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Effect of Low Molecular Weight Pectin and Metformin on the Antitumour Effect of Chemopreventive Agents in Rats with Walker's Carcinosarcoma

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Abstract:-

Introduction and Objective: Currently, the increase in the effectiveness of antitumour treatment of patients with oncological pathology of various localizations is associated with the use of multicomponent chemotherapy regimens. However, the cost of innovative drugs is excessively high and, according to experts of the World Health Organisation, no state is able to fully compensate patients for the costs of treatment with immunological agents. Therefore, there is growing interest in evidence-based pharmacocorrection as a promising way to improve the effectiveness of anti-blastoma treatment. According to the results of numerous screening studies it was found that flavonoids, alkaloids, glycosides can be used to suppress the growth of the main tumour node and dissemination process both at isolated administration and in combination with cytostatics, and water-soluble polysaccharides turned out to be the most effective. One plant remedy is low molecular weight pectin. Pectins have been found to have not only antitumour potential, but also the ability to enhance the antitumour activity of conventional cytostatics. The aim of the study is to improve the efficacy of conventional therapies for malignant diseases.

Materials and methods: The main research methods included determination of antitumour activity of preparations on models of transplanted rat tumours, morphologically and statistically. Experimental therapy was carried out on white mongrel rats and Wistar rats. The tumour was transplanted subcutaneously. Pectin and metformin were administered by probe, intragastrically. Cytostatics were administered intraperitoneally. Antitumour activity was assessed by TGI (tumour growth inhibition), ILE (increased life expectancy) and ALE (average life expectancy).

Results: The combination of metformin, pectin and chemopreparations (methotrexate and oxaliplatin) resulted in a significant antitumour effect with an increase in life expectancy. The combination of metformin and pectin with methotrexate increased the life expectancy of animals, whereas in monotherapy animals died earlier than in the control group without treatment, which indicates the ability of the combination to reduce the toxicity of cytostatics. Combinations of pectin + paclitaxel and metformin + paclitaxel showed significant antitoxic effect. When doxorubicin was combined with pectin, the TGI was 65.89 and 62.64%, which is quite high in the therapy of Walker carcinosarcoma. In experiments with fluorouracil, high doses of the drug proved toxic to tumour-bearing animals. The combination of fluorouracil with metformin showed the best TGI and ILE rates (80.65% and 89.9%, respectively). The combination of fluorouracil with pectin showed high TGI (59.7). For the combination of pectin + gemcitabine at a dosage of 50mg/kg, the TGI (89.5% to 98.48% on days 9 and 21) and ILE (134.26%) showed a mutual influence of the drugs' properties in the direction of enhancing the antitumour effect, whereas the gemcitabine monotherapy showed lower rates (TGI 66.2% and ILE 80.21%). The combination of all 3 drugs had

a good antitumour effect at gemcitabine dosage of 25mg/kg (TGI - 83.05%, ILE - 157.3%), whereas at gemcitabine dosage of 50mg/kg the TGI was 49.3% and ILE - 15.73%. Similar conclusions can be drawn when etoposide was combined with pectasol. At high TGI at the beginning of the experiments with both monotherapy and drug combination, the survival rate of rats was higher when pectasol was administered in combination with etoposide.

Keywords*: low molecular weight pectin, pectasol, Walker's carcinosarcoma, synergy, metformin, oxaliplatin, gemcitabine, paclitaxel, doxorubicin, fluorouracil*

