In Vivo Antimetastatic and Immunomodulating Activity of Phytomix-40

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Antimetastatic and immunomodulating activity of Phytomix-40, a new plant preparation containing various adaptogens has been evaluated on the model of Lewis lung carcinoma. Intragastric administration of the drug decreased the intensity of metastatic process by 57% in comparison with the control and improved survival by 43%. This correlated with immunomodulating effect of the drug, which manifested by increased functional activity of T lymphocytes.

Key Words: *plant adaptogens; Lewis lung carcinoma; antimetastatic activity; T lymphocytes; immunomodulating activity*

Phytomix-40 (PM-40), a plant preparation, has been developed as a preventive agent for cancer patients. It consists of 40 ingredients: adaptogens (ginseng root and rhisome, *Rodiola rosea*, thorny eleutherococcus, *etc.*), plants with antiinflammatory, cardiosedative, diuretic, and cholagogue properties, and raw polyvitamins. Experiments on mice and rats of both sexes demonstrated low toxicity of PM-40: LD_{50}>15 ml/kg for mice and >20 ml/kg for rats.

We investigated antimetastatic and immunomodulating effects of a new phytomixture on mice with transplanted Lewis lung carcinoma (LLC).

MATERIALS AND METHODS

Experiments were carried out on male (CBA×C57Bl/6) F_1 mice weighing 22-24 g. Control group 1 consisted of intact mice (n=10). LLC was transplanted subcutaneously by a routine method [7]. Control group 2 consisted of animals with tumors receiving no treatment (n=10). Control group 3 (n=10) consisted of animals with transplanted LLC, to which ethanol solution was administered according to the same protocol as to experimental animals (ethanol control). To experimental mice (group 4, n=14) PM-40 was administered into the stomach in a dose of 2.5 ml/kg once a day for 18 days starting from day 8 after tumor transplantation. The animals were sacrificed on day 26 after tumor transplantation. Organs (lungs, heart, liver, kidneys, testes, stomach, small and large intestine) were examined histologically on paraffin sections stained with hematoxylin and eosin and with picrofuscin according to Van-Gieson. The intensity of metastatic process was evaluated by the number of animals with lung metastases. Proliferative activity of splenic T lymphocytes (spontaneous and induced by a polyclonal activator concanavalin A, ConA, 1 μg/ml) was evaluated in the lymphocyte blastogenesis test in 5-7 mice per group. The results were expressed in stimulation index (I_S) [3].

RESULTS

Macroscopic revision of internal organs in mice treated with PM-40 showed no metastases. They were detected in lung tissue during histological studies in 6 of 14 animals (group 4) (Table 1). In groups 2 and 3 mice with LLC, lung metastases were seen macroscopically. Hence, the metastatic process was notably at-

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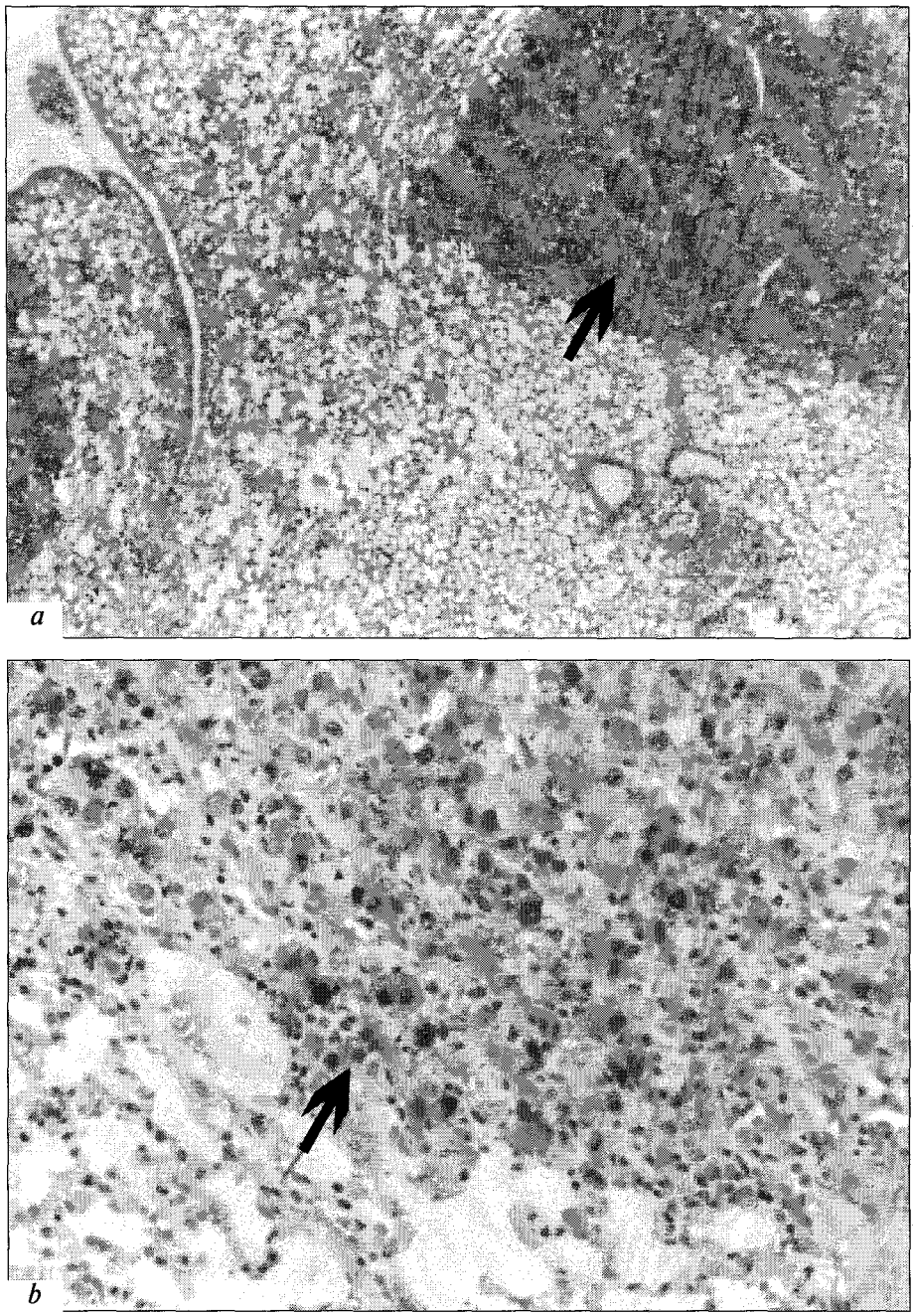

