0273-2289/00/88/0267/$11.75

Anticancer Enzyme L-Lysine α-Oxidase

Properties and Application Perspectives

Helena M. Treshalina,[1] Elena V. Lukasheva,*,[2] Ludmila A. Sedakova,[1] Galina A. Firsova,[1] Galina K. Guerassimova,[1] Natalia V. Gogichaeva,[2] and Temir T. Berezov[2]

[1]Blokhin Cancer Research Center of Russian Academy of Medical Sciences, Moscow, Russia; and [2]Department of Biochemistry, Russian Peoples' Friendship University, Moscow, Russia, E-mail: lukashev@aha.ru

Abstract

Fungal L-lysine α-oxidase (1.4.3.14) (LO) from *Trichoderma harzianum Rifai* presents an oxidoreductase with a firmly attached coenzyme—FAD. This stable enzyme catalyzes an oxidative deamination of L-lysine yielding hydrogen peroxide, ammonia, and α-keto acid. LO exhibits antitumor activity toward 5 of 12 tested transplantable tumors. The sensitive tumors were ascitic hepatoma 22 (T/C = 201%, CR = 66%); mammary adenocarcinoma Ca-755 (TGI = 96%); melanoma B-16 (TGI = 81%); AKATOL (TGI = 75%); RSHM 5 (TGI = 79%). LO therapeutic activity was observed within a wide range of doses, 35–350 U/kg, by intraperitoneal daily injections for 5 d.

Contrary to *Escherichia coli* L-asparaginase, LO demonstrates its antitumor activity by the low therapeutic doses in vivo within a wide range of optimal doses and through another antitumor spectrum. Fisher lymphadenosis L-5178y highly sensitive toward L-asparaginase appeared to be LO resistant.

The possible mechanisms of LO antitumor activity through the key biochemical processes are discussed.

Index Entries: L-lysine α-oxidase; L-amino acid oxidase; antitumor enzyme therapy; anticancer testing in vivo; schedule of L-lysine α-oxidase anticancer therapy.

Introduction

The biochemical approaches based on the specificity of cancer cell metabolism led to the elaboration of several original methods of cancer diagnostics and therapy *(1–3)*. It was determined that adenosine desaminase and 5'-nucleotidase regulating the intracellular level of adenosine play an

*Author to whom all correspondence and reprint requests should be addressed.

important role in the normal functioning of macrophages, which are believed to be responsible for antimetastatic resistance *(4,5)*.

Remarkable progress in human cancer therapy is based on the suppression of enzymes involved in the biosynthesis of polyamines, which are known to be mitogenic agents, and on the blockade of key enzymes of nucleic base synthesis. An outstanding role in cancer enzymotherapy is played by bacterial desamidases. A significant antitumor effect based on desamidation of L-asparagine was exhibited by L-asparaginase from *Escherichia coli* and *Erwinia*. In vivo experiments on mice showed that these enzymes were highly effective against Fisher lymphadenosis in mice (complete remission), but they exhibited a narrow specificity and practically did not influence the growth of other mammalian tumors. The bacterial L-asparaginase application to cancer patients significantly increased the treatment efficacy of the different types of lymphomas. Sufficient wide practice in clinical application revealed a number of common drawbacks of these bacterial enzymes together with some positive pharmacological properties. The main disadvantage is a low substrate specificity, low stability, and high immune reactivity causing the side effects restricting the perspectives of L-asparaginase application *(3)*.

This fact triggered the search for new enzymes affecting other metabolic reactions essential for cancer cell growth. L-Lysine α-oxidase (LO) (1.4.3.14) as one of the potent antitumor enzymes was isolated and purified from *Trichoderma viride Y-244-2* for the first time in Japan *(5)*. It is well known that L-lysine is very important for the human organism. It stabilizes desoxyhemoglobin and the DNA-binding proteins in chromatin are rich with lysine as well as the proteins of connective tissues. In 1991, LO was isolated in Russia from the strain *Trichoderma harzianum Rifai.* LO molecule consists of two identical subunits with the molecular weight of 60 kDa and contains flavine adenine dinucleotide as a cofactor. LO oxidatively deaminates L-lysine yielding α-keto ε-aminocaproic acid, H_2O_2, and NH_3. LO exhibits a wide pH-optimum of catalytic activity (4.5–10.0) and low K_m (0.014 m*M*) toward L-lysine *(5–9)*. It acts practically only on L-lysine (100%) and to a small extent on some positively charged amino acids, i.e., L-arginine (5.1%) and L-ornithine (5.9%). LO is characterized by sufficient stability when stored in lyophilized form at 0–4°C or frozen in solution at –20°C *(10)*. Different effective methods for LO purification have been elaborated *(5,11)*.

