International Journal of
Laboratory Medicine & Research

Original Article Open Access

Anticancer Effects of *Pulsatilla koreana, Anemarrhenae Asphodeloides Bunge, Coptis Rhizoma* or Fucoidan in Combination with Oxaliplatin, 5-Fluorouracil, Capecitabin, or Irinotecan on Human Gastric or Colon Cancer Cell Lines

A-Jin Lee[1], Hyun-Dong Chae[2], Dae-Dong Kim[2], Chang-Ho Jeon[1*]

[1]*Department of Laboratory Medicine, Catholic University of Daegu School of Medicine, Daegu, 42472, Republic of Korea*
[2]*Department of General Surgery, Catholic University of Daegu School of Medicine, Daegu, 42472, Republic of Korea*

Abstract

Background: Chemotherapeutic agents such as oxaliplatin, 5-FU, capecitabin, or irinotecan are widely used to treat metastatic gastric or colorectal cancer. Various herbal medicines may be used to reduce chemotherapy-related toxicities. In this study, we evaluated the effectiveness of 16 herbal medicines, selected herbal medicines with anticancer effect, and investigated the growth inhibitory effect of combined herbal medicines and chemotherapy agents in 5 gastric cancer cell lines or 3 colon cancer cell lines.

Methods: We evaluated anti-cancer effect of the 16 herbal medicines, then we finally selected 4 herb medicines: *Pulsatilla koreana, Anemarrhenae asphodeloides bunge,Coptis rhizoma*, and fucoidan. We evaluated the sensitivity of these herb medicines and anticancer agents including oxaliplatin, 5-FU, capecitabin, or irinotecan.

Results: Compared with chemotherapeutic agent alone, *Pulsatilla koreana, Anemarrhenae asphodeloides bunge, Coptis rhizoma* and fucoidan combined with capecitabin significantly inhibited the growth of gastric and colorectal cancer cells. The four herb medicines also inhibited significantly the growth of gastric cancer cells with combination of anti-cancer agents: *Pulsatilla koreana*+ 5-FU, *Anemarrhenae asphodeloides bunge*+ oxaliplatin, *Coptis rhizoma* + 5FU or oxaliplatin, fucoidan + irinotecan.

Conclusions: Pulsatilla koreana, Anemarrhenae asphodeloides bunge, Coptis rhizoma, or fucoidan extract can be applied as adjuvant medicinesin combination of oxaliplatin, 5-FU, capecitabin, or irinotecan to inhibit the growth of gastric cancer cells or colon cancer cells.

Publication History:

Received: May 14, 2017
Accepted: August 08, 2017
Published: August 10, 2017

Keywords:

Colon cancer, Gastric cancer, Pulsatilla koreana, Anemarrhenae asphodeloides bunge, Coptis rhizoma, Fucoidan

Introduction

Gastric cancer is the most prevalent cancer in Eastern Asia and colorectal cancer is the second most prevalent cancer globally [1]. The most common treatment for these cancers is surgery [2]. Chemotherapy is necessary to treat cancer with advanced stage. Chemotherapeutic agents such as oxaliplatin, 5-fluorouracil (5-FU), capecitabin, or irinotecan are commonly used to treat metastatic gastric or colorectal cancer [3].However, many patients may be suffer from chemotherapy-related toxicity such as diarrhea, vomiting, fatigue, neuropathy, hematological abnormalities, or immune dysfunction [3,4]. These side effects can impair quality of life of cancer patients. In addition, some cancers are chemo-resistant and systemic cytotoxic chemotherapy are minimally effective at cure of cancer [5].

Various herbal medicines have been widely used to care cancer-related symptoms and to treat cancer in China, Japan, and other Asian countries [6]. Several studies have investigated the effectiveness of herbal medicines combined with chemotherapeutic agents [7–9]. Herbal medicines have been reported to have favorable therapeutic effects in improving quality of life. However, these studies only focused on single targeted herbal medicines. In this study, we evaluated the effectiveness of many herbal medicines and selected herbal medicines with anticancer effect. And we investigated the therapeutic effect of combined herbal medicines and chemotherapy agents in gastric cancer cells or colon cancer cells.

