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Research Article

Biochemistry for better diagnosis and therapy

An in-vitro and in Silico Anticancer Study of FDA Approved Antidiabetic Drugs Glimepiride and Empagliflozin

Uzma Faridi*[1], Fahad Al-Mutairi[1], Humaira Parveen[2] and Sahar Khateeb[1]

[1]*Biochemistry Department, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia*
[2]*Chemistry Department, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia*

Abstract: Drug repurposing is the way to find the role of the drug for new disease. Mostly the drug already passes human trails and approved by FDA. Many studies confirmed the role of many drugs in the treatment of more than one disease. Present research was conducted to evaluate the anticancer potential of FDA approved antidiabetic drugs Glimepiride and Empagliflozin. Glimepiride binds to ATP-sensitive potassium channel receptors and reduces the potassium conductance and Empagliflozin is a sodium glucose co-transporter-2 (SGLT-2) inhibitor. As many anti-diabetic drugs like meantime proved to be good anticancer drug so these two drugs were selected for the present study as their pharmacodynamics and pharmacokinetics studies are already reported and both showed no side-effect in human. The purpose of this study was to repurpose the Glimepiride and Empagliflozin as anticancer agents. In-vitro anticancer study was performed on two human cancer cell-lines MCF-7 and A549. To confirm the anticancer potential, the effect of these drugs on selected apoptotic proteins was studied in-silico. Both Glimepiride and Empagliflozin have significant anticancer activity on both the cell-lines but Empagliflozin seems to have better activity in-vitro. In-silico results indicated that both the drugs have significantly high activity towards apoptotic proteins. Although both the drug showed promising results in *in-vitro* and in-silico studies but the further studies like in-vivo studies are also required to prove these drugs as anticancer as well.

Keywords: Glimepiride, Empagliflozin, Repurposing, Anticancer, Antidiabetic, Apoptosis.

***Corresponding Author**

Uzma Faridi*1 , Biochemistry Department, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia

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1. INTRODUCTION

Most of the rapidly dividing and growing human cells, especially the cancerous cells, exhibit high glucose metabolism and due to that glucose breakdown occurs for their rapid growth and survival. The carcinogenesis triggers the core signalling pathways to adapt to the growth and survival rates of cancer cells[1]. The cancers become addicted towards glucose and amino acid glutamine as they are the source of energy required for the division of cells. Thus the metabolism can be one of the most promising targets for anticancer drugs and targeting of glucose and glutamine metabolic pathway can be used as selective way to kill cancer cells [2]. Deregulated glucose metabolism, fatty acid synthesis and serine–glutamine metabolism play a significant role in cancer cell proliferation, and metastasis[3]. Although there are many anticancer drugs available in the market none of them has 100% success rate. Many drugs show positive response during preliminary stages but fail at later stages or in clinical trials. Drug repurposing is the idea of using old drugs for new diseases [4]. Drug repurposing bypasses many steps normally required for de-novo drug discovery as the chemical optimization and toxicological studies have already been performed. Most of antidiabetic drugs target the glucose metabolism as their role is to enhance the glycogen synthesis [5]. Repurposed antidiabetic drugs have been widely used for several diseases. The development of anticancer drugs is a lengthy and expensive process [6]. After a novel compound is identified or designed, preclinical and clinical data from phase I, II and III clinical trials are generated prior to approval [7]. Drug repurposing represents the identification of the novel pharmacological effects of conventional drugs. As the pharmacokinetics, pharmacodynamics and safety in humans have already been established, expanding the application of a drug to additional diseases has advantages in terms of cost and time efficiency [6]. Glimepiride and Empagliflozin (fig. 1 &2) are FDA approved anti-diabetic drugs [8,9]. Many anti-diabetic drugs already showed positive response as anticancer agents so these two drugs were selected for the present study as their pharmacodynamics and pharmacokinetics studies are already reported and both showed no side-effect in human [10]. Glimepiride is a "second-generation" sulfonylurea agent and is used to lower blood sugar. Glimepiride binds to ATP-sensitive potassium channel receptors and reduces the potassium conductance, causing depolarization of the β cells and mitochondrial membrane. Membrane depolarization stimulates calcium ion influx through voltage-sensitive calcium channels [11]. Many studies suggest that opening of ATP-sensitive potassium channel protects the cells against apoptosis [12]. At the early stage of apoptosis, the opening of MitoKAT could inhibit depolarization of mitochondrial membrane to maintain MMP[13]. Thus, the mitochondrial membrane was stabilized to prevent further apoptotic chain reaction, such as transition pore formation, cytochrome *c* release or caspase cascade activation. It is suggested that the glimepiride depolarizes the mitochondrial membrane which can initiate the apoptosis process in cancer cells. Empagliflozin is a sodium glucose co-transporter-2 (SGLT-2) inhibitor. Glucose transport by SGLT2 is responsible for bulk reabsorption of filtrated glucose in the kidney. SGLT-2 inhibitors control the glucose level in the blood by blocking the reabsorption of glucose from the kidney [14]. SGLT-2 co-transporters are responsible for reabsorption of glucose from the glomerular filtrate in the kidney. In this study a test on the cytotoxicity of these drugs on human cancer cell-lines was conducted to check the effect of these drugs on cancer cells. In-silico studies were carried out to validate the in-vitro results. The aim of the present study was to reposition/repurpose the well-established FDA approved anti-diabetic drugs Glimepiride and Empagliflozin for their anticancer potential.

