

ORIGINAL ARTICLE

Radiosensitive effect of curcumin on thyroid cancer cell death induced by radioiodine-131

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ABSTRACT

Curcumin is a natural product widely consumed by humans. It has many biological properties. In this study, we investigated the radiosensitive effect of curcumin on thyroid cancer cells against cellular toxicity induced by ¹³¹I. Human thyroid cancer and human non-malignant fibroblast cells (HFFF2) were treated with ¹³¹I and/or curcumin at different concentrations (5, 10 and 25 µg/ml) for 48 h. The cell proliferation was measured by determination of the surviving cells by using MTT assay. Our results showed that curcumin increased the killing effect of ¹³¹I on thyroid cancer cells, while it exerted no toxicity on HFFF2 cells. This result shows a promising effect of curcumin on the enhancement of therapeutic effects of ¹³¹I in patients.

KEY WORDS: ¹³¹I; curcumin; anti-proliferation; MTT; thyroid cancer cell

Introduction

Radioiodine-131 (¹³¹I) has been used as the first line of treatment for hyperthyroidism, Graves' disease and differentiated thyroid cancer. It has a physical half-life of 8.02 days and emits gamma rays and beta particles (Sawin *et al.*, 1997, Zanzonico, 1997, Robbins *et al.*, 2005). It concentrates in thyroid cells and kills tumor cells, yet it has several side effects such as sialadenitis, gastrointestinal symptoms, xerostomia, temporary bone-marrow suppression and neoplasia (Bushnell *et al.*, 1992, Noaparast *et al.*, 2013). ¹³¹I may also induce genetic damage and chromosomal instability in normal cells that may result in secondary malignancies (Baugnet-Mahieu *et al.*, 1994, Watanabe *et al.*, 2004, Hosseinimehr *et al.*, 2013). The cytotoxic effect of ¹³¹I is mainly related to beta particles. Ionizing radiation causes cellular injury mainly by producing reactive oxygen species (ROS). ROS can induce lipid peroxidation and damage to cellular membranes and critical macromolecules such as DNA (Little, 2000, Noaparast *et al.*, 2013). Curcumin is a major component of turmeric, produced from the rhizome of the plant *Curcuma longa* (Chendil *et al.*, 2004). Many studies have indicated that curcumin has strong pharmacological

activities such as anti-oxidant, anti-cancer (Kuttan *et al.*, 1985), anti-microbial effects (Negi *et al.*, 1999). Curcumin can scavenge free radicals and protect the cellular macromolecules against oxidative stress (Kalpana *et al.*, 2004, Polasa *et al.*, 2004, Singh *et al.*, 2012). Recently we showed that curcumin protected human lymphocytes against genotoxicity induced by ¹³¹I and it significantly reduced the DNA damage induced by ¹³¹I *in vitro* (Shafaghati *et al.*, 2014). Although curcumin exhibited protective effects on chromosome damage induced by ¹³¹I in normal cells, its effect on thyroid cancer cells during ¹³¹I treatment is not clear.

The aim of this study was to determine the therapeutic effect of curcumin on cell death induced by ¹³¹I in thyroid human cancer cells and human non-malignant fibroblast cells *in vitro*.

Materials and methods

Cell lines

Human non-malignant skin fibroblasts (HFFF2) and human thyroid cancer (Thr.C1-PI 33) cell line were obtained from the Iranian Pasteur Institute (Tehran). The cells were grown at 37°C and 5% CO₂ in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), penicillin 100 IU/mL, and streptomycin 100 µg/ml, all of which were obtained from Gibco (Invitrogen, USA).

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MTT assay

Thyroid cancer and HFFF2 cells were subjected to cell proliferation assay by using MTT. The MTT colorimetric assay is used for evaluation of cell toxicity. The MTT test is based on the strength of mitochondrial enzymes to decrease MTT (pale yellow) to formazan crystals (dark blue). Owing to their impenetrability through the cell membrane, formazan crystals collect in cells (Ashrafi *et al.*, 2012). Cells (20,000) were seeded in 96-well plates. After 24 h incubation, the cells were treated with various concentrations of curcumin (CM) (5, 10 and 25 $\mu\text{g}/\text{ml}$) and were incubated at 37°C and 5% CO_2 . After 48 h incubation, 20 μL of MTT (5 mg/mL in phosphate buffer saline) was added to each well, and the cells were incubated for 4 hours. After removal of the medium, dimethyl sulfoxide (DMSO) was used to solubilize the formazan compounds and the cell plates were shaken for 10 minutes. The absorbance of every culture well was read on an ELISA Reader (Biotech, USA). Cells without any treatment were used as control for comparison of absorbance and cell survival.

