



Bismuth increases hydroxyl radical-scavenging activity of histamine H₂-receptor antagonists

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Abstract:

The effects of histamine H₂-receptor antagonists, alone or in a combination with bismuth, on [•]OH-provoked degradation of deoxyribose were studied. The histamine H₂-receptor antagonists (cimetidine, ranitidine and roxatidine), themselves decreased the deoxyribose damage in Fenton-type systems. In combinations with bismuth, their inhibitory effect in Fenton system (Fe(III)/ascorbic acid + H₂O₂) was stronger. Moreover, unlike Fe(III) and Cu(II), which in the presence of ascorbic acid + H₂O₂ led to an increase in the [•]OH formation (deoxyribose damage), Bi(III) showed an opposite effect. The present results are interpreted in view of a better [•]OH scavenging activity of bismuth complexes of histamine H₂-receptor antagonists as compared to that of the corresponding drugs. These findings might be one more explanation why bismuth salts, in combination with acid-reducing agents, are more effective anti-ulcer agents.

Key words:

[•]OH radicals, bismuth, histamine H₂-receptor antagonists, bismuth/drug complexes

Abbreviations: CIM – cimetidine, De-Nol – colloidal bismuth subcitrate, DR – deoxyribose, MX₁ – roxatidine-bismuth citrate, ¹O₂ – singlet oxygen, [•]OH – hydroxyl radical, RAN – ranitidine, ROX – roxatidine, TBARSs – thiobarbituric acid reactive substances

Introduction

Two types of drugs are known for ulcer therapy: i) inhibitors of the acid secretion (histamine H₂-receptor antagonists and proton pump inhibitors) and ii) inhibitors of pathogenic flora, anti-*Helicobacter* agents (antibiotics and bismuth salts).

In the last years, the interest in using different combinations of acid-reducing and anti-*Helicobacter*

drugs, as an alternative to long-term drug maintenance therapy or elective surgery, significantly increased. This new family of anti-ulcer drugs is represented by the complex agent – ranitidine-bismuth citrate (trademark “Pylorid”), whose anti-ulcer efficacy has been shown both in experimental and clinical conditions [2, 8, 9, 11, 24, 25, 32, 33, 40, 46]. A good gastroprotective effect against stress-induced ulcers has also been found using roxatidine-bismuth citrate (code name MX₁) [27]. This substance markedly reduces the number and the size of the ulcerative lesions in a model of ethanol-induced gastric mucosal damage, reduces the acid secretion in pylorus-ligated rats and shows an anti-*Helicobacter* activity [35]. The stronger gastroprotective effect of MX₁ against acetylsalicylic acid- and indomethacin-induced ulcers, as compared to that of roxatidine (ROX) and De-Nol

(colloidal bismuth subcitrate) is attributed both to its H₂-blocking and mucin-stimulating activity [36].

Although the primary action of anti-ulcer drugs consists in inhibition of the acid secretion and pathogenic flora, some other mechanisms of action cannot be excluded. There are data that oxygen free radicals contribute to the development of gastrointestinal mucosal damage [1, 45, 51]. Histamine H₂-receptor antagonists are able to prevent gastric mucosal lipid peroxidation [26, 34, 39, 41]. Cimetidine (CIM) and ranitidine (RAN) have been found to be good $\cdot\text{OH}$ radical scavengers [6, 49]. There are data for reactions of CIM (or its derivatives) and copper/CIM complexes with O₂⁻ radicals [13, 29, 30]. However, we failed to find data about the participation of bismuth complexes with histamine H₂-receptor antagonists in reactions with free oxygen radicals. It is possible that they possess a better antioxidant activity than that of the corresponding drugs, which might be one more explanation of their better therapeutic effect.