2. MATERIALS AND METHODS

2.1 Cell culture and chemicals

Breast cancer cell-lines MCF-7 and Lung cancer cell lines A549 were maintained in MEM supplemented with 10% fetal bovine serum (Sigma-Aldrich, St Louis, Missouri, USA). Cells were grown in an incubator with 5% CO_2 at 37°C. Glimepiride and Empagliflozin were obtained from Sigma-Aldrich (St Louis, Missouri, USA), and they were dissolved in DMSO.

2.2 Cell cytotoxicity assay

About $4X10^4$ cancer cells were seeded into a 96-well plate in 100 µL of medium and grown for 24 h. In total, five different concentrations of Glimepiride and Empagliflozin were studied to calculate the inhibitory concentrations. Glimepiride and Empagliflozin were added to the culture medium, and cells were further cultured for 40 hrs. MTT solution (5 mg/mL in phosphate buffered saline was added to each well and the cells were incubated for 4 hrs at 37°C. After 4 hrs the formazan crystals were formed and dissolved in Dimethyl sulfoxide (DMSO and the plates were incubated at 37°C for 30 min. Absorbance was measured on SpectraMax M3 (Molecular Devices) microplate reader at 570 nm.

2.3 Statistical analysis

Experiments were performed at least three times using three replica each time. Mean value and standard deviation were calculated by using SPSS 21.0 software. IC50 values were calculated using Prism 7 software.

2.4 *In-silico* studies

Hex 8.0 tool was used to study the protein-drug interaction between Glimepiride and Empagliflozin and 1GJH (Human bcl-2, isoform 2), 1TUP (Tumor suppressor p53 complexed with DNA -p53), 2XYG (Caspase-3). The 3D structures of the proteins were taken from the Protein Database (PDB) and the PDB structure of Glimepiride and Empagliflozin were obtained from PubChem. [15]. The docking was analysed by the instructions provided in the hex 8.0 manual. Hex is user-friendly, freely available and an interactive tool for the calculation and display of convenient docking mode of pairs protein. Hex can also calculate the receptor and ligand interaction, assuming ligand as a rigid body [16]. PDB provides all the information regarding various proteins obtained by X-ray crystallography, NMR etc. The parameters used in the docking process are mentioned in Table 1.

