Original Article

Chinese Herb Formulae Inhibit the Proliferation of Human Colon Cancer SW480 Cells by Inducing Cell Apoptosis

Su Fu[a], Shao-Lin Qin[a], Wolf-Dieter Rausch[b]

[a]Department for Internal Medicine of Traditional Chinese Medicine, Eye Hospital China Academy of Chinese Medical Sciences, Beijing, China, [b]Department for Biomedical Sciences, Institute for Chemistry and Biochemistry, University of Veterinary Medicine Vienna, Vienna, Austria

Abstract

Objective: This study aimed to reveal the antitumor effects of Chinese herbal formulae and the underlying mechanisms in treating colorectal cancer, with a focus on developing traditional Chinese medicine (TCM) as a supplement and alternative therapeutic method for cancers. **Materials and Methods:** Human colon cancer SW480 cells were treated with three Chinese herbal formulae, Bu Zhong Yi Qi Decoction, Fuzi Lizhong Decoction, and Pulsatilla Decoction at different concentrations (50–600 μg/mL) for 24, 36, and 48 h, respectively. Cell viability was determined using the resazurin reduction assay, and cell survival rate was evaluated using a colony formation assay. After treatment with different concentrations (50–600 μg/mL) of these three formulae for 48 h, the effects of the Chinese herbal formulae on cell apoptosis were investigated using Hoechst/propidium iodide (PI) staining. The positive PI-stained cells were investigated using an EnSpire multilabel plate reader and the positive Hoechst-stained cells were observed under a fluorescence microscope for morphological changes. **Results:** Bu Zhong Yi Qi Decoction, Fuzi Lizhong Decoction, and Pulsatilla Decoction inhibited SW480 cell proliferation in a dose-and time-dependent manner and induced cell apoptosis. **Conclusion:** Chinese herbal formulae with a special prescription form of TCM with antitumor effects bring a new perspective in line with the principles of TCM in cancer treatment.

Keywords: Apoptosis, cell proliferation, Chinese herbal formulae, colorectal cancer, SW480 cells

Introduction

Colorectal cancer is one of the cancers with the highest incidence in developed countries, and the number of new cases is increasing rapidly with more diagnoses in the younger population.[1,2] In Europe, around 250,000 new cases are diagnosed annually, accounting for around 9% of all the malignancies. Meanwhile, the incidence rate of colorectal cancer has also increased rapidly in middle- and low-income countries over the past decades.[3] Currently, great progress has been achieved in the treatment of colorectal cancer: surgery has been optimized to achieve the best results with a low morbidity, and adjuvant chemotherapy has been reported to be effective for the prolongation of survival.[4] However, these side effects can cause severe pain and numerous new problems to the patients. There is currently intense research being conducted on traditional Chinese medicine (TCM) because of its excellent or supplemental therapeutic effects in colorectal cancer treatment and its relatively few side effects.[5] Clinically, TCM has been used successfully to alleviate perioperative period complications, such as intestinal obstruction,[6] and to relieve chemotherapy- and radiotherapy-associated complications, such as anemia, leukopenia, thrombocytopenia, fatigue, nausea, diarrhea, and anorexia.[7,8] The antitumor activities of many candidates have been investigated in the field of laboratory research. In general, the antitumor activities of TCM are detected in isolated TCM herbs or pure ingredients from TCM drugs. However, there are few reports on the antitumor effects of Chinese herbal formulae, a specific form of TCM based on TCM principles that is dominantly used in clinical practice. In TCM prescriptions, herbs are generally used as a formula, which is a combination of several Chinese herbs, in

Access this article online

Quick Response Code:

Website: www.wjtcm.net

DOI: 10.4103/2311-8571.369650

Address for correspondence: Prof. Wolf-Dieter Rausch, University of Veterinary Medicine Vienna, Vienna 1210, Austria. E-mail: wolf.rausch@vetmeduni.ac.at

Received: 09-03-2022, **Accepted:** 13-05-2022, **Published:** 15-02-2023

How to cite this article: Fu S, Qin SL, Rausch WD. Chinese herb formulae inhibit the proliferation of human colon cancer SW480 cells by inducing cell apoptosis. World J Tradit Chin Med 2023;9:348-54.

the belief that the combination enhances therapeutic effects when simultaneously reducing side effects.[9] This study focused on evaluating the effects of Bu Zhong Yi Qi Decoction, Fuzi Lizhong Decoction, and Pulsatilla Decoction on human colon cancer SW480 cells *in vitro* with the aim of identifying the possible antitumor activities and underlying mechanisms of Chinese herbal formulae.