It is known that the damaging effects of reactive oxygen species are attributed mainly to the more reactive species, such as hydroperoxyl radical ($\cdot\text{OH}_2$), singlet oxygen (¹O₂) and especially hydroxyl radical ($\cdot\text{OH}$) [18, 21]. In the presence of a metal reducer (ascorbic acid), Fe(III) ions added to H₂O₂ generate $\cdot\text{OH}$ radicals (Fenton system) leading to deoxyribose degradation, which could be inhibited by specific metal chelators, catalase and hydroxyl radical scavengers [23].

Thus, using the deoxyribose test, a study on the effects of histamine H₂-receptor antagonists alone and in combinations with bismuth on the deoxyribose degradation, provoked by $\cdot\text{OH}$ radicals, generated in Fenton-type systems was carried out.

Materials and Methods

Materials

ROX, De-Nol, ROX-bismuth citrate (N-[3-(1-piperidinylmethyl)phenoxy]propyl]-hydroxyacetamide-2-hydroxypropane-1,2,3-tricarboxylate-bismuth(3⁺)-citrate complex) and the other metal complexes of ROX were received from Chemical Pharmaceutical Research Institute, Bulgaria. RAN, CIM and deoxyribose (DR) were purchased from Sigma Chemical Co.;

K₂HPO₄ · 3H₂O, KH₂PO₄ and H₂O₂ were from Merck; CuSO₄ and FeCl₃ were from Reanal; Bi(NO₃)₃ was from Riedel de Haen. The other reagents were of analytical grade. All solutions were prepared with water redistilled from glass apparatus.

Methods

Deoxyribose-test (principle):

Deoxyribose (DR) binds iron ions, which bound on this detector molecule would catalyze a site-specific production of $\cdot\text{OH}$ radicals [14]. The classical $\cdot\text{OH}$ scavengers can affect $\cdot\text{OH}$ -dependent damage to detector molecule by binding iron ions. They act predominantly by this mechanism and are practically unable to protect DR against damage produced by $\cdot\text{OH}$ radicals generated at specific sites [15] under experimental conditions where they do not interact with iron ions. If a scavenger molecule has a higher binding affinity for iron than the detector, then the scavenger can protect the detector molecule, transferring the damage to itself [12], as this protection depends on the concentration of scavenger with respect to the detector molecule and on the scavenger's second-order rate constant of reaction with $\cdot\text{OH}$ [14, 23].

Hydroxyl radicals were generated by incubating the following reagents: 20 mM potassium phosphate buffer, pH 7.2, 3.4 mM DR, 0.1 mM FeCl₃ or CuSO₄ · 5H₂O, 0.1 mM ascorbic acid, 0.5 mM H₂O₂ and additions: 0.1 mM EDTA, 0.1 mM Bi(NO₃)₃, 0.1 mM citric acid, 0.1 mM De-Nol and drugs (CIM, RAN, ROX). After incubation of the samples at 37°C for 60 min the degradation of DR, as a detector of $\cdot\text{OH}$ radicals, was measured in terms of the formation of thiobarbituric acid reactive substances (TBARs) [19]. The samples were heated at 100°C for 15 min to develop the color and after cooling the absorbance at 532 nm was read against appropriate blanks. The A₆₀₀ was considered to be a nonspecific base line drift and was subtracted from A₅₃₂.

Statistical analysis

The values are expressed as the mean ± SE of n experiments. Statistical comparisons were made using the Student's *t*-test. A probability (p) value of 0.01 was taken to indicate the statistical significance.

Results

The present experiments designed to examine the effects of the histamine H₂-receptor antagonists (CIM, RAN and ROX), alone or in combinations with bismuth on [•]OH-provoked DR degradation were carried out in the presence and in the absence of EDTA. The addition of EDTA to the reaction system, generating [•]OH radicals, greatly enhances damage to DR through removing iron from the detector molecule thereby causing that more [•]OH radicals escape into free solution [12, 15]. On the other hand, the Fe-chelation to EDTA facilitates iron reduction by ascorbate [5] and affects its reactivity in this ascorbate-dependent system, producing OH radicals [20, 22].

