ISSN: 1343-4292
Volume 140, Issue 04, July, 2022

Comparison Cytotoxicity effects of metformin and paclitaxel on cervical cells and normal cell

Abeer Mansour Abdel Rasool[1], Isam Hamo Mahmood[2]

Department of Pharmacology, University of Mosul, Mosul, Iraq[1]
Department of Pharmacology, University of Al Noor, Bartella, Iraq[2]

ABSTRACT— Cervical cancer, which is generally caused by the Human Papilloma Virus (HPV) in-fection is the first prevalent gynecological cancer worldwide. Paclitaxel, a new anti-cancer agent has a wide spectrum of antineoplastic activity the first microtubule-stabilizing agent. It has in vitro cytotoxicity against human ovary, breast, cervical, pan-creas, prostate, head, and neck, colon, gastric, bladder, lung, and CNS cancers, melano-ma, and leukemia cell lines, often at concentrations lower than those achieved in the serum of patients. Metformin has been widely utilized as an anti-diabetic drug due to its excellent therapeutic effect on sugar levels and very minor adverse effects. Metformin used in present study, Human cervical cancer cell lines, Cy-totoxicity assays and Determination maximum inhibitory concentration. Current results showed that a comparison of the Influence of metformin with the similar dose of paclitaxel showed the non-significant difference between the two drug and the cytotoxic effects of paclitaxel rather than metformin on normal breast cells after 72 hours when applied to HBL100 and suggestion a therapeutic antitumor impact of metformin that is less harmful effect to normal tissues IC 50 on (HBL10048.910) in contract to IC50 of metformin (Hela 7.447). The present study confirms on the therapeutic value of metformin in patients with cervical cancer.

KEYWORDS: Cervical Cancer, Metformin, Paclitaxel, IC50.

1. INTRODUCTION

Numerous researches have advocated that metformin may has a role in the treatment of cancer. These studies have examined the possible impacts of metformin in terms of cancer therapy and prevention. When used alone or in conjunction with other drugs, Metformin has been widely utilized as an anti-diabetic drug due to its excellent therapeutic effect on sugar levels and very minor adverse effects. A well-known medication for the management of T2DM is metformin. an addition to has anticancer effects on cervical cancer both in vitro and in vivo. Thus, metformin may be used in conjunction with other treatments to treat cervical cancer [1]. One of the main gynecologic ma-lignancies, cervical cancer, is a problem for the world's public health.

The disease's stage is a major factor in determining the prognosis and best course of treatment [2]. Chemotherapy, surgical removal, radiotherapy, or a combination of these treatments are available as treatment options; nonethe-less, relapse and recurrence are possible, and the prognosis may not be posi-tive.

Higher molecular therapy choices may be developed with a better under-standing of relevant molecular biological variables. Higher molecular therapy choices may be developed with a better understanding of relevant molecular biological variables.

Galega officinalis is a plant from which the natural substance metformin, al-so known as galegine, is

obtained. Since the 1950s, it has been routinely used to treat diabetes. In populations with type 2 diabetes and congestive heart failure, the use of metformin lowers all-cause mortality [3].

Recently, researchers looked at the potential anticancer effects of metformin in non-diabetic cancer patients, including those with lung, breast, and pros-tate cancer; however, the findings are debatable [4].

Since metformin is normally exclusively prescribed to diabetic patients and the majority of cervical cancer patients are young and non-diabetic, its poten-tial impact in non-diabetic women with cervical cancer is still unknown [5].

Recent research demonstrates that metformin can enhance cellular apoptosis, stimulate the AMPK pathway, and block the mTOR/AKT pathway [6].

many studies have been conducted on the potential effects of metformin in the treatment and prevention of cancer [6]. Diabetes may contribute to the onset and development of particular types of cancer as it is possibly linked to an elevated site-specific cancer risk [7]. The meta-analysis also showed that diabetes was related to patients with cervical cancer having a worse progno-sis [8]. Not all of the research now underway, nevertheless, focus on the predictive value of metformin in cervical cancer patients. According to [5] there is no connection between taking metformin and a woman’s chance of surviving cervical cancer [8].

