Ultralow Doses of Various Drugs in Chemotherapy of Experimental Tumors

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Antitumor activity of ultralow doses of cytostatic lonidamine and effects of various biological preparations in ultralow doses (chemosensitizer, immunomodulator, and antioxidant) on the efficiency of adriamycin and cisplatin were studied in animals with transplanted tumors. High efficiency of this method was demonstrated. Nitrotriazole chemosensitizer in ultralow doses increased the sensitivity of leukemia P388/rn substrain with multiple drug resistance phenotype to mitomycin C during combination therapy.

Key Words: *experimental tumors; chemotherapy; cytostatics; ultralow doses*

Modern concept of antitumor chemotherapy is based on the use of preparations in doses close to the maximum tolerated dose (MTD), which, due to low selectivity of cytostatics, cause severe side effects impairing the quality of patient's life. In light of this the search for new methods of treatment with low and ultralow doses of antitumor preparations attracts much attention.

Recent clinical observations indicate that the efficiency of various cytostatics, including cisplatin, in ultralow doses is comparable with their effects is high (close to MTD) doses [14]. Physiologically active compounds in ultralow concentrations modulate the activity of biological systems at various levels [1,2]. There are data on high efficiency of ultralow doses of some antitumor preparations [5,8].

Here we studied the effects of ultralow doses of antitumor cytostatics on the growth of experimental tumors in animals and the effects of combination therapy with cytostatics in subtherapeutic doses and biological modifiers in ultralow doses.

MATERIALS AND METHODS

The following experimental tumors were used in the study: L1210 leukemia, P388 leukemia, P388/rn leukemia (substrain with multiple drug resistance phenotype and genotype) [4], Lewis lung carcinoma, and melanoma B16.

Tumors were transplanted routinely [7]. Experiments were performed on BDF1 mice weighing 20-22 g.

The increase in the mean lifespan (compared to controls), number of survivors, and index of suppression of metastasizing (ISM) were evaluated. ISM was calculated by the formula:

$$ISM=[(A_C \times B_C - A_E \times B_E)/(A_C \times B_C)] \times 100\%,$$

where A_C and A_E are the incidence of metastasizing and B_C and B_E are the mean number of metastases in control and experimental mice, respectively.

Each group consisted of 6-10 mice. The experiments were performed in 2 repetitions. We used cisplatin (Ebewe), mitomycin C (Kyova), adriamycin (PharmItalia), lonidamine (La Sapienza Rome University), sanazole (AK-2123, University of Kyoto), and antioxidant C16 (synthesized at the Institute of Problems in Chemical Physics). Aqueous solutions of these preparations were injected intraperitoneally.

RESULTS

Mitochondria are the target for lonidamine. This preparation selectively modulates energy metabolism in tumor cells and inhibits DNA repair [13]. In our experiments lonidamine in doses of 10^{-15}-10^{-19} mg/kg slightly but significant suppressed the formation of metastases (24-56%). The effect increased with increasing the dose of lonidamine from 10^{-6} to 50 mg/kg (69-95% inhibition, Fig. 1).

Melanoma B16 was less sensitive to lonidamine. It should be noted that this preparation in a dose of 10^{-15} mg/kg inhibited metastatic dissemination by 77%.

Thus, lonidamine exhibits antimetastatic activity not only in therapeutic, but also in low and ultralow doses.

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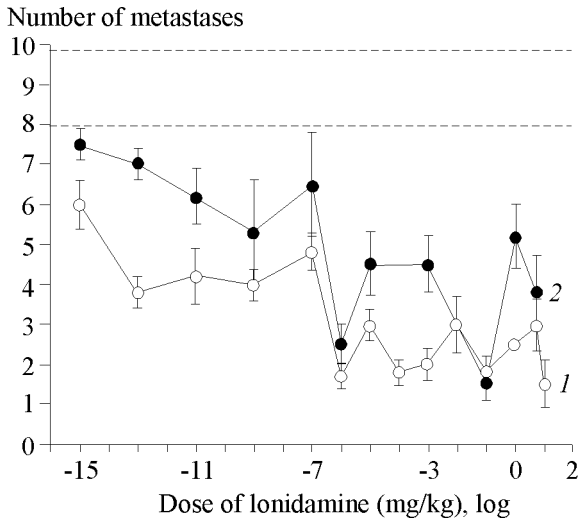

Fig. 1. Number of metastases of Lewis lung carcinoma (*1*) and melanoma B16 (*2*) in mice treated with different doses of lonidamine. Dotted lines *1* and *2*: limits of the corresponding controls.

