Purine Receptor Agonists Protect the Genome of Plant and Animal Cells from Clastogen Damage

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Purine receptor agonists adenosine, cyclohexyladenosine, phenylisopropyladenosine, dimethylaminopurine riboside, ATP, and ADP reduced the level of chromosome aberrations and the number of micronuclei induced by ethylmethane sulfonate and cyclophosphamide in plants (*Crepis capillaris*) and mice. Possible mechanisms of the protective effect of these ligands are discussed.

Key Words: *purine receptors; agonists; clastogenesis; aneugenesis; antimutagenesis*

Involvement of the nervous system in the formation of cell resistance to mutagens is confirmed by many studies [4,6,7,14]. However the mechanism of initial activation of cell antimutagenesis by the nervous system, the nature of the primary signal, the conditions and parameters essential for modification of gene-protective systems remain unclear. Pilot studies showed that neurotransmitters of the sympathetic nervous system can be the primary signal activating antimutagenesis in the cells [8]. In this context, it was important to elucidate whether ligands of other receptors, *e.g.* purinergic receptors located on the postsynaptic membranes of neuronal or effector cells [13] and modulating activity of adrenergic and cholinergic neurotransmitter systems [10], can act as the primary signal. It was interesting to study possible activation with purine receptor ligands of the genome protecting systems in cells expressing and lacking purine receptors. The aim of the present study was evaluate the possibility of suppressing (in a preventive treatment regimen) mutagenesis induced by clastogens and aneugens in plant and animal cells with purine ligands.

MATERIALS AND METHODS

The following purine receptor agonists were used: adenosine (Reanal), N^6-cyclohexyladenosine (CHA), N^6-(2-phenylisopropyl)adenosine (PIA), 6-dimethylaminopurine-9-riboside (DAP), adenosine 5'-diphosphate (ADP; all from Sigma), adenosine triphosphate sodium salt (ATP, Darnitsa). Neutral solutions were used. The anticlastogenic effect was evaluated by the number of chromosome aberrations in plant meristem and mouse bone marrow cells and antianeugenic effect by the content of micronuclei (MN) in mouse peripheral blood erythrocytes.

MN in peripheral blood erythrocytes were counted in male mice (20-25 g). The ligands were injected intraperitoneally in doses: 5 mg/kg adenosine, 10 μg/kg PIA, 0.5 mg/kg CHA, 0.5 mg/ml DAP, 300 mg/kg ATP and ADP. These doses are not toxic and are used in studies of the specific properties of ligands [3,10]. Eight hours after injection of the ligand, cyclophosphamide in a dose of 30 mg/kg was injected and after 48 h blood was collected from the caudal vein for MN analysis (3-4 mice per point; at least 100 erythrocytes from each animal were analyzed; at least 4000 erythrocytes per time point were examined). Each experiment was repeated 2-3 times. Controls were injected with normal saline in the same volume. Aneugenic activity of cyclophosphamide and ligands was evaluated in each experimental series. Chromosome aberrations in mouse bone marrow cells were analyzed as described previously [1] and according to WHO recommendations for evaluation of potential mutagenic activity of chemical compounds (Hygienic Criteria of Environmental Status, WHO, 1989). Cyclophosphamide was injected 8 h after injection of the ligands.

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This term (like in the micronuclear test) was chosen because in some control mice mutagenic activity the alkylating agent decreased after injection of normal saline, but this was not observed, if the interval between the injections was 6-8 h. Colchicine (0.01 mg/g 0.025% working solution intraperitoneally) was injected 2.5 h before the end of mutagen action. All types of chromosome aberrations except gaps were counted..

Experimental studies of anticlastogenic activity of ligands on *Crepis capillaris* were carried out in accordance with methodological recommendations [9]. Seedling meristem cells of early growing seeds were analyzed. Cells were pretreated with ligands (10^{-2}-10^{-9} M) during the presynthetic period. Ethylmethane sulfonate (1.6×10^{-5} M, Sigma) and antitumor agent cyclophosphamide (4.5×10^{-4} M, KONPO) were used as mutagens. Both agents are alkylating compounds with known mechanism of action [11]. Mutagen treatment was carried out 2 h after washing from the ligand. Pressed preparations were stained with acetocarmine. All types of chromosome and chromatide aberrations except gaps were counted. No more than 50 metaphases in each preparation were counted.

The antimutagenic effect (AE) was estimated by the formula:

$$AE = \frac{(A_M - A_C) - (A_{L+M} - A_L)}{(A_M - A_C)} \times 100,$$

where A_M is the number of aberrations or MN induced by the mutagen, A_C the number of spontaneous aberrations or MN, A_{L+M} level of aberrations or MN after treatment with ligand and mutagen, and A_L level of aberrations of MN after treatment with ligand. The results were statistically processed using Student's *t* test.

