ORIGINAL ARTICLE

Efficacy of Modulated Pectin and Metformin on Walker Carcinosarcoma Progression in Rats Undergoing Chemotherapy with Doxorubicin and Fluorouracil

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Abstract

Background: Cancer has caused nearly 10 million deaths globally in 2020, accounting for one-sixth of all fatalities. Although chemotherapy can be effective in some cases, it often fails to treat solid tumors effectively because of their biological diversity and uncontrolled growth. Chemotherapy can also be toxic, particularly when multiple drugs are used. This study aimed to investigate the potential of combining low-molecular-weight pectin and metformin with chemotherapy in Walker carcinoma cells. **Materials and Methods:** This study was conducted using white-outbred Wistar rats. Tumors were transplanted subcutaneously, and the animals were then treated with pectin and metformin administered intragastrically using a probe, whereas cytostatic agents were administered intraperitoneally. Antitumor activity was assessed based on three parameters: tumor growth inhibition (TGI), survival/increase in life span (ILS), and average lifespan. **Results:** The combination of pectin and doxorubicin had the most significant antitumor effect (TGI 88.66% and ILS 142.22%) compared to the combination of metformin and cytostatic agents. In a series of experiments with fluorouracil, a notable antitumor effect was observed with the combination of fluorouracil and metformin (TGI, 89.6%). **Conclusion:** This study demonstrated that combined therapy with modified citrus pectin and doxorubicin led to higher antitumor effects and ILS than doxorubicin monotherapy. In addition, the combination of metformin and fluorouracil showed the potential to increase survival rates, suggesting its potential detoxifying properties.

Key words: Doxorubicin, fluorouracil, metformin, pectin, Walker carcinoma

INTRODUCTION

The increasing incidence of cancer is a global concern that cannot be ignored. This deadly disease claimed almost 10 million lives in 2020, accounting for nearly one in every six deaths.[1] Although cytostatic treatments are used to combat cancer, solid tumors that are biologically diverse and capable of rapid growth and metastasis are challenging to treat. Moreover, chemotherapy can be toxic, and its toxicity increases when combined with other drugs.[2] To improve the efficacy and reduce the toxicity of cancer treatments, biologically active plant-derived substances must be incorporated into treatment regimens.

In recent years, interest in the use of plant-derived agents in combination therapies for tumors has increased. These agents have a range of regulatory effects, low toxicity, and high bioavailability and can indirectly affect the growth and metastasis of tumor cells. Low-molecular-weight pectin is a plant-derived agent that has been found to have antitumor

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potential and the ability to enhance the effectiveness of conventional cytostatics.[3] Modified citrus pectin (MCP) is pectin with a low degree of etherification, which allows it to be absorbed into the bloodstream through the epithelium of the small intestine. MCP's anti-adhesive properties, ability to stimulate apoptosis, and induction of apoptosis of MCP make it a promising candidate for targeting several critical stages of cancer metastasis.[4,5]

The exploration of MCP and metformin as means to boost the effectiveness and minimize the toxicity of chemotherapy is a promising area of research. Although the precise mechanism of action of metformin remains unclear, it is thought to affect adenosine monophosphate-activated protein kinase activity, which is associated with cancer stem cell development and tumor growth. Research has also revealed that metformin can selectively reduce the number of cancer stem cells and suppress programmed death-1 ligand-1 expression in tumor cells.[5,6] Metformin is believed to exert antitumor effects by regulating the genes that affect immunity and signaling pathways, making it a promising candidate for antitumor therapy and offering new avenues for further investigation.

The objective of this study was to evaluate the potential for enhancing the efficacy and reducing the toxicity of chemotherapy using MCP and metformin.

MATERIALS AND METHODS

This study was conducted on 180 white outbred Wistar rats with transplantable strains. Walker 256 carcinoma cells were obtained from the Kazakh Research Institute of Oncology and Radiology in Kazakhstan. A commonly accepted transplantation technique was used, with a homogenized suspension of 0.5 mL at a dilution of 1:10 administered subcutaneously in the thigh area.

