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A. V. Krementsova, V. N. Erokhin, V. A. Semenov,
O. M. Alekseeva, G. E. Zaikov

THE MELAFEN INFLUENCES TO THE MICE'S SOLID CARCINOMA LUIS DEVELOPMENT

Key words: Melafen, tumor development - Luis carcinoma – lifespan.

This investigation deals with the actions of aqua Melafen (melamine salt of bis (oximethyl) phosphinic acid) solutions, applied in agriculture, as the plant growth regulator, to the animal tumor cells in vivo. The aqua Melafen solutions influences to the growth kinetics of experimental malignant neoplasm of mice's solid tumors carcinoma Luis were tested. We measured the changes of tumor sizes, and the duration of animal's life in control and experimental groups of mice. The tested doses of Melafen (10^{-12}, 10^{-9}, 10^{-5} mol/kg) suppressed the growth of carcinoma. Notably, the rate of tumor growth decelerated, and the average tumor volume was decreased at the point of animal death time. The means of lifespan of experimental and control animals were similar. Thereby, we obtained that the Melafen had the appreciable suppressive actions to the tumor development of mice's Luis carcinoma under the all tested dozes, even under the super low dozes (10^{-12} mol/kg). The values of the latent periods of the tumor development in the experimental mice's groups had the same duration, as in control mice groups. The obtained data testify that aqua Melafen solutions slowed the mouse tumor growth, but its does not increase the life expectancy of experimental animals.

Ключевые слова: мелафен, малые дозы, апоптоз, развитие опухоли.

В работе изучалось влияние малых доз на свойства опухолевых клеток животных in vitro - на Ca^{2+}-сигнальную систему, на содержание белка регулятора p53 и антиапоптозного белка Bcl-2 в клетках асцитной карциномы Эрлиха (АКЭ) методами первичного светорассеяния и иммуноблоттинга; in vivo - на кинетику роста экспериментальных злокачественных новообразований солидных опухолей (карцинома Льюис мышей F1 C57BlxDBA). Мелафен вводили мышам в дозах 10^{-5} моль/кг, 10^{-9} моль/кг, 10^{-12} моль/кг. Развитие опухолевого процесса отслеживали по изменению линейных размеров опухоли и по продолжительности жизни животных. Полученные кинетические кривые динамики роста опухоли аппроксимировались функцией Гомперца. Наблюдалось торможение роста карциномы Льюис при всех изученных дозах мелафена: замедлялся темп роста опухоли и уменьшался средний объем опухоли на момент гибели животного, средняя продолжительность жизни не изменялась. Сочетание противоопухолевой эффективности с низкой токсичностью делает мелафен интересным для дальнейших онкологических исследований.

Introduction

Melafen (melamine salt of bis (oximethyl) phosphinic acid) is the preparation, applied in agriculture, as a plant growth regulator. Subject to concentration it can operate, as the stimulator (10^{-18} - 10^{-13} M) or as the inhibitor (10^{-9} - 10^{-3} M) of the rate of developments of the plant body. We used the aqueous solutions of Melafen from large concentrations up to ultra small ones (10^{-3} - 10^{-21} M) when studying its effects on the experimental objects. Taking into account the close interdependence of vegetation and animal bodies in nature, it was necessary to investigate the action of plant growth regulator to the fate of objects by animal's origin.

At the earlier papers [1-5] the data of Melafen influences to the cellular components by animal's origin were obtained and were discussed. At that works there were number measurements of Melafen actions to the experimental objects were provided. The model and cellular objects were selected on the principle of the forward complicating of structure and/or function. There were the lipids and proteins compounds of animal cellular membranes: multilammelar liposomes, formed by dimyristoilphosphatidylcholine or egg lecithin and erythrocytes ghost; and soluble protein - bovine serum albumin; and isolated native cells – erythrocytes and Ehrlich ascetic carcinoma cells. At first, that investigations [1] deals with the influence of Melafen, at the wide concentration range (10^{-3} - 10^{-21} M), on the structural properties of lipid membranes with different composition. The lipid-Melafen interactions were tested with differential scanning microcalorymetry and small-angle X-ray diffraction. Authors did not reveal by X-ray diffraction any noticeable structural changes of the egg lecithin membranes at concentrations of Melafen used at crop production. But on the basis of differential scanning microcalorymetry data it was concluded that the microdomains structure of dimyristoilphosphatidylcholine bilayer membrane was changed by Melafen under the polymodal manner.

