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Original article

Biochemical estimations of multidrug resistance (ferulic acid and paclitaxel) in non-small cells lung carcinoma cells *in vitro*

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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.

1. Introduction

Multidrug resistance, the principal mechanism by which many cancers develop resistance to chemotherapy drugs, is a major factor in the failure of many forms of chemotherapy. It affects patients with a variety of blood cancers and solid tumors, including breast, ovarian, lung, and lower gastrointestinal tract cancers [1]. Tumors usually consist of mixed populations of malignant cells, some of which are drug-sensitive while others are drug-resistant. Chemotherapy kills drug-sensitive cells, but leaves behind a higher proportion of drug-resistant cells [2]. As the tumor begins to grow again, chemotherapy may fail because the remaining tumor cells are now resistant [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-

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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, 1 mM sodium pyruvate, 10 mM HEPES, 1.5 g/L sodium bicarbonate, 2 mM l-glutamine, and 100 U/ml penicillin-streptomycin at 37 °C in 5% CO_2 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 100 μM) and incubated for 24 h at 5% CO_2 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

The NCI-H460 cells were 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 treatment with FA and FA-PLGA, NCI-H460 cells were harvested by trypsinization. The pellet obtained was 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 sulphydryl groups.

2.5.3. Estimation of reduced glutathione levels 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 $6.22 \times 103\ M^{-1}\ cm^{-1}$.

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 H_2O_2 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.

Table 1
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 80 μM, 60 μM and 40 μM, respectively. Values are given as mean + S.D. of six experiments in each group.

Concentration (μM)	Percentage toxicity		
	FA	PTX	FA + PTX
10	16	18	34
20	27	32	45
40	38	44	**56**
60	49	**54**	68
80	**58**	65	79
100	72	78	88

Bold specifies the IC50 values of the drugs treated.

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

Multidrug resistance is a condition enabling a disease-causing organism to resist distinct drugs or chemicals of a wide variety of structure and function targeted at eradicating the organism. Organisms that display multidrug resistance can be pathologic cells, including bacterial and tumor cells [28]. Table 1 shows the percentage cytotoxicity of PTX and PTX-PLGA (10, 20, 40, 60, 80 and 100 μM) in NCI-H460 cells. Inhibitory concentration 50 (IC50) value for FA, PTX and FA + PTX was found to be 80 μM, 60 μM and 40 μM 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 μM, less than the 88.69 μM value reported before for PTX [29].

The levels of TBARS were significantly increased in FA + PTX treated cells (Fig. 1). 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

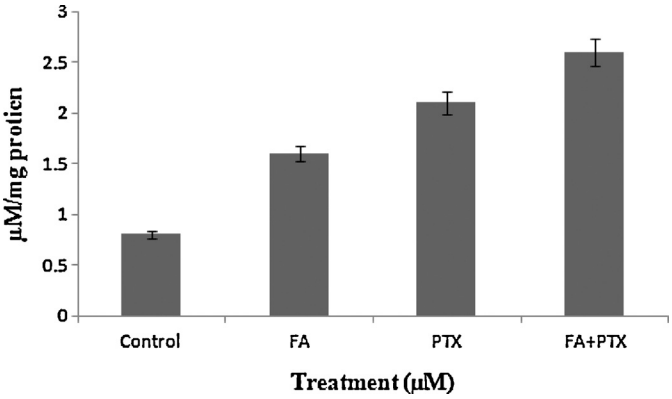

Fig. 1. Progressively increased levels of thiobarbituric acid reactive substance (TBARS) in ferulic acid (FA) + paclitaxel (PTX) treated cells when compared to FA and PTX treated cells. Values are given as mean ± S.D. of six experiments in each group.