The Egyptian Journal of Biochemistry & Molecular Biology
VOL 37(N.1&2) 95-110 December. 2019

DEXAMETHASONE IMPROVES THE RESPONSIVENESS OF HEPATOMA CELLS FOR BOTH FREE AND SOLVENT CONTAINING PACLITAXEL IN VITRO

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Received 1/9/2019 - Accepted 21/10/2019

ABSTRACT

This work was designated to explore the effect of glucocorticoid (dexamethasone) on the responsiveness of hepatoma cells to Paclitaxel (PTX) and the expression of Taxol resistance gene (Txr1) and the paclitaxel metabolizing genes. Hepatocellular carcinoma cells (HepG2) were treated with standard paclitaxel (PTX) or solvent containing paclitaxel (Taxol) in the presence or absence of dexamethasone (DEX). Cell viability and apoptosis were determined by MTT assay and flow cytometry, respectively. Also, total RNA was isolated, reverse transcribed and used to determine the expression levels of Txr1, CYP 3A4, and CYP2C8 genes.

Initially, HepG2 cells were more resistant to PTX than Taxol. Also, cells became more responsive to the standard PTX and Taxol in the presence of DEX, where the IC_{50} values decreased from 42.5 µg/ml to 13.07 µg/ml and from 6.5 µg/ml to 3.6 µg/ml, respectively. Apoptosis was the main mechanism of cytotoxicity in cells treated with PTX or Taxol. The involvement of DEX, however, decreased the percent of apoptotic cells. Moreover, the expression of Txr1 decreased by 18% and 35% in cells cotreated with PTX+DEX or Taxol+DEX. In parallel, the expression of paclitaxel metabolizing genes (CYP3A4 and CYP2C8) was increased compared to DEX free cells. This *in vitro* study reports the associations between the enhanced responsiveness of hepatoma cells to paclitaxel or Taxol in presence of dexamethasone,

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associated with a decrease in drug resistance and upregulation of the paclitaxel metabolizing genes.
Keywords: Liver cancer, Paclitaxel nanoparticles, Taxol resistance gene, CYPs.

INTRODUCTION

Cancer is the most leading cause of death worldwide. The new incidence of cancer is projected to rise from 17·0 million to 26·0 million between 2018 and 2040. Liver cancer, in particular, represents a major portion of cancer patients **(Wilson et al., 2019)**. Paclitaxel (PTX) is a naturally-occurring product isolated from the yew tree (*Taxus brevifolia*) and broadly used as an antineoplastic drug against many types of cancers, including ovarian, breast, lung, head-and-neck, prostate and pancreatic cancer **(Kampan et al., 2015)**. Also, the drug demonstrated the therapeutic potential for hepatocellular carcinoma (HCC) **(Gagandeep et al., 1999)**. The anticancer effect of PTX is mediated by promoting microtubule polymerization, making the microtubule structure stable, blocking their depolymerization into subunits, subsequently induces cell cycle arrest at G2/M phase and apoptosis **(Symmans et al., 2000)**. The poor water solubility of paclitaxel (less than 0.1 µg/ml) restricted its direct use in clinical practice. To overcome this problem, it is dissolved in a 50/50 (v/v) mixture of Cremophor EL (CrEL) and ethanol in a widely used formulation commercially known as Taxol. Although Food and Drug Administration (FDA) has approved Taxol for the treatment of ovarian and breast and some other cancers since 1992 **(Wang et al., 2004),** its clinical practice demonstrated a long list of severe solvent related adverse effects **(Holmes et al., 1991)**. In addition to the cytotoxic effect of ethanol, which has been reported decades ago, CrEL is not an inert vehicle and exerts a wide range of adverse biological effects including anaphylactic hypersensitivity reactions, hyperlipidemia, aggregation of erythrocytes and peripheral neuropathy **(Liebmann et al., 1994; Gelderblom, et al., 2001).** In order to avoid chemotherapy-associated complications, many strategies were developed including nanoformulation paclitaxel. Glucocorticoids, however, are efficacious in reducing the chemotherapy adverse effects and enhance their intrinsic anticancer activity **(Le.Vee et al., 2009)**. Dexamethasone (DEX) pretreatment, for example, increased the antitumor activity of carboplatin and gemcitabine and decreased host toxicity in nude mouse xenograft models of human cancer. The underlining protective effect of DEX was

