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Antitumor effect of avermectins

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Abstract

The effect of a mixture of naturally occurring aversectin C and avermectin B_1 on the growth of ascites and solid experimental tumors of mice was studied. It was shown for the first time that avermectins possess a pronounced antitumor action. When added at nontoxic doses, they significantly suppressed the growth of ascites Ehrlich carcinoma and P388 lympholeukemia and solid Ehrlich and 755 carcinomata. With some administration regimens, avermectins suppressed the tumor growth by 70–80%. Avermectins were most effective when injected intraperitoneally. It was also shown that avermectins enhanced the vincristine-induced suppression of the growth of Ehrlich carcinoma, melanoma B16, and P388 lympholeukemia. Avermectins produced this effect only when injected after vincristine.

Keywords: Avermectin; (Mouse); Tumor growth

1. Introduction

Avermectins, macrolytic lactones produced by the fungus *Streptomyces avermitilis*, have found wide application as pesticides and antiparasitic drugs for humans and animals (Burg et al., 1979; Campbell, 1989; Ostlind and Long, 1979). The most extensively used compounds of this class are avermectin B_1 (abamectin) and its synthetic derivative, ivermectin. The mechanism by which avermectins produce the pesticide and antiparasitic effects in invertebrates and a neurotoxic effect in vertebrates is the release of γ-aminobutyric acid (GABA) and the enhancement of its inhibitory action (Campbell, 1989). We recently showed that naturally occurring avermectins effectively inhibit the multidrug resistance of tumor cells (Korystov et al., 2004). This finding opens up the possibility of using avermectins in antitumor chemotherapy to abolish the multidrug resistance of tumors. Therefore, it would be appropriate to use avermectins in combination with antitumor agents, the substrates of transport proteins, to enhance their effectiveness. Until now, avermectins have not been considered as modifiers in tumor chemotherapy.

We studied the effect of a mixture of naturally occurring avermectins, aversectin C and avermectin B_1, on the growth of ascites and solid experimental animal tumors. We also tested whether mixtures of naturally occurring avermectins (aversectin C, aversect 3, and avermectin B_1) could modify the antitumor effect of vincristine. We showed that avermectins have a pronounced antitumor effect. Furthermore, when injected after vincristine, they enhanced the antitumor effect of vincristine.

2. Materials and methods

Male mice, 8–10 weeks old (animal collection of the Institute of Bioorganic Chemistry, Pushchino), were used. Mice were housed in groups of 20 per cage (35×25×15

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cm). Housing conditions were thermostatically maintained at 22±2 °C with a 12-h light–dark cycle. Animals were given free access to food and water. Mice were killed by cervical dislocation. The local ethics committee for animal use approved these experiments.

Experimental Ehrlich carcinoma and P388 lympholeukemia (tumors growing as both ascites and solid forms) and carcinoma 755 and melanoma B16 (solid tumors) were used. Ehrlich carcinoma was grown in SHK male mice, P388 lympholeukemia was grown in DBA2 mice, and carcinoma 755 and melanoma B16 were grown in C57/BL6 male mice. Ehrlich carcinoma was inoculated at a concentration of 5×10^6 cells/mouse and all other tumors were inoculated at a concentration of 2×10^6 cells/mouse. The effect of drugs on tumor growth was determined from the number of tumor cells in the abdominal cavity and the size of solid tumors. The cells were counted under a microscope, using a hemocytometer. The volume of the tumor was determined with the equation: $V=a\times b^2\times0.5$, where a is the length and b is the width of the tumor in mm. Tumor growth inhibition (TGI) by the action of the preparation was estimated from the tumor volume: $TGI=\{(V_c-V_e)/V_c\}\times100\%$, where V_c and V_e are the average volumes of the tumors in the control and experimental groups for a given time, respectively. For each experiment, 10–30 mice were used. The figures show average tumor growth inhibition values with root-mean-square deviations.

