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The Synergistic Effect of Cisplatin and Interferon β on Human Lung Adenocarcinoma Cell Line (A549)

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Abstract

Cisplatin is a basic therapeutic agent of lung carcinoma which is consider as one of the most common anti-cancer drug. Recently, its efficacy is limited due to toxic side effects and chemoresistance over course of time. On the other hand,

To elucidate the synergistic pattern impact of co-exposure to Cisplatin and interferon β - induced apoptosis and compare that to single exposure to each drug at three time point 24,48 and 72 respectively. Apoptosis induced by mixture of Cisplatin and interferon in human lung adenocarcinoma cell line A549 was detected through cell viability using castle violet. Low six concentrations used to show that the apoptotic effect increased in a time dependent pattern as well as concentration dependent pattern. In conclusion, induction of apoptosis to cancer cell line may be an important mechanism and promising results of anti- tumor.

Keywords -Cisplatin, interferon β, Apoptosis, lung adenocarcinoma cell line A549

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum) is well identified as a metal-based anti-cancer common drug[1] which has been broadly expended for more than a couple of decades against different sorts of cancers[2] it was reported that Cisplatin can produce a significant toxicities, and developed chemo resistance through long term treatments, therefore its usage was limited[3]. It has been reported that using cisplatin to treat cancer cells for long time would cause cell line resistance mediated with cells death [4]. The chemotherapy treatment would be inhibited when p-Akt and the NF-κB/Bcl- pathways activated, which mostly resulted in resistance to treatments [5]. NF-κB pathway activation is detected as a main mechanism that is through cisplatin resistance. Activation of NF-κB is negatively associated with cellular susceptibility for chemotherapy[6]. Several studies demonstrated that NF-κB affect would be occur through induction of Matrix Metalloproteinases (MMPs) and this in turn will encourages the migration and metastasis in some sort of cancer such as hepatocellular carcinoma cells[7] breast cancer cells[8] and cervical cancer cells[9]. Therefore, of NF-κB suppression is effective in the inhibition and healing of cancer[10]. It has been described that chemotherapy resistance is intermediated by several genes which controlled through nf-κB. Consequently, reduce the transcription factor which rises the sensitivity of cancer cells to the necrosis through the exploit of chemotherapeutic drugs [11]. For these reasons, it was suggested that agents that impeding the activation process of NF-κB activity may show the therapeutic for the suppression of carcinogenesis and cancer metastasis[12].

Interferon (INF) is an anti-tumor regulatory cytokine. It is divided into two groups included type IFN one and type IFN two [13]. While type IFN I class continent of :IFN-alpha (IFN-α); IFN-beta (IFN-β), and IFN-omega (IFN-ω), type IFN II involves IFN-gamma (IFN-γ)[14]. IFN-β expressions, in human cells, is robustly prevent cancer cell growth and prompt the apoptosis by Janus kinase1 Jak-Stat1 a signaling pathways[15]. The action of type IFN I family are facilitated through a cell surface receptor which is known as type IFN I receptor [16]. Experimental studies are suggested that grouping of IFN-β with other anti-cancer drugs will inhibit the cell growing of, especially, hepatocellular carcinoma and malignance. Another study showed that IFN-β would be synergistically enhance the anti-tumor impact with cisplatin through process done by up-regulation of p53 protein expression on mesothelioma cells[17]. Based on our knowledge no study yet investigate the influence of IFN-β and cisplatin in single and in mixture on Human lung adenocarcinoma cell line (A459). In the current study, we were hypothesized that IFN-β exposure may produce a synergistic anti-tumor outcome if merged with cisplatin.

