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Comparative efficacy of the curcumin against H_2O_2 induced ROS in cervical cancer biopsies and hela cell line

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ABSTRACT

Background: Antioxidants have played an important role in maintaining physiological homeostasis. Recent literature emphasizes potential therapeutic effects of curcumin (antioxidants) found in natural products that displayed anti-inflammatory and antioxidant effects applicable in preventing oxidative stress induced injury, which characterizes their pathogenesis.
Materials and methods: HeLa cell line and cervical cancer biopsies (CCB) were treated with varying dose of curcumin to determine its effects. Thereafter hydrogen peroxide (0-10nM) - a ROS generating compound was co-cultured with varying doses of curcumin. The effect of these compounds on Superoxide dismutase and Glutathione peroxidase activity was assessed.
Results: The activity of SOD and GPx was found to be affected by the nearly similar magnitude in cervical cancer biopsies & hela cell line.
Conclusion: Comparative efficacies of natural antioxidants were found to be similar activity in cervical cancer biopsies (CCB) and heLa cell line.
Keywords: Curcumin, SOD, GPx, HeLa and Cervical Cancer.

INTRODUCTION

Globally, cervical cancer affects approximately 490 000 women each year resulting in 270 000 mortalities (Ferlay et al., 2004). Interestingly, in recent years, cervical cancer mortality rate has declined in developed countries due to health awareness, screening programmes and advances in therapy.

Oxidative stress has been implicated in playing a crucial role in aging and in the pathogeneses of a number of diseases, including cancer (Pasupathi et al., 2009; Weinberg et al., 2009). Oxidative stress occurs due to an imbalance in pro-oxidant and antioxidant levels (Gokul et al., 2010). Reactive oxygen species (ROS) are highly reactive and may modify and inactivate proteins, lipids, DNA, and RNA and induce cellular dysfunctions. To prevent free radical-induced cellular damage, the organism has developed a defense mechanism, the antioxidative system. In this study we only emphasize on superoxide dismutases (SOD) and glutathione peroxidise (GPx) activity.

The polyphenol Curcumin (diferuloylmethane; 1,7-bis(4-hydroxy-3-methoxy-phenyl)1,6-heptadiene-3,5-dione) is an orange-yellow compound with limited water solubility that is obtained from the turmeric plant, *Curcuma longa*. Curcumin has been shown to exhibit a variety of biological effects (Maheshwari et al., 2006), such as anti-oxidant (Samuhasaneeto et al., 2009), anti-inflammatory, anti-tumor and wound-healing properties (Srivastava et al., 1995; Jurenka, 2009) and anti-proliferative property. These activities are exerted through an equally wide variety of signaling pathways, which may involve either inhibition (Chen, et al. 1998; Gaedeke et al 2004; Zhou et al., 2007) or activation (Hu et al., 2005) of specific intracellular signaling pathways. These varied beneficial effects have led to investigation of curcumin as a potential therapeutic agent in a number of disease conditions (Aggarwal et al., 2007; Reddy et al., 2005; Thangapazham et al., 2006).

In the present study, we examined the activity of glutathione peroxidase and superoxide dismutase in control biopsies (CB), cervical cancer biopsies (CCB) and HeLa cell line by using curcumin. In other part of our study, we treated the cancer biopsies and HeLa cell line with hydrogen peroxide (H_2O_2), ROS producing compound modulating with the varying concentrations of curcumin.

As the active role-played by reactive oxygen species (ROS) in the cervical cancer is well established, thus an

attempt was made to probe natural compounds having antioxidant properties in arresting ROS in cancer cells. Various workers have extensively carried out such studies on cervical cancer HeLa cell line (Shan et al., 2014, Bhimani et al., 1993), but no work was done on cervical cancer biopsies. Thus, here we observed the protective role of curcumin on SOD and GPx in cervical cancer biopsies and compared it with the HeLa cell line.

MATERIALS AND METHODS

All chemicals were obtained from Sigma-Aldrich (Milan, Italy), Curcumin purchase from Sigma Chemical Company (St Louis, U.S.A.). Cell culture plates, Dulbecco's modified Eagle's media were from Himedia (India).

