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Tetrahydrocurcumin induces G2/M cell cycle arrest and apoptosis involving p38 MAPK activation in human breast cancer cells

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ABSTRACT

Curcumin (CUR) is a major naturally-occurring polyphenol of Curcuma species, which is commonly used as a yellow coloring and flavoring agent in foods. In recent years, it has been reported that CUR exhibits significant anti-tumor activity *in vivo*. However, the pharmacokinetic features of CUR have indicated poor oral bioavailability, which may be related to its extensive metabolism. The CUR metabolites might be responsible for the antitumor pharmacological effects *in vivo*. Tetrahydrocurcumin (THC) is one of the major metabolites of CUR. In the present study, we examined the efficacy and associated mechanism of action of THC in human breast cancer MCF-7 cells for the first time. Here, THC exhibited significant cell growth inhibition by inducing MCF-7 cells to undergo mitochondrial apoptosis and G2/M arrest. Moreover, co-treatment of MCF-7 cells with THC and p38 MAPK inhibitor, SB203580, effectively reversed the dissipation in mitochondrial membrane potential ($\Delta\psi m$), and blocked THC-mediated Bax up-regulation, Bcl-2 down-regulation, caspase-3 activation as well as p21 up-regulation, suggesting p38 MAPK might mediate THC-induced apoptosis and G2/M arrest. Taken together, these results indicate THC might be an active antitumor form of CUR *in vivo*, and it might be selected as a potentially effective agent for treatment of human breast cancer.

1. Introduction

Breast cancer is the major health problem in women worldwide, regarding both its incidence and mortality, which is emphasised by the diagnosis of over one million new cases annually (Botha et al., 2003; Vaz et al., 2012). The main mode of therapy used for control of this disease is chemotherapy. However, only a minority of patients is eligible for radical treatment aimed at a curative effect. The availability of new cytotoxic drugs has led to steady improvements, but a paradigm shift is required to significantly affect the poor prognosis for most patients. In this regard, there is considerable emphasis in searching for novel agents with reduced adverse effects for prevention and treatment of breast cancer. Plant-derived drugs play an increasing role in cancer therapy due to their low toxicity and high efficacy (Zhang et al., 2012). Many natural bioactive substances found in fruits, vegetables, medicinal herbs and other plants are considered as potential anti-cancer agents. Especially, major attention has been focused on identifying dietary phytochemicals that inhibit tumor development processes (Desai et al., 2008; Landis-Piwowar et al., 2006).

CUR (diferuloylmethane) (Fig. 1A) is a polyphenol natural product isolated from turmeric, a powder produced from the rhizome of the plant *Curcuma longa*. CUR is well recognized as a dietary spice for centuries and its pharmacological activities have been studied in various animal models and clinical studies including anti-inflammatory, anti-diabetic, anti-dementia, and anti-oxidant properties (Aggarwal and Sung, 2009; Rinwa et al., 2010; Sharma et al., 2005). Particularly, it appears to have chemopreventive properties against a variety of human malignancies and currently is in clinical

Abbreviations: AO, acridine orange; cdc 2, cell division cycle 2; CDK, cyclin-dependent kinase; CDKI, cyclin-dependent kinase inhibitor; DMSO, dimethyl sulfoxide; ERK, extracellular signal-regulated kinase; FBS, fetal bovine serum; JNK, Jun N-terminal kinase; MAPK, mitogen activated protein kinase; PBS, phosphate-buffered saline; PI, propidium iodide; PMSF, phenylmethyl sulfonyl fluoride; RNase, ribonuclease; SDS–PAGE, sodium dodesyl sulfate polyacrylamide gel; TBS, Tris buffered saline.

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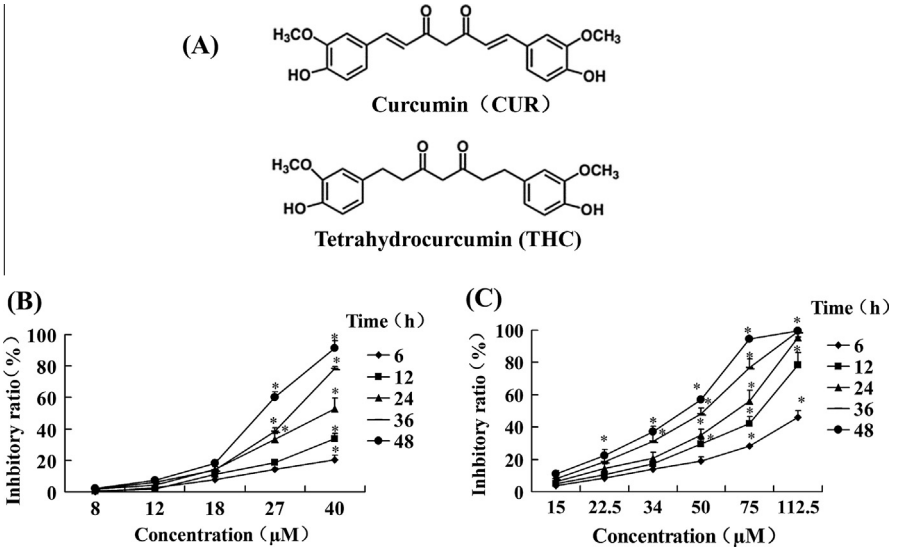

Fig. 1. Inhibitory effects of CUR or THC on cell proliferation in MCF-7 cells. (A) Chemical structures of THC and CUR; the cells were treated with different doses of CUR (B) and THC (C) for the indicated time periods, and the inhibitory ratio was measured by MTT assay. $n = 3$, Mean ± SD. Asterisk (*) statistical significance ($p < 0.01$) of growth inhibition compared to controls at the same time point.

trials as an anticancer agent (Goel et al., 2008; Zhou et al., 2011). In phase I clinical trials, it was concluded that human volunteers tolerated a CUR dose as high as 8 g/d with no side effects (Cheng et al., 2001). However, CUR shows poor oral bioavailability and undergoes extensive metabolism. Although the blood-CUR concentration is low, it still has effectiveness, which suggests that the CUR metabolites might be responsible for the pharmacological effects *in vivo* (Ireson et al., 2001; Pan et al., 1999).

