ORIGINAL ARTICLE

Enhancing the Efficacy of Radiation Therapy and Chemotherapy in Cancer Treatment with Modified Citrus Pectin and Metformin

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Abstract

Introduction: Radiation therapy, a commonly used treatment for various types of cancer, has toxic effects on the body. To counter this, researchers have developed medications that minimize the harmful effects of ionizing radiation on normal cells and tissues, while enhancing the effect of radiation on cancerous cells. These drugs are known to be radiosensitizers or radiomimetic agents. This study investigated the effectiveness of modified citrus pectin (MCP) and metformin combined with radiation therapy and chemotherapy. **Materials and Methods:** This study used 380 Wistar rats with transplantable strains and two types of cancer cells. The experimental therapy included administration of sugar beet pectin, MCP, and metformin. Effectiveness was determined by commonly accepted indicators, such as the percentage of tumor growth inhibition and increased lifespan. **Results:** MCP and sugar beet pectin had radiomodifying properties and enhanced the sensitivity of cancer cells to radiation therapy. The combination of MCP and metformin had a positive effect on cancer treatment, with complete regression or disappearance of the tumor by the 15th day, and the lifespan of the animals increased by approximately 430%. **Conclusion:** The use of pectin and MCP as radiomodifiers could potentially improve the effectiveness and reduce the toxicity of anti-tumor therapy. This study suggests that the combination of MCP and metformin with radiation therapy and chemotherapy may be a promising approach for cancer treatment.

Key words: Cancer, efficacy, metformin, modified citrus pectin, walker 256 carcinoma

INTRODUCTION

Cancer is a leading cause of death globally, with projections of 19.3 million new cases annually by 2025. Low- and middle-income countries account for more than half of cancer incidences and fatalities, and these figures are expected to rise by 2025.[1] Surgery, radiation therapy, cytotoxic chemotherapy, hormone therapy, immunotherapy, and targeted medications are current treatment options for cancer.[2] Despite these interventions, cancer remains a challenge in clinical treatment, and ongoing research is aimed at identifying effective therapies.

Cancer can be caused by a range of factors such as genetics, environment, and epigenetic elements. Other risk factors included stress, poor diet, lack of exercise, poor sleep, and low vitamin D levels. All of these can be altered in individuals. Cancer can have several symptoms, such as pain, anxiety, depression, and poor sleep,[3] which can make it difficult to complete treatment and may not be effectively managed with traditional medical treatments.

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Received: 10-02-2024
Revised: 21-03-2024
Accepted: 28-03-2024

Integrating conventional medicine with evidence-based complementary and alternative treatments, nutritional counseling, and lifestyle modifications is a successful approach for treating illnesses, such as cancer. This approach focuses on the patient as a whole, not just on the disease. Using adjunctive technologies like circulating tumor cell assays, physicians can diagnose early carcinogenesis and monitor treatment efficacy.[4-6] In addition, adjuvant therapies used alongside conventional treatments can help patients manage various symptoms and side effects associated with cancer and its treatment.

Radiation therapy is a widely used treatment for various types of cancer. However, it is also known to have toxic effects on the body. To address this issue, researchers have used medications that can reduce the harmful impact of ionizing radiation on normal cells and tissues, while enhancing the effect of radiation on cancerous cells. These drugs are referred to as radiosensitizers or radiomimetic agents.[7,8]

Cytostatic treatment is not always effective against solid tumors because of their biological heterogeneity, which allows them to grow steadily and metastasize, as well as the low percentage of dividing cells that are targeted by cytostatics. In addition to their ineffectiveness, chemotherapeutic agents are toxic and become more effective when two or more cytostatic agents are used. One solution to increase the effectiveness and reduce the toxicity of antitumor therapy is to incorporate biologically active substances derived from plant materials into treatment regimens.

