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Effects of *Coptis* extract combined with chemotherapeutic agents on ROS production, multidrug resistance, and cell growth in A549 human lung cancer cells

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**Abstract**

**Background:** Non–small cell lung cancer is associated with high expression of multidrug resistance (MDR) proteins and low production of reactive oxygen species (ROS). *Coptis* extract (COP), a Chinese medicinal herb, and its major constituent, berberine (BER), have anticancer properties. This study aims to investigate the effects of COP and BER combined with chemotherapeutic agents, including fluorouracil (5-FU), camptothecin (CPT), and paclitaxel (TAX), on cell proliferation, ROS production, and MDR in A549 human non-small cell lung cancer cells.

**Methods:** A549 cells were treated with different doses of COP and BER, combined with 5-FU, CPT, and TAX. Cell viability was measured by an XTT (2,3-bis-(2-methoxy-4- nitro-5-sulfophenyl)-2 H-tetrazolium-5-carboxanilide) assay. Intracellular ROS levels were determined by measuring the oxidative conversion of cell permeable 2′,7′-dichlorofluorescein diacetate to fluorescent dichlorofluorescein. MDR of A549 cells was assessed by rhodamine 123 retention assay.

**Results:** Both COP and BER significantly inhibited A549 cell growth in a dose-dependent manner. Combinations of COP or BER with chemotherapeutic agents (5-FU, CPT, and TAX) exhibited a stronger inhibitory effect on A549 cell growth. In addition, COP and BER increased ROS production and reduced MDR in A549 cells.

**Conclusion:** As potential adjuvants to chemotherapy for non–small cell lung cancer, COP and BER increase ROS production, reduce MDR, and enhance the inhibitory effects of chemotherapeutic agents on A549 cell growth.

**Background**

The herb *Coptis* (COP) is used to treat “damp heat” syndrome in Chinese medicine [1]. Its major constituent is berberine (BER), an isoquinoline alkaloid [2]. The anticancer effects of COP and BER on both hematological and nonhematological cancers have been well documented [3]. Since 2000, experimental studies have confirmed the cytotoxicity of BER in various cancer cell lines, including YES (esophageal carcinoma) [4], HK1 (nasopharyngeal carcinoma) [5], HeLa (cervical carcinoma) [6], HepG2 (hepatocellular carcinoma) [7]. Our previous studies [9,10] have also shown that COP inhibits the growth of breast cancer cells.

Non–small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancers, and only responds to 15%–25% single agents and 25%–40% combined chemotherapy [11]. NSCLC is typically resistant to apoptosis induced by standard chemotherapy, which causes excessive levels of reactive oxygen species (ROS), leading to impaired intracellular ionic homeostasis by damaging cellular macromolecules and inducing apoptosis [12]. Mitochondrial ROS production is crucial to NSCLC apoptosis induced anticancer agents [13]. In addition to ROS, multidrug-resistance (MDR) proteins are intrinsically expressed and functionally active in NSCLC cells [14]. Several adjuvants to chemotherapy for NSCLC are being tested, with promising results, including the antagonists of EGFR and COX-2 [15-17].
This study aims to investigate the effects of COP and BER on ROS production and MDR, and the effects of combinations of COP or BER with chemotherapeutic agents, including fluorouracil (5-FU), camptothecin (CPT), and paclitaxel (TAX) on A549 human cancer cells, which are derived from NSCLC [18].

**Methods**

**Materials**

A powder form of COP extract was made from *Coptis japonica* (Mayway Corporation, Oakland, CA, USA) by boiling the plant in water and spray drying. A solution of COP was prepared as previously described [10]. BER, 5-FU, and CPT were dissolved in dimethylsulfoxide (DMSO) (Sigma-Aldrich, USA). COP and BER and tested for cell viability with an XTT assay to examine the effects of COP and BER on cancer cell growth. As shown in Figure 1, treatment with COP significantly inhibited A549 cell growth. The maximum inhibition rates were 60% and 64% for COP and BER, respectively. A Pearson Correlation Test by Prism 4 was used to determine the correlation between the...
doses of COP or BER and the inhibitory effects on A549 cell growth. The results indicated that the growth inhibition was in a dose-dependent manner \( P = 0.0032 \) for COP; \( P = 0.0178 \) for BER.

Low and high doses of COP or BER combined with 5FU, CPT, or TAX were used to treat A549 cells to investigate the inhibitory effects of COP and BER in combination with chemotherapeutic drugs on cancer cells. As shown in Figure 2, a combination of a low dose of COP and chemotherapeutic drugs had an inhibitory effect stronger than CPT or TAX alone on cancer cell growth \( (P < 0.001 \) for both CPT and TAX), whereas high doses of COP enhanced the inhibitory effects of CPT, TAX, and 5FU on A549 cell growth \( (P < 0.001 \) for CPT, TAX, and 5FU). In addition, the combination of a high dose of BER with chemotherapeutic drugs exhibited an inhibitory effect stronger than CPT, TAX, or 5FU alone on A549 cell growth. These findings suggest a potential use of COP and BER as adjuvant therapies for NSCLC.

