ONCOLOGY

Effect of Myelopeptide-2 on the Development of Spontaneous and Urethane-Induced Tumors in Mice

A. A. Mikhailova, E. A. Kirilina, O. V. Morozova, and V. S. Turusov

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We studied the effect of endogenous immunoregulatory myelopeptide-2 on the development of spontaneous and urethane-induced lung adenomas. Long-term treatment with myelopeptide-2 (25 injections) in parallel with urethane poisoning decreased animal mortality caused by this carcinogen. Short-term treatment with myelopeptide-2 decreased the number of spontaneous and urethane-induced lung tumors in mice. Myelopeptide-2 delayed the appearance of urethane-induced tumors irrespective of the number of injections.

Key Words: *myelopeptides; urethane; carcinogen; lung adenoma*

Immunotherapy attracts now sspecial attention as a component of antitumor therapy, because tumor growth and development are usually accompanied by disorders in the immune system and can lead to immunodeficiency. Cytostatics and irradiation also suppress the immune system. Cytokines and recombinant interferons correcting the imbalance between cell proliferation and differentiation are now used in the therapy of leukemia [4]. However, these preparations cause side effects, and therefore the search for new endogenous immunomodulators without side effects is in progress. Myelopeptide-2 (Leu-Val-Val-Tyr-Pro-Trp, MP-2) is an endogenous immunoregulatory peptide initially isolated from the media conditioned by porcine bone marrow cells [5]. This peptide is characterized by antitumor activity: it restores functional activity of T lymphocytes suppressed by products of HL-60 leukemic cells [3,7] and inhibits the growth of transplanted tumors (sarcoma S-180, melanoma B-12, adenocarcinoma, Lewis lung tumor, *etc*.) in mice [2].

M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences; Institute of Carcinogenesis, N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow

Our study demonstrated the inhibitory effect of MP-2 on the development of spontaneous and carcinogen (urethane)-induced lung tumors in BALB/c mice.

MATERIALS AND METHODS

The study was carried out on 250 male BALB/c mice (from Department of Laboratory Animals, Cancer Research Center) characterized by high incidence of spontaneous lung tumors (up to 40%) and high sensitivity to pulmonotropic carcinogen urethane [6,7]. The mice were kept under standard vivarium conditions and were taken into experiment at the age of 3 months.

The mice divided into 5 experimental groups and received MP-2, urethane, or both according to one of the following protocols: 1 injection of MP-2 and after 7 days 3 injections of MP-2 every other day or alternating with injections of urethane (4 injections of MP-2; 3 injections of urethane+4 injections of MP-2); 3 injections of MP-2 every other day or alternating with injections of urethane and after 3 weeks 2 injections of MP-2 monthly with one-week interval, a total of 22 injections (25 injections of MP-

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2; 3 injections of urethane+25 injections of MP-2); 3 injections of urethane every other day.

Control group consisted of intact mice. MP-2 was dissolved in normal saline and injected intraperitoneally in a dose of 10^{-5} g/mouse. Urethane in normal saline was injected subcutaneously in a dose of 0.5 mg/kg.

The protocols of MP-2 injection were selected on the basis of experimental data on its inhibitory effect on transplanted mouse tumors [2]. The experiment was carried out for 50 weeks. Dead or sacrificed (by ether narcosis) mice were autopsied and examined. Organs were fixed in 10% formalin. After fixation, lung tumors were counted and measured, the material was dehydrated in alcohols, embedded in paraffin, and 5-μ sections were stained with hematoxylin and eosin.

The results were statistically processed using Student's *t* test and life table analysis, allowing evaluation of tumor probability with consideration for intercurrent deaths. The multiplicity index (number of tumors per group/number of mice per group) was estimated. Latent period was evaluated as the time between carcinogen injection and appearance of the first tumor.

RESULTS

The highest mortality was observed in mice receiving urethane and urethane+4 injections of MP-2, while in the group receiving 25 injections of MP-2 (with or without urethane) this parameter virtually did not differ from the control. Hence, 25 injections of MP-2 in parallel with urethane decreased animal mortality, while 4 injections of MP-2 in parallel with urethane did not improve the survival (Fig. 1). In mice receiving 4 injections of MP-2, high mortality on week 50 was due to intercurrent infection.

