Protective effects of curcumin against genotoxicity induced by 131-iodine in human cultured lymphocyte cells

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ABSTRACT

Background: 131-radioiodine has been widely used as an effective radionuclide for treatment of patients with thyroid diseases. The purpose of the present study is to investigate the radioprotective effects of curcumin as a natural product that protects against the genotoxic effects of ^{131}I in human cultured lymphocytes. **Materials and Methods:** Whole blood samples from human volunteers were incubated with curcumin at doses of 5, 10, and 50 µg/mL. After 1-hour incubation, the lymphocytes were incubated with ^{131}I (100 µCi/1.5 ml) for 2 hours. The lymphocyte cultures were then mitogenically stimulated to allow for evaluation of the number of micronuclei in cytokinesis-blocked binucleated cells. **Results:** Incubation of lymphocytes with ^{131}I at dose 100 µCi/1.5 mL induced genotoxicity shown by increase in micronuclei frequency in human lymphocytes. Curcumin at 5, 10, and 50 µg/mL doses significantly reduced the micronuclei frequency. Maximal protective effects and greatest decrease in micronuclei frequency were observed when whole blood was incubated with 50 µg/mL dose of curcumin with 52%. **Conclusion:** This study has important implications for patients undergoing ^{131}I therapy. Our results indicate a protective role for curcumin against the genetic damage and side effects induced by ^{131}I administration.

Key words: Curcumin, genotoxicity, ^{131}I, micronucleus, radioiodine

INTRODUCTION

Radioiodine-131 (^{131}I) has been widely used for treatment of patients with thyroid diseases such as hyperthyroidism and thyroid cancer. The wide usage of ^{131}I in nuclear medicine is due to its physical and radiochemical properties. It emits beta particles (E_{max} = 0.61 MeV and E_{avg} = 0.20 MeV) and gamma rays (E = 0.36 MeV), with a physical half-life of approximately 8 days. It accumulates at high concentration in the thyroid tissue with high target to nontarget ratio.[1] The therapy effect of ^{131}I is related to beta particle, which is damaging to cells with direct or indirect effects. In direct action, radiation may directly hit a particularly sensitive atom or molecule and disrupt chemical bonds in the cell. In the indirect action, radiation can damage a cell indirectly by interacting with water molecules in the body through reactive oxygen species (ROS).[2] ROS can attach to critical macromolecules such as DNA, resulting in DNA damage, chromosomal breaks, and cell death.[3] Although ^{131}I acts with this mechanism to damage the tumor cells, it can have side effects on normal tissues due to unwanted accumulation of this radionuclide in healthy organs. Short- and long-term side effects related to radioiodine therapy are nausea, sialadenitis, and hematological depression. Since iodine is secreted in salivary glands at high concentration, salivary gland dysfunction is reported in patients undergoing ^{131}I therapy.[4] In addition to these side effects, there is an induction of secondary primary cancer and genetic damage following in ^{131}I therapy. An increased risk of leukemia, bladder cancer, and colorectal cancer were reported in patients after ^{131}I therapy.[5-9] There are several studies showing that genetic damage was increased in patients after ^{131}I therapy. The frequency of micronuclei as breaks of chromosome was elevated in patients after ^{131}I therapy.[10-12] Thus, protection of DNA may reduce side effects induced by ^{131}I which reduces incidence of secondary cancer. Curcumin is a

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dietary antioxidant derived from a herb *Curcuma longa* and possesses therapeutic properties. It has been reported to scavenge free radicals and inhibit lipid peroxidation, with these mechanisms, curcumin protects critical macromolecules such as DNA from oxidative stress.[13,14] Several studies reported the radioprotective effects of curcumin against cytotoxicity induced by external gamma rays. Pretreatment with curcumin protected hepatocytes against gamma rays-induced cellular damage.[15] Curcumin protected human lymphocytes against genotoxicity induced by gamma radiation.[16] With respect to protective effects of curcumin, the aim of this study is to determine the radioprotective effects of curcumin against genotoxicity induced by ^{131}I in human peripheral blood lymphocyte cells *in vitro*.

MATERIALS AND METHODS

Chemicals

All chemicals were obtained from Merck and Sigma. Curcumin was prepared in ethanol and diluted with medium culture. [131]I Na in sterile solution was prepared from AEOI, Tehran, Iran, it was used freshly.

Irradiation protocol

The study protocol was approved by the ethical committee of the university. After obtaining written informed consent, 12 mL whole blood samples were collected in heparinized tubes from three healthy, nonsmoking male volunteers aged 25–35 years. The whole blood was divided into six 1.5-mL tubes, one for each of the six study groups: Control; ^{131}I only; curcumin at doses 5, 10, and 50 μg/mL with ^{131}I; and curcumin only. First, blood samples were incubated with curcumin for 1 hour, after which 100 μCi of ^{131}I was added to the blood samples and incubated at 37°C for 2 hours. After incubation, RPMI 1640 medium was added to each tube and the cultures were centrifuged at 1500 g for 8 minutes. To separate ^{131}I from the whole blood, the upper (less dense) solution was removed and blood was transferred for micronucleus assay.