At the next investigations authors [2, 3] did not reveal any noticeable structural changes of the proteins, which are soluble or bounded by membranes. Bovine serum albumin (soluble protein – the main component of blood serum) and proteins of erythrocyte ghost membranes were tested under the concentrations of Melafen that used at crop production. The Melafen actions to the native isolated erythrocytes were tested by the measurements the value of microviscosity and the rate of spontaneous hemolysis. Melafen had not any destruction actions to the membrane-bounded and soluble proteins under the concentrations that activate the plant growth.

Also the Melafen actions were tested on the protein's functions with aid the activation of purine-depended Ca^{2+}- transduction of transformed ascetic Ehrlich carcinoma (EAC) mouse cells [4, 5]. It was disclosed that over a wide range of concentration (from 10^{-}

^{13} M up to 10^{-3} M) the aqueous solution of Melafen oppressively influenced to the purine-depended Ca^{2+}-signal system, witch dealing with the activation of Ca^{2+}-depended K^+- and Cl^- -channels, in EAC cells. Melafen exerts the destructive effect on the functioning of transformed EAC cells under the certain region of concentration the Melafen aqueous solution (from 10^{-7} M up to 10^{-3} M).

The next logical stage of research was the study of Melafen aqua solution effects at the level of organism entire. Because the Melafen solution, in low concentrations, is powerful plant growth stimulant, and in big concentrations - the inhibitor, then logically would be suppose, that it is acting on the divided animal's cells too. As the experimental object we chose the animals with transformed cells. It is known that the constant proliferation is the main properties of tumor (malignant) cells. On this basis, the influence of solution Melafen on the development of experimental malignant neoplasm in animals was studied, over a wide range of concentrations. In earlier papers [4, 5] has been demonstrated the life activity oppression of isolated cells of short-lived ascetic Ehrlich carcinoma. As the next object for investigations the long-lived solid Lewi carcinosarcoma of mouse was chosen. Due to of long-term monitoring of tumor development and life expectancy of tumor-bearing mice we were able to come to some conclusions about influence of aqueous solutions of Melafen over a wide range of concentrations at the level of organism entire.

The solid carcinoma Lewi is the widespread model for the testing of anticancer drugs: nature substances and synthetic ones. Melafen is synthetic hydrophilic agent, derivative melamine and bisphophinic acid. It was applauded as aqueous solutions in doses (10^{-12} mol/kg, 10^{-9} mol/kg, 10^{-5} mol/kg) concerning the mice weights.

Material and methods

Carcinoma was transplanted to mice of line C57Bl and their of F1 hybrids (C57BlxDBA). For the watching of development of tumor process in mice there were recorded the change of tumor sizes and the duration of animal life in control (the tumor growth without action) and in experimental (the solution introduction of Melafen) groups.

The animals, which had been transplanted the tumor cells of Lewi, were divided on any groups on 7 animals in each group. The first: № 1 group – it was the "clean" control (the tumor developed without any actions). Second № 2 group – was the control animals, which were inputted intraperitoneally on 0,2 ml of distilled water (the water used for the preparation of Melafen solutions). Other groups consisted of animals which were inputted on 0,2 ml of solutions that containing the needed doses of Melafen. So, in third animals group (№ 3) the Melafen solution was inputted to mice body at every day in dose 10^{-5} mol/kg. Over all the 15 injections were done. In fourth animals group (№ 4) the Melafen solutions were inputted daily in dose 10^{-5} mol/kg during the entire period of animal life (up to death of last animal). In № 5- group of animal the Melafen solutions were inputted daily in dose 10^{-9} mol/kg. Over all 15 injections were done. In № 6-group of animal the Melafen solution were inputted daily in dose 10^{-9} mol/kg, during the entire period of animal life (up to death last animal). In № 7- group of animal the Melafen solutions were inputted daily in dose 10^{-12} mol/kg. Over all 15 injections was done. In № 8-group of animal the Melafen solutions were inputted daily in dose 10^{-12} mol/kg, during the entire period of animal life (up to death last animal).

