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Antioxidant properties of omeprazole

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Abstract Potential antioxidant properties of therapeutically achievable concentrations of the protonated, active form of omeprazole (OM) were investigated in vitro at specific acidic pH values to mimic intragastric conditions in the clinical setting. We found that OM is a powerful scavenger of hypochlorous acid (HOCl) even at a drug concentration of 10 μM at pH of 5.3 or 3.5. This effect is also evident in the presence of the physiological HOCl scavenger ascorbate. Moreover, 10 and 50 μM OM inhibit significantly both iron-and copper-driven oxidant damage at pH 5.3 and 3.5, respectively. Since oxidative stress is involved the gastric injury of peptic ulcer and gastritis, it may be hypothesized that some therapeutical effects of OM could also be related to its antioxidant properties.

Key words: Omeprazole; Hypochlorous acid; Iron; Copper; Oxidative stress; Antioxidant

1. Introduction

Omeprazole (OM) is an inhibitor of the proton pump of the gastric parietal cells widely used for the treatment of peptic ulcer and gastritis. OM is a lipophilic weak base [1], so that it is in its unprotonated and inactive form at physiological pH of the bloodstream, but accumulates in acidic environments [1], such as in parietal cells and, conceivably, in gastric inflammatory domains, which are characterized by low pH values and increased vascular permeability. OM, therefore, is a prodrug, which in an acidic pH becomes converted to its active form, a sulfenamide, by protonation [1]. This sulfenamide, besides an anti-secretory activity, could also have some antioxidant effects. Indeed, similar compounds, namely sulfonamides, chemically react with hypochlorous acid (HOCl) [2], which is the most toxic and abundant oxidant generated by phagocytes [2–4]. These cells are relevant in inflammatory gastric injury, especially in the presence of *Helicobacter pylori* [5,6]. Moreover, the benzimidazolic structure of OM [1] suggests possible inhibitory interactions with catalytic transition metals. We have indeed reported similar antioxidant properties for the imidazolic drug cimetidine [7]. Notably, redox-active transition metals are crucial in oxidant generation and oxidative stress [3,4,8], which play a role in the pathogenesis of peptic ulcer and gastritis [5,9–12].

The present study was designed to investigate potential antioxidant effects of the protonated, active form of OM in specific experimental models. The results show that this drug is a powerful scavenger of HOCl and, additionally, it can counteract iron-and copper-mediated oxidant injury.

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2. Materials and methods

Reagents were from Sigma Aldrich s.r.l., Milano, Italy, except for OM, which was a generous gift of Astra, Sodertalje, Sweden. Procedures were carried out in plastic or acid-washed glassware, and solutions were prepared in Chelex 100 resin-treated glass-bidistilled water. The protonated form of OM was obtained by adding the native drug to 5 mM HCl, and used at final concentrations of 10, 50 and 100 μM. Biochemical tests were specifically carried out at acidic pH values, which may correspond to those of acute gastric inflammatory areas and of the intragastric pH reached after drug treatment in vivo. In this regard, it has been shown that inflammatory phagocytic cells secrete H^+ and can acidify the pericellular environment at pH levels of about 3.5 [13]; back-diffusion of acid through damaged gastric mucosa [14] could also favour these relatively low pH values in ulcer tissues. Furthermore, it has been reported that the mean intragastric pH is approx. 5.3 after OM therapy in humans [15]. Antioxidant effects of protonated OM were not investigated at pH 7.4, since the active drug is not formed at physiological pH values of the bloodstream [1]. Possible antioxidant activity of the unprotonated form of OM (which is poorly water-soluble) was also not investigated, considering that it does not represent the active drug in the clinical setting.

2.1. HOCl scavenging

We have used the β-carotene test to assess scavenging properties of OM against HOCl [7,16]. Indeed, it is known that HOCl induces β-carotene oxidation and bleaching [4,7,16,17], which can be counteracted by HOCl scavengers [7,16]. Since ascorbic acid is present in the gastric juice [18] and it can scavenge HOCl [4], potential scavenging capacity of OM against HOCl was investigated not only with the drug alone but also in the presence of ascorbate; moreover, specific antioxidant effects of ascorbate alone were studied. In this context, mean intragastric ascorbic acid concentrations of about 20 and 70 μM have been shown in patients with peptic ulcer or gastritis and in healthy subjects, respectively [18].

Reaction mixtures contained 1.8 μM β-carotene in 20 mM K_2HPO_4-HCl, pH 5.3 or 3.5, with and without protonated OM or 20 and 70 μM ascorbic acid or OM plus ascorbate. HOCl was added at 'physiologically' relevant final concentrations of 70 μM [4,7,16] to start the reaction, followed by 10 min incubation at 25°C; the β-carotene-related absorbance values at 451 nm (A_{451}) were then recorded spectrophotometrically against appropriate drug-containing blanks to assess specific antioxidant effects.

The concentrations of NaOCl-derived HOCl were calculated using a molar extinction coefficient of 100 M^{-1} cm^{-1} at 235 nm [19].

