Pharmacokinetics and Antitumor Effects of the Drug Containing TNF-α in Nanoparticles

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 3, pp. 296-299, March, 2010
Original article submitted September 3, 2009

Antitumor activity of TNF-α incorporated in nanoparticles (VLP-TNF-α) and dynamics of its accumulation and elimination from the blood and tumor tissue were studied in ICR mice. The VLP-TNF-α preparation exhibited higher antitumor activity compared to free TNF-α, presumably due to longer circulation of the cytokine in the blood and its more intensive accumulation by tumor tissue.

Key Words: *tumor necrosis factor-α; double-strand RNA; nanoparticle; pharmacokinetics; Ehrlich carcinoma*

Prospects for the development of nanobiotechnologies, technologies for targeted creation and use of substances and materials with a structure of up to 100 nm in size, now attract much attention all over the world. In nanomedicine, the studies are focused on the creation of nanomolecular vehhicles for drug delivery to target organs for improving treatment efficiency, because incorporation of a drug into a nanoparticle results in its lesser degradation, increase of its bioavailability, and minimization of side effects.

Tumor necrosis factor-α (TNF-α) is an antitumor agent suppressing the growth of malignant tumors and causing their hemorrhagic necrosis [11]. However, the use of TNF-α as the antitumor drug is limited by a wide spectrum of side effects [9]. The search for creation of new forms and methods for the use of TNF-α is in progress all over the world. These studies are focused on reduction of its systemic toxicity, increase in TNF-α accumulation by tumor cells, and increase in therapeutic index.

Creation of drugs containing TNF-α in nanoparticles is an attempt at solution of the problem of TNF-α proteolytic resistance and increase of its accumulation in tumor tissue. Published data attest to the possibility of modifying pharmacotoxic characteristics of TNF-α by its incorporation into a nanostructure [7,10].

A technology for obtaining human recombinant TNF-α was developed at Vector Center [2,3]. Bilayer molecular constructions containing in their central part the nucleotide material (double-strand yeast RNA) enveloped in spermidine polyglucin, were created and studied [4,6]. Under physiological conditions, the polynucleotide polysaccharide construction had a spherical virus-like shape, and hence, was called a "virus-like particle" (VLP) with a size of 25-40 nm, which classified it as a nanomaterial.

We evaluated pharmacokinetics, accumulation in tumor tissue, and antitumor activity of TNF-α incorporated in VLP in comparison with free TNF-α.

MATERIALS AND METHODS

Experiments were carried out on male ICR mice (18-24 g) from Breeding Department of Vector Center. The animals were kept under standard vivarium conditions at natural light before and during the experiment.

Ehrlich carcinoma served as the tumor model. In the pharmacokinetic experiments, the tumor cells were intramuscularly transplanted to mice (5×10^5 cell/animal). On day 6 after transplantation, the mice were

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0007-4888/10/1493-0320

divided into 3 groups (30 per group). Group 1 mice received TNF-α in VLP (VLP-TNF-α) in a dose of 10 μg/kg (by TNF-α). Group 2 (reference group) received TNF-α in the same dose. The drugs were injected in a single dose into the caudal vein (0.2 ml/20 g). Intact mice with tumors served as the control (group 3). The animals were sacrificed after 0.02 (1 min), 0.08 (5 min), 0.5, 1, 4, and 24 h by cervical dislocation. Blood and tumor tissue specimens were collected for the analysis. In the control, blood and tumor tissue specimens were collected 24 h after the start of the experiment.

The serum was separated from blood cells by centrifugation (3000 rpm, 10 min, 4°C). Tissue homogenate (25%) was prepared from tumor tissue in saline using a Glass-Col homogenization system (Cole-Parmer). The homogenates were centrifuged at 4000 rpm (15 min, 4°C). The sera and tumor homogenate supernatants were stored at -20°C until analysis. Serum and supernatant concentrations of TNF-α were measured by enzyme immunoassay using alpha-TNF–EIA-BEST kits (Vector-Best).

For evaluation of antitumor activity, Ehrlich carcinoma cells were transplanted intramuscularly to mice in a dose of 10^5 cell/animal. VLP-TNF-α was injected intraperitoneally (10^2-10^4 U/20 g). The dose was selected from the range of effective doses determined in studies of antitumor activity of TNF-α preparation [1]. The reference TNF-α was used in equivalent doses (by TNF-α); controls were injected with saline. Each group consisted of 10 animals. The animals received 3 injections at 24-h interval, the course of injections started on day 7 after tumor cell transplantation.