attributed to its inhibitory effects on cytokines produced by the tumor, increasing tumor necrosis factor (TNF)· and decreasing the expression of IL-1ß and VEGF **(Trotman et al., 2007)**. Moreover, DEX administration attenuated the severity of paclitaxel-associated acute pain syndrome in patients with non-squamous lung carcinoma **(Saito, 2019)** and enhanced the anti-tumor effects of PTX in orthotopic mouse breast cancer **(Popilskiet al., 2018)**. Besides the physicochemical characteristics of the drug and its formulation, some other host (cellular) related factors greatly participate in the therapeutic efficacy. Cytochrome CYPs, enzymes are included among these factors, due to their role in hepatic metabolism of the vast majority of drugs. These enzymes represent a large superfamily of membrane-bound monooxygenases involved in phase I oxidative biotransformation of a wide range of xenobiotics including paclitaxel **(Zerilli, 1998)**. There is unlimited number of reports that investigated the expression of CYPs in both hepatic and extrahepatic tissues, with respect to their roles in drug metabolism **(Rendic and Carlo, 1997)**. Hepatic CYP3A4 and CYP2C8, for instance, metabolize PTX in phase I reactions through 2 successive hydroxylation steps **(Cresteil et al., 1994; Lindley et al., 2004).** The expression of CYP3A4 and CYP2C8 genes play a major role in cell responsiveness to chemotherapeutic compounds **(Waxman et al., 1989)**. Moreover, drug resistance participates in their therapeutic effectiveness. Taxol-resistant gene 1 (Txr1) was found to be differently expressed in many types of cancers including non-small cell lung cancer, gastric cancer, and breast cancer. Some investigators have regarded Txr1 expression as an independent prognostic factor **(Bai et al., 2010; Du et al., 2012).** Also, other studies have demonstrated the mechanistic association between Txr1 gene expression and autophagy **(Chi et al., 2019)**. Taken together, it is imperative to investigate both the cytotoxic potential of anticancer drugs and the associated regulation of genes deeply involved in their resistance and metabolism. Thus, this work was designated to investigate how far glucocorticoid cotreatment can modulate on the responsiveness of hepatoma cells to PTX in both standard and solvent-containing formulations. Also, to monitor the regulation of 3 genes directly involved in PTX resistance and metabolism in liver cells.

MATERIAL AND METHOD

PTX was purchased from Carbosynth, UK and Dimethyl sulfoxide (DMSO) from Sigma-Aldrich, (St. Louis, MO, USA). Taxol was obtained from Ebewa pharma Ges.m.b.H.Ntf.KG. Standard PTX was

dissolved in dimethyl sulfoxide (DMSO) to a stock concentration of 10 mM. Hepatoma cells were generously provided by The Department of Cancer Biology, National Cancer Institute, Cairo University. Cell culture reagents Dulbecco's modified Eagle medium (DMEM), Fetal bovine serum (FBS) and penicillin, and streptomycin) were purchased from Lonza, BioWhittaker®, USA.

Cell culture and treatment

Cells were seeded in DMEM with L glutamine, supplemented with 10% FBS, 1% Penicillin and Streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. Initially, cells were cultured in a low cell density then passaged with defined cell densities according the experimental settings. Cells were exposed to DMSO, standard PTX, Taxol, PTX+DEX and Taxol+DEX for 48 h **(Gagandeep et al., 1999)**.

Cell viability assay:

The viability of cells was determined by (3-(4,5-dimethylthiazolyl-2)-2, 5- diphenyltetrazolium bromide) (MTT) assay. Cells were seeded in 96 well plates with initial cell density 5×10^4 cells/well in 100 µl DMEM containing increasing doses of standard PTX or Taxol with or without a fixed and low concentration of DEX (15 nM). Five wells were assigned for each concentration. Cells were incubated in a humidified atmosphere at 37°C, 5% CO_2, and 95% air for 24 h. After incubation, 10 µl of the MTT labeling reagent (0.5 mg/ml) was added per well. After 4 hour incubation, 100µl of solubilization solution was added per well. After the development of the purple formazan color, the absorbance was measured at 570 nm, where the average OD (±SD) was calculated and the cytotoxicity curve was plotted to determine the IC_{50} concentrations **(Gagandeep et al., 1999).**

Apoptotic assay:

In order to determine the apoptotic effect, Annexin V FITC kit (Miltenyi Biotec, Auburn, CA, USA) was used following the manufacturer's instructions. Briefly, treated cells were detached from the plates by scraping and centrifuged at 1000 rpm for 5 min. The cell was resuspended in 1 ml PBS and then incubated with 0.25 µg/ml Annexin V reagent in 1X binding buffer for 15 min followed by two washes with Wash Buffer. Cells were resuspended in binding buffer containing 0.5 µg/ml propidium iodide (PI) then subjected to flow cytometry (BC

Novus). The data was retrieved and analyzed by Kaluza software **(Gagandeep et al., 1999)**.

Expression analysis by real-time quantitative PCR:

The expression levels of PTX resistance (Txr1), CYP3A4 and CYP2C8 genes were assessed using Qiagen Rotor-Gene Q-PCR Cycler 5 Plex. Total RNA was isolated using the GeneJET RNA purification kit, (ThermoFisher Scientific, USA) and 200 ng was used for cDNA synthesis using SensiFAST™ cDNA Synthesis Kit, (Bioline Inc, USA) following the manufacturer's protocol. 50 ng/µl (2µl) of cDNA was used as a template in cycling reactions mixture (20 µl) containing 50 nmol/µl (2 µl) of gene-specific primes where the primers sequence according to **(Bai et al., 2012)** used Txr1 was forward5-GGACCCTTCCCTCAAGTCTC-3,reverse:5-CTCTTCCCATTTCCCCTAGC-3; CYP3A4 sequence according to **(Zhang et al., 2014)** were forward: 5-CAGGAGGAAA TTGATGCAGTTTT-3, revere5-GTCAAGATACTCCATCTGTAGCACAGT-3;CYP2C8 according to **(Klose et al., 1999)** was forward : 5-AGATCAGAATTT TCTCAC CC -3, reverse: 5-AAC TTCGTGTAAGAGCAACA-3and GAPDH sequence according to **(Bai et al., 2012)** is forward: 5-CCTGCCAAGTATGATGACATCAAGA-3,reverse:5-GTAGCCCAGG ATGCCCTTTAGT-3. The reaction mix included 10 *QIAGEN SYBR green*, raised to 20 µl with ddH_2O then subjected a thermal cycling program consisted of a single step of initial denaturation and 45 cycles (each consists of single denaturation at 94 ^{O}C for 5 sec, annealing at 67 ^{O}C, 61 ^{O}C (for Txr1) and an extension step at 72 ^{O}C for 20 sec. Reactions were terminated with a single step in which the temperature was increased from 50 °C to 99 °C to produce a melt curve. In parallel, the expression of GAPDH was used as an internal control to determine the fold expression changes in target genes. The critical threshold (Ct) of target genes was normalized with quantities (Ct) of GAPDH by using the $2^{-\Delta\Delta Ct}$ **(Bai et al., 2012).**