Pectins, a type of plant polysaccharides, have been shown to possess antitumor potential and the ability to enhance the effectiveness of cytostatics. Recent studies have demonstrated that pectins have therapeutic and preventive properties against a range of diseases and are comparable to those of some medications.[9] Metformin, a commonly used antidiabetic drug, is an example of a medication that can be used to modify tumor metabolism.[10] Its mechanisms of action are multidirectional and not fully understood; however, research suggests that it may selectively reduce the number of cancer stem cells and suppress tumor development. This study aimed to investigate whether modified citrus pectin (MCP) and metformin could increase the effectiveness of radiation therapy and chemotherapy.

MATERIALS AND METHODS

This study was conducted using 380 white-outbred Wistar rats with transplantable strains. Walker 256 carcinoma and sarcoma 45 (SARC-45) cells were acquired from the Kazakh Research Institute of Oncology and Radiology in Kazakhstan. The transplantation process was carried out using a well-established technique with a homogenized suspension of 0.5 mL at a dilution of 1:10 and injected subcutaneously into the thigh area.

The experimental therapy included administration of sugar beet pectin and MCP (EcoNugenics Inc., United States) at a dose of 650 mg/kg per os (intragastric) 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. Cyclophosphamide was administered intraperitoneally at a dosage of 25 mg/kg, methotrexate (Oncotec Pharma, Germany) intraperitoneally at a dosage of 10 mg/kg and 1 mg/kg once, oxaliplatin (Kosak Pharma, Turkey) intraperitoneally at a dosage of 8-4-2 mg/kg. Paclitaxel (EBEWE Pharma, Austria) was administered intraperitoneally at dosages of 15, 25, 50, and 100 mg/kg, and doxorubicin (EBEWE Pharma, Austria) was administered intraperitoneally at dosages of 1.5 and 3 mg/kg. Fluorouracil (EBEWE Pharma, Austria) was administered once at a dosage of 15–45 mg/kg once, and etoposide (LENS-PHARM, Russia) was administered intraperitoneally at a dosage of 15–30 mg/kg. Gemcitabine (EBEWE Pharma, Austria) was administered intraperitoneally at a dose of 25–45 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, V_e – average tumor volume in the experimental group;

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

Where ILS, ALS_c in the control group, ALS_e 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. 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 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

The first set of experiments to assess the anti-tumor properties and radio-modifying potential of sugar beet pectin was conducted using Walker 256 carcinoma cells [Table 1].

TGI was observed on the 2nd, 5th, and 15th days of the experiment, with results displaying a $P < 0.01$ or 0.001. This resulted in complete regression or disappearance of the tumor by the 15th day, which affected the ALS of the animals. Animals that received both pectin and irradiation treatment survived, and their lifespan increased by approximately 430%. In subsequent experiments, the effects of MCP combined with irradiation on Walker carcinosarcoma 256 cells were investigated.

MCP treatment led to a significant reduction in tumor growth. On the 20th day after treatment, the TGI was nearly 92% and the ILS was 354% [Table 2]. The radio-modifying properties of SARC-45 cells were then tested for MCP preparation. When combined with local irradiation at 3 Gy and MCP treatment on the 7th day after irradiation, tumor node growth was inhibited by 87.5%. By the 14th day after irradiation, tumor node growth was completely inhibited compared to that in the control group [Tables 3 and 4].

In addition, MCP treatment reduced the severity of radiation injury and ILS by 357.7% and tumor regression by 87.5%, resulting in the complete survival of animals by the end of the observation period. In comparison, animals in the control group died within 2–3 weeks of tumor transplantation due to irradiation at 3 Gy.

Experiments on pectin modification revealed that, after mechanical processing, sugar beet pectin exhibited antitumor potential similar to that of MCP obtained from citrus fruits through enzymatic modification. Therefore, pectin and MCP demonstrate comparable radio-modifying abilities. These findings suggest that the antitumor activity of MCP is influenced solely by the molecular weight of the polymer. In subsequent experiments, preliminary assessments were conducted to evaluate the combined effects of metformin, MCP, and chemotherapy on SARC-45 tumors.

The combined administration of methotrexate (10 mg/kg) + pectin + metformin had an advantage over methotrexate monotherapy, with antitumor effect of 88.65% and 54.44%. When paclitaxel was administered at doses of 25 mg/kg and 50 mg/kg, the animals in the group died on the 1st day after drug administration. Animals in the group receiving the combined administration of pectin + metformin + paclitaxel also died. When the dose of paclitaxel was reduced to 10 mg/kg, the TGI for the combination of pectin + paclitaxel was 66.68 and 72.18%, and 100% on days 10, 18, and 25, respectively [Table 5].