An epidemiologic study that examined the relationship between metformin use and oncological outcomes in cervical cancer patients with type 2 diabetes found that metformin use was associated with a lower recurrence rate than metformin non-use furthermore [9]. Numerous epidemiological studies have shown a link between type 2 diabetes and a higher chance of developing several cancers, including cervical cancer. In addition, type 2 diabetes may have an effect on the prognosis for cervical cancer patients [10].

Both in vitro and in vivo, metformin has antitumor effects. Both direct and indirect effects of metformin exhibit anticancer properties. Reduced levels of insulin and glucose in the bloodstream as well as the inhibition of tumour growth are examples of direct effects. Cancer progression and tumorigenesis are two examples of indirect effects [11].

Other ways by which metformin may inhibit cancer cells include the sup-pression of the mTOR pathway and the induction of autophagy. It is essential to comprehend the molecular processes behind metformin's antitumor activity. potential metformin anticancer benefits against cervical cancer and talked about potential underlying mechanisms [12]. The bark of Taxus brevi-folia served as the source of the new anticancer drug paclitaxel, which has a broad spectrum of anti-neoplastic activity. Human ovary, breast, cervical, pancreatic, prostate, head and neck, colon, stomach, bladder, lung, and CNS cancers, as well as melanoma and leukaemia cell lines are all susceptible to it in vitro, frequently at doses lower than those found in patient serum. The first microtubule-stabilizing drug discovered, paclitaxel, is regarded as the most important development in chemotherapy in the previous 20 years. With widespread activity in a number of malignancies, including breast cancer, endometrial cancer, non-small-cell lung cancer, bladder cancer, and cervical carcinoma, it is regarded as one of the most often used antineoplastic medi-cations. It serves as a second-line treatment for Kaposi sarcoma linked to AIDS [13].

2. Materials and method

ISSN: 1343-4292
Volume 140, Issue 04, July, 2022

2.1 drug used in present study

Metformin (Samara company Iraq), Paclitaxel injection I.P(Taxeleon)® 100mg /16.67ml Neon brand. Made in India.

2.2 cell lines

Current study used Human cervical cancer cell lines (HeLa), normal human HBL100 cell lines (HBL100), cell is maintained in RPMI-1640 that has been supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 g/mL streptomycin. The cells were passaged using trypsin-EDTA. twice weekly reseeded at 50% confluence and incubated at 37°C and 5% CO2.

2.3 Cytotoxicity assays [14]

The MTT cell viability assay was performed on 96-well plates. Cell lines were planted at a density of 1* 104 cells per well. After 24 hours and when a confluent monolayer was produced, cells were treated with Metformin and Paclitaxel then reported the Cytotoxicity on normal cell & cervical can-cer cell calculated the data by the equation below:

Cell viability% = Mean OD/Control OD * 100%.

2.4 Determination maximum inhibitory concentration (IC 50)

Using non-linear regression, the half maximum inhibitory concentration (IC50) values were extracted from survival-concentration curves. Graph Pad Prism (version 8) was used to determine the IC50 for metformin after 72 hours in all cancer and healthy cell lines. then consider how metformin acts to this cell by studying other markers such as Paclitaxel injection's greater cytotoxic effects and concentration on the same cancer cell line over time and at a reasonable concentration [15].

2.5 Statistical analysis

The Statistical Software for Social Sciences (SPSS), version 24, was used to conduct the statistical analyses, and it is used to express the results. using conventional statistical methods, determine the mean and standard error. To identify significant differences between and within groups, P-values were computed using analysis of variance (ANOVA) and the Duncan test post hoc. The results were performed and presented as means SEM using the statistical software Graph Pad Prism version 8. P values * denote significant when p 0.05, and ** denote highly significant when p 0.01.

