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Metformin as a potential anti-proliferative agent against cancer cells: A comparative study elucidating the mechanism of action and anti-proliferative preference

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Abstract

Introduction: Metformin specifically inhibits the regulation of cell proliferation and survival via several mechanisms. This study aimed to investigate the anti proliferative effect of metformin against different cancer cell lines, in order to delineate its specificity and elucidate its mechanism.

Methods: The inhibitory concentration (IC50) of metformin against several cancer cell lines was calculated and statistically compared with the corresponding values of doxorubicin as a reference drug using One way ANOVA followed by Tukey Kramer post test.

Results: Metformin was effective against PC3>Caco>HeLa>Hep-2>A549>Hep-G2>MCF7 cancer cell lines. The potency of metformin as anticancer agent in reference to doxorubicin ranged between 1.1 and 8%.

Conclusions: The anti proliferative effect of metformin was attributed to its reduction of the glucose uptake and oxidation, leading to alteration of cell metabolism. Its anti proliferative effect against PC3 cell line with a minor effect against MCF7 cell line indicates that metformin helps the patients with positive androgen rather than estrogen receptor cancer cells.

INTRODUCTION

Metformin is an oral anti-diabetic drug with multiple pharmacological actions. It inhibits the function of the mitochondria, and this effect may explain its inhibitory action against the cell growth [1, 2]. Metformin specifically inhibits the regulation of cell proliferation and survival *via* its inhibitory effect against the mammalian target of rapamycin complex-1 (mTORC1) [3, 4]. Inhibition of TOR activity causes a decrease of messenger ribonucleic acid (mRNA) levels, and thereby suppresses the cell growth [4]. A recent study delineated that metformin inhibits vascular endothelial growth factor/protein kinase B/phosphatidylinositol-3-kinase (VEGF/Akt/PI3K) pathway of the human prostate cancer cell [5]. The direct cytoxic effect of metformin is linked to its inhibitory effect against adenosine monophosphaste-activated protein kinase, while its indirect anticancer effect is related to its effect against the glucose molecule [6]. It is important to mention that the anticancer effect of metformin is potentiated in the presence of other substances. Flavone, epothilone, and quercetin as antioxidants enhance the activity of metformin as anticancer against breast, ovary, and prostate cancer cell line [5, 7, 8]. On the other side, metformin lacks anticancer effect in animal models of cancer including urinary bladder, head and neck, and colon-intestine cancer [9]. Accordingly, the anticancer effect of metformin may be specific against certain cancer cells, taking into consideration its inhibitory effects against the metabolic pathways in the cancer cell. This study aimed to investigate the anti proliferative effect of using metformin alone against different cancer cell lines, in order to delineate its specificity. The inhibitory concentration (IC_{50}) of metformin against different cancer cell lines was determined and compared with doxorubicin as a reference drug.

