RESEARCH ARTICLE

# Apoptosis of Colorectal Cancer UTC116 Cells Induced by Cantharidinate

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## Abstract

**Effects of Cantharidinate on apoptosis of human colorectal cancer UTC-116 cells were investigated by means of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay, H and E staining, flow cytometry, and Raman Spectra analysis. The results showed Cantharidinate to exert inhibitory action on proliferation of human colorectal cancer UTC-116 cells, inducing apoptosis, arresting cells in G1 phase, with decline of S and G2 phases. In addition, the results of Raman spectrum showed significant changes in the UTC-116 cells chemical structure with stretching after the application of Cantharidinate. Taken together, these results suggest that the treatment of human colorectal cancer with Cantharidinate may be associated with multiple molecular mechanisms for apoptosis. Furthermore, similar to fluorouracil, Cantharidinate should be considered as novel assistant drug for controlling the growth of human colorectal cancer UTC-116 cells.**

**Keywords:** Colorectal cancer - UTC116 cells - Cantharidinate - apoptosis

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## Introduction

Colorectal cancer is the third most commonly diagnosed cancer in males and the second most commonly diagnosed cancer in females, and was estimated to account for over 1.2 million new cancer cases and 608,700 deaths in 2008 (Kotzev et al., 2008). Nowadays, researches have been substantially focused on the chemotherapy for tumors (Posner, 2005; Takiuchi, 2007). Fluorouracil is one of the standard chemotherapeutic drugs in the treatment of this disease (van Hazel et al., 2009). In the United States in 1994, Colorectal cancer was estimated to be the second most common cause of cancer deaths after lung cancer (DeCosse et al., 1994) and responsible for one in every 10 cancer deaths (Boushey, 2009).

In the past decade, treatment options for colorectal cancer have expanded and include additional chemotherapeutic agents and targeted therapies (cetuximab, panitumumab, bevacizumab) (Gerber, 2008). Proper use of these therapies can have a major impact on patients' prognoses (Dehmer et al., 2010). In recent years, emerging data in treat cancer on traditional Chinese medicine have influenced some cancer setting with regard to the identification of specific molecular markers and pathway aberrations that could guide treatment decisions (Liu and Huang, 2007). However, whether some traditional Chinese medicine may be used to aid colorectal cancer? Meanwhile, whether its effects are similar to fluorouracil? For years, Cantharidinate entered gradually our field of vision and become this article research hot spot.

In this study, we attempted to ascertain whether Cantharidinate induced apoptosis in human colorectal cancer UTC-116 cells, as well as the mechanisms of its action, using human colon cancer UTC-116 cells. In addition, we attempted to determine whether Cantharidinate induced the structural change of protein through Raman spectrum. Meanwhile, whether Cantharidinate should be considered as novel assistant drug for controlling the growth of human colorectal cancer UTC-116 cells?

## Materials and Methods

*Materials*

UTC-116, a human colorectal cancer cell line, was obtained from institute for Regenerative Medicine, Jilin University. UTC-116 cell line was routinely cultured in the Dulbecco's modified Eagle media (DMEM, GIBCO, Life Technologies, Helgerman Court, MD) under the conditions of 5% $CO_2$ at 37 °C.

*Cell Proliferation Assay*

Cell proliferation assay was carried out with the aid of a 3-(4,5-dimethiazol-2-yl)-2,5-diphenylterazaolium bromide (MTT) staining kit (Sigma Chemical Co., USA) according to the manufacture's protocol(Bromberg et al. 2010). UTC-116 cells proliferation had been determined after 24~72 h of drμg treatment before 20 μL of MTT (5mg/L) was dropped in each well. Results were obtained

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**Table 1. MTT Proliferation Rate(%) in Human Colorectal Cancer UTC-116 Cells at 24 h and 48 h with Non-medication, Serum-free, Cantharidinate and Fluorouracil Group**

| | MTT Proliferation rate(%) | |
|---|---|---|
| | 24 h | 48 h |
| Non-medication (control) | 1.28±0.02 | 1.557±0.012 |
| serum-free | 0.77±0.04* | 1.03±0.03* |
| Cantharidinate (5 μg/ml) | 0.742±0.04*# | 0.90±0.01*# |
| Cantharidinate (2.5 μg/ml) | 1.058±0.06*# | 0.932±0.01*# |
| Cantharidinate (1 μg/ml) | 1.196±0.05# | 1.112±0.02*# |
| fluorouracil (2.5 μg/ml) | 1.424±0.04*# | 1.341±0.03*# |

*Groups of Non-medication, serum-free, Cantharidinate and fluorouracil are the same as those in table.1. Values are mean±SEM. *P<0.01 vs. control group; *# P<0.01 vs. control group and serum-free group( n=5, mean ± SEM)

by measuring the absorbance value at 490 nm on an enzyme-linked immunosorbent assay system (Spectra MRTM. Dynex, USA). Each experiment was performed in triplicate.