The experimental therapy with MCP (EcoNugenics Inc., United States) was administered orally at a dose of 650 mg/kg for 7 days, starting 3 days after tumor transplantation. Metformin (Franco-Indian Pharmaceuticals, India) was administered intragastrically at a dose of 25 mg/kg for 7 days. Doxorubicin (EBEWE Pharma, Austria) was administered intraperitoneally at doses ranging from 1.5 to 3.0 mg/kg. Fluorouracil (EBEWE Pharma, Austria) was administered intraperitoneally at a single dose of 15–45 mg/kg.

The effectiveness of the treatment was determined using commonly accepted indicators, including the percentage of tumor growth inhibition (TGI%) and increased lifespan (ILS%) at 10, 14, and 17 days after tumor transplantation. The number of cured animals was also recorded, but this assessment was not conducted until at least 90 days after the completion of the treatment regimen. The antitumor effect was assessed by the difference in average tumor volumes (V_{avg}, cm^3), TGI, average lifespan (ALS, days) of animals treated with the drug compared to controls, and ILS.

1. $$TGI = \frac{Vc - Ve}{Vc} \times 100\%,$$

where TGI, V_c–average tumor volume in the control group, and V_e–average tumor volume in the experimental group

2. $$ILS = \frac{(ALSe - ALSc)}{(ALSc)} \times 100\%,$$

where ILS – increased lifespan, ALS_c – average lifespan in the control group, and ALS_e – average lifespan in the experimental group.

Statistica v8.0 (StatSoft Inc., Tulsa, USA) was employed for the statistical analysis. Data are presented as *n* (%) or mean ± standard deviation. A student's *t*-test was used to assess parameter differences. Differences were considered statistically significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$ compared to the control. The Bioethics Committee of the I.K. Akhunbaev Kyrgyz State Medical Academy (Protocol No. 2, dated February 14, 2021) approved the study and ensured confidentiality of the collected data.

RESULTS

On the 7th day after transplanting the tumor strain, the following outcomes were obtained: The TGI when doxorubicin was administered at a dosage of 1.5 mg/kg was lower than that observed with a dosage of 3 mg/kg in three measurements taken at 7-day intervals. However, the ILS showed minimal variation (135.4% vs. 139.19%). With combined therapy with doxorubicin and metformin, TGI was slightly lower than with monotherapy, but the increase in lifespan was only 28.87%. The combination of pectin and doxorubicin demonstrated a more significant antitumor effect, with a TGI of 88.66% and an ILS of 142.22% [Table 1]. The combination of all three drugs at the beginning of therapy showed a high TGI of 72.28%. However, by the 24th day, the indicators dropped to negative values (–24.34%), and animal survival rates were lower than those in the other experimental groups (ILS, –3.92%).

In subsequent experiments, we administered fluorouracil in combination with pectin and metformin to rats with Walker 256 carcinoma at various dosages. As depicted in Table 2, when fluorouracil alone was used at a dosage of 15 mg/kg on the 7th day, the TGI was 88.12%. However, this decreased to 37.6% by day 14. A high TGI was achieved at a dosage of 45 mg/kg, resulting in nearly complete inhibition; however, all animals in this group died pre-maturely, around day 14.5. The combination therapy groups using pectin and fluorouracil at two dosages (15 and 45 mg/kg) showed similar results.

The combination of fluorouracil and metformin showed remarkable antitumor effects in a series of experiments, with

Table 1: Effects of the combination and monotherapy of doxorubicin, pectin, and metformin on the growth of Walker 256 carcinoma cells

Group	Tumor volume in cm^3 and TGI, %			ALS, days and ILS, %
	Day 7	Day 17	Day 24	
Control	3.81±0.85	4.86±1.08	6.20±0.59	27.71±1.91, 3.9
Doxorubicin 1.5 mg/kg	2.02±0.27, 47.07	2.50±0.86, 48.46	1.25±0.50, 79.77	65.2±3.05, 135.4
Doxorubicin 3.0 mg/kg	1.57±0.47, 58.75	2.14±0.88, 55.90	0.72±0.24, 88.37	66.28±11.88, 139.19
Doxorubicin 1.5 mg/kg+pectin	0.90±0.27, 76.29	1.26±0.33, 73.94	0.70±0.23, 88.66	67.125±9.8, 142.22
Doxorubicin 1.5 mg/kg+metformin	1.04±0.17, 72.69	2.94±0.69, 47.64	1.41±0.94, 77.17	35.71±5.8, 28.87
Doxorubicin 1.5 mg/kg+metformin+pectin	1.05±0.38, 72.28	2.89±1.5, 40.45	7.71±3.46, –24.34	26.62±3.24, –3.92
P-value	<0.05*	<0.05*	<0.05*	