Fig. 1. Lung metastases of transplanted Lewis carcinoma in control mice. Here and in Fig. 2: arrows show the same site on the preparaton at different magnifications: *a*) ×40, *b*) ×400; hematoxylin and eosin staining.

tenuated (43%) in group 4 mice treated with PM-40, in comparison with groups 2 and 3 (100%). The efficiency of metastases inhibition was 57%. On day 26 after tumor transplantation, 93% animals in group 4 survived, *vs.* 50% controls with LLC. On the other hand, the drug did not suppress growth of primary tumor. Therefore, the complex adaptogen agent exerted antimetastatic activity.

Pathomorphological studies revealed actively proliferating zone in the tumor in group 4 mice treated with PM-40 similar to that in groups 2 and 3, however the central necrotic zone was more extensive. Micrometastases were detected in the lungs in 6 of 14 animals (group 4). These metastases were few and smaller than in the control (Fig. 1, *2*). No metastases were found in other organs, both in experimental and control animals. Changes in the liver were adaptive and presented by focal (mainly centrolobular) granular degeneration of hepatocytes and appearance of hepatocytes with nuclei of different size and giant cells. In the kidneys there were signs of granular and microfocal hydroptic (vacuolar) degeneration of nephrocytes

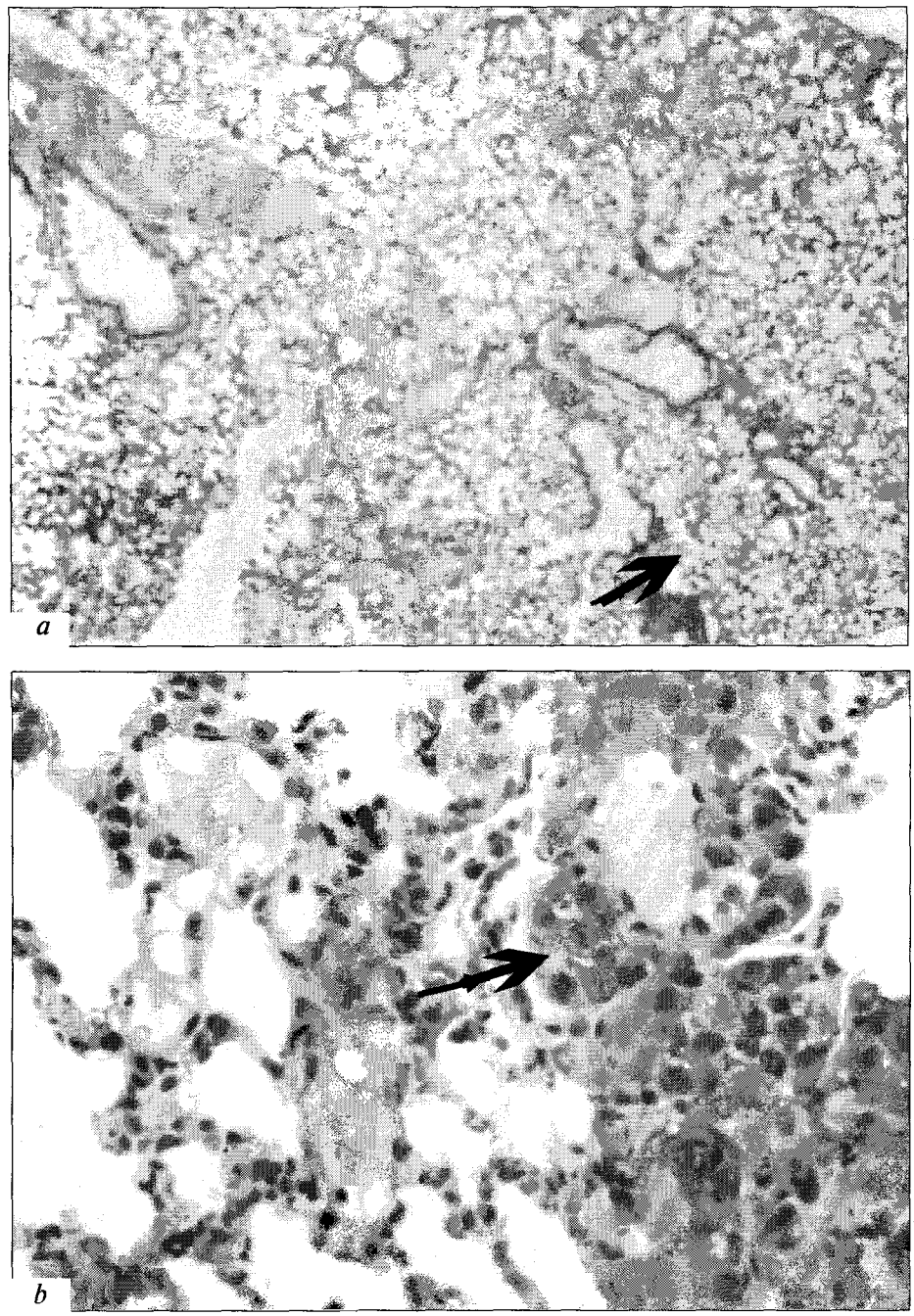

Fig. 2. Micrometastases of transplanted Lewis carcinoma in the lungs of experimental mice.

in convoluted canaliculi. Focal epithelial desquamation was observed in the gastrointestinal mucosa, as well as vacuolar degeneration in individual and grouped epithelial cells. Vacuolar degeneration was also found in individual cardiomyocytes.

These data agree with previous reports on individual adaptogens tested with various metastatic tumors (CCK sarcoma, LLC, B16 melanoma, *etc.*) [1,6,8,13].

Immunomodulating activity of PM-40 on the chosen model of metastatic tumor was analyzed by the index of functional activity of T lymphocytes. Growth and metastasizing of the tumor were associated with a sharp depression of T lymphocyte proliferation (Table 2). The results in groups 2 and 3 were the same, and therefore were united. I_S in the lymphocyte blastogenesis test was 0.05 *vs.* 3.5 in intact animals. Therapy with PM-40 in experimental group 4 increased both spontaneous and ConA-induced proliferative activity of splenic T lymphocytes. I_S reached 0.7, but remained below than in intact animals. Moreover, it should be noted that PM-40 produced an immunomodulating effect under our experimental condi-