Materials and Methods

Materials and reagents

Dulbecco's modified Eagle medium (DMEM) was purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Fetal bovine serum (FBS), penicillin–streptomycin, trypsin–ethylenediaminetetraacetic acid, and TRIzol reagent were purchased from Invitrogen Corporation (Carlsbad, CA, USA). Dulbecco's phosphate-buffered saline (DPBS) was purchased from Gibco Corporation (USA). Resazurin, crystal violet (CV), and propidium iodide (PI) were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Hoechst was purchased from Hoechst Corporation (Germany). All the other chemicals that were used, unless otherwise stated, were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA).

Preparation of the decoctions

All the three decoctions were purchased from Beijing Tcmages Pharmaceutical Co., Ltd., (Beijing, China) and prepared as TCM granules. The doses and proportions of the components were determined by TCM clinical experts. Infrared fingerprint technology and high-performance liquid chromatography technology were used in the production of granules to ensure the quality standards of the components. The stock solution of the candidates was prepared by dissolving them in phosphate-buffered saline to a stock concentration of 40 mg/mL, followed by sterilization and filtration. Working concentrations were prepared by diluting the stock solution in the cell culture medium. The components of these three decoctions are listed in Table 1.

Cell culture

Human colon cancer SW480 cells were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). The cells were grown in DMEM containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin, incubated at 37°C in a humidified atmosphere with 5% CO_2. The cells were subcultured at 80%–90% confluency.

Cell treatment

The cells were treated with different concentrations of decoctions for 24, 36, and 48 h, respectively. The working concentrations are listed in Table 1.

Evaluation of cell viability

Cell viability was evaluated using the resazurin (7-hydroxy-10-oxido-phenoxazin-10-ium-3-one) reduction assay, commonly known as the AlamarBlue assay. The SW480 cells were seeded into 96-well plates at a density of 1.5×10^4 cells/well in 0.15 mL medium. The cells were treated with various concentrations of decoctions for 24, 36, and 48 h, respectively. The cell viability was measured after the addition of a 10-fold concentrated resazurin solution (500 μM in DPBS) at 1/10 of the sample volume to all samples,

Table 1: The components, effects from the TCM perspective, clinical applications, and working concentrations of three decoctions

Name	Components	Effects from the TCM perspective	Clinical applications	Working concentrations (μg/mL)
Bu Zhong Yi Qi Decoction	Milkvetch Root, Ginseng, Largehead Atractylodes Rhizome, Roasted Licorice Root, Chinese Angelica, Dried Tangerine Peel, Largetrifoliolious Bugbane Rhizome, Chinese Thorowax Root, Fresh Ginger, Chinese Date at a ratio of 15:15:10:15:10:6:6:12:10:10	Spleen-*qi* tonic	Colorectal cancer patients with syndrome of *qi*-deficiency of the spleen and stomach, such as fatigue, shortness of breath, poor appetite, loose stool or diarrhea, and weakness	50, 100, 200, 300, 400, 500, 600
Fuzi Lizhong Decoction	Lateralis Preparata, Ginseng, Zingiber Dried Ginger, Largehead Atractylodes Rhizome, Roasted Licorice Root at a ratio of 9:9:9:9:6	Spleen-*yang* tonic	Colorectal cancer patients with syndrome of *Yang*-deficiency of the spleen and stomach with excessive pathogenic cold which is manifested as coldness of four limbs with faint pulse, vomiting, diarrhea, and stiffness of extremities	50, 100, 200, 300, 400, 500, 600
Pulsatilla Decoction	Chinese Pulsatilla Root, Colden Thread, Amur Cork-tree, Ash bark at a ratio of 15:12:6:12	Clear away damp-heat and accumulated toxins	Colorectal cancer patients with syndrome of damp-heat and accumulated toxins in the large intestine, such as abdominal pain, tenesmus, and diarrhea	50, 100, 200, 300, 400, 500, 600

TCM: Traditional Chinese medicine

followed by a 4 h incubation at 37°C. Colorimetric absorbance was measured using an ELISA reader at 570 nm (resorufin absorbance) and 600 nm (resazurin absorbance; used as a reference). Measurements were performed at 0, 1, 2, 3, and 4 h after administration of the resazurin reagent.

Colony formation

The SW480 cells were seeded into 96-well plates at a density of 1.5×10^4 cells/well in 0.15 mL medium. The cells were then treated with various concentrations for 48 h. After the AlamarBlue assay, the cells were covered with ice-cold 100% ethanol and stored at −20°C for 15 min. The staining process was carried out with 150 µL of a 0.1% CV solution for 10 min, followed by at least three washing steps with mqH_2O. Next, the dried wells were solubilized in 100 µL of 10% acetic acid, followed by a measurement at 590 nm.

Cell apoptosis

Hoechst/PI staining was performed to measure cell apoptotic changes. The cells were then treated with various concentrations for 48 h. After treatment, 30 µL of Hoechst 33342 (1:2000 in DPBS) and 30 µL PI (1:2000 in DPBS) staining solutions were added to the wells and incubated for 10 min at 37°C. The measurements were performed using an EnSpire multilabel plate reader in chronological order of green, red, and Hoechst fluorescence. The fluorescence intensity of PI was detected at excitation and emission wavelengths of 435 and 590 nm, respectively. The fluorescence intensity of Hoechst was detected at 497 nm, with an excitation wavelength of 360 nm.