Statistical data analysis

SPSS13.0 software package was used to analyze the data. All cell culture work was performed in triplicates and the average value was determined. Apoptosis levels measured and displayed as a percent of the control (untreated) cells and represented as the mean of 3 runs ± SD (standard deviation).

RESULTS

1-Dexamethasone enhances the anticancer effect of paclitaxel and Taxol

To investigate the effect of DEX on the responsiveness of HepG2 cells to standard PTX and Taxol, cells were treated with increasing concentrations of PTX or Taxol, in the presence or absence of dexamethasone (fixed concentration 15 nM). These treatments demonstrated a progressive decrease the cell viability with increasing drug concentrations to the corresponding of PTX or Taxol. Similar changes in viability patterns were observed in cells cotreated with PTX (or Taxol) with DEX (Figure 1).

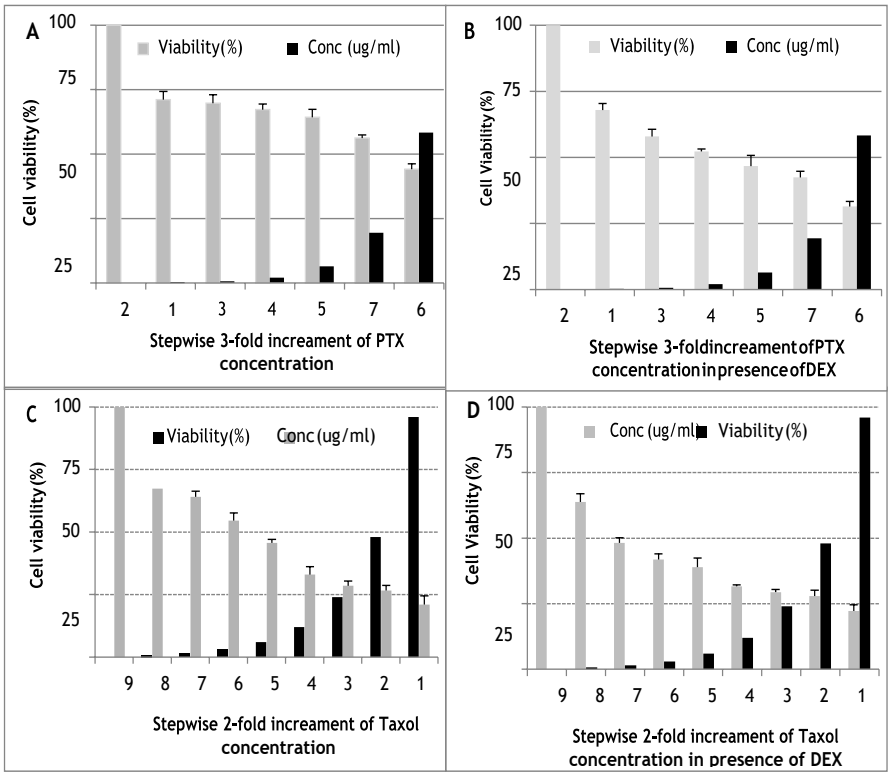

Figure 1: Paclitaxel and Taxol induced dose-dependent regression of HepG2 cells viability, in presence or absence of dexamethasone. The gradual decrease of the fraction of surviving was observed when cells treated with PTX (A), PTX+DEX (B), Taxol (C) and Taxol+DEX (D). In all panels the first left column corresponds the viability of control (untreated) cells.

The calculated IC_{50} of PTX was 42.5 µg/ml, which was reduced to13.07 µg/ml in presence of DEX. Also, Taxol demonstrated an initial low IC_{50} value (6.52 µg/ml), which further decreased to 3.64 µg/ml in presence of DEX. The decrease in IC_{50} values observed in cells cotreated with DEX was significant in both cases ($P<0.001$) (Figure 2).