Vincristine (Gedeon, Richter; Hungary) was dissolved in physiological saline supplemented with 0.9% benzyl alcohol. Alcohol solutions of mixtures of natural avermectins (Farmbiomed, Russia) contained approximately 2% of oligomycin. Aversectin C had the following avermectin composition B_1 42%, B_2 22%, A_1 13%, and A_2 23%, and aversect 3 the following composition B_1 13%, B_2 36%, A_1 13 and A_2 38%. Avermectin B_1, which is free of oligomycin, was also used. All avermectins were from Farmbiomed (Russia). The mixtures of avermectins used differ substantially in the B_1/A_2 ratio: in aversectin C, this ratio is 1.8, and in aversect 3, it is 0.34. All avermectins used were nontoxic up to a concentration of 10 mg/kg. In most experiments, the agents were injected intraperitoneally: first vincristine and 5–10 min later avermectins.

3. Results

Fig. 1 shows the effect of aversectin C on the growth of P388 ascites tumor. The first intraperitoneally injection of the agent was given 24 h after inoculation of the tumor. Control mice were injected with an appropriate volume of solvent (ethanol). Seven days after inoculation, the mice were killed, and the number of tumor cells in the abdominal cavity was measured. It can be seen from Fig. 1 that a single injection of aversectin C led to the

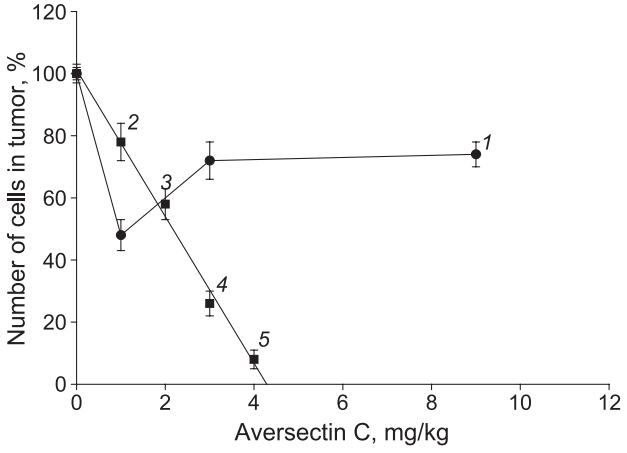

Fig. 1. Effect of aversectin C on the growth of P388 cells in the abdominal cavity of mice. Aversectin C was injected once 24 h after tumor inoculation (1), and once 2 days after inoculation (2), two times (3), three times (4), and four times (5) at intervals of 1 day. The number of cells in control tumors was taken to be 100%.

suppression of tumor growth over a narrow dose range. As the dose was increased above the optimum (about 1 mg/kg), the effect became weaker. Multiple injections of aversectin C were more effective than a single injection. We determined the regimen of drug administration at which the number of tumor cells in the tumor decreased linearly with increasing cumulative doses of aversectin C and the tumor growth was nearly completely suppressed at a cumulative dose of 4 mg/kg (Fig. 1). In the Ehrlich ascites tumor, the preparation was effective only over a narrow dose range regardless of the regimen of drug administration (data not shown).

One of the reasons for the decrease in the effectiveness of aversectin C with increasing dose may have been the suppression of the antitumor activity of leukocytes. The number of leukocytes was to the maximum on day 3 of the growth of the Ehrlich ascites carcinoma: 17±4% of the total number of cells in the peritoneal cavity. Most leucocytes (82±5%) were in contact with tumor cells. When aversectin C (1 mg/kg) was injected on day 1 after tumor inoculation, the number of leucocytes decreased to 11±3%, and the percentage of leucocytes in contact with tumor cells decreased to 36±4%. Thus, aversectin C reduced the migration of leukocytes into the tumor and the number of contacts with tumor cells. In addition, aversectin C at concentrations of 0.05–0.1 mg/kg stimulated (chemiluminescence increased by 20%), and at concentrations higher than 0.3 mg/kg inhibited the formation of reactive oxygen species by blood leukocytes. When extrapolating the last results to the in vivo situation, one should take into account that avermectins are uniformly distributed in the organism due to their transport from tissues to the blood, bile, and intestine.

