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Combined anti-cancer effects of curcumin and oxaliplatin on colon carcinoma colo205 cells using transplanted nude mice

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Abstract The objective of this study was to investigate the effects of curcumin combined with oxaliplatin on human colon carcinoma Colo205 cells and to analyze the anti-cancer mechanism. Sixty nude mice were inoculated with Colo205 cells as tumor transplanted models which were randomly divided into control group, curcumin group, oxaliplatin group and curcumin plus oxaliplatin group (n=15 in each group). The volume of tumor and the tumor suppressor rate was calculated after continuous administration of the curcumin or oxaliplatin for 10 times. The routine blood tests, the liver function and kidney tests were performed. The cell cycle, apoptosis rate and the pathological morphology of tumor tissues was observed. The expression of Bcl-2 and Bax related to apoptosis was detected. The turned out degree of tumor inhibition varied when compared to the control group while the curcumin plus oxaliplatin group served for highest inhibition rate. Statistically insignificant difference (P>0.05) was observed among the groups in routine blood, liver and kidney function tests. The tumor apoptosis rate was significantly increased in other three groups when compared to the control group. There was insignificant difference (P>0.05) in Bax expression of tumor tissue of control group, curcumin group and oxaliplatin group while in curcumin plus oxaliplatin group, it was significantly increased. The expression of Bcl-2 in oxaliplatin group was significantly lower and the value of Bcl-2/Bax in curcumin plus oxaliplatin group was decreased most obviously.

Keywords: Curcumin, tumor, colorectal carcinoma.

INTRODUCTION

Colorectal carcinoma denotes one of the most frequent causes of cancer death. Colorectal cancer is generally treated with a combination of surgery, radiation, and chemotherapy. Many anti-cancer drugs have shown efficiency in colorectal cancer, hwever metastatic stage remains mostly incurable (Terstriep & Grothey, 2006). Oxaliplatin is one of the more potent drugs used in this disease. Oxaliplatin a third-generation platinum compound that disrupts DNA replication and transcription of cancer cells (Bleiberg, 1998). Oxaliplatin has substantial anti-tumor effects in colorectal cancer (Ibrahim *et al*., 2004). The role of curcumin (a proapoptotic compound) for the treatment of cancer has been an area of growing interest. It exerts its anti-cancer effects via diverse biological characters, including suppression of nuclear factor-κB and inhibition of angiogenesis (Aggarwal *et al*., 2003). A success of the strategy for cancer treatment includes using combination therapy frequently with drugs that have differing mechanisms. Because curcumin is nontoxic, and may potentiate certain chemotherapeutic agents, it has potential for use in combination regimens especially with oxaliplatin.

Previous studies showed that oxaliplatin tends to produce toxicity in the blood and the nervous systems, thereby causing a negative impact on the treatment of cancer. Currently, there are rare reports about the application of curcumin combined with oxaliplatin as anti-tumor therapy (Rao *et al*., 2013). The purpose of this study was to explore the effects of curcumin with oxaliplatin on the growth of human colon carcinoma and to analyze the mechanism of anticancer so as to provide valuable reference for clinical treatment of tumors.

MATERIALS AND METHODS

Animals and chemicals

A total of sixty Kunming male nude mice provided by the animal experimental center of "Renmin hospital, Wuhan University, China" were selected to be studied. These mice aged 7 weeks and weighed about 17.5±0.7g. Human colon carcinoma cell line Colo205 purchased from the Chinese type culture collection. Curcumin was purchased from Nanjing Pharmaceutical Factory Co. ltd; Oxaliplatin from Beijing Union Pharm; RPMI 1640 medium from Biological Engineering (Shanghai) Limited by Share Ltd. Propidium iodide (PI): Sigma Company. Reverse transcription kit was purchased from Beijing Noble Ryder Technology Co. Ltd. Other reagents were purchased from Changzhou Xinli Pharmaceutical Chemical Co. Ltd.

Vernier caliper was purchased from Cangzhou Huarui Instrument Equipment Co. ltd. and Blood cell analyzer from Ji'nan Glett Company. Automatic biochemical analyzer was brought from Shenzhen Kubel Biotechnology Polytron Technologies Inc. Flow

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cytometry from Backman Company; Optical microscope from Shanghai biBaimu Optical Instrument Factory and PCR from Jones International Ltd. The gel imaging analysis system was of Shanghai Jia Peng Technology Co. Ltd.