We watched on change in size of tumor growing for registration of tumor process development in mice body. The measurements were made in three of mutually perpendicular directions (that corresponding to the linear dimensions x, y, z) that allowed to estimate the swelling of volume. The volume calculated from the formula:

$$D = \frac{1}{6}\pi xyz .$$

The individual kinetic curves were made for everyone animal groups. These curves reflected the dependence of change of tumor sizes from time that past after the carcinoma transplantation to mice (the longitudinal study). The obtained curves of tumor growth were approximated by the Gompertz function [6]:

$$D = D_\infty e^{-be^{-ct}},$$

where D - the tumor volume, D_∞ - fixed the maximum volume of tumor (it was chosen by the equal to D_∞=12000, on the assumption of work [6]), b - parameter on kinetic curve, determining the latent period after carcinoma transplantation to mice, c - parameter, rate-controlling of tumor growth.

Results and discussion

In figure 1 we showed the kinetic curves of tumor growth in mice body, when three different doses of Melafen solution were injected to animals.

In practice when all injection schemes of solution mice of Melafen introductions to animal's body, we observed the inhibition of growth of carcinosarcoma the Lewi. The curves, obtained for experimental groups of mouse, are located lower than curve was built for control group of mice (at the Figure it was the curve 1). There were not significant differences from control groups. Only group of mice № 8 curve was not significant different from control group.

All individual kinetic curves of tumor growth were fitted by Gompertz function and approximating parameters *b* and *c* were calculated. The corresponding values of kinetic parameters of *b* and *c* (their median data with 25%-75% intervals) are introduced on fig. 2. The significant differences between control group and administration Melafen solution to mice groups was estimated by non-parametric Mann-Whitney U test.

Estimating the parameter of *b*, characterizing the latent period after the carcinoma transplantation mice (fig. 2), it is possible to note that the animals group № 3 and the group № 4 (the Melafen solution in dose 10^{-5} mol/kg, when different injection circuits) and the group № 6 (the Melafen solution in dose 10^{-9} mol/kg at the introduction up to death of last animal) showed the significant differences from control group. Animals groups № 7 and № 8 (the most small dose of Melafen in solution, 10^{-12} mol/kg) had not shown significant differ-

ences from control. On parameter of *c*, reflecting the rate of tumor growth in mice body, significant difference were found in groups of animals № 4, № 5, № 6 and even in group № 7 (the most small dose of Melafen in solution 10^{-12} mol/kg, when 15-fold introduction). The comparing on both kinetic parameters of *b* and *c* animals groups № 1 ("clean" the control) and № 2 (the dissolvent introduction - water) demonstrated the absence of differences.

