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Effects of diphenhydramine and famotidine on lipid peroxidation and activities of antioxidant enzymes in different rat tissues

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Abstract:
The potential antioxidant activity of diphenhydramine (histamine H_1-receptor antagonist) and famotidine (histamine H_2-receptor antagonist) was studied. Diphenhydramine inhibited the spontaneous, Fe(II)-induced and Fe(II)/ascorbate-induced lipid peroxidation, while famotidine showed a biphasic concentration-dependent effect on spontaneous lipid peroxidation (a stimulation by 1mM and an inhibition by 5mM) and increased Fe(II)-induced- and inhibited Fe(II)/ascorbate-induced lipid peroxidation in the rat liver and brain. Both drugs decreased $^{\cdot}OH$-provoked deoxyribose degradation in Fenton-type systems and inhibited $O_2^{\cdot-}$-provoked reduction of nitro-blue tetrazolium and ferrycytochrome C, but famotidine effect was stronger than that of diphenhydramine. The significant famotidine-induced inhibition of nitro-blue tetrazolium reduction might be underlain by the stimulation of superoxide dismutase activity. Famotidine and diphenhydramine did not alter the catalase activity in all tissue preparations, except for its concentration of 5mM (a complete inhibition). The present results suggest a beneficial effect of histamine H_1 and H_2-blockers, especially famotidine, as antioxidants and/or metal chelators, which might be an additional explanation of their therapeutic action.

Key words:
diphenhydramine, famotidine, oxygen free radicals, lipid peroxidation, antioxidant enzymes

Abbreviations: CIM – cimetidine, DR – deoxyribose, FAM – famotidine, MEP – mepyramine, histamine H_1-blockers, $^{\cdot}OH$ – hydroxyl radical, RAN – ranitidine, histamine H_2-blockers, TBARS – thiobarbituric acid reactive substances

Introduction

Free radical processes and peroxidation of important cell structures underlie the pathogenesis of many diseases, including gastrointestinal mucosa damage [2, 9, 32, 36, 38, 44]. Oxygen free radicals have been suggested to cause gastric mucosal injury induced by ischemia-reperfusion in pylorus-ligated rats [40]. According to Kitano et al. [24] this acute mucosal injury is protected by the histamine H_2-receptor antagonist – cimetidine (CIM), which is due not only to suppression of the gastric acid secretion but also to CIM antioxidant action when it is present at a high concentration in the "intragastric environment". There are data that pretreatment with CIM prevents ethanol-induced mucosal lipid peroxidation [34] and decreases CCl_4-induced liver damage [33]. CIM and ranitidine (RAN)

have been found to be good scavengers of the •OH radicals, generated in a Fe^{2+}-H_2O_2 reaction mixture [7, 29, 43]. According to Lapenna et al. [29], the drugs scavenge •OH radicals with a very high rate constant but are ineffective in protection against H_2O_2 and O_2^-, while other authors [23, 46] suggest the possibility of reaction of CIM or of its derivatives with O_2^- radicals.

However, an increased O_2^- generation in granulocytes of patients with ulcer disease and in healthy subjects after CIM, but not after RAN or famotidine (FAM) administration has been reported [21]. Moreover, pretreatment with CIM or diphenhydramine (DPH) does not alter the ischemia-induced increase in the vascular permeability of skeletal muscles, while the pretreatment with free radical scavengers (superoxide dismutase, catalase or dimethylsulfoxide) significantly attenuates it [28].

These contradictory data about the potential antioxidant capacity of the different histamine H_2-blockers, as well as the insufficient studies on histamine H_1 antagonists in view to their antioxidant capacity, justify the present study. Its objective was to investigate the effects of DPH (a histamine H_1-blocker) and FAM (a histamine H_2-blocker) on the lipid peroxidation and activities of antioxidant enzymes in different rat tissues, as well as on the amount of •OH and O_2^- radicals, generated in model systems. Some experiments with other histamine H_1 and H_2-antagonists: the H_1-antagonist – mepyramine (MEP) and the H_2-antagonist – RAN were also carried out.