Model preparation

Human colon cancer cell line Colo205 was added to 1640 RPMI culture liquid for culture at 37°C and 5% CO_2 with saturated humidity collecting the followed by collection of the logarithmic growth phase cells for experimental treatment. The density of human colon cancer cell line Colo205 was adjusted to 5×10^6/mL. The experimental mice were first disinfected in skin and after that, they were injected subcutaneously into right axilla with 0.1mL human colon cancer Colo205 cell line suspension for inoculation followed by the breeding back in cage. The mice with the average diameter of tumors being about 5mm were used for subsequent experiments.

Grouping and Administration

The selected animals were were randomly divided into 4 groups i.e. control group (n=15), curcumin group (n=15), oxaliplatin group (n=15) and curcumin plus oxaliplatin group (n=15). Control group received 0.1mL normal saline, curcumin group were given curcumin 5mg/kg (0.1 mL), oxaliplatin group were treated Swith oxaliplatin 5mg/kg (0.1 mL) and curcumin plus oxaliplatin group were given curcumin 5mg/kg (0.1 mL) plus oxaliplatin 5mg/kg (0.1mL). Each group was administered once every day continuously for 10 days.

Body weight and tumor volume measurement

Major diameter (a) and minor diameter (b) of the tumor were measured every two days by using Vernier caliper with the tumor volume (V) formula as $V=1/2*a*b^2$.

Routine Blood and Liver plus Kidney Functions Detection

At 24h after the last time of administration, the nude mice of each group were anesthetized and their eyes were extracted for blood collection. After anticoagulation, the blood cell analyzer was applied to detect white blood cell (WBC) count, red blood cell (RBC) count and platelet (PLT) count. Plasma separation of blood samples was conducted and the automatic biochemical analyzer was used to detect the level of such indexes as alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (BUN) and creatinine (Cr).

Inhibition rate calculation

The animals were killed by cervical dislocation immediately after the bloods drawn followed by the weighing of tumor mass under sterile condition and the calculation of average tumor weight and tumor inhibition rate. The following formula was used Inhibition rate = (average tumor weight in control group - average tumor weight in administration group) /average tumor weight in control group.

Detection of tumor cell cycle by flow cytometry

Tumor tissues were cut by using scissors and filtered through the 500-pole copper screen followed by making a single cell suspension of tumor. 0.1mL single cell suspension (cell density of 1×10^6/mL) was taken, to which 1mL PI dye liquor was dropped followed by staining for 30min at 4°C and PBS washing 2 times. The cell cycle was determined by flow cytometry.

Observation of tumor tissue morphology

Under sterile conditions, the tumor tissues were immediately separated out in nude mice and a part in them were fixed by using formalin followed by paraffin embedding, slicing and HE staining. Pathological histology of tumor was observed by microscopy.

Detection of Bax and bcl-2 Gene Expression

The total RNA of tumor tissues was extracted according to instructions of the Axy Prep TM Multisource RNA mini-preparation kit and then given electrophoresis to detect its purity and quality. The RNA was reversely Tran scripted to cDNA followed by RT-PCR to detect the gene expression of bax and bcl-2 with β-actin as reference. The primer sequence was designed by Shanghai Gongda Company Limited.

Reaction conditions of PCR amplification

Pre-denaturation at 94°C for 4min; denaturation at 94°C for 30s; annealing at 52°C for 30s; extending at 72°C for 1 min; with a cycle of 30 times. Final extending at 72°C for 10min, and warm keeping at 4°C. PCR products were taken out and PCR amplification result was detected by means of 1% agarose gel electrophoresis. The various bands were scanned by gel imaging system for quantitative analysis and the ratio of target gene to the light intensity of amplified bands with internal reference was calculated to reach the relative expression of target gene, which was obtained in accordance with the following formula: $\Delta Ct = Ct_{\text{target gene}}-Ct_{\beta\text{-actin}}$, the higher the value of ΔCt, the lower the relative expression of target gene (Rao *et al*., 2013).

Ethical approval

This study was approved from the institutional ethical review board (IERB) of Taian City Central Hospital, Shandong, PR China. The Reference number is 196/TCH-Shand/2016. All the protocols were carried out as per NIH guidelines for Lab animals.

STATISTICAL ANALYSIS

SPSS 21 statistical software was used for statistical analysis. The measurement data were assessed by using the homogeneity of variance test and the difference

between the two groups were checked by t test. The data among multiple groups were assessed by single factor analysis of variance. 5% p value ($p<0.05$) was considered significantly significant.

