PHARMACOLOGY AND TOXICOLOGY

Antimutagenic Characteristics of New Diazacrown Compounds with N-Carboxyalkyl Substitutes

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Two new benzodiaza-15-crown-5 compounds containing two N-hydroxycarbonylmethyl or N-hydroxycarbonylpropyl substitutes were synthesized. The first of these compounds exhibited more pronounced protective effects towards human cells according to criteria of primary DNA injury and cell survival after exposure to γ-radiation and $CdCl_2$; by antimutagenic activity this compound was comparable to garlic extract. The antimutagenic effect of these compounds was realized not through the antioxidant mechanism.

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

Increasing environmental pollution including factors with pronounced genetic activity (chemical mutagens, radiation) necessitated creation of antimutagenic substances reducing the unfavorable effects of mutagens on humans.

The problem of antimutagenesis is now most intensively solved in two directions: search for effective antimutagens in order to prevent the effects of certain mutagens or their combinations and study of antimutagen mechanisms of action [3]. Antimutagens can be natural and synthetic; the greater part of them is characterized by antioxidant effects, which is very important, because the damaging component of many chemical mutagens (*e.g.* heavy metals) is mediated by the formation of free radicals, similarly as in irradiation [2-4]. The effects of crown compounds were studied in human cells cultured *in vitro* and high antimutagenic activity of the compounds was shown [1,5] similar to that of the garlic extract [7]. The effect of the studied crown compounds is not determined by antioxidant activity, while the mechanism of the antimutagenic effect of garlic extract is due to this effect [6].

Here we compared antimutagenic activity of new diazacrown compounds (N-hydroxycarbonylmethyl and N-hydroxycarbonylpropyl substitutes; I and II, respectively). These compounds reduced the incidence of primary DNA damage and even final events in the cell (improved cell survival).

MATERIALS AND METHODS

Compounds I and II were synthesized in two stages (Fig. 1). Benzo-4,10-diaza-15-crown-5 compound was alkylated with bromoacetic acid ethyl ester in tetrahydrofurane (THF) solution with triethylamine and thus diester was obtained (90% output). γ-Bromobutyric acid ethyl ester in boiling acetonide with sodium carbonate was used for the synthesis of diester (42% output). The purity of the resultant compounds was proven by nuclear magnetic resonance spectra, mass spectra, and element analysis data.

The study was carried out on cultured human RD cells and lymphocytes cultured by the routine

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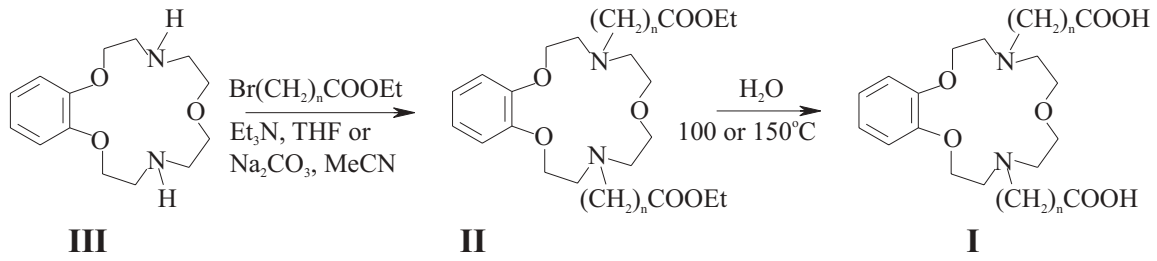

III **II** **I**

Fig. 1. Crown compounds I, II, and III.

method. Log-phase cultures were labeled with ^{3}H-thymidine (1 μCi/ml medium) for 24 h at 37°C. The cells were pretreated with crown compounds I or II or garlic extract for 20 h at 37°C before exposure to the mutagen. Before treatment with antimutagen the cells were incubated with SOD inhibitor (triethylene tetramine; triene) in a concentration of 1 μmol/ml medium for 1 h with subsequent washout. γ-Irradiation in a damaging dose (50 Gy) was carried out on a GUPOS device (Institute of General Genetics) at a dose power of 0.29 Gy/min. Treatment with $CdCl_2$ (10^{-6} M) was carried out for 20 h. Chromatography of cell lysates on columns packed with hydroxyapatite was used [2]. The number of DNA breaks after exposure to mutagens was evaluated by the decrease in the percentage of double strand DNA in comparison with the control. Cell survival was evaluated by vital staining with 3% Trypan Blue (Chemapol) in Hanks solution (1:1 ratio). The cells were counted in a Goryaev chamber. Live (unstained) and dead (blue stained) cells were counted in 8 large squares. The percentage of live and dead cells from the total number of cells (100%) in suspension was evaluated.

RESULTS

Table 1 presents data proving the protective effect of crown compounds I and II against γ-radiation. The number of DNA breaks after exposure in cells treated with these compounds was lower than in the control. The protective effects of compound I were more pronounced than of compound II: after exposure to γ-radiation in the same dose the percent of double strand DNA in the sample treated with compound I was 94-96% from the control *vs*. 89% in the sample treated with compound II. Triene, as we showed previously [4], significantly increased the number of γ-induced DNA breaks (to 53%). However, pretreatment of the cells with compound I and triene led to leveling of SOD inhibition effect, hence SOD virtually did not participate in the protection of cells pretreated with compound I before γ-irradiation. In experiments with $CdCl_2$ compound I exhibited a pronounced protective effect, transforming irreparable DNA injuries into repairable. Compound II exhibited no effect of this kind.

The formation of DNA break is the indicator of primary DNA damage, while cell death characterizes final events in the cell. Compound II exhibited higher cytotoxic effect than compound I: cell death after treatment with these compounds alone was 5.5 and 1.6%, respectively. Experiments with γ-irradiation also showed greater number of dead cells after pretreatment with compound II than with compound I. Triene had little effect on cell death from γ-irradiation.

Protection coefficients for cells pretreated with crown compounds exposed to γ-radiation and $CdCl_2$ are presented in Table 1. Garlic extract, effective in exposure to both mutagens [2,7] served as the positive control.

We conclude that compound I exhibited more pronounced protective effect than compound II in

TABLE 1. Protection Coefficients for Human Cells Pretreated with Crown Compounds I, II, and Garlic Extract

Experiment conditions	I			II			Garlic extract
	0	10^{-4} M	10^{-5} M	0	10^{-4} M	10^{-5} M	
γ-Irradiation, 50 Gy		73	79		24	62	88
Reparation, 15 min	96			96			
$CdCl_2$, 10^{-5} M		54	58		0	0	62
Reparation, 20 min	0	16		0	0	0	

Note. Protection coefficient $[(C\text{-}Ex)\text{-}C^1\text{-}Ex^1)/(C\text{-}Ex)]\times 100\%$, where C and Ex are contents of double strand DNA in control and mutagen-treated samples, C^1 and Ex^1 are the corresponding values in samples with cell protection.

γ-irradiation and exhibited a protective effect in $CdCl_2$ exposure. Its activity is just slightly lower than that of the garlic extract. Previously studied monoazacrown compounds N-(hydroxycarbonylmethyl)aza-15-crown-5 and N-(hydroxycarbonylmethyl)benzo-7-aza-15-crown-5 [6] exhibited antimutagenic effects close by their activity to compound I. Since the majority of antimutagens possess antiradical activity, it seems that crown compounds are perspective antimutagens with non-antioxidant activities.

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