3. Results

3.1 Effect of metformin and Paclitaxel on human cervical cell line(HeLa)

Using MTT tests, the cytotoxic effect of metformin on the human cervical cell line has been evaluated for 72 hours. as shown in table (1). Three repli-cates' worth of statistical analysis reveals a substantial difference in actual points. A range of metformin concentrations was applied to the concertation (0,5,10,25,35,45,65,130 µM) and the effect of paclitaxel as the same concer-tation of metformin as shown in Table (2) demonstrated a comparison of the Influence of metformin with the similar dose of paclitaxel showed the non-significant difference between the two drug.

Table (1): Effect of metformin on viability of cervical cancer cells line (Hela) after72 hours cell viability was measured by MTT assay.

Cytotoxic effect of metformin on human cervical cancer cell line (hela) cell lines after 72 hours.

Met. Conc µM	0	5	10	25	35	45	65	130	P-Value
Mean Via-bility	97.517 d	73.132 c	43.057 b	22.452	15.649 a	15.305 a	15.279 a	15.081 a	**0.000****
SEM	0.324	1.372	1.602	0.754	0.272	0.135	0.251	0.1677	

Means with standard error of mean different superscript different small letter (a, b, c, d) are significantly difference between different group in same period (Dunken Test)
*p<0.05 mean significant ** mean p<0.01 highly significant.

Table (2): A comparison of the influence of metformin with a similar dose of paclitaxel (anti-tumor) on cervical cancer cell lines (Hela) after 72 hrs.

Concentration (µM)	Metformin		Paclitaxel		*P*- value
	Mean	SEM	Mean	SEM	
0	**97.563 A**	0.3703	**98.334 A**	0.489	**0.184 NS**
5	**73.132 B**	1.372	**56.341 A**	1.733	**0.000***
10	**43.057 B**	1.602	**28.385 A**	2.036	**0.000***
15	**22.452 B**	0.754	**17.442 A**	1.131	**0.000***
35	**15.649 A**	0.272	**15.763 A**	0.621	**0.847 NS**
45	**15.305 A**	0.135	**15.059 A**	0.537	**0.716 NS**
65	**15.279 A**	0.251	**14.883 A**	0.048	**0.275 NS**
130	**15.081 A**	0.167	**14.531 A**	0.102	**0.060. NS**

* (P≤0.05) significant, NS: Non-significant.
The independent T test same letter no difference, difference letter significant difference

3.2 Effect of metformin and Paclitaxel) on HBL100 (normal breast cell) after 72 hours

Table (3) observed the impact of metformin on normal cell line (normal breast cell) after 72 hrs. incubation. It was reported slight effect on variability of normal cell line which was non-significant (p=0.0624). A demonstrated the cytotoxic effects of paclitaxel rather than metformin on normal breast cells after 72 hours when applied to HBL100. as table (4).

Table (3): Effect of Metformin on HBL100 (normal breast cell) after 72 hours

ISSN: 1343-4292
Volume 140, Issue 04, July, 2022

Cytotoxic effect of metformin on HBL100 (normal breast cell) after 72 hours.									
Conc.	**0**	**5**	**10**	**15**	**35**	**45**	**65**	**130**	***P*-Value**
Mean Viability	99.636 b	98.848 b	98.219 b	95.547 ab	94.329 a	94.132 a	93.688 a	86.518 a	**0.0624**
SEM	0.086	3.721	4.539	1.886	1.735	2.322	1.373	2.498	

Means with different superscript different small letter (a,b) are significantly difference between different group in same period (Dunken Test) *p<0.05 mean significant ** mean p<0.01 highly significant.

