ANTITUMORAL ACTIVITY OF "ERBISOL®" CLASS MEDICATIONS IN VITRO AND IN VIVO

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Summary. It is shown that the medications of "Erbisol®" class possess the cytotoxic activity *in vitro* regarding the tumor target cells (the direct linear dependence on the concentration of medication is observed), as well as the positive effect on lymphocytes, macrophages and hepatocytes. All the studied medications have highly therapeutic efficiency in mice with experimental tumor (a solid form of sarcoma-37 and Ehrlich carcinoma), significantly inhibiting the tumor growth ($p < 0.05$), and increasing the life-span of animals ($p < 0.05$). The best effect for all studied parameters was obtained with using the medication Erbisol® ULTRApharm, produced from chicken embryos. The medications of Erbisol® ULTRApharm and Erbisol®, produced from the chicken embryos, and Erbisol® (produced from the cattle embryonic tissues) can be the effective cytostatics of tumor cells, providing favorable effects on the cells of normal tissue in the same doses.

Key words: experimental tumors, medications of "Erbisol®" class, antitumor activity.

INTRODUCTION

The creation, development and implementation of natural effective medications remain one of the actual problems of modern pharmacology in the medical practice. The tissue medications, on the basis of membrane glycoproteins that are "markers of cell physiological state", signaling control body systems of pathological process presence [1], take a special place among them. Such markers are fully synthesized and have minimal immunogenicity as "own" molecule under normal cell condition. Under pathological processes the conformation of the carbohydrate component and therefore marker immunogenicity, which value is proportional to the degree of disease severity are changed. Thus, it is given the signal of "an alarm" to which the immune system is immediately responded. These markers are present in all the cells, helping the immune system to detect the pathological processes. A protein part of marker molecules is immunologically conservative for many animal species, that are evolutionally distant from each other.

If the glycoprotein markers are isolated from the tissue, where unusual processes of its normal state occur, and after appropriate treatment, necessary for eliminating the side effects, the "signal" glycopeptide section is identified, then inserting it into another organism the signal "alarm", provoking the immune system to search for pathologic foci in the recipient organism is activated. In the presence of the last, a mechanism of its elimination is started by activated immunocompetent cells [2].

The development, production and implementation of such highly effective medications of new generation of "Erbisol®" class are realized in Scientific Production Center "ERBIS" together with the privately owned enterprise "Laboratory ERBIS". Medications of "Erbisol®" class as an effective agent contain the low-molecular fragments of membrane glycoproteins, isolated from the embryonic tissue of the cattle, and the birds [3]. When administered into the body, they initiate the start of repair mechanism in the damaged tissues, and the elimination of the abnormal cells nonspecifically activating the immune system by inducing the synthesis of corresponding cytokines [4-6].

As the immunomodulators, the medications of "Erbisol®" class, on the one hand, activate the cells of macrophage series involved in the elimination of cells undergoing apoptosis or necrosis, and the regeneration of tissues and organs with restoring their functional activity, so acting as the "restorer" of the body. On the other hand, they activate the cells of killer series (NK, T-killer cells, cytotoxic $CD8^+$ T-lymphocytes) that are responsible for maintaining antigenic homeostasis by eliminating the anomalous cells (mutant, malignantly transformed, infected with viruses, acquired

autoantigenicity), acting as the "auditor". At the same time, these medications are immunocorrectors – they restore the balance activity of Th1-and Th2-lymphocytes, thus harmonizing the correlation of cellular and humoral immunity, and inhibit the autoimmune and allergic processes [7, 8].

The medications of "Erbisol®" class - Erbisol®, Erbisol® ULTRApharm, and Extra Erbisol® are protected by patents in 20 countries around the world and have found a wide application in medical practice both in the Ukraine and in several foreign countries. The medication "Erbisol®" is primarily used as the medication of accompaniment in chemo- and radiotherapy [9, 10], and the medication Erbisol® ULTRApharm – as an antitumor agent for complex treatment in oncology [11-14]. It is therefore advisable the detail study of the antitumor activity of "Erbisol®" class medications *in vitro* (a study of cytotoxic effect on tumor target cells) and *in vivo* (in animals with experimental (model) tumor).