Statistical analysis

Three independent experiments were performed, and the data were collected. All the data were presented as mean ± standard deviation. The data were analyzed using the SPSS 22.0 software. SPSS 22.0 software was purchased from International Business Machines Corporation (Armonk, NY, USA). Statistical analysis of the data was performed using analysis of variance. $P < 0.05$ was considered statistically significant.

Results

Cell proliferation

The antitumor effects of Chinese herbal formulae on SW480 cell proliferation were determined by the resazurin reduction assay to compare the cell viability of SW480 cells treated with decoctions to that of untreated control cells. The results showed that Bu Zhong Yi Qi Decoction, Fuzi Lizhong Decoction, and Pulsatilla Decoction inhibited the cell proliferation in a dose- and time-dependent manner. As shown in Figure 1a-1, 2, and 3, treatment with 100–600 µg/mL of Bu Zhong Yi Qi Decoction for 24, 36, and 48 h reduced cell viability by 5.1–46.3, 7.1–65.7, and 6.5%–70.7%, respectively, when compared with that of the untreated control cells ($P < 0.05$); treatment with 100–600 µg/mL of Fuzi Lizhong Decoction for 24, 36, and 48 h reduced cell viability by 15.2–52.0, 12.0–68.6, and 14.5%–71.0%, respectively, compared with that of the untreated control cells ($P < 0.05$); and treatment with 100–600 µg/mL of Pulsatilla Decoction for 24, 36, and 48 h reduced cell viability by 9.4–21.0, 16.7–48.0, and 20.5%–51.5%, respectively, when compared with the untreated control cells ($P < 0.05$). These results were verified using colony formation assays. As shown in Figure 1b and c-1, 2, and 3, treatment with 200–600 µg/mL of Bu Zhong Yi Qi Decoction, Fuzi Lizhong Decoction, and Pulsatilla Decoction for 48 h dose dependently reduced the survival rate by 16.6–47.8, 17.5–51.5, and 26.9%–62.1%, respectively, when compared with that of the untreated control cells ($P < 0.05$).

Apoptosis and morphological changes

The effect of the Chinese herbal formulae on cell apoptosis was investigated by Hoechst/PI staining. The number of positive PI cells treated with Bu Zhong Yi Qi Decoction, Fuzi Lizhong Decoction, and Pulsatilla Decoction was significantly different compared with that of the untreated control cells ($P < 0.05$). As shown in Figure 2a and b-1, 2, and 3, the percentage of PI-stained cells after treatment with 200–600 µg/mL of the Bu Zhong Yi Qi Decoction, Fuzi Lizhong Decoction, and Pulsatilla Decoction for 48 h dose dependently increased by 34.1–90.6, 28.4–84.8, and 24.6%–80.8%, respectively, when compared with that of the untreated control cells ($P < 0.05$). As shown in Figure 2c, Hoechst staining indicated that cells treated with these three decoctions underwent morphological changes, which could be divided into three stages: Stage I cells exhibited shrunken cell membranes and condensed cytoplasm, Stage II cells exhibited aggregated chromatin and nuclear fragments, and Stage III cells showed the formation of apoptotic bodies. These aforementioned changes appeared to be concentration dependent.

Discussion

In China, TCM, with a history of 2500–5000 years, plays an important role in the treatment of cancers. In the most important classics of TCM *Huang Di Nei Jing*, which was published more than 2000 years ago, there are records and descriptions of the pathogenesis and treatment of cancers. Before the introduction of modern Western medicine into China, TCM-based herbal medicine was the only available therapeutic method for cancers. In modern times, to improve the survival rate and quality of life of patients with cancer, a combination of modern Western medicine and TCM has become the dominant strategy used in China. However, only herbal treatment is used when Western medical intervention is unlikely to have any significant clinical benefits.

According to the theories of TCM, cancer is caused by an imbalance between endogenous physical conditions of the body and exogenous pathogenic factors. The internal conditions of the body, which include *qi*, blood, *yin*, and *yang* in the TCM theory, play a dominant role in the onset of cancer.[10,11] The harmony of *qi* and blood and the balance of *yin* and *yang* protect the body from the invasion of pathological factors, whereas the deficiency of *qi* and blood and the imbalance of *yin* and *yang* causes failure of the defense system to resist attacks by pathogenic factors, such as damp-heat,

