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Turmeric ethanolic extract possesses stronger inhibitory activities on colon tumour growth than curcumin – The importance of turmerones

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ABSTRACT

The active ingredient curcuminoid (including curcumin, demethoxycurcumin and bisdemethoxycurcumin) from the Asian medicinal and culinary herb turmeric possesses anti-tumour effects, but poor oral absorption in the intestine impedes its widespread clinical application. Our previous study showed that turmerones increased the accumulation of curcumin inside colonic cells. The present study demonstrates the enhanced anti-proliferative and anti-angiogenic activities of curcumin in the presence of turmerones in human colon cancer cells and endothelial cells, respectively. Furthermore, in HT29 tumour xenograft-bearing mice fed with curcumin alone or turmeric ethanolic extract (in which the concentration of curcumin was kept the same), the tumour burden of turmeric extract-fed mice was the lowest, suggesting turmeric extract provided better anti-tumour activities than the same amount of curcumin alone did. The superior anti-tumour effects of turmeric extract, which contains curcumin, turmerones and other constituents, were verified in tumour-bearing mice, indicating the potential use of turmeric for colorectal cancer adjuvant therapy.

1. Introduction

Colorectal cancer is the third most common cancer in men and the second in women worldwide (International Agency for Research on Cancer, 2012), but there is wide variation in incidence among different regions across the world. Diet and lifestyle may be associated with the development of colorectal cancer (Aggarwal & Shishodia, 2006). Previous epidemiological studies have suggested that turmeric contributes to the lower incidence of large-bowel cancers in Indians (Chauhan, 2002; Mohandas & Desai, 1999). Turmeric, the dried rhizome

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of *Curcuma longa* Linn. (Zingiberaceae family), is a commonly used spice and traditional medicine in Indian and Chinese cultures. Turmeric is also recommended for the prevention of cancer and other diseases (Aggarwal & Shishodia, 2006; Gupta, Patchva, & Aggarwal, 2013). The active ingredient, curcumin, has been widely tested as a chemopreventive agent for colorectal cancer (Goel, Kunnumakkara, & Aggarwal, 2008; Prasad, Tyagi, & Aggarwal, 2014).

Numerous *in vitro* studies have reported that curcumin possesses anti-inflammatory, anti-oxidant, apoptotic and transcription factor modulatory effects in various types of cancer cell lines (Akkoc et al., 2015; Kantara et al., 2014; Prasad et al., 2014; Ravindran, Prasad, & Aggarwal, 2009). Several clinical studies have also demonstrated the chemopreventive effects of curcumin in colon cancer patients (Carroll et al., 2011; Garcea et al., 2005; He et al., 2011; Sharma et al., 2001). However, the poor bioavailability of curcumin is still regarded as a major problem, and hence curcumin has not yet been approved as a therapeutic agent (Anand, Kunnumakkara, Newman, & Aggarwal, 2007). Pharmacokinetic studies in humans demonstrated that oral administration of curcumin was well tolerated without any toxicity, and trace amounts of curcumin were detected in plasma (Gupta et al., 2013). In a previous clinical trial of colorectal cancer patients who have ingested curcumin, both normal and malignant colorectal tissues were found to have taken it up (Garcea et al., 2005). Another clinical trial showed that a product containing an unknown ratio of curcumin and non-curcuminoid components of turmeric showed enhanced curcumin absorption in humans (Antony et al., 2008). The curcumin-free turmeric components have also been shown to exhibit various biological activities (Aggarwal, Yuan, Li, & Gupta, 2013).