1. Introduction

According to the World Health Organization (WHO), 9.6 million people die from malignant neoplasms per year and cancer accounts for almost one in six deaths worldwide (Stewart BW, Wild CP, editors. World cancer report 2020. Lyon: World Health Organisation (WHO). Global Health Estimates 2020: Deaths by Cause, Age, Sex, by Country and by region, 2000-2019. WHO; 2020. Accessed December 11, 2020). Today, despite the development of such areas as targeting and immunotherapy, chemotherapy is still a key step in the treatment of cancer patients. It should be noted that cytostatic treatment is not always effective against solid tumours, which, as a rule, are biologically heterogeneous, are capable of steady growth and metastasis, and have a low percentage of dividing cells that are a target for cytostatics. In addition to the lack of efficacy, another problem in the use of chemotherapeutic agents is their toxicity, which is even greater when two or more cytostatics are used. Currently, the increase in the effectiveness of antitumour treatment of patients with cancer pathology of various localizations is associated with the use of multicomponent chemotherapy regimens. However, the cost of innovative drugs is excessively high and, as experts of the WHO believe, no state is able to fully compensate patients for the costs of treatment with immunological agents. One of the approaches to solving the problem of increasing the efficacy and reducing the toxicity of antitumour therapy is the inclusion of biologically active substances isolated from plant raw materials into treatment regimens. In recent studies it has been shown that drugs that can be used to modify tumour metabolism have different indications for prescription and previously had no application in oncology. Such an example would be the use of the most common antidiabetic drug, metformin (dimethylbiguanide), in oncology. The mechanisms of action of metformin are multidirectional and not fully understood. However, the leading concept is its effect on adenosine monophosphate kinase (AMPK) activity. There is evidence of metformin's effects on cancer stem cells. It has been reported that metformin can selectively reduce the number of cancer stem cells and suppress tumour development []. The present study investigates whether low molecular weight pectin and metformin can improve the efficacy and reduce the toxicity of chemotherapy.

2. Materials And Methods

Animals. Studies were performed on 180 white mongrel rats and Wistar rats.

Transviable strain. Walker's carcinosarcoma 256 was obtained from Krasnoshtanov V.K., Research Institute of Oncology and Radiology, Ministry of Health of the Republic of Kazakhstan. The transplantation was carried out according to the generally accepted method by homogeneous suspension of 0.5 ml in dilution (1:10) subcutaneously in the thigh area.

3. Preparations and routes of administration

Experimental therapy with pectin (Pectasol - citrus low molecular weight pectin - commercial product of Econugenics, PectaSol-C) was carried out at a dose of 650 mg/kg per os (intragastrically) for 7 days, starting from 3 days after tumour transplantation. Metformin, a commercial preparation (Insufor 500mg, Turkey) was administered at a dosage of 25mg/kg intragastrically for 7 days. Cyclophosphan (Russia, Saransk) was administered intraperitoneally at a dosage of 25mg/kg, methotrexate (Onkotek Pharma, Germany) - intraperitoneally at a dosage of 10mg/kg and 1mg/kg once-daily, oxaliplatin (Kosak Pharma, Turkey) intraperitoneally at a dosage of 8-4-2 mg/kg. Paclitaxel (Ebeve, Austria) was administered intraperitoneally, in doses of 15, 25, 50, 100mg/kg, Doxorubicin (Veropharm, Russia) was administered intraperitoneally in doses of 1.5 and 3mg/kg. Fluorouracil, Ebewe pharma, ges.m.b.h.nfg.kg (Austria) - intraperitoneally in doses of 15-45

mg/kg once daily. In the repeated series of experiments paclitaxel was administered intraperitoneally in doses of 2.5 and 5 mg/kg, fluorouracil 15mg/kg, oxaliplatin 2mg/kg. Etoposide (LENS-PHARM, LLC (Russia) was administered intraperitoneally at a dosage of 15-30 mg/kg. Gemcitabine ("Ebewe" Gemcitabin "Ebewe", Austria) was administered at a dose of 25-45mg/kg intraperitoneally.

4. Methods

When assessing the antitumour potential, we used generally accepted indicators of treatment efficacy assessment: tumour growth inhibition (TGI %), increased life expectancy (ILE %) on days 10, 14, 17 after tumour transplantation, as well as counted the number of cured animals, which was carried out not earlier than 90 days after the end of the course of therapy. The antitumour effect was evaluated by the difference in mean tumour volumes (V cr, cm3), tumour growth inhibition (TGI), average life expectancy (ALE, days) of animals treated with the drug compared to the control animals and increased life expectancy (ILE).

1. Tumour growth inhibition (TGI): (Vk-Vk-Vo)/Vk×100%,

where Vk is the average tumour volume in the control group, Vo is the average tumour volume in the experimental group;

2. Increased life expectancy (ILE)=(ALEo-ALEk)/(ALEk)x100%,

where ALEk - average life expectancy in the control, ALEo - average life expectancy in the experimental group.

Statistical processing of the results was carried out using nonparametric Wilcoxon-Mann-Whitney criteria, differences were considered reliable at $p < 0.05$.