Material and Methods

Cell lines and cell culture

Human gastric carcinoma cell lines SNU-1, SNU-216, SNU-484, SNU-668 and NCI-N87 were obtained from the Korean Cell Line Bank (Seoul, Korea). Human colorectal carcinoma cell lines SNU-175 and SNU-1411 were obtained from the Korean Cell Line Bank. Another colorectal carcinoma cell lines CRC1306 was isolated from the patients of Daegu Catholic Medical Center. Cells were suspended in RPMI-1640 (Gibco/BRL, Grans Island, NY) containing 10% FBS, 25 mM HEPES, and 25 mM sodium bicarbonate (Gibco/BRL).

Reagents

A total of 16 herbal medicines were initially screened as follows: (extract of *Alnus japonica* (Jeokyang), extract of *Anemarrhenae asphodeloides bunge* (AAB, Jimo), extract of *Astragalus sinicus* (Honghwa), water extract of *Coptis rhizoma* (CR, Hwang-ryeon), curcumin, Daehwang-Mokdanpi-tang, Daesiho-tang, Dangguibohyul-tang, extract of *Duchesnea indica* (Samae), extract of *Euphorbiae Lathyridis* (Soksuja), fucoidan extract, Gyejibokryeong-hwan, Hyangsa-Pyeongwi san, extract of *Mylabris phalerata* (Banmyo), *Pulsatilla Koreana* extract (PKE, Baekduong-tang) and

***Corresponding Author:** Prof. Chang-Ho Jeon, Department of Laboratory Medicine, Catholic University of Daegu School of Medicine, Daegu, 42472, Republic of Korea, Tel: +82-53-650-4144; E-mail: chjeon@cu.ac.kr

Citation: Lee AJ, Chae HD, Kim DD, Jeon CH (2017) Anticancer Effects of *Pulsatilla koreana*, *Anemarrhenae Asphodeloides Bunge*, *Coptis Rhizoma* or Fucoidan in Combination with Oxaliplatin, 5-Fluorouracil, Capecitabin, or Irinotecan on Human Gastric or Colon Cancer Cell Lines. Int J Lab Med Res 3: 114. doi: https://doi.org/10.15344/2455-4006/2016/114

extract of *Sophora flavescens* (Gosam). Gyejibokryeong-hwan, and Daehwang-Mokdanpi-tang were purchased from Hanzung pharmaceutical company (Daejeon, Korea), curcumin and fucoidan extract was purchased from Sigma-Aldrich Co. (St. Louis, MO). The other herbal medicines were obtained from Semyung University Korean Medicine (Jecheon, Korea). Anticancer agents including oxaliplatin, 5-FU, capecitabine, and irinotecan were purchased from Sigma-Aldrich (St. Louis, MO), and was dissolved in dimethylsulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO). The tetrazolium salt MTT (3-[4,5]-dimethyl-2-thiazolyl-2,5-diphenyltetrazolium bromide) were purchased from Sigma-Aldrich (St.Louis.MO, USA).

Choice of herbal medicine with anticancer effect using MTT assay

We first screened 16 herbals, and herbal medicines were tested using MTT assay. A colorimetric assay using the tetrazolium salt MTT was used to assess the cytotoxicity of herbal medicines or anticancer agents. Equal number of colorectal cancercells (CRC1306, 2×10^4/well) cultured from a cancer patient of Daegu Catholic University Medical Center (Daegu, Korea) was inoculated into 96-well plates and treated with the herbal drugs or phosphate buffered saline (for untreated 100% survival control). Herbal medicines were added at serial diluted concentrations, with the initial drug concentration of 612μg/mL. These mixtures were cultured at 37°C and 5% CO_2 for 3 days. After that, 100 μL of MTT at concentration of 5 mg/mL was added to each well and incubated at 37°C for 4 hours. Plates were centrifuged at 450 g for five min at room temperature and culture medium removed. DMSO (500 μL) was added to each well to solubilize the crystals, and the plates were read at 540 nm on a scanning multiwall spectrometer (EVOLIS, Bio-Rad Laboratories, Hercules, CA, USA). Herbal medicines producing more than 50% reduction (50% of cell viability) in absorbance at 540 nm compared to untreated controls in an MTT assay were defined as having anticancer effect.

