# Non-Starch Polysaccharides as Correctors in Cytostatic Therapy of Experimental Tumors

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We studied the effects of low-esterified pectin, calcium pectate, and alginate on the development of transplanted Ehrlich adenocarcinoma and Lewis pulmonary carcinoma and on the efficiency of cyclophosphamide treatment in mice.

**Key Words:** *transplanted tumors; cytostatic therapy; non-starch polysaccharides*

Cytostatics are the main drugs for the treatment of oncological patients. The majority of antiblastic drugs produce toxic effect on actively regenerating cell systems, which limits their effective use. Therefore, the search for new means increasing selective effect of cytostatics is a pressing problem in tumor chemotherapy.

A variety of pharmacological properties of pectins suggest them as a potential source of new drugs: these compounds reduce the levels of serum cholesterol and LDL [6] and intensity of endogenous intoxication syndrome, bind heavy metals, stimulate excretion of bile acids [2], exhibit antimutagenic activity, inhibit proliferation of malignant cells [9], and regulate functional activity of the immune system [1].

We studied the effects of non-starch polysaccharides (low-esterified pectin, calcium pectate, and alginate) on the development of Ehrlich adenocarcinoma (EAC) and Lewis pulmonary carcinoma (3LL) in mice and on the efficiency of cyclophosphamide treatment of animals with these tumors.

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## MATERIALS AND METHODS

The study was carried out on 145 outbred mice and 170 female certified C57Bl/6 mice from Laboratory of Experimental Biosimulation, Institute of Pharmacology. EAC was transplanted intraperitoneally in a dose of $7.5\times10^6$ cells in 0.2 ml saline. Solid 3LL was transplanted intramuscularly as tumor tissue homogenate ($5\times10^6$ tumor cells in 0.1 ml saline) [3]. Low-esterified pectin was prepared by alkaline de-esterification of commercial highly esterified citrus pectin (Copenhagen Pectin A/S, Lille Skensved). Calcium alginate (77.3% alginic acids, 72.5 mg/g calcium; mol. weight 403 kDa; experimental series I), low-esterified pectin (69.0% anhydrogalacturonic acid, 1.2% esterification; mol. weight 39.3 kDa, ion exchange capacity 3.92 mg-eqv/g; experimental series II), and calcium pectate (67.3% anhydrogalacturonic acid, less than 1.2% esterification; 38 mg/g calcium; mol. weight 39.3 kDa; experimental series III), were dissolved in distilled water and administered into the stomach through a tube in doses of 50 and 100 mg/kg to mice with EAC daily for 7 days (first dose 24 h after transplantation) and to animals with 3LL for 12 days (first dose on day 7 of tumor development). According to published data, 50-250 mg/kg are affective doses for non-starch polysaccharides [4,5].

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Cyclophosphamide was injected in a single dose of 150 mg/kg to mice with EAC 72 h after transplantation and in a single dose of 125 mg/kg to mice with 3LL on day 13 of tumor development. Controls were injected with solvents. On day 3 after cytostatic injection, peripheral blood leukocytes were quantified in mice with EAC. The animals were sacrificed by cervical dislocation. The volume of tumor cells in mice with EAC was evaluated after 5-min centrifugation of ascitic fluid in a graduated tube at 3000 rpm; in mice with 3LL the weight of the first tumor node, number of metastases per animal, mean area of metastases, percent of tumor growth inhibition, incidence of metastases, and metastasis inhibition index were estimated [1,7,10]. The significance of results was evaluated using nonparametrical Mann—Whitney and exact Fisher's tests [3,7].

