BIOPHYSICS AND BIOCHEMISTRY

Effects of Methyluracil and Hydroxymethacil on Free-Radical Oxidation in Model Systems

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Three pyrimidine derivatives - methyluracil, hydroxymethacil (a new compound), and its lithium salt - were tested in model systems of differing complexity for antioxidant properties in comparison with the well-known antioxidant ionol. Tests for antiradical activity and for effects on spontaneous and Fe^{2+}-ascorbate- or NADPH-dependent lipid peroxidation revealed high antioxidant activity (comparable to that of ionol) of the hydroxymethacil lithium salt.

Key Words: *antioxidants; lipid peroxidation; methyluracil; hydroxymethacil; pyrimidine derivatives*

Some pyrimidine derivatives (PD) have been shown to possess antioxidant properties comparable in terms of efficiency with those of certain synthetic antioxidants [4,5]. For example, an inhibitory effect of 5-hydroxy-6-methyluracil (hydroxymethacil) on lipid peroxidation (LPO) *in vivo* has been demonstrated [3,5,6]. However, the mechanism of antioxidant action by methyluracil has been variously appraised by different investigators [5,6,8]. Although the lack of agreement or even opposite views regarding the antioxidant properties of this and other PD are probably attributable, in part, to differences in the design and conditions of experiments in which particular PD were studied, the antioxidant efficiency of these compounds *in vivo* is difficult to evaluate because the mechanisms of their direct action on free-radical oxidation processes are not well understood. Moreover, the antioxidant properties of PD far superior to methyluracil in biological activity [1] remain largely unknown. In view of this, the purpose of this study was to test methyluracil, hydroxymethacil, and its lithium salt for their effects on free-radical oxidation in model systems in comparison with the synthetic antioxidant ionol.

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MATERIALS AND METHODS

The antioxidant activity of the PD was tested by a chemiluminescence technique [9]. The system used consisted of an ethylbenzene:glacial acetic acid (3:2) mixture containing (at 50°C) the activator 1,4-dibromoanthracene (5×10^{-4} M), the initiator azodiisobutyronitrile (10^{-2} M), and the test compound. Constant K_7 (the rate at which ethylbenzene peroxide radicals interact with molecules of the test compound) was calculated by the formula:

$$K_7=(I/I_0 - 1)^{0.5}\times Wi^{0.5}/InH,$$

where I_0 is the chemiluminescence intensity before the test compound is added to the model system; I is the chemiluminescence intensity after its ad-

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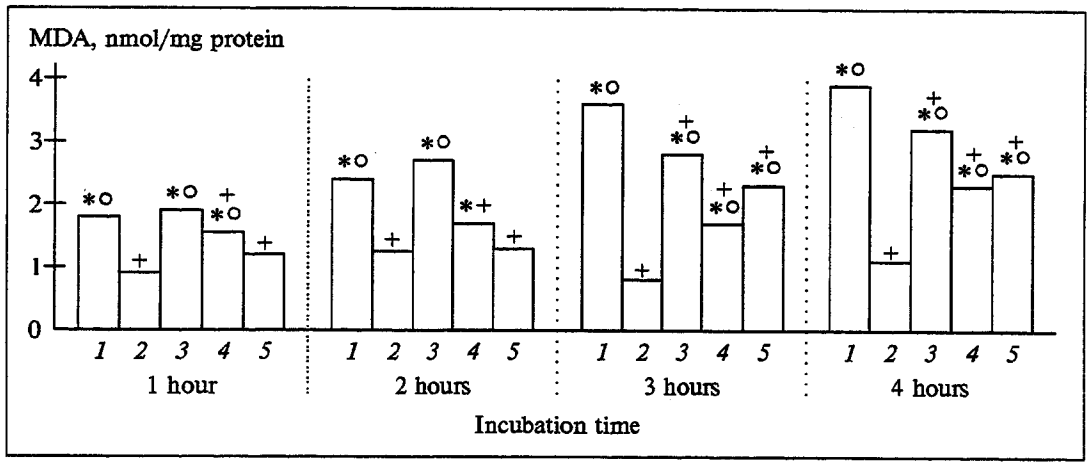

Fig. 1. Spontaneous LPO. Here and in Figs. 2 and 3: the results are shown in relation to the baseline MDA level. A significant difference from the baseline is denoted by the asterisk, that from the control tests by the plus sign, and that from the tests with ionol by the circle. *1*) control; *2*) ionol; *3*) 6-methyluracil; *4*) hydroxymethacil; *5*) Li salt of hydroxymethacil.

dition to the system; *Wi* is the initiation rate for the reaction of recombination of ethylbenzene peroxide radicals; and *InH* is the concentration of the test compound (putative inhibitor).

K_7 values of the test compounds were compared with that of ionol, a known synthetic inhibitor of free-radical oxidation.

Hydrophobicity of the test compounds was assessed by the partition coefficient *P* in the octanol-water system [10]:

$$P=r\times E_1/(E_0-E_1),$$

where *r* is the volume ratio of the aqueous to the octanol phase; E_0 is the optical density of the octanol phase before extraction; and E_1 is the optical density of the octanol phase after extraction. Optical density was measured in the absorption maxima of the test compounds with an SF-26 spectrophotometer in 10-mm quartz cuvettes.