TGI: Tumor growth inhibition, ILS: Increased lifespan, ALS: Average lifespan. Values were in % and Mean±SD. *P<0.05

Table 2: Effects of the combination and monotherapy of fluorouracil, pectin, and metformin on the growth of Walker 256 carcinoma cells

Group	Tumor volume in the control group, cm 3, and TGI, %, in experimental groups			ALS, days
	Day 7	Day 17	Day 24	
Control	1.07±0.32	2.45±0.70	2.99±1.32	14
Fluorouracil 15 mg/kg	88.12	53.18	37.64	14.3
Fluorouracil 45 mg/kg	97.87	98.51	100	14.5
Fluorouracil 15 mg/kg+pectin	89.80	76.77	55.03	19.6
Fluorouracil 45 mg/kg+pectin	100	96.7	–	14.6
Fluorouracil 15 mg/kg+metformin	92.47	77.26	89.6	41+
P-value	<0.05*	<0.05*	<0.05*	

TGI: Tumor growth inhibition, ILS: Increased lifespan, ALS: Average lifespan. Values were in Values were in %. *P<0.05

TGI values of 92.4%, 77.26%, and 89.6%. Notably, animals in this group were still alive on the 41st day of the experiment and had been effectively cured of their tumors.

DISCUSSION

The use of pectin, metformin, and doxorubicin together displayed a limited antitumor effect on Walker carcinoma 256, as evidenced by the negative TGI observed on day 24 of the study. Although the combination of doxorubicin and metformin showed significant TGI on days 7, 14, and 24, it also resulted in increased toxicity in the animal subjects (ILS, 28.8%). The most effective combination was found to be doxorubicin and pectin, as reducing the chemotherapy drug dosage by half resulted in higher antitumor effects and ILS than monotherapy with doxorubicin. Studies have also shown that modified pectin enhances the sensitivity of prostate cancer cells to doxorubicin and of ovarian cancer cells to paclitaxel.[7,8]

A study compared liposomal and non-liposomal doxorubicin treatments, both alone and in combination with tamoxifen, for Walker 256 carcinoma in white rats.[9] Liposomal doxorubicin administered 5 days after tumor inoculation resulted in a 43% reduction in tumor growth compared to the control group 17 days later, with similar inhibition between monotherapy and combination therapy. The combination of liposomal doxorubicin and tamoxifen was found to be the most effective, with a significant difference in tumor growth curves from day 5 and 32% inhibition.

Studies on fluorouracil have revealed that high doses are toxic to animals with tumors, despite showing antitumor effects within 24 days. Fluorouracil is a drug with toxic properties, particularly in the gastric and intestinal mucosa.[10] Pectin, when combined with fluorouracil, did not show any detoxifying or enhancing effects. The combination of fluorouracil (15 mg/kg) and metformin requires further investigation. TGI in this combination was 89.6%, whereas monotherapy had low TGI and ILS. It is crucial to determine the factors that contribute to the reduction in the fluorouracil toxicity of these drugs.

CONCLUSION

This study showed that combined therapy with MCP and doxorubicin resulted in higher antitumor effects and an ILS than doxorubicin monotherapy. The combination of metformin and fluorouracil also increased survival rates, suggesting the potential detoxifying properties of this combination.

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AUTHOR'S CONTRIBUTIONS

Conception, design of the work, manuscript preparation, and data acquisition: Indira Kudaibergenova, Azamat Kylchykbaev, Yulia Sitnikova, Iskander Chakeyev, Adel Asanalieva, Muratbek Orozaliev, Chethan Raj Gundoji, Sushmitha Bhavanthi, and Tugolbai Tagaev. Clinical management: Indira Kudaibergenova, Azamat Kylchykbaev, Yulia Sitnikova, Iskander Chakeyev, Adel Asanalieva, and Muratbek Orozaliev.

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