Effect of Oxipine on the Severity of Anaphylactic Shock and the Ratio of Lipid Fractions in Lymphocytes of Allergic Rabbits

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It is shown that oxipine (mexidol; a 3-hydroxypyridine-derived antioxidant) injected intravenously into rabbits in a dose of 10 or 25 mg/kg alleviates the severity of the anaphylactic reaction, lowers the anaphylactic index, and considerably reduces the percentage of anaphylaxic shock-induced mortality. The preparation causes changes in the ratio of the total lipid to phospholipid fractions of lymphocytes, which has distinctive features in intact, primed, and challenged animals. It is thought that the antiallergic effects of oxipine are connected with both inhibition of free-radical oxidation and with the ability of the preparation to alter receptor properties of the allergy target cells due to its effect on the lipid ensembles of the cell membranes.

Key Words: *oxipine; lymphocytes; allergy; total lipids; phospholipids*

Synthetic antioxidants were introduced into medical practice as preparations protecting the internal environment of the organism from damage developing due to activation of free-radical oxidation. The use of antioxidants in allergic reactions is based on the concept that membrane structures become damaged when oxidative processes and antioxidative system mechanisms are thrown into imbalance under the influence of an antigen [8,14].

It is known that the initial stage of antigen-mediated action on the cell is antigen binding with the receptors of target cells. Specific antigen-binding sites are found on mast cells, basophils, lymphocytes, monocytes, eosinophils, and macrophages [2]. The key role in the molecular organization and functioning of receptor site-bearing biological membranes belongs to lipids and phospholipids [9].

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Since the mid-1980s intensive efforts have been made to develop new 3-hydroxypyridine synthetic antioxidants. These are structural analogs of vitamin B_6 compounds (pyridoxine and others) [10].

The present study examines the effect of oxipine (mexidol; 3-hydroxypyridoxine derivative) on allergic reactions and on the lipid spectrum in rabbit lymphocytes.

MATERIALS AND METHODS

Experiments were carried out on 129 California rabbits of both sexes weighing 2-2.5 kg. Animals were sensitized by three subcutaneous injections of 1 ml/kg swine serum at one-week intervals. On the 21st day of the experiment the rabbits were injected in the ear vein with the challenging dose of antigen (1 ml/kg) [11]. The severity of anaphylactic shock was assessed in points as follows: 0, absence of shock symptoms; 1, mild shock; 2, shock of intermediate severity; 3, severe shock; 4, shock soon followed by death (within one hour) due to immediate anaphylaxis or by delayed death

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Table 1. Distribution of Rabbits according to the Severity of Anaphylactic Shock after Intravenous Injection of Oxipine and Saline

Severity of shock, points	Distribution of animals, % of total number		
	oxipine, mg/kg		saline, 1 ml/kg (n=53)
	10 (n=17)	25 (n=15)	
0	11.8	20.0	–
1	41.1	33.4	15.1
2	23.5	13.3	22.7
3	5.9	13.3	26.4
4 immediate phase	5.9	13.3	26.4
4 delayed phase	11.8	6.7	9.4
Anaphylactic index	1.76	1.80	2.83

Note. Here and in Table 2: n is the number of animals in the series of experiments.

(within 24 hours) due to a prolonged anaphylactic reaction. The anaphylactic index was calculated according to Weigle's formula [16].

In the first series of experiments, performed on 85 rabbits, we evaluated the effect of oxipine on the anaphylactic reaction. The preparation was injected in the ear vein in doses of 10 and 25 mg/kg (1% and 2.5% solution in saline) 20 min before the challenging dose of antigen. Control animals received saline in a volume of 1 ml/kg. In the second series of experiments (44 rabbits) we compared the ratio of the fractions of total lipids to phospholipids in the lymphocytes of allergized and intact animals injected with oxipine. Lymphocytes were isolated one hour after intraveneous injection of oxipine in a dose of 25 mg/kg. Blood was taken from a cannulated *a. carotis*. A 5% solution of Trilon B in a final concentration of 4.5 ml per 60 ml blood was used as a preservative. Lymphocytes were isolated on a Ficoll-Verographin density gradient [12] using a modified method [3]. Occasional admixtures of erythrocytes in the lymphocyte fraction were eliminated using the hemolytic method [4].

Lymphocyte lipids were extracted as described elsewhere [14] and fractionated using silicagel flow thin-layer chromatography [5]. Total lipids were subjected to chromatography using heptane-sulfuric

Table 2. Effect of Oxipine on Ratio of Total Lipid and Phospholipid Fractions in Rabbit Lymphocytes under Normal Conditions and during Sensitization and Anaphylaxis ($M \pm m$)