SUBJECTS AND METHODS

The linear mice BALB/c breeding in the vivarium of R. E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of Nat. Acad. of Sci. of Ukraine were used in the experiments. All the experiments were performed according to the requirements of the regional ethics committee for the use of laboratory animals. The studies were conducted on the standard models of tumor growth: sarcoma-37 (C-37) and Ehrlich carcinoma (EC), supported by the standard method. The medications of "Erbisol®" class (SPC "ERBIS", the Ukraine), produced from the embryonic tissues of cattle and chicken embryos: Erbisol®, Erbisol® ULTRApharm and Extra Erbisol® were used.

Testing the cytotoxic effect of the medications was performed *in vitro* on the target cells of C-37 and EC by MTT-test [15]. The appropriate medication in the amount of 60; 40, 20 and 10 µl was added to 100 µl suspension of the live tumor cells ($0{,}3 \times 10^6$/ml) and were incubated for 18 h at 37 ° C in 5 % CO_2 at 100 % humidity. The optical density of each sample was measured at $\lambda = 540$ using the automatic "microELISA reader" after the test in accordance with the standard procedure. The effect of the same medications on lymphocytes (5.0×10^6/ml), macrophages (1.0×10^6/ml) and hepatocytes (3.0×10^6/ml) was evaluated *in vitro* by the same method, also by the MTT-test.

In the experiment *in vivo* the solid form of tumors was used; for their induction $6.5\text{-}7.5 \times 10^5$ living cells of EC or C-37 were transplanted (inoculated) into the mouse femoral muscle in 0.2 ml of physiologic salt solution of NaCl. The animals were randomly distributed into 7 groups in each series of experiments: 6 main groups, were received the different medications, and one control group . The course of tumor process was assessed by the standard criteria: index of tumor growth inhibition, the average lifespan. The studied medications (100 µl) were administered to mice after transplantation of the tumor cells, starting with the 3 days, 2 times a week – 10-fold, then 1 time a week – 6-fold.

Statistical processing of the obtained results was performed using application program OriginLab.

RESULTS AND DISCUSSION

The results of studying cytotoxicity of the "Erbisol®" class medications according to C-37 and EC cells *in vitro* are shown in Fig. 1, a, b. The direct dependence of the cytotoxicity on a dose of the medication was noted in study of all the medications. Erbisol® ULTRApharm and Erbisol®, produced from chicken embryos (chicken), had the most pronounced cytotoxic effect on the C-37 cells. It is necessary to note that their high cytotoxicity was observed even a minimum used concentration (10 µl): 35,68 and 49,36 %, respectively. Similar results were obtained using EC as the target cells too, but in general cytotoxicity of all the preparations tested was practically lower than in relation to the C-37 cells. As in the 1st series of experiments, Erbisol® ULTRApharm (chicken) and Erbisol® (chicken) were more active: when they were used in a dose of 10 µl, cytotoxicity was 47,92 and 40,41 %, respectively, while for the other drugs – $\leq$ 10,6 %. It is necessary to pay attention to the peculiarity of the medications Erbisol® ULTRApharm, produced

from the cattle embryonic tissues (cattle), and Extra Erbisol® (chicken) that is pronounced cytotoxicity for the C-37 target cells and a weak – to the EC cells.

