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The Effect of Doxorubicin And Thioridazine on Squamous Carcinoma Cell Line

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ABSTRACT

Background: Head and neck squamous cell carcinoma (HNSCC) is one of the most common cancers occupying the sixth position worldwide. PI3k/Akt signaling pathway plays a significant role in regulating diverse cellular functions. This includes cell growth, proliferation and survival via inhibition of apoptosis, transcription and protein synthesis. One of the most acknowledged and approved chemotherapeutic agents is Doxorubicin (DOX) which is a non- selective class I anthracycline. In spite of DOX being one of the most acknowledged chemotherapeutic agents, its use has been limited by its toxic side effects and the development of chemoresistance. Nowadays, Thioridazine (TZ) which is a newly repurposed drug that was originally used in the treatment of psychosis, schizophrenia and anxiety, has been tested in the treatment of breast, ovarian, gastric cancers and leukemias.

Methods: MTT was employed to assess the effect of TZ and DOX on the separately and in combination, with different doses and durations on the HEp2 cell line. The efficacy of both drugs was evaluated separately and in combination with different doses and durations on inhibition of cell migration on the HEp2 cell line.

Results: The results of this study revealed that the highest viability was with the control group followed by the TZ group I (low dose), compared to the other groups. While it showed that the lowest mean viability was with the combination group VI (high dose) compared to other groups. Regarding the mean values of cell migration, the lowest mean value was noted in the combination group III, and the highest mean values was noted in the control group, followed by the DOX group I.

Conclusion: From the results of this study it can be concluded that combination group of DOX an d TZ is a promising treatment for HEp2 cell line.

1. INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is one of the most common cancers occupying the sixth position worldwide. More than 600,000 cases are diagnosed annually and are often lethal, with a minority of HNSCC patients being able to survive 5 years after their diagnosis [1]. In spite of the emergence of new treatment modalities, the mortality rates are still high owing to the development of chemoresistance [2,3].

Apoptosis is one of the main mechanisms by which cytotoxic drugs mediate their effect. Failure of activation of the apoptotic pathway represents an important mode of chemoresistance.

Survival signals induced by several receptors are mediated mainly by the phosphatidylinositol 3-kinase/Akt (PI3k/Akt) signaling pathway. Hence this pathway may decisively contribute to the resistant phenotype [4].

PI3k/Akt signaling pathway plays a significant role in regulating diverse cellular functions. This includes cell growth, proliferation and survival via inhibition of apoptosis, transcription and protein synthesis. The activation of Akt inhibits apoptosis, increases migration, metabolism and enhances cell cycle induction. PI3k/Akt signaling pathway is dys-regulated in many human cancers making it an appealing target for cancer therapy [5].

One of the most acknowledged and approved chemotherapeutic agents is Doxorubicin (DOX) which is a non-selective class I anthracycline. This drug is used in the treatment of various cancers among of which are lung, gastric, breast, thyroid, ovarian and Hodgkin's and non-Hodgkin's lymphoma[6,7].

In spite of DOX being one of the most acknowledged chemotherapeutic agents, its use has been limited by its toxic side effects, and the development of chemoresistance. One of the mechanisms involved in developing drug resistance is the up regulation of the PI3k/Akt pathway which transmits anti-apoptotic and survival signals[8,9].

Nowadays, Thioridazine (TZ) which is a newly repurposed drug that was originally used in the treatment of psychosis, schizophrenia, and anxiety. TZ belongs to the phenothiazine drug group and is an antagonist of the dopamine receptor D2 family of proteins. Lately, it was shown that patients suffering from schizophrenia who were receiving TZ, had a lower risk of developing cancer [10].

Recently, TZ has been tested in the treatment of breast, ovarian, gastric cancers and leukemias. Investigations showed that TZ reduced cell proliferation which was mediated through cell cycle arrest. Furthermore, it was found that TZ has an anti-proliferative effect that could be attributed to the inhibition of PI3k/Akt signaling pathway [11-13].

The emergence of TZ as an anticancer therapeutic agent is exciting with the benefit of it being moderately safe for use for over 40 years of psychosis therapy, as well as having the potential to serve as an adjuvant with anticancer agents. But further studies should be done in order to transition it a from bench to a bedside cancer treatment [11].