*H&E staining*

The human colorectal cancer UTC-116 cells with the application of Cantharidinate were assessed by light microscopy with H&E staining.

*Cell Cycle Analysis*

UTC-116 cells were cultured in 50 $cm^2$ plates ($1\times10^6$ cells//well) for 48 h, and treated with 2.5 μg/ml Cantharidinate. After incubation for 48 h, the cells were digested by 0.25% trypsin and then analyzed for the percentage of apoptosis rate and cell cycle phase distribution by flow cytometry (FACSClibur, Becton Dickinson, USA) with the help of ModFit LTTM3.0 software(Bromberg et al., 2010). Each experiment was done in duplicate and was repeated five times.

*UV-Vis Absorption Spectroscopy and Raman spectrum*

The absorption spectra of human colorectal cancer UTC-116 cells were recorded on a UV-2450 spectrophotometer in a range of A200-700 nm. The absorption spectra of human colorectal cancer UTC-116 cells were recorded on a Raman spectrometer equipped with an Olympus BX40 system microscope with a 1003 objective. Summarized spectra were corrected by polynomial baseline with Origin 6.0 software.

*Statistical analysis*

All the data obtained from, at least, three independent experiments were shown as the mean ± SEM. Statistical comparison was made with Student s P-Values of less than 0.05 were considered to be of statistical significance. Statistical analysis of the data was performed via SPSS for Windows Version 11 (SPSS, Chicago, II, USA).

## Results

*Effects on proliferation of cancer Cells*

MTT assay shows that the inhibition rates of the cell proliferation were significantly improved with the

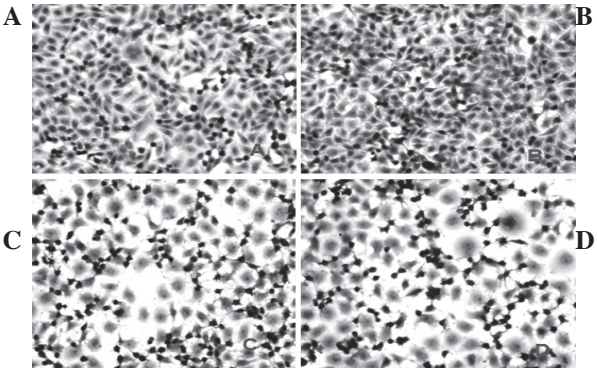


**Figure 1. H&E Staining of UTC-116 Cells.** (A)Non-medication group (control); (B) serum-free group; C) fluorouracil group; (D) Cantharidinate group

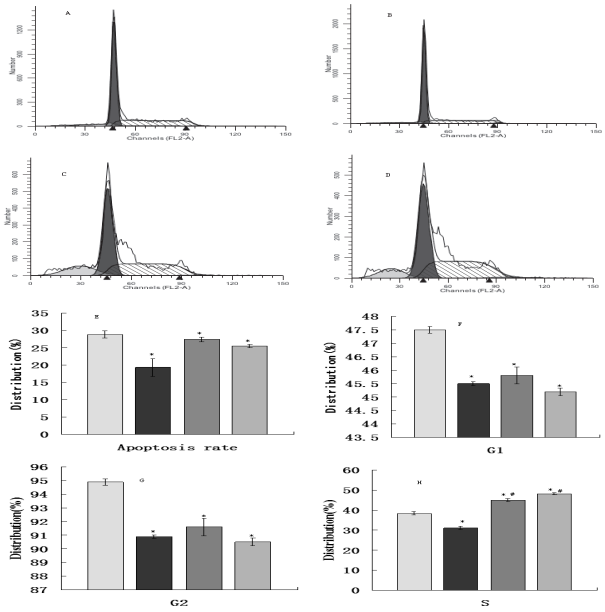


**Figure 2. Effects of Non-medication Group(control), Serum-free, Cantharidinate and Fluorouracil on Distribution of UTC116 Cells by Flow Cytometry Assay.** (A) Non-medication group(control); (B) serum-free group, c=0.5 μmol/L; (C) Cantharidinate group, c=2.5 μg/ml; (D) fluorouracil group, c=2.5 μg/ml. (E)-(H): Apoptosis rate, G1, G2 and S, *P<0.05 serum-free group vs control, *# P<0.01 Cantharidinate and fluorouracil group vs serum-free group and control