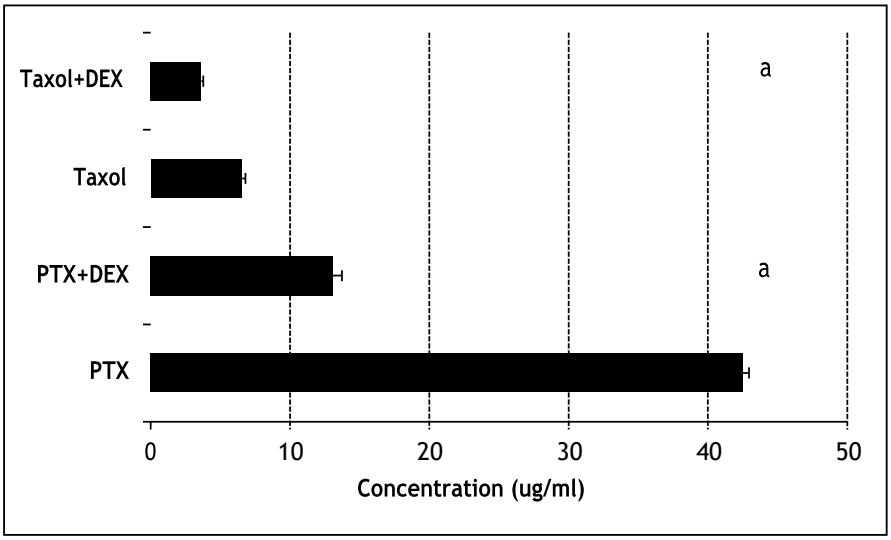

Figure 2: Cytotoxic effect of Paclitaxel and Taxol in HepG2 cells, represented as IC_{50} values. The IC_{50} values significantly decreased in cells cotreated with PTX (or Taxol) and DEX.
(a:) significant changes in the indicated cells compared to DEX free cells ($P<0.001$).

2-Determination of cell apoptosis.

The apoptotic effect of standard PTX and Taxol were investigated by flow cytometry in which treated cells were dually stained with Annexin V-FITC and Propidium iodide (PI). Figure 3 demonstrates the flow cytometric analysis, where the fraction of cells that underwent early and late apoptotic fractions are shown in the lower and upper right quadrates, respectively, whereas the percent of viable and dead cells are shown in the lower and upper left quadrants, respectively. Dexamethasone alone did not demonstrate significant apoptotic effect, where the percent of apoptotic (early plus late

apoptosis) cells was 5.4%. Standard PTX and Taxol induced apoptosis in 64.7% and 42.92% of cells, respectively.

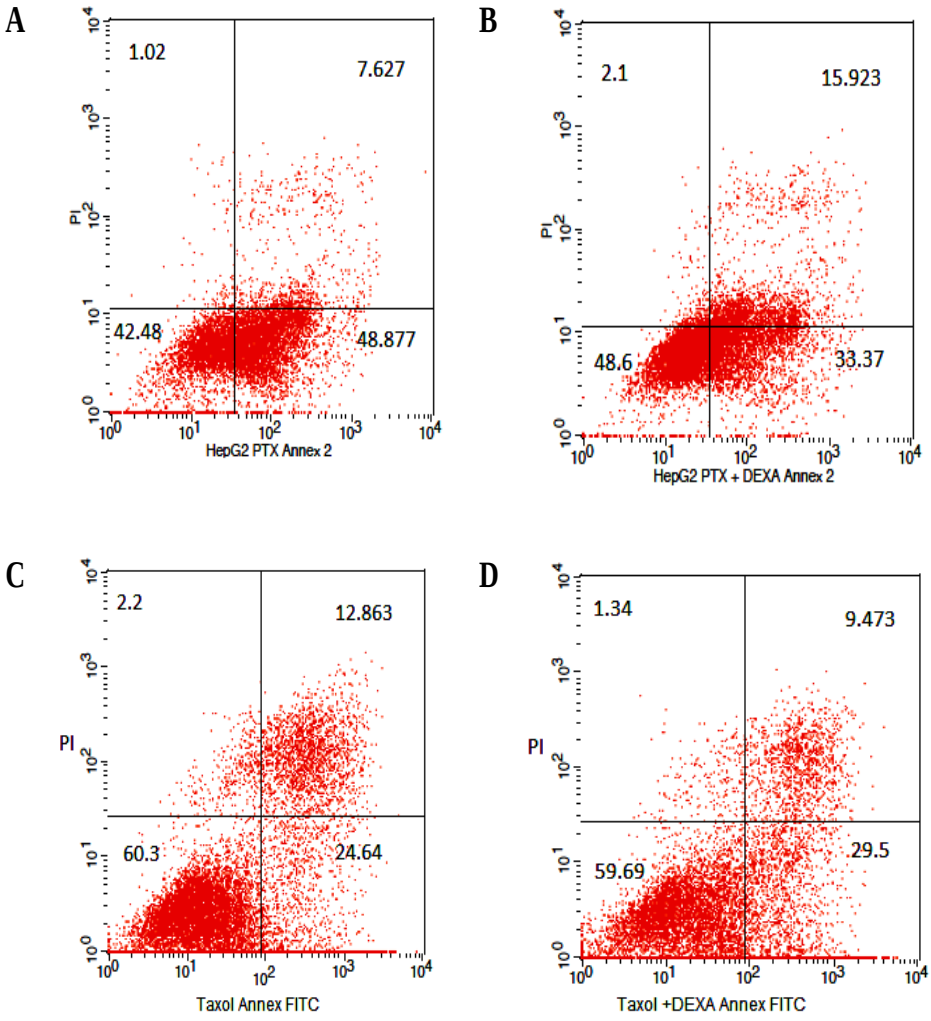

Figure 3: Flow cytometric dot plots of Annexin V-FITC and Propidium iodide stained HepG2 cells treated with standard PTX (A), PTX+DEX (B), Taxol (C) and Taxol+DEX (D). In each plot the lower right quadrant represents the fraction of the early apoptotic cells, positive for Annexin V-FITC and negative for PI (FITC+/PI-). The upper right quadrant represents the late apoptotic cells, which are positive for Annexin V-FITC binding and PI uptake (FITC+/PI+).