3. RESULTS

In order to understand the anticancer properties of Glimepiride and Empagliflozin on human cancer cell-lines i.e. MCF-7 and A549 antiproliferative study was conducted. The results indicated that Empagliflozin has better anticancer activity on both the cell-lines (Table 2 fig.3). To confirm the

anticancer targets of Glimepiride and Empagliflozin in-silico studies were performed and docking information was obtained using docking software Hex8.0. Three apoptotic proteins 1GJH (Human bcl-2, isoform 2), 1TUP (Tumor suppressor p53 complexed with DNA -p53), 2XYG (Caspase-3) were selected for in-silico studies. All the selected proteins play significant role in anticancer potential of drug induced apoptosis. E-value was calculated to check the effect of Glimepiride and Empagliflozin on apoptotic proteins. The in-silico results confirm the induction of apoptosis by Glimepiride and Empagliflozin in both lung cancer and breast cancer cells by using the proteins up-regulated in these cancer lines. The E-values of receptors 1GJH (Tumor suppressor p53 complexed with DNA -p53), (Human bcl-2, isoform 2), 1TUP 2XYG (Caspase-3) and ligand Glimepiride were -336.25, -300.66, -190.20 respectively (Table 3 and fig.4). The E value of same receptors with ligand Empagliflozin were -348.12, -300.12, -203.36 (Table 4 0and fig. 5). The negative E-value indicates a stable system so a likely binding interaction.

Table-1:The parameters used in the docking process

SNo.	Correlation type	Shape Only
1.	FTT mode	3D Fast lite
2.	Grid Dimension	0.6
3.	Receptor Range	180
4.	Ligand Range	- 180
5.	Twist Range	- 360
6.	Distance Range	- 40

Table 2: The percentage viability of the cells in presence of Glimepiride and Empagliflozin against breast cancer (MCF) and lung cancer (A549) cells.

SN0	Concentrations (µg/ml)	Glimepiride		Empagliflozin	
		MCF-7±SD	A549±SD	MCF-7±SD	A549±SD
1.	0	100± 0.538	100± 0.33	100± 0.53	100± 0.33
2.	6.25	95.16±0.518	85.32± 0.28	96.28±0.51	96.10±0.32
3.	12.25	88.47±0.498	81.43±0.27	92.56±0.47	88.92±0.29
4.	25	88.10± 0.46	79.94±0.267	86.98± 0.474	78.74±0.26
5.	50	82.34±0.43	75.14±0.251	80.85±0.443	74.25±0.248
6.	100	21.00±0.17	57.78±0.193	32.71±0.113	51.49±0.172

Table-3: Effect of Glimepiride over different targets

S.no	Targeto protein	PDB ID	E-value
1.	Tumor suppressor p53 complexed with DNA	1tup	-336.25
2.	Human bcl-2, isoform 2	1gjh	-300.66
3.	Caspase-3	2xyg	-190.20

Table-4: Effect of Empagliflozin over different targets

S.no	Targeto protein	PDB ID	E-value
1.	Tumor suppressor p53 complexed with DNA	1tup	-348.12
2.	Human bcl-2, isoform 2	1gjh	-300.12
3.	Caspase-3	2xyg	-203.36