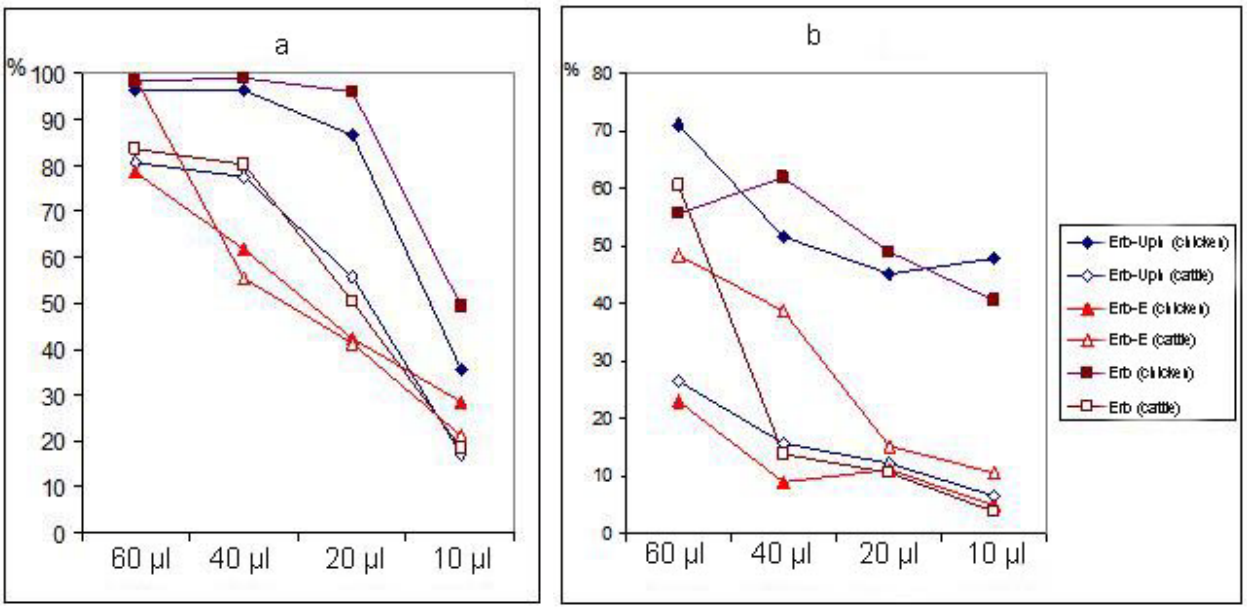

Fig. 1. Cytotoxic effect of the "Erbisol®" class medications on tumor target cells *in vitro: a* – C-37; b – EC

In general, can be concluded that there is the cytotoxic activity of "Erbisol®" class medications concerning the tumor target cells *in vitro*; thus, as a rule, there is a direct linear dependence on the concentration of medication.

In order to select the optimal concentration of "Erbisol®" class medications for use *in vivo* their parallel testing was *in vitro* conducted in the same manner on the target cells (lymphocytes, macrophages and hepatocytes) of intact mice (Fig. 2 - a, b, c). Most of the medications had a negative effect on lymphocytes in a dose of 60 µl, and in a dose of 20 µl – the positive (activating) action (see Fig. 2, a). An exception had the medication Erbisol® (produced from chicken embryos), which optimal positive doses effecting on lymphocytes (and also macrophages) consist of 40-20µl. Assessing the impact of studied medications on macrophages and hepatocytes, should also emphasize the presence of dose response. However, these cells were activated by the medications produced from the chicken embryos, also in a dose of 40 µl, and Erbisol® (chicken) – in all the dose range (See Fig. 2, a, b). The highly positive effect of the medications, isolated from the chicken embryos, on hepatocytes should be particularly emphasized: their life activity was not depressed by Erbisol® ULTRApharm (chicken) and Erbisol® even in a maximum dose of 60 µl.

The results obtained indicate that the medications of "Erbisol®" class in the therapeutic doses (10-20 µl per 100 µl of the target cell suspension) have a marked activating effect, and in higher doses – the cytostatic effect concerning the cells that are actively involved in maintaining homeostasis of the body: lymphocytes, macrophages, hepatocytes.

It should be emphasized that a significant direct cytotoxic effect of the medications Erbisol® ULTRApharm (chicken) and Erbisol® (chicken) *in vitro* concerning the tumor cells is combined with the favorable effect on the normal cells that advantageously distinguishes these medications from the majority of cytostatics used in oncology practice. Based on the data obtained, we can conclude, that the "Erbisol®" class medications of different types and origin have a different optimum of cytotoxic effect regarding to the tumor cells and stimulating effect on the cells of the immune system and hepatocytes, which requires prior testing of the medications *in vitro* before their usage *in vivo*. The medications obtained from the chicken embryos are more promising for future use because they exhibit a positive effect (activate breathing metabolism) concerning the cells of the immune system and hepatocytes (especially in the optimal concentration) in a case of expressed cytotoxic effect on the tumor cells.

