Antitumor and Antimetastatic Effects of Betulonic Acid Amides in Mice with Transplantable Lewis Carcinoma

I. V. Sorokina, T. G. Tolstikova, N. A. Zhukova, N. I. Petrenko, N. V. Uzenkova, E. E. Shul'ts, and N. A. Popova*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 7, pp. 78-81, July, 2006
Original article submitted June 24, 2005

We studied the effects of four synthetic amides of betulonic acid containing amino acid fragments (*d*,*l*-α-alanine, β-alanine, and their methyl esters) on the rate of growth and metastatic dissemination of transplantable Lewis lung carcinoma in C57Bl/6 mice. The test compounds were administered intragastrically in a single dose of 500 mg/kg. 3-[-oxo-20(29)-lupen-28-oilamino]-propionic acid suppressed primary tumor growth (by 26%) and decreased the number of lung metastases (by more than 2 times). Antitumor and antimetastatic activity of triterpenoids decreased after methylation of the amino acid fragment in betulonic acid.

Key Words: *transplantable tumor; antitumor effect; antimetastatic effect; betulonic acid; 2- and 3-[3-oxo-20(29)-lupen-28-oilamino]-propionic acid*

New lupane derivative were recently synthesized. These bioactive compounds are characterized by a wide range of pharmacological effects. Semisynthetic derivatives of betulinic and betulonic acids are lupane triterpenoids with antiviral, antibacterial, and antitumor properties [8,11]. Betulinic acid derivatives differ from natural cytostatics by low toxicity and selectivity of action on tumor cells [10]. New derivatives of betulonic acid synthesized at the Novosibirsk Institute of Organic Chemistry contain fragments of long-chain amino acids and peptides at C-28 and exhibit antiviral activity relative to human immunodeficiency virus type 1 and herpes simplex virus [4,5]. Previous experiments on cultured tumor cells of human melanoma and epidermal carcinoma showed that introduction of amino acid fragments into the molecule of betulinic acid (C-29) potentiates the cytotoxic effect of this compound [9]. Our studies showed that betulonic acid derivatives with α- or β-alanine fragments at C-28 are characterized by low toxicity after intragastric administration (mean lethal dose >5000 mg/kg) and produce an antioxidant effect in mice with toxic hepatitis [6]. These compounds decrease the severity of necrotic and dystrophic changes in the liver and kidneys of rats under conditions of combined cytostatic treatment [7]. It was interesting to evaluate whether betulonic acid derivatives exhibit *in vivo* antitumor and antimetastatic properties. We studied the effects of betulonic acid derivatives with fragments of α-alanine, β-alanine, and their methyl esters on the growth and metastatic dissemination of transplantable Lewis lung carcinoma in C57Bl/6 mice.

MATERIALS AND METHODS

We used amides of betulonic acid containing fragments of amino acids (α-alanine, β-alanine) and

Laboratory of Pharmacological Studies, N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Division of the Russian Academy of Sciences; *Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences, Novosibirsk. ***Address for correspondence:*** sorokina@nioch.nsc.ru. I. V. Sorokina

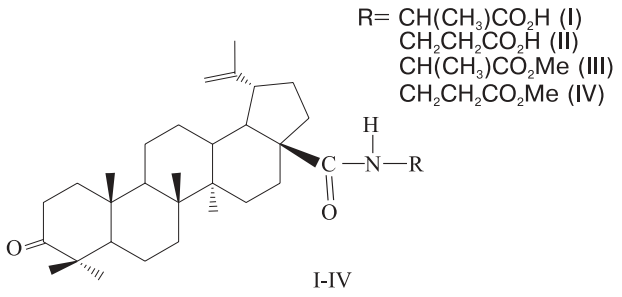

Fig. 1. Structural formulas of betulonic acid amides.

their methyl esters at C-28: 2-[3-oxo-20(29)-lupen-28-oilamino]-propionic acid (compound 1), 3-[3-oxo-20(29)-lupen-28-oilamino]-propionic acid (compound 2), methyl ester of 2-[3-oxo-20(29)-lupen-28-oilamino]-propionic acid (compound 3), and methyl ester of 3-[3-oxo-20(29)-lupen-28-oilamino]-propionic acid (compound 4, Fig. 1). The test compounds were synthesized at the Laboratory of Medical Chemistry (Novosibirsk Institute of Organic Chemistry) [3].

