

## A Potential Role for the Anti-diabetic Drug Metformin in the Treatment of Platinum Resistant Ovarian Cancer

K Morris, M Reboe, A Levy

### ABSTRACT

**Objective:** The treatment of ovarian cancer is complicated by high drug-resistance often linked to over-expression of focal adhesion kinase (FAK). Additionally, cancer cells preferentially metabolize glucose and hyperglycaemia is considered a promoter of tumour growth. In this context, the anti-diabetic drug metformin is being investigated as a potential treatment. The present study assessed the cytotoxic effects of metformin and FAK inhibitor, PF-573228, as therapeutic adjuncts with carboplatin in the treatment of platinum resistant OVCAR3 ovarian cancer cells.

**Method:** OVCAR-3 cells were maintained in eagle's minimum essential medium (EMEM) complete media (80% EMEM, 20% FBS, 1% antibiotic) with a culture environment of 5% CO<sub>2</sub> at 37 °C. Cells were exposed to metformin (5 mM, 25 mM, 50 mM), carboplatin (1 µM, 10 µM, 100 µM) and FAK inhibitor, PF-573228 (5 µM, 50 µM, 100 µM) over 24 hours in triplicates to determine IC<sub>50</sub>. Twenty-four-hour combination treatments of metformin plus carboplatin, metformin plus PF-573228 and metformin plus carboplatin plus PF-573228 were carried out in triplicates. Cytotoxicity tests were performed using the (MTT) [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay and absorbance was measured by a spectrophotometer at 570 nm.

**Results:** Metformin, carboplatin and FAK inhibitor (PF-573228), alone induced a dose-dependent cytotoxicity in OVCAR-3 cells with IC<sub>50</sub> of 26.31 mM, 57 µM and 100 µM, respectively. For combination treatments, metformin significantly enhanced the cytotoxic effects of carboplatin by 10% (p = 0.0002) and PF-573228 by 36% (p < 0.00001). The combination result of all three revealed 94% (p < 0.000001) cytotoxicity which was significantly higher than metformin only (29%, p < 0.05) or carboplatin and PF573228 only which produced 50% cytotoxicity.

**Conclusion:** Metformin potentiates the cytotoxic effects of carboplatin and PF-573228, in platinum resistant ovarian cancer cells.

**Keywords:** Cytotoxicity, metformin, ovarian cancer, platinum-resistant

## Papel Potencial del Fármaco Antidiabético Metformina en el Tratamiento del Cáncer Ovárico Resistente al Platino

K Morris, M Reboe, A Levy

### RESUMEN

**Objetivo:** El tratamiento del cáncer ovárico es complicado por la alta resistencia a los fármacos vinculada a menudo a la sobreexpresión de la quinasa de adhesión focal (FAK, siglas en inglés). Además, las células cancerosas metabolizan preferentemente la glucosa, y la hiperglucemia se considera un promotor del crecimiento tumoral. En este contexto, el fármaco antidiabético metformina ahora se está investigando como un tratamiento potencial. El presente estudio evaluó los efectos citotóxicos de la metformina y el inhibidor de la FAK, PF-573228, como complementos terapéuticos con el carboplatino en el tratamiento de las células cancerosas ováricas de la línea celular del carcinoma ovárico humano resistente al platino (OVCAR-3).

**Método:** Las células OVCAR-3 fueron mantenidas en medios completos del medio esencial mínimo de Eagle (EMEM) (80% EMEM; 20% FBS; 1% antibiótico) con un ambiente de cultivo de 5% de dióxido de carbono (CO<sub>2</sub>) a 37°C. Las células se expusieron a metformina (5 mM, 25 mM, 50 mM), carboplatino (1 µM, 10 µM, 100 µM) e inhibidor de FAK, PF-573228 (5 µM, 50 µM, 100 µM) más de 24 horas por triplicado para determinar IC<sub>50</sub>. Tratamientos combinados de 24 horas de metformina con carboplatino, metformina con PF-573228, y metformina con Carboplatino y PF-573228 se realizaron por triplicados. Las pruebas de citotoxicidad se realizaron mediante el ensayo MMT y la absorbancia se midió mediante un espectrofotómetro a 570 nm.