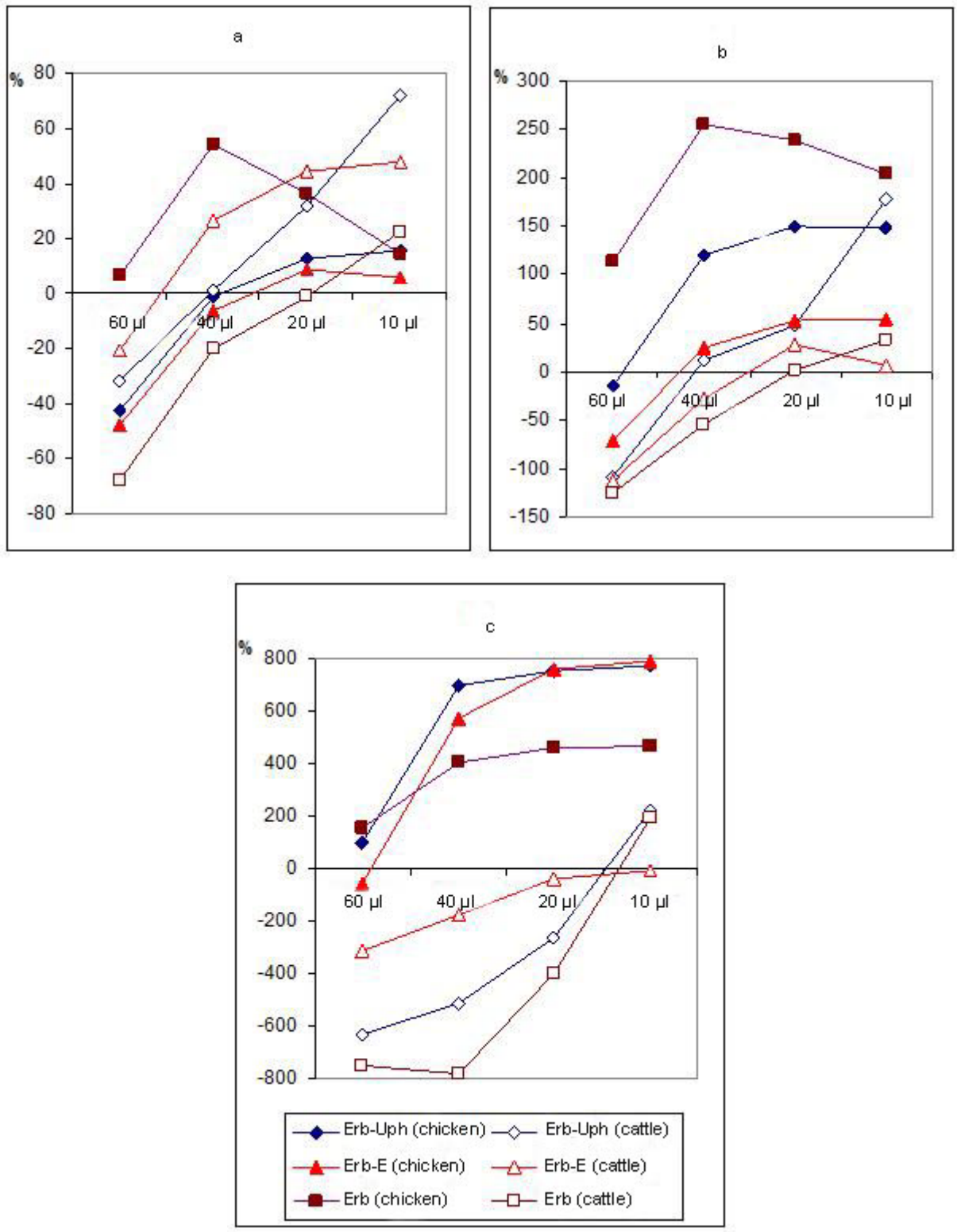

Fig. 2. Influence of "Erbisol®" class medications *in vitro* (%): *a* – on lymphocytes; *b* – on macrophages; c – on hepatocytes