Determination of the concentrations of anticancer agents

Equal number of cells (2×10^4/well) cultured from cancer tissue (CRC1306) was plated and treated with variable doses of anticancer agents alone. The MTT assay was performed as described above. To avoid the growth inhibitory effect of anticancer agent, the concentration producing a 30% reduction in absorbance at 540 nm compared to untreated controls in an MTT assay was determined. The concentrations of oxaliplatin, 5-FU, capecitabin, and irinotecan were 5 μg/mL, 12.5μg/mL, 63 μg/mL, and 6 μg/mL, respectively.

Cell viability assay of combination therapy targeting various cancer cell lines

Equal number of cells (2×10^4/well) cultured from various cancer cell lines was inoculated into each well and cultured at 37°C and 5% CO_2 for 3days. After that, the anticancer agent alone (for untreated with herbal medicine, control) or in combination of herbal medicineswere added and cultured at 37°C and 5% CO_2. The anticancer agents were added at the same concentrations described above. The initial concentration of herbal medicines was 500 μg/mL and the drugs were serially diluted. The anti-cancer combination effects were shown at the figure 1.

Statistical method

A dose-inhibition curve was drawn from the results of the MTT assay by calculating the percentage of cell viability (100 x absorbance of drug treated wells/absorbance of control wells). Statistical significances were analyzed by one-way ANOVA test in the herbal medicine-anticancer agent combinations experiments withsubsequent post hoc range tests (Turkey). Statistical analysis was performed using the SPSS software, version 21.0 (SPSS, Chicago, IL, USA). *P* value <0.05 was considered statistically significant.

Results

Screening of anti-cancer effects of 16 herbal medicines

Based on the results of cell viability, AAB, CR, Curcumin, fucoidan, *Mylabris phalerata* and PKE showed anti-cancer effect. Curcumin and *Mylabris phalerata* were excluded due to their limitation in vivo usage. AAB, CR, fucoidan and PKE were selected for the further study (Table 1).