Like mannitol (a typical [•]OH scavenger), CIM, RAN and ROX significantly decreased the DR degradation in Fenton system (Tab. 1). When the iron in this system was exchanged for copper (Fenton-like system), where [•]OH radicals were also formed [16, 17, 42, 44], the drugs decreased DR degradation anew. Thus, the results, obtained both in the presence and in the absence of EDTA showed that like CIM and RAN [6, 49] ROX had also a good [•]OH scavenging activity.

DR degradation strongly decreased, when a bismuth salt was added to the Fenton system (Tab. 2). Moreover, the bismuth presence led to an increase in the preventing effect of histamine H₂-receptor antago-

Tab. 1. [•]OH-scavenging activity of histamine H₂-receptor antagonists in Fenton-type systems

Additions	Without EDTA	0.1 mM EDTA
I. Fe(III)/ascorbic acid, H ₂ O ₂ , DR (Fenton system)		
Controls	1.163 ± 0.041	2.162 ± 0.088
+ 10 mM Mannitol	0.651 ± 0.023*	0.980 ± 0.039*
+ 10 mM Cimetidine	0.361 ± 0.039*	0.670 ± 0.052*
+ 10 mM Ranitidine	0.577 ± 0.045*	0.889 ± 0.098*
+ 10 mM Roxatidine	0.572 ± 0.030*	0.760 ± 0.049*
II. Cu(II)/ascorbic acid, H ₂ O ₂ , DR (Fenton-like system)		
Controls	0.663 ± 0.014	0.717 ± 0.020
+ 10 mM Mannitol	0.393 ± 0.017*	0.437 ± 0.023*
+ 10 mM Cimetidine	0.315 ± 0.038*	0.343 ± 0.020*
+ 10 mM Ranitidine	0.399 ± 0.012*	0.409 ± 0.016*
+ 10 mM Roxatidine	0.329 ± 0.019*	0.378 ± 0.017*

Reaction mixtures: 20 mM potassium phosphate buffer, pH 7.2, 3.4 mM deoxyribose (DR), 0.5 mM H₂O₂, 0.1 mM FeCl₃ or 0.1 mM CuSO₄, 0.1 mM ascorbic acid and additions: 0.1 mM EDTA and drugs. Incubation was carried out for 60 min at 37 °C. Values (E₅₃₂-E₆₀₀) are the mean ± SEM of 7-9 separate experiments. Significant differences vs. controls at * p < 0.01

nists on DR molecule. De-Nol and Bi/citric acid (molar ration 1:1) also increased the preventing effect of RAN and ROX on DR molecule; however, the effect of CIM was not significantly changed. In addition, the metal complexes – bismuth/ROX (MX₁), zinc/ROX and copper/ROX, but not cobalt/ROX also decreased

Tab. 2. Bismuth effects on [•]OH-scavenging activity of histamine H₂-receptor antagonists in Fenton system

Additions	–	Cimetidine	Ranitidine	Roxatidine
Fe(III)/ascorbic acid, H ₂ O ₂ , DR				
Controls	1.163 ± 0.041	0.361 ± 0.039	0.577 ± 0.045	0.572 ± 0.030
+ Bi(III)	0.572 ± 0.064*	0.212 ± 0.016*	0.342 ± 0.014*	0.246 ± 0.028*
+ Bi/citric acid [‡]	0.602 ± 0.043*	0.282 ± 0.020	0.368 ± 0.008*	0.321 ± 0.016*
+ De-Nol	0.428 ± 0.010*	0.321 ± 0.031	0.350 ± 0.005*	0.347 ± 0.006*
Fe(III)/ascorbic acid, H ₂ O ₂ , DR, EDTA				
Controls	2.162 ± 0.088	0.670 ± 0.052	0.889 ± 0.098	0.760 ± 0.049
+ Bi(III)	1.978 ± 0.085	0.465 ± 0.029*	0.571 ± 0.023*	0.696 ± 0.021*
+ Bi/citric acid	1.734 ± 0.042	0.242 ± 0.008*	0.327 ± 0.013*	0.386 ± 0.020*
+ De-Nol	1.623 ± 0.086	0.773 ± 0.044	0.928 ± 0.034	0.850 ± 0.092

