

Testing of an Antitumor Enzyme L-lysine-A-oxidase from Trichoderma Harzianum Rifai F-180

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Abstract: An enzyme L-lysine-A-oxidase has been obtained from *Trichoderma harzianum* Rifai F-180. The analysis of serum from CBA mice immunized five times with native L-lysine-A-oxidase intravenously, at a dose of 35 U/kg, showed that the preparation had virtually no effect on the immune response to T-dependent antigen. L-lysine-A-oxidase at a dose of 35 U/kg administered twice intradermally has no effect on the delayed-type hypersensitivity. Multiple, parenteral administration of the native enzyme at doses of 35 U/kg and 70 U/kg slow down the spontaneous migration of leukocytes.

Key words: L-Lysine-A-Oxidase • Immunogenicity • Trichoderma

INTRODUCTION

Previously we had conducted animal studies on the biological effects of a substance obtained. According to these studies the enzyme is polyfunctional. The substance obtained has antiviral, antitumor, antimicrobial properties, is an immunomodulator and can be used to prepare dosage forms of different therapeutic orientation [1-8].

Objective of this study is to continue some preclinical trials of L-lysine-A-oxidase from trichoderma in mice.

MATERIALS AND METHODS

In this study, we used a homogeneous substance from the strain of *Trichoderma harzianum* Rifai, a producer of an antitumor and antiviral enzyme L-lysine-A-oxidase deposited in the Russian National Collection of Industrial Microorganisms (RNCIM) under number F-180.

An enzyme was obtained according to the technique developed previously [7].

To study the effect of L-lysine-A-oxidase on the antibody-formation to L-asparaginase *E.coli*, the C57Bl mice were administered L-lysine-A-oxidase intraperitoneally three times at a dose of 35 U/kg and then L-asparaginase *E.coli* at a dose of 300 U/kg also intraperitoneally for three days. Blood sampling from mice was performed on 7, 14, 21 and 28 day after starting immunization with L-asparaginase.

The study of mouse antiserum was performed in dynamic (on 7, 14 and 21 day after starting immunization by means of polarization fluoroimmunoassay).

RESULTS AND DISCUSSION

We have studied the effect of the preparation on the immune response to T-dependent antigen (S.RBC - sheep red blood cells), as well as on the synthesis of antibodies to T-dependent antigen according to the number of hemagglutination and antibody-producing cells.

The analysis of serum from CBA mice immunized five times with native L-lysine-A-oxidase intravenously, at a dose of 35 U/kg, showed that the preparation had virtually no effect on the immune response to T-dependent

antigen. Antibody titers in highly responsive CBA mice were not significantly different from the control and experimental animals at the appropriate time and were $7.0 \pm 0.2 \log_2$ and $7.2 \pm 0.3 \log_2$ in an experiment on days 7 and 14 and $6.9 \pm 0.2 \log_2$ and $7.2 \pm 0.3 \log_2$ in control at $P > 0.05$ (the difference between experiment and control).

Effect of L-lysine-A-oxidase on the humoral immune response to T-dependent antigen was also assessed by the number of antibody-forming cells in CBA mice splenocytes. As is evident from Table 1, native and modified L-lysine-A-oxidase causes some suppression of the synthesis of IgM antibodies, however, no reliable differences between experiment and control have been detected. There is also no differences between the effect of the drug on the amount of antibody between native and modified enzyme (Table 1).

The subsequent experiments studied the effect of the native L-lysine-A-oxidase on a cell-mediated response.

We have investigated the delayed-type hypersensitivity (DTH), reflecting the state of T-cell immunity, on the model of immune inflammation induced by sensitization with sheep erythrocytes.

Native enzyme at a dose of 35U/kg was administered three times, then antigen (SRBC) was administered on the fourth day and then the preparation was double-administered.

As is evident from Table 2, the enzyme L-lysine-A-oxidase did not change DTH significant differences between the experimental and control groups, no response index has been identified on indication.

Table 1: Effect of L-lysine-A-oxidase on the humoral immune response to T-dependent antigen (S.RBC).

Preparations, Batch No. of different terms.	Number of animals in a group	Number of antibody-forming cells per 105 splenocytes.	P*
1.Control-1 (saline)	15	278.0 ± 17.47	-
2.L-lysine-A-oxidase (native-1)	12	218.8 ± 13.52	>0.05
3.L-lysine-A-oxidase (native-2)	12	220.8 ± 12.86	>0.05
4.Control-2 (saline)	14	335.8 ± 14.11	-
5.L-lysine-A-oxidase (modified)	13	352.4 ± 18.34	>0.05

Note:

1.Control-1 - refers to the group of animals receiving native enzyme

2.Control-2 - refers to the group of animals receiving modified enzyme.

