Curcumin effects on cell proliferation, angiogenesis and metastasis in colorectal cancer

Gizem Calibasi-Kocal¹*, Ahu Pakdemirli²*, Serdar Bayrak³, Nazli Mert Ozupek⁴, Tolga Sever⁵, Yasemin Basbinar¹, Hulya Ellidokuz⁶, Turkan Yigitbasi⁶

¹Dokuz Eylul University, Institute of Oncology, Department of Basic Oncology, Izmir, Turkey; ²Dokuz Eylul University, Vocational School of Health Services, Department of First and Emergency Aid, Izmir, Turkey; ³Dokuz Eylul University, Faculty of Medicine, Department of Cardiovascular Surgery, Izmir, Turkey; ⁴Dokuz Eylul University, Institute of Health Sciences, Department of Medicine, Department of Medicine, Department of Biochemistry, Istanbul, Turkey.

*These authors contributed equally to this study.

Summary

Purpose: Curcumin is a natural phytopolyphenol compound isolated from the root of turmeric (Curcuma longa) and possesses a wide range of biological properties. The purpose of this study was to evaluate the antiproliferative, wound healing, anti-invasive and anti-migrative effects of curcumin on HCT-116 and LoVo colorectal cancer cell lines.

Methods: The antiproliferative activity of 2.5-75 µM curcumin was tested on HCT-116 and LoVo colorectal cell lines and the viability of the cells was tested with WST-1 reagent by using ELISA plate reader at 450 nm. xCELLigence RTCA DP system was used for the detection of anti-invasive and anti-migrative effects of curcumin.

Results: The IC₅₀ of curcumin was 10±0.03 for HCT-116 and 20±0.05 µM for LoVo cell lines. The IC₅₀ of curcumin (10µM for HCT-116 and 20 µM for LoVo) showed anti-metastatic activity on these cell lines.

Conclusion: This study showed that curcumin could be evaluated as a promising anti-cancer agent for human colorectal cancer.

Key words: colorectal cancer, curcumin, invasion, migration, proliferation

Introduction

Curcumin ((1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione) is a hydrophobic polyphenol-structured secondary metabolite of dried rhizome of turmeric (Curcuma longa and Curcuma spp.) [1,2] and its empirical formula is C₂₁H₂₀O₆ [2]. It is a yellow-orange colored substance. Moreover, curcumin is the most abundant component of turmeric and the other metabolites found in the plant are demethoxycurcumin and bisdemethoxycurcumin [3]. Since their natural pathways are the same and they convert to each other, their biological properties are quite similar [2]. According to the experiments on both animals and humans, the daily oral intake of curcumin is up to 12 g [4]. Also, curcumin has biological activities such as anti-angiogenic, anti-inflammatory, antioxidative and anticancer [2]. Colorectal cancer is the third most common cancer type around the world which leads to cancer-related death [5]. However, if diagnosed in the early stages, the survival of colorectal cancer is increased [6]. There are several factors (age, familial cancer syndromes, inflamma-
Curcumin effects on cell proliferation, angiogenesis and metastasis in colorectal cancer

Tory bowel disease, dietary factors, and hereditary polyposis conditions) effect that can contribute to the development of colorectal cancer [7]. The main components of colorectal cancer therapy are chemotherapy (5-fluorouracil and oxaliplatin), radiotherapy and surgery [8,9]. As natural therapeutic agents, irinotecan (a derivative of camptothecin) and polysaccharide K are used for colorectal cancer [10].

The purpose of this study was to investigate the anticancer activities of curcumin, i.e. antiproliferative, anti-invasive, anti-migrative and wound healing properties on colorectal cancer cell lines in vitro.

Methods

Cell culture

Human colorectal carcinoma HCT116 (ATCC CCL-247) cell line was obtained from American Type Culture Collection (ATCC) (Rockville, CT, USA). This cell line was cultured in McCoy's-5a modified medium (Biochrom GmbH, Berlin, Germany) supplemented with 15% fetal bovine serum (FBS, Cegrogen Biotech GmbH, Stadtallendorf, Germany) and 1% penicillin/streptomycin (Biochrom GmbH, Berlin, Germany). Human metastatic colorectal adenocarcinoma, LoVo (ATCC CCL-229) cell line was obtained from ATCC (Rockville, CT, USA). This cell line was cultured in RPMI-1640 (Biochrom GmbH, Berlin, Germany) containing 10% FBS (Cegrogen Biotech GmbH, Stadtallendorf, Germany), 1% L-glutamine (Biochrom GmbH, Berlin, Germany) and 1% penicillin/streptomycin (Biochrom GmbH, Berlin, Germany). Cells were kept at 37 °C in humidified 5% CO₂ incubator.