Reaction mixtures: 20 mM potassium phosphate buffer, pH 7.2, 3.4 mM deoxyribose (DR), 0.5 mM H₂O₂, 0.1 mM FeCl₃, 0.1 mM ascorbic acid and additions: 0.1 mM EDTA, 0.1 mM Bi(NO₃)₃, 0.1 mM De-Nol, 0.1 mM Bi(III)/citric acid (1:1) and 10 mM drugs. Incubation was carried out for 60 min at 37 °C. Values (E₅₃₂-E₆₀₀) are the mean ± SEM of 9 separate experiments. Significant differences vs. controls at * p < 0.01. [‡] Compared to the corresponding controls, citric acid itself had no effect on DR damage (1.163 ± 0.041 and 1.128 ± 0.055, respectively)

Tab. 3. Effect of roxatidine/metal complexes on deoxyribose degradation in Fenton-type systems

Additions	Fenton system	Fenton-like system
Controls	1.049 ± 0.067	0.663 ± 0.014
Controls + Roxatidine	0.636 ± 0.029	0.329 ± 0.019
+ Bi/Roxatidine (MX ₁)	0.233 ± 0.017*	0.213 ± 0.019*
+ Cu/Roxatidine	0.326 ± 0.009*	–
+ Zn/Roxatidine	0.353 ± 0.044*	–
+ Co/Roxatidine	0.618 ± 0.004*	–

Reaction mixtures: 20 mM potassium phosphate buffer, pH 7.2, 3.4 mM deoxyribose (DR), 0.5 mM H₂O₂, 0.1 mM FeCl₃, 0.1 mM ascorbic acid and additions: 10 mM roxatidine and its metal complexes. Incubation was carried out for 60 min at 37 °C. Values (E₅₃₂–E₆₀₀) are the mean ± SEM of 7 separate experiments. Significant differences vs. controls (+ roxatidine) at * p < 0.01

the DR degradation and their inhibitory effect was stronger, than that of ROX itself (Tab. 3).

The inhibitory effect of bismuth, added to Fenton system as Bi(NO₃)₃, De-NoL or bismuth/citric acid complex (molar ratio 1:1) was apparent only in the absence of EDTA (Tab. 2). Similar results were obtained with MX₁. Compared with ROX and Bi(III), MX₁ inhibited DR degradation stronger in the absence of EDTA (Tab. 3), while its inhibitory effect was similar to that of ROX (0.808 ± 0.034 and 0.760 ± 0.049 with MX₁ and ROX, respectively) in the presence of EDTA. Thus, EDTA strongly decreased or completely abolished the bismuth effects in Fenton system, irrespective of the presence or the absence of the histamine H₂-receptor antagonists under study.

Tab. 4. •OH-generation in the presence of iron and bismuth

Additions	Rates of deoxyribose degradation (E ₅₃₂ – E ₆₀₀)		
	Ascorbic acid, H ₂ O ₂ , DR	Fe(III)/ascorbic acid, H ₂ O ₂ , DR	Bi(III)/ascorbic acid, H ₂ O ₂ , DR
Controls	0.403 ± 0.023	1.163 ± 0.041	0.258 ± 0.014
+ Cimetidine	0.378 ± 0.046	0.361 ± 0.039*	0.169 ± 0.043*
+ Ranitidine	0.428 ± 0.057	0.577 ± 0.045*	0.185 ± 0.012*
+ Roxatidine	0.417 ± 0.037	0.572 ± 0.030*	0.203 ± 0.009*