The efficacy of "Erbisol®" class medications was studied in monotherapy of mice with the corresponding experimental (model) tumors in experiments *in vivo*. It is shown that all the medications studied, irrespective of their type and origin, significantly inhibited the growth of C-37 (by 56-76%) and increased the average life expectancy of animals (by 57,8-72,75%), which indicates their high antitumor efficiency. The highest index of the C-37 growth inhibition (76,71 ± 0,88%) was registered in mice treated with Erbisol® ULTRApharm (produced from chicken embryos) that was significantly higher than the results of all other medications used (table 1). The

average life expectancy, of all the mice, treated by the medications of "Erbisol®" class, with a high degree of significance ($p<0.05$) exceeded that of the control animals. It should be emphasize that the effect of using medications Erbisol® ULTRApharm and Extra Erbisol®, prepared from chicken embryos, exceeded the effects of similar medications from the cattle embryonic tissues, while the results of using medications Erbisol® (chicken) and Erbisol® (cattle) practically did not differ (Table 1).

Table 1

Effectiveness of the "Erbisol®" class medications in mice with a solid form of C-37

N	Group	n	Average percentage of tumor growth inhibition	The average life expectancy, days		
				X ± m	t	IM (%)
1	Erb-Uph (chicken)	13	76,71±0,88 [2,3,4,5,6]	83,54 ± 5,80 [7]	5,12	+72,75
2	Erb-Uph (cattle)	14	71,76±0,70 [1,3*, 4,5*]	77,07 ± 3,58 [7]	6,02	+59,37
3	Erb-E (chicken)	10	69,21±0,59 [1,2*,4]	81,70 ± 7,09 [7]	4,45	+68,94
4	Erb-E (cattle)	16	56,13±1,19 [1,2,3,5,6]	76,31 ± 4,44 [7]	4,76	+57,80
5	Erbisol (chicken)	10	69,36±0,82 [1,2 *, 4]	80,30 ± 8,55 [7]	4,08	+66,04
6	Erbisol (cattle)	11	70,41±0,90 [1,4]	77,18 ± 5,20 [7]	4,86	+59,59
7	Control	11	–	48,36 ± 2,85		

1 – $p<0.05$ compared to the index of corresponding group, 1* – $0.1<p<0.05$ compared to the index of corresponding group.

Similar results were also obtained in mice with solid EC (Table 2). In mice treated with Erbisol® ULTRApharm (chicken), the inhibition of tumor growth reached up 71,25 ± 0,42 % and significantly exceeded the effectiveness of rest medications used (except Erbisol®, produced from chicken embryos), including the same medication from the cattle embryos (50,48 ± 1,38 %). It should be noted the high index of tumor growth inhibition with Erbisol® (cattle) administration to mice in a dose of 100 µl – 58,85 ± 0,84 %. A more accurate comparison makes it possible to analyze the indices of the average life expectancy that are significantly higher than the same index of the control group. Significant differences ($p<0.05$) between Erbisol® ULTRApharm (chicken) and Erbisol® ULTRApharm (cattle) (67,29 ± 2,81 and 59,67 ± 1,90 days, respectively), as well as Erbisol® ULTRApharm (cattle) and Extra Erbisol® (cattle) (59,67 ± 1,90 and 66,00 ± 2,40 days, respectively) were found. It should be noted that the overall increase in the average life expectancy in mice with EC in the main groups was smaller than in the experiment with C-37, however the significant increased index (22-38 %) compared to control animals was noted in this series of experiments.

Table 2

Effectiveness of the "Erbisol®" class medications in mice with a solid form of EC

N	Group	n	Average percentage of tumor growth inhibition	The average life expectancy, days		
				X ± m	t	IM (%)
1	Erb-Uph (chicken)	14	71,25±0,42 [2,3,4,6]	67,29±2,81 [2,7]	5,90	+38.14
2	Erb-Uph (cattle)	15	50,48±1,38 [1,4,6]	59,67±1,90 [1,4,7]	4,56	+22.50
3	Erb-E (chicken)	15	46,45±1,79 [1,4,6]	61,07±3,38 [7]	3,15	+25.37
4	Erb-E (cattle)	15	56,51±1,10 [1,2,3]	66,00±2,40 [2,7]	6,09	+35.50
5	Erbisol (chicken)	15	60,27±0,92 [1,2,3]	66,12±2,53 [2,7]	6,18	+36.04
6	Erbisol (cattle)	11	58,85±0,84 [1,2,3]	64,45±3,14 [7]	4,91	+32.31
7	Control	14	–	48,71±1,43		

1 – $p<0.05$ compared to the index of corresponding group

Summarizing the trial of the "Erbisol®" class medications *in vivo*, it can be concluded of their high efficiency: an index of C-37 growth inhibition was 56-76 %, and EC – 46-71 % (Fig. 3).