Fig. 2 shows the growth kinetics of solid Ehrlich carcinoma in the control and after the intraperitoneally injection of different doses of aversectin C 24 h after tumor

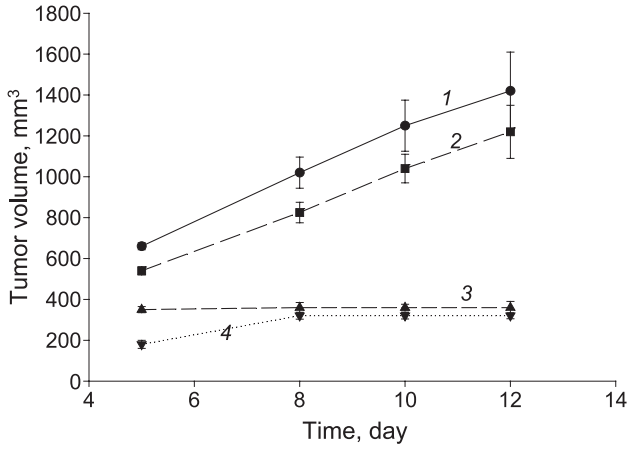

Fig. 2. Growth of Ehrlich carcinoma in the control (1) and after intraperitoneal injection of 1 (2), 3 (3), and 9 μg/g (4) of aversectin C 24 h after tumor inoculation.

inoculation. Aversectin C suppressed tumor growth, and the effect increased with dose up to a concentration of 9 mg/kg. The most effective was the intraperitoneally injection of aversectin C; the administration of aversectin C per os was the least effective (Fig. 3). When injected once 5 days after tumor inoculation, aversectin C was less effective than when injected 24 h after tumor inoculation: the maximum tumor growth inhibition was no higher than 50% (data not shown).

We also tested whether aversectin C has a therapeutic effect against large tumors (1000 mm^3). The first injection was given on day 13 after tumor inoculation. Then aversectin C was injected daily 10 times and after a 5-day interval another five times (Fig. 4). Aversectin C was injected intraperitoneally at a dose of 1 mg/kg. It can be seen from Fig. 4 that 13 days after the beginning of the course of aversectin C injections, the tumor had regressed. On day 23 (after 10 injections), the administration of aversectin C was terminated. The tumor started to growth again after 27 days, but repeated administration of aversectin C (5 injections, one injection per day) did not

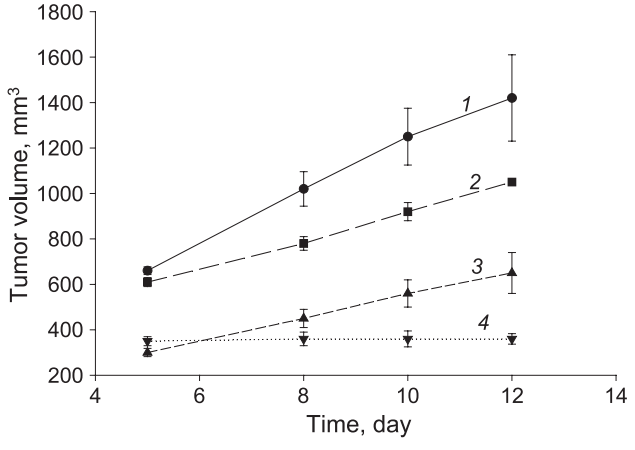

Fig. 3. Growth of Ehrlich carcinoma in the control (1) and after injection of 3 mg/kg of aversectin C 24 h after tumor inoculation. Aversectin was given per os (2), subcutaneously (3) and intraperitoneally (4).

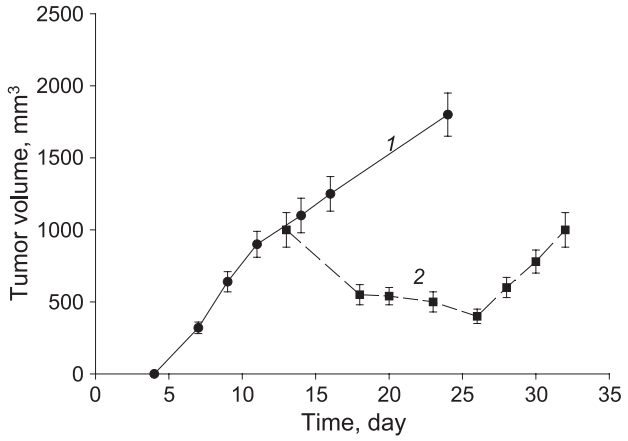

Fig. 4. Growth of Ehrlich carcinoma in the control (1) and after multiple injections of 1 mg/kg of aversectin C. The first injection was given on day 13 after tumor inoculation, and then 10 injections were given, one injection per day, and after a 5-day interval, five more injections were given.

affect tumor growth. The resumption of tumor growth after the regression period can be explained by the selection of cells resistant to aversectin C.

