A Brief Review on the Experimental Aspects of Bojungikki-Tang in Cancer

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Abstract

Bojungikki-tang (BJIKT, Hochuekkito in Japanese, Bu-zhong-yi-qi-tang in Chinese) has been widely used as a traditional herbal formula in Korea, Japan, and China to improve the function of digestive systems, the quality of life and nutritional status. Currently there are numerous basic and clinical studies to find anticancer drugs from herbal medicine. Modern biomolecular tools and approaches have contributed to understand the role and mechanism of action of herbal medicine that exhibited anticancer activity. BJIKT have also gained much attention in recent years as adjuvant treatment for cancer. This review will introduce the experimental approaches to understand the pharmacological actions of BJIKT and overview the potential for cancer treatment.

Keywords: Bojungikki-Tang, Cancer, Fatigue, Skeletal Muscle Wasting

1. Introduction

Bojungikki-tang (BJIKT, Hochuekkito in Japanese, Bu-zhong-yi-qi-tang in Chinese) has been widely used as a traditional herbal formula in Korea, Japan, and China. BJIKT was first described in Pi Wei Lun (Treatise on Spleen and Stomach) written by Li Gao (1180–1251 A.D. in the Chinese Yuan Dynasty)1. Li Gao stated that conditions based on psychological or physical problems, such as excessive emotional changes, immoderate drinking, irregular eating pattern and overwork, that lead to qi deficiency are recommended for BJIKT to treat symptoms like dyspepsia, anorexia, and fatigue.2,3 The crude ingredients typically include Angelicae Gigantis radix, Astragali radix, Atractylodis rhizome, Bupleuri radix, Cimicifugae rhizome, Citriunshipericarpium, Ginseng radix alba and Glycyrrhizae radix.4 This herbal prescription has been identified as an effective drug for its potential impact on improving the function of digestive systems, the quality of life and nutritional status of patients.5–8 BJIKT is useful to treat conditions such as general fatigue, poor appetite, spontaneous sweating, and intermittent fever. Numerous research studies have reported the immunomodulatory and anti-inflammatory effects of BJIKT. Kiyohara’s group have reported that oral administration of BJIKT, to early aged BALB/c mice given a intranasal inoculation of influenza hemagglutinin vaccine, results in stimulating the mucosal immune system of upper respiratory tract.10 Yang’s group shows that BJIKT treatment suppress the responses of polymorphonuclear neutrophils to IL-4-stimulation in patients with perennial allergic rhinitis.11 Gou’s group have suggested that BJIKT inhibits the apoptosis and necrosis induced by 5-fluorouracil in mouse intestinal mucosal epithelia by reducing the inflammatory factors.12 BJIKT have also gained much attention in recent years as adjuvant treatment for cancer.13 Kuroda’s group demonstrate that BJIKT had clinical effects on cachexia for genitourinary cancer patients.14 Kim’s group...
reports the radioprotective effects of BJIKT in mouse exposed with high and low doses of gamma-rays. Jeong's group shows that BJIKT has beneficial effects on patients with cancer-related fatigue and quality of lives in cancer patients. Currently there are numerous basic and clinical studies to find anticancer drugs from herbal medicine. Modern biomolecular tools and approaches have contributed to understand the role and mechanism of action of herbal medicine that exhibited anticancer activity. This review will introduce the experimental approaches to understand the pharmacological actions of BJIKT in cancer.

