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Antimutagenic effect of α -lipoic acid on three model test systems

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Some natural cellular metabolites which are antioxidants may reduce or inhibit the mutagenic activities of mutagens [1]. The control of cellular mutability by antimutagens may provide ways for preventing mutations which could result in cancer as well as diseases caused by genotoxic agents [2]. The antioxidant properties of α -lipoic acid (LA) and dihydrolipoic acid (DHLA), a reduced form of LA, have been demonstrated in different biological sys-

tems [3–5]. LA is present in all kinds of prokaryotic and eukaryotic cells and functions as a cofactor of multienzyme complexes that catalyze the oxidative decarboxylation of α -keto acids [6]. It is proposed that the antioxidant activity of LA might play an important role in its pharmacotherapeutic efficacy [5]. Since LA is a potent antioxidant its potential antimutagenic effect was investigated on the basis of its ability to inhibit the mutagenicity of direct-acting prototype mutagens/carcinogens and promutagens/procarcinogens. The antimutagenic potential of LA was evaluated in three model test systems: the *Salmonella*/microsome assay [7], the yeast toxicity and mutagenicity assay [8], and the *Vicia faba* chromosome aberration assay [9]. The experimental data revealed that LA exhibited an antimutagenic effect against 9-aminoacridine (9-AA)-induced mutagenicity in the bacterial strain *Salmonella typhimurium* TA97 (Table 1), 4-nitroquinoline-1-oxide (4-NQO)-induced mutagenicity in the strain *S. typhimurium* TA98 (Table 2), and against 2-aminofluorene (2-AF)-in-

Table 1: Antimutagenic effect of LA against 9-AA and 2-AF applied to bacteria *S. typhimurium* TA97

Compound tested	Concentration (μ g/plate)	Frequency of his ⁺ revertants/plate
– S9 mix		
Control (DMSO)		91 \pm 4.6
LA	250	90 \pm 4.9
LA	100	94 \pm 3.5
LA	50	102 \pm 11.1
9-AA	50	276 \pm 27.1*
9-AA + LA	50 + 250	104 \pm 13.1 ⁺
9-AA + LA	50 + 100	129 \pm 17.6 ⁺
9-AA + LA	50 + 50	138 \pm 14.5 ⁺
+ S9 mix		
Control (DSMO)		92 \pm 4.6
LA	250	69 \pm 9.8* ^a
LA	100	93 \pm 6.9
LA	50	94 \pm 6.7
2-AF	100	349 \pm 18.0
2-AF + LA	100 + 250	240 \pm 15.4 ⁺
2-AF + LA	100 + 100	259 \pm 10.7 ⁺
2-AF + LA	100 + 50	271 \pm 16.3 ⁺

* significant difference in comparison with control (DMSO) at $p < 0.05$;

⁺ significant difference in comparison with 9-AA or 2-AF at $p < 0.05$;

^a this value is such a low one due to increased toxicity of the highest concentration of LA in the presence of S9 mix

Table 2: Antimutagenic effect of LA against 4-NQO and 2-AF applied to bacteria *S. typhimurium* TA98

Compound tested	Concentration (μ g/plate)	Frequency of his ⁺ revertants/plate
– S9 mix		
Control (DMSO)		30 \pm 2.2
LA	250	32 \pm 1.1
LA	100	25 \pm 2.2
LA	50	29 \pm 2.3
4-NQO	50	324 \pm 38.4*
4-NQO + LA	0.2 + 250	188 \pm 18.2 ⁺
4-NQO + LA	0.2 + 100	221 \pm 27.1 ⁺
4-NQO + LA	0.2 + 50	230 \pm 31.2 ⁺
+ S9 mix		
Control (DSMO)		32 \pm 2.9
LA	250	28 \pm 1.9
LA	100	26 \pm 2.6
LA	50	35 \pm 2.7
2-AF	100	523 \pm 38.4*
2-AF + LA	100 + 250	375 \pm 182 ⁺
2-AF + LA	100 + 100	400 \pm 27.1 ⁺
2-AF + LA	100 + 50	426 \pm 31.2 ⁺

* significant difference in comparison with control (DMSO) at $p < 0.05$;

⁺ significant difference in comparison with 4-NQO or 2-AF at $p < 0.05$;

Table 3: Antimutagenic effect of LA against 4-NQO applied to yeast *S. cerevisiae* D7