RESULTS

Comparison of tumor growth among groups

The turned out of control group was of different degree for tumor inhibition in comparison to curcumin, oxaliplatin and curcumin plus oxaliplatin group in which the inhibition rate of curcumin plus oxaliplatin group was the highest and statistically significant ($P<0.05$), as shown in table 1.

Comparison of routine blood and liver plus kidney function among groups

When statistical tool was used then it was concluded that there was no significant difference ($P>0.05$) in routine blood, liver and kidney function of control group, curcumin group, oxaliplatin group and curcumin plus oxaliplatin group, as shown in table 2.

Comparison of tumor cell cycle

The apoptosis rate of tumor cells in curcumin group, oxaliplatin group and curcumin plus oxaliplatin group was significantly increased when compared with the control group. Particularly higher cellular proportion in S phase in which cellular proportion in G2/M phase in curcumin plus oxaliplatin group was increased most noticeably as shown in table 3.

Comparison of pathological observation of tumor tissue among groups

The range of tumor cells was irregular with marked pleomorphism in control group. They were mainly rounded or with an ellipse of varying shape and size. The cells arranged closely with deep nuclear chromatin, moderately larger nucleus and nuclear division phase. In curcumin group, oxaliplatin group and curcumin plus oxaliplatin group, the cell size was even with the presence of hydropic degeneration and the change of bright cellular plasma, which was followed by karyopycnosis, dissolution and cell disintegration. Some tumor tissues were subjected to serious degeneration and necrosis with less nuclear division phase. The apoptosis of some scattered tumor cells was visible with the generation of apoptotic body as shown in fig. 1.

Comparison of bax and bcl-2 Gene Expression

It was observed that there was insignificant ($P>0.05$) difference in Bax expression of tumor tissue of control

Table 1: Comparison of Tumor Growth among Groups (n=15)

Group	Tumor Volume (mm^3)	Tumor Weigh (Gm)	Tumor Inhibition Rate (%)
Control	1655.23±113.46	1.27±0.62	00
Curcumin	1455.75±227.75	1.01±0.24	61.02
Oxaliplatin Group	1404.62±315.45	1.21±0.36	49.15
Curcumin+ Oxaliplatin	1078.03±274.05	0.54±0.13	77.9
F	12.42	13.51	15.07
P	<0.05	<0.05	<0.05

Table 2: Comparison of routine blood and liver plus kidney function (n=15)

Groups	WBC ($\times10^9$/L)	RBC ($\times10^{12}$/L)	PLT ($\times10^9$/L)	ALT (U/L)	AST (U/L)	BUN (mmol/L)	Cr (μmol/L)
Control	3.71±0.6	8.9±1.0	101.66±8	52.21±2	51.72±37	13.65±1.4	97.03±9.3
Curcumin	3.5±0.4	7.83±1.0	101.5±7.6	60.5±2	53.08±34	15.93±1.2	101.65±1.04
Oxaliplatin	4.3±0.4	8.12±1.0	109.81±6	69.93±1	287.56±45	17.03±0.8	103.85±15.7
Curcumin+ Oxaliplatin	3.2±0.3	8.5±0.6	105.21±4	61.57±1	53.34±37	14.95±1.7	93.5±22.64
F	2.29	2.99	2.21	3.61	2.1	1.20	1.1
P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Table 3: Comparison of Tumor Cell Cycle among Groups (n=15)

Groups	G_0/G_1 period (%)	S period (%)	G_2/M period (%)	Apoptosis rate (%)
Control Group	69.18±0.30	17.34±1.65	4.24±0.05	3.02±0.56
Curcumin Group	61.44±12.32	30.78±10.04	3.99±1.03	18.87±3.22
Oxaliplatin Group	62.92±4.7	27.43±6.51	4.02±1.78	13.01±1.51
Curcumin + Oxaliplatin Group	58.52±2.99	26.77±1.35	10.68±1.22	24.57±1.47
F	14.04	16.9	21.48	19.62
P	<0.05	<0.05	<0.05	<0.05