MATERIAL AND METHOD

Experiment design: human lung adenocarcinoma cell (A549) were purchased from LONZA Biologics (Slough, UK)\ and seeded at $2X10^2$ density in RPMI medium which supplemented with 5% of bovine serum and 1% of pencillin /streptomycin and incubated at 37C. Cells planted in 96 well plates and incubated for 24h in present multiple doses of interferon or Cisplatin in order to screen the dose response. After a couple of experiments to determine the does' toxicity, it has been decided to use six doses of each agent single. (1.562,3.125,6.25,12.5,25,50) µM of Cisplatin was used for 24,48,72h. Moreover, minimum amount of interferon also used (0.039,0.0781,0.56,0.312, 0.625, 1.25) µM.

Crystal Violet assays

Human lung cancer cells A549 seeded in 96 wll plate at $1x10^6$ cells/ml and the all plates were incubated at 37°C. 100 µl) of PBS were used to wash the cells with for 5 minutes. Cells were fixed with 10% formalin at room temperature, after fixation to 20 minutes the solution was removed. Cells fixed and ready for staining. Cells were marked with 100 µl of 0.1% aqueous crystal violet solution for 20 minutes. Dye was diluted by adding 200 µl of 95% ethanol. the spectrophotometer was used for measuring the immersion at a wavelength 540 nm. The results showed after been normalized to the control.

Statistical analysis

Data were expressed as the (mean ± SD) and the differences using tow way ANOVA. Statistical analyses were performed by SPSS software followed by graph pad prims program. *P*-value of <0.05 was reflected as significant difference.

RESULTS

Six doses for each chemical drug was used. The Cisplatin used at very low concentrations grade from 1.562µM to 50µM. The result of the present study showed that there isno a significant reduction in cells viability after 24h Cis exposure at 1.56µM (107.9±2.45), 3.125µM (99.07±0.93), 6.25µM(95.81±4.185),12.5µM(96.74±3.25) and 25µM(99.07±0.93). the only significant can be noticed at 24h was at 50µM (71.63±28.37) .

after 48h exposure the effect was different where the cell availability reduced starting from 12.5µM (71.97±3.03), 25µM(70.07±4.106) and 50µM(49.04±2.98). longer exposure appeared even more toxicity comparing with 24h and 48. At 72h exposure, there was a statically different at 6.25µM(70.06±4.60),12.5µM (71.97±3.92), 25µM (70.7±4.106) and 50µM (49.04±5.14) Figure (1)

Regarding to Interferon (INF) concentration. We used 0.039, 0.078, 0.156.0.312, 0.625 and 1.25µM. Single exposure to INF exhibited a toxicity which are significant comparing to the control at 0.625µM (49.03±4.41), 1.25µM(31.16±2.53). keep the cells with INF for 48h exposed clear reduction in number of life cells even at 0.156µM(71.66±4.75), 0.312µM (41.08±9.88), 0.625µM (85.35±14.32), 1.25µM (14.81±14.3). longer single exposure to INF displayed more cell death and a significant drop cell viability at 0.156µM (79.1±5.79), 0.312µM (22.83±3.65), 0.625µM (13.94±3.89) and 1.25µM (15.66±2.45). Number of cells at higher does went sincerely low comparing with control at this time point.

The mixture exposure presented different pattern of toxicity which was not displayed with any of the single exposure. The reduction was significant at mixing with low concentrations where we did not see any change at these concentrations. The combination included 0.039INF+ 1.562 Cis which was significantly different from control at 72h exposure (88.42±3.257) but not at 48h (94.39±2.75) or 24h (91.84±11.16). Rise both concentrations to double 0.078INF+3.125Cis. even though, there was no significant different with single exposure to each of drugs, the combination was significantly different from control at 24 (69.31±30.69), 48(72.15±2.725) and 72 (78.95± 3.257) and). 0.156INF+6.25Cis showed a significant different at 24h (80.42±19.58) 48h (67.51±2.725) 72h (74.74±3.257). 0.312 INF+12.5Cis which is represent twofold of both concentrations showed higher impact on cell viability not at 24h (97.35±2.64), 48(47.26±2.725) and 72h (64.21±32.79). Fifth concentrations showed different influence comparing to both drug alone at 24h where no significant different noticed (80±10.05). long time exposure exhibited even more influence on number of the cells at 48h (27.43±1.53) and 72h(52.63±47.37). Finally, tow highest concentrations applied showed low number of the cells at 24(37.04±62.96), 48(8.439±1.84) and 72h (22.63±77.37) figure (3).

