrum. OMP binds to a hydrophobic pocket located near the catalytic site of CYP2C19, displacing a water molecule coordinated to the heme iron at the proximal side. This binding shifts the formed five-coordinated heme iron from a low-spin to high-spin state and shifts the Soret band.

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**Table 2**

| Drug Combination | $K_{m}$ (M) | $V_{max}$ (M/min/$\mu$M) | $CL_{int}$ (mL/min/$\mu$mol) |
|------------------|-------------|---------------------------|-------------------------------|
| OMP-alone        |             |                           |                               |
| OMP-CLR          |             |                           |                               |
| OMP-TND          |             |                           |                               |
| OMP-AMX          |             |                           |                               |
| OMP-MET          |             |                           |                               |
| OMP-CLR-TND      |             |                           |                               |
| OMP-AMX-MET      |             |                           |                               |

*Fig. 4. Michaelis–Menten Plots for OMP Metabolism by CYP2C19*

Metabolic activity of CYP2C19 on OMP (0–40 min) and velocities plotted against OMP concentrations (filled circles). Data are fit to a theoretical curve from the Michaelis–Menten Eq. 2. OMP metabolism in the presence of AMX or CLR was calculated by subtracting one from the ratio of the $CL_{int}$ of OMP in the presence of AMX to the $CL_{int}$ of OMP alone. Next, the effects of the other drugs—AMX, TND, and MET—on OMP metabolism were also investigated, and enzymatic parameters were measured similarly to those for CLR. Finally, the degrees of inhibition were determined to be 28, 66, and 40%, for AMX, TND, and MET, respectively. The obtained enzymatic parameters are summarized in Table 2.

**Effect of Triple Combination Therapy on Metabolism of OMP by CYP2C19** The effect of triple combination therapy on OMP metabolism was measured for two sets of combinations: OMP-CLR-TND and OMP-AMX-MET. In the case of the CLR-TND combination with OMP, the $V_{max}$ decreased to 3.1 $\mu$M/min/$\mu$M of CYP2C19 and the $K_{m}$ increased to 36 $\mu$M (Fig. 5). These values resulted in a $CL_{int}$ of 86 mL/min/$\mu$mol of CYP2C19 and suggested that the combination resulted in 76% inhibition of OMP metabolism. Similarly, the AMX-MET combination resulted in $K_{m}$ and $V_{max}$ values of 24 $\mu$M and 4.4 $\mu$M/min/$\mu$M of CYP2C19, respectively (Table 2). These values indicated that the combination inhibited OMP metabolism by 48%. A comparison of double and triple combination therapies on OMP metabolism by CYP2C19 is represented in Fig. 6.

**Fig. 5. Effects of Triple Combination Therapies on OMP Metabolism by CYP2C19**

OMP metabolism was studied in the presence of AMX-MET (open rhombuses) or CLR-TND (filled rhombuses). OMP alone (filled circles) is provided again for comparison. AMX: amoxicillin, CLR: clarithromycin, MET: metronidazole, OMP: omeprazole, TND: tinidazole, $V$: initial reaction velocity.

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**Fig. 6. Comparison of Calculated $CL_{int}$ of OMP by CYP2C19 in the Absence (—, White Bars) or Presence of Co-existing Drugs in Either Double (Gray Bars) or Triple (Black Bars) Combination Therapy**

These values were statistically analyzed between $CL_{int}$ of OMP in the absence and the presence of co-existing drugs. Also, statistical analyses were performed between double and triple combination therapies, and significance for the differences were marked with asterisks (*$p<0.05$ or **$p<0.01$) in accordance with the summarized values in Table 2. Some metabolic activities were assessed in the presence of 1.75% (v/v) acetonitrile (9). AMX: amoxicillin, $CL_{int}$: intrinsic clearance, CLR: clarithromycin, MET: metronidazole, OMP: omeprazole, TND: tinidazole.
to a shorter wavelength. This binding type makes the heme more easily reduced by CYP reductase, thereby initiating the catalytic cycle of oxidative metabolism. OMP can be considered a CYP2C19 substrate; the low $K_d$ of CYP2C19 for OMP (8.6 M) indicates their high affinity for each other. Subsequently, enzyme kinetic parameters were measured to evaluate the rate of metabolism of OMP by CYP2C19. CYP2C19 had high $V_{max}$ and $C_{cat}$ values for OMP, suggesting that, in agreement with in vivo studies, CLR is responsible for metabolism of OMP.

CLR, AMX, TND, and MET Binding Affinities for CYP2C19 The binding affinity of CYP2C19 for CLR and the associated spectral changes were measured and traced. Upon titration of CYP2C19 with CLR, only a decrease around 420 nm (Soret band) was observed, indicating that CLR could bind to a more remote location within the CYP2C19 active sites to coordinate weakly to the heme. This spectral change was similar to that previously reported for CYP2C19 with cimetidine, an inhibitor of CYP2C19 and a typical type II ligand. This suggests that CLR can act as an inhibitor of CYP2C19.