## RESULTS

Cyclophosphamide injected to female mice with EAC promoted a significant (1.7-2.5 times) decrease in the volume of tumor cells in ascitic fluid in comparison with untreated animals. Isolated treatment with calcium alginate or pectate inhibited tumor growth only in a dose of 100 mg/kg: tumor cell volume decreased significantly 1.3 and 1.9 times, respectively, in comparison with the control (Table 1). Low-esterified pectin did not modify the

**TABLE 1.** Effects of Non-Starch Polysaccharides on the Development of EAC in Outbred Mice and Efficiency of Cyclophosphamide Treatment

| Group | Dose/interval | Leukocyte count (g/liter; $X\pm m$) | Tumor cell volume (ml; $X\pm m$) | Tumor growth inhibition, % |
|---|---|---|---|---|
| Calcium alginate (series I) | | | | |
| control ($n$=10) | — | 17.78±1.42 | 1.03±0.06 | — |
| cyclophosphamide ($n$=10) | 150 mg/kg/72 h (1)[x] | 2.75±0.19* | 0.42±0.07* | 59 |
| Ca alginate-50 ($n$=10) | 50 mg/kg/24 h (7) | 18.30±0.64 | 0.86±0.09 | 17 |
| cyclophosphamide+Ca alginate-50 ($n$=10) | 150 mg/kg/72 h (1)<br>50 mg/kg/24 h (7) | 3.28±0.29 | 0.62±0.08** | 40 |
| Ca alginate-100 ($n$=10) | 100 mg/kg/24 h (7) | 18.52±0.77 | 0.82±0.08* | 20 |
| cyclophosphamide+Ca alginate-100 ($n$=10) | 150 mg/kg/72 h (1)<br>100 mg/kg/24 h (7) | 3.35±0.31 | 0.79±0.07** | 23 |
| Calcium pectate (series II) | | | | |
| control ($n$=5) | — | 58.63±7.99 | 1.20±0.09 | — |
| cyclophosphamide ($n$=7) | 150 mg/kg/72 ч (1) | 16.20±2.22* | 0.70±0.14* | 42 |
| Ca pectate-50 ($n$=7) | 50 mg/kg/24 h (7) | 81.73±6.04 | 0.89±0.11 | 26 |
| cyclophosphamide+Ca pectate-50 ($n$=9) | 150 mg/kg/72 h (1)<br>50 mg/kg/24 h (7) | 6.92±1.30** | 0.67±0.17 | 44 |
| Ca pectate-100 ($n$=7) | 100 mg/kg/24 h (7) | 78.57±5.30 | 0.63±0.17* | 48 |
| cyclophosphamide+Ca pectate-100 ($n$=7) | 150 mg/kg/72 h (1)<br>100 mg/kg/24 h (7) | 7.23±0.91** | 0.63±0.14 | 48 |
| Low-esterified pectin (series III) | | | | |
| control ($n$=5) | — | 58.63±7.99 | 1.20±0.09 | — |
| cyclophosphamide ($n$=7) | 150 mg/kg/72 h (1) | 16.20±2.22* | 0.70±0.14* | 42 |
| pectin-50 ($n$=7) | 50 mg/kg/24 h (7) | 74.73±13.92 | 1.30±0.12 | +8 |
| cyclophosphamide+pectin-50 ($n$=8) | 150 mg/kg/72 h (1)<br>50 mg/kg/24 h (7) | 10.18±2.22** | 0.85±0.24 | 29 |
| pectin-100 ($n$=8) | 100 mg/kg/24 h (7) | 90.73±3.04* | 1.26±0.16 | +5 |
| cyclophosphamide+pectin-100 ($n$=8) | 150 mg/kg/72 h (1)<br>100 mg/kg/24 h (7) | 8.67±2.7** | 0.41±0.06** | 66 |

**Note.** Here and in Table 2: $n$: number of animals. [x]In parentheses: number of injections. $p<0.05$ *vs.* *control, **cyclophosphamide group.

growth of EAC, but being administered in a dose of 100 mg/kg in combination with the cytostatic improved treatment efficiency: the volume of tumor cells decreased 1.7 times ($p<0.05$) in comparison with animals injected with the antiblastic drug alone. On the other hand, the use of calcium pectate in combined therapy did not modulate the antitumor effect of the cytostatic, while calcium alginate abolished the effect of cyclophosphamide on the tumor.