The first lung tumor induced by urethane was detected 23 weeks after the start of the experiment

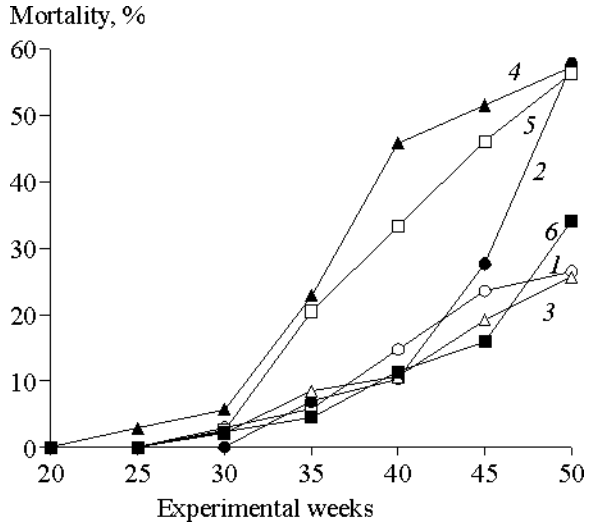

Fig. 1. Mortality of male BALB/c mice treated with urethane and myelopeptide-2 (MP-2). *1*) control; *2*) 4 injections of MP-2; *3*) 25 injections of MP-2; *4*) urethane; *5*) urethane+4 injections of MP-2; *6*) urethane+25 injections of MP-2.

in the group treated with urethane alone (Table 1). Seven weeks later the first tumors were detected in mice receiving urethane+4 injections of MP-2 and urethane+25 injections of MP-2. Hence, MP-2 delayed the appearance of the first induced tumor in comparison with the group treated by urethane alone irrespective of the number of injections.

No positive effect of MP-2 on the latency and number of spontaneous tumors was noted. The first lung tumor in animals receiving 25 injections of MP-2 appeared more early than in the control (30 *vs.* 50 weeks).

Four injections of MP-2 reduced the number of spontaneous pulmonary adenomas in comparison with the control and with the group receiving 25 injections of MP-2. Moreover, the multiplicity index of urethane-induced tumors was lower in mice receiving 4 injections of MP-2 (Table 1). This effect was not observed after 25 injections of MP-2. Hence,

TABLE 1. Incidence and Multiplicity of Lung Tumors in Male BALB/c Mice Treated with Urethane and MP-2 (*M*±*m*)

Parameter	Control	MP-2		Urethane	Urethane+MP-2	
		4 injections	25 injections		4 injections	25 injections
Appearance of the first tumor, weeks	50	50	30	23	31	30
Mean latency, weeks	50±0	50±0	44.8±8.9	42.4±10.9	44.8±8.9	47.7±5.6
Number of animals surviving by the first tumor	34	37	47	35	39	44
Number of mice with tumors (%)	6 (17.6)	2 (5.4)	8 (17.0)	35 (100)*	37 (94.9)*	43 (97.7)*
Multiplicity index	0.24±0.02	0.05±0.04*	0.21±0.07	5.91±0.06**	4.21±0.39**+	5.70±0.44**

Note. *p<0.001, **p<0.05 *vs.* control. Here and in Table 2: +p<0.05 *vs.* urethane and urethane+25 injections of MP-2 groups.

TABLE 2. Size Distribution of Lung Tumors in Male BALB/c Mice Treated with Urethane and MP-2

Tumor size, mm	Control (*n*=8)	MP-2		Urethane (*n*=207)	Urethane+MP-2	
		4 injections (*n*=2)	25 injections (*n*=10)		4 injections (*n*=164)	25 injections (*n*=251)
<1	5 (62.5)	1 (50)	5 (50)	155 (74.9)	117 (71.3)	137 (54.6)
1-3	2 (25)	1 (50)	5 (50)	41 (19.8)	38 (23.2)	87 (34.7)
3-5	1 (12.5)	—	—	6 (2.9)	4 (2.4^{+})	20 (7.5)
5-7	—	—	—	4 (1.9)	4 (2.4)	4 (1.59)
>7	—	—	—	1 (0.5)	1 (0.03)	3 (1.2)

Note. *n*: number of tumors; percentage of the total number of tumors in the group is given in parentheses.

short-term treatment with MP-2 had a positive effect on the multiplicity index of both spontaneous and urethane-induced tumors.

The percentage of small (<3 mm) adenomas was comparable in all groups (Table 2). The incidence of urethane-induced lung adenomas was significantly lower in animals receiving 4 injections of MP-2 compared to the group receiving urethane alone or in combination with 25 injections of MP-2. Solitary large adenomas were found only in mice treated with urethane.

Hence, the effect of MP-2 on the number of urethane-induced tumors manifests only when the tumor reaches a certain size (3-5 mm). This is in line with the data that this immunoregulatory peptide restores functional activity of T lymphocytes suppressed by tumor cell products. Functional activity of T lymphocytes is suppressed when the tumor is sufficiently large.

Hence, multiple injections of MP-2 in parallel with urethane decreased animal mortality by 20-30%. Short-term treatment with MP-2 decreased the number of both spontaneous and urethane-induced pulmonary adenomas in mice. MP-2 prolonged the latentcy of the first urethane-induced tumors irrespective of the duration of treatment.

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