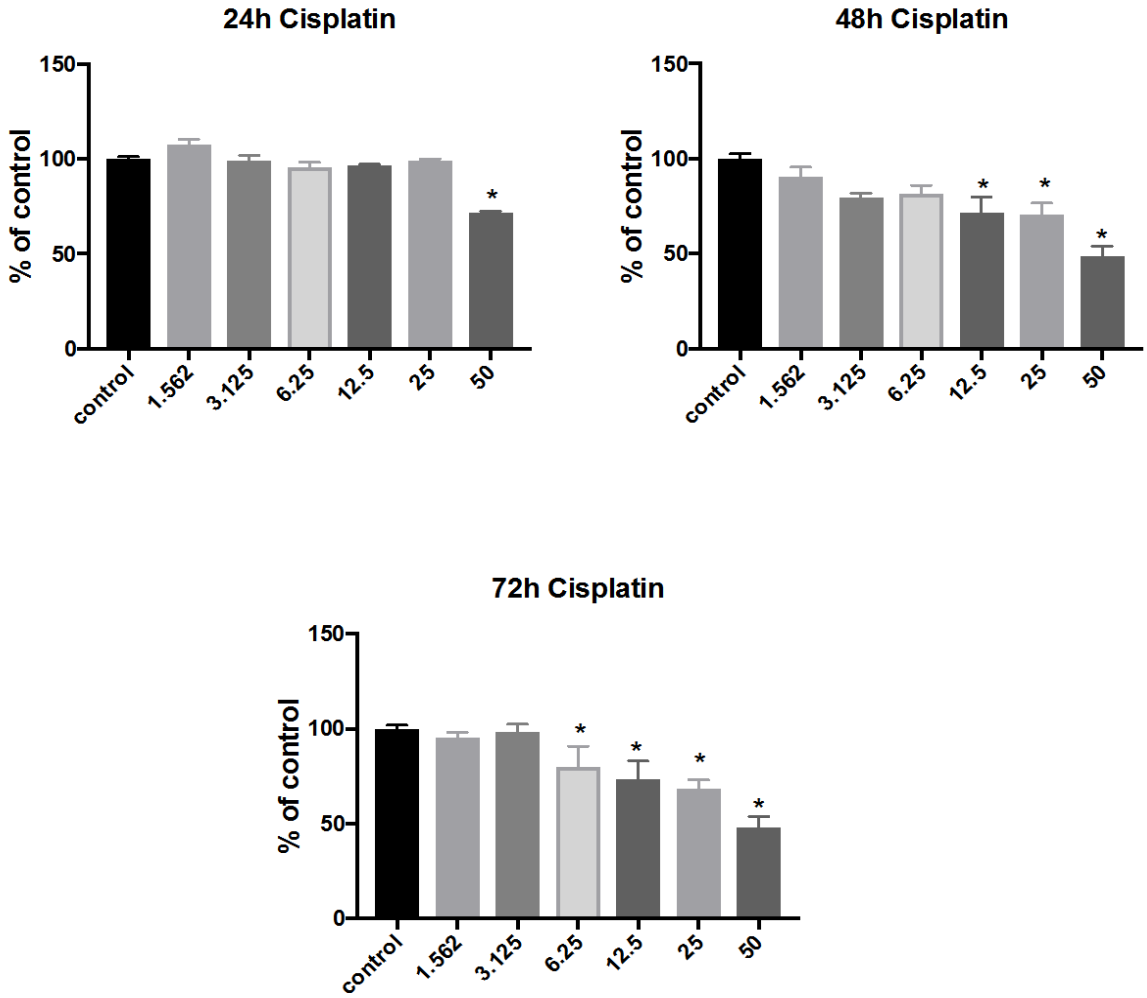

Figure 1: The effect of cisplatin on lung adenocarcinoma cell line (A549). The reduction in cell number at 24h was at 50µM but at 48h was at 12.5. 72h Cis single exposure the decrease was at 6.25.