Our previous *in vitro* study using colonic Caco-2 cell monolayers demonstrated that the presence of turmerones, the non-polar constituents in turmeric ethanolic extract, could increase the accumulation of curcumin inside colonic cells (Yue et al., 2012). Furthermore, our studies also showed that aromatic-turmerone exhibited immunostimulatory effects in human peripheral blood mononuclear cells (Yue et al., 2010b), as well as anti-angiogenic activities in human endothelial cells and in mouse model (Yue et al., 2015a). Aromatic-turmerone (Ar-turmerone) has been reported to induce apoptosis in various leukaemia and lymphoma cell lines (Lee, 2009; Sandur et al., 2007) and attenuate invasion in breast cancer cells (Park, Kim, Kim, & Lee, 2012). Based on the previous findings, we hypothesised that more curcumins can be absorbed into the colonic cells in the presence of turmerones so that curcumin could exhibit its anti-tumour and anti-inflammatory properties towards the colorectal malignant cells. Besides, the anti-angiogenic and immunomodulatory effects of other constituents in turmeric extract (e.g. aromatic-turmerone) might have contributed to the modulation of the intestinal immunity, and hence the anti-tumour effects of curcumin could be enhanced.

Despite curcumin has been studied alone in colon cancer cell lines and endothelial cells, the pharmacological activities of curcumin plus turmerones and turmeric ethanolic extract have not been compared. In the present study, the proliferative and angiogenic activities of human colon cancer cells (HT29 and HCT116) and human umbilical vein endothelial cells (HUVEC) were assessed after different curcumin preparation treatments (added alone or with turmerones, or in turmeric ethanolic extract). Since there are few reports of the anti-tumour efficacies comparison between curcumin alone and turmeric ethanolic extract, colon xenograft-bearing mice were thus treated with curcumin alone or turmeric ethanolic extract, and the haematological parameters, tumour growth and blood vessel growth in tumours were determined. Hereby, we demonstrated that turmeric ethanolic extract provided better anti-tumour activities than the same amount of curcumin alone did, indicating the potential use of turmeric extract instead of curcumin alone for colorectal cancer adjuvant therapy.

2. Materials and methods

2.1. *Turmeric ethanolic extract preparation and chemical analysis*

Dried rhizomes of *Curcuma longa* Linn. (also known as turmeric, Curcumae Longae Rhizoma or jianghuang) were purchased from a renowned supplier (Wing Seng Hong, Hong Kong, China). The raw herb originated from the Guangxi province of China and authenticated by both morphological and chemical methods in accordance with Chinese Pharmacopoeia 2010 (Chinese Pharmacopoeia Commission, 2010). An authenticated voucher specimen (Number: 3353) was deposited in the museum of the Institute of Chinese Medicine, The Chinese University of Hong Kong. The dried rhizome of *Curcuma longa* was powdered, soaked in 95% ethanol for 30 min, and then extracted under reflux using 95% ethanol for 1 h and the extraction was repeated. Following filtration, the crude ethanolic extract was centrifuged at 3717 × *g* (Allegra X-15R, Beckman Coulter, Indianapolis, IN, USA) to remove undissolved particles, and then evaporated under reduced pressure at 60 °C to dryness. The percentage yield of the turmeric ethanolic extract was 12.7% (w/w). The turmeric ethanolic extract (named "turmeric extract" used throughout the manuscript) was stored at 4 °C and protected from light. It was dissolved in 95% ethanol to give 100 mg/mL and reconstituted in appropriate media prior to cell culture experiments or in distilled water containing 1% methylcellulose for animal studies. The vehicle control cultures received the vehicle solvent (0.5% v/v, ethanol).

Quantification of curcumin and aromatic-turmerone (Sigma-Aldrich, St. Louis, MO, USA) in turmeric extract was achieved by Waters Acquity UPLC system (Milford, MA, USA) coupled with a Waters Acquity UPLC BEH C_{18}, 2.1 mm × 100 mm packed with 1.7 μm hydrophobic bonded C_{18} phase, accompanied with a guard column of 2.1 mm × 5 mm, 1.7 μm (Waters Acquity UPLC C18 VanGuard Pre-Column) maintained at 25 °C. The details of UPLC analysis are presented in Supplementary information Table S1. The contents of curcumin and Ar-turmerone in turmeric ethanolic extract were 18.7% (w/w) and 5.3% (w/w), respectively.

2.2. *Isolation of α/β-turmerone and aromatic-turmerone*

For the isolation of pure compounds, curcumin was purified from the commercially available crude curcumin (purity ≥ 65%,