Compound tested	Concentration	Survival (%)	Mitotic Crossing-Over ^a	Total aberrants ^b (%)	Number of revertants/10 ⁵ survival cells	Number of revertants/10 ⁶ survival cells
Control (DMSO)		100.00 (65 670)	– (0)	0.239 (19)	3.5 (280)	1.88 (75)
LA	1%	25.08 (244 201)	– (0)	0.21 (50)	7.32 (147)	3.52 (33)
LA	1.2%	16.57 (18 225)	– (0)	0.27 (50)	8.43 (122)	5.57 (35)
4-NQO	5 \times 10 ^{–3} mM	13.44 (14 655)	0.30 (44)	3.06 (449)	403.06 (4671)	181.62 (1001)**
4-NQO + LA	5 \times 10 ^{–3} mM + 1%	19.39 (13 248)	0.20 (27)	1.58 (209)	229.10 (3228)	46.56 (356) ⁺⁺
4-NQO + LA	5 \times 10 ^{–3} mM + 1.2%	11.98 (8938)	0.26 (23)	1.62 (145)	97.06 (968) ⁺⁺	27.58 (148) ⁺⁺

** significant difference in comparison with control (DMSO) at $p < 0.01$; ⁺⁺ significant difference in comparison with 4-NQO at $p < 0.01$;

^a Mitotic crossing-over between centromere and *ade2* locus is manifested as twin spotted (pink/red) colonies among white colonies;

^b Total aberrants are further changes observed in *ade2* locus (pink; red; white/pink; white/red; white/pink/red colonies) which reflect cumulative mitotic segregation due to mitotic gene conversion, chromosome loss or smaller structural deletions and point mutations

Numbers in parentheses indicate the actual numbers of colonies.

Table 4: Anticlastogenic effect of LA against MH applied to *V. faba*

Compound tested	Concentration (%)	Number of cells analyzed in ana-telophase	Chromosome aberrations (%)
Control (DMSO)		1000	0.60 ± 0.25
Control (SB)	0.0066 M	1627	0.61 ± 0.19
LA	0.00025	1544	0.45 ± 0.17
MH	0.00112	1694	5.08 ± 0.53**
MH	0.00056	1560	2.82 ± 0.42**
MH + LA	0.00112 ± 0.00025	1553	1.80 ± 0.34 ⁺⁺
MH + LA	0.00056 ± 0.00025	1503	1.37 ± 0.30 ⁺

SB Sørensen buffer pH 6; ** significant difference in comparison with control (SB) at $p < 0.01$; ++ significant difference in comparison with MH at $p < 0.01$; + significant difference in comparison with MH at $p < 0.05$

duced mutagenicity in both bacterial strains after metabolic activation (Table 1 and 2). LA might act as a competitive inhibitor of the frameshift mutagens used. As is illustrated in Table 3, inhibition of the mutagenic activity of 4-NQO in *Saccharomyces cerevisiae* caused a significantly reduced frequency of revertants at the *ilv1* locus and gene convertants at the *trp5* locus. Data summarized in Table 4 demonstrated the anticlastogenic effect of LA. LA significantly reduced the frequency of chromosome aberrations induced by maleic hydrazide (MH). Prophylaxis by some antioxidants against the genotoxicity of MH was also indicated in the *Allium micronucleus* assay [10]. The development of research in the area of antimutagenesis has led to the identification of a broad range of inhibitors belonging to different classes, and acting through multiple mechanisms [11, 12]. Our experimental data provided evidence for antimutagenic and anticlastogenic effects of LA. It may be included in a group of natural antimutagens of great pharmacological importance, probably acting in a desmutagenic manner.

Experimental

1. Chemicals

9-AA, Mr = 248.7 (Serva); 4-NQO, Mr = 190.2 (Sigma); 2-AF, Mr = 181.2 (Sigma); LA, Mr = 206.3 (Serva). These chemicals were dissolved in dimethyl sulfoxide (DMSO). MH, Mr = 112.1 (Sigma) was dissolved in 0.0066 M Sørensen buffer pH 6.

2. Procedures

Antimutagenic activity of LA was assessed by the *Salmonella* plate incorporation revised assay described in [7]. The toxicity and mutagenicity test was used for evaluation of the antimutagenic effect of LA on *S. cerevisiae* D7 strain [8]. The cytogenic *Vicia faba* test was employed for assessment of the anticlastogenic effect of LA [9]. The data were evaluated using Student's t-test.

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