Antioxidant properties of the PD were compared with those of the standard free-radical oxidation inhibitor ionol under conditions of spontaneous and induced LPO. A 20% rat liver homogenate was used as a model system. The PD and ionol were added to the incubation medium as 10^{-4} M alcohol solutions (the alcohol concentration did not exceed 0.01%). LPO was evaluated by the accumulation of malonic dialdehyde (MDA), whose quantity was estimated by the reaction with 2-thiobarbituric acid [7]. LPO was induced by the Fe^{2+}-ascorbate system containing 6 μM Mohr's salt and 0.5 mM ascorbate. NADPH-dependent LPO was induced by adding 1 mM NADPH, 6 μM Mohr's salt, and 0.2 mM sodium pyrophosphate to the incubation medium.

Hydroxymethacil and its lithium salt were synthesized by V. P. Krivonogov at the Institute of Organic Chemistry of the Ufa Research Center, Russian Academy of Sciences.

RESULTS

The data in Table 1 indicate that hydroxymethacil exhibits pronounced antiradical activity. In the reaction with peroxide radicals in the two-phase system, hydroxymethacil's efficacy was more or less the same as that of ionol. Methyluracil has a negative K_7 value, which may be an indication of its prooxidant activity. One reason for the differential activities of these structurally similar uracil

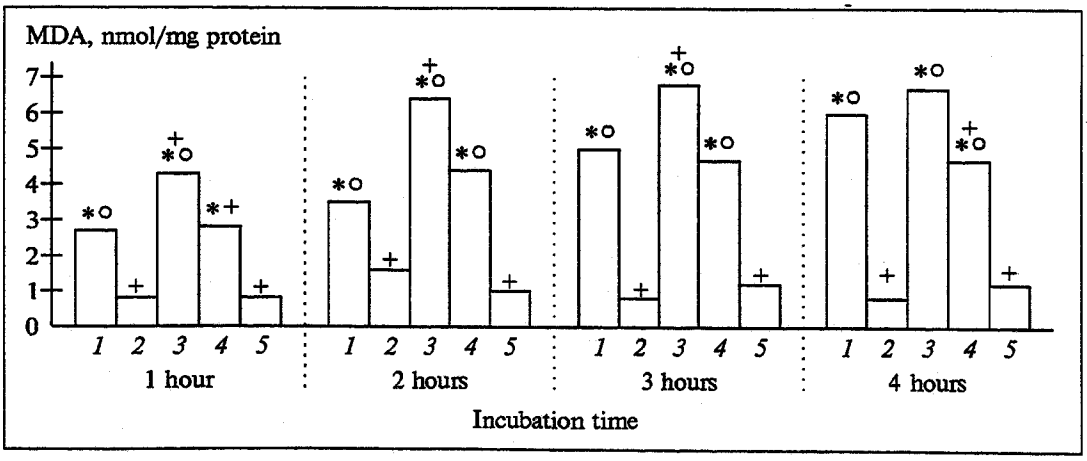

Fig. 2. Ascorbate-dependent LPO.

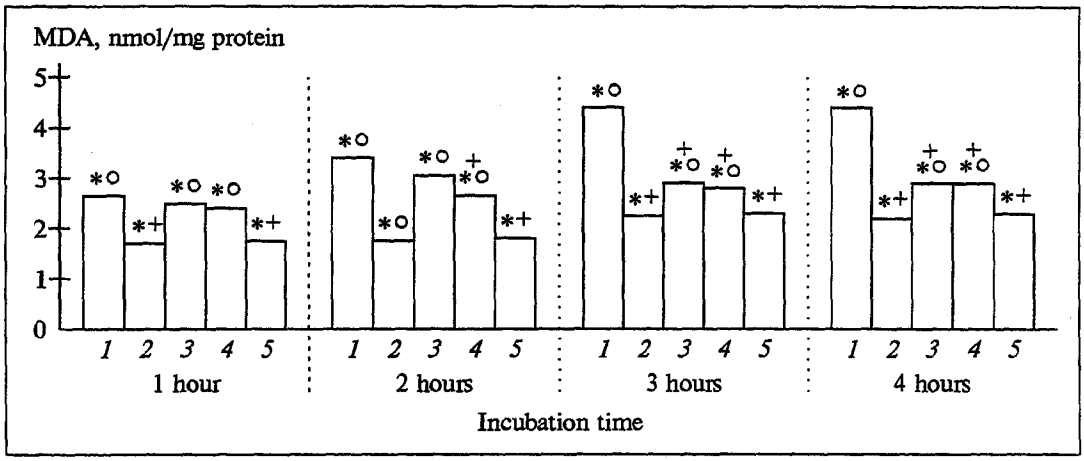

Fig. 3. NADPH-dependent LPO.

analogs may be the absence of a reactive OH group in position 5 of the methyluracil molecule. Modification of the hydroxymethacil structure through substitution of lithium for the hydrogen atom of the OH group in position 5 led to some decrease in the K_7 value. It follows from Table 1 that the direct influence of hydroxymethacil and its lithium salt on LPO processes may be rather strong and should be taken into account when evaluating results obtained *in vivo*.