Materials and Methods

Materials

The drugs and deoxyribose were from Sigma Chemical Co., NADP(H) and GSH were purchased from Boehringer (Mannheim, Germany); $K_2HPO_4 \cdot 3H_2O$, KH_2PO_4 and H_2O_2 were from Merck (Darmstadt, Germany), 2-thiobarbituric acid and riboflavine were from Fluka. All reagents used were of analytical grade. All solutions were prepared in water redistilled from glass apparatus.

Animals

Male Wistar rats, weighing 180–200 g, were given free access to tap water and a standard lab diet. The animals were starved 24 h before sacrificing and were killed by exsanguination under light ether anesthesia.

Preparations

Stomach mucosa homogenate. The stomach was opened and washed with cooled 0.15 M KCl. The mucosa was scraped from the glandular part of the stomach, suspended in 5.0 ml of cooled 0.15 M KCl-10 mM potassium phosphate buffer, pH 7.4, containing 0.1% Triton X-100 and centrifuged at 1,000 × g for 10 min.

Liver homogenate. The liver was perfused with cooled 0.15 M KCl. The 10% homogenate in 0.15 M KCl-10 mM potassium phosphate buffer, pH 7.4 was centrifuged at 3,000 rpm for 10 min.

Brain homogenate. The whole brain was washed with cooled 0.15 M KCl and its homogenate in 15 ml of cooled 0.15 M KCl-10 mM potassium phosphate buffer, pH 7.4 was centrifuged at 3,000 rpm for 10 min. The postnuclear tissue homogenates were used for measuring of lipid peroxidation, while the frozen (for one night) residues of them – for measuring of the enzyme activities.

Analytical procedures

The lipid peroxidation was determined by the amount of thiobarbituric acid reactive substances (TBARS) formed in the fresh tissue preparations [20]. After incubation at 37°C for 60 min (in the presence or absence of inducers of lipid peroxidation and drugs), the reaction was stopped by the addition of 0.25 ml of 40% trichloroacetic acid (w/v), 0.125 ml of 5 M KCl, 0.25 ml of 2% of thiobarbituric acid (w/v in 50 mM NaOH). The samples were heated at 100°C for 15 min to develop the color and after cooling, the absorbance was read at 532 nm against appropriate blanks. The 600 nm absorbance was considered to be a nonspecific baseline drift and was subtracted from A_{532}. The amount of TBARs was expressed in nmoles of malondialdehyde/mg of protein using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

The Cu, Zn-superoxide dismutase activity (SOD) was determined according to Beauchamp and Fridovich [4] and was expressed as units per milligram of protein. A unit of SOD activity is the amount of the

Tab. 1A. Effects of drugs on spontaneous lipid peroxidation in tissue homogenates

Drug (mM)	Liver	Brain	Gastric mucosa
Controls	2.5 ± 0.16	4.6 ± 0.22	0.6 ± 0.07
Histamine H_2-receptor antagonist			
FAM (0.1 mM)	2.6 ± 0.19	5.1 ± 0.32	0.5 ± 0.05
FAM (1.0 mM)	3.2 ± 0.17*	6.2 ± 0.49*	0.4 ± 0.01
FAM (5.0 mM)	0.6 ± 0.07*	1.2 ± 0.23*	0.5 ± 0.05
Histamine H_1-receptor antagonist			
DHP (0.1 mM)	2.2 ± 0.14	4.4 ± 0.33	0.5 ± 0.05
DHP (1.0 mM)	1.8 ± 0.06*	3.7 ± 0.25*	0.4 ± 0.01
DHP (5.0 mM)	0.4 ± 0.03*	0.7 ± 0.05*	0.4 ± 0.02

The amount of TBARs was measured in tissue homogenates after incubation for 60 min at 37 C in the presence of different concentrations of the drugs. Results (nmoles MDA/mg of protein) are the mean ± SE of 7 separate experiments. * Statistically significant differences *vs.* controls at $p < 0.01$

enzyme producing 50% inhibition of nitro-blue tetrazolium (NBT) reduction.

The catalase activity (CAT) was determined according to Aebi [1] and was expressed as A_{240} per minute per milligram of protein.

