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ORIGINAL ARTICLE

Investigation of Cytotoxic Effects of Empagliflozin on Caco-2, LNCaP and A2780 Human Cancer Cell Lines

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Abstract

Background: Cancer is a major health concern with high morbidity and mortality rates, necessitating new treatment strategies. Despite the effectiveness of chemotherapy, drug resistance and side effects pose significant challenges. In recent years, the drug repurposing approach has emerged as a promising strategy for new therapeutic options. SGLT2 inhibitors, originally developed for diabetes treatment, have gained attention for their potential anticancer effects. In this study, the cytotoxic effects of empagliflozin on Caco-2 (colorectal adenocarcinoma), A2780 (ovarian carcinoma), and LNCaP (prostate adenocarcinoma) cell lines were investigated.

Methods: In this study, the A2780, LNCaP, and Caco-2 cell lines were utilized. Each cell line was exposed to empagliflozin at varying concentrations of 1, 5, 25, 50, and 100 μM for a duration of 48 hours. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was employed to evaluate changes in cell viability following treatment. From the cytotoxicity data, the half-maximal inhibitory concentration (IC50) and logIC50 values of empagliflozin were determined to assess its inhibitory effects on cell viability.

Results: Empagliflozin significantly reduced cell viability in Caco-2, A2780, and LNCaP cell lines. The most pronounced cytotoxic effect was observed in LNCaP cells, followed by Caco-2 and A2780 cell lines, respectively.

Conclusion: These findings suggest that Empagliflozin exhibits cytotoxic effects in certain cancer cell lines and may have potential as an anticancer agent.

Keywords: Cancer, Empagliflozin, SGLT2 Inhibitors, Cytotoxicity, MTT Assay.

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INTRODUCTION

According to data from the World Health Organization, approximately 20 million new cancer cases were diagnosed worldwide in 2020, and nearly 10 million people lost their lives due to this disease. Cancer is considered one of the most significant contributors to the global disease burden today. This burden is expected to increase further in the coming years, particularly due to the aging population, environmental factors, and lifestyle changes (1). Current chemotherapeutics have extended the lifespan of cancer patients; however, factors such as treatment-related side effects, varying tumor responses, and drug resistance necessitate the discovery of new strategies and compounds for cancer treatment (2). The traditional drug development process involves preclinical research and clinical trials, typically taking 10–15 years to complete. This process is highly costly, with an estimated expense of approximately $1–2 billion. Moreover, only a very small fraction (<1%) of drug candidates successfully reach clinical trials and gain market approval (3). Drug repurposing offers a solution by investigating the use of approved drugs for the treatment of new diseases, thereby reducing both the cost and duration of drug development (4). Notably, this approach is frequently adopted in cancer research, where the antiproliferative, pro-apoptotic, or anti-metastatic effects of existing drugs are evaluated to identify novel therapeutic options (5). In this context, the oncological potential of certain drugs originally developed for diabetes treatment is also being investigated. In particular, sodium-glucose cotransporter 2 (SGLT2) transporters have attracted attention in cancer cell biology due to their effects on metabolic processes (6). SGLT2 is primarily expressed in the first and second segments of the proximal convoluted tubule in the kidney and plays a crucial role in glucose reabsorption from urine, driven by the sodium concentration gradient (7). SGLT2 inhibitors lower blood glucose levels by reducing glucose reabsorption in the kidney. Initially developed for the treatment of type 2 diabetes mellitus (T2DM), SGLT2 inhibitors such as dapagliflozin, empagliflozin, and canagliflozin have gained attention over time due to their beneficial effects on cardiovascular and renal health. As a result, these drugs have been utilized not only for T2DM treatment but also in managing heart failure and chronic kidney disease (8, 9). Studies have shown that certain tumor cells express SGLT2 transporters (10). Kaji et al. demonstrated that the pharmacological inhibition of SGLT2 transporters suppressed the proliferation of liver cancer cells by reducing glucose uptake, lactate production, and intracellular ATP levels (11). Similarly, it has been reported that empagliflozin exhibits cytotoxic effects in breast cancer (MCF-7), lung cancer (A549) (12), and cervical cancer (HeLa) cell lines (13). Based on these findings, we analyzed the cytotoxicity of empagliflozin against human cancer cell lines, including Caco-2 (colorectal adenocarcinoma), A2780 (ovarian carcinoma), and LNCaP (prostate adenocarcinoma), using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