Table (4): Comparison the effect of metformin with the same dose of paclitaxel (anti-tumor) on HBL100 (normal breast cell) after 72 hours

Concentration (Mm)	**Metformin**		**Paclitaxel**		***P*- value**
	Mean	**SEM**	**Mean**	**SEM**	
0	**99.636**	0.086	**98.775**	0.229	0.025 *
5	**98.848**	3.721	**66.593**	3.743	0.000 *
10	**98.219**	4.539	**45.851**	0.773	0.000 *
15	**95.547**	1.886	**32.872**	0.320	0.000 *
35	**94.329**	1.735	**32.811**	0.962	0.000 *
45	**94.132**	2.322	**32.569**	0.468	0.000 *
65	**93.688**	1.373	**31.901**	0.696	0.000 *
130	**86.518**	2.498	**31.356**	0.457	0.004*

* ($P \leq 0.05$) significant, NS: Non-significant.
The independent T test same letter no difference, difference letter significant difference

3.3 Determination maximum inhibitory concentration (IC 50) metformin on human cervical cell line(HeLa)

To assess the effect of metformin on cell proliferation, Low survival cell value shows damaging effect if survival cell values are less than 60%, according to IC50 value measurements in normal and cancer cell lines. The- se medications were select for additional dose-response studies in cancer cell lines. The metformin's IC50 values were used to measure its activity. The active compounds were those with IC50 values less than 10 M. In this inves- tigation, the metformin IC 50 on (Hela 7.447). This would suggest a thera- peutic antitumor impact that is less harmful effect to normal tissues IC 50 on (HBL10048.910). and as showed in figure (1) the effect of metformin on Hela &normal cell HBL100.

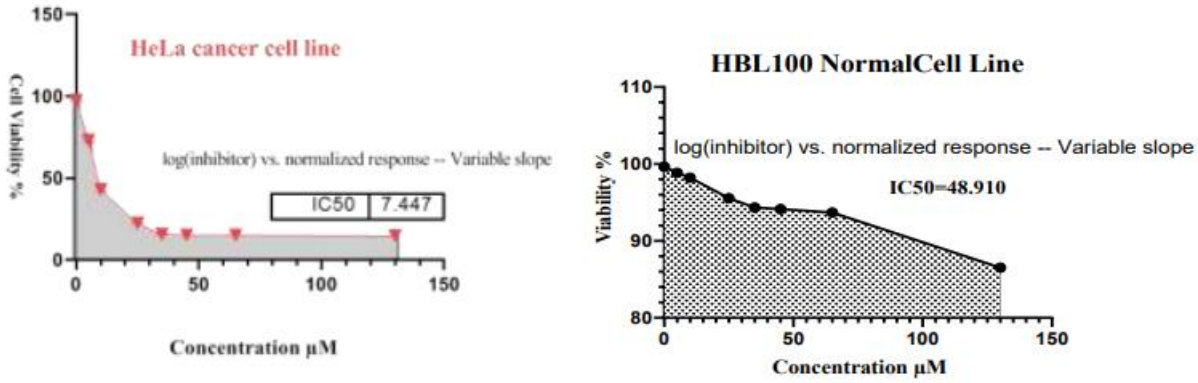

Figure (1): Demonstrated the IC50of metformin on Hela and on the normal cell

4. Discussion

Because of its excellent therapeutic effect on sugar levels and relatively mild side effects, metformin has been used extensively as an anti-diabetic medi- cine. metformin is a recognized drug for the treatment of T2DM [16]. Con- sidering that metformin is hydrophilic and cationic at physiological pH. It is exceedingly unlikely that it rapidly diffuses over the cell membrane and af- fects how cells operate.

Metformin is anticipated to have favorable financial impacts and raise the likelihood that cancer patients will survive because of its affordable market pricing in comparison to other cancer treatment drugs and its adaptability in how it is delivered in cancer therapy [17].

Because MTT is a colorimetric, enzyme-based method for evaluating the ac- tivity of mitochondrial dehydrogenase in cells, it is frequently used to assess the viability and cytotoxicity of cells. It is also safe, easy, and sensitive [18]. The fourth leading cancer in women and the fourth leading cause of cancer death is cervical cancer [14].