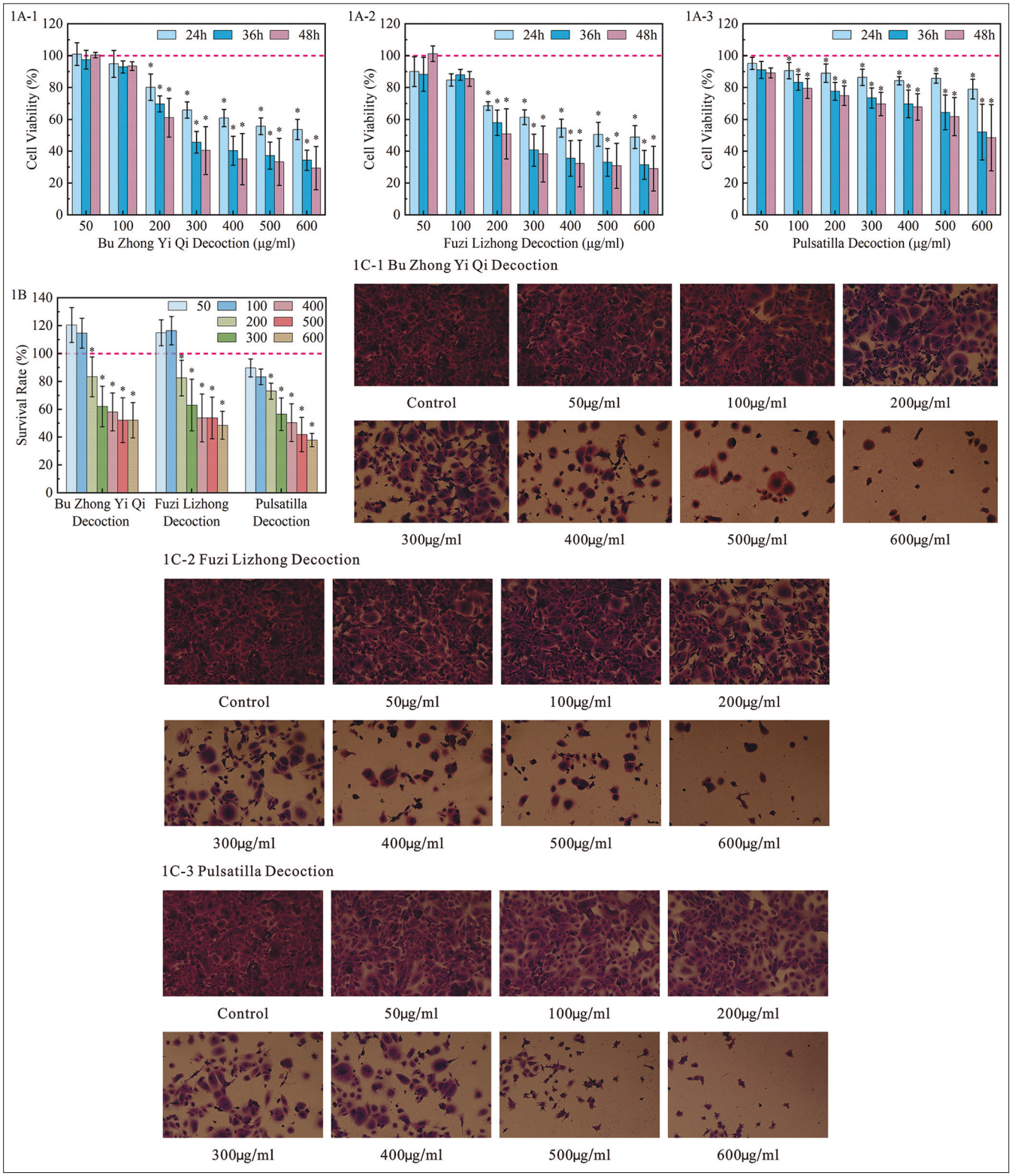

Figure 1: Effects of Chinese herb formulae on the proliferation of SW480 cells. After treatment with different concentrations of three decoctions for 24, 36, and 48 h, the cell viability was determined by the resazurin reduction assay, respectively (1A-1,2,3). After treatment with different concentrations of three decoctions for 48 h, the cell survival rate was evaluated using a colony formation assay (1B), and stained by crystal violet (1C-1,2,3). *$P<0.05$ vs. controls

toxins, phlegm, and blood stasis in Chinese medicine terms. Thus, cancer treatment is considered the improvement of the body's ability to eliminate pathogenic factors. From the TCM perspective, cancer carcinogenesis is associated with *qi*