The study of LO biological properties shows that it exhibits a sufficient level of cytotoxicity toward tumor cells *(5,12)*, but contrary to L-asparaginase, LO shows antileukemic activity and antimethastatic effect in vivo at rather low efficient doses *(4,5,14)*. The above mentioned properties enable us to consider LO as a potent antitumor agent. The spectrum of LO antitumor activity and its possible noncoincidence with that of L-asparaginase have not been investigated so far. The goal of the present work was the study of LO antitumor activity in comparison with L-asparaginase from *E. coli.*

Materials and Methods

The experiments were carried out on mice and rats with transplanted tumors; 540 female mice of different lines (18–20 g body weight) and 100 female regular rats (150–180 g) were used in our experiments.

The frozen tumor strains were purchased from the Laboratory of Tumor Strains of the Blokhin Cancer Research Center, Russia, and transplanted in vivo to animals of indicated strains. The tumors were inoculated intraperitoneally or subcutaneously using a standard method. The following tumor strains were used: Fisher lymphadenosis L-5178y on mice $BDF_1(C_{57}Bl/j6 \times DBA_2)$; leukemia P388 on mice strain DBA_2; leukemia L1210 on hybridic mice BDF_1 ($C_{57}Bl/j6 \times DBA_2$); plasmacytoma MOPC-406 on mice BALB/c; hemoblastosis La on mice $C_{57}BL_6$; ascitic hepatoma 22 on mice C_3H; Walker ascitic carcinosarcoma W-256 on rats; mammary adenocarcinoma Ca-755 on mice BDF_1; colon adenocarcinoma AKATOL on mice BALB/c; melanoma B-16 on mice $C_{57}BL_6$; sarcoma 180 on tetrahybridic mice CBWA; squamous cells cervix carcinoma RSHM-5 on mice CBA.

Lyophilized LO was manufactured at the Institute of Applied Enzymology (Lithuanian Republic) by the method given in ref. *11*. The treatment of the transplanted solid and ascitic tumors was started after 48 h; and 24 h after tumor inoculation in the case of hemoblastosis. The treatment course was 5 d with 24-h intervals; the route of administration used was intraperitoneal injections. LO powder was dissolved in sterile physiological NaCl solution (2–20 U/mL) and injected to animals in 35–1000 U/kg doses. L-Asparaginase (commercial product of Latvian Republic) was dissolved *ex temporae* in distilled *pro injectionibus* water to 20–50 U/mL concentrations and injected into mice in 1000 U/kg dose for 10 d.

The anticancer efficacy was estimated by the commonly used criteria: tumor growth inhibition (TGI, %) and the increase of life span (T/C, %). The mice were regarded cured if they were alive for 120 d after the tumor inoculation. We used the standard statistic Fisher–Student method. The comparison between LO and *E. coli* L-asparaginase antitumor activity was studied on the mice with L-5178y.

The tumors were regarded sensitive if the effectiveness of treatment by each of the enzymes satisfied the following requirements: TGI ≥ 70%, T/C ≥ 150, and cured (complete remission, CR).

Results and Discussion

LO activity toward hemoblastosis and ascitic mice and rat tumors is presented in Table 1. LO has a weak effect on mice hemoblastosis. Lymphadenosis L-5178y highly sensitive toward L-asparaginases did not respond to LO therapy. In L-1210, La, and P-388, the effect (T/C) was within the range 136–154%. In MOPC-406, LO activity was practically absent. Walker carcinosarcoma W-256 also proved to be LO nonsensitive; T/C was no more than 119%.