Production of reactive oxygen species in A549 cells

The intracellular levels of ROS production were measured after treatment with low or high doses of COP and BER. As shown in Figure 3, low doses of COP and BER increased ROS production by approximately 50\% \( (P < 0.05) \), relative to the control group, and ROS production in the cells incubated with high doses of COP and BER was nearly 3 times that of the control group \( (P < 0.01, n = 4) \).

Our results show that both COP and BER significantly increased ROS levels in A549 cells, in a dose-dependent manner, and enhanced the inhibitory effect of chemotherapeutic drugs on A549 cells. The present study agrees with previous findings that ROS production is increased in cancer cells \([21]\), which sensitizes the cancer cells to drugs \([13,22]\) and to radiotherapy \([23]\). It has been reported \([24]\) that BER enhances the anticancer effect of irradiation by increasing ROS production in human hepatoma cells.

Inhibition of MDR in A549 cells

Rhodamine 123 retention in A549 cells was tested to determine whether COP and BER affect MDR. As shown in Figure 4, both low and high doses of COP and BER enhanced dye retention by as much as 40\% \( (P < 0.05) \). Because elevated dye retention levels are inversely
related to MDR [20], this suggests that the inhibitory effects of COP and BER on A549 cells were enhanced due to the prolonged intracellular retention of the chemotherapeutic drugs.

Discussion
NSCLC is extremely difficult to treat because of its low therapeutic and long-term survival rates [11]. This study demonstrates that a combination of COP or its major constituent BER with chemotherapeutic drugs including 5-FU, CPT, and TAX exhibits a stronger inhibitory effect on the growth of A549 human lung cancer cells than any individual treatment. These findings suggest a potential use of COP and BER in the adjuvant treatment of NSCLC.

ROS levels are elevated in cells exposed to various stressors, including anticancer drugs, leading to apoptosis by stimulating pro-apoptotic signaling molecules (e.g., P53, MAPK, etc.) [25]. Some studies [13,22] have shown that increasing the production of ROS may sensititize cancer cells to drugs. Our results show that both COP and BER

![Figure 2](image2.png)

**Figure 2** Growth inhibition by combined use of COP/BER and chemotherapeutic agents in A549 cells. Effects of COP or BER, combined with 5-fluorouracil (5-FU), camptothecin (CPT), or paclitaxel (TAX), on the growth of A549 cells. COP-L: 1.6 μg/mL; COP-H: 6.4 μg/mL; BER-L: 0.5 μg/mL; BER-H: 4 μg/mL. *P < 0.05, compared to drugs alone. Values are means ± SD of 4 independent assays.

![Figure 3](image3.png)

**Figure 3** Effects of COP and BER on ROS production in A549 cells. Cells were treated with COP-L (1.6 μg/mL), COP-H (6.4 μg/mL), BER-L (0.5 μg/mL), or BER-H (4 μg/mL) for 24 h and harvested for ROS determination. *P < 0.05 compared to control. Values are means ± SD of 4 independent assays.
Our previous study [9] showed that COP and BER markedly inhibit cell proliferation and induce apoptotic cell death of MCF-7 cells through up-regulation of interferon-β, an important cytokine that regulates cell growth and death. COP and BER also enhance the anticancer effect of estrogen receptor antagonists, including tamoxifen and fulvestrant, likely by regulating multiple cancer-related genes, e.g., EGFR, HER2, bcl-2, COX-2, and p21 [10]. In this study, we observed some differences in efficacy between COP and BER. It may be that there were components in COP other than BER that contributed to its anticancer effect in agreement with our previous studies [9,10]. Further studies are required to discover the pathways targeted by COP and BER.

**Conclusions**

This study demonstrated that combinations of COP or BER with chemotherapeutic drugs (5-FU, CPT, and TAX) are more effective in inhibiting the growth of A549 cells than that of any single-agent therapy, possibly due to increased production of ROS and reduce MDR.

**Abbreviations**

COP: Coptis extract; BER: Berberine; 5-FU: Fluorouracil; CPT: Camptothecin; TAX: Paclitaxel; NSCLC: Non-small cell lung cancer; ROS: Reactive oxygen species; MDR: Multidrug resistance; DMSO: Dimethyl sulfoxide; DCFH-DA: 2′,7′-dichlorofluorescin diacetate; DCF: Dichlorofluorescein; Pgp: P-glycoprotein.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

JK conceived of the study, designed the study, and wrote the manuscript. CH designed the study, performed the experiments, analyzed the data and wrote the manuscript. RR performed the experiments and wrote the manuscript. JL performed the experiments, assisted the study design and data analysis. JW performed the experiments. KZ provided the materials and designed the study. All authors read and approved the final manuscript.

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**References**

1. Yin J, Zhang H, Ye J. Traditional Chinese medicine in treatment of metabolic syndrome. Endocr Metab Immune Disord Drug Targets 2008, 8(9):91–111.

2. Chang YC, Chen LY. Determination of berberine in coptis. Yao Xue Xue Bao 1962, 13:418–423.
8. Soule HD, Vazquez J, Long A, Albert S, Brennan M: The extract of huanglian, a
berberine and Coptidis rhizoma as novel antineoplastic agents: a review of traditional use and
biomedical investigations. J Ethnopharmacol 2009, 126:5–17.

