

**BIOCHEMICAL PROPERTIES ON CHEMOSENSITATION OF
FERULIC ACID AND PACLITAXEL IN NON-SMALL CELLS LUNG
CARCINOMA CELLS *IN VITRO***

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Article Received on
29 August 2013,

Revised on 18 Sept. 2013,
Accepted on 16 October 2013

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ABSTRACT

Ferulic acid (FA) is a phenolic phytonutrient, which possesses strong anticancer effect. However, its prominent application in cancer is limited due to poor bioavailability at the tumor site. Paclitaxel (PTX) is a semi synthetic drug which is used for cancer treatment. The aim of the study was to investigate the multidrug resistance of FA and PTX. It was noticed that anticancer potential of FA + PTX was greater than that of FA and PTX treatment alone. Further, FA + PTX exhibits increased TBARS, Catalase and SOD, altered GSH and GPx in NCI-H460 cells when compared to bulk FA and PTX treatment alone. Our results indicate that FA + PTX demonstrated increased anticancer property in cancer cells than FA and PTX treatment alone.

Keywords: Ferulic acid (FA), Paclitaxel (PTX), Anticancer, Chemotherapy.

1. INTRODUCTION

Chemoresistance and subsequent tumor recurrence are often the outcome of such therapies. An example of this all too common event is the use of taxanes (paclitaxel and its semisynthetic analogue, docetaxel) in the treatment of a variety of cancers including ovarian, breast, prostate, and non-small cell lung cancers [1]. While surgery along with taxane- and platinumbased chemotherapy for advanced ovarian cancer has allowed up to 80% of women to achieve a clinical response [2], cancers in most patients initially diagnosed with late stage disease eventually recure. Lung cancer is one of the most lethal cancers and causes second

most common cancer in both men and women [3]. Lung cancer is one of the most lethal cancers and causes second most common cancer in both men and women [4]. Non-small cell lung cancer (NSCLC), which constitutes 75–80% of all lung cancers, is one of the most frequent tumors in the world [5].

The long-term survival rate of lung cancer patients treated by conventional modalities such as surgery, radiation, and chemotherapy remains far from satisfactory [6]. Lung cancer cells are only the modestly responsive or even non-responsive to the cytotoxic effects of chemotherapeutic agents [7]. Paclitaxel (PTX) is one of the most active anticancer drugs used in chemotherapy [8]. PTX has shown significant activity against a variety of solid tumors, including lung cancer [9–12]. It disrupts the dynamic equilibrium within the microtubule system and arrests cells in the late G2 phase and M phase of the cell cycle, thereby inhibiting cell replication [13]. However, the success of its clinical application is limited by its low therapeutic index, low solubility in water [14]. PTX also showed neurotoxicity, nephrotoxicity, and effects on endothelium and vascular muscles, leading to vasodilatation, labored breathing, lethargy, and hypotension [15]. Ferulic acid (FA), a polyphenol, is an antioxidant nutrient commonly found in fruits and vegetables such as tomatoes, sweet corn, and rice bran [16]. Ferulic acid accounts for 90% of the total phenolic acids in common flour [17]. It exhibits beneficial effects against various diseases like cancer, diabetes, cardiovascular and neurodegenerative disorders [18].

Recent evidence suggests that polyphenols-like curcumin is a highly pleotropic molecule that interacts physically with its diverse range of molecular targets including transcription factors, growth factors and their receptors, cytokines, enzymes, and genes regulating cell proliferation and apoptosis [19,20]. FA possesses antioxidant, anti-inflammatory, anticarcinogenic, and antimicrobial properties, and suppresses proliferation of a wide variety of tumor cells [21].

2. MATERIALS AND METHODS

2.1. Chemicals

Ferulic acid (FA), paclitaxel (PTX), thiobarbituric acid (TBA), phenazinemethosulphate (PMS), nitrobluetetrazolium (NBT), 5, 5-dithiobis 2-nitrobenzoic acid (DTNB), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), heat inactivated fetal calf serum (FCS), RPMI-1640 medium, glutamine–penicillin–streptomycin solution, ficollhistopaque 1077, trypsin-EDTA were purchased from Sigma Chemicals Co., St. Louis, USA.