The content of leukocytes in the peripheral blood of mice with EAC treated with low-esterified pectin (100 mg/kg) was 1.5 times higher ($p<0.01$) than in controls (Table 1). The growth of EAC in mice (experimental series II and III) was associated with pronounced leukocytosis: the content of white blood cells reached 58.63±7.99 g/liter on day 7 after transplantation. Chemotherapy 3.6-fold decreased the leukocyte count ($p<0.01$) in comparison with the control. Combined treatment with cyclophosphamide and calcium pectate or low-esterified pectin promoted decrease this parameter to values observed in intact animals (7-8 g/liter).

Cyclophosphamide inhibited tumor dissemination in all experiments on C57Bl/6 females with 3LL and inhibited the growth of the main tumor node in experimental series II and III.

Monotherapy with low-esterified pectin and calcium pectate in a dose of 100 mg/kg significantly decreased the weight of the main tumor node, but did not inhibit dissemination of 3LL tumor. Treatment with calcium alginate in the same dose increased the number of metastases in the lungs in comparison with the control (Table 2).

Cyclophosphamide treatment caused no statistically significant decrease in the number of animals with metastatic involvement of the lung tissue (series II and III), while addition of calcium pectate (50 mg/kg) and low-esterified pectin (50 and 100 mg/kg) to the treatment protocol led to a significant reduction in the incidence of metastases in comparison with controls, with a trend to a decrease in the number and area of metastatic nodes. Similar effects were observed after treatment with a combination including calcium alginate (50 mg/kg): the number and area of metastases decreased by 2.3 and 5.8 times, respectively. The incidence of 3LL metastases in this group was minimum (56%, Table 2). The highest index of inhibition of the metastatic process was observed in animals receiving cyclophosphamide with calcium pectate (50 mg/kg), calcium alginate, and low-esterified pectin.

Thus, calcium alginate and pectate used alone in a high dose (100 mg/kg) inhibited tumor growth in mice with EAC, but did not modulate the antitumor effect of cyclophosphamide. Low-esterified pectin alone did not modify the development of this tumor, but used in a dose of 100 mg/kg in combination with the cytostatic stimulated its antiblastic effect. Monotherapy with low-esterified pectin and calcium pectate in a dose of 100 mg/kg inhibited the growth of the main tumor node in animals with metastasizing 3LL. Combined treatment with cyclophosphamide and calcium pectate (50 mg/kg) and low-esterified pectin (in both doses) reduced the number of animals with metastases in the lungs in which the cytostatic failed to reduce the incidence of metastases. Combination of the cytostatic with calcium alginate (50 mg/kg) decreased the area of metastatic involvement, the incidence of metastases was minimum.

Calcium alginate and pectate produced a significant inhibitory effect on the growth of the primary tumor node only in a dose of 100 mg/kg. The same dose of these compounds potentiated the antitumor effect of the cytostatic. On the other hand, both doses of non-starch polysaccharides increased the antimetastatic activity of cyclophosphamide.

Presumably, the capacity of non-starch polysaccharides to inhibit the growth of transplanted tumors or potentiate the effect of cytostatic therapy is mediated through the function of galectin-3, which promotes migration and morphogenesis of endothelial cells, vascularization, and angiogenesis [8, 10]. Pectins can directly block adhesion of galectin-3 and tumor endothelial cell receptors. This interaction is confirmed by reduction of the number of tumor-associated blood vessels under the effect of some pectins [10]. High antimutagenic activity of pectins was demonstrated in experiments with nitroaromatic compounds [11]. Hence, our findings are in line with published data on the capacity of pectins to inhibit the growth of primary tumor node and metastases in induced and transplanted tumors [11]. The status of the blood clotting system plays an important role in the dissemination process. Non-starch polysaccharides due to their anticoagulant effects normalize enhanced blood clotting, thus reducing the intensity of metastatic process. An important factor in the mechanism of antimetastatic effect is pectin capacity to reduce cholesterol and plasma fibrinogen content, which results in modification of the qualitative characteristics of fibrin network, becoming more permeable and prone to lysis. The increase in the efficiency of cytostatic therapy under the effect of non-starch polysaccharides observed in our study is presumably mediated through immune system activation in animals with tumors [1].