Cells co-treated with DEX, however, demonstrated significantly less apoptosis compared to cells treated alone with PTX or Taxol (Figure 4).

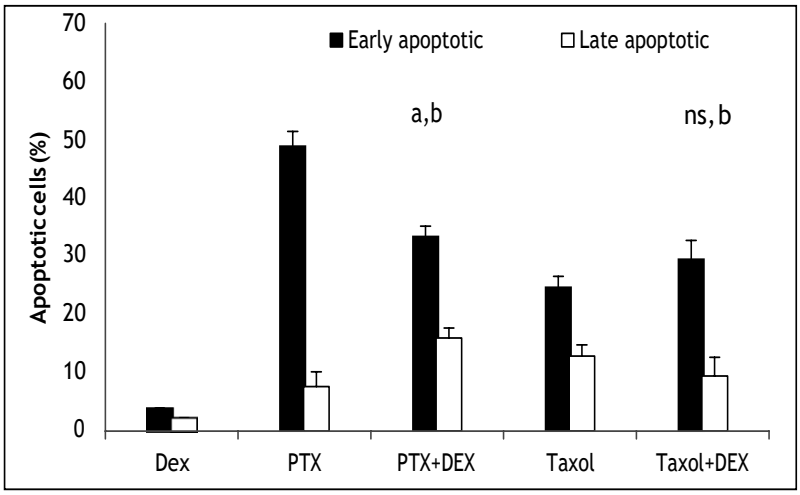

Figure 4: Dexamethasone minimized the apoptotic effect of Hepg2 cells cotreated with PTX or Taxol.
(a,b): Significant decrease in the indicated cells versus DEX free cells (P<0.001). (*ns*): Insignificant decrease versus DEX free cells (P>0.05).

3-Expression analysis

The initial expression of Txr1 in PTX treated cells was lower than the control gene (0.73), and further downregulation was observed in cells cotreated with PTX+DEX. A similar changing profile was seen in cells treated with Taxol with or without DEX (Figure 4). The expression of CYP3A4 and CYP2C8 demonstrated the upregulation in cells treated with PTX or Taxol (2.7 and 3.6, respectively). Cotreatment with DEX led to further increase in their expression, where CYP3A4 expression showed 2-fold increase, whereas CYP2C8 showed 4 fold increase (2.7-8.815) Figure 5.

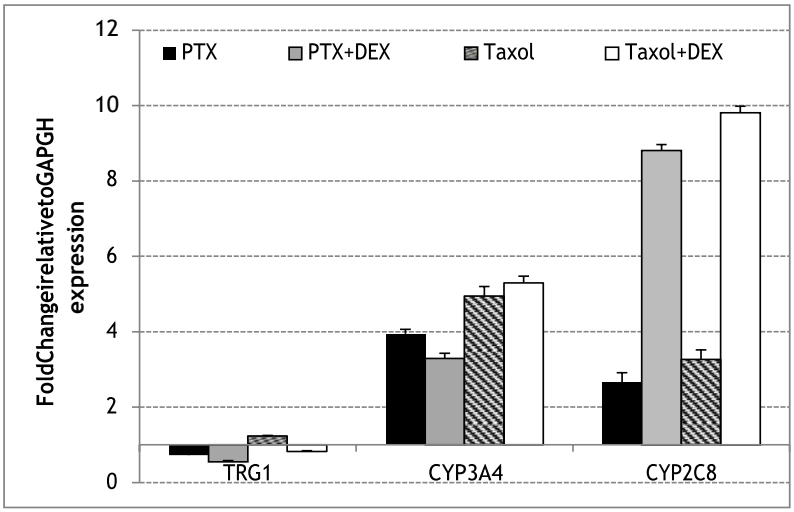

Figure 5: Fold changes in the expression of Taxol resistance gene 1 (Txr1), and paclitaxel metabolising genes (CYP3A4 and CYP2C8) in HepG2 cells treated with the IC_{50} values of standard PTX or PTX+DEX.

DISCUSSION

Many anticancer drugs demonstrate poor therapeutic effectiveness duo to their water insolubility and/or instability **(Xu et al., 2013).** PTX is predominantly used in treatment of many types of cancers including liver cancer. The pure compound, which is water-insoluble, is commercially formulated in Cremophor EL and ethanol, to which the side effects of PTX are attributed. Another major drug-related concern is represented by drug resistance, where PTX is challenged by multi-drug resistance (**Reshma et al., 2019)** and expression of PTX metabolizing enzymes. This raises the importance of monitoring the regulation of genes involved in PTX metabolism and resistance. As it was previously reported **(Yadav et al., 2014)**, Paclitaxel has induced a dose dependent decrease in cell viability, where the survival rate of hepatoma cells intervened by pure PTX decreased with the increasing its concentration (with IC_{50} value 42.5%). The responsiveness of cells to PTX combined with DEX was markedly increased, with about 3-fold decrease in the IC_{50} value. It seems that a substantial part of the cytotoxic effect of Taxol is attributed to the solvents it contains (Cremophor EL and ethanol), where the IC_{50} of Taxol was much

lower than the corresponding IC_{50} of pure PTX (6.52μg/ml), which further diminished to 3.6 μg/ml in presence of DEX. Increasing the responsiveness of hepatoma cells by dexamethasone is not the only gained part. Many reports have demonstrated the protective effect of DEX in ovarian cancer patients receiving PTX (**Kumar et al., 2018**). Also, DEX protected rats against the hepatotoxic effect of other anticancer drugs **(Donald et al., 2003).** In agreement with previous reports, DEX may induce antiapoptotic effect, where the percent of apoptotic cells was lower in cells treated with DEX combined with PTX or Taxol relative to cells treated only with the anticancer drug.