Table 1
Increasing of Life Span of Animals with Leukemias and Ascitic Transplantable Tumors After L-Lysine α-Oxidase Treatment

	Maximal efficacy, T/C (%)						
Preparation	L-5178y	P-388	L-1210	La	MOPC-406	Hep-22a	W-256[a]
L-lysine α-oxidase	112	154	136	140	119	201[b]	111
L-asparaginase	200[b]	100	100	110	100	121	113

[a]Rat tumor (others are murine tumors).
[b]Complete remission 29–66%.

Table 2
Inhibition of Solid Transplantable Tumors Growth After L-Lysine α-Oxidase Treatment

	Maximal efficacy, TGI (%)				
Preparation	Ca-755	B-16	AKATOL	RSHM-5	S-180
L-lysine α-oxidase	96	81	75	79	61
L-asparaginase	12	0	46	24	15

The best results were obtained on ascitic hepatoma 22. The LO treatment increased the life span substantially—T/C = 201%, CR = 29–66%.

L-Asparaginase was highly effective only in the case of L-5178y treatment: T/C was 200% and 70% cured. For other hemoblastosis and ascitic tumors, the effect was either insignificant or absent.

Among the solid tumors, the pronounced LO antitumor effect was obtained for Ca-755; TGI reached 96%. For melanoma B-16, AKATOL, and RSHM-5 the effects were 81, 75, and 79%, respectively. The lower activity was observed for sarcoma 180, TGI = 61% (Table 2). L-Asparaginase practically did not inhibit the growth of the studied solid tumors.

Using the accepted criteria of therapy effectiveness, the list of the LO-sensitive tumors included: ascitic hepatoma 22; mammary adenocarcinoma Ca-755; melanoma B-16; squamous cell cervix carcinoma; colon adenocarcinoma AKATOL.

Effective enzyme doses were determined by intraperitoneal daily single injections during 5 d using different dose values up to the toxic ones (Table 3). It is possible to attain a considerable antitumor effect for hepatoma 22 (T/C criterion) using 35–750 U/kg single doses (course doses 175–3750 U/kg). A range of LO optimal therapeutic doses for ascitic-hepatoma-bearing mice was determined as 35–300 U/kg. Doses higher than 1000 U/kg were toxic.

For mice with Ca-755 the range of single effective doses was found to be 200–350 U/kg. The single lethal dose for the mice with this tumor was significantly lower than for the mice with hepatoma 22 and comprised 500 U/kg. On

Table 3
Range of L-Lysine α-Oxidase Therapeutic Doses for Mice

	Tumor strain, efficacy[a]		
Single dose, U/kg	Hepatoma 22a T/C, %	Ca-755 TGI, %	Melanoma B-16 TGI, %
35	283	55	47
70	350	60	56
150	414	64	59
200	442	90	58
300	347	91	63
350	215	95	81[a]
500	447	96[a]	—
750	301	—	—
1000	78[a]	—	—

[a]Toxicity.