Micronuclei assay

From each sample, an aliquot of 750 μL (control and irradiated groups) was added to 4.15 mL of RPMI 1640 culture medium (Gibco, USA), which contained 10% fetal calf serum, 0.1 mL/5 mL phytohemagglutinin (Gibco, USA), antibiotics (penicillin 100 IU/mL, streptomycin 100 μg/mL), and 2 mM glutamine (Sigma, USA) at final concentration. All cultures were set up in duplicate and incubated at 37 ± 1°C in a humidified atmosphere of 5% CO_2/95% air. Cytochalasin B (Fluka, final concentration: 6 μg/mL) was added after 44 hours of culture incubation. At the end of 72 hour of incubation, the cells were collected by centrifugation and resuspended in 0.075 M cold potassium chloride for 8 min at 1500 g. They were then immediately treated with a fixative solution three times (methanol: acetic acid). Fixed cells were dropped onto clean microscopic slides, air-dried, and stained with Giemsa solution (20%). All slides were coded by an individual other than the scorer, and were evaluated at × 100 magnification for the micronuclei frequency in cytokinesis-blocked binucleated cells with well-preserved cytoplasm. To be scored as micronuclei, candidates had to have a diameter between 1/16th and 1/3rd of main nuclei, be non-refractile, and not be linked to or overlap with the main nuclei.[17] At each blood collection time, at least 1000 binucleated cells from duplicate irradiated and control cultures from each volunteer were examined; then, the frequency of micronuclei was recorded.

Statistical analysis

At each blood collection, the prevalence of micronuclei was recorded for each volunteer. The data were analyzed using analysis of variance (ANOVA) with Tukey's HSD Post-hoc test.

RESULTS

The mean percentage of micronuclei in the lymphocytes of volunteers treated with 100 μCi of ^{131}I was 3.38 ± 0.33, whereas the percentage of nontreated control lymphocytes was 1 ± 0.6 ($P < 0.001$) [Table 1]. The frequency of micronuclei (an indication of the genotoxic effects of internal irradiation) after preincubation with curcumin at doses of 5, 10, and 50 μg/mL were, 2.44 ± 0.49, 2.0 ± 0.61, and 1.63 ± 0.21, respectively [Figure 1]. The data demonstrate that the frequencies of micronuclei found in the curcumin-treated samples were significantly lower than that of samples cultured with only [131]I. Whole blood samples incubated with 5, 10, and 50 μg/mL of curcumin, and then exposed *in vitro* to ^{131}I radiation, exhibited a significant decrease in micronuclei frequency compared with samples incubated with only [131]I ($P < 0.01$). Total micronuclei values were 28, 41 and 52% less in the samples treated with curcumin at concentrations of 5, 10, and 50 μg/mL, respectively than in controls [Table 1]. Curcumin alone did not cause genotoxicity in cultured lymphocytes at concentrations of 50 μg.

A typical depiction of binucleated cells with micronuclei is given in Figure 2.

DISCUSSION

In the present study, we show that curcumin significantly protects lymphocytes against genotoxicity induced by [131]I.

Table 1: The percentages of micronuclei induced *in vitro* by ^{131}I on cultured blood lymphocytes from human volunteers and protective effects of curcumin

Volunteer Group	Binucleate cells with micronuclei (%)*					
	Control	I	I+Cur5	I+Cur10	I+Cur50	Cur50
Volunteer 1	0.2	3.05	2.21	1.7	1.4	1
Volunteer 2	0.6	3.7	2.1	2.7	1.7	1.08
Volunteer 3	1	3.38	3	1.6	1.8	1.33
Mean±SD	0.6±0.4	3.38±0.33	2.44±0.49	2.0±0.61	1.63±0.21	1.14±0.17

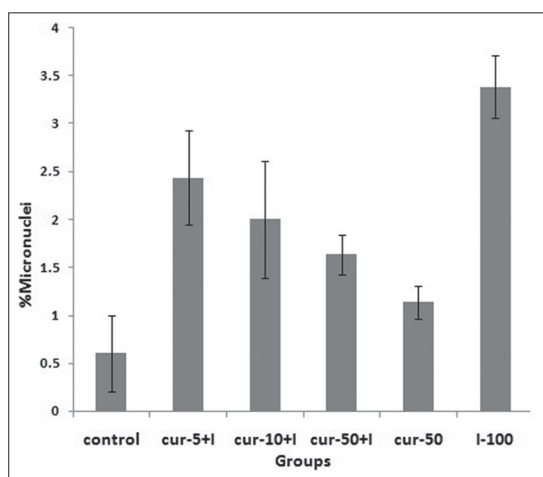Cur=curcumin, I=131I, *1000 BN cells were examined in each culture, SD: Standard deviation

Figure 1: *In vitro* protection by curcumin (Cur) at different concentration (5, 10 and 50 µg) against radiation-induced genetic damage induced by 131I (I) in cultured whole blood lymphocyte. The data represent average ± standard deviation of three human volunteers. $P<0.001$: Sample at control compared with similarly irradiated lymphocytes from the blood sample treated with I. $P<0.05$: I sample compared to I-Cur-5+I, Cur-10+I and Cur-50+I samples. $P>0.05$: Cur-50 sample compared control sample. $P>0.05$: Between groups of I-Cur-5+I, Cur-10+I and Cur-50+I samples

