Carnosine and Related Compounds Inhibit Chemiluminescence of Human Plasma Lipoproteins *In Vitro*

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The antioxidant activity of carnosine and related compounds (5 and 10 mM) is assessed from the chemiluminescence curve using a model of Fe-initiated lipid peroxidation of human serum apoB-lipoproteins. These agents lower to different degrees the amplitudes of "fast" and "slow" bursts, the reaction rate, and the chemiluminescence light sum; the latency is prolonged, which may occur upon reduction of lipid hydroperoxides and/or inactivation of free radicals.

Key Words: *carnosine-related compounds; antioxidant activity; chemiluminescence; blood lipoproteins; man*

For a long time a high pH-buffer capacity was the only known function of the histidine-containing dipeptide carnosine (β-alanyl-L-histidine) [1]. Since the discovery of the antioxidant activity of carnosine [3], this compound has been studied in models revealing different reactive oxygen species such as peroxyl and hydroxyl radicals [1,10,14], singlet oxygen [8], or the total cellular response of activated leukocytes [4]. On the other hand, the antioxidant activity of naturally occurring derivatives of carnosine has hardly been studied, even though it has interesting medical applications [13].

Recently, it was demonstrated that carnosine (0.1-2.5 mM) reduces the Cu-catalyzed oxidation of low density lipoproteins without affecting latency and maximum oxidation [12]. In a monolayer membrane model, carnosine shortened the latency of the slow burst of chemiluminescence (CL) of Fe-induced lipid peroxidation (LPO) [5].

It thus makes sense to investigate the *in vitro* antioxidant activity of carnosine-related compounds in a model of Fe^{2+}-activated LPO in apoB-containing lipoproteins isolated from human serum.

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MATERIALS AND METHODS

Blood was taken from the cubital vein of patients with cerebrovascular diseases during the rehabilitation period. After spontaneous sedimentation of blood cells, the sera were pooled, and the total lipoprotein fraction of low density and very low density lipoproteins was isolated [7]. The Fe^{2+}-initiated CL in the lipoprotein fraction was recorded using a Luminometer-1251 apparatus (LKB) [9]. The following parameters of the CL curve were analyzed (Fig. 1):

- the amlplitude of the fast CL burst appearing immediately after the addition of Fe^{2+} and lasting several seconds, being proportional to the baseline hydroperoxide content in the sample;
- the amlplitude of the slow burst, which gradually increases, reaches the maximum, and slowly decreases to a steady-state level after a certain time (it reflects the maximum possible intensity of LPO in the examined sample);
- the latency between the fast and slow bursts, the duration of which depends on the ratio between anti- and pro-oxidants in the system;
- the time from the moment when the slow burst starts to increase to the moment when it reaches the maximum;

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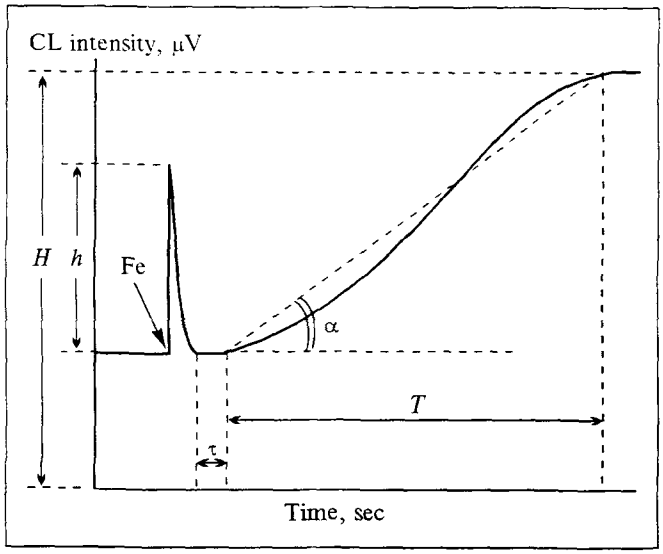

Fig. 1. Kinetics of lipoprotein CL during Fe^{2+} – initiated LPO. *h* and *H* are the amplitudes of fast and slow burst; τ is the slow burst latency, *T* is the time taken for the slow burst to reach the maximum; tg α is the angle determining the reaction rate.

- the tangent of the slope angle during the time that the amlplitude of the slow burst characterizing the LPO rate increases;
- the light sum: the area under the CL curve from the moment when the amlplitude of the slow burst starts rising till the moment it reaches the maximum (calculated from the following formula: amlplitude of the slow burst×time period/2). It reflects the number of peroxide radicals per Fe ion [6].

The following compounds were studied: imidazole, β-alanine, histidine, carnosine, acetylcarnosine, anserine, acetylanserine, homocarnosine, and ofidine. The solutions were diluted to a final concentration of 5 and 10 mM and added together with compensating volumes of phosphate buffer to the studied sample 30 sec prior to the addition of Fe. Alterations in the studied parameters were expressed as percentages related to the control values for the samples to which the corresponding volumes of buffer were added.

The results were processed with the use of Statgraph IMB PC software.

RESULTS

The studied compounds had no effect on the intensity of spontaneous CL of the samples. However, almost all the parameters determined from the curve of Fe-initiated CL were altered in their presence (Table 1).