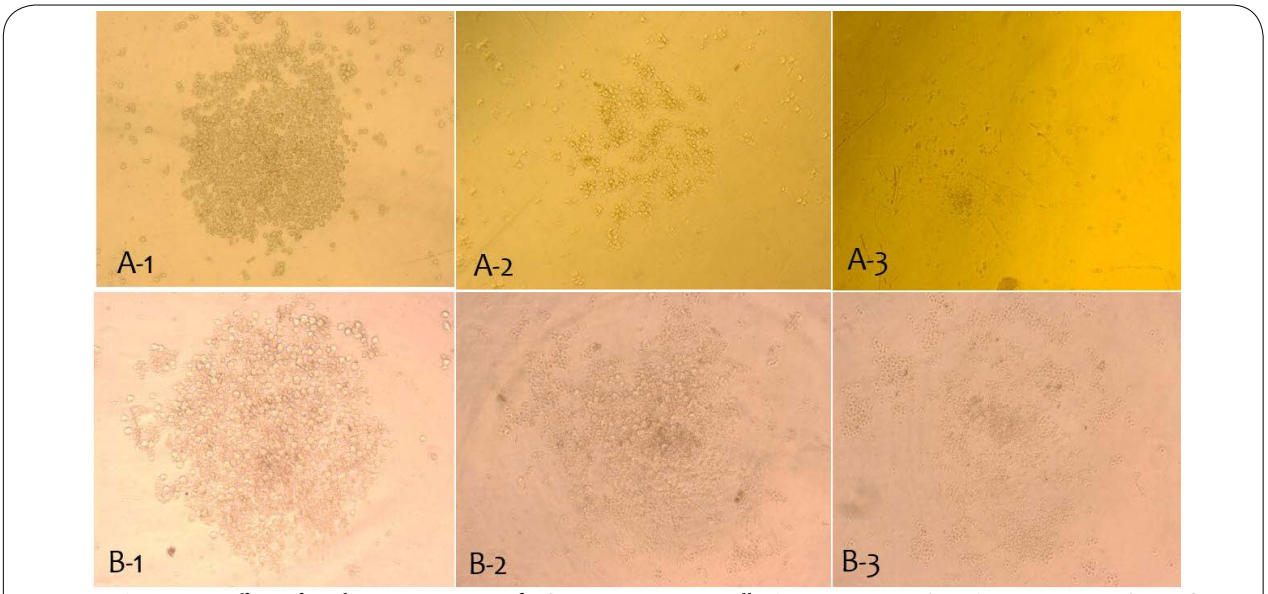

Figure 1. Anti-cancer effects of combination treatments for SNU1 gastric cancer cells, A1 Irinotecan 6ug/mL, A2 Irinotecan 6ug/mL + *Coptis rhizoma* 125ug/mL, A3 Irinotecan 6ug/mL + *Coptis rhizoma* 500ug/mL, B1 Oxaliplatin 5ug/mL, A2 Oxaliplatin 5ug/mL + *Pulsatilla koreana* 125ug/mL, A3 Oxaliplatin 5ug/mL + *Pulsatilla koreana* 500ug/mL.

Herbal medicines (μg/mL)	Cell viability (%)				
	0	76.5	153	306	612
Alnus japonica	95	90	90	90	80
Anemarrhena asphodeloides Bunge	90	90	80	40	10
Astragalus sinicus	90	90	90	90	90
Coptidis Rhizoma	90	30	10	10	0
Curcumin	90	0	0	0	0
Daehwang-Mokdanpi-tang	90	90	90	90	90
Daesiho-tang	90	95	95	95	95
Dang gui bohyul-tang	90	80	80	80	80
Duchesnea indica	90	90	90	90	90
Euphorbia lathyris	90	90	90	90	70
Fucoidan	90	90	80	60	30
GyejiBokryeong-hwan	90	90	95	90	95
Hyangsa-Pyeongwi san	90	95	90	90	95
Mylabris phalerata	95	0	0	0	0
Pulsatilla koreana extract	90	80	60	40	20
Sophora flavescens	90	90	90	90	90

Table 1: Anti-cancer effects of 16 herbal medicines measured using MTT assay.

Cytotoxicity of *Pulsatilla koreanaon* Gastric and Colorectal Cancer Cell Lines (Figure 2)

PKE combined with 5-FU or capecitabin, significantly inhibited the growth of gastric cancer cells (P<0.05), while combined with irinotecan or oxaliplatin, inhibited the growth of gastric cancer cells but statistically not significant (P=0.096 and 0.056 respectively, Figure 2a). PKE combined with capecitabin inhibit the growth of colorectal cancer cells (P<0.05), while combined with 5-FU, irinotecan or oxaliplatin did not inhibit the growth of colorectal cancer cells (Figure 2b).

Cytotoxicity of AAB on Gastric and Colorectal Cancer Cell Lines (Figure 3)

AAB combined with capecitabin or oxaliplatin significantly inhibited the growth of gastric cancer cells (P<0.05). AAB combined with capecitabin inhibit the growth of colorectal cancer cells (P<0.05).

Cytotoxicity of CR on Gastric and Colorectal Cancer Cell Lines (Figure 4)

CR combined with 5-FU, capecitabin and oxaliplatin, significantly inhibited the growth of gastric cancer cells (P<0.05), while combined

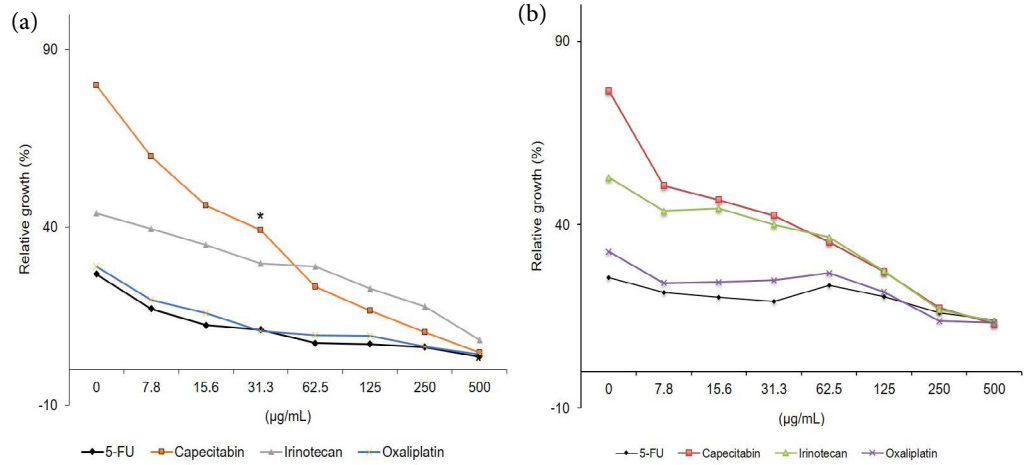

Figure 2: Growth inhibitory effects of Pulsatilla koreana in combination with chemotherapeutic agents on gastric cancer cells (A) or colorectal cancer cells (B). * Capecitabin and 5-FU shows significant difference from the chemotherapeutic agent alone group (P<0.05).