application of Cantharidinate and fluorouracil at 48 h (Table 1). There were significant differences in inhibition rate in this two groups (P<0.01). The results show that both Cantharidinate and Fluorouracil inhibit the cell proliferation of human colorectal cancer UTC-116 cells in dose-dependent and time-dependent manners (Table 1). These are significant differences in the inhibition rate between the Cantharidinate group and the non-medication, serum free group (P<0.01) at 48 h. In addition, the results show that the Cantharidinate can exert a synergistic effect, and it may reduce chemotherapeutic time of the non-medication group and subsequently decrease the risk of the treatment of human colorectal cancer.

*H&E staining of cancer cells*

The pathological changes of human colorectal cancer UTC-116 cells in different groups were shown in Figure 1. From our experiment, we found that the quantity of

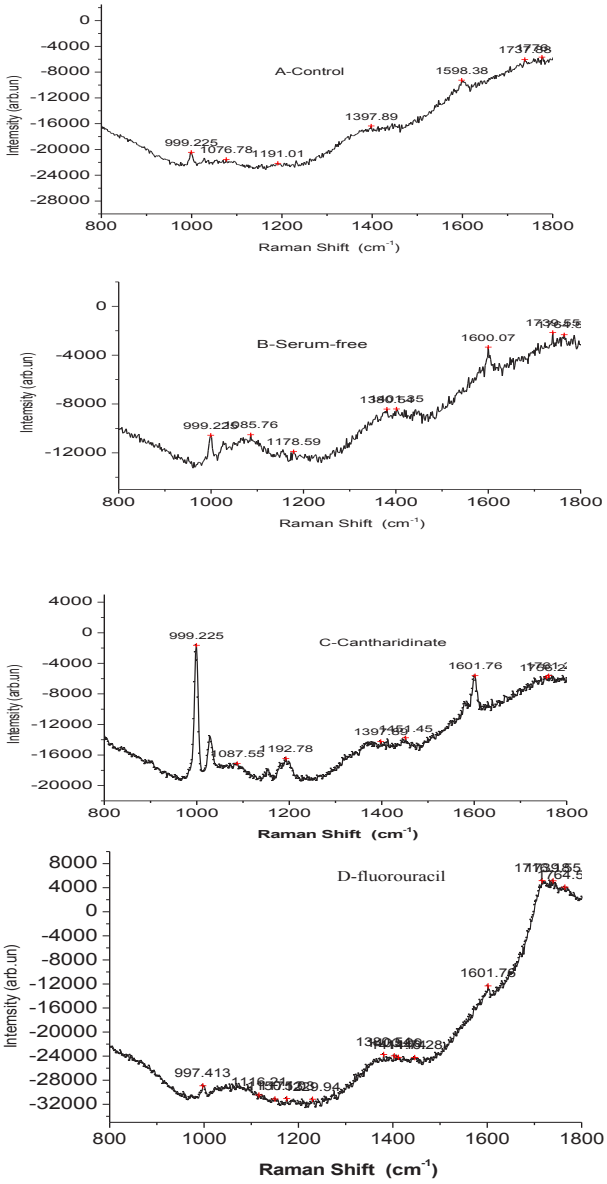


**Figure 3. A~D Raman Absorption Peak of UTC116 Cells.** (A) Non-medication group (control); (B) serum-free group; (C) Cantharidinate group; (D) fluorouracil group

human colorectal cancer UTC-116 cells were decreased the application of Cantharidinate and fluorouracil at 48h (Figure1 C, D). These are significant differences in the inhibitory quantity of UTC-116 cells among the Cantharidinate, fluorouracil group and the Non-medication (control), serum-free group. The results show that the Cantharidinate may exert an accessorial effect, and it may reduce chemotherapeutic time of Non-medication and subsequently decrease the risk of the treatment of human colorectal cancer.

*Effects on cancer apoptosis and cell cycle*

In order to further evaluate the effects of Cantharidinate, flow cytometry assay was performed with 2.5 μg/ml acting upon UTC-116 cells for 48 h. The results show that the apoptosis ratio of UTC-116 cells was significantly increased for the Cantharidinate group (Figure 2). There are significant differences in apoptosis rate between the Cantharidinate group and Non-medication group, and there are significant differences between the Cantharidinate group and Non-medication group (P<0.01).The results show that the Cantharidinate induces the cell apoptosis and should be considered as an effective therapeutic strategy for controlling the growth of human colorectal cancer UTC116 cells.