According to the effect produced on the given parameters, the compounds were assigned to the following groups:

1) β-alanine - induces a small but significant change only in the amlplitude of the fast burst that reflects the baseline content of hydroperoxides;

2) imidazole and acetylcarnosine - have a considerable effect on the amlplitude of the fast burst and on the latency. A reduction in latency may indicate chelation of Fe ions [5], but imidazole exhibits no complexing activity. Consequently, imidazole accelerates the reaction via another mechanism, though Fe complexation may cause the effect of acetylcarnosine;

3) carnosine, homocarnosine, ofidine, and acetylcarnosine - reduce the amplitudes of the fast and slow bursts 4- and 2-fold, respectively. The first two compounds also lower the reaction rate 3-fold, judging from the tangent of the slope angle;

4) anserine and histidine - are the most potent inhibitors of lipoprotein oxidation. Moreover, histidine inhibits the initial fast CL burst 10-fold.

At a concentration of 5 mM these compounds had less pronounced effects. Their effect on the

TABLE 1. Alterations in the CL Curve Caused by the Tested Compounds (10 mM) Expressed as a Percentage of the Control Values

Compound	*h*, μV	*H*, μV	τ, sec	*T*, sec	tg α	*S*, μV×sec
b – Alanine	72*	92	142	112	83	103
Imidazole	22*	80	46*	78	102	61
Acetylcarnosine	22*	84*	45*	120	71	100
Carnosine	24*	42*	138	118*	36*	52*
Homocarnosine	24*	78*	167	167*	34*	84
Ofidine	27*	53*	198	107	51*	61*
Acetylanserine	24*	78*	167	107	73	84
Anserine	68	16*	250	72	21*	11*
Histidine	11*	11*	183	211	7*	28*

Note. *h* and *H* are the amplitudes of fast and slow bursts; τ is the latency of the slow burst; *T* is the time taken for the slow burst to reach the maximum; tg α is the angle determining the reaction rate; *S* is the light sum. Here and in Table 2: an asterisk indicates values different from the control at $p<0.05$.

slow CL burst was analyzed in a separate set of experiments (Table 2).

Taken together, these findings indicate that β-alanine is practically ineffective in the chosen model. Imidazole and acetylcarnosine change the reaction kinetics, diminishing latency and amlplitude. The light sum and the reaction rate remain virtually unchanged. The other compounds reduce (or tend to reduce) the amplitudes of the fast and slow bursts, the reaction rate and the light sum, and prolong the time parameters of the reaction kinetics, which hinders the formation of the luminescent products and causes LPO inhibition.

These alterations in kinetics are characteristic of inhibitors of free-radical reactions and reducers of hydroperoxides which prevent branching of the reaction chain after the insertion of an initiator in the model, thereby inhibiting LPO.

Consequently, naturally occurring carnosine derivatives, like carnosine itself, possess an antioxidant activity, which in the case of histidine and anserine is higher than that of homocarnosine, ofidine, and acetylanserine. Presumably, the differences in the efficiency of these compounds are associated with their different reducing activities, which in turn depend on the ability of the imidazole ring to donate a hydrogen atom.

The ability of these compounds to inhibit the development of the fast CL burst seems to be their most important property, which may indicate that they interact with hydroperoxides preexisting in the model system and may have a certain significance for the use of naturally occurring derivatives of carnosine in medicine.

TABLE 2. Effect of Carnosine and Related Compounds on the Slow Burst of Lipoprotein Chemiluminescence

Compound	Amplitude of slow burst of lipoprotein CL (% of control) at compound concentration, mM	
	5	10
β−Alanine	96	92
Acetylcarnosine	–	84*
Acetylanserine	86	78*
Imidazole	78*	72*
Ofidine	75*	54*
Homocarnosine	62	54*
Carnosine	58*	42*
Histidine	65	11*
Anserine	22	16*

REFERENCES

1. A. Boldyrev, E. Kurella, A. Rubtsov, *et al., Biokhimiya,* **57**, 1360-1365 (1992).
2. Yu. A. Vladimirov and A. I. Archakov, in: *Lipid Peroxidation in Biological Membranes* [in Russian], Moscow (1972), p. 252.
3. A. Dupin, A. Boldyrev, Yu. Arkhipenko, and V. Kagan, *Byull. Eksp. Biol. Med.,* **98**, № 8, 186-188 (1984).
4. S. Egorov, E. Kurella, A. Boldyrev, and A. Krasnovskii, *Bioorg. Khimiya,* **18**, 142-144 (1992).
5. Li Ho Ik, Yu. A. Vladimirov, and A. I. Deev, *Biofizika,* № 1, 82-85 (1990).
6. Yu. M. Lopukhin, Yu. A. Vladimirov, M. N. Molodenkov, and G. I. Klebanov, *Byull. Eksp. Biol. Med.,* **95**, № 2, 61-63 (1983).
7. Yu. M. Lopukhin, M. N. Molodenkov, Yu. A. Vladimirov, *et al., Ibid.,* **93**, № 4, 101-102 (1982).
8. O. Tyulina, V. Kagan, and A. Boldyrev, *Ibid.,* **118**, № 11, 463-465 (1994).
9. T. N. Fedorova, O. Yu. Rebrova, and E. G. Larskii, *Lab. Delo,* № 3, 37-39 (1991).
10. O. Aruoma, M. Laughton, and B. Halliwell, *Biochem. J.,* **264**, 863-869 (1989).
11. E. Bate-Smith, *J. Physiol (Lond.),* **92**, 336-343 (1938).
12. S. L. Bogardus, G. A. Boissonneault, and E. A. Decker, *FASEB J.,* **6**, № 5, A1387 (1992).
13. A. Boldyrev, *Int. J. Biochem.,* **22**, 129-132 (1990).
14. R. Kohen, Y. Yamamoto, K. Cundy, and B. Amnes, *Proc. Nat. Acad. Sci. USA,* **85**, 3175-3179 (1988).