Licorice Preparations Improve Efficiency of Chemotherapy and Surgical Treatment of Transplanted Tumors

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Experiments on animals with Lewis lung carcinoma and Ehrlich tumor showed that licorice (glycyrrhiza) extract and glyciram prepared from this plant improved the antitumor effect of cyclophosphamide. Glyciram reduced the toxic effect of the cytostatic on peripheral blood leukocytes. Licorice extract inhibited the growth of Ehrlich tumor and development of metastases in mice with Lewis lung carcinoma. Glyciram administered to mice after removal of Lewis lung carcinoma produced an antimetastatic effect and prevented relapses.

Key Words: *transplanted tumors; licorice extract; glyciram*

Licorice alone and in combination with other plants is used as a diuretic, diaphoretic, antiseptic, and antiallergic preparation. The main pharmacological property of licorice preparations is their antiinflammatory effect determined by the presence of triterpenes close by their structure to adrenal cortex hormones. Licorice roots and rootstocks contain triterpenoids glycyrrhizin and glycyrrhizinic acid (glycyrrhetic acid in hydrolysate), flavonoids liquiritin, isoliquiritin, and liquiritoside, carbohydrates, organic acids, volatile oils, steroids, tannins, coumarins [7]. Glyciram isolated from licorice is a monoammonium salt of glycyrrhizinic acid. The effects of triterpenoids from Glycyrrhiza glabra on tumor growth were previously studied on animals with transplanted tumors. It was found that these substances suppress the growth of Zaidel hepatoma, sarcoma 37, and Ehrlich tumor. Published data on the antitumor effects of licorice preparations and wide spectrum of their pharmacological activity prompted us to evaluate experimentally the effects of licorice extracts and glyciram on the efficiency of chemiotherapic and surgical methods of treatment of transplanted tumors.

MATERIALS AND METHODS

The experiments were carried out on outbred, C57Bl/6, and F_1(CBA×C57Bl/6) mice (n=353, conventional certified mouse strain obtained from the Department of Biomedical Modeling, Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Science). The animals were kept in accordance with European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasburg, 1986). Ehrlich tumor (ET) and Lewis lung carcinoma (LLC) were transplanted routinely [10]. LLC was removed under ether narcosis. Dry licorice extract and glyciram were obtained from State Research Center of Drugs (Khar'kov), liquid extract was prepared by percolation on a V. L. Komarov Mountain Taiga Station, Far Eastern Branch of Russian Academy of Sciences (Vladivostok).

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Cyclophosphamide (CP, Biokhimik company) was injected intramuscularly (to mice with ET) or intraperitoneally (to mice with LLC). The doses and schedule of treatment are shown in Tables 1-3.

For evaluation of treatment efficiency, the weight of the tumor or the volume of tumor cells, percent of inhibition of tumor growth, number of metastases in the lungs, their area, frequency of metastasizing, weight of metastases, inhibition of metastasizing, and index of metastasis inhibition (IMI) were determined [1,10]. The results were processed using nonparametric Mann—Whitney (*U*) test and Fisher angular transformation (f).

RESULTS

Treatment with CP considerably reduced the volume of tumor cells in mice with ET (2.2±0.2 *vs.* 3.6±0.3 ml in the control, $p<0.01$). Addition of liquid licorice extract (10 ml/kg) to the treatment scheme increased antitumor activity of CP (1.4±0.3 ml; $p<0.05$), the inhibition of tumor growth was 61% (*vs.* 39% in mice receiving CP alone). Combined use of CP and dry extract (100 mg/kg) also inhibited tumor growth (by 50%), but only a tendency to the decrease in the volume of tumor cells was observed in animals of this group (1.8±0.3 ml) compared to the control. Dry licorice extract in a dose of 100 mg/kg inhibited tumor growth, as was seen from the decrease in cell volume in ascetic fluid (2.2±0.3 ml; $p<0.01$), the index of tumor growth inhibition being 39%. Dry extract in a dose of 500 mg/kg and liquid extract in a dose of 10 ml/kg administered alone had no significant effects on the growth of ET in mice.

Glyciram administered in combination with CP did not modulate the efficiency of cytostatic therapy in mice with ET (2.1±0.2 ml in the control, 0.8±0.2 ml in mice treated with CP, $p<0.01$; 0.6±0.2 ml in mice receiving CP+glyciram), but considerably reduced the toxic effect of the cytostatic on the blood: the number of leukocytes in animals treated with CP in combination with glyciram was 1.9-fold higher than in animals receiving CP alone (7.6±1.9 vs. 3.9±0.4 G/liter, $p<0.05$). The volume of tumor cells considerably decreased in animals receiving glyciram alone (1.6±0.3 ml; $p<0.05$).

