PHARMACOLOGY

Antimutagenic and Antioxidant Activities of Crown Compounds in Comparison with the Effects of Garlic Extract

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Antimutagenic activity of N-carboxyalkyl derivatives of aza- and benzoazacrown compounds was revealed and antimutagenic activity of garlic extract was confirmed. Specific genoprotective effect of crown compounds towards the effects of various mutagens was demonstrated. The antimutagenic effect of these compounds was not realized via antioxidant mechanisms, while the protective effect of garlic extract was associated with its antioxidant and reparative activities.

Key Words: *antimutagens; antioxidants; crown compounds; plant extracts*

The search for new synthetic and natural antimutagens attracts much attention because of good prospects of their practical use for the prevention of delayed negative effects of induced mutagenesis in humans, the main of which are high prevalence of hereditary diseases and cancer [1,4,11].

The major obstacles to practical use of antimutagens are high specificity of the protective effects of antimutagens towards certain mutagens, target organs, cell cycle stages, *etc.*, and poor understanding of the possible mechanisms of their protective effects [3,4].

We previously revealed antimutagenic activity of N-carboxyalkyl derivatives of aza- and benzoazacrown compounds and confirmed the antimutagenic activity of garlic extract (GE) [2,5]. Now we compared the effects of two crown compounds and GE on the intensity of DNA damage induced by γ-irradiation, $CdCl_2$, and 4-nitroquinolone-1-oxide [2,6] and evaluated the antimutagens antioxidant activity which can underlie the protective action.

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MATERIALS AND METHODS

Crown compounds B (2-(1,4,7,10-tetraoxa-13azacyclopentadecanyl)acetic acid) and C (2-(2,3,5,6,8,9,11,12-octahydro-7H-1,4,10,13,7-benzotetraoxazacyclopentadecine-7-yl)acetic acid) were synthesized at Photochemistry Center [5,9].

DNA injuries and repair were studied using cultured human RD cells. The cells were labeled with ^{3}H-thymidine (specific activity 19.6 Ci/mol) during the logarithmic phase of growth. Crown-containing compounds (10^{-5} M) or GE (170 μg/ml medium) were added 24 h before mutagen treatment ($CdCl_2$ in a dose of 5×10^{-5} M, 24 h; 4-nitroquinolone-1-oxide, 5×10^{-5} M, 2 h; and γ-radiation in a dose of 75 Gy). The number of DNA breaks was evaluated chromatographically (on hydroxyapatite columns) by the decrease in the

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relative content of double-stranded DNA in cells in comparison with the control [10].

Antioxidant effects of the studied crown-containing compounds (final dilutions 10^{-6}-10^{-4} M) and GE were evaluated by chemiluminescence (CL) on a Bioorbit 1251 luminometer in human blood leukocyte suspension.

Leukocytes were isolated from heparinized donor blood by centrifugation in a Histopaque gradient 1.119 (3000 rpm, 30 min) and washed twice with Hanks' solution. The cells were kept at 0°C (thawing ice) for 4 h.

SOD-like activity was measured in the xanthine-xanthine oxidase system containing 0.1 M K-phosphate buffer (pH 7.4), xanthine oxidase (0.25 U/ml), lucigenin (8×10^{-5} M) at 25°C. The reaction was triggered by adding xanthine (50 μM) and the maximum CL intensity was recorded. The preparation was added simultaneously with xanthine oxidase. The effect of the test drug on the production of $OH^{\bullet}$ was evaluated by Fenton reaction carried out in 0.01 M K-phosphate buffer (pH 7.4) containing 2×10^{-4} M luminol and 5×10^{-4} M $FeSO_4$; the reaction was triggered by adding 40 μl 0.01% H_2O_2 at 25°C. The effect on the leukocyte respiratory burst was evaluated at 37°C. The cuvette contained Hanks solution, leukocyte suspension (final concentration of 5×10^5 cells/ml), and luminol (2×10^{-4} M). Spontaneous CL was recorded for 2 min, after which 10 nM phorbol myristate acetate was added and the maximum CL intensity was recorded. All measurements were repeated several times, the error was no more than 10%. The results were expressed in percent of control (sample containing no test drug).

