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Effects of 5-Pyrimidinol Derivative SNK-41 on Cytokine Profile of Mice with Lewis Lung Carcinoma

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We studied the effects of SNK-411, a new 5-pyrimidinol derivative, on serum cytokine profile of C57Bl/6 mice with Lewis lung carcinoma. The compound was injected intraperitoneally in doses of 25 and 50 mg/kg. A significant (by 3.5 times) increase in serum IL-4 content was detected in mice with tumors on day 9 of tumor development. In mice receiving SNK-411 in doses of 25 and 50 mg/kg, IL-4 content significantly decreased (by 4.0 and 3.6 times) on days 2-8 of carcinoma development; IL-2 content decreased by 1.4 and 1.2 times and IL-6 content decreased by 2.7 and 1.6 times, respectively, in comparison with control mice with tumors. Injections of SNK-411 in the same doses on days 8-15 of carcinoma development led to a significant decrease in IL-4 levels (by 2.2 and 4.5 times, respectively, in comparison with control mice with tumors) and did not affect serum levels of other cytokines.

Key Words: *SNK-411; cytokines; interleukin-4; Lewis lung carcinoma; cancer immunotherapy*

Analysis of immunogenic mechanisms of carcinogenesis revealed the key role of cytokines in the regulation of tumor growth and metastasizing and in the formation of antitumor immune response [7]. Cytokines activate cytotoxic T cells, NK cells, and macrophages and serve as negative factors of tumor cell growth [1,2,7]. Sometimes, intratumor clonal heterogeneity and high rate of tumor clone selection determine tumor insensitivity to the overwhelming majority of cytokines and its capacity to produce and use the immune system factors for growth stimulation. This leads to imbalance in the production of various IL types: Th-1 (IL-2, IL-12, IL-15, and IFN-g) and Th-2 (IL-1, IL-4, IL-5, IL-6, IL-10, and IL-13). Regulation of cytokine content in the microenvironment is now regarded as the target for drug correction of malignant growth [1,3,8].

Hence, the search and creation of drugs correcting the cytokine profile under conditions of tumor development is an important problem in the development of approaches to immunotherapy of cancer.

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We evaluate the effects of SNK-411, a 5-pyrimidinol derivative, previously exhibiting antitumor activity *in vitro* and *in vivo* [4], on cytokine levels on the model of transplanted Lewis lung carcinoma (LLC) in C57Bl/6 mice.

MATERIALS AND METHODS

The study was carried out on 80 male C57Bl/6 mice (18-20 g) from Stolbovaya Breeding Center. The animals were kept in vivarium of V. V. Zakusov Research Institute at 12:12 h day:night schedule with free access to water and standard balanced fodder at natural light and 20-21°C. All animal experiments were carried out in accordance with international regulations (Directive 86/609/EEC) and regulations for studies on animals approved by the Ethic Committee of V. V. Zakusov Research Institute of Pharmacology.

LLC cells from Cell Culture Bank (Research Institute of Experimental Diagnosis and Therapy of Tumors, N. N. Blokhin Russian Cancer Research Center) were subcutaneously implanted in the armpit in a dose of 30-60 mg cell suspension in 0.3-0.5 ml Hanks fluid per animal. The day of LLC cell trans-

plantation was taken for day 0 of tumor development [5]. Animals with implanted LLC were randomized into 2 groups and received SNK-411 intraperitoneally in doses of 25 and 50 mg/kg daily on days 2 through 8 (series I) or 8 thorough 15 (series II) of tumor development. The choice of doses, protocols, and routes of SNK-411 administration was based on the data of previous studies of its pharmacological activity carried out at V. V. Zakusov Research Institute of Pharmacology [4].

Measurements of serum concentrations of 10 cytokines (TNF-α, IL-6, IL-10, IL-1α, IL-2, IL-4, IL-5, IL-17, IFN-g, and GM-CSF) in mice were carried out on an EPICS XL 4 colors laser flow cytometer (Beckman Coulter) by multiplex evaluation of fluorescent particles (FlowCytomix 10-plex, eBioscience) according to manufacturer's instruction. The blood and sera were collected after decapitation of mice on days 9 and 16 of LLC development, the specimens were stored at -30°C until cytometry analysis.

The results were processed by FlowCytomixPro 3.2 software and expressed in pg/ml serum as the arithmetic mean and error of the mean. Normality of data distribution was verified by the Shapiro–Wilk test, the data were statistically processed by the Student's *t* test for independent samples. The differences were considered significant at $p<0.05$.

RESULTS

On day 9 of tumor growth, the content of IL-4 in mouse serum increased by 3.5 times in comparison with the control ($p<0.05$).

These results are in line with published and clinical data on the immunopathological role of IL-4 in carcinogenesis of solid tumors [13,14]. For instance, high levels of IL-4 were found in tumor microenvironment and these parameters positively correlated with the intensity of malignant growth [8,12]. According to published data, IL-4 improves survival and proliferation of tumor cells and stimulate the development of their resistance to endogenous mechanisms of CD95-dependent apoptosis and activation of the mitogen-activated proteinkinase (Erk, p38, JNK) cascade [6,11-15].