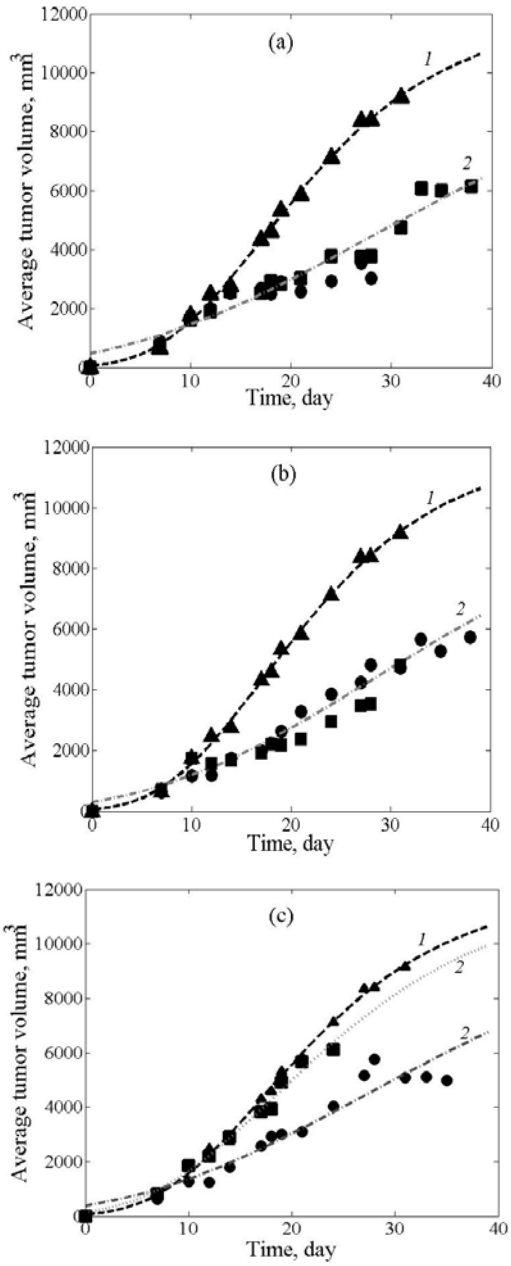

Fig. 1 - Kinetic curves of changes in average tumor volume in mice at the control ("triangle", regression - curve 1) and at the administration Melafen solution to mice (regression - curve 2) in different doses: (a) - 10^{-5} mol/kg, group № 3 – "points", group № 4 – "squares"; (b) - 10^{-9} mol/kg, group № 5 – "points", group № 6 – "triangles"; (c) - 10^{-12} mol/kg, group № 7 – "points", group № 8 – "squares"

So that, we indicated that the Melafen solution (in concentrations from 10^{-12} M up to 10^{-5} M) slows the rate of the tumor growth at experimental mice compared to checking, i. e. influences the kinetics of developmental expression of carcinosarcoma Lewi.

If address to results of comparison average tumor volume at death point of animals (fig. 3), then it should be noted that the tumor size in animals, which inputted the Melafen solution in groups № 3 and № 4 (10^{-5} mol/kg) and groups № 5 and № 6 (10^{-9} mol/kg) were significant smaller than in control. The Melafen solution even in most small dose (10^{-12} mol/kg), used for animals in groups № 7 and № 8, is likely also decreased average the tumor size in mice, but the data sets were not significant different.

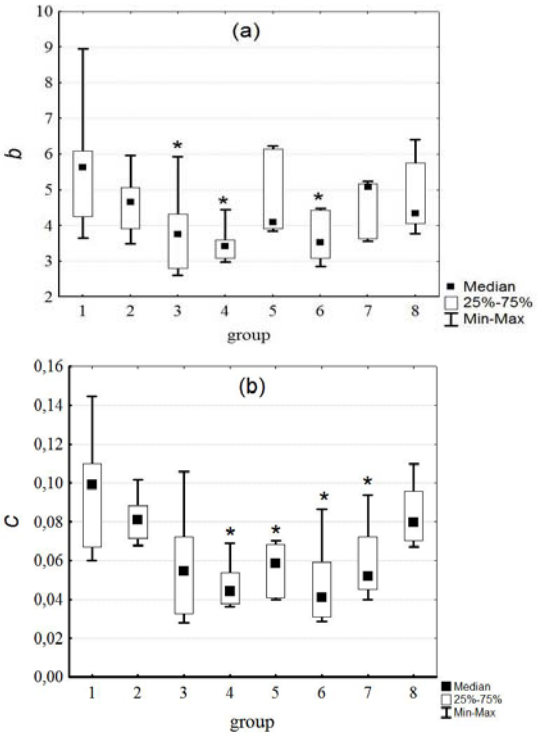

Fig. 2 - The Melafen influence on the kinetic parameters of tumor growth. The median, 25%-75% intervals, minimum and maximum values of parameters. (a) - latent period of tumor development b; (b) - rate of tumor growth c. Sing (*) were denoted significant difference by Mann-Whitney U test from control set ($p<0.05$)

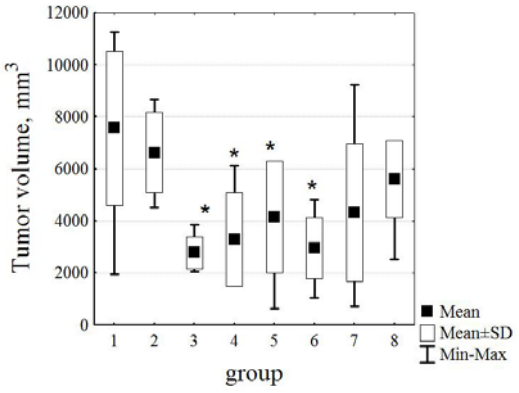

Fig. 3 - The Melafen influence on tumor volume in groups of animals. Average tumor volume in mice, standard deviation, minimum and maximum values of tumor volume (mm^3) at the time of animal's death. Sing (*) were denoted significant difference by Mann-Whitney U test from control set ($p<0.05$)

Most important finding of antitumor activity of preparation – is the increasing of average duration of animal life. In our work we estimated the mean life span of checking and experimental mice (fig. 4).