Reaction mixtures: 20 mM potassium phosphate buffer, pH 7.2, 3.4 mM deoxyribose (DR), 0.5 mM H₂O₂, 0.1 mM ascorbic acid and additions: 0.1 mM FeCl₃, 0.1 mM Bi(NO₃)₃ and 10 mM drugs. Incubation was carried out for 60 min at 37 °C. Values (E₅₃₂–E₆₀₀) are the mean ± SEM of 7 separate experiments. Significant differences vs. controls at * p < 0.01

It might be suggested that the Bi(III)-induced inhibition of •OH-provoked DR degradation in Fenton system is due to: i) a competition between bismuth and iron for binding sites on the detector molecule (DR) and/or ii) an inability of bismuth to generate •OH-radicals in the presence of H₂O₂.

For understanding of whether or not free •OH radicals are formed in the presence of bismuth and H₂O₂, the iron in the Fenton reaction (Fe(II) + H₂O₂ → Fe(III) + •OH + •OH) was exchanged with bismuth. Unlike iron, which led to a significant production of •OH radicals, bismuth showed an opposite effect (Tab. 4). Moreover, •OH-scavenging drugs strongly decreased DR damage in Fenton system (Fe(III)/ascorbic acid, H₂O₂, DR), but had a very slight inhibitory effect in a bismuth-containing system (Bi(III)/ascorbic acid, H₂O₂, DR).

Discussion

Helicobacter pylori has emerged as an important factor, which can cause or predispose to development of ulceration [4, 31, 47, 48]. Its elimination results in healing of ulcers and low recurrence rate [4]. Being inhibitors of pathogenic flora, bismuth salts have been found to be very effective for ulcer therapy, especially in combinations with antisecretory agents [3, 7, 28, 38, 52]. It has already been mentioned that "Pylorid" and MX₁ have better gastroprotective effect, as compared to that of the corresponding drugs. The treatment of the gastric ulcer with De-NoL elicited also a better gastroprotective effect than the treatment with acid-reducing drugs [28]. According to Peterson [43], a direct protective effect of bismuth on mucosa, rather than its antimicrobial activity against *Helicobacter pylori* [50] is responsible for its therapeutic effect.

The present study showed that ROX, like CIM and RAN, had a good •OH scavenging activity, which strongly increased in the presence of bismuth. The bismuth-induced decrease in •OH-provoked DR degradation in Fenton system might be due to a preventing role of bismuth ions and/or BiO• (bismuthyl radical) in iron ions binding to the detector molecule (DR).

The behavior of iron and bismuth was opposite in the presence of H₂O₂, i.e. iron increased DR damage (a result of •OH generation), while bismuth decreased it. It seems that different mechanisms underlie the re-

action of H_2O_2 with iron and bismuth ions. Two basic mechanisms for this reaction have been described: the bridged mechanism and the outer-sphere mechanism [10]. Because the Fenton-type reaction is unlikely to take place by the outer-sphere electron transfer mechanism [37], the bismuth behavior in the presence of H_2O_2 might be interpreted on the basis of the above-described mechanism, i.e. considering that bismuth, unlike iron, is not able to generate $\cdot OH$.

The present results justify further studies of the effects of histamine H_2 -receptor antagonists and their metal complexes, especially bismuth complexes ("Pylorid" and MX_1) on lipid peroxidation and antioxidant enzyme activities in biological preparations in relation to ulcer-induced free radical processes. These studies would allow for considering the therapeutic action of these metal/drugs complexes in a new light.

In conclusion, the better bismuth effects on the $\cdot OH$ -scavenging activity of histamine H_2 -receptor antagonists might be one more explanation of the better gastroprotective effect of the complex agents, like RAN-bismuth citrate ("Pylorid") and ROX-bismuth citrate (MX_1), as compared to corresponding drugs. Moreover, the inclusion of other suitable metal ions in the drug molecules would be of interest for medicine.

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