Effect of a Novel Antineoplastic Drug Olipifat on Antitumor Immunity in Mice

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Olipifat is an antineoplastic drug containing pyrophosphate and a product of special lignin processing. Donor C57Bl/6J mice with syngeneic B16 melanoma received a single 5-day course of olipifat. Effect of olipifat on antitumor resistance was evaluated by local neutralization test [3]. In animals with rapid melanoma growth, splenic cells from intact donors stimulated tumor growth. Olipifat abolished this growth-stimulating effect of splenocytes. In animals with slow melanoma growth, splenocytes had no effect on the growth of melanoma or Lewis lung cancer. In this case, splenocytes from olipifat-treated donors completely arrested the growth of melanoma B16 and decelerated the growth of Lewis lung carcinoma.

Key Words: *antitumor immunity; modulator of antitumor immunity; B16 melanoma; Lewis lung carcinoma*

Olipifat is a dark brown multicomponent fluid containing 50 mg/ml active substance including pyrophosphate and product of special lignin processing. In experiments, olipifat showed a pronounced antineoplastic effect and low toxicity [2]. This effect was not mediated by direct cytotoxic influence on tumor cells. No immunotoxic activity of the drug was revealed. There are no data on the mechanisms of the effect of olipifat. Therefore, it was interesting to examine the effect of olipifat on mouse immune response to inoculated syngeneic tumors.

MATERIALS AND METHODS

Two-3-month-old C57Bl/6J (B6) mice were obtained from the Kryukovo breeding center, Russian Academy of Medical Sciences. The mice were kept under standard vivarium conditions and fed granulated food in accordance with recommendations of the Ethical Committee of the Russian Academy of Medical Sciences.

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Transplanted tumors B16 melanoma and Lewis lung carcinoma [1] stored in liquid nitrogen at -196°C were maintained under syngeneic conditions on B6 mice by subcutaneous passages every 2 weeks. The day of inoculation of tumor cells was considered as day 0. Tumor growth was evaluated by the weight of tumors isolated from sacrificed mice or *in vivo* by tumor size and animal survival. For *in vivo* evaluation, tumor weight was calculated by the formula:

$$CTW=0.5(L\times W\times H),$$

where CTW is calculated tumor weight in mg; L, W, and H are three maximum perpendicular diameters in mm.

Olipifat (lot 79, manufactured 01.10.99) was injected intramuscularly (right thigh) in a dose of 0.04 ml (≈100 mg/kg) once a day for 5 days.

For local neutralization test [3], donor spleen and tumor cells were mixed at a ratio 50:1/100:1 immediately before inoculation. The cell suspension containing 10^6 tumor cells was inoculated subcutaneously in a volume of 0.2 ml. The mice received this mixture or only tumor cells.

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The results were processed statistically. Significance of differences was evaluated by Student *t* test. Differences in animal survival were evaluated using Mann—Whitney *U* test. Differences were considered significant at $p<0.05$.

RESULTS

The effect of olipifat on mouse resistance against B16 melanoma transplanted to syngeneic mice was evaluated in 2 different experimental series.

In series I, female B6 mice received subcutaneous right-side injections of 0.2 ml 20% suspension (weight/volume) of B16 melanoma cells on day 0. Group I mice (n=10) were not treated, while group II mice received 0.04 ml olipifat (intramuscular injections into the right thigh) from day 3 to day 7 of the experiment. In this experimental series rapid tumor growth was observed in all mice. By the moment of donor killing (day 11) the only appreciable effect of olipifat was a significant increase in the weight of the contralateral (left) inguinal lymph node (Fig. 1). No inhibition of tumor growth was observed by day 11 (the mean tumor weights in control and olipifat-treated mice were 268± 92 and 242±64 mg, respectively), which agrees with the data on antineoplastic activity of olipifat [2].

In series II, female B6 mice received subcutaneously (into the right side) 10^6 B16 melanoma cells on day 0. Group I mice (n=8) received only tumor cells (control of tumor growth), group II and III mice (n=6 each) received tumor cells mixed with splenocytes from untreated and olipifat-treated donors, respectively. The ratio between the spleen and tumor cells was 50:1. The mice were sacrificed on day 13. Tumor