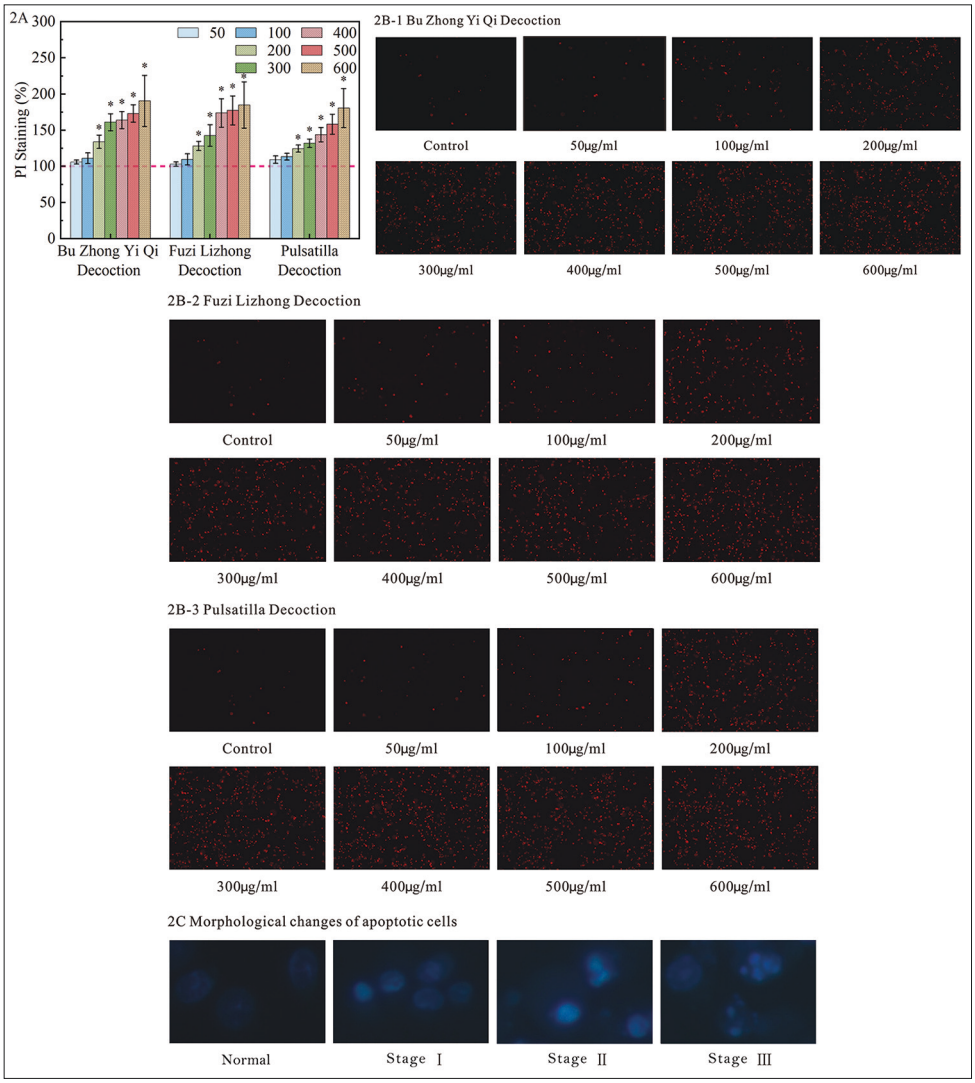

Figure 2: Effects of Chinese herb formulae on the apoptosis of SW480 cells. After treatment with different concentrations of three decoctions for 48 h, the positive PI-stained cells were investigated using an EnSpire multilabel plate (2A,2B-1,2,3) and the Hoechst-stained cells were observed under a fluorescence microscope for morphological changes (2C). *$P<0.05$ vs. controls

and blood deficiency, *yin* and *yang* deficiency, and redundant damp-heat, toxins, phlegm, and blood stasis. *Qi* is defined as a vital energy force in the human body and is associated with the function, activity, and movement of the body, as well as the production and movement of blood. The primary function of *qi* combined with blood is to circulate through the whole body to nourish and promote the physiological functions of the organs and tissues. The deficiency of *qi* causes a deficiency of blood, decline of physical functions, and destruction of the immune system. Clinical research has revealed that *qi* deficiency is closely related to cancer-related fatigue and the quality of life in patients with cancer.[12] The most basic theory of TCM is based on the principles of *yin* and *yang*. *Yin* and *yang* are two opposing and unified complements of the human body. A balance and a harmony of *yin* and *yang* maintain the healthy state of the body; however, an imbalance due to the internal or external factors ultimately leads to diseases. In cancers, *yin* is involved in cell apoptosis and promotes cancers, whereas *yang* is involved in cell survival, growth, proliferation, and maintenance of the normal state.[13] Exogenous pathogenic factors, such as redundant damp-heat, toxins, phlegm, and blood stasis, contribute to the obstruction of the circulation, the deficiency of *qi* and blood, and the imbalance of *yin* and *yang*, which lead to the onset, formation, and development of cancers.[14] Furthermore, according to the basic theory of TCM, spleen-*qi* deficiency, spleen-*yang* deficiency, damp-heat, and accumulated toxins in the large intestine are related to the occurrence, development, and metastasis of colorectal cancer.