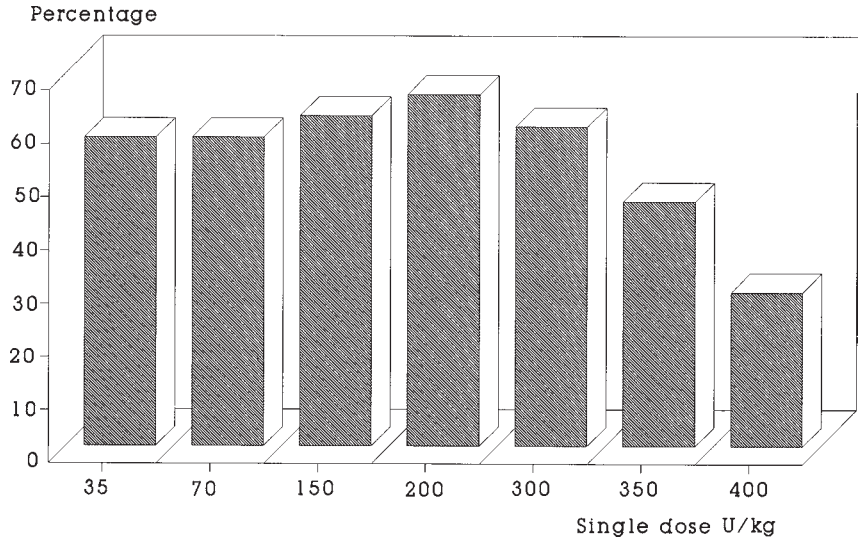

Fig. 1. Complete remission of murine ascitic hepatoma 22 after L-lysine α-oxidase therapy. Course of treatment: daily injections during 5 d. Route of administration: intraperitoneally.

melanoma B-16 the appreciable effect was observed only by the application of 350 U/kg dose (course dose 1750 U/kg), which caused toxic death (Table 3). Figure 1 shows that in the case of daily intraperitoneal LO injection in single 35–200 U/kg dose, there exists a linear dependence of the antitumor effect on the value of LO single dose. The increase in the single doses up to 200–350 U/kg was not accompanied by the proportional growth of effectiveness. The single doses 350–1000 U/kg proved to be toxic for the mice with different tumors. The LO application in the single doses higher than 350 U/kg caused the mice death on the d 5–7 after the treatment course without visual pathological changes.

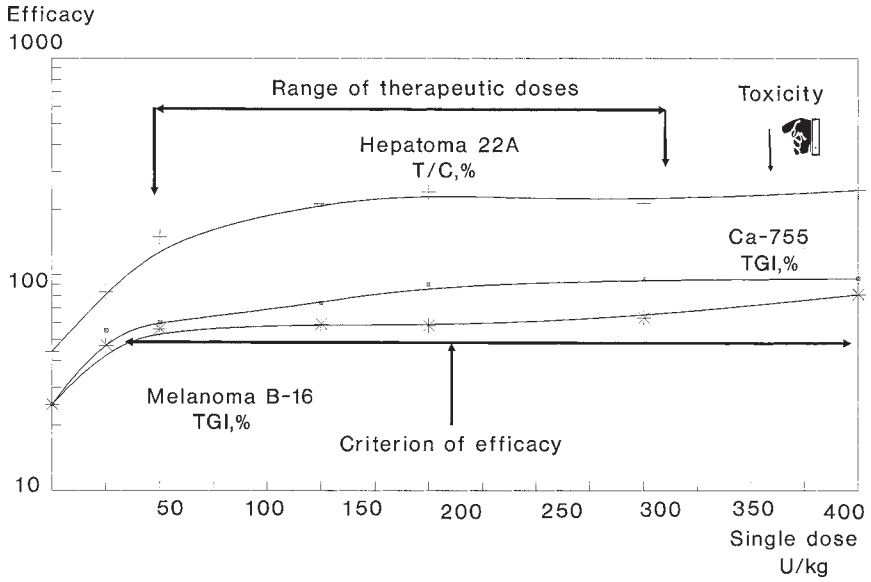

Fig. 2. Dependence between L-lysine α-oxidase antitumor activity and its dose range. Course of treatment: daily injections during 5 d. Route of administration: intraperitoneally. TGI, tumor growth inhibition; T/C, increasing of life span.

It was shown that LO exhibits an antitumor effect toward 5 of the 12 tested tumors. The study of the antitumor spectrum in comparison with that for L-asparaginase revealed an original and wider spectrum of antitumor action of LO with application of rather low therapeutic doses. Ascitic hepatoma 22 appeared to be the most sensitive to LO (Fig. 2). The pronounced effect was detected for mammary adenocarcinoma Ca-755; moderate tumor growth inhibition was observed for three other tumors.

It is important for the evaluation of clinical perspectives of LO that its antitumor action is observed within a wide range of efficient doses and it is not accompanied by pronounced toxicity. It is peculiar that for the mice with highly sensitive ascitic hepatoma, the range of effective doses was 35–350 U/kg, but for the mice with moderately sensitive solid tumors it was 200–300 U/kg. The lower the sensitivity of tumor toward LO treatment, the shorter was the interval of effective doses. This fact may be connected with different availability of tumor cells for LO. For instance, the direct contact with tumor cells was attained in the case of ascitic hepatoma intraperitoneal treatment.

