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Combinational effect of Paclitaxel and Clotrimazole on human breast cancer: Proof for synergistic interaction

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ABSTRACT

Study was aimed to determine the anti-cancerous co-effect of paclitaxel (PAX) with clotrimazole (CLZ) on breast cancer cells and to explore the mechanism involved. Cell viability was evaluated through MTT assay followed by CompuSyn simulations to evaluate, whether the effect of PAX and CLZ in combination was additive, synergistic or antagonistic. Nuclear morphology was examined through DAPI/PI staining and AO/EtBr staining. Level of H_2O_2 and NO were evaluated to detect reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation as an effect of drug treatment. Comet assay was performed to determine genotoxicity followed by the analysis of cellular glucose uptake. Cell viability assay and CompuSyn simulations confirms synergistic effect of combination at low doses of PAX-12.5 nM and CLZ–25 μM (PACL) against MCF-7 and MDA-MB-231 cells, with minimal effect on normal HEK-293 cells. We observed significant nuclear damage with PACL treated cells through DAPI/PI and AO/EtBr staining, further confirmed by comet assay, where significant DNA damage was observed in MCF-7 (50%) and MDA-MB-231 (73%). NO level increased by 2.6 fold (MCF-7) and 2.4 fold (MDA-MB-231) and H_2O_2 level also surged by 4.5 fold for both the cell line as an aftermath of PACL treatment. Interestingly, PACL exhibited glucose uptake upto 15% and 20% in MCF-7 and MDA-MB-231 cells respectively. These findings suggest that, PACL acts synergistically against breast cancer cells and its potential can be attributed to increased oxidative stress, reduced glucose uptake and enhanced genotoxicity.

1. Introduction

Breast cancer is the second most lethal cancer in women, causing morbidity or mortality worldwide. Among the available cancer treatments, radiation therapy, hormonal therapies, surgery and chemo or poly-chemotherapeutic regimens are most successful with higher survival rate of cancer patients [1,2]. Most frequently prescribed chemotherapeutic agent for the treatment and management of breast carcinomas is Paclitaxel (PAX), also acknowledged as Taxol [3,4]. PAX acts through unique mechanism of enhancing polymerization of tubulin, which further hyperstabilizes the microtubules, leading to obstruction in cell propagation and induces programmed cell death [5,6]. Although, PAX is effective alone, but, it is frequently used with other drugs (mainly anthracycline-based chemotherapy regimens) in combination to reduce its side effects and enhance its cytotoxicity against breast cancer [7,8]. These combinations of PAX come at a cost of increased risk of nonspecific cytotoxicity such as, doxorubicin leads to cardiotoxicity [9]; docetaxel causes bone marrow suppression and peripheral neurotoxicity [10]; cyclophosphamide induces neutropenia [11] etc. Likewise, other chemotherapeutic agents (cisplatin, 5-fluorouracil, mitoxantrone, etc.) are also known to induce non-specific toxicity. But obvious solution to this prevailing problem seems to conjoin PAX with some other potent and nontoxic moieties.

Clotrimazole (CLZ) is a clinically used antifungal drug, possessing profound anti-proliferative effect on tumorigenic and metastatic cells while having minimal effect on non-tumoral cells [12,13]. Mechanism through which CLZ act is by inhibition of major glycolytic regulatory enzymes viz. 6-phosphofructo-1-kinase (PKF) and hexokinase (HK), which are involved in cancer biology, by altering glucose metabolism and energy production [13,14]. More importantly, CLZ is known to induce cell cycle arrest by inhibiting cellular growth in G1- and M-phases of cell cycle [15].

Cancer cells demonstrate the most common physiological hallmark of increased glucose utilization and aberrant glucose metabolism during proliferation phase. Increased fermentative glycolytic flux is the distinguishing characteristic possessed by cancer cell, even with the high oxygen supply, a phenomenon known as “Warburg effect” [16,17]. As a consequence of the

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Warburg effect, there is over activation of PFK, which confers several advantages for the growth and invasion of tumour cells [18,19]. Studies suggest that blocking glycolysis reduces tumour progression and furthermore enhances the efficacy of chemotherapy [12,20].

Individually, both PAX and CLZ have the ability to kill cancer cells at doses which are associated with several side effects. Moreover previous findings [12,13,20] also support the hypothesis that, the combinational cancer therapies designed to arrest the cell cycle and inhibit glucose metabolism may provide a useful biochemical rationale for the treatment and management of breast carcinoma. Keeping this in mind, current study was designed to investigate whether or not low dose combination of PAX and CLZ (combo) possess significant toxicity against breast cancer (MCF-7 and MDA-MB-231) and to explore the underlying mechanism involved.

2. Material and method

2.1. Materials

PAX was obtained as a gift sample from Samarth life science Pvt. Ltd; (H.P. India) and CLZ was procured from Optimum Pharmaceuticals Pvt. Ltd; (H.P. India). Unless specified all the reagents and chemicals were procured from Sigma Aldrich.