The xanthine oxidase activity was measured in 50 mM potassium phosphate buffer, pH 7.8, 1×10^{-5} M ferricytochrome C, 0.4 mM xanthine and 0.012 U/ml xanthine oxidase and the urate formation was measured at 295 nm.

The hydroxyl radicals were generated in 20 mM potassium phosphate buffer, pH 7.2, supplemented with 3.4 mM deoxyribose, 0.1 mM $FeCl_3$, 0.1 mM ascorbic acid, 0.5 mM H_2O_2 and additions: 0.1 mM EDTA and drugs. After incubation of the samples at 37°C for 60 min, the degradation of deoxyribose (a detector of $^{\bullet}OH$ radicals) was measured in terms of the formation of TBARSs [16, 19].

The O_2^- radicals were generated in: i) 50 mM potassium phosphate buffer, pH 7.8 with 1.17×10^{-6} M riboflavin, 0.2 mM methionine, 2×10^{-5} M KCN and 5.6×10^{-5} M NBT and O_2^- provoked photoreduction of NBT was measured at 540 nm [3] and ii) 50 mM potassium phosphate buffer, pH 7.8 with 1×10^{-5} M ferricytochrome C, 0.4 mM xanthine and 0.012 U/ml xanthine oxidase and O_2^--provoked ferricytochrome C reduction was measured at 550 nm [3].

The protein content was determined by the method of Lowry et al. [30].

Statistical analysis

The results were statistically analyzed by one-way ANOVA (Dunnett *post hoc* test), $p < 0.01$ being accepted as the minimum level of statistical significance of the established differences.

The experiments have been performed according to the "Principles of laboratory animal care" (NIH publication No. 85–23, revised 1985), and the rules of the Ethics Committee of the Institute of Physiology, Bulgarian Academy of Sciences (registration FWA 00003059 by the US Department of Health and Human Services).

Results

As shown in Table 1A, DPH (1 mM and 5 mM) inhibited the spontaneous lipid peroxidation in the rat liver and brain, while FAM showed a biphasic concentration-dependent effect: stimulation by 1 mM and inhibition by 5 mM. DPH and FAM also differed in their effects on Fe^{2+}-induced lipid peroxidation in these tissues: a strong inhibition by 5 mM DPH and a significant stimulation by 5 mM FAM (Tab. 1B). Both drugs were similar only in their effects on Fe/ascorbate-induced lipid peroxidation: an inhibition by 5 mM drugs was observed (Tab. 3C).

Tab. 1B. Effects of drugs on Fe(II)-induced lipid peroxidation in tissue homogenates

Drug (mM)	Liver	Brain	Gastric mucosa
Controls	6.1 ± 0.41	12.9 ± 087	0.5 ± 0.05
Histamine H_2-receptor antagonist			
FAM (0.1 mM)	6.3 ± 0.40	12.7 ± 0.98	0.6 ± 0.09
FAM (1.0 mM)	8.2 ± 0.49*	14.6 ± 0.99	0.5 ± 0.07
FAM (5.0 mM)	14.2 ± 1.29*	17.7 ± 1.27*	0.4 ± 0.05
Histamine H_1-receptor antagonist			
DHP (0.1 mM)	5.7 ± 0.44	12.4 ± 0.83	0.5 ± 0.05
DHP (1.0 mM)	6.5 ± 0.35	12.2 ± 0.85	0.5 ± 0.04
DHP (5.0 mM)	0.7 ± 0.12*	0.5 ± 0.15*	0.4 ± 0.04

The amount of TBARs was measured in tissue homogenates after incubation for 60 min at 37°C in the presence of 0.05 mM $FeSO_4$ (a LP inducer) and different concentrations of the drugs. Results (nmoles MDA/mg of protein) are the mean ± SE of 7 separate experiments. * Statistically significant differences *vs.* controls at $p < 0.01$

Tab. 1C. Effects of drugs on Fe/ascorbic acid-induced lipid peroxidation in tissue homogenates

