ORIGINAL ARTICLE

Protective effect of curcumin on cisplatin-induced genotoxicity in human leukocytes culture

Yogamaya D. Prabhu, Nikhila Ramesh, Nishu Sekar, Kaviyarasi Renu, Shalaka S. Ramgir, V. G. Abilash

Department of Biomedical Sciences, School of Biosciences and Technology, VIT University, Vellore, Tamil Nadu, India

Abstract

Aim: Curcumin known for its anti-inflammatory and antioxidant properties was studied for its protective effect against genotoxicity when administered in conjunction with cisplatin. **Materials and Methods:** Cytokinesis-block micronucleus assay was performed to determine whether the genotoxicity caused by cisplatin is reduced in human leukocyte cells when administered with curcumin. **Results:** Cells in the control (C1 and C2) uniformly exhibited a large amount of mononucleated and binucleated cells, but there were significant differences between the cells scored in the test and radioprotective samples. **Conclusion:** Two-factor ANOVA was performed. $F = 4.089605735$ and $F_C = 3.07246699$ between samples and $F = 1.505376$ and $F_C = 2.4875777$ show that there is significant variation in the action of curcumin both between the samples and within the samples.

Key words: Cisplatin, curcumin, cytokinesis-block micronucleus, genotoxicity, protective effect

INTRODUCTION

Chemotherapy is a type of cancer treatment that uses drugs to kill or slow down the division of cancer cells. Cisplatin (platinum coordination complex- cis - diamminedichloroplatinum (II)) is an antitumor drug that is widely used to treat testicular and ovarian cancer due to its high success rate. It binds to deoxyribonucleic acid (DNA) in the nucleus and subsequently interferes with normal transcriptional and DNA replicating mechanisms and eventually causes cell death by triggering cytotoxic processes. Despite its effectiveness, a large amount of cisplatin is lost as it is inactivated due to high reactivity of platinum against the sulfur group of amino acids. It also binds to various other non-DNA targets such as phospholipids, glutathione, and RNA. The main mechanism of cisplatin in the nucleus is to cause the formation of DNA adducts and cross-links that are poorly repaired by nuclear repair enzymes[1,2]. As a result of these malformations, there is a marked decrease in the stability of DNA leading to cell dysfunction, and eventually cell death. Cisplatin also causes cell death by binding to ubiquitin molecules which triggers a cascade of cytotoxic processes[1]. Even though the usage of cisplatin has become common in chemotherapy, as with all chemotherapy drugs, it kills both cancerous cells and normal cells. Therefore, it comes with severe side effects such as nephrotoxicity, infertility, neurotoxicity, and ototoxicity[1]. Its genotoxic effect on DNA may cause secondary cancers, and there have also been some cases where it caused drug resistance[3]. To ameliorate the side effects, cisplatin is almost always combined with other drugs; however, there is a need to reduce the toxicity of cisplatin without reducing its effectiveness. Several compounds have been analyzed for a protective effect against cisplatin-induced genotoxicity[4,5]. Here, curcumin, a natural compound found in the rhizoid turmeric (*Curcuma longa*), was studied for a protective effect against DNA damage by cisplatin. Curcumin has two methoxy groups and two phenolic hydroxy groups that have been found to promote antiproliferative and anti-inflammatory activities. It has been found to be generally

Address for correspondence:
Dr. V. G. Abilash,
Department of Biomolecules and Genetics,
School of Bio Sciences and Technology (SBST),
VIT University, Vellore - 632 014, Tamil Nadu, India.
E-mail: abilash.vg@vit.ac.in

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chemotherapeutic in nature[3-6]. In this study, the aim is to show that curcumin lends a protective effect against the genotoxicity caused by cisplatin in human leucocyte cells by performing cytokinesis-block micronucleus (CBMN) assay. The CBMN assay blocks cell division at cytokinesis stage to observe DNA damage by scoring for the presence of DNA damage biomarkers[7]. This was achieved by collecting blood samples and performing two cultures. After culturing for 24 h, at the 24th h, the drug was introduced into both cultures at different concentrations. Two different concentrations of curcumin are also added along with cisplatin at 24th h to the second culture alone. At 44th h, cytochalasin B, a chemical that blocks cytokinesis, is added to both cultures. Finally, at the end of 72nd h, the cultures are harvested without the addition of colchicine. The samples are then stained with Giemsa and observed under the microscope for characteristic DNA damage markers.

MATERIALS AND METHODS

Curcumin (IUPAC name (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), the natural compound studied in this project for protective against genotoxicity, has the molecular formula $C_{21}H_{20}O_6$ and a molar mass of 368.38 g/mol.

