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Assessment of cell viability, lipid peroxidation and quantification of DNA fragmentation after the treatment of anticancerous drug mitomycin C and curcumin in cultured human blood lymphocytes

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Abstract

Mitomycin C (MMC) is an antineoplastic agent used to fight a number of different cancers including cancer of the stomach, colon, rectum, pancreas, breast, lung, uterus, cervix, bladder, head, neck, eye and oesophagus. It is a potent DNA cross-linker. The prolonged use of the drug may result in permanent bone marrow damage and other various types of secondary tumors in normal cells. The toxic effect of anticancerous drugs may be reduced if supplemented with natural antioxidants/plant products. With this view, the effect of 5, 10 and 15 μM of curcumin was studied against the genotoxic doses of MMC, i.e. 10 and 20 μM, in cultured human lymphocytes using cell viability, lipid peroxidation and DNA damage quantification as parameters. The treatment of curcumin with MMC results in a significant dose-dependent increase in cell viability and decrease in lipid peroxidation and DNA damage suggesting a protective role of curcumin against the anticancerous drug mitomycin C.

Keywords: Mitomycin C; Curcumin; Cell viability; Lipid peroxidation; DNA damage

Introduction

Mitomycin C is an antitumor, antibiotic compound that has a range of genotoxic effects including the inhibition of DNA synthesis, mutagenesis and clastogenesis (Tomasz, 1995). It is a direct acting clastogen requiring only intracellular reductive activation to initiate its potent DNA cross-linking action (Gresolia, 2002). Implications of genotoxic effects of compounds include the initiation of carcinogenicity, the generation of hereditary defects via germ cell mutations and the teratogenicity (Mitchelmore and Chipman, 1985). Genotoxicity can result in a general decline in physiological health referred to as the "genotoxic disease syndrome" (Kurelee, 1993). Increase in the genotoxicity is associated with an increased overall risk of cancer (Hagmar et al., 1998), and any decrease in the genotoxicity is an indication of the decrease in the overall risk of cancer (Albertini et al., 2000). It has been well-established that secondary cancers are complication of traditional treatments with chemotherapy (Meadows et al., 1985). For these reasons, utilization of anticancerous nutrients could play a vital role in protecting those exposed to chemotherapeutic agents (Siddique and Afzal, 2009). Curcumin (diferuloylmethane), the main yellow bioactive component of turmeric is shown to have a

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wide spectrum of biological actions useful for humankind (Chattopadhyay et al., 2004). In the present study, the effect of curcumin was studied on the cell viability, lipid peroxidation and DNA damage induced by mitomycin C in cultured human blood lymphocytes.

Materials and methods

Chemicals

Mitomycin C (CAS: 50-07-07, Sigma); RPMI 1640, fetal calf serum, phytohaemagglutinin-M, antibiotic–antimycotic mixture (Gibco), Giemsa stain (Merck); curcumin (Sigma); 1-methyl-2-phenylindole (Sigma); acetonitrile (SRL); methanol (Qualigens, India); HCl (Qualigens, India), Tris (SRL, India); trypan blue (Loba, India); EDTA (SRL, India); Triton X-100 (SRL, India); trichloroacetic acid (SRL, India); diphenylamine (SRL, India).

Human lymphocyte culture

Duplicate peripheral blood cultures of two healthy male donors were treated according to Carballo et al. (1993). Briefly, heparinized, blood sample (0.5 ml) was obtained from a healthy donor and was placed in a sterile culture tube containing 7 ml of RPMI 1640 medium supplemented with 10% fetal calf serum, 10% antibiotic–antimycotic mixture and 1% phytohaemagglutinin of the final volume of cell culture. The cultures tubes were placed in an incubator at 37 °C for 24 h.

Cell viability assay by trypan blue exclusion

Cell viability assay was performed according to the method of Cook and Mitchell (1989). After 24 h, the treatments of mitomycin C at 10 and 20 μM were given separately. About, 10 μM of MMC was also treated with 5, 10 and 15 μM of curcumin separately. Similarly 20 μM of MMC was treated with 5, 10 and 15 μM of curcumin. After 48 h, the cells were centrifuged at 800 rpm for 10 min. The supernatant was removed and the cell suspension was used for the assay. The cell suspend from each of the treatments was made to about containing $\sim 5 \times 10^4$ cells. The equal amount of 0.2% trypan blue (in phosphate buffer saline) was mixed and allowed to incubate for 1–2 min at room temperature. The samples were loaded onto the hemacytometer (Neubauer type) and the total number of cells and the number of unstained cells in five major section of the hemacytometer were counted and the percent viability was calculated by the following formula

$$\%\ \text{Viability} = \frac{\text{Number of unstained cells}}{\text{Total number of cells}} \times 100$$

Lipid peroxidation assay

The present method of lipid peroxidation assay is based on the reaction of malonaldehyde with two molecules of 1-methyl-2-phenylindole at 45 °C at 586 nm (Gerard-Monnier et al., 1985). The estimation of lipid peroxidation by Suryawanshi et al. (2006) was based on the same reaction. After 24 h, the treatments of mitomycin C at final concentrations of 10 and 20 μM were given separately and kept for another 48 h at 37 °C in an incubator. Untreated were also run simultaneously. Simultaneously, 10 μM of MMC was treated with 5, 10 and 15 μM of curcumin and similar treatment was given with 20 μM of MMC.

Preparation of buffers

Reagent 1 (RI) 10 mM 1-methyl-2-phenylindole in acetonitrile was dissolved in 30 ml of acetonitrile and finally 10 ml of methanol was added to make 40 ml of volume.

Reagent 2 (RII) 37% HCl.

Procedure for treated cells

After incubation of 48 h, the treated blood cultures were centrifuged at 3000*g* for 20 min and the supernatant was collected. To a fresh tube, 1.3 ml of R1 was taken. About 1 ml of supernatant was diluted 10 times with Tris buffer (20 mM; pH 7.4) and about 200 μl of diluted supernatant was taken from each of the treated culture along with 200 μl of distilled water separately and vortexed. To the tube about 300 μl of R2 was added and vortexed. The tubes were incubated at 45 °C for 40 min. The tubes were cooled on ice and centrifuged at 15000*g* for 10 min at 4 °C. The readings were noted at 586 nm on digital photo colorimeter (Metzer).

Quantitative assay for DNA fragmentation

The quantification of DNA fragmentation was performed according to the protocol of Burton (1956). After 24 h of incubation, the treatments were given similarly as described earlier in the text. After incubation of 48 h, the cell suspension containing $1-10 \times 10^6$ cells in a 1 ml volume was prepared for each of the treated culture. About 0.8 ml of cell suspension was transferred to a microcentrifuge tube and 0.7 ml of ice cold lysis buffer (5 mM Tris Cl, pH 8.0/20 mM EDTA/0.5% (v/v Triton X-100) was added. The tubes were vortexed and allow to lysed for 30 min at 4 °C. The tubes were centrifuged for 15 min at 15000*g* (4 °C) and