Cell viability

The antiproliferation activity of curcumin (C7727, Sigma-Aldrich, St Louis, MO, USA) was tested on HCT-116 and LoVo cell lines. These cell lines were seeded in 96-well plates (1×10⁴ cells/well). After 48 h of incubation with 2.5-75 µM curcumin, cell viability was evaluated with water soluble tetrazolium salt-1 (WST-1) assay (Version 16 Cell Proliferation Reagent WST-1, Roche Diagnostics, Indianapolis, USA). The assay was performed according to the manufacturer’s protocol.

Wound healing assay

Cell migration capability of HCT-116 and LoVo cells were also detected by using wound healing assay. These cell lines (30×10⁴ cells/well) were seeded in 6-well plates. After 90% confluence of cells on the plates, the wells were scratched with a pipette tip across the center of the well and the IC50 and IC10 doses of curcumin were added. McCoy’s-5a modified medium (Biochrom GMBH, Berlin, Germany) was used for HCT-116 cell line and RPMI-1640 (Biochrom GMBH, Berlin, Germany) was used for LoVo cell line as control. JuLI Br live cell movie analyzer was used for monitoring the wounds. ImageJ software 1.49 was used for measuring the wound gaps.

Cell invasion and migration assay

Cell invasion and migration activity were measured by using xCELLigence RTCA Dual Plate (RTCA-DP) instrument according to the manufacturer’s recommendations with CIM-plate 16 (Roche Diagnostics GmbH, Mannheim, Germany). For detection of cell migration, electrical impedance changes were measured at a gold microelectrode plated on the bottom of a membrane

![Figure 1. A: Dose-dependent cytotoxicity of curcumin on HCT-116 (orange) and LoVo (gray) cells incubated with increasing concentrations of curcumin (p<0.05). B: IC50 and IC10 of curcumin on HCT-116 and LoVo cell lines.](image-url)
Curcumin effects on cell proliferation, angiogenesis and metastasis in colorectal cancer

Figure 2. Invasion effect of curcumin on A: HCT-116 and B: LoVo cell lines after 24-h incubation (p<0.05).

Figure 3. Cell invasion results of curcumin on A: HCT-116 and B: LoVo cell lines at 24 h (orange control, blue curcumin). The results were obtained from xCelligence RTCA DP.
separating the upper and lower chambers. The lower compartment was supplemented with 10% FBS-containing medium for migration assay. 4×10⁴ cells suspended in serum-free medium were supplemented to the upper compartment of the plate. The upper compartment was coated with 1:40 diluted Matrigel (Basement Membrane Matrix, growth factor reduced, BD Biosciences, Erembodegem, Belgium) and the lower compartment was supplemented with 10% FBS-containing medium for invasion analysis. IC₅₀ of curcumin found by WST-1 assay analysis was added to the upper compartments for both invasion and migration assays for each cell line. The impedance was recorded per 15 min and the total analysis time was set at 48 h. xCELLigence RTCA software vs.1.2.1 was used for data analysis. At 6th, 12th and 48th h migration and invasion of cells were calculated as the percentage of CI compared to control group.

Statistics

Mann-Whitney U test was used to determine the significance between curcumin and its activity. SPSS (Version 15.0; SPSS, Inc., Chicago, IL, USA) was used for all analyses. The level of statistical significance was set at p<0.05. The data in the figures represent the mean±standard error of the mean (SEM). In this study, all tests were performed in triplicate.

Results

The effect of curcumin on cell proliferation of colorectal cancer cells

The in vitro proliferation activity doses of curcumin on HCT-116 and LoVo cells were measured by using WST-1 reagent. After 48-h incubation with curcumin, IC₅₀ values were 10 µM for HCT-116 and 20 µM for LoVo cell lines. Also, IC₁₀ values were 5 µM for HCT-116 and 2.5 µM for LoVo cell lines (Figure 1A AND 1B).

Wound healing activity of curcumin on colorectal cancer cell lines

After 24-h incubation, the control group exhibited 16.2% closure for HCT-116 cell line. IC₁₀ dose of curcumin (5 µM) showed 25.6% closure. On the other hand, the wound opened up 14.2% at IC₅₀ dose

Figure 4. Cell migration results of curcumin on A: HCT-116 and B: LoVo cell lines at 24 h (Orange control, blue curcumin). The results were obtained from xCelligence DP (p<0.05).
of curcumin (10 μM). For the control group of LoVo cell line, IC_{50} dose of curcumin (2.5 μM) and IC_{50} dose of curcumin (20 μM) showed 10.1, 18.4 and 7.3% closure, respectively. The results are shown in Figure 2A and B.

**Cell invasion and migration analysis**

The invasion and migration activities of curcumin on HCT-116 and LoVo cell lines were investigated at the 48th hour. According to these results, curcumin showed anti-migrative effect on HCT-116 and LoVo cell lines. (Figures 3 and 4).