2.2. Iron-and copper-driven oxidant injury

The redox-active transition metals iron and copper can oxidize the sugar deoxyribose [4,20,21], which is an integral component of DNA [4]. Iron-and/or copper-related oxidizing species generated at target molecular sites are relevant in conditioning oxidant injury in vivo [4,8,22,23], and they are involved in metal-dependent deoxyribose oxidation in the absence of the non-physiological chelator EDTA but in the presence of phosphates [4,20,21], which are physiological transition metal complexing agents [24]. Thus, molecules able to bind and inactivate catalytic transition metals, removing them from deoxyribose, can counteract specifically metal-mediated sugar oxidation [4,20,21]. Reaction mixtures contained, in a final volume of 1.0 ml, the following reagents at the final concentrations stated: 10 mM K_2HPO_4-HCl, pH 5.3 or 3.5, 4.0 mM deoxyribose, 6 μM $FeCl_3$ or $CuCl_2$, 50 μM H_2O_2 and 20 μM ascorbic acid, with or without protonated OM. After 30 min incubation at 37°C, 1.0 ml of 2.5% trichloroacetic acid and 1.0 ml of 0.6% aqueous solution of thiobarbi-

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Table 1
Effects of protonated OM with and without ascorbic acid on HOCl-induced β-carotene bleaching at pH 5.3

	A_{451}
1.8 μM β-carotene alone	0.139 ± 0.007
Control (1.8 μM β-carotene plus 70 μM HOCl)	0.023 ± 0.005
Control plus OM	
10 μM	0.073 ± 0.007[a,d]
50 μM	0.140 ± 0.009[a–d]
100 μM	0.141 ± 0.010[a,c,d]
Control plus 20 μM ascorbate	0.037 ± 0.006[a]
Control plus 20 μM ascorbate and OM	
10 μM	0.089 ± 0.008[a,d,e]
50 μM	0.142 ± 0.011[a–d]
100 μM	0.143 ± 0.012[a,c,d]

Means ± S.D. of 5 experiments. [a]$P < 0.05$ vs control; [b]$P < 0.05$ vs preceding values; [c]P = NS vs β-carotene alone; [d]$P < 0.05$ vs control plus ascorbate; [e]$P < 0.05$ vs control plus OM (ANOVA plus Student–Newman–Keuls test). See sections 2 and 3 for further explanations.

turic acid (TBA) were added, followed by 15 min heating at 95°C. After cooling, the pink chromogen was read at 532 nm on a double beam Varian DMS 200 spectrophotometer against an appropriate blank. The drug gave no interference in the TBA test. Results were expressed as nmol TBA reactants (TBA-R)/ml, using for calculations a molar extinction coefficient of 1.54×10^5 M^{-1} cm^{-1}.

2.3. Statistics

Data were calculated as means ± S.D. of 5 different experiments for each biochemical test and specific pH value. Specific drug effects were evaluated via the one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test [25]. $P < 0.05$ was regarded as statistically significant [25].

3. Results

3.1. Effects of protonated OM and ascorbic acid on HOCl-induced β-carotene bleaching

As shown in Table 1, protonated OM antagonized significantly HOCl-induced β-carotene bleaching at pH 5.3, resulting in about 43% inhibition of β-carotene oxidation at 10 μM drug concentration, and in total inhibition at 50 and 100 μM. Similar results were observed at pH 3.5; indeed, the drug gave approx. 39% inhibition of HOCl-induced β-carotene bleaching at 10 μM, and total inhibition beginning from 50 μM (data not shown). HOCl scavenging effects of protonated OM were also striking in the presence of ascorbic acid. We will report the results obtained at pH 5.3, but a similar experimental trend was observed at pH 3.5. Interestingly, 20 μM ascorbate afforded only little protection against HOCl-induced β-carotene bleaching (Table 1), suggesting that the intragastric ascorbate concentrations of patients with peptic ulcer and/or gastritis are poorly effective at counteracting HOCl-driven oxidant injury. In the presence of 20 μM ascorbate and 10 μM OM, however, marked inhibition of β-carotene oxidation was observed, which was greater than that mediated by each compound alone; moreover, OM, even at 10 μM, was by far more effective than ascorbate in HOCl antagonism (Table 1). Hence, the HOCl scavenging effects of ascorbate and protonated OM are apparently additive, although those of the drug are superior. OM, at 50 and 100 μM, was also totally inhibitory with 20 μM ascorbate (Table 1). Under our conditions, 70 μM ascorbic acid gave a near total (i.e. about 92%) antioxidant protection, which became effectively total in the presence of each OM concentration (not shown). These results are indicative of the HOCl scavenging capacity of protonated OM even in the presence of the physiological HOCl scavenger ascorbic acid.

