

COMPARATIVE STUDY OF ANTICANCER EFFICACY OF ACONITINE-CONTAINING AGENT BC1 AGAINST ASCITE AND SOLID FORMS OF EHRlich'S CARCINOMA

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Aim: To study anticancer activity of aconitine-containing agent BC1 *in vivo*. **Methods:** BC1 water solution was administered *per os* to mice bearing ascite or solid form of Ehrlich's carcinoma. Anticancer effect of BC1 administered *per os* was evaluated by the indexes of tumor growth inhibition and average life span of experimental animals. **Results:** BC1 didn't show anticancer activity in the case of ascite form of Ehrlich's carcinoma. At the same time treatment with BC1 resulted in 77.3% growth inhibition of solid form of Ehrlich's carcinoma ($p < 0.05$). **Conclusion:** BC1 is active *in vivo* against tumor with angiogenesis-dependent growth. **Key Words:** aconitine-containing agent BC1, anticancer activity, Ehrlich's carcinoma.

Hypothesis that vascular system feeding cancer cells could be targeted by anticancer therapy was firstly stated in 1970th by Folkman, who showed that there is no sufficiently developed blood vessels system in dormant metastatic nodes [1].

Now it is postulated that growth and progression of solid tumor greatly depends on its angiogenesis. The new strategy for treatment of cancer patients based on inhibition of blood vessels growth has been developed. Different anticancer drugs possessing antiangiogenic properties are currently at different stages of clinical trials. Although the fast development of molecular biology and chemistry allowed to generate *de novo* synthetic drugs, plants are still traditionally used as a sources of biologically active substances: about 35% of all modern anticancer drugs were obtained directly or indirectly from natural products. Recently a number of studies are focused on diterpenoid alkaloids, some of which showed high anticancer activity and significant antiangiogenic effects [2–6]. Aconitine, which belongs to the group of neurotoxins, belongs to diterpenoid alkaloids and represents the substance of plant origin. Anti-inflammatory, analgetic and in some cases antineoplastic effects of *Aconitum* species are known for many years, especially in the Far East countries, where aconitine-containing plants grow [7–9].

Previously we have studied the antineoplastic efficacy of aconitine containing agent BC1 on the model of Lewis lung carcinoma (LLC) and its *cis*-diammine-dichloroplatinum (*cis*-DDP)-resistant variant LLC/R9 [10]. It was shown that LLC and LLC/R9 forms have different metastatic potential and proangiogenic ability. We found that parental LLC cells were highly metastatic and weakly proangiogenic, however its LLC/R9 counterpart is characterized by weak metastatic activity and rather high proangiogenic ability [11]. Moreover, LLC is resistant to BC1 action, while for LLC/R9 significant inhibition of tumor growth and metastasis upon met-

ronomic administration of BC1 at non-cytotoxic doses was observed. Such difference in anticancer activity of BC1 could be explained by possible antiangiogenic mechanism of its anticancer action. To test this hypothesis the comparative study of anticancer activity of BC1 for tumors with angiogenesis dependent and independent growth has been carried out.

The solid and ascite forms of Ehrlich's carcinoma were used as models of tumors with angiogenesis dependent and independent growth.

Investigations were carried out on white inbred female mice weighting 19–22 g 2–2.5 months old, bred by vivarium of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of National Academy of Sciences of Ukraine (Kyiv, Ukraine). All investigations with animals were approved by the Regional Animal Ethics Committee.

Cells of Ehrlich's carcinoma (10^6 cells/mice) were either inoculated subcutaneously into the leg at the volume of 0.1 ml of physiologic solution (for solid form), or injected intraperitoneally at the volume of 0.5 ml of physiologic solution (for ascite form). Next day mice were divided according to their weight and separated into control and experimental groups (11 mice per group).

BC1 is a new herbal preparation produced on the base of extract of *Aconitum* species [10]. The calculation of BC1 dose was based on the content of aconitine as a main active substance. For anticancer therapy BC1 was dissolved in water *ex tempore*. For mice with ascite and solid tumors, beginning on the 2nd day after tumor cell inoculation, the drug was administered *per os* (0.4 ml aconitine animal) on 5 consecutive days followed by 2 drug-free-days (5-days-per-week schedule) for 2 weeks. The total dose of BC1 per animal was 18 μ g. The animals from control group received 0.4 ml water *per os*. Mice were under daily observation, and their weight was registered triply per week.

In the case of solid form of Ehrlich's carcinoma tumor size was recorded (with caliper) triply per week for 22 days. Anticancer activity of BC1 against solid form of Ehrlich's carcinoma was characterized by growth inhibition index GI (at the end of therapy and

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Abbreviations used: ALS – average life span; LLC – Lewis lung carcinoma.

in a week after its completion), which was calculated using formula:

$$GI = \frac{(V_k - V)}{V_k} \cdot 100\%$$

where V , V_k — average tumor volume for experimental and control groups correspondingly.

The average life span (ALS) for animals bearing ascite tumors was determined for control and experimental groups. The weight of animals was monitored during the study, and was used as an additional criterion of BC1 anticancer activity.

Statistical analysis of the data was done using descriptive statistic and parametric t -criteria in the framework of Microsoft Excel and Microcal Origin.

Kinetic analysis of survival of mice bearing ascite form of Ehrlich's carcinoma showed that BC1 therapy didn't result in the increase of ALS (Fig. 1): ALS of mice from experimental group was 19.9 ± 1.2 days and did not differ statistically from that of mice from control group (17.5 ± 0.9 days). However, it can be noted that a minor tendency towards increase of ALS for mice with ascite form of Ehrlich's carcinoma treated with BC1 was observed.