Experimental data were statistically processed using standard parametrical methods.

TABLE 1. DNA Breaks in Human Cells Incubated with Crown-Containing Compounds (*M*±*m*)

Concentration, M	Double-stranded DNA, % of control	
	compound B	compound C
10^{-6}	100±1	90±2
10^{-5}	100±1	91±3
10^{-4}	88±3	89±2

RESULTS

Incubation of cultured human cells with crown-containing compounds for 24 h had practically no effect on DNA structure (Table 1), *i.e.* these compounds exhibit no DNA damaging activity.

The content of double-stranded DNA in untreated cultures exposed to γ-radiation was 78% (Table 2). The damaging effect of irradiation was less pronounced in cultures treated with compounds C or B; GE produced a similar effect.

The content of double-stranded DNA after 24-hour incubation with $CdCl_2$ little differed from that observed immediately after addition of this agent; hence, this compound induced unrepairable DNA damage. Virtually no DNA damage was observed in $CdCl_2$-treated cultures after treatment with GE of compound B, *i.e.* these preparations protected DNA. Presumably, $CdCl_2$-induced non-repairable damages to DNA were transformed into repairable under the effect of compound B; compound C showed no activity of this kind.

It is noteworthy that compound B was ineffective in experiments with 4-nitroquinolone-1-oxide and did not stimulate resynthesis, like in experiments with GE (Table 2).

TABLE 2. DNA Breaks Induced by Different Mutagens in Human Cells Pretreated with Crown-Containing Compounds B, C (10^{-5} M) and GE (*M*±*m*)

Mutagen	Antimutagen	Double-stranded DNA, % of control	
		after exposure	after repair
γ-Radiation	—	78±2	98±3*
	B	88±1	98±3*
	C	91±1	98±3*
	GE	86±4	98±3*
$CdCl_2$	—	79±5	72±6**
	B	95±5	94±5**
	GE	92±5	98±3**
4-Nitroquinolone-1-oxide	—	58±2	69±4***
	B	50±3	60±3***
	GE	58±6	98±3***

Note. *15 min postincubation in growth medium, **24 h postincubation, ***4 h postincubation.