Sample Collection

Fresh blood was collected from healthy individuals with the help of a sterile heparinized syringe, and 0.5 ml of blood was transferred to 10 tubes (for two cultures with 5 tubes each) containing 5 ml of RPMI 1640 media which is supplemented with fetal bovine serum, phytohemagglutinin-M, and L-glutamine under aseptic conditions. The culture tubes were then incubated at 37°C and samples were drawn from the tube to determine DNA damage.

Pre-analysis Sample Preparation

After culturing for 24 h, 2.5 and 5 µl of cisplatin was introduced into both cultures (two tubes of each concentration as test or T-samples). 25 and 50 µl of curcumin (two tubes each) was added along with cisplatin (radioprotective or RP samples) at 24th h to the second culture alone. In both cultures, one tube was kept as control without the addition of either cisplatin or curcumin. At 44th h, 40 µl of cytochalasin-B, a chemical that blocks cytokinesis, is added to all the tubes. Finally, at the end of 72nd h, the cultures are harvested without the addition of colchicine.

Harvesting

After 72 h, the culture tubes were centrifuged at 1500 rpm for 5 min after which the supernatant was carefully discarded. After gently tapping the cell button, 6 ml of cold hypotonic solution (0.565% KCl) was added to the tubes and the tube contents were gently mixed. The tubes were incubated for 6 min, followed by 5 min of centrifugation at 1500 rpm. The supernatant was again removed carefully, and the cells were washed with carnoy's fixative by mixing vigorously. Further, 2-3 drops of formaldehyde were added to preserve the cytoplasm. The tubes were kept at room temperature for 2 h after which a wash of fixative was given before slide preparation. Sides were prepared by dropping 1-2 drops of cell button mix obtained on chilled slides followed by immediately drying them on a hot plate (40°C). The slides were stained by keeping them in a Coplin jar with 4% Giemsa solution followed by destaining by keeping them in a Coplin jar with double-distilled water for 1 min. Scoring was performed under ×100 oil immersion objective of the (Labomed) microscope.

Statistical Analysis

Statistical analysis was performed to check the scored DNA damage biomarkers (nucleoplasmic bridges [NPBs], nuclear buds [NBUDs], and micronuclei [MN]) to confirm protective effect against genotoxicity. NPBs are biomarkers of DNA misrepair and/or telomeric end fusions. NBUDs are biomarkers of amplified DNA and DNA repair complexes while MN is markers of chromosome breakage or whole loss. Mononucleated, binucleated, and multinucleated cells denote the mitotic status of the cells.

RESULT

While the cells in the controls (C1 and C2) uniformly exhibited a large amount of mononucleated and binucleated cells [Figure 1], there were significant differences between the cells scored in the test and radioprotective samples. The images below show observed biomarkers such as BN

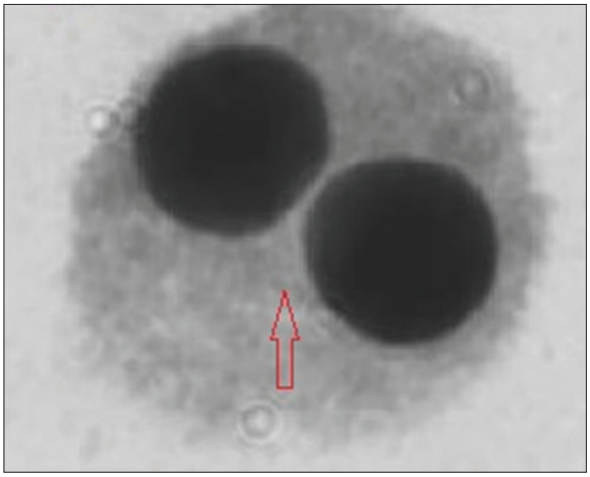

Figure 1: Binucleated cell scored in cytokinesis-block micronucleus assay

and multinucleated cells [Figure 2]. Percentage of DNA damage biomarkers observed in T1 was 13.33% of total cells scored. Percentage of a total number of biomarkers in T1 was mean ± standard deviation [SD]: 0.5 ± 1. Percentage of DNA damage biomarkers observed in T2 was 15.38% of total cells scored. Percentage of a total number of biomarkers in T2 was mean ± SD: 0.5 ± 1. Percentage of DNA damage biomarkers observed in RP1 was 72.22% of total cells scored. Percentage of a total number of biomarkers in RP1 was mean±SD: 3.25 ± 3.95. Percentage of DNA damage biomarkers observed in RP2 was 63.64% of total cells scored. Percentage of a total number of biomarkers in RP2 was mean ± SD: 1.75 ± 2.06. Percentage of DNA damage biomarkers observed in TT1 was 33.33% of total cells scored. Percentage of total number of biomarkers in TT1 was mean ± SD: 1.25 ± 1.8). Percentage of DNA damage biomarkers observed in TT2 was 14.29% of total cells scored. Percentage of total number of biomarkers in TT2 was mean ± SD: 0.25 ± 0.50. Percentage of DNA damage biomarkers observed in RPRP1 was 10.34% total cells scored. Percentage of a total number of biomarkers in RPRP1 was (mean ± SD: 0.75 ± 0.96). Percentage of DNA damage biomarkers observed in RPRP2 was 45.83% total cells scored. Percentage of a total number of biomarkers in RPRP2 was (mean ± SD: 2.75 ± 3.20) graphically represented in Figure 3. Two-factor ANOVA was performed and $F = 4.089605735$ and $F_C = 3.07246699$ were obtained indicating significant differences between the samples. $F = 1.505376$ and $F_C = 2.4875777$ within the samples show the significant variation in the action of curcumin within the samples.