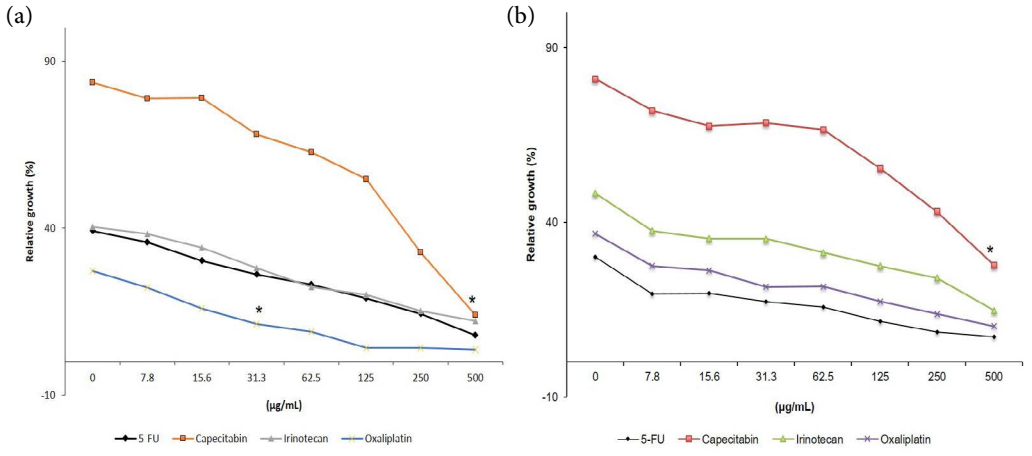

Figure 3: Growth inhibitory effects of extract of *Anemarrhenae asphodeloides bunge* in combination with chemotherapeutic agents on gastric cancer cells (A) or colorectal cancer cells (B). * Capecitabine (for gastric and colorectal cancer) and oxaliplatin shows the significant different concentration from the chemotherapeutic agent alone group (*P*<0.05).

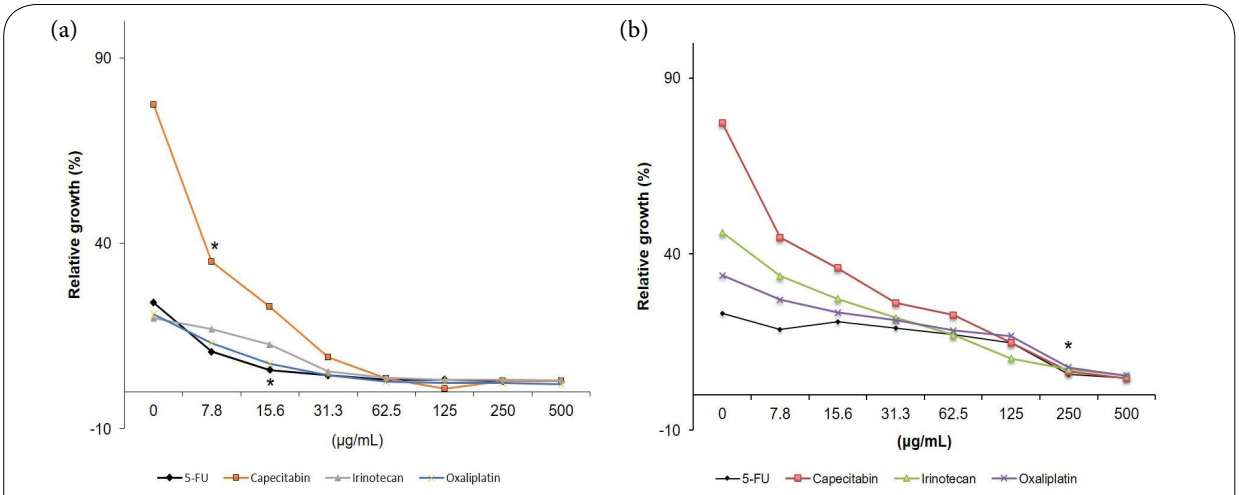

Figure 4: Growth inhibitory effects of water extract of *Coptis rhizoma* in combination with chemotherapeutic agents on gastric cancer cells (A) or colorectal cancer cells (B). * Capecitabin (gastric and colorectal cancer), 5-FU and oxaliplatin shows the significant different concentration (P<0.05) from the chemotherapeutic agent alone group.