The experiments were performed on 50 male C57Bl/6 mice obtained from the vivarium of the Institute of Cytology and Genetics. The animals were maintained in a vivarium under standard conditions. All experimental manipulations were conducted according to the European Convention on Humane Care and Use of Laboratory Animals.

The suspension of Lewis lung carcinoma cells was transplanted into the thigh muscle (6×10^6 cells in 0.1 ml physiological saline). Ten days after transplantation the animals were divided into 5 groups (10 mice per group). During this period the size of the primary tumor reached 1 cm^3. The test compounds were administered intragastrically in a single dose of 500 mg/kg (aqueous Tween 60 solution, ICN; 0.2 ml per 10 g body weight). Control animals received an equivalent volume of aqueous Tween solution. Tumor size was measured in 3 perpendicular planes daily over 10 days after treatment with the test compounds. The animals were killed by cervical dislocation after 10 days. The lungs were fixed in neutral formalin. Metastatic nodes were counted under a binocular loupe. To estimate the area of metastases, the lung tissue was treated by the standard histological method on a MICROM automatic system (Zeiss) and stained with hematoxylin and eosin. Histological examination of samples was performed to determine the volume density of metastases (ratio of the area of metastases to section area) [1]. The antitumor effect of triterpenoids was studied by changes in primary tumor growth over 10 days after treatment with the test compounds. The index of tumor growth inhibition (TGI) was calculated as the ratio of the difference

TABLE 1. Effect of Single Treatment with Betulonic Acid Amides on Growth of Transplantable Lewis Lung Carcinoma in C57Bl/6 Mice

Compound	Tumor size after treatment with compounds, cm^3						
	day 1	day 2	day 3	day 4	day 6	day 8	day 10
Control	1.42±0.10 (100)	2.08±0.15 (146)	2.44±0.16 (172)	2.60±0.20 (183)	3.73±0.22 (263)	4.77±0.35 (336)	5.91±0.35 (416)
1	1.42±0.11 (100)	1.50±0.11* (106)	1.84±0.12* (130)	1.96±0.17* (138)	3.18±0.13 (224)	4.36±0.17 (308)	5.87±0.33 (413)
2	1.49±0.11 (100)	1.53±0.13* (103)	1.93±0.14* (129)	2.20±0.15 (148)	2.97±0.22* (199)	3.96±0.28 (266)	5.61±0.33 (376)
3	1.24±0.10 (100)	1.57±0.08* (128)	2.15±0.10 (173)	2.39±0.11 (193)	3.03±0.17* (244)	4.27±0.29 (344)	5.78±0.10 (466)
4	1.50±0.17 (100)	1.71±0.23 (114)	2.07±0.27 (138)	2.04±0.27 (136)	3.22±0.53 (215)	4.23±0.82 (282)	5.50±1.03 (367)

Note. Percentage of the initial level is shown in brackets. Here and in Table 2: *$p<0.05$ compared to the control.

between the average tumor size in control and treated mice to tumor size in control animals [2].

The results were analyzed using Statistica 6 software. The differences were significant at $p<0.05$.

RESULTS

Table 1 shows the dynamics of primary tumor growth in mice over 10 days after administration of the test compounds.

Comparative study showed that the test compounds inhibit the growth of Lewis lung carcinoma. Tumor growth delay was most pronounced over the first 4-6 days. Betulonic acid derivatives with fragments of amino acids had a persistent and significant effect. For example, compounds 1 and 2 inhibited tumor growth by 40-45 and 35-43%, respectively, compared to the control. Methyl esters of these triterpenoids were less active (compound 3) or their activity varied in a range surpassing the interval corresponding to significant difference from the control group (compound 4).

Index TGI was estimated for each compound. TGI for betulonic acid amides (compounds 1 and 2) reached 26-28% (Fig. 2). For compounds 3 and 4 TGI was lower over the first days, but than underwent significant variations, which made impossible to make final conclusion about the effectiveness of these compounds.

Examination at low magnification showed that compound 2 twofold decreases the number of metastatic nodes in the lungs. Other triterpenoids had little effect on this parameter (Table 2). Morphometry of histological samples showed that compound 2 has a strong antimetastatic effect manifesting in a 3-fold decrease in the volume density of metastases. Another carboxylated amide (compound 1) tended to decrease this parameter. However, the number of metastatic nodes was relatively high in animals of this group. Compounds 3 and 4 exhibited no antimetastatic activity.