Thus, aversectin C had an antitumor action toward both ascites and solid tumors. This effect may have been due to the presence in the preparation of oligomycin, which is toxic to cells. To test this possibility, we studied the effect of avermectin B_1, which contains no oligomycin. It was found that this preparation also possessed the antitumor activity, comparable to that of aversectin C. Thus, the tumor growth inhibition value after a single injection of 1 mg/kg of avermectin B_1 into mice bearing a solid Ehrlich carcinoma 5 days after tumor inoculation was 50%. A similar result was obtained for aversectin C (see above). Avermectin B_1 at a concentration of 1 mg/kg inhibits also the growth of carcinoma 755, and the tumor growth inhibition value (60%) reached a maximum when avermectin B_1 was injected on day 3 after tumor inoculation. These results indicate that although avermectin B_1 at doses of up to 1 μg/ml exhibits no cytotoxicity in vitro (Korystov et al., 2004), it suppresses the growth of experimental animal tumors in vivo. The mechanism of this phenomenon is unknown. Presumably this is not due to a direct effect of avermectin B_1 on tumor cells but to the involvement of avermectin B_1 in the interactions of tumor cells with the organism.

Taking into consideration that avermectins inhibit the multidrug resistance of tumors (Korystov et al., 2004), we studied the combined effect of avermectins and vincristine on tumor growth. Fig. 5 shows the dependence of the maximum inhibition of growth of Ehrlich carcinoma on the dose of vincristine injected alone or in combination with avermectin B_1 and aversectin C. The agents were injected once on day 5 after tumor inoculation. The maximum tumor growth inhibition value was found to increase linearly with increasing dose of vincristine. Avermectins substantially increased the tumor growth inhibition, the

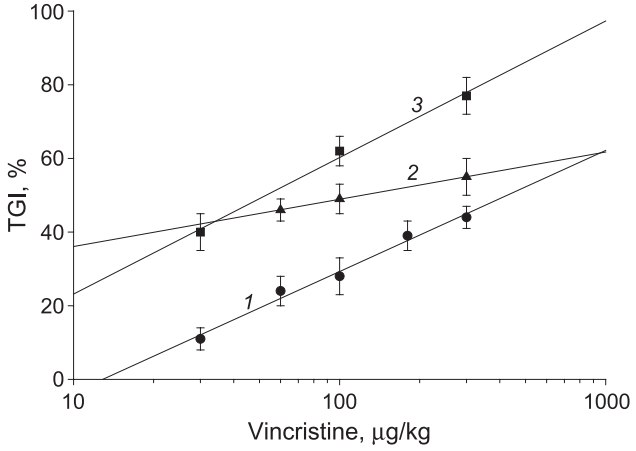

Fig. 5. Dependence of the maximum inhibition of growth of Ehrlich carcinoma on the concentration of vincristine injected alone (1) and in combination with 1 mg/kg of avermectin B_1 and aversectin C (3).

effect of aversectin C being more pronounced than that of avermectin B_1.

Fig. 6 presents the data on the growth of melanoma B16 treated with vincristine alone and vincristine in combination with avermectins. The agents were injected three times, on days 3, 7, and 12 after tumor inoculation. Vincristine C was injected at doses of 0.3 (day 3), 0.6 (day 7), and 0.9 mg/kg (day 12). Avermectins were injected in all cases at a dose of 1 mg/kg. It is evident from Fig. 6 that vincristine alone had little affect on tumor growth, whereas the addition of avermectins substantially slowed tumor growth. Avermectins without vincristine did not affect tumor growth (data not shown).