Tetrahydrocurcumin (THC) (Fig. 1A) is an active metabolite of CUR. In the liver, CUR is reduced by endogenous reductase systems to hexahydrocurcumin, THC, and hexahydrocurcuminol, among of which THC has been demonstrated to be the major metabolite (Ireson et al., 2001; Pan et al., 1999). Therefore, THC might also play a crucial role in CUR-induced biological effects. It is notable that THC is stable in phosphate buffer and in saline at various pH values, which is quite different from CUR (Yodkeeree et al., 2008). Particularly, THC is easily absorbed through the gastrointestinal tract, which suggests that THC might be easier as a potential candidate for the development of anticancer agent.

Apoptosis is a fundamental and complex biological process in which cells play an active role in their own death. Dysregulation of apoptosis is the hallmark of all cancer cells and the agents that activate programmed cell death could be valuable anticancer therapeutics (Hu and Kavanagh, 2003). Mitochondria play critical roles in apoptotic cell death, and it is becoming one of the principal targets in screening therapeutic agents against cancer. Thus, many studies also focused to find compounds which could affect mitochondria for anticancer agents (Armstrong, 2007; Danial and Korsmeyer, 2004; Kumar and Sabbioni, 2010).

Cell cycle control is one of the major regulatory mechanisms of cell growth. Many anticancer agents have been reported to arrest the cell cycle at a specific checkpoint and thereby induce apoptotic cell death (Lu et al., 2007; Murray, 2004). The eukaryotic cell division cycle is regulated in large part by cyclin/cyclin-dependent kinase (CDK) complexes, which are in turn modulated by CDK inhibitors (CKIs), such as p21WAF1/Cip1 (referred to as p21 hereafter), that bind to specific cyclin/CDK complexes (Israels and Israels, 2001; Vermeulen et al., 2003). In contrast to the inhibitory effects of p21 on CDK complexes, cdc25c activates tyrosine-phosphorylated CDKs by dephosphorylating the tyrosine residues, thereby permitting step-cell entry into mitosis. It has been shown that cdc25c is negatively regulated by phosphorylation of its Ser-216 residue, which prevents the activation of cyclin B1/CDK1 complex, thereby leading to the arrest of the G2/M transition (Takizawa and Morgan, 2000).

Mitogen activated protein kinase (MAPK) is an important intracellular signal transduction system and participates in a series of physiological and pathological processes, including cell growth, differentiation and apoptosis (LaChapelle et al., 2007). The most prominent members of MAPKs family are c-Jun-N-terminal kinase (JNK), p38 MAPK and extracellular-regulated protein kinase (ERK). Ample evidences suggest that antitumor agents can change the activities of MAPK members in most cancer cell lines (Lee et al., 2011).

Some of studies have indicated that CUR has chemopreventive effects on breast cancer *in vivo* (Masuelli et al., 2013; Nagaraju et al., 2012; Sinha et al., 2012). Since THC is one of the major metabolites of CUR, we speculate that THC might be one of the active forms which are responsible for CUR antitumor pharmacological effects *in vivo*. It would be interesting to identify the efficacy and associated mechanism of action of THC in human breast cancer cells. Our results presented here suggested that THC significantly induced G2/M arrest and apoptosis *via* p38 MAPK in human breast cancer MCF-7 cells. THC inhibited the proliferation of the human breast cancer cells and might, therefore, potentially serve as a therapeutic agent in cancer treatment.

2. Materials and methods

2.1. Drugs and reagents

Curcumin was isolated from *Curcuma longa*, and was identified by comparing its physical and spectroscopic (^{1}H NMR, ^{13}C NMR) data with those reported in the literature (Feng and Liu, 2009). The purity was measured by HPLC [column: 4.6 mm × 150 mm, Inertsil ODS-SP, 5 μm; solvent phase, methanol: H_2O, 70: 30, t_R = 17.6 min] and determined to be 99.6%. THC was obtained by hydrogenation of curcumin with $NaBH_4$, followed by purification with silica gel column chromatography using petroleum ether/EtOAc 3:1 as the eluent (Feng and Liu, 2009). Curcumin and THC were dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution, respectively. The DMSO concentration was kept below 0.05% in all the cell cultures so that it had no detectable effect on cell growth or cell death.

Fetal bovine serum (FBS) was obtained from TBD Biotechnology Development (Tianjin, China). MEM medium was obtained from Gibco/BRL (Gaithersburg, MD, USA). Acridine orange (AO), propidium iodide (PI), rhodamine-123, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), ERK inhibitor PD98059, JNK inhibitor SP600125 and p38MAPK inhibitor SB203580 were purchased from Sigma Chemical (St. Louis, MO, USA). Polyclonal antibodies against Bax, Bcl-2, ICAD, PARP, caspase-9, caspase-3, cytochrome *c*, cyclinB1, cdc2, p-cdc2, cdc25c,