Irradiation protocol

Cells were seeded in 96-well plates. After 24 h incubation, the cells were treated with various concentrations of CM (5, 10 and 25 $\mu\text{g}/\text{ml}$) and incubated at 37°C and 5% CO_2 . After 2h incubation, the diluted solution of ^{131}I was added

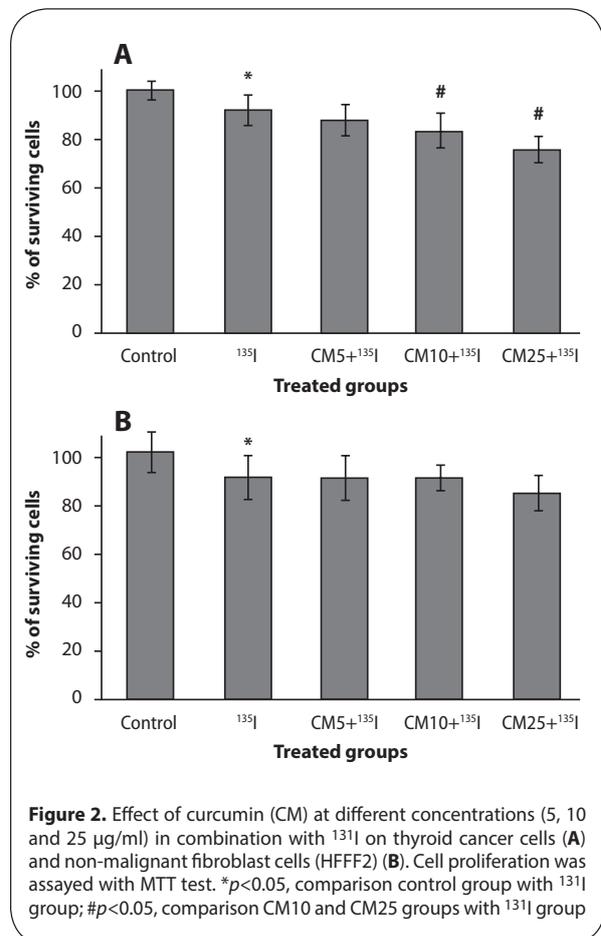
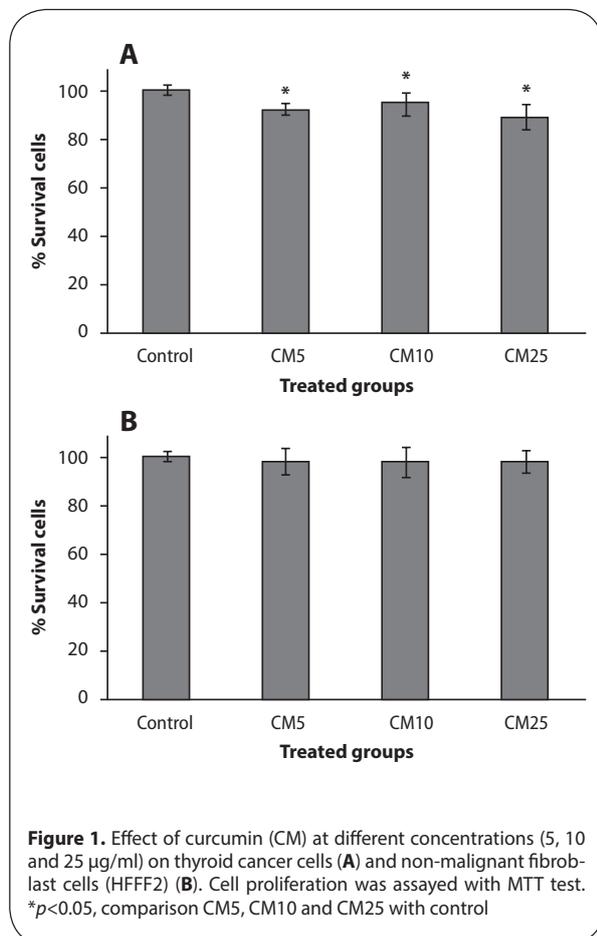
at the dose of 10 μCi (100 μl) to each well and incubated for 48 h. MTT assay was performed according to the above protocol.

Statistical analysis

Data were presented as mean \pm standard deviation (SD) of four experiments. Data were compared and the differences were considered significant if the p -value <0.05 .