The main principle of TCM is treatment based on symptom pattern differentiation, which is the basic principle of understanding and treating diseases in TCM theories. The TCM syndrome is a pathological condition that integrates the information on pathogenesis and clinical manifestations of TCM. Syndrome pattern differentiation is a process that summarizes and evaluates the symptoms and signs (such

as pulse and tongue) collected by the four diagnostic methods (observe, listen, ask, and feel the pulse) to analyze, synthesize, and distinguish the cause, nature, and location of diseases. After diagnosing the syndrome, the corresponding treatment method is administered according to the results of syndrome pattern differentiation. For colorectal cancer, TCM doctors distinguish the symptom pattern based on the imbalance of the state of the whole body and the invasion of the pathogenic factors and make treatment strategies to improve the whole body and eliminate the pathogens. Spleen-*qi* deficiency, spleen-*yang* deficiency, damp-heat, and toxin accumulation are the main symptoms of colorectal cancer reported by TCM doctors.

In TCM prescriptions, several herbs with each having different therapeutic effects are combined into a formula, which provides a comprehensive, integrated treatment through different pharmacological actions and multiple targets. Each formula contains a principal herb and a few of adjuvant herbs, according to the therapeutic aims of the formula and the pharmacological effects of the ingredients. In the past decades, owing to the resistance and side effects of chemotherapeutic drugs, the therapeutic value of TCM-based herbal medicine as an alternative therapy has received great interest, as its clinical application for thousands of years has confirmed that it has not only antitumor activities, but also relatively less toxicity and fewer adverse effects.[15,16] Several laboratory studies have confirmed that TCM herbs and pure ingredients from TCM herbs can play an antitumor role by inhibiting cell proliferation, telomerase activity, tumor invasion and metastasis, and angiogenesis, inducing differentiation, apoptosis, and autophagy, regulating the immune state, and affecting the cell growth cycle.[17-22] However, research on the antitumor effects of Chinese herbal formulae is very limited, even though they represent the real manifestation of TCM in clinical practice. Through synergistic interactions, Chinese herbal formulae have been shown to have better curative effects and fewer adverse effects than isolated herbs and pure ingredients. The absence of strong evidence-based research is the most important obstacle to the modernization of TCM.

In our research, the three selected candidates are well-known and commonly used formulae for the treatment of patients with the aforementioned TCM syndromes of colorectal cancer. In clinical settings, these formulae are widely used to palliate cancer-related fatigue, nausea, diarrhea, and anorexia to prolong survival time and improve the quality of life of patients with colorectal cancer. The effects of the decoctions from the TCM perspective and their clinical applications are listed in Table 1. Bu Zhong Yi Qi Decoction is a classic TCM formula with a spleen-*qi* tonic function. *Radix Astragali* (Milkvetch Root), the principal herb in this formula, has antifatigue, anemia improvement, immune regulation, and antibacterial effects. Clinical studies have revealed that Bu Zhong Yi Qi Decoction alleviates the chemotherapy-related fatigue[23] and inhibits gastric cancer progression when combined with 5-fluorouracil.[24] Laboratory practices have shown that Bu Zhong Yi Qi Decoction has a protective effect on 5-fluorouracil-induced intestinal mucositis in mice,[25] enhances cisplatin-induced apoptosis in HeLa cells,[26] and inhibits the proliferation of hepatoma cell lines by inducing apoptosis.[27] Fuzi Lizhong Decoction is a Chinese herbal formula with a spleen-*yang* tonic function. *Radix Aconiti* (*Lateralis Preparata*), the principal herb in this formula, has circulation improvement, anti-inflammatory, and analgesic actions. Laboratory studies revealed that Fuzi Lizhong Decoction can interpose carcinoma transformation in colitis,[28] and a study based on molecular docking and network pharmacology showed that Fuzi Lizhong Decoction has multitarget antitumor effects in advanced gastric cancer.[29] Pulsatilla Decoction has the functions of clearing away damp-heat and accumulated toxins, which are exogenous pathogenic factors of colorectal cancer. *Radix Pulsatilla* (Chinese Pulsatilla Root), the principal herb in this formula, has antitumor, antidiarrheal, antibacterial, and analgesic functions. *In vitro* research has shown that Pulsatilla Decoction combined with 5-fluorouracil triggers immunogenic cell death in colorectal cancer cells.[30]

Cancer cells are characterized by an uncontrolled increase in cell proliferation.[31] Therefore, inhibiting excessive proliferation of cancer cells is an important strategy for the study and development of antitumor drugs. The overall cell viability was evaluated using the resazurin reduction assay, commonly known as the AlamarBlue assay. Resazurin is a nontoxic and cell-permeable redox dye that displays colorimetric and fluorometric changes that are directly proportional to the cellular activity. CV is widely used for monolayer cell staining, and its color intensity is directly proportional to the number of cells.[32] Therefore, CV is an uncomplicated assay that is suitable for obtaining quantitative information. Using the AlamarBlue assay and CV staining, it was demonstrated that Bu Zhong Yi Qi Decoction, Fuzi Lizhong Decoction, and Pulsatilla Decoction inhibited the cell proliferation of SW480 cells in a dose- and time-dependent manner.