The combined effect of vincristine and avermectins on P388 lympholeukemia was studied by measuring the tumor growth and the average lifetime of mice carrying tumors. It was found that vincristine injected 24 h after tumor inoculation inhibited tumor growth: the dose at which the number of tumor cells in the abdominal cavity decreased

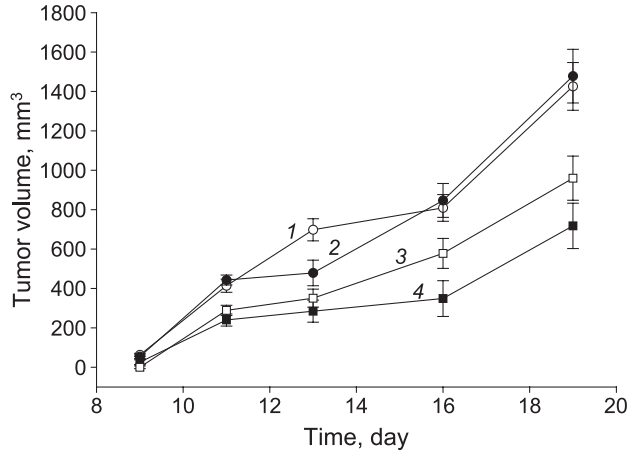

Fig. 6. Growth of melanoma B16 in the control (1) and after the injection of (2) vincristine (0.3+0.6+0.9 mg/kg), (3) vincristine in combination with aversectin C (3×1 mg/kg), and (4) aversect 3 (3×1 mg/kg). Aversectins without vincristine did not affect tumor growth (data not shown).

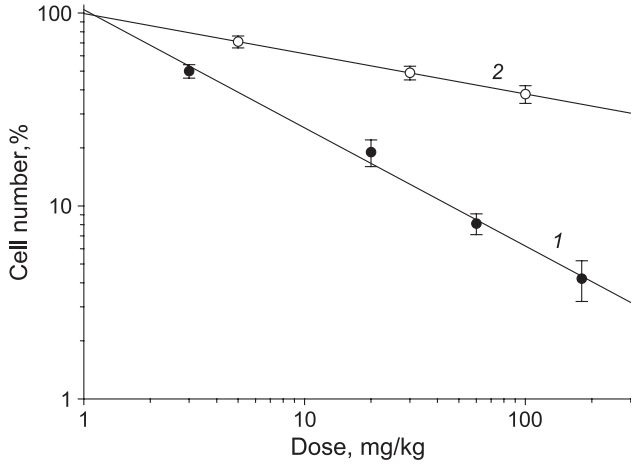

Fig. 7. The effect of vincristine (1) and (2) vincristine (60 μg/kg) in combination with avermectin B_1 on P388 lympholeukemia growth. 100% is the cell number in the control for line 1, and for line 2 100% is the cell number at the vincristine dose of 60 μg/kg.

twofold was about 3 μg/kg (Fig. 7). Avermectin B_1 increased the activity of vincristine at doses that did not affect tumor growth by themselves. Thus, the dose of avermectin B_1 that enhanced the effect of vincristine twofold was about 30 μg/kg (Fig. 7).

We also tested the combined effect of vincristine and avermectins on vincristine-resistant P388VR lympholeukemia. The average lifetime of control mice with P388VR tumors was 11±0.5 days. A single injection of 1 mg/kg of vincristine increased average lifetime to 12.9±0.3 days; after the addition of 1 mg/kg of avermectin B_1 or aversectin C, the average lifetime did not change (13±0.2 days). At a dose of vincristine of 1.5 mg/kg, the average lifetime was 13±0.4 days; however, if vincristine was injected together with aversectin C at a dose of 1 mg/kg, the average lifetime

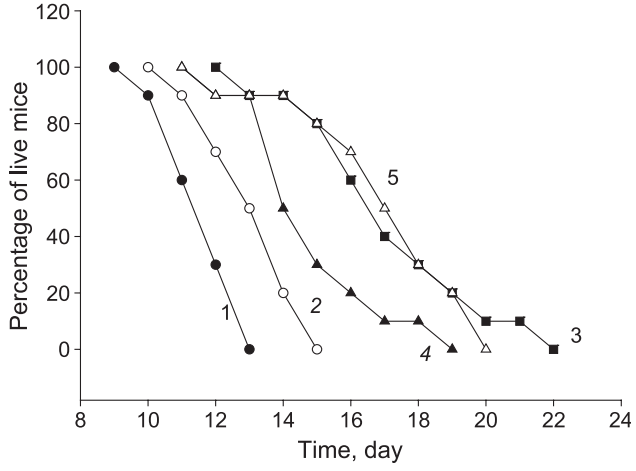

Fig. 8. The death of DBA2 mice with P388VR lympholeukemia in the control (1-●) and after injection of (2-○) vincristine (1.5 mg/kg 1 day after tumor inoculation), (3-■) vincristine in combination with aversectin C (1.5+1 mg/kg 1 day after tumor inoculation), (4-▲) vincristine (10×0.2 mg/kg every day and (5-△) vincristine in combination with aversectin C (10×0.2+10×0.3 mg/kg every day).