In mice with LLC, treatment with licorice extract in a dose of 50 mg/kg considerably decreased the incidence of metastasizing (from 82 to 27%, $p<0.005$), number of metastases (0.6±0.4 *vs.* 3.6± 1.3 in the control; $p<0.05$) and their area (0.06± 0.03 *vs.* 0.84±0.34 mm^2 in the control; $p<0.05$), IMI being 95%. Administration of the extract in a higher dose (100 mg/kg) had no effect on tumor growth.

In mice with LLC, the effect of dry licorice extract in a dose of 50 mg/kg and glyciram in doses of 50 and 100 mg/kg on the efficiency of chemotherapy was studied. When the treatment was started on day 4, the antimetastatic activity of CP increased. Metastases in mice receiving combined therapy were absent, whereas 25% mice treated with CP alone had metastases in the lungs. In mice receiving combined treatment (cytostatic+extract simultaneously starting from day 10), the weight of primary node was significantly lower (by 1.5 fold) than in animals receiving monochemotherapy (Table 1).

Glyciram administered in both tested doses starting from day 10 significantly improved the antitumor effect of the cytostatic: in mice receiving

TABLE 1. Effect of Licorice Preparations on the Development of LLC in F_1(CBA×C57Bl/6) Female Mice

Group	Number of animals	Weight of tumor, g	Inhibition of tumor growth, %	Incidence of metastasizing, %	Number of metastases	Area of metastases, mm^2
Control	15	5.7±0.2		93	2.47±0.32	0.64±0.26
CP (100 mg/kg) twice (on days 10 and 14)	12	3.8±0.3*	33	25*	0.25±0.13**	0.03±0.02**
CP+licorice extract (50 mg/kg) 14 times (starting from day 4)	12	3.9±0.3	32	0^{++}	0	0
CP+licorice extract (50 mg/kg) 9 times (starting from day 10)	12	$2.5±0.4^{++}$	56	8	0.08±0.08	0.01±0.01
CP+glyciram (50 mg/kg) 9 times (starting from day 10)	12	$2.8±0.4^{++}$	51	0^{+}	0	0
CP+glyciram (100 mg/kg) 9 times (starting from day 10)	11	$2.0±0.4^{++}$	65	27	0.27±0.14	0.18±0.16

Note. The tumor was transplanted intramuscularly ($1-2\times10^6$ cells in 0.1 ml physiological saline); the efficiency of treatment was evaluated on day 20. *$p<0.01$, **$p<0.05$ compared to the control, $^{+}p<0.01$, $^{++}p<0.05$ compared to animals receiving CP (100 mg/kg).

TABLE 2. Effect of Glyciram on Metastasizing of LLC after Removal of Tumor Node in C57Bl/6 Female Mice

Group	Number of animals	Weight of tumor, g	Number of mice with relapses, %	Weight of lungs, mg	Incidence of metastasizing, %	Number of metastases	Area of metastases, mm^2
Area of metastases, mm^2							
Control	15	3.9±0.3		331.7±44.3	100	23.1±4.4	98.37±26.37
Surgery on day 11	9	1.6±1.1	22	346.8±58.5	78*	4.3±1.3*	69.03±26.11
Surgery+glyciram (25 mg/kg) 19 times (starting from day 11)	10	1.4±0.4	50	230.5±32.1+	70	6.3±2.3	36.61±20.85**
Surgery+glyciram (50 mg/kg) 19 times (starting from day 11)	11	3.6±0.7	27	295.5±45.1	82	11.9±5.2	37.37±10.98**

Note. Treatment schedules are presented: dose and number of injections. The tumor was transplanted subcutaneously ($4\text{-}6\times10^6$ cells in 0.2 ml physiological saline). Experiment was continued until day 31 after transplantation. The weight of metastases was determined by the difference between the weight of the lungs in experimental and healthy animals (148.0±6.3 mg). *$p<0.01$, **$p<0.05$ compared to the control, +$p<0.05$ compared to operated animals.

50 and 100 mg/kg glyciram with CP the weight of the tumor was 1.4- and 1.9-fold lower than in animals treated with CP alone. In animals treated with CP+ 50 mg/kg glyciram, no metastases in the lungs were found (Table 1).

These results suggest that licorice extracts and glyciram improved the efficiency of cytostatic treatment, glyciram reduced the toxic effect of CP on leukocytes. Previous experiments showed that glyciram considerably increased the antitumor effect of CP in mice with B-16 melanoma, while in rats with Walker-256 carcinosarcoma it potentiated the antimetastatic effect of CP by inhibiting lymphogenic metastasizing [9]. It should be noted that glyciram stimulated recovery of bone marrow granulomonocytopoiesis and, to a lesser extent, other hemopoietic stems under conditions of hemosuppressions caused by cytostatics with different mechanisms of action [3,4]. The presented results provided the basis for further investigation of the antitumor effects of glyciram under conditions of surgical removal of the primary tumor. Removal of the primary LLC tumor on day 11 of its development inhibited dissemination process and significantly reduced the number and incidence of metastatic nodes in the lungs (by 5.4 times) compared to unoperated control, IMI was 85% (Table 2).