2. In vitro Studies

Kao's group studied the Granulocyte Colony-Stimulating-Factor (G-CSF) and Tumor Necrosis Factor-a (TNF-a) production by Peripheral Blood Mononuclear Cells (PBMC) isolated from healthy volunteers and hepatocellular carcinoma patients. Various concentrations of BJIKT were added to the prepared mononuclear cells. BJIKT stimulated PBMC to produce G-CSF and TNF-a, that are beneficial to the biological defensive system. Kao's group also reported that BJIKT suppressed the proliferation of 3 human hepatoma cell lines, Hep3B, HepG2, and HA22T cells. BJIKT induced cell cycle arrest at the G0/G1 phase and apoptosis in Hep3B cells to inhibit tumor cell proliferation. Interestingly, growth-inhibitory effects were higher in BJIKT compared to individual major compounds of BJIKT. Kao's group suggest that major compounds from BJIKT might act in a synergistic or additive pathway to inhibit Hep3B proliferation. Zhu's group evaluated growth inhibition of 4 herbal medicine (Sho-saiko-to, Hochu-ekki-to, Juzen-taiho-to, and Ninjin-yoei-to) on six human ovarian cancer cells (KF-1, MN-1, A2780 and their respective cisplatin-resistant sublines KF-r, MN-r, A2780cp) . However, BJIKT even at high concentrations did not show growth inhibition and apoptosis on all six cancer cell lines. Kuo's group investigated the anti-tumor effect of BJIKT, and two other herbal formulas in human gastric cancer MKN-74 cells. BJIKT most highly induced cytotoxicity in combination with mitomycin C (MMC) through a non-apoptotic mechanism. Exposure to BJIKT first, followed by MMC treatment induced more cell death compared to MMC first, followed by BJIKT treatment. From these data, BJIKT and chemotherapy administration sequence may alter cytotoxicity and cell death. Sato's group studied the effect of BJIKT in low doses (50 µg/ml) to prevent non-specific cytotoxic effects attributed to saponins or detergent-like compounds. The present study observed that BJIKT augment the apoptotic impact of cisplatin by increasing caspase-3 activation and Bax/Bcl-2 ratio in human cervical cancer cell-line HeLa cells. Data shows that BJIKT followed by cisplatin decreased protein levels of p-Akt, a cell survival factor, and increased protein levels of p53, a tumor suppressor. Moreover, the interplay between Akt and p53 would explain the stimulation of cisplatin-induced cell death by BJIKT in HeLa cells. Yu's group also reported that BJIKT enhanced the cytotoxicity of cisplatin in non-small cell lung cancer (NSCLC) cells. The study focused on the cultured human lung carcinomacisplatin resistant variant A549/DDP cells, BJIKT and cisplatin co-treatment induced apoptosis and autophagy via accumulation of reactive oxygen species (ROS). BJIKT and cisplatin co-treatment increasedBax/Bcl-2 ratio, cleaved caspase 3, and PARP cleavage in A549/DDP cells. In addition z-VAD-FMK, a broad spectrum caspase inhibitor, partially limited the BJIKT and cisplatin-induced cell death. Taken together, these data indicate that BJIKT and cisplatin co-treatment mediates cell death partially through caspase-dependent intrinsic pathway. Also, BJIKT and cisplatin co-treatment induced cytotoxic autophagy by increasing in LC3 fluorescent puncta formation, autophagic vacuoles, and LC3-II and ATG7 levels. Therefore, the results suggest that both autophagy and caspase-dependent apoptosis contribute to the antitumor effect of BJIKT and cisplatin co-treatment.

3. In vivo studies

Harada's group treated mice bearing the syngeneic fibrosarcoma, Meth-A, with BJIKT and demonstrated that the enhancement of cytostatic activity mediated antitumor immunity. Li's group using the B16 murine melanoma cell line xenograft on C57BL/6 (B6) mice, under restraint stress, reported that BJIKT administration normalized the serum levels of corticosterone, IL-12, and the expression of CD80 and CD86. These data support that BJIKT augmented antitumor immune response in stress-burdened, tumor-bearing mice.
Onogi's group performed endometrial carcinogenesis, in ICR mice, induced by N-Methyl-N-Nitrosourea (MNU) into the left uterine tube and normal saline was injected into the opposite side. Diet with 17β-estradiol (E2) alone, E2 plus BJIKT, was given and incidence of prenoplasic and neoplastic mouse endometrial lesions were measured. The incidence of adenocarcinoma was significantly less in BJIKT treated mice. In addition, it was suggested that decrease in expression of c-Jun, TNF-α, ER-α and ER -β mediates the inhibitory effect of BJIKT on endometrial carcinogenesis. Tsuneoka’s group studied on the effect of BJIKT on N-nitrosobis(2-oxopropyl) amine (BOP) induced biliary carcinomas in bilioenterostomized hamsters. Histological data show that BJIKT administration significantly decreased the average number of intrahepatic bile duct carcinomas per animal from 11.4 to 3.9, and also the total percentage of animals with carcinoma from 88% to 47%. PCNA labeling index of the biliary epithelium was measured, and BJIKT significantly decreased cell proliferation from 9.6% to 6.4%. The study shows the cancer preventative effect of BJIKT on BOP-induced biliary carcinogenesis in hamsters. Yae’s group used BALB/c mice bearing the Colon-26 (C26) adenocarcinoma to evaluated the effect of BJIKT. The data shows that BJIKT did not suppress tumor growth, but significantly decreased serum IL-6 level, and increased the serum triglyceride level, weights of the gastrocnemius muscle and fat tissue around the testes in tumor-bearing mice in the terminal stage. IL-6 expression from tumor tissues was evaluated by immunohistochemistry, and indicates that BJIKT treatment did not alter the IL-6 expression in cancer cells. Interestingly, immunohistochemical analysis of IL-6 production by macrophages in the tissues surrounding tumors was significantly reduced in BJIKT treated C26-bearing mice. Moreover, BJIKT inhibits IL-6 secretion from cultured THP-1 and RAW264.7 macrophage cell lines.