When metformin + paclitaxel were administered, the TGI for the combination was 55.5%, 69.96%, and 70.2 on days 10, 18, and 25 after transplantation, respectively. Combination therapy with doxorubicin + metformin, with fairly high TGI on days 7, 14, and 24, was more toxic for animals in the experimental group (ILS 28.87%). The combination of doxorubicin+ pectin was optimal. When the dose of chemotherapy was reduced by 2 times, the antitumor effect and ILS were higher than those in the group treated with doxorubicin monotherapy. A good antitumor effect was noted for the combination of fluorouracil + metformin (TGI 77.26% and 89.6%). The animals in this group survived on the 74th day of the experiments and were cured of the tumor (ILS, 242%).

With combined therapy with gemcitabine (50 mg/kg) + pectin, the TPO was 98.48% on day 21, and the ILS was 134.26%. In this group, 3 out of 7 experimental animals survived without tumors. With combined therapy with etoposide (30 mg/kg) + pectin, the TGI was 100% and 98.013% on days 9 and 14 after tumor transplantation, respectively. At follow-up on day 21, one rat in the etoposide group survived, while 4 rats in the combination therapy group survived. Animals in the control group died on days 15–19.

DISCUSSION

MCP and sugar beet pectin were found to enhance the sensitivity of Walker 256 carcinoma and SARC-45 cells to radiation therapy and exhibited radio-protective properties,

Table 1: Results of irradiation of Walker's carcinosarcoma with oral administration of sugar beet pectin

Indicators	Tumor size in cm³ and TGI in %				ALS (days)
	Day 2	Day 5	Day 7	Day 15	
Irradiation (3 Gy)	4.1±0.2	9.9±0.5	21.7±1.9	*	14.3±1.2
Sugar beet pectin+irradiation	1.9±0.1	6.8±0.3	17.8±0.9	0	90.0±3.2
TGI (%)	53.7	31.3	18.0	100	
o-value	<0.001	<0.01	>0.05	<0.001	
ILS (%)			429.4		
P			<0.001		

TGI: Tumor growth inhibition, ILS: Increased lifespan, ALS: Average lifespan. Values were in Mean±standard deviation. *death of animals

Table 2: Results of irradiation of Walker's carcinosarcoma with oral administration of the MCP

Indicators	Tumor size in cm^3 and TGI in %		ALS (days)
	Day 13	Day 20	
Irradiation	8.3±0.4	6.1±0.3	22.3±1.9
MCP+irradiation	2.6±0.1	0.5±0.03	79.0±4.1
TGI (%)	68.7	91.8	
P	<0.001	<0.001	
ILS (%)		354.2	
P		<0.001	

MCP: Modified citrus pectin, TGI: Tumor growth inhibition, ILS: Increased lifespan, ALS: Average lifespan. Values were in Mean±standard deviation

Table 3: Results of irradiation of SARC-45 with oral administration of the MCP

Group of animals	Tumor size after irradiation in cm^3		TGI (%)	
	Day 7	Day 14	Day 7	Day 14
Control+ irradiation	0.545±0.27	2.11±0.029	–	–
MCP+ irradiation	0.068±0.02	0.041±0.039	87.5	98.05

MCP: Modified citrus pectin, TGI: Tumor growth inhibition, ILS: Increased lifespan. Values were in Mean±standard deviation, SARC-45: Sarcoma 45

Table 4: Effect of MCP on ILS in irradiated animals with SARC-45

Group of animals	ALS (days)	ILS (%)	Survived without tumor (%)
Control+irradiation	17.5±1.63	–	–
MCP+irradiation	62.6±11.47	357.7	87.5

MCP: Modified citrus pectin, ILS: Increased lifespan ALS: Average lifespan. Values were in Mean±standard deviation, SARC-45: Sarcoma 45

as demonstrated by an increase in the TGI and ILS in experimental animals. The radio-modifying capabilities of MCP and sugar beet pectin were similar.