The effect of the drug on tumor development was evaluated by changes in the tumor node weight after the end of the experiment (day 15 after tumor transplantation). The tumor growth inhibition (TGI) percentage after drug therapy was calculated by the formula:

$$TGI(\%)=(Wc-We)\times 100/Wc,$$

where Wc is the mean tumor weight in the control group and We is the mean tumor weight in the experimental group.

The results were processed by methods of variational statistical using Statgraphics 5.0 software (Statistical Graphics Corp.). The significance of differences was evaluated by Student's *t* test. The differences were considered significant at $p \leq 0.05$. The normality of distribution was verified using Student's test.

RESULTS

Analysis of the dynamics of TNF-α concentration in the blood showed that injection of TNF-α incorporated in nanoparticles prolonged the period of its circulation in the blood (Table 1). One minute after intravenous injection of VLP-TNF-α, its concentrations were 61% higher than after injection of the reference drug and 1 h after injection this parameter 2-fold surpassed the corresponding value in the reference group. The difference between the experimental and reference groups persisted over 4 h after injection.

The specific content of TNF-α in tumor tissue 1 min after its injection was 7050±550 pg/g and persisted at this level over 1 h, after which it decreased to the basal level (Fig. 1). The concentration of TNF-α in tumors of mice injected with VLP-TNF-α virtually

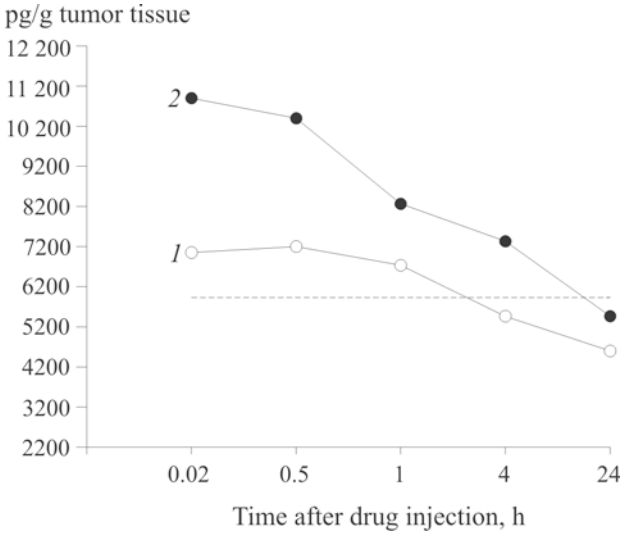

Fig. 1. Dynamics of specific content of TNF-α in Ehrlich transplanted carcinoma after a single intravenous injection of TNF-α and VLP-TNF-α in a dose of 10 μg/kg to mice with tumors. Here and in Fig. 2: dashed line: control; *1*) TNF-α; *2*) VLP-TNF-α.

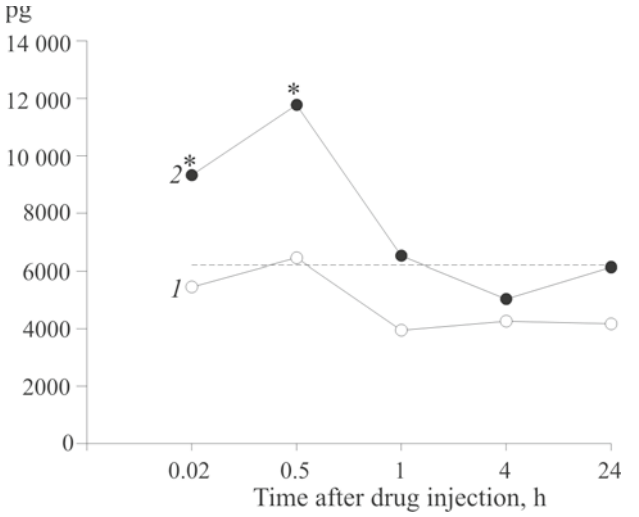

Fig. 2. Dynamics of TNF-α accumulation and elimination from Ehrlich transplanted carcinoma tissue after a single intravenous injection of TNF-α and VLP-TNF-α to mice in a dose of 10 μg/kg. Here and in Fig. 3: *$p<0.05$ compared to the control.

did not differ from the values in the reference group throughout the experiment. However, a trend to more intensive accumulation of TNF-α in the tumor during the first hour after injection of VLP-TNF-α *vs.* TNF-α was noted. The values in mice injected with VLP-TNF-α were 10,900±2892 pg/g 1 min after injection, which was 1.6 times higher than in the reference group.