4. Discussion

Most of the data derived from in vitro studies used BJIKT combined with chemotherapeutic agent. Few studies have explored the chemopreventive/anti-tumor effects of BJIKT as a single agent. BJIKT, in human hepatoma cells, a dose-dependent anti-proliferative effect was achieved. BJIKT-only treatment inhibits cell growth by 20% in A549/DDP cells at high dose (1000 μg/ml). In contrast, BJIKT-only treated human ovarian cancer cells, even at high dose (1000 μg/ml) did not inhibit cell growth. The reported differences might be explained by differences in the organ used by different groups. However, at high doses, BJIKT can have non-specific effects and cause cytotoxicity. These issues need to be explored in larger studies to examine the role of BJIKT in growth inhibition. Studies examining the effects of BJIKT on cell death have reported contradictory results. A majority of studies have reported on the apoptotic effect of BJIKT in Hep3B cells, HeLa cells, and A549/DDP cells. In contrast, apoptosis was not involved in the increased cell death by BJIKT in combination with MMC on MKN-74 cells. Interestingly, a study by Yu’s group reported that BJIKT and cisplatin co-treatment contribute to cell death by both apoptosis and autophagy in A549/DDP cells. It provides an interesting link to the non-apoptotic mechanism found in MKN-74 cells as suggested by Yu’s group.

Studies identifying the effect of BJIKT in vivo are mostly based on chemical induced carcinogenesis and xenograft tumor models. However, the anti-tumor effect of BJIKT was evaluated mainly by histopathological evaluation, and the molecular studies from Onogi’s group are based on ovarectomized mice. Understanding the anti-tumor effects of BJIKT and the underlying molecular mechanisms still remains to be investigated. Mouse models are particularly valuable for cancer research to examine interactions among tumor cells and between the tumor cells and their host environment. Transgenic mouse tumor model enable researchers to develop novel targeted therapies and chemopreventive agents. Unfortunately, despite the benefits of transgenic models, BJIKT has yet been conducted with genetic manipulation techniques.

It has been suggested by Li’s group that BJIKT increased the antitumor immune responses under stressed conditions and by Yae’s group that BJIKT ameliorate the serum triglyceride level and weight of fat tissue in tumor-bearing mice. Interestingly, Ouyang’s group reported that BJIKT enhanced chemotherapy-related fatigue in 4T1 breast cancer mice. Data shows that, combination of BJIKT and paclitaxel (PTX) treated group gradually increased weight loaded swimming time compared to PTX-only treated group. Moreover,
BJIKT and PTX co-treatment up-regulated muscle superoxide dismutase activity and decreased the levels of malondialdehyde compared to PTX treatment\(^{28}\). Growing evidence indicates that directly or indirectly production of ROS is a mechanism shared by numerous chemotherapeutic agents, and cause muscle weakness and develop of fatigue\(^{28,29}\). In the aggregate, these findings suggest that BJIKT could improve cancer induced fatigue and skeletal muscle wasting by preventing ROS generation.

### 5. Conclusion

The in vitro and in vivo studies based on BJIKT suggest that BJIKT is a potential therapeutic agent for cancer (Table 1). The anti-tumor effect of BJIKT is maximized when combined with chemotherapeutic drug. Improving survival time, body weight and fatigue affected by cancer represent another promising role for BJIKT. A role that BJIKT, has been well defined from its long history of traditional use.

| Experimental model | Tumor type | Combination | Action | References |
|--------------------|------------|-------------|--------|------------|
| in vitro           | peripheral blood mononuclear cells | PTX | produce G-CSF, TNF-α | 16 |
|                    | hepatoma cells | no action | cell cycle arrest, apoptosis | 17 |
|                    | ovarian cancer cells | PTX | no action | 18 |
|                    | gastric cancer cells | mitomycin C | increase non-apoptotic cell death | 19 |
|                    | cervical cancer cells | cisplatin | decrease cell survival factor | 20 |
|                    | non-small cell lung cancer cells | cisplatin | increase tumor suppressor | |
|                    | cervical cancer cells | cisplatin | induce apoptosis | 21 |
| in vivo            | fibrosarcoma | cisplatin | inhibition of cell growth | 22 |
|                    | melanoma | restraint stress | antitumor immunity | 23 |
|                    | endometrial carcinoma | cisplatin | decrease in tumor incidence | 24 |
|                    | biliary carcinoma | cisplatin | decrease cell proliferation | 25 |
|                    | colon carcinoma | cisplatin | decrease carcinoma per animal | |
|                    | mammary carcinoma | cisplatin | decrease percentage of tumor bearing animal | |
|                    | mammary carcinoma | cisplatin | increase serum triglyceride level | 26 |
|                    | mammary carcinoma | cisplatin | increase gastrocnemius muscle weight | |
|                    | mammary carcinoma | cisplatin | increase fat weight around testicles | |
|                    | mammary carcinoma | cisplatin | prevent ROS production | 28 |

### 6. Conflicts of Interest

All authors have no conflicts of interest to declare.

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