increased to 16.5±0.3 days (Fig. 8). We also did experiments with repeated daily injection of small doses of vincristine (0.2 mg/kg) in combination with aversect 3 (0.3 mg/kg) (Fig. 8). The course of injections was begun 24 h after tumor inoculation and continued for 10 days. When vincristine was injected alone, the average lifetime was 14.7±0.2 days (33% higher than in the control), and when it was injected together with aversect 3, the average lifetime was 16.7±0.3 days (52% higher than in the control).

Thus, avermectins enhance the antitumor effect of vincristine in a variety of tumors. However, this effect was evident only when avermectins were injected after vincristine. We studied the antitumor effect on solid Ehrlich carcinoma of vincristine (1 mg/kg) in combination with avermectin B_1 (1 mg/kg) and whether this effect was dependent on the order of their injection and the interval between the administration of the preparations. The agents were injected intraperitoneally once 5 days after tumor inoculation. When avermectin B_1 was injected after vincristine, tumor growth slowed down, but the kinetics of tumor growth inhibition being dependent on the interval between the agent injections. Thus, if B_1 was injected not later than 5 min after vincristine injection, the tumor growth inhibition value reached a maximum (30–40%) as early as day 7 after tumor inoculation and remained unchanged over a period of 17 days. After this, it began to decrease. If B_1 was injected 15–30 min after vincristine injection, the tumor growth inhibition value increased to 30–40% for 22–25 days. If B_1 was injected before the addition of vincristine, tumor growth increased compared with the control: by day 7 after tumor inoculation, the tumor growth inhibition value was initially very low (−50%) and then gradually increased. These results indicate that avermectin B_1 increases the antitumor effect of vincristine only when it is injected after vincristine.

4. Discussion

Here, we showed for the first time that avermectins have a pronounced antitumor effect. When used at nontoxic doses, they substantially suppress the growth of Ehrlich ascites carcinoma and P388 lympholeukemia as well as the growth of solid tumors such as Ehrlich carcinoma and carcinoma 755. With some injection regimens avermectins suppress tumor growth by 70–80%. Avermectins were most effective when injected intraperitoneally. We also showed that avermectins enhanced the antitumor effect of vincristine on Ehrlich carcinoma, melanoma B16, and P388 lympholeukemia. Avermectins produced this effect only if they were injected after vincristine.

The mechanism of the growth suppression of experimental animal tumors is unknown. Presumably this is not due to a direct effect of avermectins on tumor cells but to the involvement of avermectins in the interactions of tumor cells with the organism.

References

Burg, R.W., Miller, B.M., Baker, E.E., Birnbaum, J., Currie, S.A., Hartman, R., Kong, Y.L., Monaghan, R.L., Olson, G., Putter, I., Tunac, J.B., Wallick, H., Stapley, E.O., Oiwa, R., Omura, S., 1979. Avermectins, new family of potent anthelmintic agents: producing organism and fermentation. Antimicrob. Agents Chemother. 15, 361–367.

Campbell, W.C. (Ed.), Ivermectin and Abamectin. Springer-Verlag, New York. 363 pp.

Korystov, Yu.N., Ermakova, N.V., Kublik, L.N., Levitman, M.Kh., Shaposhnikova, V.V., Mosin, V.A., Drinyaev, V.A., Kruglyak, E.B., Sterlina, T.S., 1979. Avermectins inhibit the multidrug resistance of tumor cells. Eur. J. Pharmacol. 493, 57–64.

Ostlind, S.C., Long, R., 1979. Insecticidal activity of the antiparasitic avermectins. Vet. Rec. 105, 168–173.