Metformin blocks HeLa cell expansion It was hypothesized that synthetic drugs with an IC50 (the potency of the drug, in inhibiting cancer cell lines) of less than 10 M could be used as anticancer drugs in vitro. The IC50 is a quantitative measure that identifies the concentration of a specific inhibitory substance needed to stop the growth of cancer cell lines, such as metformin. As a result, our findings show that the IC50 value for metformin is 7.365 M, which could Cytotoxicity [5].

these result were in agreement with certain studies [19], [20] but additional studies were disagree with [21].

Explanation of agreement study as the following Metformin is an anti- diabetic It is currently receiving a lot of attention as an anti-tumor therapy. especially after multiple study teams confirmed its ability to fight cancer by boosting apoptosis [22].

Metformin's role in cervical cancer's mechanism Insulin resistance, which results in secondary hyperinsulinemia, is the mechanism most usually postulated to explain the link between diabetes and cancer. Additionally, in- sulin may exert mitogenic effects through the insulin-like growth factor 1 (IGF-1) receptor [11].

Metformin exhibits anticancer actions via both direct and indirect ef- fects. Understanding the molecular mech-anisms underlying the anticancer effect of metformin is crucial. Metformin may inhibit cancer cells through various other mechanisms, such as mTORpathway inhibition and autophagy induction [23].

However, excessive doses of metformin may be damaging to normal cells because there was a significant decline in cell viability over a period of 72 hours compared to the control group. The current investigation found that metformin did not restrict the proliferation of normal cells at therapeutically possible levels.

These outcomes following Metformin exposure conflict with those of a different study by [5]. It utilized regular human gall bladder cells (GBC), According to these findings, metformin inhibits cell growth and promotes apoptosis. It has also demonstrated the potential to selectively target cancer cells without harming healthy ones. by another research [24]. It was shown that although metformin does not produce ROS formation in normal cells, it increases superoxide production in pancreatic cancer cells through inhibiting mitochondrial complex I activity [25].

5. Conclusion

The present study confirms on the therapeutic value of metformin in pa- tients with cervical cancer with not impact on the normal cancer when compare with paclitaxel. metformin for the treatment of type 2 diabetes, metformin is a proven, secure, and well-tolerated medication. Considerable research has suggested that metformin may be used to treat cancer.

6. Reference

[1] Bray F, Ferlay J, Soerjomataram I, Siegel LR, Torre AL, Ahmedin DVM. GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Glob cancer Stat 2018.

[2] Ch PN, Gurram L, Chopra S, Mahantshetty U. The management of locally advanced cervical cancer. Curr Opin Oncol. 2018;30(5):323–9.

[3] Flory J, Lipska K. Metformin in 2019. Jama. 2019;321(19):1926–7.

[4] Chen K, Li Y, Guo Z, Zeng Y, Zhang W, Wang H. Metformin: current clinical applications in nondiabetic patients with cancer. Aging (Albany NY). 2020;12(4):3993.

[5] Takiuchi T, Machida H, Hom MS, Mostofizadeh S, Frimer M, Brunette LL, et al. Association of metformin use and survival outcome in women with cervical cancer. Int J Gynecol Cancer. 2017;27(7).

[6] Lu C-C, Chiang J-H, Tsai F-J, Hsu Y-M, Juan Y-N, Yang J-S, et al. Metformin triggers the intrinsic apoptotic response in human AGS gastric adenocarcinoma cells by activating AMPK and suppressing mTOR/AKT signaling. Int J Oncol. 2019;54(4):1271–81.

[7] Suissa S, Azoulay L. Metformin and cancer: mounting evidence against an association. Diabetes Care. 2014;37(7):1786–8.

[8] Hanprasertpong J, Jiamset I, Geater A, Peerawong T, Hemman W, Kornsilp S. The effect of metformin on oncological outcomes in patients with cervical cancer with type 2 diabetes mellitus. Int J Gynecol Cancer. 2017;27(1).