Drug (mM)	LiveR	Brain	Gastric mucose
Controls	14.7 ± 1.70	14.9 ± 1.40	0.7 ± 0.09
Histamine H_2-receptor antagonist			
FAM (0.1 mM)	14.5 ± 1.75	14.7 ± 1.32	0.7 ± 0.09
FAM (1.0 mM)	14.4 ± 1.85	15.9 ± 0.83	0.6 ± 0.05
FAM (5.0 mM)	1.1 ± 0.14*	2.0 ± 0.34*	0.4 ± 0.05
Histamine H_1-receptor antagonist			
DHP (0.1 mM)	13.6 ± 1.77	14.3 ± 1.59	0.6 ± 0.05
DHP (1.0 mM)	11.5 ± 1.73	11.5 ± 1.60	0.6 ± 0.04
DHP (5.0 mM)	0.8 ± 0.08*	1.3 ± 0.12*	0.5 ± 0.02

The amount of TBARs was measured in the tissue homogenates after incubation for 60 min at 37 C in the presence of 0.05 mM Fe/0.05 mM ascorbic acid (a LP inducer) and different concentrations of the drugs. Results (nmoles MDA/mg of protein) are the mean ± SE of 7 separate experiments. * Statistically significant differences *vs.* controls at $p < 0.01$

Unlike liver and brain, the stomach mucosa manifested a great stability to peroxidative changes even in the presence of Fe^{2+} and Fe/ascorbate (inductors of lipid peroxidation) and we failed to establish any drug effects on lipid peroxidation in this preparation (Tab. 1 A, B, C).

The effects of FAM and DPH on the SOD activity, studied in all tissue preparations were also different. An increase in the enzyme activity in the presence of FAM and a slight inhibition in the presence of DPH were measured (Tab. 2A). However, both FAM and DPH did not alter the catalase activity, except for their concentration of 5 mM, which completely inhibited this enzyme (Tab. 2B).

To explain the different effects of FAM and DPH on the lipid peroxidation and on SOD activity, model systems generating $^{\bullet}OH$ and O_2^- radicals, were also used in the present study.

To study of the potential $^{\bullet}OH$ scavenging activity of FAM and DPH, a deoxyribose (DR) test was used. It is known that in the presence of ascorbic acid (a metal reducer), Fe^{3+}, added to H_2O_2 leads to generation of $^{\bullet}OH$ radicals: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + HO^-$ (Fenton reaction). These radicals provoke DR degradation, which might be inhibited by metal chelators and $^{\bullet}OH$ scavengers [16].

To obtain more clear drugs effects, EDTA was also added to the reaction mixture. It is known that in Fenton system EDTA increases the amount of free $^{\bullet}OH$ radicals [11, 12], facilitates Fe^{3+}-reduction by ascorbic acid [5] and affects Fe reactivity in this ascorbate-dependent system, generating $^{\bullet}OH$ radicals [17, 18].

As shown in Table 3A, FAM and DPH decreased DR degradation in Fenton system, as more clear-cut dose-dependent inhibitory effect was observed in the presence of EDTA. When copper instead of iron was added to the reaction mixture, where $^{\bullet}OH$ radicals were also formed [14, 15, 35, 37], these drugs also in-

Tab. 2A. Effects of famotidine and diphenhydramine on superoxide dismutase activity in tissue homogenates

Drug (mM)	Liver	Brain	Gastric mucosa
Controls	51.2 ± 1.99	14.4 ± 0.30	24.6 ± 1.18
Histamine H_2-receptor antagonist			
FAM (0.1 mM)	55.6 ± 1.67	16.3 ± 0.60	–
FAM (1.0 mM)	57.0 ± 1.45*	19.1 ± 0.46*	26.1 ± 1.59
FAM (5.0 mM)	57.4 ± 1.75*	20.9 ± 0.67*	39.4 ± 2.14*
Histamine H_1-receptor antagonist			
DHP (0.1 mM)	45.9 ± 4.35	14.1 ± 0.71	–
DHP (1.0 mM)	46.8 ± 4.52	14.7 ± 0.57	26.7 ± 2.54
DHP (5.0 mM)	33.1 ± 3.32*	12.6 ± 0.29*	26.4 ± 3.19

The superoxide dismutase activity was measured in tissue homogenates after incubation for 30 min at 0 C in the presence of different drug concentrations. Results (U/mg of protein) are the mean ± SE of 5 separate experiments. * Statistically significant differences *vs.* controls at $p < 0.01$