5. Research results

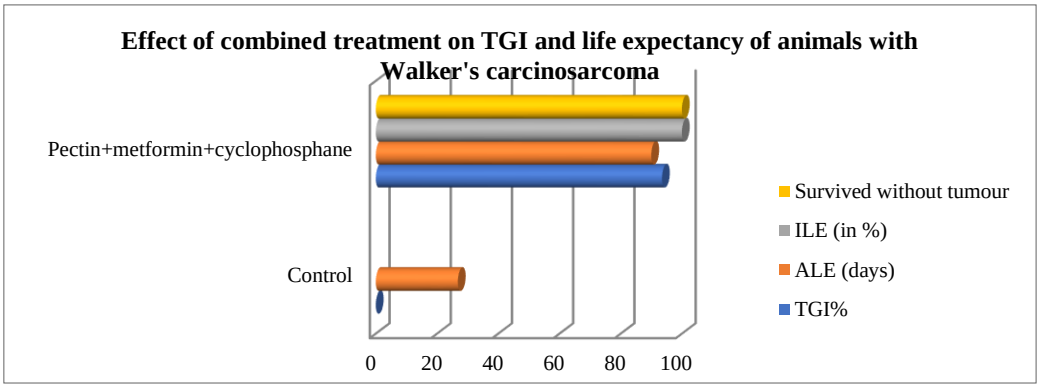
Combined effects of pectin, metformin and cyclophosphane on Walker's carcinosarcoma

Combined and separate effects of metformin, pectin and cyclophosphan on Walker's carcinosarcoma tumour were tested. Metformin was administered intragastrically via a probe at a dose of 25 mg/kg for 6 days. It was combined with pectin and cyclophosphan. The results of the experiments are presented in Table1.

Table 1. Effect of combined treatment on TGI and life expectancy of animals with Walker's carcinosarcoma.

Group of animals	TGI%	ALE (days)	ILE (in %)	Survived without tumour
Control	--	26,8		
Pectin+metformin+cyclophosphane	93,5	90,0	100	100

Diagram 1. Effect of combined treatment on TGI and life expectancy of animals with Walker's carcinosarcoma.



When metformin, pectin and cyclophosphan were administered, the TGI was 93.54%, 100%, 100% on days 10,18 and 25 after tumour transplantation, respectively. Earlier in our studies, we observed high TGI rates with combined administration of pectin and cyclophosphane, or with tumour exposure to pectin. In this case, the high TGI results with metformin monotherapy required repeated experiments for reproducibility of the results, although there are

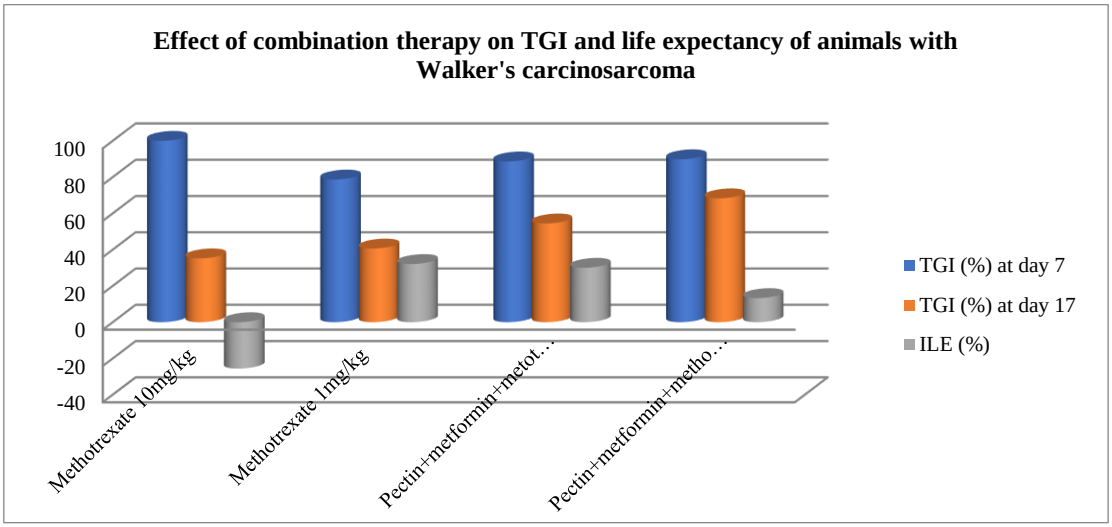
no studies in the literature yet, with administration of high doses of metformin to animals with inoculated tumour. The TGI with the combined treatment was 100% (Table 5).