Group of rabbits	Total lipid fractions, % of total lipids					Phospholipid fractions, % of total phospholipids						
	PL	Ch	FFA	TG	ChE	LPC	LPEA	SPM	PC	PI	PS	PEA
Control (saline, 1 ml/kg)												
Normal state (n=7)	30.4± 0.4	26.6± 0.4	2.1± 0.13	18.6± 0.5	23.3± 0.3	2.32± 0.12	9.64± 0.17	19.9± 0.2	44.4± 0.2	6.21± 0.13	0.83± 0.10	16.7± 0.3
Sensitization (n=8)	31.7± 0.4	22.0± 0.6	3.70± 0.16	19.1± 0.3	23.5± 0.3	3.06± 0.15	10.83± 0.12	17.0± 0.3	39.8± 0.4	7.21± 0.16	2.80± 0.14	19.3± 0.3
Anaphylaxis (n=7)	36.6± 0.4	22.5± 0.3	4.30± 0.14	17.0± 0.3	19.6± 0.1	3.97± 0.14	19.68± 0.14	22.6± 0.4	24.1± 0.3	8.61± 0.4	4.34± 0.19	16.7± 0.3
Experiment (oxipine, 25 mg/kg)												
Normal state (n=8)	32.2± 0.6*	26.5± 0.5	2.90± 0.15*	19.1± 0.5	19.3± 0.5*	2.51± 0.21	8.66± 0.16*	19.9± 0.3	43.1± 0.4*	7.29± 0.17*	1.04± 0.12	17.5± 0.3*
Sensitization (n=8)	31.8± 0.6	24.2± 0.3*	3.20± 0.16*	20.9± 0.6*	19.9± 0.3*	2.78± 0.19	8.83± 0.17*	18.7± 0.4*	41.6± 0.4*	7.54± 0.22	1.85± 0.16*	18.7± 0.4
Anaphylaxis (n=6)	34.1± 0.7*	25.6± 0.5*	3.40± 0.17*	18.7± 0.6*	18.2± 0.3*	3.03± 0.24*	12.68± 0.41*	19.8± 0.4*	42.1± 0.4*	6.74± 0.24*	2.65± 0.21*	13.0± 0.3*

Note. *: Results of analogous subgroups in experiment and control differ reliably (p<0.05). Lipid fractions: PL – phospholipids; Ch – cholesterol; FFA – free fatty acids; TG – triglycerides; ChE – cholesterol esters; LPC – lysophosphatidylcholines; LPEA – lysophosphatidylethanolamines; SPM – sphingomyelins; PC – phosphatidylcholines; PI – phosphatidylinositols; PS – phosphatidylserines; PEA – phosphatidylethanolamines.

ether-ethylacetate (12:3:0.6 v/v). The separation of phospholipids was performed using a chloroform-methanol-7 N ammonium hydrate system (13:5:1 v/v). Identification of fractions was performed using reference spots and dye reactions [6,7]. Chromatograms were developed by charring lipids in the vapor of a chromium mixture at 200°C during 30 min. The percentage content of individual lipid fractions was recorded on a Bian-170 densitometer.

The treatment of the results included calculation of the arithmetic mean and its deviation. Significance was estimated using Student's t test [1].

RESULTS

As can be seen in Table 1, oxipine alleviated the severity of anaphylactic shock in the rabbits. Administration of antioxidant in a dose of 10 mg/kg abolished all signs of anaphylaxis in 1 out of 10 animals on average. A dose of 25 mg/kg inhibited the anaphylactic response to antigen in 20% of the animals. The preparation increased the percentage of rabbits with mild symptoms of shock and reduced immediate and delayed anaphylactic reaction-induced mortality by 2 and 1.8 times, respectively. The overall picture of animal distribution according to the severity of anaphylaxis is characterized by an anaphylactic index that was 1.6 times lower in the rabbits of both groups as compared with the control.

The effect of oxipine on the ratio of lipid fractions in the lymphocytes was observed at all stages of the experiment and showed peciliarities in the intact and allergized rabbits at the stage of latent sensitization and anaphylaxis (Table 2). Injection of oxipine into intact rabbits resulted in a significant increase of the lymphocyte content of phospholipids, free fatty acids, phosphatidylinositols, and phosphatidylethanolamines. On the other hand, the relative content of cholesterol, lysophosphatidylethanolamine, and phosphatidylcholine was reduced. Certain peculiar features were noted in the effect of the antioxidant in allergized rabbits. Thus, in the lymphocytes of animals that had received oxipine during sensitization a reliable rise in the content of cholesterol, triglycerides, sphingomyelin, and phosphatidylcholine was accompanied by a drop in the content of cholesterol esters, lysophosphatidylethanolamine, and phosphatidylserine, as compared to the data concerning cells of sensitized rabbits from the control group. During anaphylaxis, in the lymphocytes of rabbits injected with antioxidant there was a rise in the relative content of cholesterol, triglycerides, and phosphatidylcholine, and a drop in the content of the fractions of phospholipids, free fatty acids, cholesterol esters, lysophosphatidylcholines, lysophosphatidylethanolamines, sphingomyelins, phosphatidylinositols, and phosphatidylserines, and phosphatidylethanolamines, as compared with the results for control animals subjected to anaphylaxis.

It may be concluded that oxipine-induced inhibition of free-radical oxidation is one of the factors alleviating the allergic reaction. The capacity of the antioxidant to alter the lipid composition of lymphocytes suggests that the preparation induces modifications of the cell receptor and transport systems participating in the allergic reaction.

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