**Resultados:** La metformina, el carboplatino y el inhibidor de la FAK (PF-573228) indujeron una citotoxicidad dependiente de la dosis en las células OVCAR-3 con un IC<sub>50</sub> de 26.31 mM, 57 µM y 100 µM, respectivamente. Para los tratamientos combinados, la metformina mejoró significativamente los efectos citotóxicos del carboplatino en un 10% (p = 0.0002) y PF-573228 en 36% (p < 0.00001). El resultado de la combinación de los tres reveló 94% (p < 0.000001) de citotoxicidad, que fue significativamente más alto que la metformina solamente (29% p < 0.05) o el carboplatino y PF573228 sólo, que produjeron un 50% de citotoxicidad.

**Conclusión:** La metformina potencia los efectos citotóxicos del carboplatino y PF-573228, en las células cancerosas ováricas resistente al platino.

**Palabras clave:** Citotoxicidad, metformina, cáncer de ovario, resistente al platino

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### INTRODUCTION

Globally, ovarian cancer is the eighth most common cancer in women and the 18th most common cancer overall with an overall five-year survival rate of 45% (1). In Jamaica, cancer of the ovary is the 8th most frequent cancer in women with a rate of 4.6 per 100 000 for the period of 2003–2007 (2), and in younger females, less than 25 years of age, ovarian cancer is the commonest cause of cancer related deaths (2).

The high mortality rates associated with ovarian cancer is linked to the development of resistance

to first-line chemotherapy with platinum compounds such as cisplatin and carboplatin (3). More than 50% of patients with advanced stage ovarian cancer experience recurrence due to drug-resistance within two years of disease remission (4). Resistance to platinum based therapy has been attributed to increased activity of focal adhesion kinase (FAK) which promotes metastasis and invasiveness (5, 6). Focal adhesion kinase is a tyrosine kinase activated *via* the Y397 site by integrin signalling in response to multiple factors within the tumour micro-environment (7). Focal adhesion kinase inhibition has

also been shown to increase sensitivity to platinum based therapy (7). We therefore, explored the role of FAK inhibition in combination with metformin in this study.

Metformin is a well characterized and widely used anti-diabetic drug. In recent years, metformin has emerged as a possible anti-cancer agent owing to *in vitro* and *in vivo* studies depicting its anti-proliferative actions in prostate (8), oesophageal squamous cancer (9) and lung cancer cells (10). One study reported a reduction in ovarian cancer tumour size in mice with metformin administration (11). Additionally, epidemiological studies noted a reduction in cancer incidences in metformin treated patients possibly due to the reduction in insulin levels and glucose production which inhibits cancer cell growth (12). Our study is the first to determine the *in vitro* effects of metformin in ovarian cancer cells. The objective of the present study was to evaluate the cytotoxic effect of metformin in platinum resistant ovarian cancer cells and its ability to enhance the effects carboplatin and the FAK inhibitor PF-573228 in this setting. The implication being a potential role for metformin in advanced ovarian cancer treatment.

## SUBJECTS AND METHODS

### Cell culture

The platinum resistant ovarian cancer cell line; OVCAR-3 was obtained from American Type Culture Collection (ATCC) and maintained in 80% RPMI-1640 medium supplemented with 20% fetal bovine serum and 1% penicillin/streptomycin antibiotics. Cells were propagated as adherent monolayers and maintained in a humidified incubator of 5% CO<sub>2</sub> at 37 °C. Cells were sub-cultured at approximately 80% confluence and harvested for the various studies after brief trypsinization. Cell viability by trypan blue assay was > 95%.

### Dose response curves and combination treatments

OVCAR-3 cells were plated at a density of 1 x 10<sup>6</sup> cells and treated for 24 hours at various concentrations of carboplatin, metformin and PF-573228 obtained from Sigma Aldrich, United States of America. Carboplatin was dissolved in media and added to the cells at final concentrations of 1 µM, 10 µM, 100 µM (carboplatin), 5 mM, 25 mM, 50 mM (metformin), 5 µM, 50 µM, 100 µM (PF-573228). Media only treated cells were used as controls. For combination treatments, metformin (23.31 mM) and PF 573228 (57 µM) were used in combination with carboplatin (100 µM). All treatment protocols were conducted in triplicate.

### MTT assay

Cytotoxicity was assessed using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT) assay. Cells (1 x 10<sup>6</sup>) were seeded into 96-well plates and left to adhere for 24 hours. Cells were exposed to the various concentrations of cytotoxic agents for 24 hours. After treatment, the medium was replaced with 100 µl of MTT solution and incubated at 37 °C for two hours. Following solubilisation, a spectrophotometer was used to measure absorbance at 570 nm. Percentage cell death was determined using the formula (average test value – average blank) / (average control – average blank) × 100.