The partition coefficient P in the two-phase octanol-water system carries significant information about the likely site of action of compounds in biomembranes. Methyluracil and hydroxymethacil proved to be largely hydrophilic compounds and were located for the most part in the aqueous phase. The lithium salt of hydroxymethacil has hydrophobic properties and was mainly located in the hydrocarbon phase.

The results of testing the PD *vs.* ionol for antioxidant properties in the model system of natural origin are presented in Fig. 1. In the case of spontaneous LPO, ionol (10^{-4} M) inhibited MDA accumulation in the rat liver homogenate almost completely after just 1 h of incubation, and its inhibitory effect remained virtually unchanged after 2, 3, and 4 h of incubation. Methyluracil inhibited LPO after 3 h of incubation. Hydroxymethacil was more inhibitory: its effects were observed after 2, 3, and 4 h of incubation.

The actions of the PD and ionol on Fe^{2+}-ascorbate-dependent (nonenzymatic) LPO are illustrated in Fig. 2. Note the powerful antioxidant effect produced by hydroxymethacil lithium salt, even though its antiradical activity was lower than that of ionol (Table 1).

Methyluracil and hydroxymethacil exerted opposite effects on nonenzymatic LPO after 3 and 4 h of incubation: the former compound exhibited prooxidant and the latter, antioxidant activity. In contrast, NADPH-dependent LPO was inhibited by all three PD (as well as by ionol), but the lithium salt was at all times more inhibitory than methyluracil or hydroxymethacil (Fig. 3).

The results of this study indicate that the activity of methyluracil, hydroxymethacil, and its lithium salt depends on their chemical structure and certain physicochemical characteristics. The small difference in chemical structure between methyluracil and hydroxymethacil (presence of an OH group in position 5 of the pyrimidine ring in the latter compound and its absence in the former) has a marked effect on their antiradical activity, as judged by the K_7 values. Although hydroxymethacil and its lithium salt have similar K_7 values, their antioxidant efficiencies in a more complex biological system (rat liver homogenate) differ markedly. The antioxidant activity of free-radical oxidation inhibitors in heterophase systems appears to depend not so much on the kinetic characteristics or reactivity of the inhibitor as on the oxidation conditions. An important role in determining the antioxidant activity of an inhibitor is played by the coefficient of its partition between the polar (aque-

TABLE 1. Antiradical Activity (Constant K_7) and Partition Coefficients (P) of Pyrimidine Derivatives

Compound	K_7, mol/liter×sec	$K_{7\text{compound}}/K_{7\text{ionol}}$	P
6-Methyluracil	$-(3.0\pm0.9)\times10^{1}$	-1.3×10^{-3}	0.264
Hydroxymethacil	$(2.6\pm0.8)\times10^{4}$	1.1	0.179
Li salt of hydroxymethacil	$(2.9\pm0.9)\times10^{3}$	0.13	1.272
Ionol	$(2.3\pm0.6)\times10^{4}$	–	7.2

ous) and nonpolar (lipid) phases. This explains the high antioxidant activity *in vitro* of the hydroxymethacil lithium salt, which is comparable to the activity of the lipid antioxidant ionol.

REFERENCES

1. D. N. Lazareva and E. K. Alekhin, *Immunomodulators* [in Russian], Moscow (1985).
2. G. F. Lakin, *Biometry* [in Russian], Moscow (1990).
3. V. A. Myshkin, S. A. Bashkatov, A. F. Vakaritsa, *et al.*, *Pat. Fiziol.*, № 2, 47-49 (1993).
4. V. A. Myshkin, D. V. Srubilin, A. F. Vakaritsa, *et al.*, in: *Contributions to Public Health by Scientists of the Bashkir Medical Institute* [in Russian], Ufa (1992), pp. 73-77.
5. S. A. Silaeva, N. V. Gulyaeva, V. Ya. Khatsernova, *et al.*, *Byull. Eksp. Biol. Med.*, **109**, № 2, 180-182 (1990).
6. S. A. Silaeva, V. A. Golenchenko, A. V. Gavril'chak, *et al.*, *Biokhimiya*, **57**, № 9, 1366-1372 (1992).
7. I. D. Stal'naya and T. G. Garishvili, in: *Modern Methods in Biochemistry* [in Russian], Moscow (1977), p. 66.
8. L. N. Shishkina, Yu. P. Taran, and S. V. Eliseeva, *Izv. Akad. Nauk SSSR. Ser. Biol.*, № 2, 350-357 (1992).
9. V. Ya. Shlyapintokh, O. N. Karpukhin, *et al.*, *Chemiluminescence Methods for the Study of Slow Chemical Processes* [in Russian], Moscow (1966).
10. K. Hanch, *Khim.-Farm. Zh.*, № 10, 15-30 (1980).