

CHAPTER I

INTRODUCTION

Rationale and significance of the study

Colorectal cancer is cancer which occurs in the cells of colon or rectum that become abnormal, and divides without control. Currently, colorectal cancer is a major cause of cancer death in many countries. Over-expression of cyclooxygenase-2 (COX-2) is found in inflammation tissues, and involved in development of many cancer types including colorectal cancer both *in vitro*, and *in vivo* studies (Yamazaki, et al., 2002; Janssen, et al., 2006; Williams, et al., 1996 Sunayama, et al., 2002).

5-fluorouracil (5-FU) is a chemotherapeutic drug widely used in treating the colorectal cancer. However, the toxicity of 5-FU to normal cells, and resistance to this drug are major barriers to successful cancer chemotherapy. Therefore, combined treatment of 5-FU with several other regimens or chemopreventive medicine are often used for reduce 5-FU toxicity and enhance the efficacy of 5-FU chemotherapeutic drug. Previous study reported that combination of 5-FU with genistein, soy-derived phytoestrogen, markedly induced apoptosis and down-regulated the COX-2 expression and prostaglandin in HT-29 colon cancer cells more than 5-FU treatment alone (Hwang, et al., 2005). Furthermore, 5-FU combined with geraniol, a component of plant essential oils, caused reduction of thymidylate synthase (TS) expression in colon cancer cells and caused 53% reduction of tumor volume on human colon tumor xenografts, but no effect when treated with 5-FU alone (Carnesecchi, et al., 2004). Moreover, 5-FU in combination with curcumin (CUR) showed synergistic effect on growth inhibition, and reduced COX-2 protein expression in HT-29 human colon cancer cell line (Du, et al., 2006).

CUR is the major yellow pigment in turmeric which is derived from rhizome of the herb, *Curcuma longa* Linn has been traditionally used in cooking in India and Southeast Asia (Chuang, et al., 2000), and used as a naturally occurring substance in the treatment of cancer both *in vitro* and *in vivo* studies of a wide variety of cancers, such as ovarian (Wahl, et al., 2007), oral (Sharma, et al., 2006), lung (Chen, et al., 2003), skin (Kakar and Roy, 1994), and colorectal cancer (Hanif, et al., 1997;

Kawamori, et al., 1999; Chauhan, 2002). CUR specifically inhibited mRNA and protein expression of COX-2, which highly expressed in a variety of human cancers (Zhang, et al., 1999; Lee, et al., 2005; Lev-Ari, et al., 2007) including colorectal cancer, but not suppressed the cyclooxygenase-1 (COX-1), housekeeping enzyme that maintains body homeostasis (Plummer, et al., 1999; Goel, et al., 2001). Therefore, CUR possible safe and non-toxic agent for prevention, and treatment colorectal cancer in human, but CUR characteristics are unsuitable for drug design, such as poor solubility, and poor absorption. After oral administration of CUR about 60% of the dose was absorbed and remained 38% in the large intestine of rat (Ravindranath and Chandrasekhara, 1980), and also low in humans (Cheng, et al., 2001). Furthermore, less than 20% of CUR rapidly decomposed within 1 hour in cell culture medium containing 10% fetal calf serum, and in human blood (Wang, et al., 1997). Based on these results may suggest that the systemic bioavailability of CUR is low, so that its pharmacological activity may be mediated by CUR metabolites. Several studies investigated the effects of major CUR metabolites, such as tetrahydrocurcumin (THC) and hexahydrocurcumin (HHC). Recently studies showed that THC was more stable than CUR in 0.1 M phosphate buffer, pH 7.2 (37°C) (Pan, et al., 1999). Moreover, THC exhibited stronger antioxidant activity than CUR (Somparn, et al., 2007) and significantly decreased number of aberrant crypt foci (ACF), preneoplastic lesion of colorectal cancer, as compared to none treated mice (Kim, et al., 1998). HHC inhibits the biosynthesis of prostaglandin E₂ (PGE₂) in lipopolysaccharide (LPS) - stimulated macrophages (Shao, et al., 2003) and decreasing PGE₂ levels on phorbol ester-induced PGE₂ production in human colonic epithelial cells (HCECs) (Ireson, et al., 2001). PGE₂ is a major product of COX-2 enzymes implicates in colorectal carcinogenesis and has been shown to stimulate the growth of human colorectal carcinoma cells (Williams, et al., 1996; Sunayama, et al., 2002; Janssen, et al., 2006). However, whether HHC is responsible for the reported anti-colon carcinogenic effects is still to be determined.