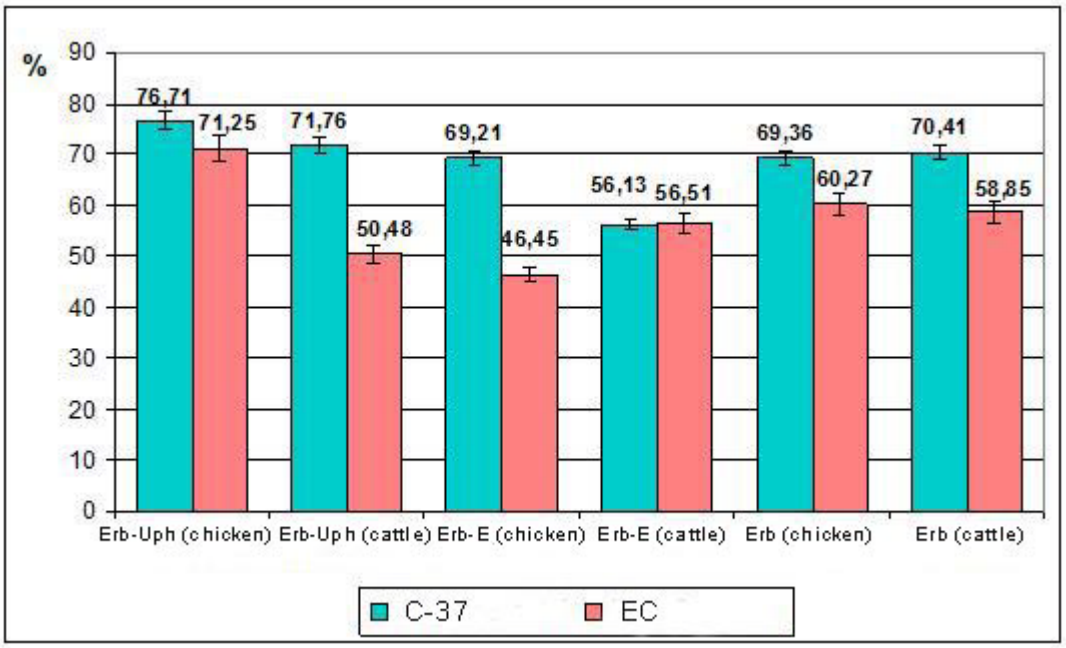

Fig. 3. Inhibition of tumor growth in mice with the solid form of C-37 and EC treated by the medications of "Erbisol®" class as a monotherapy

The most significant effect of two experimental (model) dimensional systems was induced by the use of Erbisol® ULTRApharm produced from chicken embryos (76,71 and 71,25 %, respectively). While talking about a significantly higher efficiency of the "Erbisol®" class medications on the C-37 model, it is important to emphasize that in all the cases when using medications of "Erbisol®" class a significant increase in the animal lifespan was registered: in C-37 – by 57-72 %, in EC – by 22-38 % (Fig. 4).