MATERIALS AND METHODS

Cell Culture

To evaluate the anticancer potential of empagliflozin, LNCaP (American Type Culture Collection [ATCC]), A2780 (ATCC), and Caco-2, (ATCC) cancer cell lines were used. Initially, cells retrieved from the liquid nitrogen tank were seeded into 75 cm² culture flasks under appropriate culture conditions to ensure adaptation and proliferation. For optimal proliferation, LNCaP and A2780 cells were grown in Roswell Park Memorial Institute 1640 medium, whereas Caco-2 cells were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F12 medium. To enhance cell growth and viability, all media were supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. The cells were incubated at 37°C in a humidified environment containing 5% CO_2 (14). When the cells reached 80–90% confluence, trypsin-ethylenediaminetetraacetic solution was applied to detach adherent cells from the flask surface. Following the preparation of the cell suspension, staining was performed using 0.4% trypan blue, and cell viability was analyzed under an inverted microscope (SOIF, XDS-1B). Cells with a viability rate above 90% were included in the experimental phase (15).

Determination of Cell Viability

The MTT assay is a commonly employed colorimetric technique to evaluate cell viability and proliferation. This method relies on the conversion of MTT tetrazolium salt into insoluble purple formazan crystals by mitochondrial dehydrogenase enzymes, reflecting cellular metabolic activity. Since this biochemical conversion occurs exclusively in metabolically active mitochondria, the amount of formazan formed is directly correlated with cellular metabolic activity and, consequently cell viability (16). In the experimental procedure Caco-2, A2780, and LNCaP cells were seeded into 96-well culture plates at a density of 15.000 cells per well and incubated under standard conditions. Subsequently, the cells were exposed to media containing 1, 5, 25, 50, and 100 μM empagliflozin and incubated for 48 hours at 37°C with 5% CO_2. At the end of the incubation period, the culture medium was removed from each well, and the cells were incubated with 50 μL of MTT solution (0.5 mg/mL) (M2128, Sigma-Aldrich, USA) prepared in phosphate-buffered saline. The cells were maintained under dark conditions in an incubator at 37°C for 3 hours. After incubation, the supernatant was removed, and 100 μL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formed formazan crystals. The absorbance was then recorded at 570 nm using a microplate reader (BioTek Synergy-HTX, USA) (17). Control cells were maintained in culture medium under the same conditions as treated cells, without empagliflozin or vehicle. Empagliflozin stock solutions were initially prepared in DMSO and subsequently diluted to the required concentrations. The obtained data were analyzed by comparing each experimental group with its respective control group to evaluate the potential cytotoxic effects of empagliflozin on different cell lines (18, 19).

Statistical Analysis

All statistical analyses were carried out using IBM SPSS software. The Shapiro-Wilk test was employed to assess the normality of the data distribution. Comparisons across groups were conducted using the Kruskal–Wallis H test. Post hoc analyses were then performed using the Mann-Whitney U test with Bonferroni correction applied. Descriptive statistics are presented as mean ± standard deviation, and a p-value of less than 0.05 was considered statistically significant. Additionally, dose-response relationships were analyzed by calculating the half-maximal inhibitory concentration (IC_{50}) and LogIC50 values using nonlinear regression modeling in GraphPad Prism, based on MTT assay data.

RESULTS

Empagliflozin inhibited the proliferation of Caco-2 cells in a dose-dependent manner. At lower concentrations (1 μM: 88.0 ± 8.1%; 5 μM: 77.9 ± 9.2%; 25 μM: 74.7 ± 8.1%), the reduction in cell viability was not statistically significant. However, treatment with 50 μM (56.3 ± 6.1%) and 100 μM (46.2 ± 4.3%) resulted in a significant decrease compared to the control group ($p < 0.05$, Figure 1).