2.2. Cell lines and culture conditions

The present work was carried out in non-small cell lung carcinoma cell line (NCI-H460). Cell line was obtained from National Centre for Cell Science (NCCS), Pune, India. The cells were grown as monolayer in RPMI-1640 medium supplemented with 10% FCS, 1mMsodiumpyruvate, 10mMHEPES, 1.5 g/L sodiumbicarbonate, 2mM l-glutamine, and 100U/ml penicillin-streptomycin at 37 °C in 5% CO₂ incubator.

2.3. Drug treatment and dose fixation study

Cells were treated with different concentration of FA, PTX and FA+ PTX (10, 20, 40, 60, 80 and 100µM) and incubated for 24 h at 5% CO₂ incubator. Cytotoxicity was observed by MTT assay by the method of Mosmann, 1983 [22]. IC₅₀ values were calculated and the optimum dose was used for further study.

2.4. Experimental groups

TheNCI-H460 cellswere divided into four experimental groups: group 1: untreated control cells, group 2: FA treatment, group 3: PTX treatment and group 4: FA+ PTX treatment.

2.5. Biochemical estimations

2.5.1. Determination of thiobarbituric acid reactive substance (TBARS)

After treatmentwith FA and FA-PLGA, NCI-H460 cellswere harvested by trypsinization. The pellet obtainedwas suspended in PBS and sonicated. The supernatant was taken for the measurement of TBARS, according to the procedures described elsewhere [23].

2.5.2. Determination of reduced glutathione levels (GSH)

The total reduced glutathione (GSH) content was measured by the method of Ellman, 1959 [24]. This method was based on the development of yellow colour when 5, 5-dithiobis 2-nitrobenzoic acid was added to compound containing sulphhydryl groups.

2.5.3. Estimation of glutathione peroxidase (GPx)

Glutathione peroxidase activity was assayed by the method of Mohandas et al., 1984 [25].Disappearance of NADPH at 340 nm was recorded at 25 °C. Enzyme activity was calculated as nmol NADPH oxidized/min/mg protein using a molar extinction coefficient of $97.622 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

2.5.4. Catalase activity

Catalase activity was measured by the method of Clairborne 1985 [26]. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated as nmol H₂O₂ consumed min⁻¹ mg⁻¹ protein.

2.5.5. Superoxide dismutase activity (SOD)

Superoxide dismutase activity (SOD) activity was estimated by the method of Kakar et al. [27]. Amount of chromogen formed was measured by recording color intensity at 560 nm. Results are expressed in units/mg protein.

2.6. Statistical analysis

Statistical analysis was performed by one-way ANOVA followed by DMRT taking $P < 0.05$ to test the significant difference between groups.

3. RESULTS AND DISCUSSION

Chemoresistance is a major obstacle in cancer treatment. Targeted therapies that enhance cancer cell sensitivity to chemotherapeutic agents have the potential to increase drug efficacy while reducing toxic effects on untargeted cells. [28]. Table 1 shows the percentage cytotoxicity of PTX and PTX-PLGA (10, 20, 40, 60, 80 and 100 μ M) in NCI-H460 cells. Inhibitory concentration 50 (IC₅₀) value for FA, PTX and FA + PTX was found to be 80 μ M, 60 μ M and 40 μ M respectively, and it was used for further experiments. Furthermore, we evaluated the anticancer activity of FA, PTX and FA + PTX in NCI-H460 cell line. It was found that FA + PTX could greatly inhibit the NCI-H460 cell growth. The IC₅₀ of FA and PTX were significantly lower than that of the FA + PTX treatment. The reason for increased cytotoxicity observed in the FA + PTX group might be due to increased cellular uptake. Enhanced cytotoxicity during FA + PTX treatment indicates that the cells were achieving greater cytotoxicity. IC₅₀ values for FA + PTX in our study were 40 μ M, less than the 88.69 μ M value reported before for PTX [29]. The levels of TBARS were significantly increased in FA + PTX treated cells (Table 2). FA + PTX treated cells showed progressively increased levels of TBARS when compared to FA and PTX treated cells. The levels of GSH were found to be greatly decreased in FA + PTX treated cells when compared to FA and PTX treatment alone (Table 2). We observed significant increase in lipid peroxidation indices in FA+ PTX treated cancer cells.