2.2. Cell lines and cell culture

The human breast cancer cell line (MCF-7 and MDA-MB-231) and normal epithelial cell line (HEK-293) were procured from NCCS Pune India. MCF-7 and HEK-293 cells were cultured in DMEM (Dulbecco's modified Eagle's medium; Invitrogen), supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS; Invitrogen) and 1% antibiotics (100 U/mL penicillin and 0.1 mg/mL streptomycin; Gibco) maintained at 37 °C with 5% CO_2. MDA-MB-231 cells were cultured in L-15 (Leibovitz's; Himedia) media supplemented with 10% FBS and 1% antibiotic, maintained at 37 °C without CO_2 atmosphere.

2.3. Cell proliferation assay and selection of drug combination dose

The cytotoxicity of PAX and CLZ against MCF-7 cells was evaluated by MTT exclusion assay. The cells were seeded in 96-well plates (1×10^4) and incubated at 37 °C till 70% confluency was achieved. Culture medium was replaced with 200 μl of fresh serum free media containing varying concentration of PAX (6.25–100 nM) and CLZ (6.25–100 μM) and cells were further incubated for 24 h. Eventually cells were incubated with MTT (20 μl) at 37 °C for 4 h. DMSO was added to each well to solubilise the formazan product and absorbance (A) was recorded at 570 nm test wavelength and 630 nm reference wavelength to test relative cell viability using a microplate reader (Bio-Rad). Triplicate wells were assayed for each

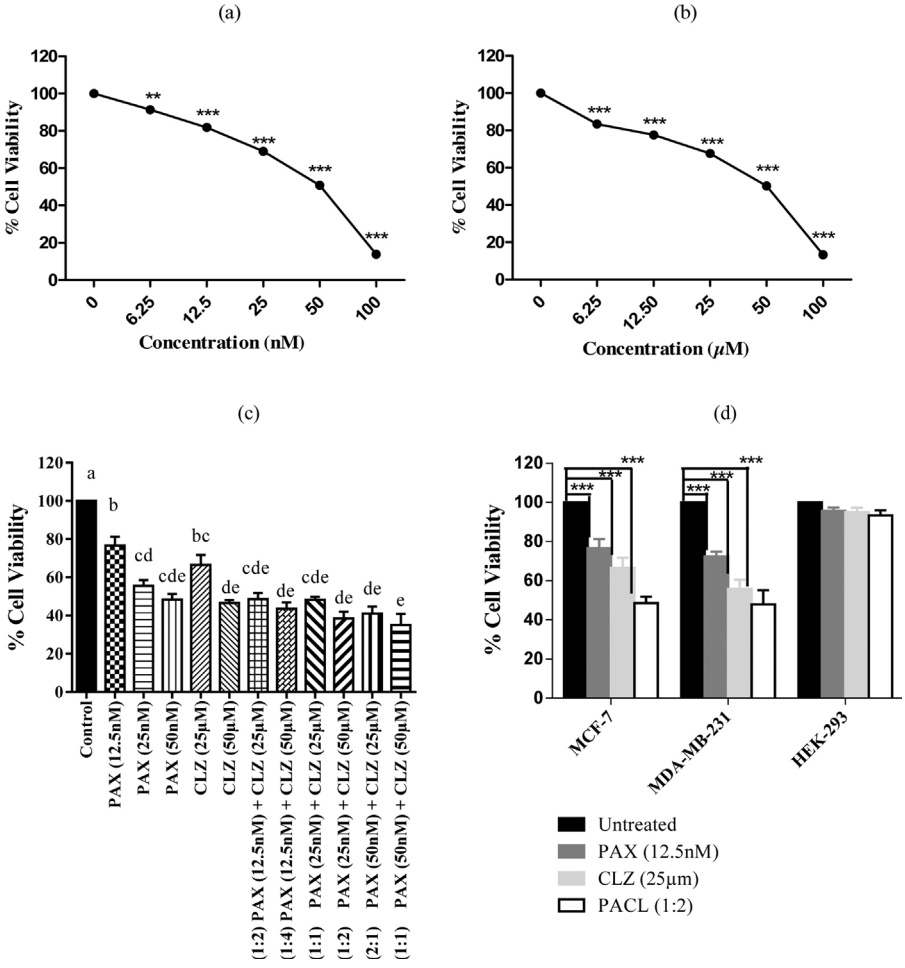

Fig. 1. Dose dependent effects of PAX and CLZ on viability of MCF-7 cells (a and b) in the form of percent cell viability relative to untreated control cells. Co-effect of selected PAX and CLZ doses in combination (c) and bars not sharing the same letters are significantly different with $p < 0.05$. Comparative analysis of selected combo on MCF-7, MDA-MB-231 and HEK-293 at 24 h (d) and *** indicates $p < 0.001$ when compared with untreated group. All data is presented as mean ± SEM of three independent experiments.