Apoptosis, a programmed cell death pathway, is important for maintaining the stability of the internal environment and the cell survival/death balance. Defects in apoptosis can cause cancer.[33] Cells undergoing apoptosis show specific morphological changes, such as shrunken cell membranes, condensed cytoplasm, aggregated chromatin, and apoptotic body formation. The induction of cell apoptosis is one of the most important mechanisms of antitumor activity.[34] The PI staining is an effective method for the detection of apoptotic cells, while Hoechst staining is an outstanding method for observing the morphological changes during apoptosis.[35] Based on this study, it was demonstrated that Bu Zhong Yi Qi Decoction, Fuzi Lizhong Decoction, and Pulsatilla Decoction all induced cell apoptosis, which might be the mechanism underlying their antitumor activities.

Conclusion

From this study, we conclude that the three Chinese herbal formulae, Bu Zhong Yi Qi Decoction, Fuzi Lizhong Decoction,

and Pulsatilla Decoction, inhibit the proliferation of human colon cancer SW480 cells by inducing cell apoptosis. In our study, the antitumor activities of Chinese herbal formulae were investigated, which represents a perspective in line with the principles of TCM in cancer treatment.

Acknowledgments

This study was financially supported by the Austrian Federal Ministry of Education, Science, and Research (GZ 402.000/00015-WF/V/6/2016) granted to Dr. Wolf-Dieter Rausch. The project was named *Sino-Austrian TCM research on lifestyle-related diseases: The potential of TCM for the treatment of colorectal cancer*. The authors would also like to thank the Eurasia-Pacific Uninet of the Austrian Agency for International Cooperation in Education and Research for the supports to PhD. Su FU in academic communication in Austria.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Baidoun F, Elshiwy K, Elkeraie Y, Merjaneh Z, Khoudari G, Sarmini MT, *et al.* Colorectal cancer epidemiology: Recent trends and impact on outcomes. Curr Drug Targets 2021;22:998-1009.
2. Brenner H, Kloor M, Pox CP. Colorectal cancer. Lancet 2014;383:1490-502.
3. Patel SG, Ahnen DJ. Colorectal cancer in the young. Curr Gastroenterol Rep 2018;20:15.
4. Siegel RL, Jakubowski CD, Fedewa SA, Davis A, Azad NS. Colorectal cancer in the young: Epidemiology, prevention, management. Am Soc Clin Oncol Educ Book 2020;40:1-14.
5. Li N, Lu B, Luo C, Cai J, Lu M, Zhang Y, *et al.* Incidence, mortality, survival, risk factor and screening of colorectal cancer: A comparison among China, Europe, and northern America. Cancer Lett 2021;522:255-68.
6. Dekker E, Tanis PJ, Vleugels JL, Kasi PM, Wallace MB. Colorectal cancer. Lancet 2019;394:1467-80.
7. Yu L, Cao X, Tao W, Li M, Li X, Chen L. Antioxidant activity and potential ameliorating effective ingredients for high altitude-induced fatigue from Gansu Maxianhao (Pedicularis Kansuensis Maxim). J Tradit Chin Med 2020;40:83-93.
8. Sun Q, He M, Zhang M, Zeng S, Chen L, Zhao H, *et al.* Traditional Chinese medicine and colorectal cancer: Implications for drug discovery. Front Pharmacol 2021;12:685002.
9. Li W, Li C, Zheng H, Chen G, Hua B. Therapeutic targets of traditional Chinese medicine for colorectal cancer. J Tradit Chin Med 2016;36:243-9.
10. Yang G, Li X, Li X, Wang L, Li J, Song X, *et al.* Traditional Chinese medicine in cancer care: A review of case series published in the Chinese literature. Evid Based Complement Alternat Med 2012;2012:751046.
11. Zhang ZJ, Zhang M, Wu XT, Cui Q, Guo YJ. Zhengyuan capsule for the treatment of cancer-related fatigue in lung cancer patients undergoing operation: A study protocol for a randomized controlled trial. J Tradit Chin Med 2021;41:486-91.
12. Liao S, Jia XL, Yang Y, Sun YX, Gong SM, Li M. Efficacy and safety of traditional Chinese medicine decoction combined with chemotherapy in the treatment of advanced colorectal cancer: A protocol of a systematic review and meta-analysis. Medicine (Baltimore) 2021;100:e23952.