Our study was conducted to evaluate the effect of TZ and DOX separately and in combination, with different doses and durations on the HEp2 cell line.

2. MATERIALS AND METHODS

Materials

Doxorubicin hydrochloride (1mg), 98.0-102.0% (HPLC), Thioridazine hydrochloride (5g), 99% were purchased from sigma Aldrich. The HEp2 cell line was supplied from the Cell Culture Department of the holding company for biological products and vaccines-VACSERA-Egypt. Human laryngeal squamous cell carcinoma, HEp2, cell line was purchased from the CellCulture Unit - VACSERA, Egypt. HEp2 cells were imported from the "American Type Culture Collection (ATCC)" in the form of frozen vials.

Cell Culture

HEp2 cells were cultured in Dulbeco"s Modified Eagle's Medium (DMEM) supplemented in 10% fetal bovine serum (FBS) (Cambrex BioScience, Copenhagen, Denmark), and 1% streptomycin-penicillin. The cells were grown at 37°c in a humidified atmosphere of 5% CO2 in air.

Cell Viability Assay

The HEp2 cell line was divided into 20 groups. Groups I and II were the control groups after 24, and 48 hrs, respectively. Groups from 3 to 8 were treated with different doses (low dose, intermediate dose and high dose) of TZ for 24 and 48 hours. Groups 9 to 14 were treated by different doses (low dose, intermediate dose and high dose) of DOX for 24 and 48 hours. Groups from 15 to 20 were treated with different doses (low dose, intermediate dose, and high dose) of combined TZ and DOX, for 24 and 48 hours.

Cells were incubated with or without TZ, DOX and combinations of TZ and DOX, in 96-well plates ($5x10^3$ cells / well) for 24 h, cell viability was evaluated using the MTT assay. The dosages of each drug were selected based on previous studies.

Cell migration assay

HEp2 cell were treated with or without TZ, DOX, combination of TZ and DOX (0.5, 5, 10 µg/ml) for 24 hrs in six-well plates, then were harvested and resuspended in serum DMEM with 0.1% bovine serum albumin. About 200µL of the cell suspension was added onto the insert filter and then placed into the lower well containing 500µL of 10% FBS/DMEM. Transwell plates were incubated at 37˚C with 5% CO2 for 24 hrs. Unmoved cells on the filters were removed using cotton swabs, and cells under the filters were fixed with dehydrated alcohol and stained with crystal violet. The number of migrated cells was counted under a microscope.

3. RESULTS

Using ANOVA, control group V showed statistically significant differences, compared with all other groups.

There was no statistically significant difference upon comparing TZ group VI with TZ group V and DOX group IV, V. There was no statistically significant difference between TZ group V, TZ group VI, DOX group VI, V, and combination groups IV. TZ group VI showed statistically significant difference between all groups except DOX group IV, V, VI and combination groups IV, V and VI (they showed statistically significantly lowest mean viability).

There was a statistically significant difference between DOX group VI with all the other groups except DOX group V, and combination group IV. On comparing DOX group V with all the other groups, there was a statistically significant difference with all the other groups except DOX group VI and combination groups VI, V and VI. There was a statistically significant difference between combination group VI with all the other groups except combination group V and VI.

Regarding the comparison of viability between all studied groups at 24 and 48 hours, all the drugs at different concentrations showed no statistically significant change in mean viability after 48 hours using the Wilcoxon signed-rank test.