METHODS

Metformin was generously provided as a gift from Jordan/Swedish company, Jordan. Tissue culture Laboratory (Vacsera, Dokkey, Giza, Egypt) generously provided the cancer cell lines: Hepatoma G2 (HepG2 cells, Organism; Homo sapiens, human tissue: liver, cell type: epithelial, culture properties: adherent, disease: hepatocellular carcinoma); Human epithelial type 2 (Hep2 cells, Organism; Homo sapiens, human tissue: HeLa contaminant: cell type: epithelial, culture properties: adherent, disease: carcinoma); Henrietta Lacks (HeLa cells, Organism; Homo sapiens, human tissue: cervix, cell type: epithelial, culture properties: adherent, disease: adenocarcinoma); Prostate cancer cell line 3 (PC3 cells, Organism; Homo sapiens, human tissue: prostate; derived from metastatic: bone, cell type: epithelial, culture properties: adherent, disease: grade IV, adenocarcinoma); Michigan cancer foundation 7 (MCF7 cells, Organism; Homo sapiens, human tissue: mammary gland; derived from metastatic site: pleural effusion, cell type: epithelial, culture properties: adherent, disease: Adenocarcinoma, ATCC: HTB-22); Adenocarcinoma of the colon 2 (Caco2 cells, Organism; Homo sapiens, human, Tissue: colon, cell type: epithelial, culture properties: adherent, Disease: Colorectal adenocarcinoma, ATCC: ATB-37) and; Human alveolar epithelial cells (A549 cell, Organism; Homo sapiens, human, tissue: Lung, cell type: epithelial, culture properties: adherent, Disease: carcinoma). The cells were maintained in Roswell Park Memorial Institute RPMI-1640 medium with 2% serum and the cultures supplemented with 0.5% ampicillin and 0.5% streptomycin in 5% CO_2 at 37°C. Several trials of cell reactivation were done in order to obtain monolayered cells in a specific falcon (volume 25 ml), verified by microscopical visualization. Once the growth of monolayer cells has been confirmed, the old growth media was discarded, washed with phosphate buffer saline (PBS) buffer once, followed by addition of 0.5–1 ml trypsin-versin solution, and 10 ml sterile growth media (supplemented with 2% serum). The dispersed cells were transferred into a sterile microplate (a total volume of 100 µl cells suspension is transferred into the wells of the microplate), incubated at 37°C for 24 h to achieve a monolayer cell growth. The cells were plated at 1×10^5 cells per well in 100 µl of complete culture medium containing metformin (10000 µg/ml with a serial dilution to 78.12 µg/ml) or doxorubicin (1000 µg/ml with a serial dilution to 7.81 µg/ml) as a reference positive control, incubated at 37°C for 24 h.

The viability of the cells was determined by the MTT (3-[4,5-dimethylth-iazol-2-yl]-2,5-diphenyl tetrazoliumbromide assay. A 20µl of prepared tetrazolium dye (5 mg/ml in phosphate buffer solution) was added to each well in a dark room (to avoid oxidation of the dye) and placed on a horizontal shaker (150 rpm for 5 minutes) to allow homogenous distribution of MTT into the media. The microplate was then incubated for 1-5 h at 37°C (5% CO_2) in the incubator for 1-5 hours to allow the MTT to be metabolized. Then, whole wells' content was discarded, and the MTT metabolic product formazan was dissolved in 100 µl dimethyl sulfoxide, shaken at 150 rpm for 15 min using a horizontal shaker before recording the absorbance of each well at 560 nm and subtracting the background absorbance at 620 nm by Enzyme linked immunosorbent assay ELISA reader.

Statistical analysis

Results were expressed as number, percentage and mean. A dose-response curve using log transformation of drug concentration was constructed to determine the inhibitory concentration (IC_{50}). Excel software program (IC_{50} calculation) for Windows (applied in calculation the IC_{50} of metformin and doxorubicin.

RESULTS

As shown in Figure 1, the dose-effect curve of doxorubicin against Caco cell and HepG2 cell was shifted to the left, while its curve against MCF7 cell was shifted to the right, indicating that doxorubicin was less effective against MCF7. According to the inhibitory concentration (IC_{50}), the order of effectiveness of doxorubicin against cancer cells was Caco>HepG2>A549>PC3>Hep-2>HeLa> MCF7 (Table 1).

On the other hand, Figure 2 shows that metformin was more effective against the PC3 cells, and as with doxorubicin it was less effective against MCF cells. According to the IC_{50} values, the order of effectiveness of metformin against the cancer cells was PC3>Caco>HeLa>Hep-2>A549>HepG2> MCF7 (Table 1).

The potency of metformin as anticancer agent in reference to doxorubicin ranged between 1.1 and 8%, according to the following order starting from the highest values; PC3> HeLa> Hep-2> Caco>A549>HepG2> MCF7.

Table 1: The inhibitory concentration 50% (IC_{50}) of doxorubicin and metformin against cancer cell and the potency of metformin in reference to doxorubicin

Cell line	Doxorubicin (IC_{50}) (µg/ml)	Metformin (IC_{50}) (µg/ml)	Potency of metformin to doxorubicin (%)
HepG2	32.9	2242.5	1.47
Hep-2	45.9	1824.1	2.52
HeLa	71.5	1430.6	5.0
PC3	41.9	522.3	8.02
Mcf7	87.9	7921.1	1.11
Caco	28.5	1321.2	2.16
A549	36.2	1916.2	1.89