In the next series of experiments, preliminary tests of the combined and separate effects of **metformin, methotrexate and pectasol** on the Walker carcinosarcoma tumour were performed.

Table 2. Effect of combination therapy on TGI and life expectancy of animals with Walker's carcinosarcoma.

Group of animals	TGI (%) at day 7	TGI (%) at day 17	ILE (%)
Methotrexate 10mg/kg	100	35,2	-25,6
Methotrexate 1mg/kg	78,67	40,62	32,06
Pectin+metformin+metotrexate (10mg/kg)	88,65	54,44	30
Pectin+metformin+methotrexate (1mg/kg)	89,84	68,3	13,29

Diagram 2. Effect of combination therapy on TGI and life expectancy of animals with Walker's carcinosarcoma.



Combined administration of methotrexate (10 mg/kg), pectin and metformin had an advantage over methotrexate monotherapy as the antitumour effect was 88.65%, 54.44% and 53.88% on days 10, 17 and 24 respectively.

Combined effects of pectin, metformin and paclitaxel on Walker's carcinosarcoma

The results of the experiments are presented in Table 3.

Table 3: Determination of toxic doses of paclitaxel in monotherapy and in combination with pectin and metformin

Group	Average tumour size in cm3			Animal deaths after drug administration
	Day 8	Day 16	Day 25	
Control	2,209 ±0,42	14,2±1,22	14,72±3,18	100% alive at day 25
Paclitaxel 10mg/kg	–	–	–	62,5% fell at days 1-4
Pectin + paclitaxel 10mg/kg Inhibition of tumour growth %	0,736±0,19	3,95±0,91	0	50% tumour free 50% fell at day 23 день
	66,68%	72,18%	100%	
Metformin+ paclitaxel 10mg/kg Inhibition of tumour growth %	0,98±0,30	4,26±2,43	4,37±2,10	57,1% fell at day 23
Inhibition of tumour growth %	55,50%	69,96%	70,27%	

Pectin+ paclitaxel 25mg/kg	–	–	–	100% fell at days 3-6
Pectin+ metformin+paclitaxel 25mg/kg	–	–	–	100% fell at day 1
Pectin+ metformin+paclitaxel 10mg/kg	–	–	–	100% fell at days 1-4
Pectin+ metformin+paclitaxel 25mg/kg	–	–	–	100% fell at days 1-3
Level of significance p	<0,05*			

* Wilcoxon-Mann-Whitney test

When paclitaxel was administered at doses of 25 and 50mg/kg, the animals of the group fell on the first day after the drug administration. Animals of the group of combined administration of pectin, metformin and paclitaxel also fell. When the dose of paclitaxel was reduced to 10 mg/kg, the TGI for the pectin + paclitaxel combination was 66.68, 72.18%, and 100% on days 10, 18 and 25 of Walker tumour transplantation, respectively. When metformin and paclitaxel were administered, the TGI for the combination was 55.5, 69.96%, and 70.2 on days 10, 18 and 25 of Walker tumour transplantation respectively.

Combined effects of pectin, metformin and doxorubicin on Walker's carcinosarcoma.
The following series of experiments tested the combined and separate effects of **doxorubicin, pectasol and metformin** on Walker's carcinosarcoma tumour.

Table 4. Combined effect of doxorubicin (1.5 mg/kg), pectasol and metformin (200 mg/kg) on the growth of Walker's carcinosarcoma tumour

	No.	Day 10	Day 18	Day 25
		cm3 volume	cm3 volume	cm3 volume
Pectin+ doxorubicin+metformin	1	2,5935	0,48	
	2	1,536	3,96	4,4
	3	0	8,7	10,56
	4	0,968	0,7425	15,84
	5	1,078	0	
	6	0,0625	0,588	
	7	0,108	0	
	8	0		
Total		6,345	14,47	30,8625
Average		0,793	2,41	7,715625
Standard devaiation		0,880893143	11,51996438	0,0625
Representativeness error		0,331830744	1,282851978	3,46000517
TGI%		71,10%	30,74%	-152,067381

Table 5. Combined effects of pectin (650mg/kg) and doxorubicin (1.5mg/kg) on Walker's carcinosarcoma

