Effects of antidiabetic drug on the anticancer activity of Cisplatin

Ahmed Ibrahim Rashid\textsuperscript{1}, Kaiser N. Madlum\textsuperscript{2}\textsuperscript{*}, Rana Ghaleb\textsuperscript{2}, Hamid Naji Obied\textsuperscript{1}, Sabah H. Enayah\textsuperscript{3}

\textsuperscript{1}. Department of Pharmacology, Collage of Medicine, University of Babylon, Hilla, Iraq.
\textsuperscript{2}. Department of Human Anatomy, Collage of Medicine, University of Babylon, Hilla, Iraq.
\textsuperscript{3}. Department of Biology / Collage of Science / Thi-Qar University, Thi-Qar, Iraq.

\* Corresponding author:
Dr. Kaiser N. Madlum
Department of Human Anatomy/
Collage of Medicine/
University of Babylon,
Hilla, Iraq.
E-mail: kaiser.madlum@gmail.com

Abstract

Although its mechanism of action on cancer cells not well understood, Metformin (Met) is widely used nowadays to improve the anticancer activity of some drugs. Metformin has also been shown to decrease the growth of breast cancer cells and pancreatic cancer in hamsters and delays other types of tumors. The aim of this study was to compare the effects of Cisplatin (Cis) alone and Metformin alone on colon cancer cell line SW480. The results showed that the effect of Metformin on cell proliferation is concentration-dependent. Metformin enhances the proliferation and attachment of colon cancer at lower concentrations. Results showed a significant decrease in cell proliferation and attachment of colon cancer cells after Cisplatin treatment. Results of this study revealed that Cisplatin treatment decreased both proliferation and cell adhesion to the matrix. Combination therapy (Met+Cis) showed promising synergism and enhancement of anticancer activity of Cisplatin on colon cancer cells. We are strongly recommended more investigations to be more specific for using Metformin as general anticancer.

Keywords: Antidiabetic, Anticancer activity, Metformin, Cisplatin.

How to cite this article: Madlum KN, Rashid AI, , e al (2019): Effects of antidiabetic drug on the anticancer activity of Cisplatin, Ann Trop & Public Health; 22(10): S280. DOI: http://doi.org/10.36295/ASRO.2019.221012

Introduction

Metformin is widely used to treat diabetes mellitus type 2\textsuperscript{[1]}, and was shown recently to inhibit different types of cancer stem cell proliferation. Metformin has also been shown to decrease the growth of breast cancer cells\textsuperscript{[2][3]} and pancreatic cancer in hamsters and delays other types of tumors\textsuperscript{[4]}. It is thought to affect glucose production by increasing insulin sensitivity and glucose use by peripheral tissues\textsuperscript{[5]}. Cisplatin is a well-known anticancer drug used for the treatment of various types of cancers. Cisplatin drugs induced apoptosis by increasing the intracellular ROS which is acts as a common
trigger to induce apoptosis in cancer cells. The problem of multidrug resistance still at least understood, and most unpredictable factor affecting Cisplatin. Colon cancer cells show natural abilities in resistance to apoptosis which can be induced by Cisplatin this depends on their spontaneous apoptotic phenotypes. The natural apoptotic phenotypes of colon cancer cells correlate with their chemoresistance can lead to malignancies. However, clinical application is limited by the development of Cisplatin resistance and the toxic side effects of it. About 50% of patients are still not response to Cisplatin due to drug resistance. The response of patient response and development of alternative treatments is an importance in the colon cancer field. Metformin could represent an alternative to conventional Cisplatin. The mode of action of Metformin in colon cancer cells has not yet understood. However, several reports have demonstrated that Metformin induces apoptosis through LKB1-mediated AMPK signaling pathway.

The aim of this study was to compare the effects of Cisplatin (Cis) alone and Metformin alone on colon cancer cell line SW480.

Materials and Methods

Cell line

The colon cell line SW480, was purchased from LONZA Biologics (Slough, UK). Cells were cultured in DMEM medium supplemented with 5% bovine serum (v/v), 1% penicillin - streptomycin. The viable cells number were determined after centrifuging the cells at 1000 rpm for five min and resuspending the cells in fresh growth medium at a cell density of 2 X10^5 cells per ml into vented 50 ml Erlenmeyer flasks with 15 ml of DMEM media (Corning,NY, USA).