Discussing the LO antitumor spectrum and the dose dependence of this enzyme, it is possible to suppose that if the elimination of L-lysine from lysine-sensitive-tumors metabolism is fatal for their proliferation, the enzyme with high substrate specificity toward L-lysine should lethally inhibit this proliferation. If this supposition is correct, the LO effectiveness in extremely low doses is quite explainable.

We can propose some other reasons for LO biological action: (a) LO catalyzed reaction of L-lysine deamination yields H_2O_2, which is known to be a cytotoxic agent; (b) LO administration causes the decrease in adenosine concentrations in tissues resulting in the inhibition of metastasis growth observed for Lewis lung carcinoma *(4)*.

A new fungal enzyme L-lysine α-oxidase from *Trichoderma harzianum Rifai* characterized in this work is a low toxic substance with the original antitumor activity principally different from that of L-asparaginase both by the tumor spectrum and by the possible mechanism of action. The enzyme with this combination of properties could be useful for oncological patients in different clinical situations. It is necessary to clarify the precise mechanism of its antitumor action for the therapeutical perspectives in oncology.

References

1. Berezov, T. T. (1971), *Vestnik Akademii Nauk USSR.* **N11,** 35–46 (in Russian).
2. Berezov, T. T. (1984), *Vestnik Akademii Nauk USSR.* **N8,** 11–24 (in Russian).
3. Chabner, B. A. and Loo, T. T. (1996), *Enzyme Therapy: L-Asparaginase, Cancer Chemotherapy and Biotherapy*, 2nd ed., Lippincott-Raven Publishers, Philadelphia, pp. 485.
4. Umanski, V. J., Khaduev, S. Kh., Zaletok, S. P., Balitzki, K. P., Berdinskih, N. K., and Berezov, T. T. (1990), *Bull. Exper. Biol. Med*. **109,** 458–459 (in Russian).
5. Kusakabe, H., Kodama, K., Kuninaka, A., Yoshino, H., Misono H., and Soda, K. (1980), *J. Biol. Chem*. **255,** 975–981.
6. Berezov, T. T. and Lukasheva, E. V. (1988), *Biochem. Int.* **17,** 529–534.
7. Lukasheva, E. V., Smirnova, I. P., and Berezov, T. T. (1987), *Voprosi Med. Khimii* **33,** 127–132.
8. Lukasheva, E. V., Vesa, V. S., Korpela, T. K., and Berezov, T. T. (1991), *Voprosi Med. Khimii* **37,** 68–70 (in Russian).
9. Lukasheva, E. V. and Berezov, T. T. (1988) *Prikladnaia Biokhimia i Microbiologia* **24,** 459–466 (in Russian).
10. Lukasheva, E. V., Dzhukovskii, A. P., and Berezov, T. T. (1994), in *Modern Enzymology: Problems and Trends*, (Kurganov, B. I., Kochetkov, S. N., and Tishkov, V. I., eds.), Nova Science Publishers, NY, pp. 593–597.
11. Laugalene, N. F., Vesa, V. S., Jankevechene. R. P., et al. (1990), *Voprosi Med. Khimii* **36,** 88–90.
12. Khaduev, S. Kh., Dzhukova, O, S., Dobrinin, J. V., Soda, K., and Berezov, T. T. (1986) *Bull. Exper. Biol. Med*. **101,** 603,604 (in Russian).
13. Khaduev, S. Kh., Glazkova, T. Ju., Vesa, V. S., Laugalene, N. F., Puodjute, S. P., Denis, G. I., Jurchenko, N. J., and Berezov, T. T. (1989), *Bull. Exper. Biol. Med*. **108,** 476–477 (in Russian).
14. Kusakabe, H., Kodama, K., Kuninaka, A., Yoshino, H., and Soda, K. (1980), *Agricultural Biol. Chem*. **44,** 387–392.