Cell culture

The human cervical cancer cell line HeLa was obtained from the National Centre for Cell Science, Pune, India. Cervical cancer biopsy was collected from the Gynaecology ward of Jawaharlal Nehru Medical College, Aligarh, India. The cells were maintained in Dulbecco's modified Eagle's medium (Sigma, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Hyclone), and antibiotic (Ampicillin) in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C.

Glutathione peroxidase assay

Control biopsies, cervical cancer biopsies and Hela cell line were treated with curcumin (50 µg/ml) and H_2O_2 (10 nM) for 24 hr to estimate the activity of SOD and glutathione peroxidase (GPx). The activity of GPx was measured as described elsewhere (Young JF et al., 2002, Yang CS et al 2002, Zhang G et al., 2000). Cancerous cell line were co-cultured for 24 hours with or without curcumin. Thereafter, cells were gently scraped with lysis buffer containing protease inhibitors (50 mM Tris/HCl, pH 7.4; 1 mM EDTA; 500 mM PMSF). The cell suspension was homogenized and centrifuged at 10,000 rpm for 10 minutes at 4°C. Protein concentration of supernatant was determined by the method of Bradford with BSA as the standard, and were subjected to GPx activity determination. The reaction mixture (1.0 ml) containing 50 mM potassium phosphate (pH 7.0), 1 mM sodium azide, 2 mM GSH, 0.2 mM NADPH, 1 unit/ml glutathione reductase, and 20–100 µl of samples was incubated at 25°C for 5 min. The reaction was initiated by the addition of NADPH. The kinetic change was recorded spectrophotometrically at 340 nm (37°C) for 3 minutes. GPx activity was calculated, as µmol of NADPH oxidized/minute/mg protein (U/mg protein).

Superoxide Dismutase Assay

SOD was measured using superoxide dismutase assay kit provided by Oxis Research, USA SOD-525 method. The BIOXYTECH is based on the SOD-mediated increase in the rate of autoxidation of 5, 6, 6a,11b-tetrahydro-3, 9, 10-trihydroxybenzo [c] fluorene R1 in aqueous alkaline solution to yield a chromophore with maximum absorbance at 525 nm (Maheshwari et al., 2006).

Statistical analysis

Results are expressed as mean ± SD of five individual experiments. Standard deviation (SD) was calculated using Microsoft excel.

RESULTS

Effect of H_2O_2 and Curcumin on the SOD and GPx activity in control biopsies: Control biopsies cell culture (NB) treated with varying doses of curcumin (0, 10,20,30,40 and 50 µg/ml) for 24h has shown similar activity of SOD and GPx, so a concentration of 50 µg/ml was selected of study and we also used a reported dose of H_2O_2 (10 nM). Conversely, the control biopsies cells were treated with H_2O_2, the activity of SOD & GPx was decreased by 30.22% and 39.85% respectively. Thereafter we treated the H_2O_2 induced control biopsies with curcumin (50 µg/ml) for 24 h, shows that activity of SOD & GPx ameliorate by 22.99% and 29.97% respectively (Table 1).

Table 1. Effects of curcumin on H_2O_2 induced normal biopsies

S. No.	SOD	GPx
CB	86.04 ± 2.74	84.22 ± 2.64
CB + H_2O_2	56 ± 4.16 (- 30.22%)	50.65 ± 2.33 (-39.85 %)
CB + H_2O_2 + Curcumin	66 ± 3.60 (+ 22.99 %)	70.23 ± 2.69 (+29.97 %)

CB: Control biopsies; H_2O_2: Hydrogen peroxide; SOD: Superoxide dismutase; GPx: Glutathione peroxidase.

Values are expressed as Mean ± S.E.M. of 5 experiments, the value in parentheses show the percentage decrease and increased with respect to control. The value of significances ($p<0.05$)

Table 1. Competitive effect of hydrogen peroxide (H_2O_2) (10 nM) versus curcumin (50 µg/ml), on the SOD and GPx activities in control biopsies (CB) cell culture after 24 h.