RESULTS

All the studied ligands protected the cell genome from aneugenic and clastogenic effects of cyclophosphamide (Fig. 1). Adenosine showed the highest AE: it decreased the number of MN and chromosome aberrations by 95.6 and 91.7%, respectively. ATP had the lowest AE (41.0 and 46.8%, respectively). The protective effects of other ligands were intermediate. ADP and PIA possessed similar efficiency in decreasing the number of MN and chromosome aberrations. DAP showed high AE in MN assay and was 1.3-fold less potent towards chromosome aberrations. The difference in AE can be explained by different realization of the protective properties of the ligands under conditions of different intracellular mechanisms of chromosome rearrangements and MN formation.

All ligands protected mouse erythroid cell and lymphocyte genome from ethylmethane sulfonate- and cyclophosphamide-induced damage. The mechanism of this phenomenon is ambiguous and most likely determined by mechanisms of mutagenesis induced by these agents. Mutations can result from direct alkylation of DNA by clastogen molecules or induction of active oxygen forms attacking DNA [2]. Hence, the protective effects of ligands can be realized via several routes. For instance, the level of free radical damage to DNA induced by secondary mutagens can be considerably reduced by uric acid, a product of adenosine cell metabolism [3]. It was shown that uric acid present in human blood plasma in concentrations of

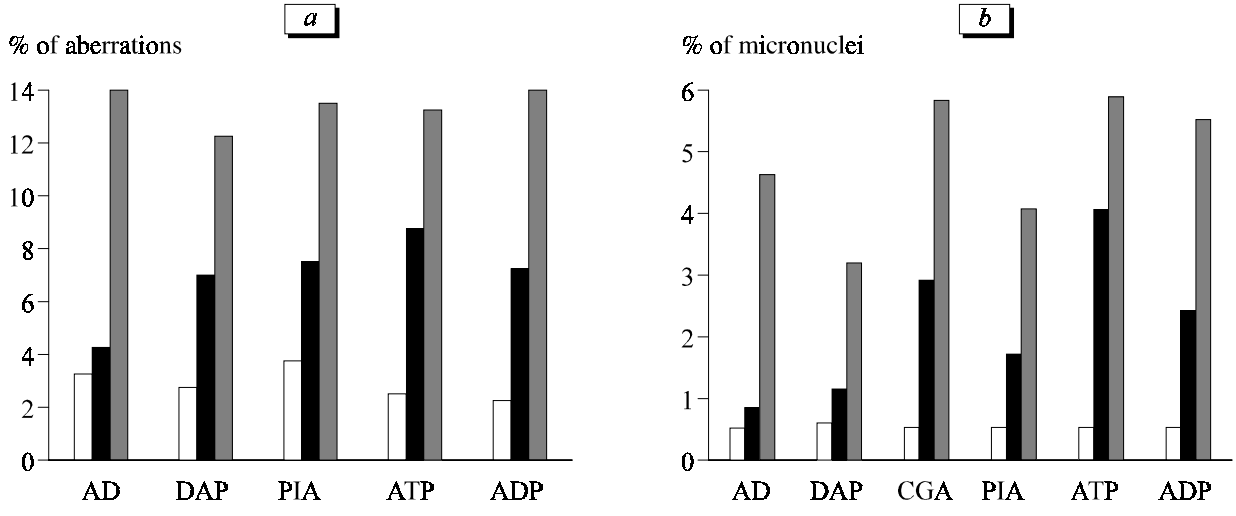

Fig. 1. Modification of clastogenic (*a*) and aneugenic *b*) effects of cyclophosphamide with purine receptor agonists. Light bars: control; dark bars: CP+ligand; cross-hatched bars: CP. Here and in Fig. 2: AD: adenosine; DAP: 6-dimethylaminopurine-9-riboside; CGA: N^6-cyclohexyladenosine; PIA: N^6-(2-phenylisopropyl)-adenosine.