The data obtained by calculation of total TNF-α content in tumors of experimental animals are presented (Fig. 2). Total level of TNF-α in tumors of animals injected with the reference drug and its specific concentration did not differ from the values in the control. On the other hand, the values in animals injected with VLP-TNF-α were significantly (1.5 times) higher than in the control as soon as just 1 min after the drug injection. A significantly higher content of TNF-α in tumors of experimental mice persisted over 0.5 h after the drug injection.

Hence, the results of pharmacokinetic studies indicate that injection of TNF-α in VLP led to longer circulation of the antitumor drug in the blood and its more intensive accumulation in the tumor in comparison with free TNF-α.

Prolongation of TNF-α circulation in the blood seems to be due to its higher resistance to proteolysis, which was described for some other proteins [8]. More intensive accumulation of the cytokine in tumor tissue during the first hours after injection can be due to specific features of the pathological vascular wall, permeable for large molecules with a molecular weight >40 kDa and for small particles [5].

Comparative study of antitumor activities of the drugs showed that injection of the reference TNF-α preparation did not inhibit tumor growth in any of the studied doses (Fig. 3). A trend to a reduction of the mean weight of the tumor was noted only in the group of mice receiving the drug in the highest dose (36 μg/kg), TGI in this case did not surpass 20%.

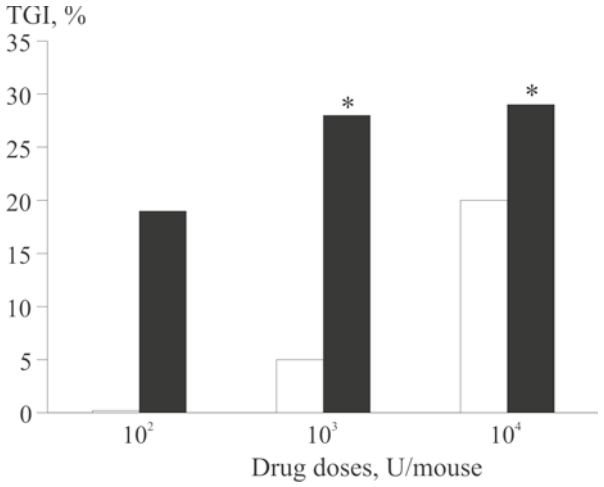

Fig. 3. Effects of VLP-TNF-α and TNF-α in doses of 0.36-36 μg/kg (10^2-10^4 Unit/20 g) on the growth of Ehrlich transplanted carcinoma. Light bars: TNF-α; dark bars: VLP-TNF-α. Control level corresponds to 0%.

In contrast to TNF-α, VLP-TNF-α inhibited tumor growth by 19% even in the minimum dose of 0.36 μg/kg (by TNF-α, Fig. 3). Three injections of VLP-TNF-α in doses of 3.6 and 36 μg/kg caused a statistically significant inhibition of tumor growth. The mean weight of the tumor node in experimental groups was lower than in the control by 28 and 29%, respectively ($p<0.05$).

In other words, tumor growth inhibition in response to VLP-TNF-α was observed after its injections in 10-100-fold lower doses than after injections of free TNF-α.

The high antitumor effect of the nanopreparation is presumably explained by longer presence of TNF-α in the blood and its intensive accumulation in tumor tissue. Moreover, double-strand RNA (dRNA) constituting the core of the construction is characterized by immunomodulating and antitumor effects [1], and

TABLE 1. Dynamics of Serum Concentration of TNF-α after Intravenous Injections of VLP-TNF-α and Reference TNF-α

Time after injection, h	Serum concentration of TNF-α (pg/ml) after injection	
	TNF-α	VLP-TNF-α
0.02 (1 min)	159,000±13,500	256,000±13,900*
0.08 (5 min)	59,400±9500	98,500±21,700*
0.5	7490±1000	11,100±600*
1	1700±290	3370±620*
4	18±6	52±7*
24	0±0	4±3

Note. *$p<0.05$ compared to TNF-α.

therefore mutual potentiation of the TNF-α and dRNA effects cannot be excluded.

Hence, experiments on mice with transplanted Ehrlich carcinoma showed higher antitumor activity of VLP-TNF-α in comparison with TNF-α, its longer circulation in the blood, and more intense accumulation in the tumor tissue early after injection.

The study was supported by the Federal Targeted Program for Science and Technology (State Contract No. 02.513.11.3372, November 26, 2007).

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