3.P*- is given in comparison with the corresponding controls

4.Experiments were performed on CBA mice which were administered the drug in doses of 35 U/kg i.v., quintuple.

Table 2: Effect of the native L-lysine-A-oxidase on the delayed-type hypersensitivity to SRBC.

Preparation	Dose U/kg	Protein mg/kg	Number of animals in a group	Response index, %	P*	P**
Controlsaline	-	-	15	18.0 ± 1.2	-	-
L-lysine-A-oxidase (native).	35	0.35	17	16.5 ± 1.4	> 0.05	-
L-lysine-A-oxidase (native).	125	1.25	14	17.6 ± 1.3	>0.05	>0.05

Note:

1.P*- is given in comparison with the control

2.P*- is given in comparison with different doses

3.Experiments performed on CBA mice

4.The drug was administered at a dose of 35 U/kg and 125/kg i.v., quintuple.

5. Native L-lysine-A-oxidase.

Table 3: Effect of the native L-lysine-A-oxidase on the development of delayed-type hypersensitivity (DTH).

Preparations	Testing time		P*
	6 hours	24 hours	
	Size of edema, mm		
Control (Hank's solution)	$1.2 \pm 0.13 \text{ n} = 15$	$1.3 \pm 0.21 \text{ n} = 15$	>0.05
L-lysine-A-oxidase	1.3 ± 0.15	1.2 ± 0.13	>0.05

Note:

1.P*- level of differences significance shown in comparison with the corresponding control.

2.n - number of animals in a group

3.Experiments performed on HP mice

Table 4: Effect of L-lysine-A-oxidase on spontaneous peripheral blood leukocyte migration in CBA mice.

No.	Preparation	Enzyme dose	Scale division value of micrometric ruler	P*
1	Control 1 n-8	Saline	8.1 ± 1.1	—
2	L-lysine-A-oxidase n-10	7 U/kg, 1/5 TD	7.0 ± 0.8	>0.5 (1-2)
3	L-lysine-A-oxidase n-10	14 U/kg 1/2 TD	9.5 ± 0.7	>0.5 (1-3)
4	Control 2 n-9	Saline	14.1 ± 3.5	—
5	L-lysine-A-oxidase n-10	35 U/kg TD	11.9 ± 1.2	>0.5 (4-5)
6	L-lysine-A-oxidase n-10	70 U/kg 2 TD	9.2 ± 2.2	>0.5 (4-6)

Note:

1.P*- is given in comparison with the corresponding control.

2.Control 1 - the animals not treated with the preparation, refers to drug-treated group at a dose of 7 and 14 U/kg.

3. Control 2 - the animals not treated with the preparation, refers to drug-treated group at a dose of 35 and 70 U/kg.

3.n - number of animals.

4.TD - therapeutic dose.

The following series of experiments studied the sensibilizing effect of L-lysine-A-oxidase on a model of delayed type hypersensitivity (DTH), but in this case the drug was used, unlike the previous experiment. The results of studying the modulation of DTH by the enzyme have shown that L-lysine-A-oxidase at a dose of 35 U/kg administered twice intradermally has no effect on the delayed-type hypersensitivity (Table 3).

Subsequent experiments have studied the effects of the preparation L-lysine-A-oxidase on the functional activity of T-lymphocytes, on the ability of T-lymphocytes to produce factor depressing the migration of leukocytes, as well as effect of the preparation on mitogen-induced lymphocyte proliferation in experiments in vivo and in vitro.

The results of studying the effect of L-lysine-A-oxidase on the ability of leukocytes migration showed that multiple, parenteral administration of the native enzyme at doses of 35 U/kg and 70 U/kg slowed a spontaneous migration of leukocytes.

Migration of leukocytes in control and in experiment at a dose of 70 U/kg was 14.1±3.5 and 9.2±2.2, respectively.

However, no significant differences were detected (Table 4).

Another ratio was observed when studying the inhibition of leukocyte migration. When using the optimal dose of tuberculin 40 µg/ml the inhibition of migration -24.39±3.73 was observed in control and -14.25±1.79 10.11±2.73 in cell cultures excreted from animals treated with L-lysine-A-oxidase in doses of 7 and 14 U/kg, respectively, repeatedly intravenously, at P <0.05.

CONCLUSION

Therefore, the analysis of serum from CBA mice immunized five times with native L-lysine-A-oxidase intravenously, at a dose of 35 U/kg, showed that the

preparation had virtually no effect on the immune response to T-dependent antigen. L-lysine-A-oxidase at a dose of 35 U/kg administered twice intradermally has no effect on the delayed-type hypersensitivity. Multiple, parenteral administration of the native enzyme at doses of 35 U/kg and 70 U/kg slowed a spontaneous migration of leukocytes.

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