	No.	Day 10	Day 18	Day 25
		cm3 volume	cm3 volume	cm3 volume
Pectin+ doxorubicin	1	0,924	1,701	0,256
	2	0,0625	0,336	0,576
	3	0	2,394	0,605
	4	3,872	2,7405	1,12
	5	0	0,56	1,6
	6	1,0725	0,256	0,0625
	7	0	1,17	
	8	1,56	0,972	
Total		7,49	10,129	4,2195
Average		0,936	1,266	0,70325
Standard devaiation		1,774219625	0,873549567	0,32285598
Representativeness error		0,470932536	0,330444694	0,23196838
TGI%		65,89%	62,64%	77,0250128

Tumour growth inhibition when doxorubicin at a dosage of 1.5mg/kg and pectin 650mg/kg was administered was 65.89% and 62.64% on days 10 and 17 after tumour transplantation, respectively (Table 5). The TGI when doxorubicin 1.5mg/kg and metformin 200mg/kg were administered was 67.46% and -25.05% on days 10 and 17 after tumour transplantation respectively (Table 4). Thus, the findings suggest that co-exposure to pectin, metformin and doxorubicin has a weak anti-tumour effect on Walker 256 carcinosarcoma, with a negative TGI on day 24 of the study. The combined therapy of doxorubicin with metformin, with sufficiently high TGI on days 7, 14 and 24, was more toxic for animals of the experimental group (ILE 28.87%). The doxorubicin+pectin combination turned out to be the most optimal. When the dose of the chemopreventive agent was reduced by 2 times, the antitumour effect and ILE were higher than in the group with doxorubicin monotherapy.

6. Discussion

Summarising the results of the experiments, we can note the results of the combined effect of the chemopreventive drug, pectin and metformin. Combinations of pectin + paclitaxel and metformin + paclitaxel showed a significant antitoxic effect when administering large doses of the drug. When the dosage of paclitaxel was decreased, antitumour effect of both drug combinations was observed. The results of the experiment demonstrated a clear effect of paclitaxel toxicity reduction when administered in high doses in combination with pectasol. The animals of the experimental groups remained alive. Further, it is necessary to select the doses of the drug in combination with pectin and metformin to obtain high TGI and ILE. And also to select the least toxic doses of paclitaxel with good antitumour effect in combination with therapy modifiers.

Doxorubicin at 1.5 mg/kg dosage showed poor antitumour effect (TGI 26.38-28.35%), at 3 mg/kg dosage the TGI was higher (42.64 and 47.35%), whereas when combined with pectin the TGI was 65.89 and 62.64%, which is quite high in the therapy of Walker carcinosarcoma. Combined use of doxorubicin and metformin and all 3 drugs (doxorubicin, pectin, metformin) on day 10 showed good antitumour effect (67.46% and 71.1%), but on day 18 and 25 the rates dropped, for metformin (- 25.05%), for the combination of 3 drugs -152.06%. At combination of all 3 drugs on day 25 of therapy the effect of stimulation of tumour growth was noted, which is probably connected with minimal doses of chemopreparations. These data require repeated experiments to exclude random factors influencing the results of experiments. Nevertheless, it can be stated that the combination of pectin with doxorubicin showed the effect of enhancing the antitumour effect. In the future, it is necessary to find out the directionality of the combination effect (synergy, additivity).

7. Conclusion

Combination therapy of pectin with metformin in several series of experiments revealed a high antitumour effect. The combination of metformin, pectasol and chemopreventive agents showed a significant antitumour effect with an increase in life expectancy. Monotherapy with methotrexate at a dosage of 10 mg/kg was toxic. Animals of this group died earlier than rats of the control group (ILE -24, 26%), whereas in the group of combined therapy (pectasol, methotrexate and metformin) ILE was 30%, which indicates a pronounced ability of the combination of pectin with metformin to reduce the toxicity of chemopreparations. Combinations of pectasol + paclitaxel and metformin + paclitaxel showed a significant antitoxic effect when administering high doses of the drug (10, 15, 25 mg/kg). The combinations, compared to the control, statistically significantly increased the ALE of rats. The minimum effective dose of paclitaxel (2 mg/kg) in combination with pectasol was selected (TGI - from 41% to 98.88%). When combined therapy with doxorubicin, the combination of doxorubicin+pectasol proved to be the most optimal. When the dose of the chemopreventive agent was reduced by 2 times, the antitumour effect and ILE were higher than in the group with doxorubicin monotherapy.

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