Tab. 2B. Effects of famotidine and diphenhydramine on catalase activity in tissue homogenates

Drug (mM)	Liver	Brain	Gastric mucosa
Controls	14.5 ± 0.73	0.31 ± 0.032	0.19 ± 0.007
Histamine H_2-receptor antagonist			
FAM (0.1mM)	13.5 ± 0.96	0.31 ± 0.028	–
FAM (1.0mM)	14.0 ± 0.96	0.26 ± 0.026	0.16 ± 0.018
FAM (5.0mM)	0*	0*	0*
Histamine H_1-receptor antagonist			
DHP (0.1mM)	13.5 ± 1.03	0.31 ± 0.036	–
DHP (1.0mM)	14.4 ± 1.03	0.32 ± 0.033	0.17 ± 0.014
DHP (5.0mM)	0*	0*	0*

The catalase activity was measured in tissue homogenates after incubation for 30 min at 0 C in the presence of different drug concentrations. Results (E_{240}/mg of protein) are the mean ± SE of 5–7 separate experiments. * Statistically significant differences *vs.* controls at $p < 0.01$

Tab. 3A. Effects of drugs in Fenton system

Drugs	– EDTA	+ EDTA
	E_{532}–E_{600}	
Controls	1.077 ± 0.041	2.271 ± 0.047
Histamine H_2-receptor antagonists		
FAM (0.1 mM)	0.720 ± 0.058*	1.946 ± 0.023*
FAM (0.5 mM)	0.652 ± 0.045*	1.442 ± 0.021*
FAM (1.0 mM)	0.621 ± 0.066*	1.249 ± 0.029*
FAM (5.0 mM)	0.376 ± 0.038*	0.870 ± 0.022*
RAN (0.1 mM)	1.035 ± 0.050	2.001 ± 0.028*
RAN (0.5 mM)	1.064 ± 0.058	1.701 ± 0.021*
RAN (1.0 mM)	1.083 ± 0.077	1.510 ± 0.039*
RAN (5.0 mM)	0.699 ± 0.028*	1.045 ± 0.020*
Histamine H_1-receptor antagonists		
DPH (0.1 mM)	1.146 ± 0.012	2.112 ± 0.017*
DPH (0.5 mM)	1.143 ± 0.054	1.476 ± 0.022*
DPH (1.0 mM)	0.894 ± 0.017*	1.318 ± 0.015*
DPH (5.0 mM)	0.215 ± 0.007*	0.590 ± 0.008*
MEP (5.0 mM)	0.362 ± 0.005*	0.806 ± 0.023*

Reaction mixture: 20 mM potassium phosphate buffer, pH 7.2 supplemented with 3.4 mM deoxyribose, 0.5 mM H_2O_2, 0.1 mM $FeCl_3$, 0.1 mM ascorbic acid and additions: 0.1 mM EDTA and drugs. Incubation: 60 min at 37°C. Results are the mean ± SE of 5–7 separate experiments. * Statistically significant differences *vs.* controls at $p < 0.01$

Tab. 3B. Effects of drugs in Fenton-like system

Drugs	– EDTA	+ EDTA
	E_{532}–E_{600}	
Controls	0.633 ± 0.028	0.731 ± 0.039
Histamine H_2-receptor antagonists		
FAM (0.1mM)	0.429 ± 0.008*	0.581 ± 0.015*
FAM (0.5mM)	0.278 ± 0.007*	0.523 ± 0.008*
FAM (1.0mM)	0.254 ± 0.005*	0.485 ± 0.015*
FAM (5.0mM)	0.227 ± 0.005*	0.451 ± 0.018*
RAN (0.1mM)	0.471 ± 0.020*	0.722 ± 0.022
RAN (0.5mM)	0.464 ± 0.016*	0.675 ± 0.025
RAN (1.0mM)	0.443 ± 0.017*	0.667 ± 0.032
RAN (5.0mM)	0.315 ± 0.016*	0.483 ± 0.019*
Histamine H_1-receptor antagonists		
DPH (0.1mM)	0.605 ± 0.010	0.666 ± 0.046
DPH (0.5mM)	0.605 ± 0.020	0.731 ± 0.039
DPH (1.0mM)	0.562 ± 0.017	0.743 ± 0.020
DPH (5.0mM)	0.283 ± 0.009*	0.374 ± 0.011*
MEP (5.0mM)	0.302 ± 0.007*	0.445 ± 0.009*