*UV-vis absorption spectroscopy and Raman spectroscopy*

UV-vis absorption spectra of human colorectal cancer UTC-116 cells were obtained in the A200-700 nm range on a UV-3101 spectrophotometer after the UTC-116 cells were cultivated for 48h. Meanwhile, the spectrum for each group appears at 490 nm.

Raman spectroscopy has been used for the investigation of living cells and tissues (Chan and Lieu, 2009; Krafft et al., 2009). It is a non-invasive method, which does not require any sample processing (Scalfi-Happ et al., 2007). In this experiment, we tested if Raman spectroscopy can be employed as a non-invasive tool to distinguish between Non-medication group and serum-free, Cantharidinate and fluorouracil groups (Figure 3). For our analyses, we focused on the spectral fingerprint range from 800 $cm^{-1}$ to 1800 $cm^{-1}$. In order to identify the therapeutic benefits of Cantharidinate for cancer, we analyzed the Raman spectra, the difference spectra and loading values as displayed in Figure 3. Accordingly, we identified major differences in the Raman spectra of UTC-116 cells after the application of Cantharidinate and fluorouracil when compared to Non-medication group. These results show the Raman spectra of UTC-116 cells after the application of Cantharidinate and Fluorouracil moved from 1598 $cm^2$~1602 $cm^2$ when compared to Non-medication group (Figure 3). In addition, the therapeutic benefits of Cantharidinate is similar to compare with fluorouracil group. In that particular, Cantharidinate should be considered as novel assistant drug for controlling the growth of human colorectal cancer UTC-116 cells.

## Discussion

A desirable property of an anticancer drug is to induce the death of tumor cells with little effect on normal cells. Our previous study showed that Cantharidinate inhibited the proliferation of human colorectal cancer UTC-116 cells with an IC50 value~2.5 μM. Very recently, it hasn't been reported that Cantharidinate had little cytotoxicity in human normal cells. Cantharidinate could inhibit proliferation of human colorectal cancer UTC-116 cells with an IC50 value~2.5 μM in our previous study. It seems that the effects of Cantharidinate are similar to fluorouracil.

Colorectal cancer, commonly known as bowel cancer, is a cancer from uncontrolled cell growth in the colon or rectum (parts of the large intestine), or in the appendix (Kaminski et al., 2010). Symptoms typically include rectal bleeding and anemia which are sometimes associated with weight loss and changes in bowel habits (Canzi et al., 2012).

Most colorectal cancer occurs due to lifestyle and increasing age with only a minority of cases associated with underlying genetic disorders (Levin et al., 2002). It typically starts in the lining of the bowel and if left

untreated, can grow into the muscle layers underneath, and then through the bowel wall (Fattori et al., 2011). Screening is effective at decreasing the chance of dying from colorectal cancer and is recommended starting at the age of 50 and continuing until a person is 75 years old (Jones et al., 2010). Localized bowel cancer is usually diagnosed through sigmoidoscopy or colonoscopy (Ericson et al., 2008). At present Fluorouracil can be used topically (as a cream) for treating actinic (solar) keratoses and some types of basal cell carcinomas of the skin (Cunningham et al., 1955). However, whether some traditional Chinese medicine may be used to aid cancer? More and more studies indicate that Cantharidinate was a reasonable and effective treatment for liver cancer. For years, Cantharidinate entered gradually our field of vision and become this article research hot spot.

In this study, we demonstrated that Cantharidinate had the effects on the enhancement of the apoptosis of human colorectal cancer UTC-116 cells. Cantharidinate can be not only harnessed cell proliferation and promote apoptosis and can improve the structure of the cancer cells. The effects of Cantharidinate are similar to fluorouracil. It may likely be associated with the multiple molecular mechanisms for inducing cell apoptosis and inhibiting cell proliferation. Furthermore, Cantharidinate should be considered as novel additional drug for controlling the growth of human colorectal cancer UTC116 cells and for encouraging the apoptosis of human colorectal cancer UTC116 cells.

In conclusions, Cantharidinate can be not only harnessed cell proliferation and promote apoptosis and can improve the structure of the cancer cells. Collectively, these results indicated that Cantharidinate can be considered as novel additional drug for controlling the growth of human colorectal cancer UTC116 cells and for encouraging the apoptosis of human colorectal cancer UTC116 cells.

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