DEX antiapoptotic effect involves the inhibition of the mitochondrial apoptosis pathway **(Lee et al., 2017).**This may indicate the dual therapeutic role of DEX, in addition to its safe use, where it doesn't induce apoptotic or necrotic effects. Apoptosis was the key mechanism observed in cells challenged by PTX or Taxol. PTX induced more apoptotic effect compared to Taxol, whereas cotreatment with DEX did not significantly affect the percent of cells underwent apoptosis.

The genes panel, chosen in the present study, includes 3 genes directly involved in PTX resistance and metabolism. Txr1 is known to actively pumps PTX out of cells **(Bai et al., 2012).** The observed decrease in the expression of TxrI in presence of DEX will predictively decrease HepG2 cells resistance to both pure and solvent containing PTX. The upregulation of the multidrug resistance gene (MDR1) to taxans (reported in other studies) which acts as a substrate of P-glycoprotein, led to its pumping out of the cell **(Miyoshi et al., 2005)**. This triggered the notion of using the expression of Txr1 as an indicator to evaluate the potential treatment and prognosis **(Chi et al., 2019)**. Other intervening factor that greatly modulates the performance of drugs is represented by the xenobiotic metabolizing machinery in liver, especially cytochromes (CYPs). CYP3A4 and CYP2C8 are mutually metabolize PTX through 2 successive hydroxylations to form dihydroxypaclitaxel **(Wang et al., 2015**). CYP3A4 enzyme is the most potent and abundant catalytic cytochrome in liver. Although their role in hepatic metabolism is well reported, the expression levels of their genes and the activity of coded enzymes in solid tumors may play a role in their hepatic biotransformation and potentially affecting the intrinsic PTX susceptibility of these tumors **(Neis et al., 2019)**. Other

anticancer drugs (such as cyclophosphamide, vinblastine sulfate, daunorubicin hydrochloride, teniposide, and docetaxel) were able to downregulate the expression of CYP3A4 gene, some other drugs including PTX did not show similar down regulatory effect **(Baumhäkel et al., 2001)**. Herein, PTX treated cells demonstrated higher expression of CYP 3A4 and CYP 2C8, relative to the expression level of the housekeeping gene GAPDH (used as an internal control). Further increase in mRNA levels of both genes was observed when cells exposed to PTX or Taxol combined with DEX.

Conclusion: The PTX resistance-relieve and enhancing PTX metabolism in hepatoma cells may predictively participate in improving the enhancing the sensitivity of hepatoma cells to PTX and subsequently minimize the unsatisfactory therapeutic outcomes related by high doses or the cytotoxic solvents.

Author contribution:

Conceptualization: **MH** and **TS**; Methodology and investigations: **MH SS, TD** and **SK**, Original draft preparation: **MH** and SK; Review and editing, **MH** and **TD**.

Conflict of interest: The authors have no conflict of interest.

REFERENCES

Bai, Z.-G., Qu, X., Han, W., Ma, X.-M., Zhao, X.-M. and Zhang, Z.-T. (2010):Expression of taxol resistance gene 1 correlates with gastric cancer patient clinical outcome and induces taxol resistance. Molecular medicine reports, 3(6): 1071-1078.

Bai, Z., Zhang, Z., Qu, X., Han, W. and Ma, X. (2012):Sensitization of breast cancer cells to taxol by inhibition of taxol resistance gene 1. Oncology letters, 3(1): 135-140.

Baumhäkel, M., Kasel, D., Rao-Schymanski, R., Böcker, R., Beckurts, K., Zaigler, M., Barthold, D. and Fuhr, U. (2001):Screening for inhibitory effects of antineoplastic agents on CYP3A4 in human liver microsomes. International journal of clinical pharmacology and therapeutics, 39(12): 517-528.

Chi HM, Du JD, Cheng J, Mao HD (2019):Taxol-Resistant Gene 1 (Txr1) Mediates Oxaliplatin Resistance by Inducing Autophagy in Human Nasopharyngeal Carcinoma Cells. Medical science monitor: international medical journal of experimental and clinical research, 25: 475-483.