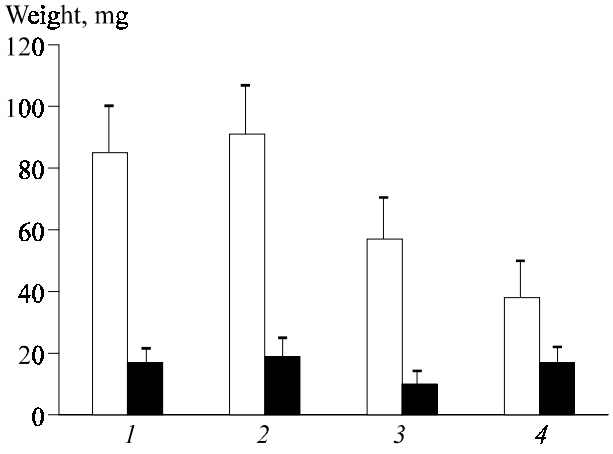

Fig. 1. Effect of olipifat on lymphatic organs in mice with B16 melanoma. Open bars: without treatment, dark bars: olipifat. *1*) spleen, *2*) thymus, *3*) regional lymph node, *4*) contralateral lymph node.

weight in mice receiving splenocytes from olipifat-treated donors was significantly lower than in mice receiving splenocytes from untreated donors (413± 167 and 897±180 mg, respectively compared to 419± 73 mg in group I, $p<0.05$).

The data of local neutralization test point to a positive effect of olipifat on the formation of antitumor resistance in tumor-bearing mice. Cells accelerating tumor growth were found in the spleen of mice with intensely growing tumors on day 11 after inoculation, while in olipifat-treated mice these cells were absent. The numbers of macrophages/monocytes and small lymphocytes in splenocyte suspension obtained from olipifat-treated donors were 1.5- and 2.3-fold higher, respectively, than in suspensions from untreated donors. Further studies are required for iden-

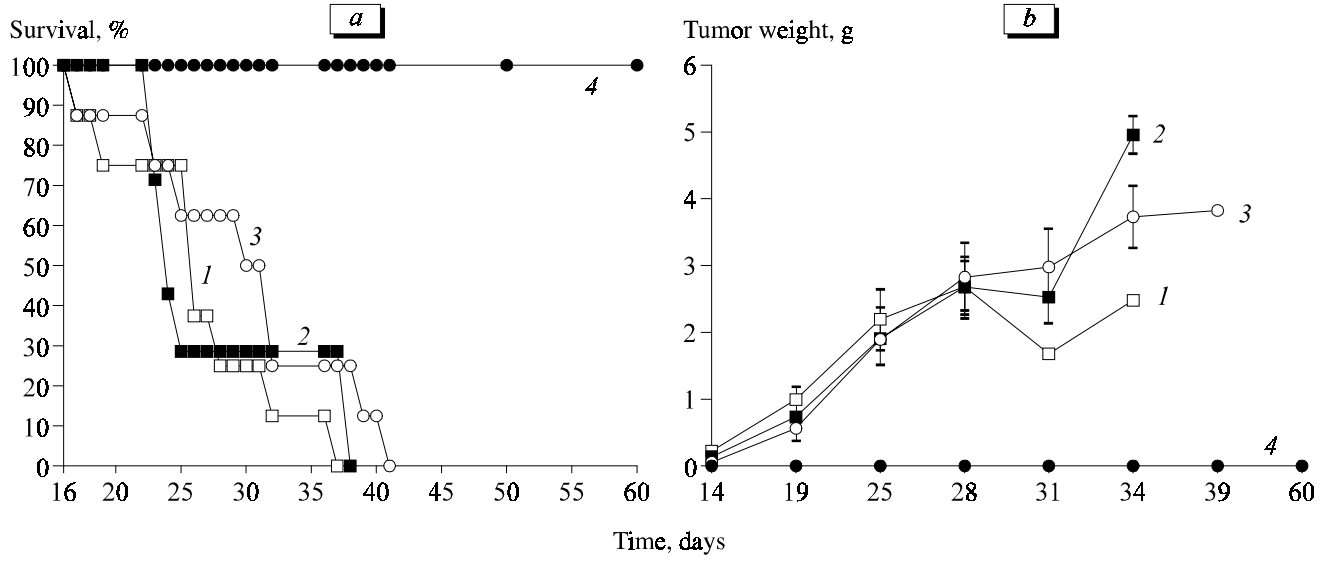

Fig. 2. Effect of donor splenocytes on survival of B16 melanoma-bearing mice (*a*) and on growth of B16 melanoma (*b*). Here and in Fig. 3: *1*) control of tumor growth, *2*) splenocytes from intact mice+tumor cells, *3*) splenocytes from mice with B16 melanoma+tumor cells, *4*) splenocytes from olipifat-treated mice+tumor cells.