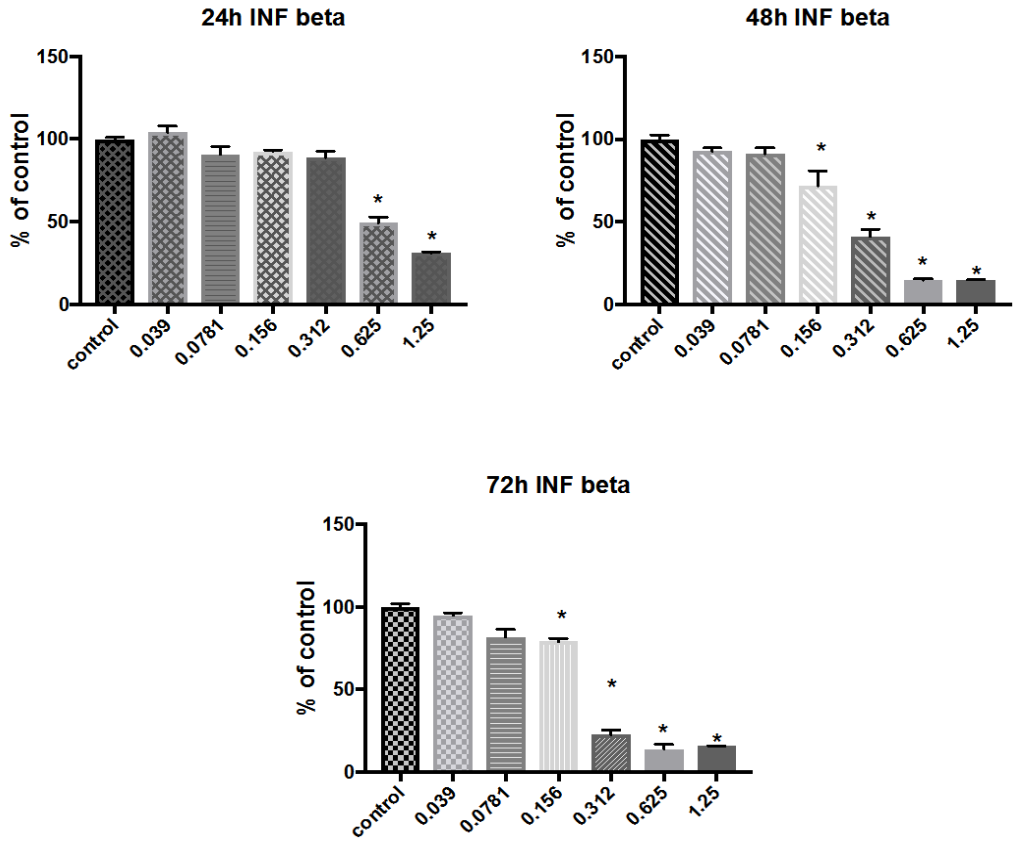

Figure 2: Lung cancer cells exposed to interferon (INF) alone. 24h showed reduction with higher two concentration used. The longer exposure had more impact where the reduction was with higher 3 concentrations used. The 72h exposure was even harder on the cell where the reduction was significant at last fourth concentration included in the current study.