Results**Effect of curcumin on cell proliferation in thyroid cancer and HFFF2 cells**

The effect of curcumin on cell proliferation in thyroid cancer and HFFF2 cells is shown in Figure 1. In thyroid cancer cells, a statistically significantly reduced cell proliferation was observed in curcumin treatments at concentrations of 5, 10 and 25 $\mu\text{g}/\text{ml}$ ($p<0.02$). The percentage of survival in thyroid cancer cells was 92.5 ± 2.4 , 95 ± 4.9 and 89.4 ± 5.3 at concentrations of 5, 10 and 25 $\mu\text{g}/\text{ml}$, respectively. A statistically significant difference was observed between the doses of 5, 10 and 25 $\mu\text{g}/\text{ml}$ of curcumin with control for cellular anti-proliferation (Figure 1A). No significant toxicity was observed in HFFF2 cells treated by any of the doses of curcumin (Figure 1B).



Effect of curcumin and ¹³¹I combination on cell proliferation in thyroid cancer and HFFF2 cells

The combination effects of curcumin and ¹³¹I on the percentage of cell proliferation in control, curcumin-pretreated, and/or ¹³¹I treated thyroid cancer and HFFF2 cells are shown in Figure 2. ¹³¹I significantly reduced the survival rate in thyroid cancer cells by 91%. Thyroid cancer cell proliferation was reduced in pre-treated curcumin groups. Curcumin reduced the percentage of cell survival to 87±6%, 83±7% and 75±5% at concentrations 5, 10 and 25 µg/ml, respectively. Curcumin significantly increased cell death in the dose of 10 and 25 µg/ml in combination with ¹³¹I as compared to ¹³¹I alone ($p < 0.05$). These results show that curcumin has a synergistic effect with ¹³¹I on cell growth inhibition in thyroid cancer cells; it is related to the radiosensitive effect of curcumin on thyroid cancer cells treated with ¹³¹I. Interestingly, curcumin at all doses of 5, 10 and 25 µg/ml did not show any enhancement of toxicity on HFFF2 cells in combination with ¹³¹I.

Discussion

In this study, we observed that curcumin exerted a radiosensitive effect on thyroid cancer cells; it reduced significantly cell growth in combination with ¹³¹I. Curcumin did not exhibit any cellular toxicity in non-malignant fibroblast cells (HFFF2) treated at the same doses with ¹³¹I. Iodine-131 is widely used for the treatment of thyroid-related diseases. High-dose radioiodine treatment is associated with dose-limited side effects. ¹³¹I emits gamma and beta rays; the latter ones have a short range board with higher destroying effects on cells as compared to gamma rays. Induction of oxidative stress is one of the main mechanisms for therapeutic and /or side effects of ¹³¹I. Oxidative stress may cause DNA damage. Several studies showed that curcumin exerted radioprotective effects on normal cells such as human lymphocytes and fibrosis in the rat lung. Protective effects of curcumin are related to free radical scavenging and enhancement of enzymatic and non-enzymatic antioxidants like GSH in cells treated with curcumin (Srinivasan *et al.*, 2006, Cho *et al.*, 2013).

Recently we showed that curcumin significantly protected human lymphocytes from genotoxicity induced by ¹³¹I. Curcumin reduced micronuclei frequency in lymphocytes in combination with ¹³¹I (Shafaghathi *et al.*, 2014). In this study we tried to evaluate the effect of curcumin on thyroid cancer cell, because it was hypothesized that curcumin could enhance cellular toxicity induced by ¹³¹I in thyroid cancer cells. Our results showed that curcumin increased radiation toxicity in thyroid cancer cells and it was showed no toxicity on non-malignant human cells induced by ¹³¹I. These results are promising for using this natural product in combination with ¹³¹I therapy in patients. Curcumin has been shown to affect mediated several cell signaling pathways such as apoptosis (activation of caspases and down regulation of anti-apoptotic gene products) (Agrawal *et al.*, 2010). Also, curcumin

sensitized human cancer cells on exposure to external gamma radiation, which is a dual benefit t of curcumin in patients with cancer therapy (Kunnumakkara *et al.*, 2008, Goel *et al.*, 2010, Lopez-Jornet *et al.*, 2011).

Our findings indicate that curcumin is a promising natural product for patients on radioiodine therapy by radiosensitizing thyroid cancer cells in combination with ¹³¹I.

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Conflict of interest statement

The authors declared no potential conflict of interest with respect to the authorship, and/or publication of this study.