Therefore, the present study needs to investigate the anti-colon carcinogenic effects of HHC and in combination with 5-FU to enhance the anti-colon carcinogenic properties, specially focusing on the expression of COX-2 both *in vitro* and *in vivo* studies.

Purpose of the study

Invitro studies

1. To determine the inhibitory effect of HHC alone or in combination with 5-FU on growth of HT-29 human colon cancer cells.
2. To determine the inhibitory effect of HHC alone or in combination with 5-FU on COX-2 mRNA and protein expression in HT-29 human colon cancer cells.
3. To determine the inhibitory effect of HHC alone or in combination with 5-FU on induction of apoptosis of HT-29 human colon cancer cells.

Invivo studies

1. To determine the combination effects of HHC with 5-FU on formation of aberrant crypt foci (ACF) in rat colon tissues induced by 1,2-dimethylhydrazine (DMH).
2. To determine the combination effects of HHC with 5-FU on expression of COX-2 in rat colon tissues induced by DMH.
3. To determine the combination effects of HHC with 5-FU on induction of apoptosis in rat colon tissues induced by DMH.

Scope of this study

The present study investigate the combination effects of HHC with 5-FU on the proliferation, COX-2 mRNA and protein expression, and induction of apoptosis in HT-29 human colon cancer cells culture and to investigate the combination effects of HHC with 5-FU on formation of ACF, expression of COX-2 and induction of apoptosis in colon carcinogenesis of Wistar rats induced by DMH.

Hypotheses

In vitro studies

1. If HHC combined with 5-FU exert a synergistic effect, the HT-29 human colon cancer cells which treated with combination of HHC and 5-FU should have lower cell viability than HHC alone.
2. If HHC combined with 5-FU exert a synergistic effect, the HT-29 human colon cancer cells which treated with combination of HHC and 5-FU should have

lower expression of COX-2 mRNA and protein in HT-29 human colon cancer cells than HHC alone.

3. If HHC combined with 5-FU exert a synergistic effect, the HT-29 human colon cancer cells which treated with combination of HHC and 5-FU should have induce apoptosis of HT-29 human colon cancer cells than HHC alone.

Invivo studies

1. If HHC combined with 5-FU exert a synergistic effect, the formation of ACF in rat colon carcinogenesis induced by DMH colon carcinogenesis which treated with the combination of HHC and 5-FU should have lower formation of ACF than treated with HHC alone.

2. If HHC combined with 5-FU exert a synergistic effect, the expression of COX-2 in rat colon tissues induced by DMH-induced colon carcinogenesis which treated with the combination of HHC and 5-FU should have lower expression of COX-2 than treated with HHC alone.

3. If HHC combined with 5-FU exert a synergistic effect, the induction of apoptosis in rat colon tissues induced by DMH-induced colon carcinogenesis which treated with the combination of HHC and 5-FU should have higher induction of apoptosis than treated with HHC alone.

Anticipated Outcomes

1. To provide the information about the combination effect of HHC with 5-FU on proliferation, expression of COX-2 mRNA and protein and apoptosis of HT-29 human colon cancer cells.

2. To provide the information about combination effect of HHC with 5-FU on ACF formation, expression of COX-2 and apoptosis of rat colon induced by DMH.

3. To provide the information about the anti-carcinogenic effect of natural substance combined with chemotherapeutic drug in cancer chemotherapy.