Animals with implanted LLC treated with SNK-411 in doses of 25 and 50 mg/kg exhibited significantly lower levels of IL-4 (by 4.0 and 3.6 times), IL-2 (by 1.4 and 1.2 times), and IL-6 (by 2.7 and 1.6 times lower, respectively) (Table 1).

It has been previously shown that injections of SNK-411 in doses of 25 and 50 mg/kg to LLC-bearing mice on days 2-8 of tumor development led to significant inhibition of tumor growth (by 1.8 and 2.2 times, respectively) [4].

Hence, the results indicate that previously detected [4] tumor growth inhibition in C57Bl/6 mice in response to SNK-411 treatment was in good agreement with a significant decrease of IL-4, IL-2, and IL-6 levels.

In mice receiving injections of SNK-411 on days 8-15 of tumor development, we observed a marked decrease in serum levels of IL-2 (by 2.7 times) and IL-6 (by 3 times; $p<0.05$) on day 16 in comparison with control (Table 2). It is known that cytokines, including IL-6 and IL-2, act as mediators connecting chronic inflammation and cancer; for this reason, they serve as a potential pharmacological target and as prognostic factors [9]. Our results are in line with the data on a significant reduction of IL production at the late stages of tumor process, which correlates with exhaustion of antitumor immune response [7].

A significant decrease of IL-4 level (2.2 and 4.5 times) was also detected on day 16 of tumor development in LLC-bearing animals treated with SNK-411 in doses of 25 and 50 mg/kg, respectively. The fact of SNK-411 regulation of the level of IL-4 (tumor growth factor) was in line with previous results indicating prolongation of the life span of mice with tumors in response to treatment with this compounds

TABLE 1. Effects of SNK-411 Administered on Days 2-8 of LLC Development on Serum Cytokine Levels in C57Bl/6 Mice (*M*±*m*)

Group	Cytokines, pg/ml		
	IL-2	IL-4	IL-6
Control	116.4±19.4	175.0±65.9	220.2±46.3
LLC+1% starch solution	102.9±12.8	614.8±95.9*	155.0±48.9
LLC+SNK-411, 25 mg/kg	73.7±16.9*+	153.9±13.8+	56.9±20.3*+
LLC+SNK-411, 50 mg/kg	86.7±16.2*+	171.1±60.6+	98.8±26.7*+

Note. Here and in Table 2: $p<0.05$ in comparison with *control, +group LLC+1% starch solution.

TABLE 2. Effects of SNK-411 Administered on Days 8-15 of LLC Development on Serum Cytokine Levels in C57Bl/6 Mice (*M*±*m*)

Group	Cytokines, pg/ml		
	IL-2	IL-4	IL-6
Control	116.4±19.4	175.0±65.9	220.2±46.3
LLC+1% starch solution	43.9±19.1*	158.5±42.5	73.2±29.2*
LLC+SNK-411, 25 mg/kg	43.1±12.6*	72.6±19.6*+	85.3±26.6*
LLC+SNK-411, 50 mg/kg	77.1±17.6*	35.2±10.5*+	181.4±33.8

during various periods of tumor growth. Previous studies demonstrated life span prolongation in mice with tumors (1.8 and 1.2 times) after treatment with SNK-411 in doses of 25 and 50 mg/kg on days 8-15 of LLC development [4].

Treatment with SNK-411 in doses of 25 and 50 mg/kg virtually did not change the levels of IL-2 and IL-6 and serum concentrations of other cytokines measured in our study (Table 2).

Analysis of the results indicated a pronounced immunopharmacological activity of SNK-411 in doses of 25 and 50 mg/kg on the model of transplanted LLC in C57Bl/6 mice.

The capacity of SNK-411 in doses of 25 and 50 mg/kg to regulate the levels of IL-2, IL-4, and IL-6 is demonstrated for the first time. It proves the key role of cytokines in the immunopathological process of tumor development in the studied LLC model in mice [7,9,10]. Shift of the cytokine profile in the lung carcinoma model in C57Bl/6 mice confirms the known shift of the cytokine balance towards Th-2 and the promoter role of IL-4 in malignant growth [10,11]. According to clinical data, IL-4 is regarded as a new pharmacological target for immunotherapy for cancer [11]. Our study showed that SNK-411 administered by different treatment protocols reduced serum level of IL-4 in LLC-bearing mice.

Previous studies have demonstrated a direct cytotoxic effect of SNK-411 *in vitro* towards K-562 erythromyeloid cells in the MTT test and a pronounced stimulatory effect towards the percentage of cytotoxic T-lymphocytes and NK cells [4]. Our results disclose one of the probable mechanisms of antitumor activity of SNK-411, consisting in inhibition of IL-4 production and correlating with LLC growth suppression and life span prolongation in mice.

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