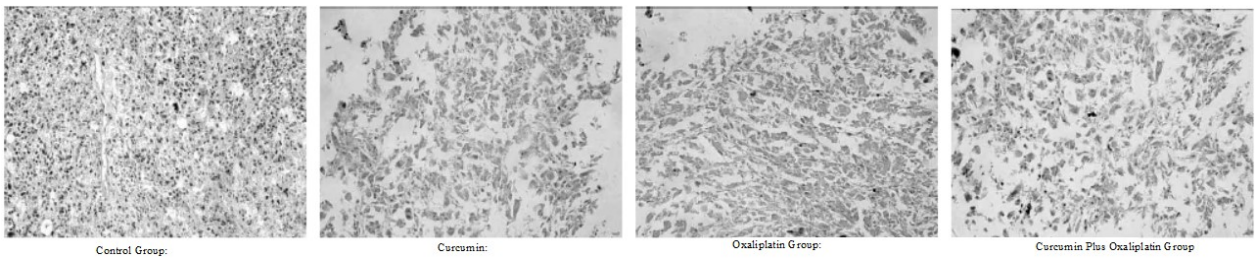

Fig. 1: Pathological Observation of Tumor Tissue among Groups (×200, HE)

group, curcumin group and oxaliplatin group while in curcumin plus oxaliplatin group it was significantly increase ($P<0.05$), as shown in fig. 2.

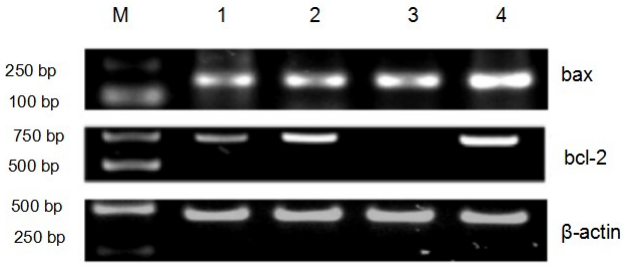

Fig. 2: Comparison of bax and bcl-2 Gene Expression among Groups

DISCUSSION

Plant based anticancer drugs are using since long and considered effective medicines. Very popular plant based drugs are Vinca alkaloids and paclitaxel are plant based. The plant based compounds have the advantage of having a promising therapeutic-to-toxicity index.

The current study evaluated the preclinical antitumor activity of curcumin with oxaliplatin in colorectal cancer. Colorectal cancer is the most common tumor disease with gradual rising of incidence in recent years and ranks the third place in gastrointestinal cancer (Cote *et al*., 2015; Mahmoud *et al*., 2014). It has been included in the key objects of current prevention and treatment in China (Cunniff *et al*., 2016). Drug combination is a major trend in treatment of cancer disease and the combined chemotherapy showed an effective antitumor effect on colon cancer (Shen *et al*., 2015). The present research studied the anti-effect of curcumin combined with oxaliplatin on colon cancer and found that compared with the control group, there turned out was with different degree of tumor inhibition in curcumin group, oxaliplatin group and curcumin plus oxaliplatin group in which the inhibition rate of curcumin plus oxaliplatin group was the highest, which suggests the combined treatment of curcumin and oxaliplatin have no obvious toxicity on blood system, liver and kidney. We also established synergism between curcumin and oxaliplatin at a ratio 5mg/kg body weight in Colo205 cell treated mice. The association of apoptosis with cell cycle is relatively close, so cell cycle may be a new target for tumor therapy (Grégoire *et al*., 1994). The present study found that, the apoptosis rate of tumor cells in other three groups was significantly increased when compared with the control group. Anti-apoptotic gene bcl-2 and pro apoptotic gene bax are involved in the development and progression of tumor diseases. They may form heterodimer or dimer with the ratio closely related to the regulation of apoptosis (Wallgren *et al*., 2013). The results of this study showed that there was no significant difference in bax expression of tumor tissue in nude mice among control group, curcumin group and oxaliplatin group while in curcumin plus oxaliplatin group it was significantly increased The expression of bcl-2 in oxaliplatin group was significantly lower and the rate of bcl-2/bax in curcumin plus oxaliplatin group was decreased most obviously indicating that the combination of curcumin and oxaliplatin was able to down regulate bcl-2/bax value, induced apoptosis and then played an anti-tumor effect.

There are many publications documenting the potent preclinical antitumor effects of curcumin and oxaliplatin and our results are in agreement to many previous studies like Kawamori *et al.* (1999) and Li *et al.* (2004).

CONCLUSION

From the findings of this study, it can be concluded that combinations of anti-cancer drugs affecting multiple pathways and therefore most likely to be effective. We have shown that antitumor effects of combined drugs against colorectal cancer *in vivo* are better than those of oxaliplatin alone.

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