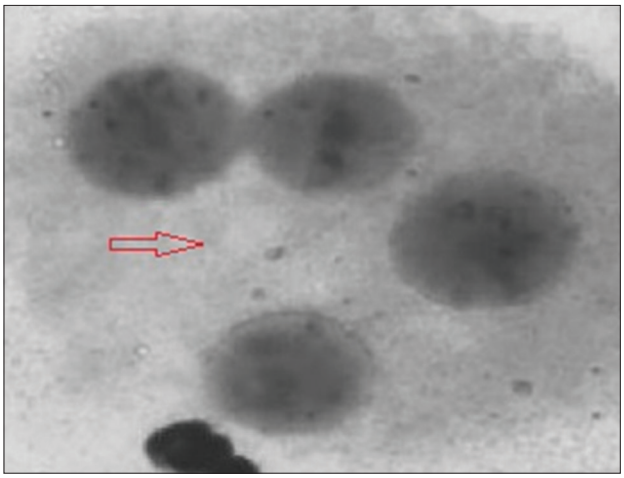

Figure 2: Multinucleated cells scored in cytokinesis-block micronucleus assay

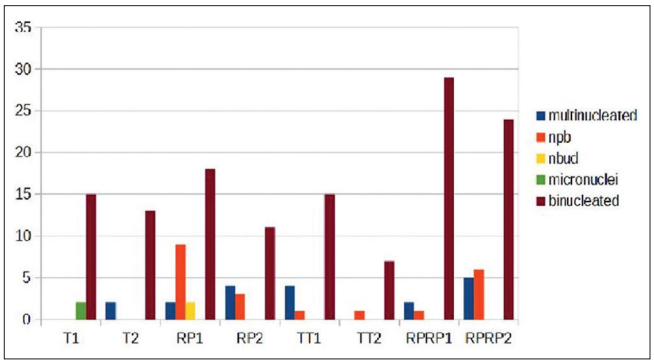

Figure 3: Aberrations observed sample wise

DISCUSSION

This study aimed to study the protective effect of curcumin against cisplatin-induced genotoxicity in human leukocytes. Similar work has been done by Mendonça *et al*. in PC12 cells lines to assess the possible genotoxic/antigenotoxic effects of curcumin in PC12 cells exposed to a range of different concentrations of cisplatin. Using MTT and MN assay, they showed that curcumin by itself in high quantities showed a great decrease in cell viability and increase in genotoxicity. However, when used in conjunction with cisplatin, the protective effect of curcumin was dose dependent. In higher doses, the genotoxicity of curcumin (5 µg/ml) + cisplatin (0.1 µl) was actually higher[8]. Another study by Notarbartolo *et al*. to check the potential capability of curcumin both alone as well as in combination with cisplatin to inhibit DNA synthesis in hepatic cancer cells was determined using a colorimetric immunoassay which suggested that curcumin may increase the antitumor activity of cisplatin by inducing apoptosis[9]. Studies performed by Sharma *et al*. suggest that the antitumor activity of curcumin is due to its strong antioxidant properties that inhibit several cell-signaling pathways[10]. Caspase studies by Khar *et al*. have shown that curcumin's antitumor activity takes place by causing apoptosis by inducing caspase-3 pathway[4]. Therefore, the role of curcumin is highly variable depending on the dosage, the type of cells studied, and the scientific procedures used. As concluded by Al-Moundhri *et al*., in their study, of the effect of curcumin against cisplatin-induced neurotoxicity in rats, large experiments with a wide dose range of both cisplatin and curcumin in different types of cells are required to arrive at the conditions, in which curcumin will provide a protective effect against cisplatin-induced genotoxicity[11].

CONCLUSION

Protective effect of curcumin with cisplatin is highly dose sensitive and requires further experiments with large dose range of both curcumin and cisplatin to arrive at the same by trial and error method.

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