### Statistical analysis

All experiments were performed in triplicate and data were presented as means ± SEM. Student's *t*-test (SPSS software version 20) was used for analysis of the difference between treatment and the control groups and a value  $p < 0.05$  considered statistically significant.

## RESULTS

Using the MTT assay, the cytotoxic effects of PF-573228, metformin and carboplatin on OVCAR-3 cells were evaluated. All three agents, PF-573228 ( $p \leq 0.02$ ), metformin ( $p \leq 0.02$ ) and carboplatin ( $p \leq 0.001$ ) significantly reduced cell viability in a dose dependent manner when compared to controls. The IC<sub>50</sub> values of metformin, PF573228 and carboplatin were 26.31 mM (Fig. 1a) 100 µM (Fig. 1b), 57 µM (Fig. 1c), respectively.

For combination treatments, metformin (26.31 mM) significantly enhanced the cytotoxic effects of carboplatin by 10% ( $p = 0.0002$ ) and PF-573228 by 36% ( $p < 0.00001$ ). The combination result of all three revealed 94% ( $p < 0.000001$ ) cytotoxicity which is significantly higher than metformin only 29% ( $p < 0.05$ ) or when compared to carboplatin or PF-573228 only which produced a 50% cytotoxicity as shown in Fig. 2.

## DISCUSSION

The first-line chemotherapeutic intervention in ovarian cancer is platinum based therapy which often fails due to resistance. Therefore, there is an urgent need for more effective therapies that are capable of combating resistance and improving patient survival. The present study is the first to establish a cytotoxic effect of metformin in platinum resistant OVCAR-3 cells with an IC<sub>50</sub> value of 26.31 mM. In further studies, we will determine the mechanism/s of cell death induced by metformin. Previous studies have shown that metformin is capable of inhibiting cell growth and proliferation *via* regulation

of the AMPK/mTOR pathway and inhibition of STAT-3 phosphorylation (13–16).

In this study, we also found that metformin enhanced carboplatin induced cytotoxicity in platinum resistant ovarian cancer cells by 10%. It is well established that carboplatin induces death of OVCAR-3 cells by alkylating DNA (17) and this therefore, is the likely mechanism utilized in this study. Because carboplatin resistance is linked to up-regulation of FAK, we further examined the ability of metformin to increase sensitivity to carboplatin with and without the presence of the FAK inhibitor. The FAK inhibitor, PF-573228, was chosen as it is a well-established inhibitor of FAK phosphorylation at the

397-site resulting in inhibition of cell survival, migration and proliferation (18). We found that metformin enhanced the cytotoxic activity of PF-573228 by 36%. The triple combination of metformin plus PF-573228 plus carboplatin produced an overall cell death of 94% representing a greater than 20% increase over the metformin plus carboplatin and metformin plus PF-573228 only treatment.

Our results confirm that the presence of metformin re-sensitizes resistant OVCAR-3 cells to the effects of carboplatin and also increases the sensitivity to FAK inhibition. While it is tempting to speculate that metformin cooperates with PF-573228 to down-regulate

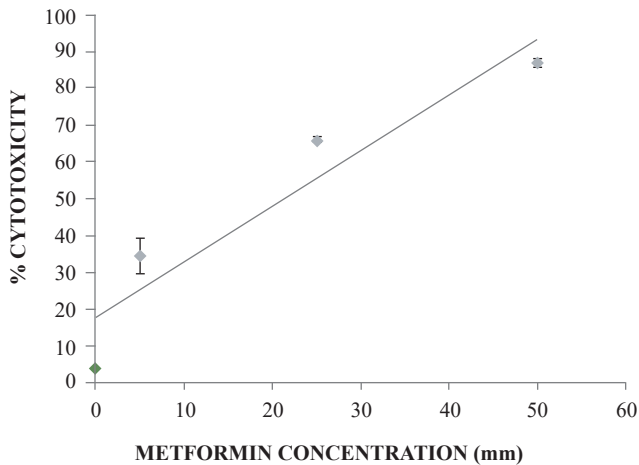


Fig. 1a: Cytotoxic effect of metformin (5 mM, 25 mM, 50 mM) following 24-hour treatment. Data represents triplicate experiments ± SEM. Carboplatin produced an IC<sub>50</sub> value of 26.31 mM.