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**TABLE 2.** Effects of Non-Starch Polysaccharides on 3LL Development in C57Bl/6 Mice and Efficiency of Cyclophosphamide Treatment

| Group | Tumor weight (g; $X \pm m$) | Tumor growth inhibition or stimulation (+), % | Incidence of metastases, % | Number of metastases per mouse ($X \pm m$) | Area of metastases per mouse ($mm^2$; $X \pm m$) | Lung weight (mg; $X \pm m$) | Metastasis inhibition index, % |
|---|---|---|---|---|---|---|---|
| Calcium alginate (series 1) | | | | | | | |
| control ($n$=10) | 5.69±0.47 | — | 100 | 13.40±1.71 | 19.26±4.15 | 209.90±0.51 | — |
| cyclophosphamide 125 mg/kg (1)$^x$ ($n$=9) | 4.70±0.35 | 17 | 78* | 3.78±1.33* | 2.54±1.33* | 208.78±13.18 | 78 |
| Ca alginate 50 mg/kg (12) ($n$=10) | 5.83±0.31 | +2 | 100 | 20.90±2.24* | 24.18±4.16 | 226.30±8.39 | -56 |
| cyclophosphamide+Ca alginate 50 mg/kg (12) ($n$=9) | 4.47±0.28* | 21 | 56* | 1.67±0.87* | 0.44±0.23** | 184.00±6.09* | 93 |
| Ca alginate 100 mg/kg (12) ($n$=10) | 5.91±0.26 | +4 | 100 | 24.50±2.49* | 53.03±13.85* | 255.90±18.85 | -83 |
| cyclophosphamide+Ca alginate 100 mg/kg (2) ($n$=10) | 4.89±0.25 | 14 | 90 | 3.00±0.76 | 2.62±0.80 | 204.55±6.76 | 80 |
| Calcium pectate (series 2) | | | | | | | |
| control ($n$=9) | 6.29±0.25 | — | 100 | 24.00±0.36 | 45.05±11.24 | 252.00±0.39 | — |
| cyclophosphamide 125 mg/kg (1) ($n$=8) | 4.39±0.46* | 30 | 88 | 5.00±1.66* | 3.02±1.55* | 186.38±2.99* | 82 |
| Ca pectate 50 mg/kg (12) ($n$=9) | 5.44±0.61 | 14 | 100 | 24.67±4.02 | 36.22±11.71 | 235.44±23.25 | +3 |
| cyclophosphamide+Ca pectate 50 mg/kg (12) ($n$=10) | 4.75±0.30 | 24 | 70* | 4.10±1.54 | 2.09±0.81 | 187.50±6.49 | 88 |
| Ca pectate 100 mg/kg (12) ($n$=11) | 5.06±0.43* | 20 | 100 | 21.18±2.80 | 33.67±10.00 | 231.27±13.17 | 12 |
| cyclophosphamide+Ca pectate 100 mg/kg (12) ($n$=8) | 4.29±0.36 | 32 | 88 | 5.75±1.77 | 4.14±1.37 | 213.88±12.12 | 79 |
| Low-esterified pectin (series 3) | | | | | | | |
| control ($n$=9) | 6.29±0.25 | — | 100 | 24.00±0.36 | 45.05±11.24 | 252.00±0.39 | — |
| cyclophosphamide 125 mg/kg (1) ($n$=8) | 4.39±0.46* | 30 | 88 | 5.00±1.66* | 3.02±1.55* | 186.38±2.99* | 82 |
| pectin 50 mg/kg (12) ($n$=9) | 6.21±0.27 | 1 | 100 | 18.44±4.79 | 31.73±10.58 | 213.89±13.77 | 23 |
| cyclophosphamide+pectin 50 mg/kg (12) ($n$=11) | 3.81±0.37 | 39 | 82* | 3.91±1.51 | 3.55±1.64 | 199.91±5.77 | 87 |
| pectin 100 mg/kg (12) ($n$=11) | 5.58±0.24* | 11 | 100 | 25.60±3.40 | 52.99±22.84 | 245.81±13.71 | -7 |
| cyclophosphamide+pectin 100 mg/kg (12) ($n$=9) | 5.19±0.37 | 17 | 78* | 4.44±1.87 | 2.67±0.98 | 201.33±5.67 | 86 |

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