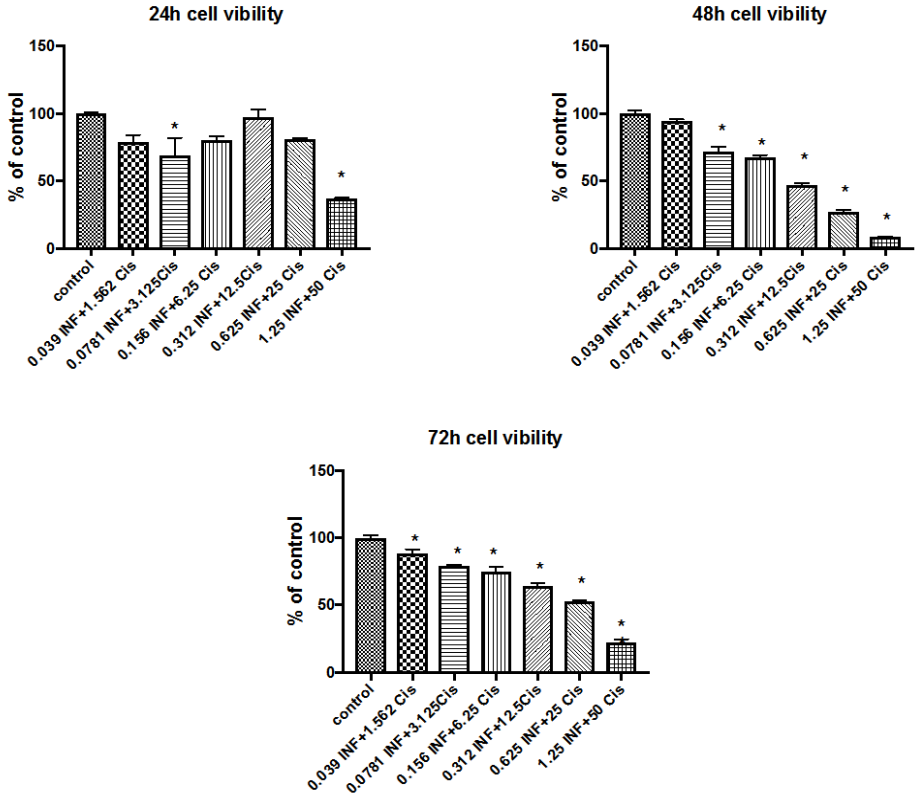

Figure 3: co-exposure to INF and Cis 24, 48 and 72h. the combination displayed toxicity at 48 with all the concentration used in the current study. 72h exposure to mixture presented reduction at 0.093 INF and 1.562 Cis.

DISCUSSION

Cisplatin is drug which is commonly used as a first-line as cancer therapy. It has been utilized either alone or in grouping with other anticancer agents [18]. Lately, several of in vivo and in vitro studies suggested that the combination agents will demonstrate an alternate treatment option to decrease the toxicity and expansion the effectiveness of single agent [2]. Moreover, a large number of studies reported that the doublet is superior to a solitary agent or even triple combination. In the current study, we investigated the cytotoxic properties of cisplatin with several dosages of IFN-β. Based on the author knowledge, this is the first study which displayed the synergistic combinatory consequence of IFN-β with the first line anticancer agent cisplatin lung cancer cells.

It has been reported that Cis able to form high reactive and charged platinum compound by binding to nucleophilic groups in DNA. As well as, Cis is capable to produce DNA cross links as well as DNA protein cross links. Consequently, apoptosis and cell growth inhibition will be induced [19]. On the other hand, INF- β has anti-proliferatice effect though arresting cell cycle at G1 phase and obstructing the phosphorylation of the tumor inhibitor protein pRB[20]. Accordingly, INF- β would synergistically, even with small dose, enhanced cell growth inhibitory impact of Cis on human lung cells that treated in combination of INF- β and Cis for 48 and 72h.

The current study come along with study conducted on human breast adenocarcinoma MDA which known as MB231 cells and approved that there is synergistic anti- carcinogenic effect of same combination by arresting the cell and decrease expression of Bcl-2 or increase expression of Bax protein that stimulated cytochrome c release. Cytochrome c, in turn, activates caspase 9 and 3 activation[17].

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