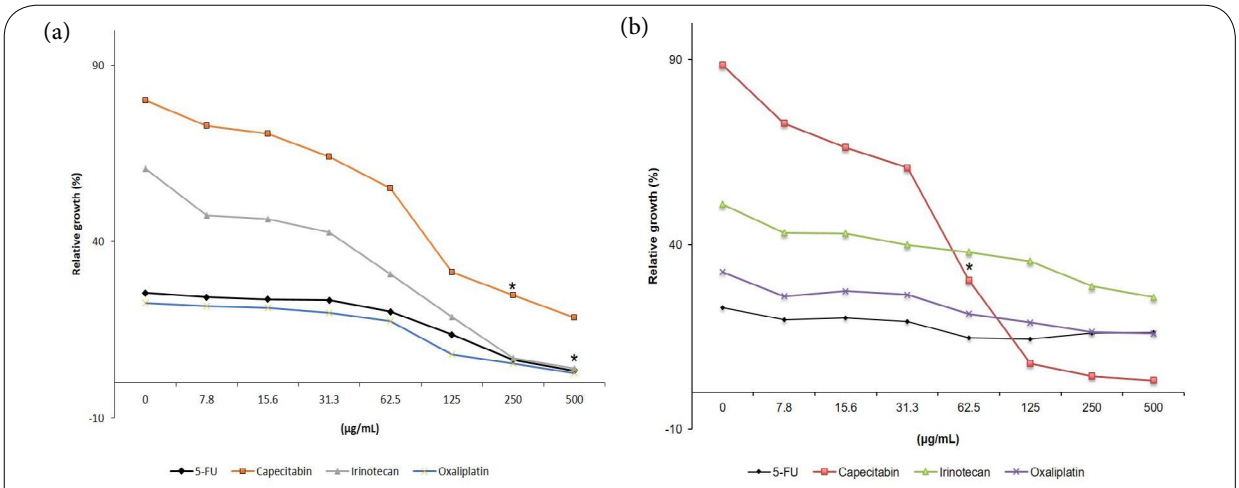

Figure 5: Growth inhibitory effects of fucoidan extract in combination with chemotherapeutic agents on gastric cancer cells (A) or colorectal cancer cells (B). * Capecitabin (gastric and colorectal cancer) and irinotecan shows the significant difference from the chemotherapeutic agent alone group.

with irinotecan inhibited the growth of gastric cancer cells but statistically not significant (P=0.179, Figure 4a). CR combined with capecitabin, significantly inhibited the growth of colorectal cancer cells, while combined with 5-FU, irinotecanor oxaliplatin did not inhibit the growth of colorectal cancer cells. (Figure 4b).

Cytotoxicity of Fucoidan Extract on Gastric and Colorectal Cancer Cell Lines (Figure 5)

Fucoidan combined with capecitabin and irinotecan, significantly inhibited the growth of gastric cancer cells (P<0.05, Figure 5a), while combined with 5-FU and oxaliplatin did not inhibit the growth of gastric cancer cells. Fucoidan combined with capecitabin, inhibit the growth of colorectal cancer cells. (P<0.05, Figure 5b).

Discussion

To decrease chemotherapy-related cytotoxicity, many herbal medicines were evaluated whether to have anticancer effects. To date, several studies have investigated the effect of single herbal agent and single chemotherapeutic agent. However, few studies have screened several herbal medicines and evaluated combination effect of herbal medicines when combined with anticancer agents. Especially, the effects of combination therapy on gastric cancer cells were rarely evaluated. We screened several herbal medicines and chose the agents with good response. And we examined the sensitivity of PKE, AAB, CR, or fucoidan extract in combination of oxaliplatin, 5-FU, capecitabine, or irinotecan on various gastric cancer cell or colorectal cancer cell lines.