13. Deng S, Hu B, An HM. Traditional Chinese medicine syndromes and treatment in colorectal cancer. J Cancer Ther 2013;3:888-97.
14. Hsu CH, Lee CJ, Chien TJ, Lin CP, Chen CH, Yuen MJ, *et al.* The relationship between Qi deficiency, cancer-related fatigue and quality of life in cancer patients. J Tradit Complement Med 2012;2:129-35.
15. Huang S, Kurubanjerdjit N, Xu W. Two opposing effects (Yin and Yang) determine cancer progression. Crit Rev Oncog 2017;22:143-55.
16. Jia L. Cancer complementary and alternative medicine research at the US National Cancer Institute. Chin J Integr Med 2012;18:325-32.
17. Xiang Y, Guo Z, Zhu P, Chen J, Huang Y. Traditional Chinese medicine as a cancer treatment: Modern perspectives of ancient but advanced science. Cancer Med 2019;8:1958-75.
18. Wang Y, Zhang Q, Chen Y, Liang CL, Liu H, Qiu F, *et al.* Antitumor effects of immunity-enhancing traditional Chinese medicine. Biomed Pharmacother 2020;121:109570.
19. Yan Z, Lai Z, Lin J. Anticancer properties of traditional Chinese medicine. Comb Chem High Throughput Screen 2017;20:423-9.
20. Wang CY, Bai XY, Wang CH. Traditional Chinese medicine: A treasured natural resource of anticancer drug research and development. Am J Chin Med 2014;42:543-59.
21. Lee GY, Lee JS, Son CG, Lee NH. Combating drug resistance in colorectal cancer using herbal medicines. Chin J Integr Med 2021;27:551-60.
22. Wang K, Chen Q, Shao Y, Yin S, Liu C, Liu Y, *et al*. Anticancer activities of TCM and their active components against tumor metastasis. Biomed Pharmacother 2021;133:111044.
23. Ouyang M, Liu Y, Tan W, Xiao Y, Yu K, Sun X, *et al.* Bu-zhong-yi-qi pill alleviate the chemotherapy-related fatigue in 4 T1 murine breast cancer model. BMC Complement Altern Med 2014;14:497.
24. Xu R, Wu J, Zhang X, Zou X, Li C, Wang H, *et al.* Modified Bu-zhong-yi-qi decoction synergies with 5 fluorouracile to inhibits gastric cancer progress via PD-1/PD- L1-dependent T cell immunization. Pharmacol Res 2020;152:104623.
25. Gou H, Gu LY, Shang BZ, Xiong Y, Wang C. Protective effect of Bu-Zhong-Yi-Qi decoction, the water extract of Chinese traditional herbal medicine, on 5-fluorouracil-induced intestinal mucositis in mice. Hum Exp Toxicol 2016;35:1243-51.
26. Sato T, Kita K, Sato C, Kaneda A. Hochu-ekki-to (Bu-zhong-yi-qi-tang), a herbal medicine, enhances cisplatin-induced apoptosis in HeLa cells. Mol Med Rep 2015;12:6215-20.
27. Yu N, Xiong Y, Wang C. Bu-Zhong-Yi-Qi decoction, the water extract of Chinese traditional herbal medicine, enhances cisplatin cytotoxicity in A549/DDP cells through induction of apoptosis and autophagy. Biomed Res Int 2017;2017:3692797.
28. Jian JN, Jing HL, Wang YD, You FM, Yan R. Research progress in the intervention of carcinoma transformation of colitis with Fuzi Lizhong decoction. J Oncol Chin Med 2021;3:63-6.
29. Xiong JH, Hou EC, Tang YM, Zheng JH, Xu X, Luo FF. Study on the mechanism of Fuzi Lizhong decoction in the treatment of advanced gastric cancer based on molecular docking and network pharmacology. China J Chin Med 2020;35:164-71.
30. Jie Y, Yang X, Chen W. Pulsatilla decoction combined with 5-fluorouracil triggers immunogenic cell death in the colorectal cancer cells. Cancer Biother Radiopharm 2021;5:34042519.
31. Feoktistova M, Geserick P, Leverkus M. Crystal violet assay for determining viability of cultured cells. Cold Spring Harb Protoc 2016;2016:343-6.
32. Sankari SL, Masthan KM, Babu NA, Bhattacharjee T, Elumalai M. Apoptosis in cancer – An update. Asian Pac J Cancer Prev 2012;13:4873-8.
33. Goldar S, Khaniani MS, Derakhshan SM, Baradaran B. Molecular mechanisms of apoptosis and roles in cancer development and treatment. Asian Pac J Cancer Prev 2015;16:2129-44.
34. Hassan M, Watari H, AbuAlmaaty A, Ohba Y, Sakuragi N. Apoptosis and molecular targeting therapy in cancer. Biomed Res Int 2014;2014:150845.
35. Atale N, Gupta S, Yadav UC, Rani V. Cell-death assessment by fluorescent and nonfluorescent cytosolic and nuclear staining techniques. J Microsc 2014;255:7-19.