**Statistical analysis**

P<0.05 was used to denote significant difference between curcumin and control groups for each cell line. As shown in Figure 3, curcumin inhibited the invasion of HCT-116 (Figure 3A) and LoVo (Figure 3B) cell lines at 24th h. For invasion, curcumin had the best invasion inhibition activity on HCT-116 cell line (Figure 3A). In Figure 4, the migration activity of curcumin on HCT-116 and LoVo cell lines is shown. Similarly, curcumin inhibited the migration of HCT-116 (Figure 4A) and LoVo (Figure 4B) cell lines at 24th h. For migration, curcumin had a significant activity on each cell line.

**Discussion**

In this study, the antiproliferative, anti-metastatic and wound healing activity of curcumin was studied. Previous studies have revealed that NF-KB activation is critical for the proliferation, survival and metastasis of colon cancer cells; therefore, inhibition of NF-KB activation is a potential antitumor strategy [11].

Curcumin has been suggested to inhibit the STAT-3 and NF-KB signal pathways (key components on cancer progression and development) [12]. Guo et al. have shown that curcumin induces apoptosis by affecting the ubiquitin-proteasome pathway. The released LDH from LoVo cells was increased along with the increase of curcumin. Curcumin could activate caspase-3 and caspase-9 in LoVo cells. Therefore, curcumin-induced apoptosis of LoVo cells was accompanied by increased activities of caspase-3 and caspase-9, which then stimulated the downstream apoptotic molecular cascade [13]. Also, this molecule plays an important role in colorectal cancer by reducing CD24 expression [2]. Curcumin up-regulates the E-cadherin expression and so, it can be characterized as an inhibitor of epithelial-mesenchymal transition. In colorectal cancer, curcumin down-regulates Sp-1 and FAK. Not only curcumin but also curcumin-related compounds decrease phospho-extracellular signal-regulated kinase (Erk)1/2 and phospho-Akt in different cell lines [14].

The main way of the cytotoxic activity of a compound is inhibiting or blocking mitosis or growth of cancer cells. In this study, the cytotoxic activity of curcumin in colorectal cancer cell lines was also studied. According to the results, IC_{50} of curcumin in HCT-116 and LoVo cell lines were 10 μM and 20 μM, respectively. In the study of Ono et al., after 72-h incubation, IC_{50} of curcumin was 25 μM on HAG-1 gallbladder adenocarcinoma [15]. Khatwani et al. carried out a study on the antiproliferative activity of curcumin, tetrahydrocurcumin, bisdemethoxycurcumin and dimethoxycurcumin on DLD-1 colon cancer cell line [16]. Their results revealed that IC_{50} values of bisdemethoxycurcumin, tetrahydrocurcumin and dimethoxycurcumin were 10 μM, 50 μM and 2 μM, respectively [17]. Also, the results of the study of Kaverimanian et al. showed that IC_{50} of curcumin was 12.5 μM in DLD-1 colon cell line [17]. Chen et al. carried out a study on seven different colorectal cancer cell lines. Their results revealed that the IC_{50} of curcumin on HCT-116 cell line was almost 50 μM after 24-h incubation [18]. As it is shown, the results presented in this study are well in-line with the literature.

One of the most dangerous parts of cancer biology is metastasis. Anti-metastatic agents’ investigation from natural sources is fundamental. In this study, the activity of curcumin on invasion and migration was also studied. According to the results, curcumin had the highest anti-invasion and anti-migration activity in HCT-116 cell line. Chen et al. studied the anti-migration activity of curcumin in colorectal cancer cell lines and observed that the highest anti-migration levels were shown in HCT-116 cell line [18]. Their results are quite similar to ours.

In this study, wound healing assay was performed to observe the effect of curcumin on wound healing and migration. Curcumin exerted its function against wound healing (a kind of migration assay) on two different colorectal cancer cell lines. After 24-h incubation, the results showed that IC_{50} of curcumin closes the wounds. A similar study by Chen et al. showed that curcumin inhibits the migration on HCT-116 cell line [18].

**Conclusion**

A synergistic combination of curcumin and resveratrol has been also described by Majumdar et al. Both agents, acting together, inhibited the constitutive activation of EGFRs and IGF-1R in HCT-116 colorectal cancer cells. A test with a xenograft mouse model of colorectal cancer showed that the
Curcumin effects on cell proliferation, angiogenesis and metastasis in colorectal cancer

According to the results, curcumin showed antiproliferation activity in HCT-116 and metastatic colorectal cancer cell lines. Also, curcumin inhibited migration and invasion on HCT-116 and LoVo cell lines. The wound healing assay revealed that curcumin plays an important role in wound healing on HCT-116 and LoVo cell lines. In conclusion, curcumin is a promising agent in metastasis and cancer.

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Conflict of interests

The authors declare no conflict of interests.

References