Effect of H_2O_2 and curcumin on the SOD and GPx activity in cervical cancer biopsies

The activity of SOD & GPx in cervical cancer biopsies treated with H_2O_2 suppressed by 38.54 % and 57.04 % respectively. Thereafter we treated the H_2O_2 induced cancer biopsies with curcumin (50 µg/ml) shows that activity of SOD & GPx ameliorates significantly by 117 % and 264.2% respectively (Table 2).

Table 2. Effects of curcumin on H_2O_2 induced cervical cancer biopsies.

S. No.	SOD	GPx
CCB	39.104 ± 1.24	37.78 ± 1.72
CCB + H_2O_2	22.62 ± 0.892 (- 38.54 %)	16.36 ± 0.60 (- 57.04 %)
CCB + H_2O_2 + Curcumin	45.45 ± 1.286 (+ 117 %)	54.76 ± 1.81 (+264.2 %)

CCB- Cervical Cancer Biopsies, H_2O_2 – Hydrogen peroxide, SOD- Superoxide dismutase, GPx-Glutathione peroxidase

Values are expressed as Mean ± S.E.M of 5 experiments, the value in parentheses show the percentage decrease and increased with respect to control. The value of significances ($p<0.05$)

Table 2. Competitive Effect of Hydrogen peroxide (H_2O_2) (10 nM) versus curcumin (50 µg/ml) on the SOD and GPx activities in Cervical cancer biopsies (CCB) cell culture after 24 h.

Competitive effect of H_2O_2 and curcumin on the SOD and GPx activity in Hela cell line

The activity of SOD & GPx in Hela cell treated with H_2O_2 suppressed by 52.57 % and 58.19 % respectively. Thereafter we treated the H_2O_2 induced Hela cells with curcumin (50 µg/ml) shows that activity of SOD & GPx ameliorates significantly by 135.55 % and 221.53% respectively (Table 3).

Table 3. Effects of curcumin on H_2O_2 induced HeLa cell line.

S. No.	SOD	GPx
Hela	39.89 ± 1.38	40.61 ± 1.68
Hela + H_2O_2	19.08 ± 1.20 (- 52.57 %)	17.60 ± 2.78 (-58.19 %)
Hela + H_2O_2 + Curcumin	45.91 ± 1.85 (+ 135.55 %)	52.04 ± 6.73 (+ 221.53 %)

H_2O_2 – Hydrogen peroxide, SOD- Superoxide dismutase, GPx-Glutathione peroxidase

Values are expressed as Mean ± S.E.M of 5 experiments, the value in parentheses show the percentage decrease and increased with respect to control. The value of significances ($p<0.05$)

Table 3. Competitive Effect of Hydrogen peroxide (H_2O_2) (10 nM) versus curcumin (50 µg/ml) on the SOD and GPx activities in HeLa cell line after 24 h.

DISCUSSION

Many recent studies indicate that Curcumin exert inhibitory effect on the activity of several enzymatic and metabolic pathways of relevance to the development and progression of cancer (Mohandas et al., 1984). Curcumin, by virtue of its ability to induce apoptosis selectively in cancer cells and not in normal cells, is potentially an important cancer chemopreventive agent. Thus, in the present study, Curcumin a natural antioxidants were employed to explore the potential chemopreventive mechanism in cervical cancer. These polyphenol compounds have antioxidative and anti-mutagenic properties. A variety of antioxidants and chemopreventive agents are cytotoxic to cancer cells. Recently, anti-proliferative and anti-cancer action of Curcumin has been reported in cancer cell line (Tharakan et al., 2010; Watson et al., 2010; Shi et al., 2009). A vast variety of naturally occurring substances are known to protect against experimental carcinogenesis. It is becoming increasingly evident that certain phytochemicals, particularly those included in our daily diet, may have important cancer chemopreventive properties (Sanaha et al., 1997). Some anti-inflammatory chemopreventive agents have been found to suppress growth and proliferation of transformed or malignant cells through induction of programmed cell death or apoptosis (Bellosillo et al., 1998). Various researchers have extensively carried out studies on cervical cancer HeLa cell line in multiple directions (Shan et al., 2014; Bhimani et al., 1993) but no work has been done on cervical cancer biopsies. Thus, the present study involves investigations on the effect of curcumin on two cervical cancer model systems, biopsies from cervical cancer patients and cervical cancer HeLa cell line. The active role-played by reactive oxygen species (ROS) in the cervical cancer is well established, thus an attempt was made in the present study to probe natural compounds having antioxidant properties in arresting ROS in cancer cells.