REFERENCES

- Agrawal DK, Mishra PK. (2010). Curcumin and its analogues: potential anti-cancer agents. *Med Res Rev* **30**: 818–860.
- Ashrafi SA, Hosseinimehr SJ, Varmira K, Abedi SM. (2012). Radioimmunotherapy with (131)I-bevacizumab as a specific molecule for cells with overexpression of the vascular endothelial growth factor. *Cancer Biother Radiopharm* **27**: 420–425.
- Baugnet-Mahieu L, Lemaire M, Leonard ED, Leonard A, Gerber GB. (1994). Chromosome aberrations after treatment with radioactive iodine for thyroid cancer. *Radiat Res* **140**: 429–431.
- Bushnell DI, Boles MA, Kaufman GE, Wadas MA, Barnes WE. (1992). Complications, sequela and dosimetry of Iodine-131 therapy for thyroid carcinoma. *J Nucl Med* **33**: 2214–2221.
- Chendil D, Ranga RS, Meigooni D, Sathishkumar S, Ahmed MA. (2004). Curcumin confers radiosensitizing effect in prostate cancer cell line Pc-3. *Oncogene* **23**(8): 1599–607.
- Cho YJ, Yi CO, Jeon BT, Jeong YY, Kang GM, Lee JE *et al.* (2013). Curcumin attenuates radiation-induced inflammation and fibrosis in rat lungs. *Korean J Physiol Pharmacol* **17**: 267–274.
- Goel A, Aggarwal BB. (2010). Curcumin, the golden spice from indian saffron, is a chemosensitizer and radiosensitizer for tumors and chemoprotector and radioprotector for normal organs. *Nutr Cancer* **62**: 919–930.
- Hosseinimehr SJ, Shafaghathi N, Hedayati M. (2013). Genotoxicity induced by iodine-131 in human cultured lymphocytes. *Interdiscip Toxicol* **6**: 74–76.
- Kalpana C, Menon VP. (2004). Curcumin ameliorates oxidative stress during nicotine-induced lung toxicity in Wistar rats. *Ital J Biochem* **53**: 82–86.
- Kunnumakkara AB, Diagaradjane P, Guha S, Deorukhkar A, Shentu S, Aggarwal BB *et al.* (2008). Curcumin sensitizes human colorectal cancer xenografts in nude mice to gamma-radiation by targeting nuclear factor-kappa-regulated gene products. *Clin Cancer Res* **14**: 2128–2136.
- Kuttan R, Bhanumathy P, Nirmala K, George MC. (1985). Potential anticancer activity of turmeric (*Curcuma longa*). *Cancer Lett* **29**: 197–202.
- Little JB. (2000). Radiation Carcinogenesis. *Carcinogenesis* **21**: 397–404.
- Lopez-Jornet P, Camacho-Alonso F, Gomez-Garcia F. (2011). Effect of curcumin and irradiation in Pe/Ca-Pj15 oral squamous cell carcinoma. *Acta Odontol Scand* **69**: 269–273.
- Negi PS, Jayaprakasha GK, Jagan Mohan Rao L, Sakariah KK. (1999). Antibacterial activity of turmeric oil: A byproduct from curcumin manufacture. *J Agric Food Chem* **47**: 4297–4300.
- Noaparast Z, Hosseinimehr SJ. (2013). Radioprotective agents for the prevention of side effects induced by radioiodine-131 therapy. *Future Oncology* **9**: 1145–1159.

- Polasa K, Naidu AN, Ravindranath I, Krishnaswamy K. (2004). Inhibition of B(a)P induced strand breaks in presence of curcumin. *Mutat Res-Gen Tox En* **557**: 203–213.
- Robbins RJ, Schlumberger MJ. (2005). the evolving role of 131I for the treatment of differentiated thyroid carcinoma. *J Nucl Med* **46**: 285–375.
- Sawin Ct, Becker DV. (1997). Radioiodine and the treatment of hyperthyroidism: The early history. *Thyroid* **7**: 163–176.
- Shafaghati N, Hedayati N, Hosseinimehr SJ. (2014). Protective effects of curcumin against genotoxicity induced by 131I-iodine in human cultured lymphocyte cells. *Pharmacognosy Magazine*: **10**: 106–110.
- Singh G, Sharma S, Choudhary N, Yadav S, Chauhan R, Dwivedi J. (2012). evaluation of radioprotective properties of curcuma longa rhizome extract: a cytogenetic analysis in cancer. *Pharmacognosy Communications* **2**: 44–49.
- Srinivasan M, Rajendra Prasad N, Menon VP. (2006). Protective effect of curcumin on gamma-radiation induced dna damage and lipid peroxidation in cultured human lymphocytes. *Mutat Res* **611**: 96–103.
- Watanabe N, Kanegane H, Kinuya S, Shuke N, Yokoyama K, Kato H *et al.* (2004). The radiotoxicity of 131I therapy of thyroid cancer: assessment by micronucleus assay of b lymphocytes. *J Nucl Med* **45**: 608–611.
- Zanzonico PB. (1997). radiation dose to patients and relatives incident to 131I therapy. *Thyroid* **7**: 199–204.