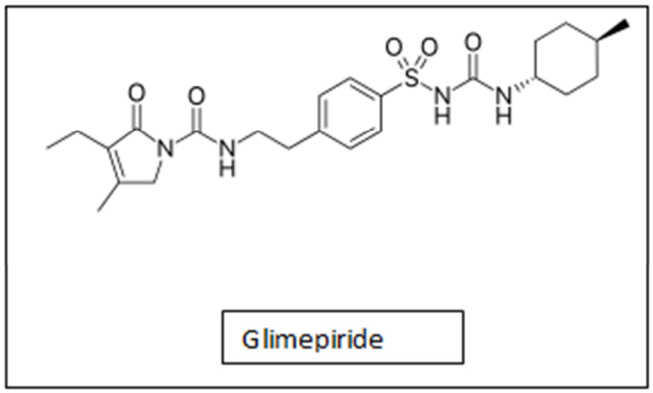

Fig 1. The structure of Glimepiride

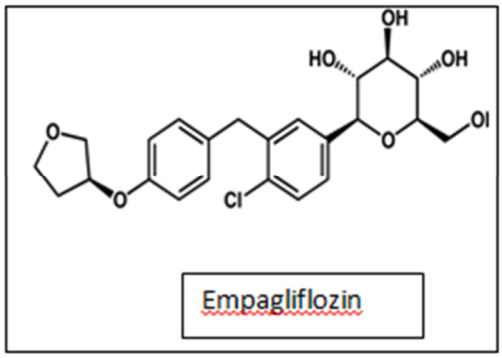

Fig 2. The Structure of Empagliflozin

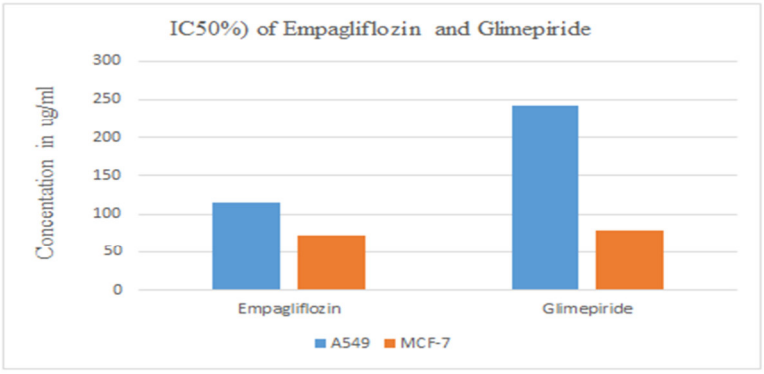

Fig 3. The IC 50 value of Glimepiride and Empagliflozin against breast cancer (MCF) and lung cancer (A549) cell-lines. Values are mean± SD (n=3) P<0.05 when compared to control