Reaction mixture: 20 mM potassium phosphate buffer, pH 7.2 supplemented with 3.4 mM deoxyribose, 0.5 mM H_2O_2, 0.1 mM $CuSO_4 \cdot 5H_2O$, 0.1 mM ascorbic acid and additions: 0.1 mM EDTA and drugs. Incubation: 60 min at 37°C. Results are the mean ± SE of 5–7 separate experiments. * Statistically significant differences *vs.* controls at $p < 0.01$

hibited DR degradation (Tab. 3B). RAN and MEP showed similar effects on DR degradation in Fenton- and Fenton-like systems, but it might be mentioned that the inhibitory effect of FAM was stronger than that of DPH or RAN. In summary, the results, obtained in Fenton- and Fenton-like systems suggested an $^{\bullet}OH$ scavenging activity for the histamine H_1 and H_2 receptor antagonists under study.

Discussion

It is accepted that compounds possessing chelating activity can be used in the treatment of "free radical" pathologies [8, 26, 27], especially diseases associated with iron overload [6, 25, 27, 39]. According to Van Zyl et al. [45], the histamine H_2-receptor antagonists are efficient chelators of iron.

It is well known that the toxic effects of iron ions are a consequence of a catalytic decomposition of hydrogen peroxide or hydroperoxides by ferrous ions to form active hydroxyl and oxyl radicals. It is assumed that these reactions are the initiation step of many free-radical damaging processes, including lipid peroxidation.

A FAM- and DPH-induced inhibition of Fe/ascorbic acid-induced lipid peroxidation, where the $^{\bullet}OH$ radicals played a significant role was established. That is why we turned our attention to the study of the potential $^{\bullet}OH$ scavenging activity of the drugs. It was found that like CIM and RAN [7, 29, 43] DPH and FAM were also able to scavenge $^{\bullet}OH$ radicals.

In Fenton-type reactions, substances with metal-binding affinity higher than those of the detector molecule (DR) are known to be more efficient $^{\bullet}OH$

Tab. 4. Effects of drugs in O_2^- - generating systems

Drugs (mM)	O_2^- - provoked reduction of nitro-blue tetrazolium		O_2^- - provoked reduction of ferricytochrome C	
	– EDTA	+ EDTA	– EDTA	+ EDTA
Controls	100	100	100	100
Histamine H_2-receptor antagonists				
FAM (0.1 mM)	60 ± 4.7*	76 ± 1.1*	60 ± 2.4*	55 ± 8.2*
FAM (0.5 mM)	21 ± 1.1*	42 ± 1.6*	29 ± 5.2*	23 ± 3.1*
FAM (1.0 mM)	9 ± 1.3*	23 ± 0.6*	24 ± 4.1*	18 ± 5.1*
FAM (5.0 mM)	5 ± 0.5*	3 ± 0.5*	0*	0*
RAN (0.1 mM)	84 ± 4.2*	94 ± 1.2*	109 ± 19.1	109 ± 12.7
RAN (0.5 mM)	74 ± 4.2*	78 ± 0.9*	95 ± 4.2*	91 ± 3.2*
RAN (1.0 mM)	51 ± 3.2*	57 ± 1.9*	91 ± 4.3*	82 ± 3.3*
RAN (5.0 mM)	10 ± 0.5*	9 ± 0.5*	71 ± 7.1*	73 ± 4.1*
Histamine H_1-receptor antagonists				
DPH (0.1 mM)	92 ± 2.1*	99 ± 1.7	100 ± 2.0	96 ± 5.1
DPH (0.5 mM)	77 ± 1.2*	92 ± 0.8*	86 ± 3.2*	86 ± 4.2*
DPH (1.0 mM)	76 ± 2.6*	89 ± 2.2*	71 ± 3.1*	68 ± 5.1*
DPH (5.0 mM)	62 ± 3.7*	87 ± 0.9*	57 ± 2.5*	68 ± 3.3*
MEP (5.0mM)	86 ± 3.1*	95 ± 1.9*	71 ± 2.2*	55 ± 6.3*