Regarding the mean values of migrated cells after 24 hrs, the lowest mean value of migrated cell was noted in the combination group III, and the highest mean value was noted in the control group I followed by the DOX group I (fig.1-3)

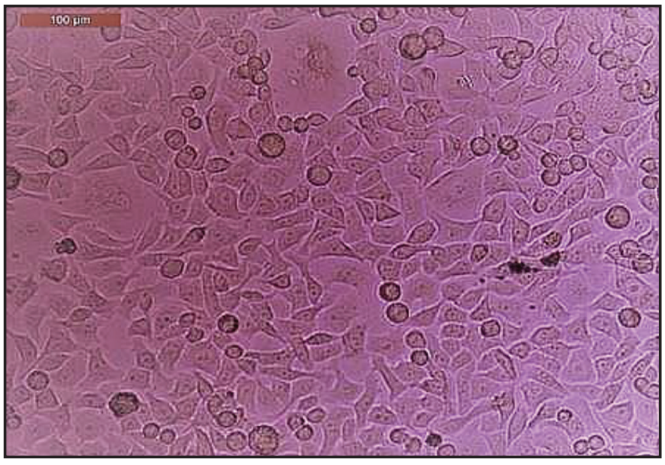

Figure1— A photomicrograph of the untreated HEp2 cells after 24 hrs

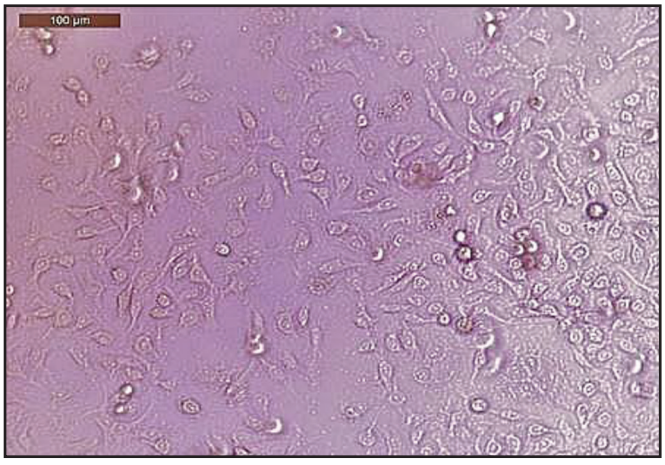

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Figure 2 — A photomicrograph of the migrated cells after treatment with DOX (DOX group I)

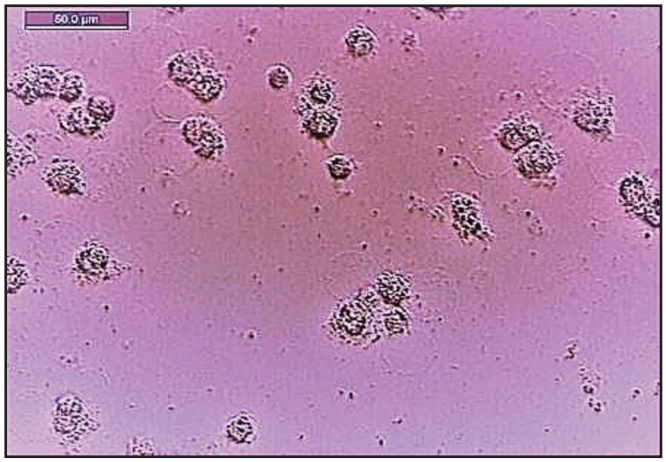

Figure 3 — A photomicrograph of the migrated cells after treatment with combination of DOX and TZ (comb III)

4. DISCUSSION

TZ a phenothiazine is an antipsychotic agent used in treating psychosis and schizophrenia [12]. Yet, there are an increasing number of studies demonstrating the anticancer effects of TZ. These anticancer effects include TZ induced apoptosis' inhibition of angiogenesis and metastasis in cancer cells˙ Moreover, TZ was shown to be a selective inducer of CSC differentiation. Researchers have attributed its effects to the inhibition of the PI3K/Akt and the mTOR signaling pathways and the antagonism of the D2 family.

Twenty groups of the HEp2 cell line were included in this study: two control groups. Six groups were treated with different doses of TZ. Another six groups were treated with different doses of DOX. Additionally, six groups were treated with different doses of combined TZ and DOX. All groups were evaluated after 24 and 48 hours. The doses were prepared according to the calculated IC50 value as referenced in the study group.

The value of the doses used in this study were calculated based on the previous studies [12-18] and the doses used were estimated as follows: a dose of (0.5, 5 and 10 µg) concentration of TZ, a dose of (0.5, 5 and 10 µg) concentration of DOX, and a dose of (0.5, 5 and 10 µg) of a combination of TZ and DOX.