PKE has been known to have the anticancer effect against various cancers including colon cancer, hepatocellular carcinoma, thyroid cancer, melanoma and ovarian cancer [10–14]. It has been reported that PKE induced the apoptosis of p53 positive HCT-116 cells, colon cancer cell line through G2/M phase arrest and intracellular redox status [10]. In the current study, PKE showed the additive growth inhibitory effect on gastric cancer cell lines when combined with 5-FU and capecitabin. In the present study, PKE also showed the growth inhibitory effect on themost of gastric cancer cell lines with conventional anti-cancer agents.

AAB, when combined with capecitabin, showed the anticancer effect on gastric and colon cancer cells. AAB combined with oxaliplatin inhibited significantly the growth of gastric cancer cells. These results correspond well with those of the earlier study which reported that AAB induces apoptosis in HT-29 human colon cancer cells through mitochondria/caspase pathway [15]. In the current study, AAB also showed the growth inhibitory tendency on cancer cell lines with 4 anti-cancer agents.

CR had the growth inhibitory effect on most gastric cancer cell lines and colon cancer cell lines when administered in combination with anticancer agent. CR has been reported to have the anticancer effect against gastric cancer cells, colon cancer cells and breast cancer cells [16–18]. It has been demonstrated that CR induces apoptosis through caspase3 pathway in SNU-668 human gastric cancer cells[16]. In the previous study, CR exhibited the anticancer effect on SNU-81 colon cancer cells through ErbB3-regulated signal transduction pathway [17].

Fucoidan extract also had the growth inhibitory effect on most gastric cancer cell lines and colon cancer cell lines when administered in combination with capecitabin or irinotecan. Oh et al. reported that fucoidan in combination with tyrosine kinase inhibitor lapatinib exerted synergistic or antagonistic effect on EGFR/ERBB2-amplified cancer cells [19]. In the present study, there was no antagonistic effect when fucoidan extract given in combination with oxaliplatin, 5-FU, capecitabin, or irinotecan. Fucoidan extract has been known to reduce the toxicities of oxaliplatin plus 5-FU/leucovorin or irinotecan plus 5-FU/leucovorin and improve survival for patients with advanced or recurrent colorectal cancer (n = 2) in clinical trial [20].

Although drug concentrations of oxaliplatin, 5-FU, capecitabin, or irinotecan were much lower than optimal concentrations, addition of herbal medicines led to satisfactory growth inhibitory result. Lower concentrations of cancer drug may reduce drug-related side effects.

There are limitations in this study. The experiments were performed not in vivo, but *in vitro*. Drug sensitivity can be different between *in vitro* and *in vivo*. Animal experiments and clinical trials should be done in the further study. Clinical trials should be performed whether to reduce chemotherapeutic agent-related toxicities. Another limitation is that prescriptions of herb medicine are not standardized worldwide and difficult to use in West.

In conclusion, PKE, AAB, CR, or fucoidan extract can be applied as adjuvant medicines in combination of oxaliplatin, 5-FU, capecitabin, or irinotecan to inhibit the growth of gastric cancer cells or colon cancer cells. Most of them showed additive growth inhibitory effects in combination with the conventional chemotherapeutic agents. Molecular study is needed to understand the mechanism of growth inhibitory effect.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contribution

C. Jeon designed the study, wrote the study protocol and conducted the experiment. H. Chae and D. Kim supplied the patients' sample. C. Jeon and A. Lee prepared the paper. All the authors read and approved the final paper.

Funding

This study was supported by grant of Korea of Health & Welfare, Republic of Korea (Project no. CIMI-13-01-023).