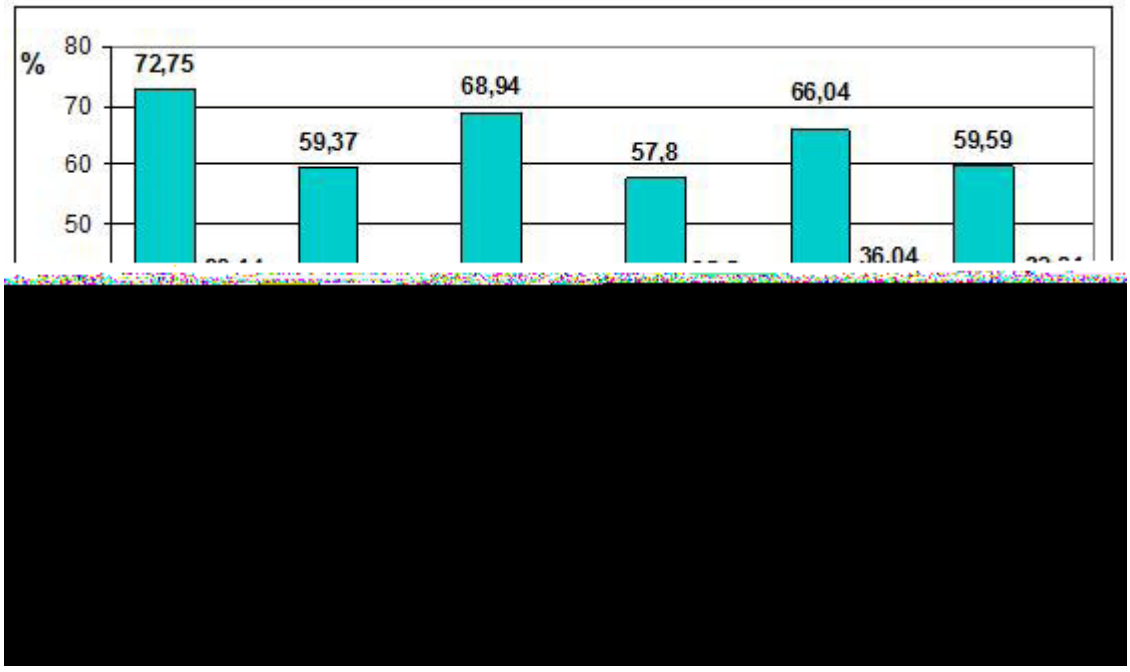

Fig. 4. Increased life expectancy in mice with the solid form of C-37 and EC treated by the medications of "Erbisol®" class (in percentage in comparison with the indices of control group) as a monotherapy

CONCLUSIONS

1. The medications of "Erbisol®" class are possessed (within the dose range of 60-10µl /100 µl of the target cell suspension) an expressed cytotoxic activity *in vitro* regarding the C-37 and EC tumor cells, as well as exhibited significant antitumor activity in mice with homologous experimental (model) tumors (the solid form of C-37 and EC).

2. The medications of "Erbisol®" class (within the dose range of 40-10 µl /100 µl of target cell suspension) not only does not have a cytotoxic effect on the cells of the immune system and hepatocytes *in vitro*, but activate them that favorably distinguishes the medications studied from the majority of cytostatics.

3. The cytotoxic activity *in vitro* with respect to the tumor target cell of the "Erbisol®" class medications shows a direct linear dependence on the concentration of the medication. A positive effect on the immune system cells (lymphocytes, macrophages and hepatocytes) also is dose-dependent (inverse dependence), but the medications derived from the chicken embryos, activate hepatocytes even in the maximum of the doses used (60 µl).

4. It was determined that the medications derived from chicken embryos, are more perspective for future use, because they exhibit a positive impact concerning the normal cells at the pronounced cytotoxic effect on the tumor cells *in vitro*.

5. The medications of "Erbisol®" class have high therapeutic efficiency in mice with a experimental (model) tumor (the solid form of C-37 and EC), significantly inhibiting tumor growth (by 56-76 and 46-71 %, respectively, $p < 0.05$) and increasing the average life expectancy of animals (by 57-72 and 22-38 %, respectively, $p < 0.05$). In this case, the best effect is obtained using Erbisol® ULTRApharm produced from chicken embryos. The "Erbisol®" medications produced both from the chicken and cattle embryos have somewhat smaller but high enough effect.

6. The medications of Erbisol® ULTRApharm (chicken), "Erbisol®"(chicken) and Erbisol® (cattle) may be effective antitumor cytostatics thus have a benefit effect on the cells of normal tissues.

Abbreviations:

Erbisol® ULTRApharm (produced from chicken embryos) – Erb-Uph (chicken)
Erbisol® ULTRApharm (produced from the cattle embryonic tissues) – Erb-Uph (cattle)
Erbisol® Extra (produced from chicken embryos) – Erb-E (chicken)
Erbisol® Extra (produced from the cattle embryonic tissues) – Erb-E (cattle)
Erbisol®(produced from chicken embryos) – Erb (chicken)
Erbisol®(produced from the cattle embryonic tissues) – Erb (cattle)

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