Similarly, titration of CYP2C19 with each of AMX, TND, and MET resulted in a decrease only around 420 nm (Soret band). This spectral change suggested weak coordination of each of AMX, TND, and MET to CYP2C19—similarly to that observed with CLR. Therefore, AMX, TND, and MET might also be considered inhibitors of CYP2C19.

Effect of Double Combination Therapy on OMP Metabolism by CYP2C19 In order to investigate the effect of CLR, AMX, TND, and MET on metabolism of OMP by CYP2C19, high concentrations of each of the investigated drugs (5-fold higher than the corresponding $K_d$ values) were combined with OMP during the measurement of OMP metabolism by CYP2C19. These high drug concentrations were used to ensure complete saturation of CYP2C19 wild type (WT) enzyme with co-existing investigated drugs to enable us to compare the inhibitory effect of each investigated drug on OMP metabolism by CYP2C19 WT. Due to the low solubility of CLR in 100 mM KPi buffer, CLR (62.9 µM) was first dissolved in acetone, then diluted with 100 mM KPi buffer. Acetone was confirmed to have no effect on the determination of metabolic activity by measuring the OMP-CYP2C19 metabolic activity in the presence of 1.75% (v/v) of acetone (Fig. 6). In comparison to the effect of CLR and AMX on OMP metabolism, CLR had a higher binding affinity to CYP2C19—as indicated by its lower $K_d$ and a higher inhibitory effect—as indicated by its lower $V_{max}$. In addition, MET had a lower binding affinity to CYP2C19 than did TND and also had a lower inhibitory effect. Previously, OMP was reported to enhance the antibacterial activity of AMX by altering intragastric acidity, and $C_{max}$ of AMX was reported to be 16 µmol/L. Further, CLR was studied with OMP and the $C_{max}$ value was determined to be 3.8 µg/mL. In this study, we studied the inhibitory effect of co-existing drugs on OMP metabolism with higher concentrations than those reported to clearly monitor the effect of co-existing drugs. Although further in vivo studies are necessary to confirm whether the inhibitory effect could occur or not, we could clarify that the double combination therapy of AMX and MET had lower inhibitory effects on OMP metabolism by CYP2C19 than did that of CLR and TND.

Effect of Triple Combination Therapy on Metabolism of OMP by CYP2C19 Triple combination therapy consisting of OMP with CLR-TND or OMP with AMX-MET—is also recommended for efficient eradication of *H. pylori* due to its low cost, good therapeutic effect, and mild side effects. Therefore, the effects of CLR-TND and AMX-MET on the metabolism of OMP by CYP2C19 were also investigated. Although CLR and TND inhibited OMP metabolism by 69 and 66%, respectively, the combination of both drugs led to 76% inhibition of OMP metabolism. This could be because the CLR-TND combination resulted in a decreased $V_{max}$ of 3.1 µM/min/µM of CYP2C19 and an increased $K_m$ of 36 µM—nearly equal to what was observed with CLR alone and TND alone, respectively. Therefore, CLR-TND double combination therapy led to an overall more inhibitory effect than was observed with each drug alone. Similarly, the AMX-MET double combination therapy led to a higher inhibitory effect than was observed for either drug alone.

OMP can interact with CYP2C19 as a substrate and competitive inhibitor. Diazepam clearance is decreased by 26–54% in the presence of OMP, indicating that omeprazole and diazepam compete for the same site on CYP2C19. The metabolism of CYP2C19 substrates could be decreased through competitive inhibition at the active site. Thus, the DDIs that occur during *H. pylori* eradication therapy could be because of CLR, AMX, TND, and MET competing with OMP to bind to the active site of CYP2C19. In addition, the binding of these drugs to the CYP2C19 active site may enhance the water coordination to the heme iron, thereby reducing oxidation of the OMP substrate. In this case, the co-administered drug may stabilize water coordination through formation of a hydrogen bonding network.

Conclusion OMP metabolism and its required dose for eradication of *H. pylori* are not only affected by CYP2C19 genotype but are also greatly affected by drugs used in combination with OMP. The presence of AMX, MET, TND, or CLR led to inhibition of OMP metabolism by CYP2C19 of 28, 40, 66, or 69% respectively. Moreover, triple combination therapy resulted in inhibition of OMP metabolism by 76 (for the OMP-CLR-TND combination) and 48% (for the OMP-AMX-MET combination). Based on this study, DDIs can have a great effect on OMP concentration and, hence, on eradication of *H. pylori*. In addition, this provides the basis for OMP dose adjustment in double and triple combination therapy for optimum therapeutic effect in *H. pylori* eradication. These drugs could improve the efficacy of OMP in the eradication of *H. pylori* owing to not only their antibacterial effects, but also their inhibition of OMP metabolism by CYP2C19—leading to a higher drug plasma level. Finally, the DDIs resulting from CYP2C19 enzyme inhibition during *H. pylori* therapy are of great concern and may alter the therapeutic outcomes of treatment.

Acknowledgments TZA was financially supported by the Egyptian government through postdoctoral fellowship.

Conflict of Interest The authors declare no conflict of interest.

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