Cresteil, T., Monsarrat, B., Alvinerie, P., Tréluyer, J. M., Vieira, I. and Wright, M. (1994):Taxol metabolism by human liver microsomes:

identification of cytochrome P450 isozymes involved in its biotransformation. Cancer research, 54(2), 386-392.
Donald, S., Verschoyle, R. D., Greaves, P., Gant, T. W., Colombo, T., Zaffaroni, M., Frapolli, R., Zucchetti, M., D'Incalci, M. and Meco, D. (2003):Complete protection by high-dose dexamethasone against the hepatotoxicity of the novel antitumor drug yondelis (ET-743) in the rat. Cancer Research, 63(18), 5902- 5908.
Du, L., Subauste, M. C., DeSevo, C., Zhao, Z., Baker, M., Borkowski, R., Schageman, J. J., Greer, R., Yang, C.-R. and Suraokar, M. (2012):miR-337-3p and its targets STAT3 and RAP1A modulate taxane sensitivity in non-small cell lung cancers. PloS one, 7(6), e39167.
Gagandeep, S., Novikoff, P. M., Ott, M. and Gupta, S. (1999):Paclitaxel shows cytotoxic activity in human hepatocellular carcinoma cell lines. Cancer letters, 136(1): 109-118.
Gelderblom, H, Verweij, J., Nooter, K., Sparreboom. A (2001): Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation. Eur J Cancer. 37(13):1590-8.
Holmes, W. E., Lee, J., Kuang, W.-J., Rice, G. C. and Wood, W. I. (1991):Structure and functional expression of a human interleukin-8 receptor. Science, 253(5025): 1278-1280.
Horwitz, S. B. (1992): Mechanism of action of taxol. Trends in pharmacological sciences, 13, 134-136.
Kampan, N. C., Madondo, M. T., McNally, O. M., Quinn, M. and Plebanski, M. (2015):Paclitaxel and its evolving role in the management of ovarian cancer. BioMed research international, 2015:413076.
Klose, T. S., Blaisdell, J. A. and Goldstein, J. A. (1999):Gene structure of CYP2C8 and extrahepatic distribution of the human CYP2Cs. Journal of biochemical and molecular toxicology, 13(6), 289-295.
Kumar, A., Solanki, S. L., Gangakhedkar, G. R., Shylasree, T. and Sharma, K. S. (2018):Comparison of palonosetron and dexamethasone with ondansetron and dexamethasone for postoperative nausea and vomiting in postchemotherapy ovarian cancer surgeries requiring opioid-based patient-controlled analgesia: A randomised, double-blind, active controlled study. Indian journal of anaesthesia, 62(10): 773797.
Le Vee, M., Lecureur, V., Stieger, B. and Fardel, O. (2009): Regulation of drug transporter expression in human hepatocytes exposed to the proinflammatory cytokines tumor necrosis factor-α or interleukin-6. Drug Metabolism and Disposition, 37(3): 685-693.
Lee, J.H., Oh S.H., Kim, T.H., Go, Y.Y., Song, J.J. (2017): Anti-apoptotic effect of dexamethasone in an ototoxicity model. Biomater Res. 21:2-6.
Liebmann, J., Cook, J. A., Lipschultz, C., Teague, D., Fisher, J. and Mitchell, J. B. (1994):The influence of Cremophor EL on the cell cycle

effects of paclitaxel (Taxol®) in human tumor cell lines. Cancer chemotherapy and pharmacology, 33(4): 331-339.
Lindley, J., Dale, A. and Dex, S. (2004):Ethnic differences in women's demographic, family characteristics and economic activity profiles, 1992 to 2002. Labour Market Trends, 112(4): 153-166.
Miyoshi, Y., Taguchi, T., Kim, S. J., Tamaki, Y. and Noguchi, S. (2005):Prediction of response to docetaxel by immunohistochemical analysis of CYP3A4 expression in human breast cancers. Breast cancer, 12(1): 11-15.
Neis, E. P., van Eijk, H. M., Lenaerts, K., Damink, S. W. O., Blaak, E. E., Dejong, C.
H. and Rensen, S. S. (2019):Distal versus proximal intestinal short-chain fatty acid release in man. Gut, 68(4): 764-765.
Popilski, H., Abtew, E., Schwendeman, S., Domb, A. and Stepensky, D. (2018):Efficacy of paclitaxel/dexamethasone intra-tumoral delivery in treating orthotopic mouse breast cancer. Journal of controlled release, 279, 1-7.
Rendic, S. and Carlo, F. J. D. (1997):Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. Drug metabolism reviews, 29(1-2): 413-580.
Reshma, P., Unnikrishnan, B., Preethi, G., Syama, H., Archana, M., Remya, K., Shiji, R., Sreekutty, J. and Sreelekha, T. (2019):Overcoming drug-resistance in lung cancer cells by paclitaxel loaded galactoxyloglucan nanoparticles. International journal of biological macromolecules,136:266-274.
Saito, Y., Kobayashi, M., Yamada, T., Sakakibara-Konishi, J., Shinagawa, N., Kinoshita, I., Dosaka-Akita, H. and Iseki, K. (2019):Efficacy of additional dexamethasone administration for the attenuation of paclitaxel-associated acute pain syndrome. Supportive Care in Cancer, 1-7 [Epub ahead of print].
Symmans, W. F., Volm, M. D., Shapiro, R. L., Perkins, A. B., Kim, A. Y., Demaria, S., Yee, H. T., McMullen, H., Oratz, R. and Klein, P. (2000):Paclitaxel- induced apoptosis and mitotic arrest assessed by serial fine-needle aspiration: implications for early prediction of breast cancer response to neoadjuvant treatment. Clinical Cancer Research, 6(12): 4610-4617.
Trotman, L. C., Wang, X., Alimonti, A., Chen, Z., Teruya-Feldstein, J., Yang, H., Pavletich, N. P., Carver, B. S., Cordon-Cardo, C. and Erdjument-Bromage, H. (2007): Ubiquitination regulates PTEN nuclear import and tumor suppression. Cell, 128(1): 141-156.
Wang, X., Ling, M. T., Guan, X.-Y., Tsao, S. W., Cheung, H. W., Lee, D. T. and Wong, Y. C. (2004): Identification of a novel function of TWIST, a