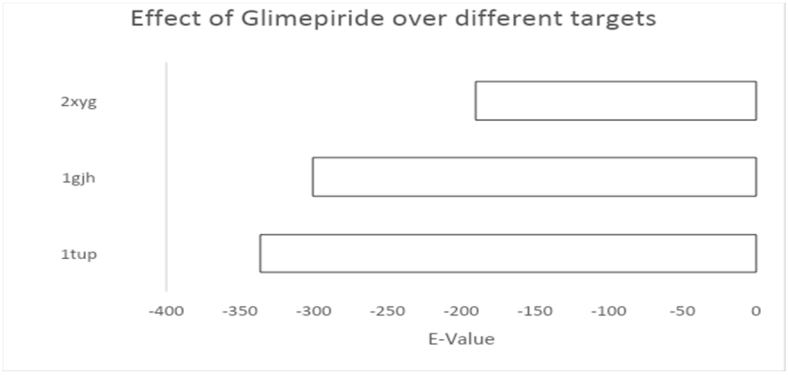

Fig 4. Effect of Glimepiride over different targets

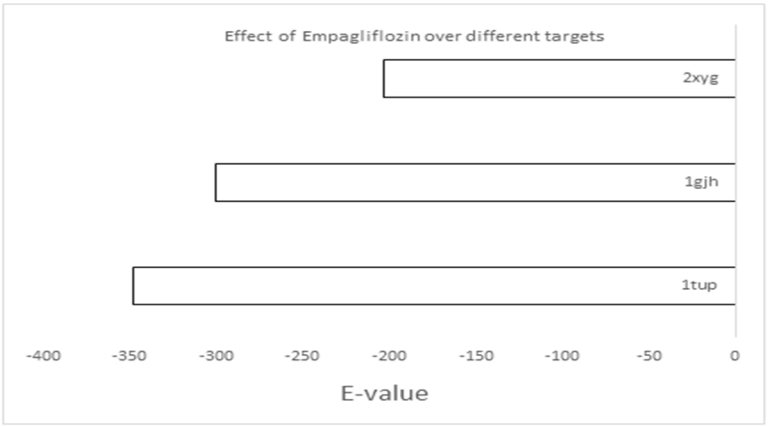

Fig 5. Effect of Empagliflozin over different targets

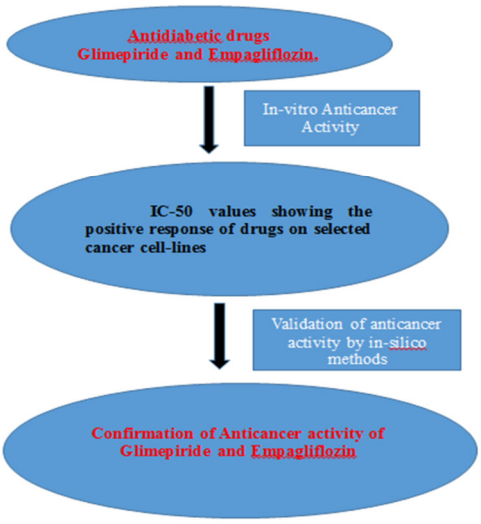

Fig 6 Pictorial representation of confirmation of anticancer activity of glimepride and empagliflozin

4. DISCUSSION

Drug discovery and development is a very expensive and time consuming process with high failure rate [17,18]. Drug 'repurposing' is the use of old drug for new therapeutic purpose instead of finding new drug. Repurposing allows skipping of many time taking and expensive steps. In the present study, two antidiabetic drugs Glimepiride and Empagliflozin, were used. These drugs were investigated for anticancer activity. Glimepiride is a common drug used in type-2 diabetics. It targets the K-ATP channel of cell membrane and mitochondrial membrane and depolarises them. As earlier studies suggested that the Ion channels especially the sodium and potassium channels play a significant role in many diseases like diabetics, CVD and cancer, because of their effect on the control of many diseases, these can be used as therapeutic targets as well [19]. The ATP-sensitive potassium (K_{ATP}) channels are heteropolymers that contain at least two types of subunit. One of them forms pore and other unit forms the receptor. The K-ATP inhibitors alter the cell proliferation by depolarisation of cell membrane and finally lead to apoptosis in cancer cells [20]. As the mechanism of action of Glimepiride is well established in diabetics by maintaining the normal glucose level this study tried to confirm its activity on cancer cells as well as the cancer cells need high glucose for their growth. The other drug selected for anticancer study was Empagliflozin. Empagliflozin is also a well know antidiabetic medicine. Empagliflozin is the SGLT2 inhibitor. It enters the blood and decreases renal reabsorption of glucose by blocking the SGLT2 channel. Glucose metabolism plays a major role in cancer cell proliferation so the glucose uptake systems and glucose metabolic enzymes can be used as potential targets for anticancer drugs. In many types of cancer the expression of SGLT2 becomes very high which provides an increased concentration of glucose in cancer cells. These studies indicate that Empagliflozin can be used as anticancer drug as it's a SGLT2 blocker. As it is earlier that the antidiabetic drugs can be repurposed as anticancer medicine as well, these two FDA approved antidiabetic drugs Glimepiride and Empagliflozin were studied for antiproliferative activity and the results indicated that both the drugs (especially Empagliflozin) show good activity against breast cancer cells and lung cancer cells. The antiproliferative molecules finally cause apoptosis in the cells so apoptosis was confirmed by in-silico studies. 3 of the most common apoptotic proteins 1GJH (Human bcl-2, isoform 2), 1TUP (Tumor suppressor p53 complexed with DNA -p53), 2XYG (Caspase-3) were selected. The insilico results showed the apoptotic response of these two drugs in both the cancer cells lines.

5. CONCLUSION

Glimepiride and Empagliflozin are considered to be the FDA approved antidiabetic drugs. The anticancer activity of Glimepiride against Breast cancer cell-lines MCF-7 and Lung cancer cell lines A549 was performed. The results indicated that both the drugs have anticancer potential in-vitro Although Empagliflozin had better activity than Glimepiride. The in-silico validation of the results confirmed the in-vitro results.

6. ACKNOWLEDGMENT

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7. AUTHORS CONTRIBUTION STATEMENT

Dr. Uzma Faridi conceptualized and gathered the data with regard to this work. Dr. Fahad and Dr. Humaira helped in data analysis writing manuscript. Dr. Sahar helped in MTT assay analysis.

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9. CONFLICT OF INTEREST

Conflict of interest declared none.

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