Superoxide dismutase and glutathione directly reacts with ROS, and GPx catalyzes the removal of hydrogen peroxide (Hu et al., 2005). Decreased activity of SOD and GPx indicate the impairment of hydrogen peroxide-neutralizing mechanisms (Aggarwal et al., 2007). Co-culturing of cancerous (cervical) cells with curcumin seems to induce apoptosis or acted as anti-inflammatory. Furthermore, apart from the above, a decline in GPx activity was observed in cervical cancer biopsies and cervical HeLa cells that were untreated or treated with H_2O_2 thereby correlating with earlier reports that substantial amounts of ROS are being generated in cancerous cells due to cellular activation (Reddy et al., 2005). Surprisingly, amelioration in SOD and GPx activity were observed when curcumin was co-cultured, indicating curcumin to be an effective natural antioxidant combating ROS, generated as a consequence of cellular activation in cancerous cell. When compared to control biopsies, cervical cancer Biopsies as well as HeLa cell line exhibited an appreciable H_2O_2-mediated suppression in the SOD and GPx activity. Interestingly, curcumin was found to possess a higher potential to ameliorate the SOD and GPx activity in cervical cancer biopsies than in HeLa cell line.

CONCLUSION

The present study, the currently available modern medicines for treating cancers is very expensive, toxic, and less effective in treating the disease (Thangapazham et al., 2006), thus it is essential to investigate further in detail the agents derived from natural sources for the prevention and treatment of cancer. Voluminous clinical trials are needed to validate the usefulness of these agents either alone or in combination with existing therapy.

CONFLICT OF INTEREST

None declared.

REFERENCES

Aggarwal BB, Sundaram C, Malani N and Ichikawa H: Curcumin: The Indian solid gold. Advances in Experimental Medicine and Biology. 2007; 595: 1–75.

Bellosillo B, Piqué M, Barragán M, Castaño E, Villamor N, Colomer D, Montserrat E, Pons G and Gil J: Aspirin and salicylate induce apoptosis and activation of caspases in B-cell chronic lymphocytic leukemia cells. Blood. 1998; 92: 1406-1414.

Bhimani RS, Troll W, Grunberger D and Frenkel K: Inhibition of oxidative stress in HeLa cells by chemopreventive agents. Cancer Research. 1993; 53(19): 4528-33.

Chen YR and Tan TH: Inhibition of the c-Jun N-terminal kinase (JNK) signaling pathway by curcumin. Oncogene. 1998; 17:173–178.

Ferlay J, Bray F, Pisani P and Parkin DM: GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide IARC Cancer, 2004 Base No 5, version 2.0. Lyon, France: IARC Press.

Gaedeke J, Noble NA and Border WA: Curcumin blocks multiple sites of the TGF-beta signaling cascade in renal cells. Kidney International. 2004; 66: 112-20.

Gokul S, Patil V, Jailkhani R, Hallikeri K and Kattappagari K: Oxidant-antioxidant status in blood and tumor tissue of oral squamous cell carcinoma patients. Oral Diseases. 2010;16 (1):29-33.

Hu M, Du Q, Vancurova I, Lin X, Miller EJ, Simms HH and Wang P: Proapoptotic effect of curcumin on human neutrophils: activation of the p38 mitogen-activated protein kinase pathway. Critical Care Medicine. 2005; 33:2571–2578.

Jurenka JS: Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: a review of preclinical and clinical research. Alternative Medicine Review. 2009; 14(2):141-153.

Maheshwari RK, Singh AK, Gaddipati J and Srimal RC: Multiple biological activities of curcumin: a short review. Life Sciences. 2006; 78: 2081-7.