As it can be seen from Fig. 4, the mean life span of animals, which was inputted the Melafen solution, when different dosage schedules of preparation, in practice has not changed. Furthermore, in group № 3 (the Melafen solution 10^{-5} mol/kg that was inputted until to the moment of death of last animal) the toxicity being discussed material was found: the experimental animals were killed significant earlier than checking mouse.

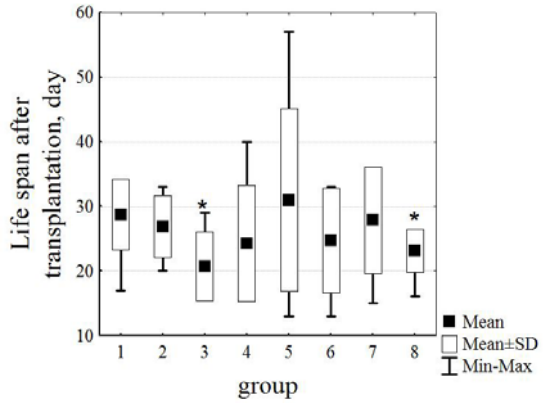

Fig. 4 - The Melafen influence on life span of mice in groups of animal after carcinoma transplantation. Mean life span per days (day), standard deviation, and minimum and maximum life span after transplantation of tumor cells to mouse body. Sing (*) were denoted significant difference by Mann-Whitney U test from control set (p<0.05)

Obtained data testify that for Melafen solution the non-linearity of dose-response relationship has been found, that corresponds famous perceptions of effect dependence from Professor E.B. Burlakova [7].

Conclusion

So that, the action of aqueous solution of Melafen, over a wide range of concentrations, from 10^{-12} M up to 10^{-5} M, at the level of whole animal organism, as an example of model the carcinosarcoma Lewi was studied. When administered to an aqueous solution of the drug at various doses Melafen, the tumor size in animals was significantly lower in all experimental groups compared to tumor size in animals control group. This suggests concluding that the Melafen solution in doses 10^{-12} mol/kg, 10^{-9} mol/kg, 10^{-5} mol/kg slows tumor growth in sick animals.

The values of latent period of tumor development after the carcinoma transplantation to mice body in all experimental groups of animals in practice was not different from one for animals in control groups. But the tumor size reduced when action of solution Melafen. It is possible to suggest that the Melafen solution, without being toxic to cells transformed solid Lewis lung carcinoma, to some extent, has a depressing effect on their livelihoods.

The duration of life when different dosage schedules of Melafen solution, over a wide range of concentrations, in experimental groups of mice compared to duration of animal life in control group has not significant changed for most of animals groups. Perhaps this is due to changes in cellular regulation in tumor when exposed to it Melafen.

As its were demonstrated in the preceding papers [3-5], the Melafen solution oppressively influence to the purine-dependent calcium signaling in cells with uncontrolled growth - cells of short-lived ascetic Ehrlich carcinoma. In these works was presented the scheme of initiation of Ca^{2+} -signaling when activating of ATP-dependent metabotropic purine receptors P2Y, and also store operated Ca^{2+}-channels (SOC). P2Y and SOC were being as integral proteins at EAC cells plasmalemma. According to set out of scheme and kinetic curves of cell answers to additions of Melafen solution, over a wide range of concentrations, it is possible believe that the Melafen solution, while acting directly or is mediated through the metabolic pathways on Ca^{2+}-channels, changes in cells Ca^{2+}-transitions, that influence the receptors functioning, enzymes activity and channels gates etc.. This, are likely, brings about not only to life activity suppressing of individual insulated from cells organism of short-lived tumor of ascetic Ehrlich carcinoma of mice, but, it is possible, and to growth retardation of such growth, as the solid malignancy of carcinosarcoma the Lewi in whole organism of mice.

The obtained data provide any evidence rather about regulator role of Melafen solution, used over a wide range of concentrations, when proliferation of the cells with uncontrolled growth in animals, than about antitumor data activity of preparation.

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© **A. V. Krementsova -** к.ф.-м.н., науч. сотр. Институтf биохимической физики РАН, Москв **V. N. Erokhin -** к.б.н., ст. науч. сотр. того же ин-та; **V. A. Semenov -** науч. сотр. того же ин-та; **O. M. Alekseeva -** науч. сотр. того же ин-та; **G. E. Zaikov** – д.х.н., проф. каф. технологии пластических масс КНИТУ, ov_stoyanov@mail.ru.