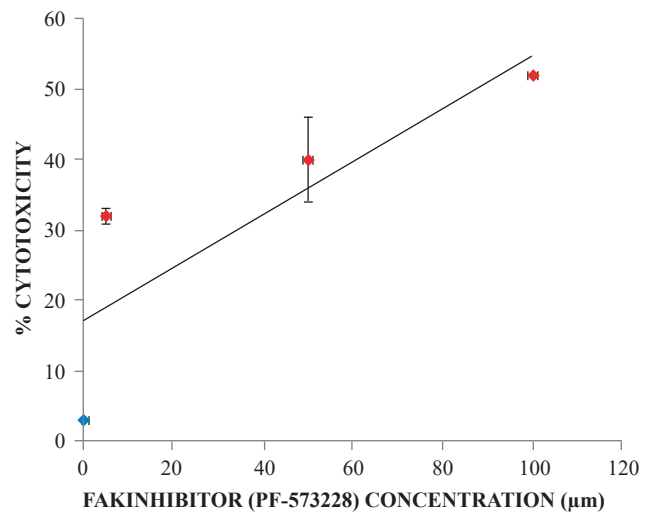


Fig. 1b: Cytotoxic effect of focal adhesion kinase inhibitor PF-573228 (5 µM, 50 µM, 100 µM) for 24 hours. Data represents trials performed in triplicates ± SEM. PF-573228 produced an estimated IC<sub>50</sub> value of 100 µM.

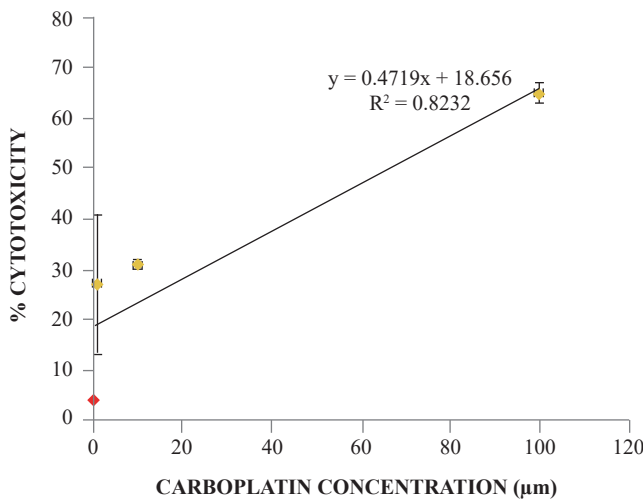


Fig. 1c: Cytotoxic effect of carboplatin (1 µM, 10 µM, 100 µM) following 24-hour treatment. Data represents triplicate experiments ± SEM. Carboplatin produced an IC<sub>50</sub> value of 57 µM.

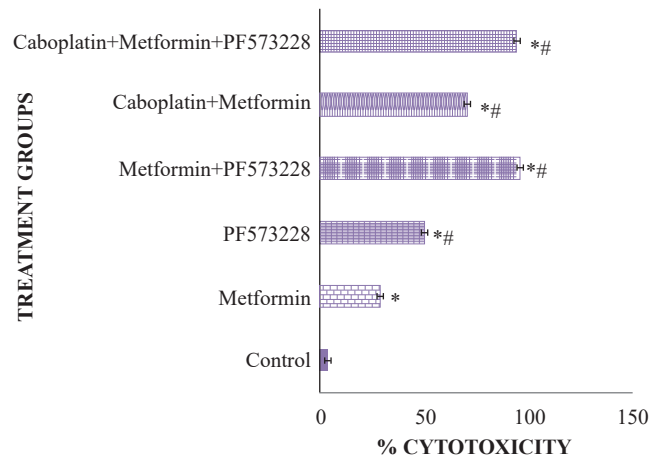


Fig. 2: Cytotoxic effect of carboplatin in combination with metformin and of focal adhesion kinase inhibitor PF-573228 in OVCAR-3 cells. Data represents trials performed in triplicate ± SEM. Asterisks (\*) represent significance ( $p \leq 0.05$ ) as compared to the control only group, (#) represents significance as compared to metformin.

FAK activity, this has to be validated in further studies that will evaluate the effect of metformin on FAK activity. Metformin has also been shown to alter several aspects of metabolism including adenosine monophosphate-activated protein kinase activity, glycolysis and lipid synthesis in OVCAR-3 cells (19) and it is therefore, possibly that these mechanisms might explain how it increases sensitivity to carboplatin. In further studies, we will explore mechanism of metformin induced cell death to shed light on the molecular processes involved.

## CONCLUSION

The results from this study establish the cytotoxic efficacy of metformin in OVCAR-3 cells and its ability to enhance sensitivity to carboplatin and PF-573228 in platinum resistant ovarian cancer. The novelty of our finding suggests that metformin warrants further investigation for use as an ovarian cancer therapeutic agent.

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