tions, when primary tumor was not removed, and therefore the predominant suppression of immunity was not eliminated. Even under these conditions functional activity of T lymphocytes, virtually completely suppressed in animals with tumors (1.4%), increased to 20% under the effect of PM-40 (T lymphocyte activity in intact animals was taken as 100%).

Experimental and clinical studies indicate that the capacity of plant adaptogens to prevent metastases correlates with their immunomodulating activity. *In vitro* and *in vivo* animal experiments demonstrated that phytoadaptogens normalized phagocytic and cytolytic activities of macrophages and polymorphonuclear leukocytes [1,9,10], the content and functions of helper and cytotoxic T lymphocytes [11,12,14,15]. Clinical studies demonstrated normalization of the immune status, primarily T-cell immunity in patients with breast cancer, ovarian cancer, skin melanoma, *etc.* [2,4,5].

Thus, we revealed immunomodulating activity of PM-40 with respect to T-cell immunity, which correlates with its antimetastatic effect and survival of animals with tumors.

These results suggest the possibility of using PM-40 for prevention of tumor metastases and relapses, a process which has much in common with metastatic process. In other words, PM-40 can be recommended for cancer patients during rehabilitation after specific radical treatment. This work can serve as the basis for clinical trials of PM-40.

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TABLE 1. Effect of PM-40 on LLC Growth and Metastases in Male (CBA×C57Bl/6) F_1 Mice

Groups of animals	Day 26 of experiment		
	survival	size of primary tusmor, mm^3	number of animals with lung metastases (%)
1 (n=10)	10	—	—
2 (n=10)	5	15.5±3.6	10 (100)
3 (n=10)	5	15.2±3.5	10 (100)
4 (n=14)	13	13.1±1.6	6 (43)*

Note. *Metastases were detected by histological analysis.

TABLE 2. Effect of PM-40 on Functional Activity of Splenic T Lymphocytes in Mice with LLC

Groups of animals	Number of blasts per sample, %		
	with ConA	without ConA	I_s
1	12.4±0.8	2.8±0.2	3.5±0.3
Mice with LLC			
2-3 (control)	2.2±0.1	2.1±0.1	0.05±0.04
4	3.8±0.2	2.2±0.04	0.7±0.07

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