Our results show that 3-[3-oxo-20(29)-lupen-28-oilamino]-propionic acid inhibits tumor growth rate and metastatic dissemination in mice with transplantable Lewis lung carcinoma. Antitumor and antimetastatic activities of the test drugs after intragastric administration decrease after methylation of the amino acid fragment in betulonic acid, which was probably associated with lower rate of methyl ester metabolism in the liver.

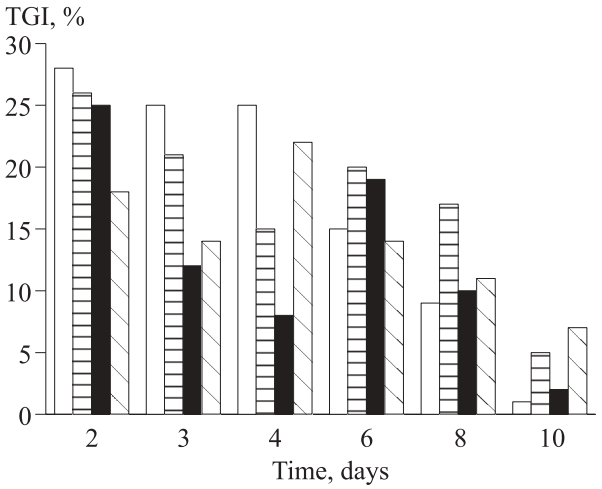

Fig. 2. Inhibition of Lewis lung carcinoma growth under the influence of betulonic acid amides. Light bars, 2-[3-oxo-20(29)-lupen-28-oilamino]-propionic acid; horizontal shading, 3-[3-oxo-20(29)-lupen-28-oilamino]-propionic acid; dark bars, methyl ester of 2-[3-oxo-20(29)-lupen-28-oilamino]-propionic acid; slant shading, methyl ester of 3-[3-oxo-20(29)-lupen-28-oilamino]-propionic acid.

This study was performed within the framework of the Integration Program of the Siberian Division of the Russian Academy of Sciences (N 146) "Development of Medicinal and Prophylactic Drugs for Medicine. Fundamental Bases and Their Realization".

REFERENCES

1. G. G. Avtandilov, *Medical Morphometry* [in Russian], Moscow (1990).
2. E. D. Gol'dberg and E. P. Zueva, *Plant Preparations in Combination Therapy of Malignant Neoplasms* [in Russian], Tomsk (2000), p. 42.
3. N. I. Petrenko, N. V. Elantseva, V. Z. Petukhova, *et al.*, *Khim. Prirodn. Soed.*, No. 4, 276-283 (2002).
4. A. G. Pokrovskii, O. A. Plyasunova, T. N. Il'icheva, *et al.*, *Khimiya Interesakh Ustoichivogo Razvitiya*, **9**, 485-491 (2001).

TABLE 2. Effect of Single Treatment with Betulonic Acid Amides on the Number and Volume Density of Lung Metastases in Mice with Transplantable Lewis Lung Carcinoma

Parameter of metastatic dissemination	Control	Compound			
		1	2	3	4
Number of nodes	16.70±2.82	14.90±1.17	8.40±2.04*	14.80±2.68	11.70±3.97
Volume density, %	3.4±1.0	1.4±0.3	1.1±0.3*	2.4±0.8	6.7±1.7

5. E. A. Semenova, O. A. Plyasunova, N. I. Petrenko, *et al*., *Dokl. Akad. Nauk*, **391**, No. 4, 556-558 (2003).
6. I. V. Sorokina, T. G. Tolstikova, E. B. Bubnova, *et al*., *Nauch. Vestn. Tyumensk. Med. Akad. Bioantioksidanty*, No. 1, 60-61 (2003).
7. I. V. Sorokina, T. G. Tolstikova, N. A. Zhukova, *et al*., *Dokl. Akad. Nauk*, **399**, No. 2, 274-277 (2004).
8. R. H. Cichewicz and S. A. Kouzi, *Med. Res. Rev*., **24**, 90-114 (2004).
9. H. J. Jeong, H. B. Chai, S. Y. Park, *et al*., *Bioorgan. Med. Chem. Lett*., **9**, 1201-1204 (1999).
10. D. S. Kim, J. M. Pezzuto, and E. Pisha, *Ibid*., **8**, 1707-1712 (1998).
11. A. M. Nadureira, J. R. Ascenso, L. Valdeira, *et al*., *Nat. Prod. Res*., **17**, 375-380 (2003).