Previous report suggests that similar phenolic acid has been reported to stimulate hydroxyl radical formation and reduce ferrylmyoglobin which suggests their potential prooxidant action. The effect of caffeic acid on the Fenton reaction has been documented using the ESR spin trapping technique [30]. The phenol ring-containing compounds are oxidized by peroxidase/ROS to phenoxyl radicals that co oxidize GSH to form a thyl radical (GS•), which then reacts with GSH to form a disulfide radical anion. The carboxylic acid group with the adjacent unsaturated C–C double bond can contribute to the stability of the radical via resonance or by providing additional attack sites for free radicals [31]. This might be the reason for decreased GSH levels in FA+ PTX treated NCI-H460 cells. Previous studies have shown that phytochemicals depleted intracellular antioxidants, hereby induced cancer cell death [32]. The GPx levels were significantly decreased in FA+ PTX treated cells when compared to FA and PTX treatment alone. The level of GPx in FA was decreased when compared to PTX was shown in table 2. Antioxidant defense enzyme glutathione peroxidase protect the cellular macromolecules against oxidative damage by detoxifying carcinogens either by destroying their reactive centers or by conjugating them with endogenous ligands facilitating their excretion [33]. Table 2 shows the CAT activity observed in the treated cells were considerably increased in FA, PTX and FA+ PTX treated cells when compared to normal cells. The activities of SOD in the treated cells were significantly increased in FA, PTX and FA+ PTX treatment when compared with control was shown in Table 5. In this study, the level of CAT and SOD were significantly higher in treatment groups than in the control group. This may show the presence of increased oxidative stress. It is likely that sustained oxidative stress may increase SOD and CAT activity. Decreased antioxidant defense probably exist later in patients under chronic treatment with neuroleptics [34,35]. The identified increase in SOD and CAT activity could be an adaptive response of these enzymes to increased production of oxygen following oxidative decomposition of catecholamines. The changes in activities of antioxidant enzymes might offer some important clues to explain pathologic mechanism of abnormal free radical metabolism. The molecular mechanisms of oxyradical mediated cellular pathogenesis are well understood. OH• and HO₂ radicals are involved in cell damage by their actions on phospholipids, proteins and nucleic acids. The reaction products of these molecules have been used as indices of oxyradical injury. Increased levels of thiobarbituric acid reaction products have been found in the cerebrospinal fluid of neuroleptic treated patients [36,37] and also in plasma of schizophrenic patients with or without tardive dyskinesia [38].

Table 1. shows the optimum dose fixation study by MTT assay. Inhibitory concentration 50 (IC₅₀) value for ferulic acid (FA), paclitaxel (PTX) and FA + PTX was found to be 40 μ M, 60 μ M and 80 μ M, respectively. Values are given as mean \pm S.D. of six experiments in each group.

Concentration (μ M)	FA	PTX	FA-PTX
10	16	19	35
20	28	31	46
30	38	45	57
60	47	58	68
80	57	68	77
100	69	79	89

Table 2. shows the levels of thiobarbituric acid reactive substance (TBARS), reduced GSH, glutathione peroxidase (GPx), . Catalase activity and Superoxide dismutase activity (SOD) in NCI-H460 cells. Values are given as mean \pm S.D.of six experiments in each group.

Treatments (μ M)	TBARS	GSH	GPx	Catalase	SOD
Control	0.7 \pm 0.02	7.9 \pm 1.4	8.2 \pm 1.5	0.2 \pm 0.01	1.8 \pm 0.04
FA (80 μ M)	1.5 \pm 0.04	6.8 \pm 1.2	6.8 \pm 1.2	0.7 \pm 0.02	2.6 \pm 0.06
PTX (60 μ M)	2.1 \pm 0.05	5.7 \pm 1.13	5.9 \pm 1.13	1.2 \pm 0.03	3.8 \pm 0.065
FA-PTX (40 M)	2.9 \pm 0.06	4.4 \pm 1.0	4.7 \pm 1.1	1.7 \pm 0.036	5.2 \pm 0.07

4. CONCLUSION

Concluding our results, we found differences in the viability of the treated cells depending on the test agent and the cytotoxicity assay used. For this reason, it is important to consider what effect is expected, respective of what cell death mechanism is predicted. Assays like TBARS, GSH, GPx, CAT and SOD were done and concluded that FA + PTX had more activity than FA and PTX. These conclude that chemosensitisation process is very good for the treatment of cancer.

DISCLOSURE OF INTEREST

The authors declare that they have no conflicts of interest concerning this article.

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