Reaction mixture: 50 mM potassium phosphate buffer, pH 7.8 with 1.17×10^{-6} M riboflavine, 0.2 mM methionine, 5.6×10^{-5} M NBT and additions (0.1 mM EDTA and drugs). II *reaction mixture*: 50 mM potassium phosphate buffer, pH 7.8 with 1×10^{-5} M ferricytochrome C, 0.4 mM xanthine, 0.012 U/ml xanthine oxidase and additions (0.1 mM EDTA and drugs). Results (% *vs.* controls) are the mean ± SE of 9 separate experiments. * Statistically significant differences *vs.* controls at $p < 0.01$

scavengers [12]. It is known that the chelating agents are able either to promote or inhibit the Fenton reaction, depending on the solubility of the complex, the redox potential of the Fe^{3+}/Fe^{2+} couple and the presence of free coordination sites in the complex [11, 12]. Moreover, they can initiate or abolish the iron pro-oxidant effect on lipid peroxidation [13, 41, 42], depending on the type of chelator and on the molar metal/chelator ratio. For example, EDTA, unlike ADP, exhibits a biphasic effect on the lipid peroxidation in brain microsomes [31].

Hence, if FAM and DPH possess a different-type chelating activity, they would have different effects on the spontaneous (induced by endogenous metal ions, mainly iron ions) and on the Fe-induced lipid peroxidation. Indeed at the concentrations used, FAM showed a biphasic concentration-dependent effect on the spontaneous and increased Fe-induced lipid peroxidation in the rat liver and brain, while DHP only inhibited them.

Using the indirect NBT and ferricytochrome C assays, a SOD-like activity of copper/cimetidine complexes has been reported [10, 22]. The studied by us drugs also inhibited the reduction of NBT and ferricytochrome C. It might be suggested that these drugs, especially FAM, are able to scavenge the O_2^- radicals generated in the reaction mixture. In a previous study [23] it was reported that CIM decreased dose-dependently O_2^--provoked reduction of ferricytochrome C and NBT, as well as the amount of O_2^-, directly generated by KO_2. Using the same experimental models, an inhibition of NBT and ferricytochrome C reduction by FAM, RAN, DPH and MEP was also found, but the FAM effect was stronger (Tab. 4). It could be assumed that the decrease in FAM-induced ferricytochrome C reduction was not due to an inactivation of the enzyme xanthine oxidase, since 5 mM FAM completely inhibited the ferricytochrome C reduction, but had no effect in the xanthine-xanthine oxidase enzyme-system (data not shown). O_2^- and H_2O_2 produced in xanthine-xanthine oxidase system can collaborate with the production of $^{\bullet}OH$ radicals [3]; hence, the higher FAM effectiveness in affecting ferricytochrome C reduction, as compared to that of the other drugs, might be connected to its stronger $^{\bullet}OH$ scavenging activity (Tab. 3A). Thus, in the presence of FAM, the amount of O_2^-, taking part in the ferricytochrome C reduction would be smaller.

The significant FAM-induced inhibition of NBT reduction would also explain the stimulation of the SOD activity, observed in the present study, as well as the *in vivo* stimulated SOD activity after CIM, RAN or FAM administration [21, 40]. This SOD-like activity might be due to the drugs themselves and/or to their *in vitro* and *in vivo* formed metal complexes.

FAM and DPH did not change the activity of catalase in the rat liver, brain and stomach mucosa, except for their concentration of 5 mM, which completely inhibited it. If the drugs are ineffective in influencing the catalase substrate – H_2O_2 [29], this inhibition might be a result of an inactivation of the enzyme iron, as a consequence of the metal-chelating capacity of the drugs.

The present results suggested that histamine H_1 and H_2 receptor antagonists, especially FAM, possess an antioxidant activity, which might be due to their abil-

ity to scavenge oxygen free radicals and/or to act as metal chelators. Their antioxidant activity could be an additional mechanism of therapeutic action and so, they could be used in the treatment of "free radical" pathologies, especially diseases associated with metal overload.

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