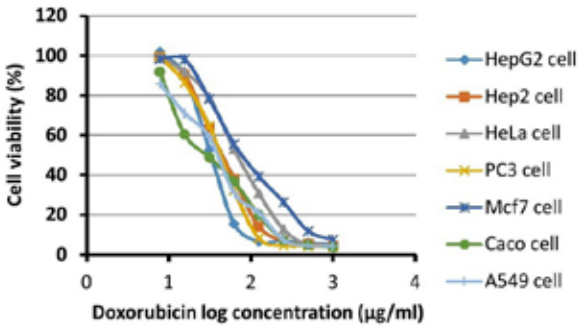

Figure 1: Effect of doxorubicin on the different cancer cell lines

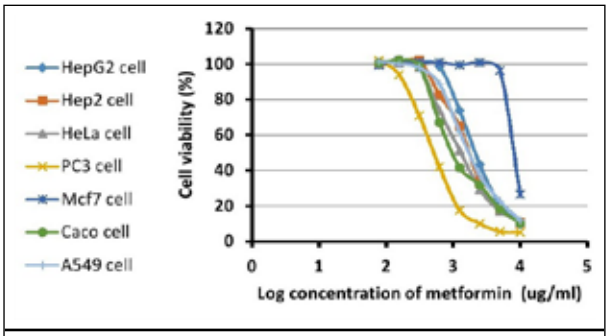

Figure 2: Effect of metformin on the different cancer cell lines

DISCUSSION

Results of this study demonstrate that metformin has anti-proliferative effect against cancer cell-line ranging from 1.1% to 8% of the corresponding effect of doxorubicin. The anti-proliferative effect of metformin against PC3 cell line is higher than others. Previous studies demonstrated the effectiveness of metformin against cancers of kidney, urinary bladder, and prostate [10]. The mechanism of anti proliferative action of metformin against the PC3 cell line could be ascribed to the down-regulation of androgen receptor protein or androgen dependent insulin like growth factor [11, 12]. Metformin through its inhibitory effect against glucose oxidation shifts the metabolism of the prostate cancer cells into glutamine metabolism leading to decreasing the cell energy [13]. The possible mechanistic action of metformin against the proliferation of Caco cell line may be related to its inhibitory effect against the panel of proinflammatory cytokines [14]. Its effect against AMP-dependent protein kinase (AMPK) activity is also possible, as metformin inhibits the glucose uptake by cancer cells leading to increase the activity of AMPK [15]. This mechanism could also explain the anti proliferative effect of metformin against HeLa cell [16]. A recent study demonstrated that metformin inhibits HepG2 cancer cell proliferation thorough its hypoglycemic effect which indirectly activates the AMPK/mTOR (mammalian target of rapamycin) pathway [17]. Moreover, the anti-proliferative effect of metformin against HepG2 cell line was not related to its effect against angiogenesis as rapamycin [18]. The highest IC_{50} value of metformin against the A549 cell line reported in our study came in accordance with other studies which showed ineffectiveness of metformin against the A549 cell, because metformin did not act directly on cell-cycle proliferation [19]. The effect of metformin against breast cancer cell (represented in this study by MCF7 cell line) was negligible, suggesting that metformin did not influence the phosphatidylinositol 3-kinase (PI3K)/serine/threonine kinase (AKT) signaling pathway in the tumor cell [7]. The minor anti proliferative effect of metformin against MCF7 cell was due to glucose uptake inhibition effect, by acting directly against glucose transport-1 [20]. This study suggests that metformin used alone without any form of pharmaceutical or nutraceutical combinations was effective against different cell lines. Moreover, the cancer cell lines showed variability in their pathways of proliferation, indicating different preferences for metformin towards cancer cells.

CONCLUSIONS

We conclude that metformin *per se* showed anti proliferative effect against cancer cells thorough its hypoglycemic effect. It reduces the glucose uptake and oxidation leading to alteration of cell metabolism. Moreover, its anti proliferative effect against PC3 cell line was more than MCF7 cell line indicating that metformin can help the patients with positive androgen rather than estrogen receptor cancer cells.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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AUTHORS' CONTRIBUTIONS

The authors contributed equally to this work.

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