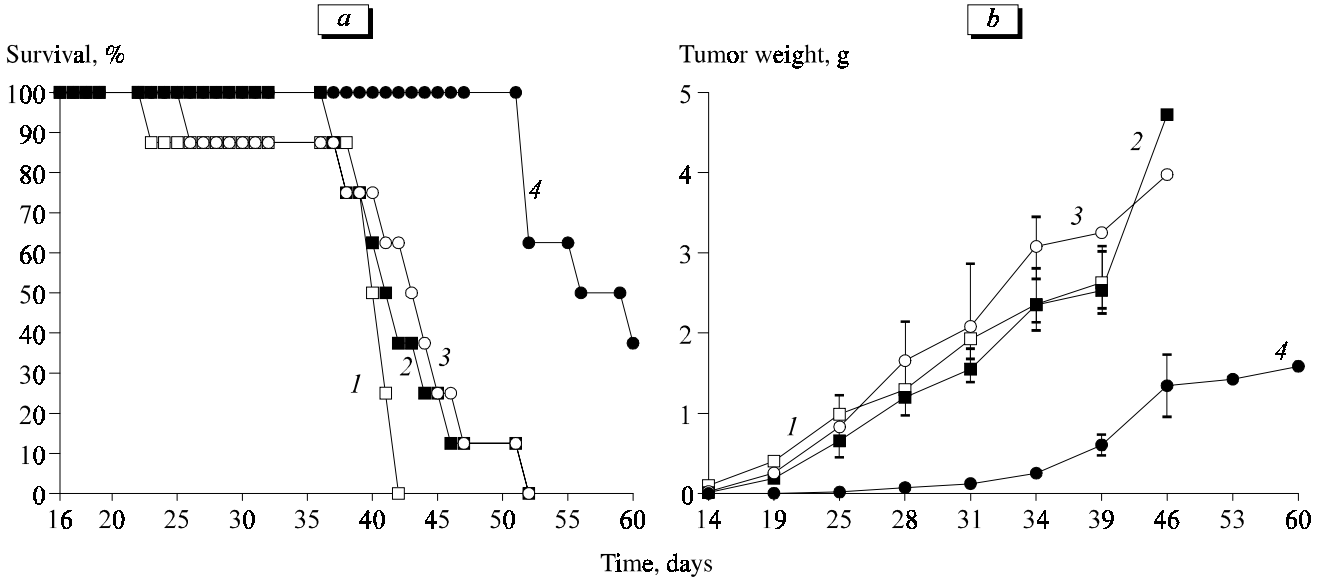

Fig. 3. Effect of donor splenocytes on survival rate in mice inoculated with Lewis lung carcinoma (*a*) and on the growth of Lewis lung carcinoma (*b*).

tification of target cells in the spleen of mice with intensively growing B16 melanoma.

In series III, B6 mice were also used as splenocyte donors, but tumor growth was significantly lower than in experimental series I. The donors were sacrificed on day 22, but tumors developed only in 2 of 12 untreated and 3 of 12 olipifat-treated mice, respectively. These mice received the same olipifat course as in experimental series I, but on days 6-10 after inoculation. In these experiments, an additional intact control group (untreated mice without tumor transplants) was used. Specificity of donor splenocyte reactivity was also tested. To this end, 50% recipients received B16 melanoma and 50% Lewis lung carcinoma syngeneic for B6 mice. The results of local neutralization test are shown on Fig. 2 and 3. Splenocytes from intact or untreated donors had no effect on the growth of B16 melanoma, while splenocytes from mice receiving a single course of olipifat completely inhibited tumor growth: all mice survived and none of them developed tumors during the observation period (60 days). Splenocytes obtained from olipifat-treated donors with B16 melanoma produced similar, but weaker antineoplastic effect on Lewis lung carcinoma. In this case, tumor development started only 3 weeks after adoptive transfer and by the end of observation (60 days) 50% animals (4 of 8) survived, but had large tumors.

Thus, olipifat corrected antitumor immune resistance in mice inoculated with syngeneic tumors. Immunocorrection is probably associated with inhibition of cells suppressing tumor rejection and stimulation of proliferation of effector cells inhibiting the growth of inoculated syngeneic tumor cells. The maximum effect was observed in the same tumor, which was inoculated to olipifat-treated donors, but it was also pronounced in other syngeneic tumors. It should be emphasized that donor splenocytes induced transformation of inoculated Lewis carcinoma cells into latent forms. In most control mice, the tumors were palpable and can be measured on day 14 of the experiment, while in mice receiving splenocytes from olipifat-treated donors the tumors became palpable only after 19-46 days after inoculation. These mice can serve as a model of "minimum residual disease" and can be used for elucidation of the mechanisms of control over latent tumor cells in cancer patients and for elaborating new methods for stimulation of these mechanisms. Olipifat can be regarded as a potential corrector of antitumor resistance of an organism.

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