[9] Anastasi E, Filardi T, Tartaglione S, Lenzi A, Angeloni A, Morano S. Linking type 2 diabetes and gynecological cancer: An introductory overview. Clin Chem Lab Med. 2018;56(9):1413–25.

[10] Thakkar B, Aronis KN, Vamvini MT, Shields K, Mantzoros CS. Metformin and sulfonylureas in

relation to cancer risk in type II diabetes patients: a meta-analysis using primary data of published studies. Metabolism. 2013;62(7):922–34.

[11] Weinstein D, Simon M, Yehezkel E, Laron Z, Werner H. Insulin analogues display IGF-I-like mitogenic and anti-apoptotic activities in cultured cancer cells. Diabetes Metab Res Rev. 2009;25(1):41–9.

[12] Chen Y-H, Yang S-F, Yang C-K, Tsai H-D, Chen T-H, Chou M-C, et al. Metformin induces apoptosis and inhibits migration by activating the AMPK/p53 axis and suppressing PI3K/AKT signaling in human cervical cancer cells. Mol Med Rep. 2021;23(1):1.

[13] Montero P, Pérez-Leal M, Pérez-Fidalgo JA, Sanz C, Estornut C, Roger I, et al. Paclitaxel Induces Epidermal Molecular Changes and Produces Subclinical Alterations in the Skin of Gynecological Cancer Patients. Cancers (Basel). 2022;14(5):1146.

[14] Al-Shammari AM, Alshami MA, Umran MA, Almukhtar AA, Yaseen NY, Raad K, et al. Establishment and characterization of a receptor- negative, hormone-nonresponsive breast cancer cell line from an Iraqi patient. Breast Cancer Targets Ther. 2015;7:223.

[15] Rasool A, Mahmood IH. Evaluation of Cytotoxic Effect of Metformin on a Variety of Cancer Cell Lines. Clin Schizophr Relat Psychoses. 2021;15(3).

[16] Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017;60(9):1577–85.

[17] Cano L, Soto-Ospina A, Araque P, Caro-Gomez MA, Parra-Marin MV, Bedoya G, et al. Diffusion Mechanism Modeling of Metformin in Human Organic Cationic Amino Acid Transporter One and Functional Impact of S189L, R206C, and G401S Mutation. Front Pharmacol. 2021;11:587590.

[18] Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. Int J Mol Sci. 2021;22(23):12827.

[19] Saraei P, Asadi I, Kakar MA, Moradi-Kor N. The beneficial effects of metformin on cancer prevention and therapy: a comprehensive review of recent advances. Cancer Manag Res. 2019;11:3295.

[20] Top WMC, Kooy A, Stehouwer CDA. Metformin: A narrative review of its potential benefits for cardiovascular disease, cancer and dementia. Pharmaceuticals. 2022;15(3):312.

[21] Muñoz-Pinedo C, Ruiz-Ruiz C, de Almodóvar CR, Palacios C, López- Rivas A. Inhibition of glucose metabolism sensitizes tumor cells to death receptor-triggered apoptosis through enhancement of death- inducing signaling complex formation and apical procaspase-8 processing. J Biol Chem. 2003;278(15):12759–68.

[22] Chen Y-H, Wang P-H, Chen P-N, Yang S-F, Hsiao Y-H. Molecular and cellular mechanisms of metformin in cervical cancer. Cancers (Basel). 2021;13(11):2545.

[23] Ford, Brian Agius L, E., Chachra SS. The metformin mechanism on gluconeogenesis and AMPK activation: The metabolite perspective. Vol. 21, International Journal of Molecular Sciences. MDPI AG;

2020.

[24] Duo J, Ma Y, Wang G, Han X, Zhang C. Metformin synergistically enhances antitumor activity of histone deacetylase inhibitor trichostatin a against osteosarcoma cell line. DNA Cell Biol. 2013;32(4):156–64.

[25] Sullivan LB, Chandel NS. Mitochondrial reactive oxygen species and cancer. Cancer Metab. 2014;2(1):1–12.