Cytotoxicity assay

SW480 cell line was cultured in 96-wellplates and incubated for 24 hours in the presence of different doses of Metformin and Cisplatin in order to detect the effect of a dose response on cell attachment. While the cells cultured in 96-wellplates for 24 hours and then the Metformin and Cisplatin were added to the cultured cells to investigate the effects of these chemicals on their proliferation. The Metformin concentrations used ranged from 32 to 500µg/ml, respectively. While the Cisplatin concentrations used ranged from 12.5 to 200 µg/ml, respectively. The plated cells were divided into the following groups: Group 1 was the control group (not treated). Group 2 received serial dilutions (32, 65, 125, 250, and 500µg/ml) of Metformin. Group 3 received serial dilutions (12.5, 25, 50, 100, 200 µg/ml) of Cisplatin. The cells were incubated at 37°C for 24 hours before counting. Group 4 was cultured in a culture media containing (32 ug/ml) of Metformin for 24 hours then received serial dilutions (12.5, 25, 50, 100, 200 µg/ml) of Cisplatin.

Cell viability assay

Cell viability was measured by crystal violate cytotoxicity assay. Two hundred microliters of target cells with 1x10^6 cells/ml were cultured in 96 well plates and the plate was incubated at 37°C. The media was removed from the plate and washed with 100 µl PBS for 5 minutes. Then the cells were fixed with 10% formalin at room temperature, after 20 minutes the fixative solution was removed. Then the cells were stained with 100µl of 0.1% aqueous crystal violet solution for 20 minutes at room temperature. The dye was eluted by adding 200 µl of 95% ethanol. Absorbance was then measured for each well by using spectrophotometer at a wavelength 540 nm. The cytotoxic effect was expressed as the percent growth inhibition, which was calculated as follows:

Percentage of growth inhibition = 100x (1 - A_t/A_u)

Where A_t and A_u are the values for treated and untreated cells.
Statistical analysis

All data were expressed as mean ± standard deviation (SD) and analyzed by the SigmaPlot 12.0 software. One-way ANOVA was used to establish the existence of significant differences among groups. The $P$ value of less than 0.05 was considered statistically significant.

Results

Effect of Cisplatin on Cell proliferation and attachment

The results showed a significant decrease ($p<0.05$) in cell proliferation and attachment after Cisplatin treatment as shown in (Figure1).

![Figure 1](image1.png)

**Figure 1**: Effect of Cisplatin at different concentrations on SW480 colon cancer cells proliferation

![Figure 2](image2.png)

**Figure 2**: Effect of Cisplatin at different concentrations on SW480 colon cancer cells attachment
Effect of Metformin on Cell proliferation and attachment

Figure 3 shows the effect of Metformin treatment on cell proliferation and attachment. There was a significant elevation on cell growth particularly at lower concentrations (32 ug/ml).

![Figure 3](image)

**Figure 3:** Effect of Metformin at different concentrations on SW480 colon cancer cells proliferation

Effect of Cisplatin on Cell proliferation after pre-treatment with Metformin

Figure 5 shows the cytotoxicity profile of Cisplatin after 24 hours of pre-treatment of the cells with Metformin at a concentration of (32 ug/ml). As shown in the Figure, Cisplatin cytotoxicity was significantly increased indicating the presence of a synergistic interaction between the two drugs.

![Figure 4](image)