Sanazole (3-nitrotriazole) is used in clinical practice as a hypoxic radiosensitizer [12]. Our previous studies showed that sanazole exhibits high antimetastatic activity [10] and produces potent chemosensitizing [11] and immunomodulatory effects [6]. The degree of inhibition of metastatic process after administration 10^{-12} and 50 mg/kg sanazole was similar (ISM 93%, Fig. 2). Sanazole in a dose of 10^{-10} mg/kg acted as a potent chemosensitizer during the therapy of leukemia P388 with cisplatin. Combination therapy with cisplatin and sanazole more significantly increased the mean lifespan (to 240%) and number of survivors (to 78%) compared to treatment with cisplatin alone (76 and 62%, respectively).

The antioxidant preparation in ultralow doses produced a chemosensitizing effect. Preparation C16 in a dose of 10^{-10} mg/kg possessed no antitumor activity, but combination therapy with preparation C16 in this dose and adriamycin in a dose of 4 mg/kg increased the mean lifespan (by 150%) and number of survived mice with leukemia L1210 (to 60%). In animals receiving adriamycin alone these parameters were 110 and 18%, respectively.

The results of experiments on drug-resistant tumors are of considerable interest.

Multiple drug resistance of tumors markedly reduces the efficiency of chemotherapy. Leukemia P388/rn is insensitive to mitomycin C. This preparation increased the mean lifespan of mice by 50-55%, while after combination therapy with mitomycin C and sanazole in ultralow doses the mean lifespan of animals with leukemia P388/rn increased to 175% (Fig. 3).

Our results indicate that various drugs in ultralow doses produce the antimetastatic and chemosensitizing effects.

The mechanisms of changes produced by physiologically active compounds in ultralow doses are now extensively studied. Since metastatic dissemination is an immune-dependent process, it can be hypothesized that the antimetastatic effect of sanazole and lonidamine is realized via the influence on immune-dependent stages of dissemination. It is well known that intensive immune reactions can be triggered by negligible influences. The phenomenon of metabolic cooperation between various cells was described [9]. The cell can act as a recipient of molecules and their donor for other cells. Secondary metabolic cooperation can trigger chain reaction of transduction of molecules. It can be hypothesized that metabolic cooperation between cells is a general biological process and the

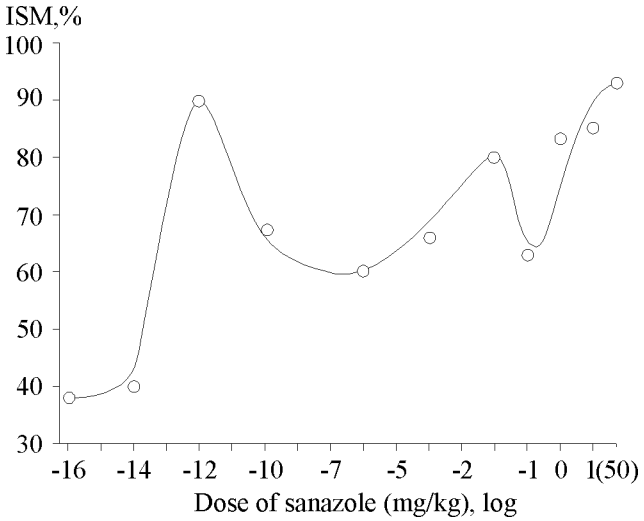

Fig. 2. Dose dependence of the antimetastatic effect of sanazole. ISM: index of suppression of metastatic dissemination.

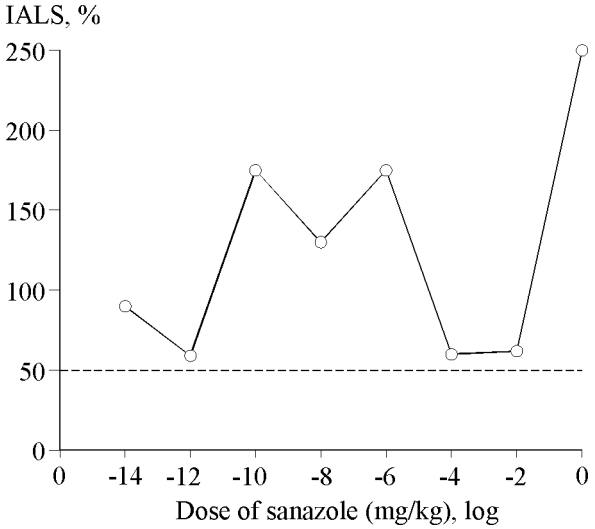

Fig. 3. Effect of sanazole on sensitivity of leukemia P388/rn with multiple drug resistance phenotype to mitomycin C in a dose of 1.5 mg/kg. IALS: increase in the mean lifespan. Dotted line: mitomycin C. Curve: mitomycin C and sanazole.

effect of ultralow doses of various agents can be explained from this viewpoint. It is hypothesized that cell regulation is a complex hierarchical process in which reactions to weak and strong factors are realized via various mechanisms [3].

Physiologically active compounds in ultralow doses hold much promise for chemotherapy of tumors and their effects require further investigations.

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