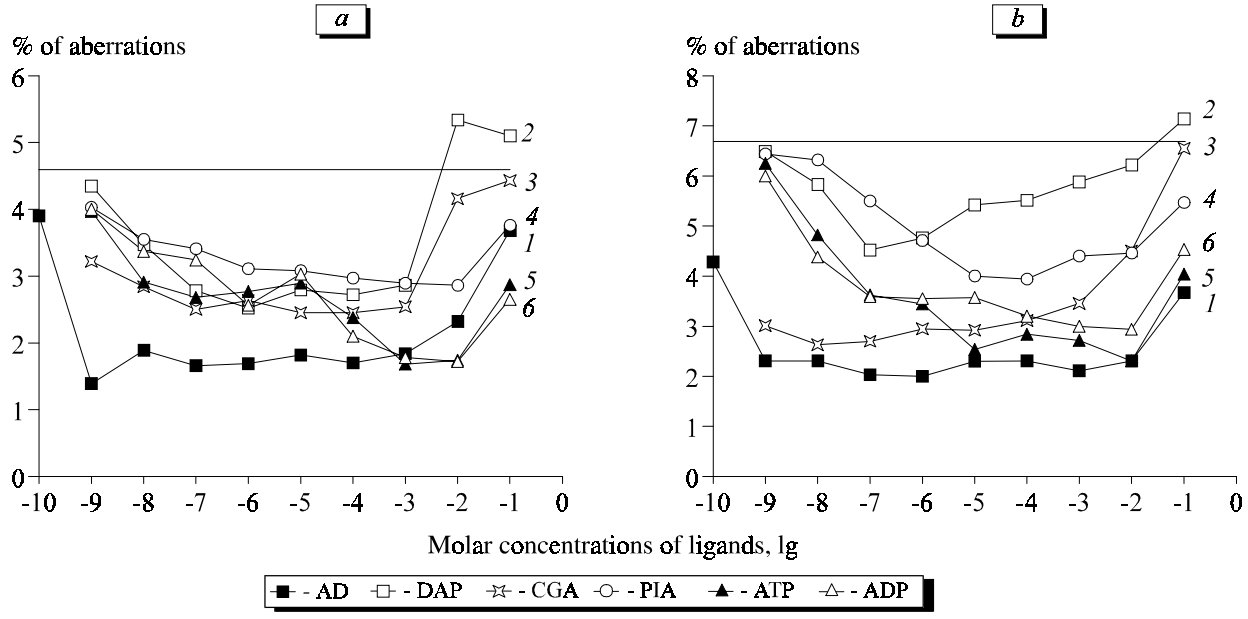

Fig. 2. Modification of clastogenic effects of ethylmethane sulfonate (*a*) and cyclophosphamide (*b*) on *Crepis capillaris* cells with ligands.

0.12-0.45 mM is an effective acceptor of singlet oxygen and hydroxyl radicals [5]. It cannot be excluded that adenosine can also serve as an effective "trap" for hydroxyl radicals [3]. Repair of primary DNA breaks can be ineffective, if the pool of nucleotides and ATP in the cell is exhausted; under these conditions cell treatment with adenine bases creates additional reserve for correct repair. The above mentioned pathways imply realization of ligand activity through certain metabolic routes. However, a receptor mechanism, *i.e*. activation of the natural antimutagenic defense systems by ligand-receptor interactions cannot be ruled out [7]. Presumably, the protective effect of ligands observed in the MN test is realized by this pathway. MN are formed after contact of the mutagen with red bone marrow cells. It can be hypothesized that cyclophosphamide either directly attacks erythroid cells or activates respiratory burst in adjacent macrophages [6]. In the later case, the genome of erythroid cell is damaged by APC produced by macrophage and transported across the cell membranes [2]. Similar damage to blood cell genome by APC generated by macrophages can be found in lymphocytes of lymphoid organs, where macrophages and lymphocytes closely contact and even form cytoplasmic bridges. Under these conditions purine receptor agonists via purine receptors on macrophage, erythroblast, and lymphocyte membranes inhibit the generation of superoxide radicals by macrophages [3] and increase the level of endogenous cAMP in erythroblasts and leukocytes [3] through activation of intracellular antimutagenesis systems [7]. Moreover, cyclophosphamide can provoke the release of histamine, a potent endogenous mutagen, from basophils, and in this case adenosine prevents the release of the mutagen from blood cells. It was shown that this effect is mediated via A_2 receptors on the basophil membrane [3]. If the receptor mechanism predominates in antimutagenesis, ligand treatment of cells lacking purine receptors will be inefficient.

In light of this, the protective effect of purine ligands was studied on plant cells expressing no purine receptors.

Experiments on *Crepis capillaris* showed that all ligands attenuated the mutagenic effects of ethylmethane sulfonate and cyclophosphamide to a different degree (Fig. 2). If we evaluate the ligand protective effect by the range of its active concentrations and degree of AE, two factors are significant. First, ligands with a high AE index have a wide range of active concentrations and vice versa, ligands with a low AE have a narrow range of active concentrations. In all other ligands the relationship between the maximum AE and the range of active concentrations was less expressed. Second, some ligands (adenosine, ADP, DAP) more effectively inhibit the clastogenic activity of ethylmethane sulfonate, others (CGA, PIA) are more active towards cyclophosphamide, while ATP similarly decreases the damaging effects of both mutagens. The detected differences and similarities in the protective effects of ligands can be due to similarity of their chemical structure; for example, both inductors can belong to single-center mutagens, to the same chemical groups (alkylating agents), *etc.* On the other hand, different functional activity (ethylmethane sulfonate is a monofunctional compound, while bis-(β-chloroethyl)-amine, active metabolite of cyclophosphamide, is

a bifunctional compound), different reaction groups, and other differences determine not only the induction of different primary DNA damage, but also different mechanisms of their fixation in apparent rearrangements. It is clear that under these conditions the systems of genome protection from mutagen damage are different and individually modulated by the ligands. We found no clear-cut dose-effect relationships for the majority of ligands. The number of induced aberrations decreased in the range of low ligand concentrations, then attained a plateau, and increased again at high concentrations (Figs. 1 and 2). This can be explained by changes in translocation of substances through the cell membrane and saturation of receptor molecules on the membrane by the ligand.

Hence, our findings suggest the existence of two mechanisms of the antimutagenic effect of the ligands: receptor-mediated and receptor-independent. The interaction of ligands with membrane receptors activates natural defense systems of the cell, including antimutagenesis.

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