**Figure 4:** Effect of Metformin at different concentrations on SW480 colon cancer cells attachment
Discussion

Metformin is an anti-diabetic drug which now is drawing much attention as anti-tumor. Specially, after several groups of researchers approved its activity as anti-cancer by increasing the apoptosis\cite{14}. Apoptosis is well known as an essential homeostatic mechanism that acts to equalize cell division and cell death to sustain the appropriate cell number in the whole body. Through the process of apoptosis, the cells will be shrinkage, blebbing of the plasma membrane and chromatin condensation without any lysis of the cells. Not only apoptosis induction would be hopeful for cancer treatment, Cell cycle deregulation also is reported as one of the major hallmark characters of cancer cell developments\cite{15}. Obviously, any chemical, which would be able efficiently lead to cycle arrest of cancer cells, would be great candidate for suppressing cancer progress, consequently this will increase the mortality of cancer cells\cite{16}. The results of the current study showed that Cisplatin is significantly reduced the proliferation while Metformin increase. The results from Metformin exposure disagrees with another study used human gall bladder Cells (GBC). These finding clearly declared that Metformin alone suppresses cell proliferation and encourage cell apoptosis\cite{15}. In another study about Metformin impaction where Annexin staining used V-FITC/PI caused cell cycle arrest in G0/G1 phase by accumulative p27, p21 and reducing Cyclin D1. In addition, the outcomes of their study also showed that accumulation of cell population at G0/G1 would lead to apoptosis\cite{14}. Moreover, in vitro study demonstrated that Metformin may activate ERK/MAPK pathways. ERK is signaling pathway which has been well documented to have major role in regulation proliferation and apoptosis\cite{17}. Furthermore, an experiment used pancreatic cancer cell where Metformin inhibits cell proliferation suggest that the biological impact of Metformin is mostly mediated in re-expression of specific genes\cite{18}. Different cell lines or different concentrations would be the main reason for different respond of colon cancer cells to Metformin in the present study compared to the previous study. The current results approved that Cisplatin would decrease the proliferation of cells which are consistent with previous works where determined that Cisplatin enters the cell cycle and reset the cells at G1/S phase. Cells in general are attached through the integrin family of cell surface receptors. These receptors are mainly consisting of $\alpha$ and $\beta$ subunits with big domains on two sides of the cell membrane. The internal side (cytoplasmic) interacts with the F-actin fibers of cell
cytoskeleton across membrane protein (vinculin), while the extracellular part of domain linkage with extracellular matrix. For these reasons, damage F-actin or any change in extracellular integrity would cause detachment for the cells. Kruidering and his colleagues investigated whether changes in F-actin or extracellular domain impacted in Cisplatin treatment. The data showed that Cisplatin exposure induced cells losing their attachment through alteration in F-actin expression. Some other studies also reported that Cisplatin exposure would lead to alteration of adhesion properties for cancer cells. The result of our study showed a reduction in cell attachment and that comes along with the previous studies. Because the data on Metformin's influence on cell adhesion is scarce, we decided to elucidate the role of Metformin at different concentrations on the attachment of colon cancer cells. The results revealed that Metformin increased the attachments which agreed with previous study about vascular endothelial cells. Metformin exposure inhibited the cytokine which suppresses NF-kB activity through AMPK activation. NF-Kb in turn induced the expression of adhesion molecule genes. In addition to their anticancer effects including colorectal, pancreatic, breast and ovarian cancers, and its role against tumorigenesis and angiogenesis, some studies reported a role of metformin in enhancing the anticancer effect of cisplatin in breast and ovarian cancers, while other studies reported adverse effects. Thus, it is reasonable to conclude that the effect of metformin on cisplatin may be dependent on the cell type and possibly to the type of cancer. Bi and his colleagues studdied the anticancer effect of Met+Cis combination. This treatment significantly increased the number of cells arrested in G0/G1 phase. The combination of the two drugs resulted in a significantly greater proportion of cells in G0/G1 phase compared with either drug alone. Analysis using Western blotting revealed that the protein of Cyclin D1 was significantly reduced after co-treatment with Cisplatin and Metformin. In contrast, the levels of P21 and P27 were significantly increased in Cis plus Met group compared with the control groups. Jaevotic et al., showed that metformin could diminish the antineoplastic effect of Cisplatin in SHY5Y, C6, U251, L929, and HL-60 cell lines through the oxidative stress and caspases suppression which disagrees with many other reports. Thus, it appears that the effect of Met on Cis may be cell type or cancer dependent. Yu et al demonstrate the synergistic effect of Met and Cis in ECA109 esophageal cancer cell line in low glucose medium. Metformin inhibits the phosphorylation of AKT causing sever diminishing of DNA repair process. Since Cisplatin affects DNA via purinecrosslinking and DNA repair inhibition leading to DNA damage and apoptosis induction, it appears that the interaction of these two mechanisms produces the synergistic anticancer effect. Moreover, it was found that Met reduces the ability of cancer cells to develop resistance to Cis treatment.

Conclusions

Altogether the findings of the present study revealed that Metformin may have possible therapeutic value in several cancer treatments but not in colon cancer where we noticed an increase in cell attachment that company with raising the average of cell proliferation. In contradiction, the data explained that Cisplatin treatment decreased both proliferation and cell adhesion to the matrix. Combination therapy (Met+Cis) showed promising synergism and enhancement of anticancer activity of Cisplatin on colon cancer cells. We are strongly recommended more investigations to be more specific for using Metformin as general anticancer.

Conflict of interest

None of the authors have any conflicts of interest relevant to this research subject.

Ethical Approval

Ethical Committee at the University of Babylon, college of Medicine, approved the study.
References

22. Wang L. et al. (2014). Downregulation of miR-133 via MAPK/ERK signaling pathway involved in nicotine-induced cardiomyocyte