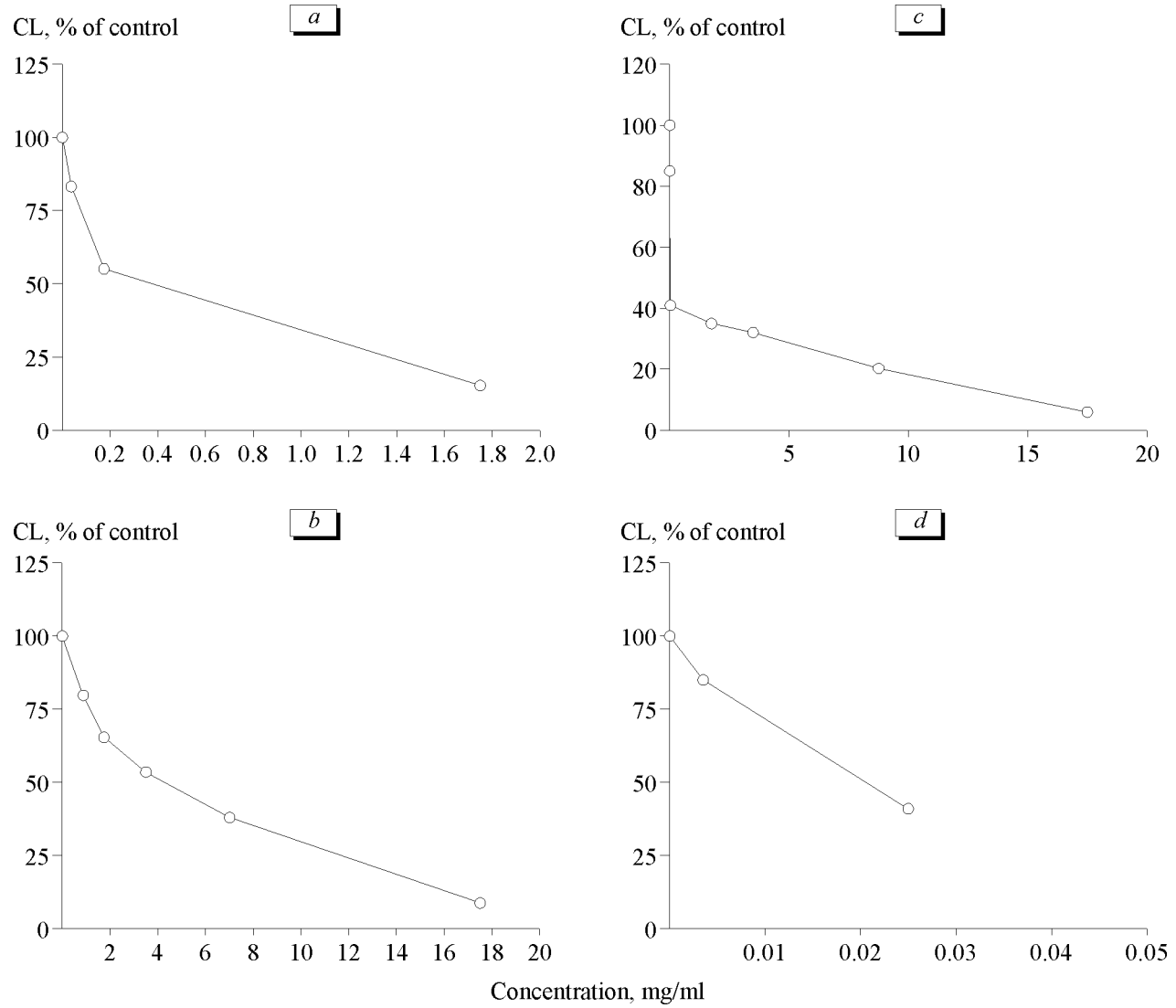

Fig. 1. Effect of garlic extract on luminal (*a*, *b*) and lucigenin (*c*, *d*) chemiluminescence (CL). *a*) CL of isolated leukocytes activated by phorbol myristate acetate; *b*) CL in Fenton system; *c*) CL in xanthine-xanthine oxidase system (general aspect); *d*) the same, C_{50} area.

Thus, our experiments revealed specificity of the studied antimutagens: compound B protects the cells from $CdCl_2$ and to a lesser extent from γ-radiation; compound C effectively protected the cells from γ-radiation, but not from $CdCl_2$; crown compounds did not induce resynthesis, while GE stimulated DNA repair processes.

Genotoxic effects of γ-radiation and cadmium salts are associated with induction of oxidative stress [4,8]. Hence, antimutagenic activity of crown-containing compounds and GE can be associated with their antioxidant properties. Crown compounds can bind metals of alternating valency, playing the key role in realization of the genotoxic effects of oxidative stress [4], while garlic contains numerous compounds with antioxidant activity [7].

It was found that GE suppresses the development of respiratory burst in leukocyte in response to phorbolmyristate acetate (Fig. 1), inhibited the formation of superoxide and hydroxyl radicals, which attests to pronounced antioxidant activity of this preparation. On the other hand, compounds B and C (10^{-4}, 10^{-5}, and 10^{-6} M) showed no antioxidant activity in these model systems. This indicates that the protective effect of the studied crown compounds is not due to their effects on the production of free oxygen radicals. The latter fact is very important, because the overwhelming majority of the known antimutagens realize the protective effect by the antioxidant mechanism [2,4], and the absence of this mechanism in the studied crown compounds discriminates them from the majority of genoprotectors.

Hence, compounds B and C and GE possess antimutagenic activity realized via principally different mechanisms. Antimutagenic activity of GE is due to its antioxidant and repair-inducing activity, while the antimutagenic effect of crown compounds is not as-

sociated with antioxidant activity towards the effects of prooxidant mutagens.

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