MTT results showed the highest viability with the control group followed by the TZ group I (low dose), compared to the other groups. While it showed the lowest mean viability with the combination group VI (high dose) compared to other groups. Even though the best effect on the cell viability were obtained by combination group VI (high dose), there was no significant difference between combination group I (low dose) and combination group VI (high dose). Further studies are required to see the feasibility of using low doses of the drugs in order to avoid high dose induced toxicity of DOX.

The highest viability in the control group (untreated HEp2 cell line) could be attributed to the number of acquired accumulated mutations such as P53 mutations, in activating mutations in NOTCH1 and dys-regulation of PI3k/Akt pathway which has been associated with multiple biological functions, such as regulation of self-renewal capacity, cell cycle exit and survival. All these factors probably resulted in uncontrolled cell proliferation and decreased cell apoptosis that was reflected on the MTT results in this study [19-21].

On the other hand, the least viability that was noted in the combination group VI, could be attributed to the growth inhibitory effects of TZ which include anti-proliferative and anti-apoptotic properties such as the down regulation of cyclin D1 and cyclin dependent kinase 4 (CDK4), which are associated with the transition from G1 to S phase, the up -regulation of the CDK inhibitors p16 and p27, which interrupts cell cycle procession at the G1 or G2/M phase. Also, it up- regulates anti-apoptotic markers such as Bax and p53 [22-26].

Furthermore, the cell migration ability results showed that the control group had the highest migration cell count compared to other groups followed by the DOX group I. This could be explained by the fact the DOX treatment alone was found to enhance metastasis. This was because DOX caused up regulation of EMT related genes[27,28]

The least migration ability was noted in the combination group III, this was attributed to the fact that TZ was found to suppress stmeness genes such as CD133 and OCT4. In addition, it inhibited cell migration and cell motility through suppression of EMT related genes.

In conclusion, in spite of the fact that DOX markedly reduced the viability of cancer cells, it didn't inhibit metastasis. Where the difference in the migration ability of the cells between DOX treated and control groups was insignificant. However, the combination group was able to overcome DOX's drug limitations by inhibiting PI3K/Akt pathway, inhibiting metastasis via TZ's anticancer effects. Consequently, the combination group enhanced DOX's cytotoxic effects in addition to the anticancer effects of TZ (acting synergistically). Leading to the best outcome compared to other groups in eradicating cancer cells.

5. REFERENCES

1- Leemans C., Braakhuis B. and Brakenhoff B.: The molecular biology of head and neck cancer. Nat Rev Cancer, 2011; 11: 9-22.

2- Goon P., Stanley M., Ebmayer J., Steinstrsässer L., et al.: HPV and head and neck cancer: a descriptive update. Head Neck Oncology, 2009; 14: 1-36.

3- Husain S. and Puri R.: Identification and characterization of cancer stem cells in human head and neck squamous cell carcinoma. Bio Med Central Cancer, 2014; 14:1-11.

4- Fresno A., Casado E., de Castro J., et al.: PI3K/Akt signaling pathway and cancer. Cancer Treat Rev, 2004; 30:193-204.

5- Wan X., Harkavy B., Shen N., et al.: Rapamycin induces feedback activation of Akt signaling through an IGF-IR dependant mechanism. Oncogene, 2007; 26: 1932-1940.

6- Carvalho C., Santos X., Cardoso S, et al.: Doxorubicin: the good, the bad and the ugly effect. Curr Med Chem, 2009; 16: 3267-3285.

7- Kang W. and Yan S.: Piperlongumine reverses doxorubicin resistance through the PI3K/Akt signaling pathway in K562/A02 human leukemia cells. Exp Ther Med, 2015; 9: 1345-1350.

8- Swift P., Rephaeli A., Nudelman A., et al.: Doxorubicin- DNA adducts induce a non-topoisomerase II- mediated form of cell death. Cancer Res, 2006; 66: 4863-4871.

9- Tacara O., Sriamornsakb P. and Dass C.: Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol, 2012; 65: 157-170.