Mohandas J, Marshall JJ, Duggin GG, Horvath JS and Tiller DJ: Low activities of glutathione-related enzymes as factors in the genesis of urinary bladder cancer. Cancer Research. 1984; 44: 5086–5091.

Pasupathi P, Saravanan G, Chinnaswamy P and Bakthavathsalam G: Effect of chronic smoking on lipid peroxidation and antioxidant status in gastric carcinoma patients. Indian Journal of Gastroenterology. 2009; 28:65-7.

Reddy RC, Vatsala PG, Keshamouni VG, Padmanaban G and Rangarajan PN: Curcumin for malaria therapy. Biochemical and Biophysical Research Communications. 2005; 326:472–474.

Sanaha HS, Kelloff GI, Steel V, Rao CV and Reddy BS: Modulation of apoptosis by sulindac, curcumin, phenylethyl-3-methylcaffeate, and 6-phenylhexyl isothiocyanate: apoptotic index as a biomarker in colon cancer chemoprevention and promotion. Cancer Research. 1997; 57: 1301-1305.

Shan X, Li Y, Meng X, Wang P, Jiang P and Feng Q: Curcumin and (-)-epigallocatechin-3-gallate attenuate acrylamide-induced proliferation in HepG2 cells. Food and Chemical Toxicology. 2014; 66:194-202.

Shi Q, Shih CC and Lee KH: Novel Anti-Prostate Cancer Curcumin Analogues that Enhance Androgen Receptor Degradation Activity. Anti-Cancer Agents in Medicinal Chemistry. 2009; 9(8):904-12.

Srivastava KC, Bordia A and Verma SK: Curcumin, a major component of food spice turmeric (Curcuma longa) inhibits aggregation and alters eicosanoid metabolism in human blood platelets. Prostaglandins, Leukotrienes and Essential Fatty Acids. 1995;.52:223-7.

Suchittra Samuhasaneeto, Duangporn Thong-Ngam, Onanong Kulaputana, Doungsamon Suyasunanont, and Naruemon Klaikeaw: Curcumin Decreased Oxidative Stress, Inhibited NF-κB Activation, and Improved Liver Pathology in Ethanol-Induced Liver Injury in Rats. Journal of Biomedicine and Biotechnology. 2009; 2009:981963.

Thangapazham RL, Sharma A and Maheshwari RK: Multiple molecular targets in cancer chemoprevention by curcumin. AAPS Journal. 2006; 8: 443–449.

Tharakan ST, Inamoto T, Sung B, Aggarwal BB and Kamat AM: Curcumin Potentiates the Antitumor Effects of Gemcitabine in an Orthotopic Model of Human Bladder Cancer through Suppression of Proliferative and Angiogenic Biomarkers. Biochemical Pharmacology. 2010; 79(2): 218-28.

Watson JL, Greenshields A, Hill R, Hilchie A, Lee PW, Giacomantonio CA and Hoskin DW: Curcumin-induced apoptosis in ovarian carcinoma cells is p53-independent and involves p38 mitogen-activated protein kinase activation and downregulation of Bcl-2 and survivin expression and Akt signaling. Molecular Carcinogenesis. 2010; 49(1): 13-24.

Weinberg F and Chandel NS. Reactive oxygen species-dependent signaling regulates cancer. journal Celluar and Molecular Life Sciences. 2009; 66 (23):3663-73.

Yang CS, Maliakal P and Meng X: Inhibition of carcinogenesis by tea. Annual Review of Pharmacology. 2002; 42: 25–54.

Young JF, Dragsted LO, Haraldstottir J et al: Green tea extract only affects markers of oxidative status postprandially, lasting antioxidant effect of flavonoid-free diet. British Jounal of Nutrition 2002; 87: 343-355.

Zhang G, Miura Y and Yagasaki K: Suppression of adhesion and invasion of hepatoma cells in culture by tea compounds through antioxidative activity. Cancer Letter. 2000; 159: 169-173.

Zhou Y, Zheng S, Lin J, Zhang QJ and Chen A: The interruption of the PDGF and EGF signaling pathways by curcumin stimulates gene expression of PPAR γ in rat activated hepatic stellate cell in vitro. Laboratory Investigation. 2007; 87:488–498.