9. Wang N, Feng Y, Zhu M, Tsang CM, Man K, Tong Y, Tsao SW: Berberine
induces autophagic cell death and mitochondrial apoptosis in liver cancer cells: the cellular mechanism. J Cell Biochem 2010, 111:1426–1436.

10. Soule HD, Vazquez J, Long A, Albert S, Brennan M: A human liver cell line from a pleural effusion derived from a breast carcinoma. J Natl Cancer Inst 1973, 51(5):1409–1416.

11. Kang JX, Liu J, Wang J, He C, Li FP: The extract of huanglian, a medicinal herb, induces cell growth arrest and apoptosis by upregulation of interferon-beta and TNF-alpha in human breast cancer cells. Cancer Chemother Pharmacol 2000, 26:1934–1939.

12. Liu J, He C, Zhou K, Wang J, Kang JX: Coptis extracts enhance the anticancer effect of estrone receptor antagonists on human breast cancer cells. Biochem Biophys Res Commun 2009, 378:174–178.

13. Delbaido C, Michels S, Syz N, Soria JC, Le Chevalier T, Pignon JP: Benefits of adding a drug to a single-agent or a 2-agent chemotherapy regimen in advanced non-small-cell lung cancer: a meta-analysis. J Am Med Assoc 2004, 292:470–484.

14. Simon HU, Haji-Yehia A, Levi-Schaffer F: Role of reactive oxygen species (ROS) in apoptosis induction. Apoptosis 2000, 5:415–418.

15. Gallego MA, Ballot C, Kluza J, Hajji N, Martoriati A, Castera L, Cuevas C, Formstecher P, Joseph B, Kroemer G, Bailly C, Marchetti P: Overcoming chemoresistance of non-small cell lung carcinoma through restoration of an AIF-dependent apoptotic pathway. Oncogene 2008, 27:1981–1992.

16. Young LC, Campbell BG, Cole SP, Deely RG, Gerfach JH: Multidrug resistance proteins MRPS, MRP1, and MRP2 in lung cancer: correlation of protein levels with drug response and messenger RNA levels. Clin Cancer Res 2001, 7:1804–1804.

17. Cancer Collaborative Group: Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data from individual patients from 52 randomised clinical trials. Non-small Cell Lung Tumour Med J 1995, 311:899–909.

18. Giaccone G, Herbst RS, Manegold C, Scagliotti G, Rosell R, Miller V, Natale RB, Schiller JH, Von Pawel J, Pizzocarri A, Gabrijelcic U, Groux J, Ochs JS, Averbuch SD, Wolf MK, Rennie P, Fandi A, Johnson DH: Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial-INTACT 1. J Clin Oncol 2004, 22:777–784.

19. Brown JR, Dubois RN: Cyclooxygenase as a target in lung cancer. Clin Cancer Res 2004, 10:4266–4269.

20. Giard DJ, Arsonson SA, Todaro GI, Amstein P, Kersey JH, Dosik H, Parks WP: In vitro cultivation of human cells: formation of a series of solid tumors. J Natl Cancer Inst 1973, 51(4):1377–1421.

21. Fontaine M, Eloumijt W, Miller DW: Use of rhodamine 123 to examine the functional activity of P-glycoprotein in primary cultured brain microvascular endothelial cell monolayers. Life Sci 1996, 59:1521–1531.

22. Brouty-Boye D, Kolonias D, Wu CJ, Savaari N, Lampidis TJ: Relationship of multidrug resistance to rhodamine-123 selectivity between carcinoma and normal epithelial cells: taxol and vinblastine module drug efflux. Cancer Res 1995, 55:1633–1638.

23. Burdon RH: Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. Free Radic Biol Med 1995, 18:757–794.

24. Liu B, Wang G, Yang J, Pan X, Yang Z, Zang L: Berberine Inhibits Human Hepatoma Cell Invasion without Cytotoxicity in Healthy Hepatocytes. PLoS One 2011, 6(6):e1416.

25. Benhar M, Engelberg D, Levitzki A: ROS, stress-activated kinases and stress signaling in cancer. EMBO Rep 2002, 3:420–425.

26. Longley DB, Harkin DP, Johnston PG: S-fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 2003, 3(5):330–338.

27. Wall ME, Wani MC, Cook CE, Palmer KH, McPhail AI, Sim GA: Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from camptotheca acuminata. J Am Chem Soc 1966, 88(16):3888–3890.

28. Wani M, Taylor H, Wall M, Coggon P, McPhail A: Plant antitumor agents. II. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. J Am Chem Soc 1971, 93(9):2325–2327.

29. Kim SA, Kwon Y, Kim JH, Muller MT, Chung IK: Induction of topoisomerase II-mediated DNA cleavage by a protoberberine alkaloid, berberubine. Biochemistry 1998, 37(1636):16324.

30. Lin TH, Kuo HC, Chou FP, Liu FJ: Berberine enhances inhibition of glioma tumor cell migration and invasiveness mediated by arsenic trioxide. BMC Cancer 2008, 8:58.

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