References

1. Bray F, Ren JS, Masuyer E, Ferlay J (2013) Global estimates of cancer prevalence for 27 sites in the adult population in 2008. Int J Cancer 132: 1133-1145.
2. Huang XE, Wang L, Ji ZQ, Liu MY, Qian T, et al. (2015) Safety of Lienal Polypeptide Injection Combined with Chemotherapy in Treating Patients with Advanced Cancer. Asian Pac J Cancer Prev 16:7837-7841.
3. Bazarbashi S, Aljubran A, Alzahrani A, Mohieldin A, et al. (2015) Phase I/II trial of capecitabine, oxaliplatin, and irinotecan in combination with bevacizumab in first line treatment of metastatic colorectal cancer. Cancer Med 4: 1505-1513.
4. Shurin MR, Naiditch H, Gutkin DW, Umansky V, Shurin GV (2012) ChemoImmunoModulation: immune regulation by the antineoplastic chemotherapeutic agents. Curr Med Chem 19: 1792-1803.
5. Urruticoechea A, Alemany R, Balart J, Villanueva A, Viñals F, et al. (2010) Recent advances in cancer therapy: an overview. Curr Pharm Des 16: 3-10.
6. Chung VC, Wu X, Hui EP, Ziea ET, Ng BF, et al. (2015) Effectiveness of Chinese herbal medicine for cancer palliative care: overview of systematic reviews with meta-analyses. Sci Rep 5: 18111.
7. Xie X, Huang X, Li J, Lv X, Huang J, et al. (2013) Efficacy and safety of Huachansu combined with chemotherapy in advanced gastric cancer: a meta-analysis. Med Hypotheses 81: 243-250.
8. Guo Z, Jia X, Liu JP, Liao J, Yang Y (2012) Herbal medicines for advanced colorectal cancer, in: The Cochrane Collaboration (Ed.), Cochrane Database Syst. Rev., John Wiley & Sons, Ltd, Chichester, UK.
9. Chen M, May BH, Zhou IW, Xue CC, Zhang AL (2014) FOLFOX 4 combined with herbal medicine for advanced colorectal cancer: a systematic review. Phytother Res 28: 976-991.
10. Kim J, Moon G, Park C, Lee J, Ji H (2010) Studies on the Anti-cancer Effect and the Mechanism of Apoptosis by Baekduong-tang in Human Colon Cancer Cell Line HCT-116. Korean J Orient Int Med 31: 273-289.
11. Hong SW, Jung KH, Lee HS, Choi MJ, Zheng HM, et al. (2012) Apoptotic and anti-angiogenic effects of Pulsatilla koreana extract on hepatocellular carcinoma. Int J Oncol 40: 452-460.
12. Park BH, Jung KH, Son MK, Seo JH, Lee HS, et al. (2013) Antitumor activity of Pulsatilla koreana extract in anaplastic thyroid cancer via apoptosis and anti-angiogenesis. Mol Med Rep 7: 26-30.
13. Bang SC, Lee JH, Song GY, Kim DH, Yoon MY, et al. (2005) Antitumor activity of Pulsatilla koreana saponins and their structure-activity relationship. Chem Pharm Bull (Tokyo) 53: 1451-1454.
14. Kim Y, Kim SB, You YJ, Ahn BZ (2002) Deoxypodophyllotoxin; the cytotoxic and antiangiogenic component from Pulsatilla koreana. Planta Med 68: 271-274.
15. Kim TH, Kim PH, Jeon BK, Yoon JR, Woo WH, et al. (2011) Effect of Anemarrhenae Rhizoma Ethanol Extract on Apoptosis Induction of HT-29 Human Colon Cancer Cells. J Korean Med Ophthalmol Otolaryngol Dermatol 24:16-24.
16. Park HJ, Kim YJ, Leem K, Park SJ, Seo JC, et al.(2005) Coptis japonica root extract induces apoptosis through caspase3 activation in SNU-668 human gastric cancer cells. Phytother Res PTR 19: 189-192.
17. Yoo TM, Kim BS, Yoo BC, Yoo HS (2009) Monitoring the Change of Protein Expression in Human Colon Cancer Cell SNU-81 treated with the Water-Extract of Coptis japonica. J Korean Inst Herb Acupunct 12: 5-12.
18. Liu J, He C, Zhou K, Wang J, Kang JX (2009) Coptis extracts enhance the anticancer effect of estrogen receptor antagonists on human breast cancer cells. Biochem Biophys Res Commun 378:174-178.
19. Oh B, Kim J, Lu W, Rosenthal D (2014) Anticancer effect of fucoidan in combination with tyrosine kinase inhibitor lapatinib. Evid Based Complement Alternat Med 2014: 865375.
20. Ikeguchi M, Yamamoto M, Arai Y, Maeta Y, Ashida K, et al. (2011) Fucoidan reduces the toxicities of chemotherapy for patients with unresectable advanced or recurrent colorectal cancer. Oncol Lett 2: 319-322.