bHLH protein, in the development of acquired taxol resistance in human cancer cells. Oncogene, 23(2): 474-482.
Wang, Y., Jiang, Y., Fan, X., Tan, H., Zeng, H., Wang, Y., Chen, P., Huang, M. and Bi, H. (2015): Hepato-protective effect of resveratrol against acetaminophen- induced liver injury is associated with inhibition of CYP-mediated bioactivation and regulation of SIRT1–p53 signaling pathways. Toxicology letters, 236(2): 82-89.
WAXMAN, D. J., MORRISSEY, J.J. and LEBLANC, G. A. (1989):Female-predominant rat hepatic P-450 forms j (IIE1) and 3 (IIA1) are under hormonal regulatory controls distinct from those of the sex-specific P-450 forms. Endocrinology, 124(6): 2954-2966.
Wilson, B. E., Jacob, S., Yap, M. L., Ferlay, J., Bray, F. and Barton, M. B. (2019):Estimates of global chemotherapy demands and corresponding physician workforce requirements for 2018 and 2040: a population-based study. The Lancet Oncology, 20(6), 769-780.
Xu, X., Wang, L., Xu, H.-Q., Huang, X.-E., Qian, Y.-D. and Xiang, J. (2013):Clinical comparison between paclitaxel liposome (Lipusu®) and paclitaxel for treatment of patients with metastatic gastric cancer. Asian Pacific Journal of Cancer Prevention, 14(4): 2591-2594.
Yadav, D., Anwar, M. F., Garg, V., Kardam, H., Beg, M. N., Suri, S., Gaur, S. and Asif, M. (2014):Development of polymeric nanopaclitaxel and comparison with free paclitaxel for effects on cell proliferation of MCF-7 and B16F0 carcinoma cells. Asian Pac J Cancer Prev, 15: 2335-2340.
Zerilli, A., Lucas, D., Dreano, Y., Picart, D. and Berthou, F. (1998):Effect of Pyrazole and Dexamethasone Administration on Cytochrome P450 2E1 and 3A Isoforms in Rat Liver and Kidney: Lack of Specificity of p-Nitrophenol as a Substrate of P450 2E1. Alcoholism: Clinical and Experimental Research, 22(3): 652-657.
Zhang, Y.-w., Bao, M.-h., Wang, G., Qu, Q. and Zhou, H.-h. (2014):Induction of human CYP3A4 by huperzine A, ligustrazine and oridonin through pregnane X receptor-mediated pathways. Die Pharmazie-An International Journal of Pharmaceutical Sciences, 69(7): 532-53

الملخص العربي

الديكساميثاسون يحسن إستجابة خلايا الكبد السرطانية لعقار الباكليتاكسيل الخالى و المحتوى على المذيب

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صممت هذه الدراسة لإستكشاف أثر الديكساميساسون على إستجابة خلايا الكبد السرطانية لتأثير عقار الباكليتاكسيل وما يصاحبه من تغير فى تعبير جين مقاومة الباكليتاكسيل وجينات أيضه. حيث تم علاج خلايا سرطان الكبد بعقار الباكليتاكسيل النقى أو المذاب(فى مذيب عضوى و المعروف بالتاكسول) في وجود أو عدم وجود الديكساميساسون ، حيث تم تقدير حيوية الخلايا والموت المبرمج بواسطة إختبار MTT والتدفق الخلوى على التوالي، كما تم إستخلاص الرنا (RNA) من الخلايا وإستخدامة لتقدير مستويات تعبير الجينات Txr1و CYP3A4 و CYP2C8.

أظهرت النتائج زيادة مقاومة الخلايا للباكليتاكسيل النقى عن التاكسول، كما أن الخلايا كانت أكثر إستجابة للباكليتكسل من التاكسول فى وجود الديكساميساسون، حيث قلت قيمة الـ (IC_{50}) من ٤٢,٥ إلى ١٣,٠٧ ميكروجرام/مل و من ٦,٥ إلى ٣,٦ ميكروجرام/مل على التوالي، وكان الموت المبرمج للخلايا هو الآلية الرئيسية للتسمم الخلوي في الخلايا المعالجة بـالباكليتاكسيل أو التاكسول. كما أن وجود الديكساميساسون قلل نسب الخلايا التى تموت عبر الموت المبرمج ، علاوة على ذلك إنخفض تعبير جين مقاومة الباكليتاكسيل (Txr1) بنسبة ١٨٪ و ٣٥٪ في الخلايا المعالجة تزامنياً بالباكليتاكسيل مع الدكساميساسون أو تلك المعالجة بالتاكسول مع الديكساميساسون على التوالى، وبالتوازى لوحظت زيادة فى تعبير جينات أيض الباكليتاكسيل (CYP3A4 و CYP2C8) مقارنة بالخلايا التى لم تعالج بالديكساميساسون.

وتخلص هذه الدراسة المعملية لإقرار مصاحبة زيادة إستجابة خلايا الكبد السرطانية إلى الباكليتاكسيل أو التاكسول في وجود ديكساميساسون مع إنخفاض مقاومة الخلايا للعقارين مع زيادة نشاط جينات المسؤولة عن إيض الباكليتاكسيل.