The use of pectin from Walker's transplanted tumors as monotherapy has been demonstrated to consistently prevent tumor development in numerous trials. The experimental outcomes accurately reflect the current state of practical oncology, despite the survival rate of the animals in the experimental group being significantly higher than that of the control group.[11,12] While antitumor treatment can be beneficial by stabilizing the process or causing the tumor to recede in certain individuals, it typically has only a limited and temporary impact on a substantial portion of patients.

Table 5: Effect of combination therapy on TGI and ILS of animals with SARC-45

Group of animals	TGI (%) on day 7 (%)	TGI (%) on day 17 (%)	ILS (%)
Pectin+metformin+ methotrexate (10 mg/kg)	88.65	54.44	30
Pectin+paclitaxel 10 mg/kg	66.68	72.18	160
Metformin+ paclitaxel	55.50	69.96	147
Doxorubicin 1.5 mg/kg+pectin	76.29	73.94	142.2
Doxorubicin 1.5 mg/kg+metformin	47.64	77.17	28.87
Fluorouracil 45 mg/kg+pectin	96.7	–	4.2
Fluorouracil 15 mg/kg+metformin	77.26	89.6	242
Gemcitabine 50 mg/kg+pectin	91.55	70.23	134.26
Gemcitabine 50 mg/kg+metformin	74.06	–	-42
Gemcitabine 25 mg/kg+ pectin+metformin	92.65	83.05	157.3
Etoposide 30 mg/kg+pectin	100	98.01	123.5

MCP: Modified citrus pectin, TGI: Tumor growth inhibition, ILS: Increased lifespan. Values were in %, SARC-45: Sarcoma 45

The study conducted by Yethindra *et al*. aimed to investigate the potential of liposomal and non-liposomal doxorubicin, either alone or in combination with tamoxifen, to exhibit antitumor effects. The results revealed that administering a prophylactic and therapeutic regimen of liposomal doxorubicin in conjunction with tamoxifen significantly suppressed tumor growth and was relatively safe.[13]

These results corroborate the hypothesis that the antitumor activity of MCP is entirely dependent on the molecular weight of this polymer. When pectin, metformin, and chemotherapy were combined, a limited antitumor effect on SARC-45 was observed when paired with doxorubicin, with a negative TGI on the 24th day of the study. However, the combination of doxorubicin and pectin was the most effective. The combination of pectin and metformin significantly reduced the toxicity of chemotherapy drugs, as evidenced by a 30% decrease in ILS. Moreover, the combination of fluorouracil (15 mg/kg) and metformin with a TGI of 89.6% was the most effective, whereas monotherapy produced low rates of TGI and ALS. Interestingly, the combination of pectin and gemcitabine or etoposide resulted in ILS in experimental animals. Therefore, combining pectin with different anticancer medications may offer cancer patients a promising new approach to overcoming drug resistance.[7,8]

CONCLUSION

The use of multiple treatment methods in cancer therapy has several advantages, such as enhanced treatment outcomes, potential synergistic effects, reduced resistance, personalized treatment plans, better management of side effects, and higher survival rates. This approach aims to optimize successful cancer treatment while improving the patient's quality of life.

AUTHOR CONTRIBUTIONS

Conception, design of the work, manuscript preparation, and data acquisition: Indira Kudaibergenova, Sharipa Zhorobekova, Yulia Sitnikova, Lyudmila Serikova, Azamat Kylchykbaev, Iskander Chakeyev, Viktor Prokhorenko, Sai Praneeth Duvvuri, Krishna Chaitanya Meduri, Yethindra Vityala. Clinical management: Indira Kudaibergenova, Sharipa Zhorobekova, Yulia Sitnikova, Lyudmila Serikova, Azamat Kylchykbaev, Iskander Chakeyev, Viktor Prokhorenko.

ACKNOWLEDGMENT

None.

CONFLICTS OF INTEREST

None.

FUNDING

None.

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Source of Support: Nil. **Conflicts of Interest:** None declared.