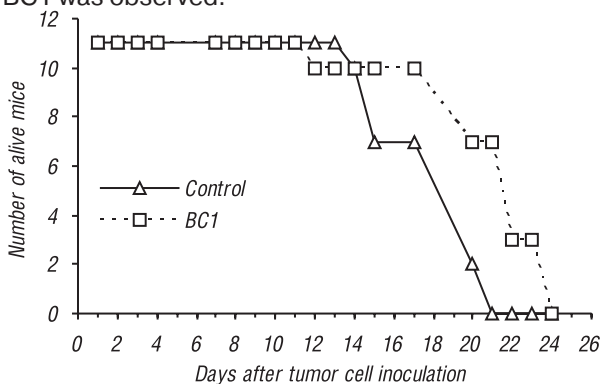


Fig. 1. Survival of mice bearing ascite form of Ehrlich's carcinoma (control and experimental groups)

The low/undetectable level of BC1 anticancer activity in the case of ascite form of Ehrlich's carcinoma was confirmed also by analysis of animals weight: by this parameter no significant difference between two groups was registered (Fig. 2).

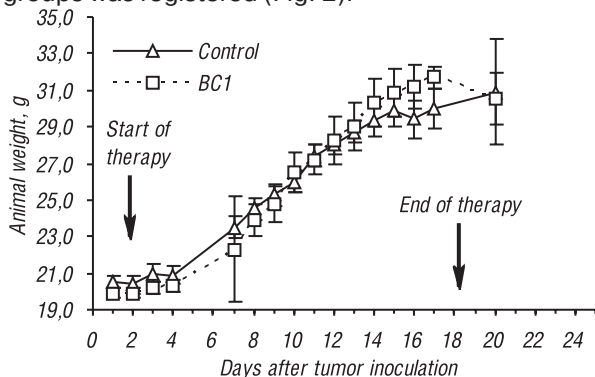


Fig. 2. Dynamics of weight changes in mice with ascite form of Ehrlich's carcinoma (control and experimental groups)

Completely different anticancer activity of BC1 was observed in the case of solid form of Ehrlich's carcinoma. Growth kinetic analysis showed that administration of BC1 resulted in substantial retardation

of tumor growth (Fig. 3). Interestingly, there were no differences in the tumor volume in mice in control and experimental group till 15th day. However after that the significant tumor growth inhibition was observed in mice in experimental group, while the tumors in control mice were growing with the constant rate. Moreover, after 15th day the growth inhibition index constantly increased reaching 38.4% at the end of BC1 therapy (18 days after cancer cell inoculation) and 77.3% — in a week after therapy completion (Table).

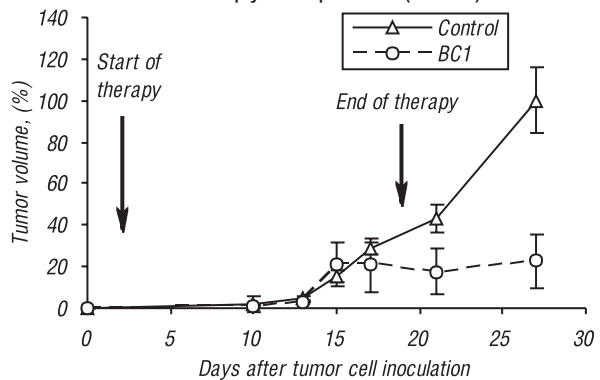


Fig. 3. Growth kinetics of solid form of Ehrlich's carcinoma during therapy with BC1. * $p < 0.05$.

Table. Growth inhibition index (%) of solid form of Ehrlich's carcinoma caused by BC1 therapy

Days after cancer cell inoculation	Growth inhibition index (%)
18	38.4
26	77.3

Results of our investigations revealed, that BC1 has marked anticancer activity in the case of solid form of Ehrlich's carcinoma. At the same time ascite form of Ehrlich's carcinoma was not sensitive to BC1 therapy. Such difference in BC1 anticancer activity cannot be explained by direct effect of this agent on cancer cells and, most likely, is associated with the differences in angiogenesis of these two forms Ehrlich's carcinoma. In contrast to ascite tumor the growth of solid form of Ehrlich's carcinoma is an angiogenesis dependent process. Thus, significant anticancer efficacy of BC1 for tumors with angiogenesis dependent growth confirmed our hypothesis about the antiangiogenic mechanism of BC1 anticancer action.

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СРАВНИТЕЛЬНЫЙ АНАЛИЗ ПРОТИВООПУХОЛЕВОЙ АКТИВНОСТИ АКОНИТИНСОДЕРЖАЩЕГО АГЕНТА ВС1 ПО ОТНОШЕНИЮ К АСЦИТНОЙ И СОЛИДНОЙ ФОРМАМ КАРЦИНОМЫ ЭРЛИХА

Цель: изучить противоопухолевую активность аконитинсодержащего агента ВС1 *in vivo*. **Методы:** асцитную и солидную формы карциномы Эрлиха перевивали белым беспородным мышам внутрибрюшинно и подкожно соответственно. Противоопухолевую активность ВС1 оценивали по показателям ингибирования роста опухоли и средней продолжительности жизни животных. **Результаты:** ВС1 не оказывал противоопухолевого действия в отношении асцитной формы карциномы Эрлиха, но был активен против солидной формы, приводя к 77,3% ингибированию роста опухоли ($p < 0,05$). **Выводы:** ВС1 оказывает выраженное противоопухолевое действие в отношении злокачественных новообразований с ангиогенеззависимым ростом. **Ключевые слова:** аконитинсодержащий растительный агент ВС1, противоопухолевая активность, карцинома Эрлиха.