Glyciram in a dose of 25 mg/kg considerably reduced the weight of the lungs; inhibition of metastasizing calculated by the weight of metastases was 55%. Glyciram 2.7-fold decreased the area of metastatic spreading compared to mice of the control group, while in the group of operated animals only a tendency to a decrease in this parameter was observed. Increasing the dose of the preparation to 50 mg/kg improved the efficiency of surgical treatment (Table 2).

In the next experimental series, glyciram was administered in a dose of 25 mg/kg. LLC was removed on days 12 and 16 after transplantation. On day 12 after removal of the tumor node, inhibition of the dissemination process was observed, which led to reliable decrease in the number (by 3.3 times) and area (by 2.1 times) of metastases, and weight of the lungs with metastases. IMI was 73%, 90% mice had relapses. Treatment with glyciram starting from day 12 after transplantation reduced the number of animals with metastases to 43%. The preparation had no effect on tumor dissemination, IMI was 74% (Table 3).

Removal of the tumor on day 16 after transplantation considerably decreased the number of metastatic nodes in the lungs (by 1.8 times), IMI was 44%, relapses were found in 50% mice. Glyciram produced an appreciable antoimetastatic effect: IMI increased to 75%; we also observed a significant decrease in the number of metastases (by 2 times), their area (by 4.6 times), and weight of the lungs with metastases (by 1.4 times) compared to the corresponding parameters in operated mice. In animals treated with glyciram, inhibition of metastasizing was 63% (Table 3).

Thus, experiments showed that licorice preparations improve the efficiency of cytostatic therapy and inhibit the development of tumor process after removal of the major tumor node, thus producing antimetastatic effect or preventing relapses. Licorice extract inhibits the growth of ET and suppresses the development of metastases in mice with LLC. Antitumor activity of licorice was also demonstrated by other authorities [8].

TABLE 3. Effect of Glyciram on Metastasizing of LLC after Removal of Tumor Node in C57Bl/6 Male Mice

Group	Number of animals	Weight of tumor, g	Number of mice with relapses, %	Weight of lungs, mg	Incidence of metastasizing, %	Number of metastases	Area of metastases, mm^2	IMI, %
Surgery on day 12 after tumor transplantation								
control	15	2.9±0.2		563.7±48.7	100	71.7±7.3	202.51±45.36	
surgery	10	1.5±0.2	90	389.0±42.4*	90	21.9±5.1*	95.67±34.85**	73
surgery+glyciram (25 mg/kg) 15 times (starting from day 12)	14	2.2±0.4	43+	376.0±31.1	100	18.7±3.9	80.39±24.73	74
Surgery on day 16 after tumor transplantation								
control	20	3.9±0.4		382.8±25.1	100	52.4±7.1	99.89±19.59	
surgery	14	1.1±0.2	50	363.4±37.6	100	29.1±8.2*	93.28±33.91	44
surgery+glyciram (25 mg/kg) 12 times (starting from day 16)	18	2.1±0.5	61	264.5±11.1++	94	14.2±3.6+	20.38±4.69	75

Note. Treatment schedules are presented: dose and number of injections. The tumor was transplanted subcutaneously (4-6*10^6 cells in 0.2 ml physiological saline). Experiment was continued until days 30 (term 1) and 31 (term 2) after transplantation. The weight of metastases was determined by the difference between the weight of the lungs in experimental and healthy animals (196.0±7.2 mg). *$p<0.01$, **$p<0.05$ compared to the control, +$p<0.01$, ++$p<0.05$ compared to operated animals.

Licorice preparations exhibit immunomodulating activity: increase proliferative activity of splenocytes, stimulate the formation of cytotoxic T cells in the spleen, increase cytotoxic activity of peritoneal macrophages, and alleviate cytostatic-induced immunosuppression [5,6]. It was demonstrated the glcyrrhizin *in vitro* stimulates IL-2 secretion by T cells and enhances expression of receptors for this cytokine. Glycyrrhizinic and glycyrrhetic acid inhibit corticosteroid metabolism and produce an anti-inflammatory effect. Tumor growth is associated with the formation of an inflammatory focus around the tumor node and increased level of proinflammatory cytokines. In light of this, we can hypothesize that the decrease in hormone content and attenuation of inflammation can lead to activation of immunocompetent cells. This in turn leads to enhanced production of glucocorticoids and immunosuppression. Tumor progression is associated with enhanced production of free radicals and reduced activity of the antioxidant system. These conditions provoke activation of free-radical oxidation. The improvement of antitumor resitance of the organism under the effect of licorice preparations can be determined by antioxidant properties of glycyrrhizinic acid. Study of antiradical activity of this substance and its effect on oxidase activity of neutrophils showed that it decreased generation of reactive oxygen species by neutrophils, while preincubation of cells with glycyrrhizinic acid reduced the formation of free radicals and increased the level of reduced glutathione [2]. Glyciram can decrease vascular permeability, which can considerably modulate the dissemination process.

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