Figure 2: A typical binucleated lymphocyte with micronuclei in our study

In vitro incubation of human blood with the natural compound curcumin reduced the frequency of micronuclei induced by internal irradiation by the radiopharmaceutical [131]I. Measures of genotoxicity have been used to estimate the risk of damage induced by internal irradiation from the radiopharmaceutical. Genotoxic agents can cause cancer, hereditary disorders, and abnormalities in developing embryos.[18]

In this study, we have observed genotoxic effects induced by ^{131}I at dose 100 µCi in lymphocytes. Gil *et al*.,[19] assessed DNA damage, namely micronuclei in peripheral lymphocytes in patients with thyroid cancer after ^{131}I therapy. The number of micronuclei in cells increased significantly 1 month after the treatment.[19] Ballardin *et al*.,[10] reported a 4-fold increase in the frequency of micronuclei at 7 days after radioiodion therapy in patients; however, micronuclei frequency declined slowly and reached baseline at 180 days after ^{131}I therapy.[10] Other studies reported a significantly higher frequency of micronuclei in patients a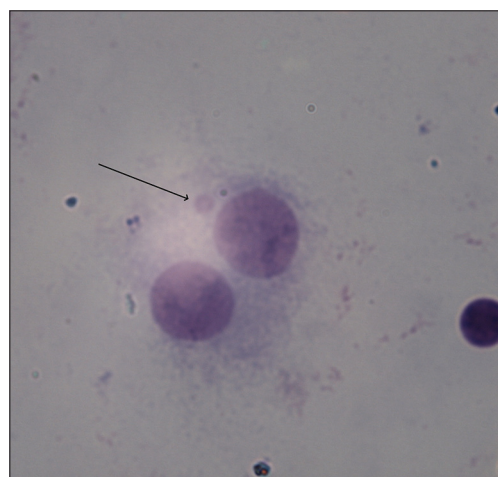fter ^{131}I therapy.[12,20] The micronuclei frequency assay can be used as a valuable endpoint and sensitive method for studying radiation biology of ^{131}I in patients for assessment of genetic damage. However, ^{131}I is considered a very useful radionuclide in reducing thyroid activity, nevertheless, as a genotoxic agent that may produce secondary cancer incidence in patients.[21-23] Incidence of leukemia was significantly increased in patients after iodine therapy.[23] [131]I emits gamma and beta rays. Beta rays have short range board with higher destroying effects on cells as compared with gamma rays. Induction of oxidative stress is one of the main mechanisms for therapeutic and/or side effects of [131]I. Oxidative stress may be related to DNA damage.[19] In this study, we showed that curcumin protects human lymphocytes against genotoxicity induced by internal irradiation. For all three volunteers, the ^{131}I-treated lymphocytes incubated with curcumin showed a reduction in micronuclei frequency as compared with ^{131}I-treated samples without curcumin. This finding suggests that curcumin acts effectively as a free radical scavenger. Curcumin treatment at 50 µg/mL provides maximal leukocyte protection. Curcumin has been reported to have several beneficial health effects, including anti-inflammatory, neuroprotective, antioxidant, anticancer, and radiosensitizer.[24-30] Curcumin has been shown to affect several cell signaling pathways, including apoptosis (activation of caspases and downregulation of anti-apoptotic gene products), proliferation (HER-2,

EGFR, and AP-1), angiogenesis (VEGF), and inflammation (NF-κB, TNF, IL-6, IL-1, COX-2, and 5-LOX).[24] Also, it has been shown to sensitize human cancer cells to gamma radiation, which is a dual benefit effect of curcumin in patients for cancer therapy.[28,31,32]

Curcumin has been shown to have antioxidant activity against the cellular oxidative stress associated with diseases. Curcumin showed a powerful capacity for scavenging intracellular small oxidative molecules such as H_2O_2, HO•, ROO•. Curcumin can readily transfer electron or easily donate H-atom from two phenolic sites to scavenge free radicals. The excellent electron transfer capability of curcumin is because of its unique structure and different functional groups, including a β-diketone and several π electrons that have the capacity to conjugate between two phenyl rings.[33,34] Pharmacokinetics parameters of curcumin were measured in patients after the consumption of 8 g of curcumin. No toxicity was observed in patients with curcumin ingestion.[35,36] Curcumin plasma level range was from 29 to 419 ng/mL,[36] and 22 to 42 ng/mL after 2–6 hours after single dose.[35] Curcumin has poor oral bioavailability, recently many investigations focused on nanoformulation and water soluble derivatives of this natural product to increase absorption by intestinal tract.[37-39]

Because curcumin has been studied extensively as a promising pharmacological drug, it may be a useful protective candidate for patients undergoing ^{131}I therapy. In this study, we showed that curcumin significantly protects human lymphocytes against genotoxicity induced by [131]I.

ACKNOWLEDGMENTS

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