10- Chou F., Tsai K., Su C., et al.: The incidence and relative risk factors for developing cancer among patients with schizophrenia: a nine-year follow-up study. Schizophr Res, 2011; 129: 97-103.

11- Sachlos E., Risueño R., Laronde S., et al.: Identification of drugs including a dopamine receptor antagonist that selectively target cancer stem cells. Cell, 2012; 149: 1284-1297.

12- Cragg G., Grothaus P., Newman D. et al.: New horizons for old drugs and drug leads. J Nat Prod, 2014; 77: 703-723.

13- Nitulescu G., Van De Venter M., Nitulescu G. et al.: The Akt pathway in oncology therapy and beyond. Int J Oncol, 2018; 53: 2319-2331.

14- Spengler G., Molnar J., Viveiros M. et al.: Thioridazine induces apoptosis of multidrug-resistant mouse lymphoma cells transfected with the human ABCB1 and inhibits the expression of P-glycoprotein. Anticancer Res, 2011; 31: 4201-4205.

15- Chien W., Sun Q., Lee K. et al.: Activation of protein phosphatase 2A tumor suppressor as potential treatment of pancreatic cancer. Molecular Oncol, 2015; 9: 889-905.

16- Harbottle, A., Daly A., Atherton K. et al.: Role of glutathione S-transferase P1, P-glycoprotein and multidrug resistance-associated protein 1 in acquired doxorubicin resistance. Int J Cancer, 2001; 92: 777-783.

17- Fic M., Podhorska-Okolow M., Dziegiel P. et al.: Effect of melatonin on cytotoxicity of doxorubicin toward selected cell lines (human keratinocytes, lung cancer cell line A- 549, laryngeal cancer cell line Hep-2). In Vivo, 2007; 21: 513-518.

18- Al-Ghamdi S.: Time and dose dependent study of doxorubicin induced DU-145 cytotoxicity. Drug metab Lett, 2008; 2: 47-50.

19- Kovaříková, P., Michalova E., Knopfová L. et al.: Methods for studying tumor cell migration and invasiveness. Klin Onkol, 2014; 27:1-10.

20- Pei S., Wang J, Wang X. et al.: Correlation of survivin, p53 and Ki-67 in laryngeal cancer Hep-2 cell proliferation and invasion. Asian Pac J Trop Med, 2015; 8: 636-642.

21- Shen N, Zhou X. et al.: ShRNA lentivirus mediated knockdown of RECQL5 enhances the tumor malignancy of Hep-2 laryngeal squamous carcinoma cells. Int J Clin Exp Pathol 2017;10:5545-5551.

22- Gao H. , Hai D , Guijun L. et al.: Combined treatment with acetazolamide and cisplatin enhances chemosensitivity in laryngeal carcinoma Hep-2 cells. Oncol Lett, 2016; 15:9299-9306.

23- Meng Q., Sun X., Wang J. et al.: The important application of thioridazine in the endometrial cancer. Am J Transl Res, 2016; 8: 2767-2775.

24- Chen T., Hu Y., Liu B. et al.: Combining thioridazine and loratadine for the treatment of gastrointestinal tumor. Oncol Lett, 2017; 14: 4573-4580.

25- Otręba M., Beberok A., Wrześniok D. et al.: Effect of thioridazine on antioxidant status of HEMn-DP melanocytes. Naunyn-Schmiedebergs Arch Pharmacol, 2015; 388: 1097- 1104.

26- Baig M., Roy A., Saqib U.: Repurposing Thioridazine (TDZ) as an anti-inflammatory agent. Sci Rep, 2018; 8: 1-10.

27- Han R, Xiong J, Xio R, Liu Y, Wang J, Xu H, et al. Activation of β-catenin signaling is critical for doxorubicin-induced epithelial– mesenchymal transition in BGC-823 gastric cancer cell line.Tumor Biol2013; 34:277- 284.

28- Zhong Z, Tan W, Tian K, Yu H, Qiang W, Wang Y. Combined effects of furanodiene and doxorubicin on the migration and invasion of MDA-MB-231 breast cancer cells in vitro.Oncol Rep 2017; 37:2016-2024.

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