3.2. Effects of protonated OM on iron-and copper-driven oxidant injury

When oxidative reactions were performed at pH 5.3, protonated OM showed significant antioxidant effects beginning from 10 μM drug concentration, which resulted in about 20 and 14% inhibition of iron-and copper-dependent deoxyribose oxidation, respectively (Table 2). At pH 3.5, however, protonated OM resulted in a significant antagonism of either iron-

Table 2
Effects of protonated OM on iron-and copper-dependent deoxyribose oxidation at pH 5.3

	Iron	Copper
Control	8.55 ± 0.65	3.15 ± 0.25
Control plus OM		
10 μM	6.85 ± 0.55[a]	2.7 ± 0.32[a]
50 μM	5.15 ± 0.42[a,b]	2.2 ± 0.17[a,b]
100 μM	4.5 ± 0.28[a]	1.9 ± 0.15[a]

Results are means ± S.D. of 5 experiments, and are expressed as nmol TBA-R/ml.
[a]$P < 0.05$ vs control; [b]$P < 0.05$ vs the values that precede (ANOVA plus Student–Newman–Keuls test). See section 2 for methodological explanations.

Table 3
Effects of protonated OM on iron-and copper-dependent deoxyribose oxidation at pH 3.5

	Iron	Copper
Control	3.3 ± 0.3	2.65 ± 0.2
Control plus OM:		
10 μM	2.97 ± 0.27	2.47 ± 0.22
50 μM	2.7 ± 0.23[a]	2.1 ± 0.16[a,b]
100 μM	2.03 ± 0.25[a,b]	1.85 ± 0.14[a,b]

Results are means ± S.D. of 5 experiments, and are expressed as nmol TBA-R/ml.
[a]$P < 0.05$ vs control; [b]$P < 0.05$ vs the values that precede (ANOVA plus Student–Newman–Keuls test). See section 2 for methodological explanations.

or copper-mediated deoxyribose oxidation at 50 and 100 μM drug concentrations (Table 3). Notably, in our model system, iron was markedly more prooxidant at pH 5.3 than at pH 3.5; a similar phenomenon, although much less evident, was observed for copper. Thus, the inhibitory effects of protonated OM against iron-and copper-mediated oxidant damage are more pronounced at pH values of 5.3, which correspond to the intragastric ones observed after OM therapy in humans [15]. On the other hand, it has been reported that the effects of chelating compounds on catalytic transition metal-driven oxidative damage may be pH-dependent [3,8].

4. Discussion

The present study shows that the protonated, active form of OM has significant antioxidant effects against HOCl-and catalytic transition metal-mediated oxidative injury.

Phagocyte-derived HOCl oxidizes biologically relevant molecules, such as β-carotene [4,7,16,17], which is decreased in the gastric juice of patients with inflammatory gastric diseases associated with *H. pylori* [26], a bacterium stimulating neutrophil oxidant generation [6]. Decreased gastric β-carotene levels may play a role in gastric oxidant damage and carcinogenesis [27]. Our data suggest that protonated OM might preserve gastric β-carotene in vivo through a direct HOCl antagonism, and show significant HOCl scavenging effects of OM also in the presence of the physiological HOCl scavenger ascorbic acid. Notably, with a concentration of ascorbate representative of the mean intragastric concentration of patients with peptic ulcer or gastritis, i.e. 20 μM [18], the drug continues exerting marked HOCl scavenging activity, suggesting its specific antioxidant effects in the clinical setting. Scavenging of HOCl by OM could also prevent formation of monochloramines; these cytotoxic compounds are generated from the interaction of HOCl and ammonia [2,3,28], which is largely produced by *H. pylori* [28].

It is remarkable that protonated OM can also bind-inactivate both iron and copper, presumably through its benzimidazole ring. Indeed, some imidazolic drugs, such as cimetidine, bind iron and copper [7,29,30]. Iron is relevant in gastric oxidative injury [9,31,32]. Copper is another powerful catalytic metal, which typically mediates site-specific oxidant damage [4,21,22]. Transition metal availability in a free and catalytically active form should be favoured in inflammatory diseases, such as peptic ulcer and gastritis. In fact, especially under acidic conditions, white blood cell prooxidants induce metal delocalization from iron-and copper-containing proteins [3,4,8,33–36], thus promoting oxidative stress. Consistently, neutrophil depletion prevents experimental gastric ulceration [37], and metal chelators antagonize oxidative injury to gastric cells [9,31,32].

Under a pharmacological and therapeutical profile, it should be noted that, even if the concentrations of protonated OM in selected sites, such as in parietal cells and in acidic gastric inflammatory areas, are unknown, relatively high concentrations, conceivably in the millimolar range, may be expected, because of drug accumulation in acidic environments and of inflammation-induced increased vascular permeability. On the other hand, drug concentrations of about 10 μM have been shown even in the plasma environment after intravenous administration of 40 mg OM in patients with peptic ulcer [38], and plasma OM levels up to 14 μM have been reported in subjects given OM by mouth after 7 days of treatment [1]. Since OM has inibitory effects on neutrophil function [39], the final antioxidant potential of the drug may be even higher in vivo, resulting from 'direct' and 'indirect' antioxidant mechanisms. OM antioxidant properties, therefore, could be significant in the clinical setting of peptic ulcer and gastritis, which are oxidative stress-related conditions [5,9–12]. Indeed, it is noteworthy that antioxidant administration improves treatment outcomes in patients with peptic ulcer [11]. Finally, OM antioxidant properties should be taken into account when investigating the levels of oxidizable antioxidants, such as β-carotene, in the human gastric mucosa and juice.

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