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 131-I. Human thyroid cancer and human non-malignant fibroblast cells (HFFF2) were treated with 131-I and/or curcumin at different concentrations (5, 10 and 25 μg/ml) for 48h. 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 131-I 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 131-I in patients.

KEY WORDS: 131-I; 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. The 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, Noaparas 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).
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 μg/ml) and were incubated at 37°C and 5% CO₂. 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 (Biotecck, 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 μg/ml) and incubated at 37°C and 5% CO₂. After 2 h incubation, the diluted solution of ¹³¹I 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 ± 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 μg/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 μg/ml, respectively. A statistically significant difference was observed between the doses of 5, 10 and 25 μg/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).

![Figure 1. Effect of curcumin (CM) at different concentrations (5, 10 and 25 μg/ml) on thyroid cancer cells (A) and non-malignant fibroblast cells (HFFF2) (B). Cell proliferation was assayed with MTT test. $p$<0.05, comparison CM5, CM10 and CM25 with control](image1)

![Figure 2. Effect of curcumin (CM) at different concentrations (5, 10 and 25 μg/ml) in combination with ¹³¹I on thyroid cancer cells (A) and non-malignant fibroblast cells (HFFF2) (B). Cell proliferation was assayed with MTT test. $p$<0.05, comparison control group with ¹³¹I group; $p$<0.05, comparison CM10 and CM25 groups with ¹³¹I group](image2)
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 radio-sensitive 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 radiiodine 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 (Shafaghati 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 of curcumin in patients with cancer therapy (Kunnunakkara 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.

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