Empagliflozin inhibited the proliferation of LNCaP cells in a concentration-dependent manner. Cell viability gradually decreased with increasing concentrations (1 μM: 90.2 ± 8.7%; 5 μM: 84.9 ± 6.9%), and a statistically significant reduction was first observed at 25 μM (61.7 ± 7.8%) and 50 μM (53.7 ± 7.1%) compared with the control group ($p < 0.05$). The strongest inhibitory effect was detected at 100 μM (42.2 ± 4.8%), which was also significantly lower than both 25 and 50 μM groups ($p < 0.05$, Figure 2).

In A2780 cells, empagliflozin showed minimal effects at lower concentrations (1 μM: 90.4 ± 6.1%; 5 μM: 92.2 ± 5.5%; 25 μM: 88.8 ± 4.6%; 50 μM: 81.0 ± 5.7%), with no statistically significant differences compared to the control group. A significant reduction in cell viability was observed only at 100 μM (57.5 ± 4.2%) ($p < 0.05$, Figure 3).

Table 1 presents the calculated IC_{50} and $LogIC_{50}$ values for A2780, LNCaP, and Caco-2 cells following empagliflozin treatment. Among the tested cell lines, LNCaP cells exhibited the greatest sensitivity, with the lowest IC_{50} value (52.32 μM, $LogIC_{50}$ = 1.719), indicating the most pronounced reduction in cell viability. Caco-2 colon cancer cells showed intermediate sensitivity (IC_{50} = 67.63 μM, $LogIC_{50}$ = 1.830), while A2780 ovarian cancer cells were the least sensitive, with the highest IC_{50} value (156.5 μM, $LogIC_{50}$ = 2.195).

Table 1. LogIC$_{50}$ and IC$_{50}$ (μM) values of the Empagliflozin in A2780, LNCaP, and Caco-2 cell lines		
LogIC50/IC50 (μM)		
A2780	LNCaP	Caco-2
2,195/156,5	1,719/52.32	1,830/67,63

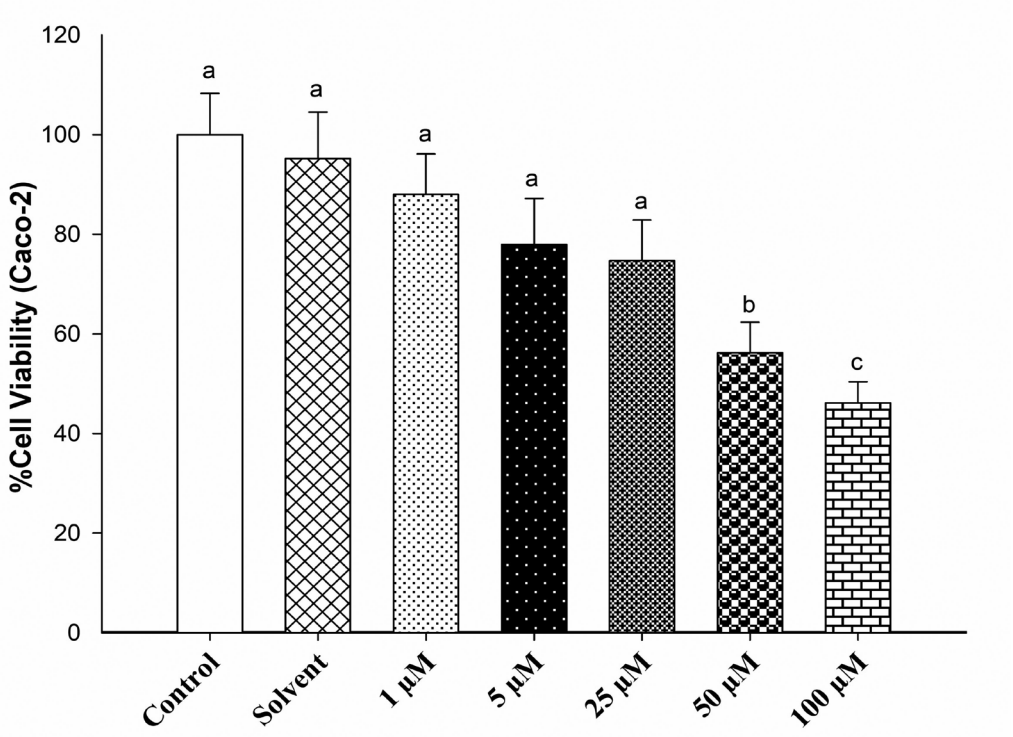

Figure 1: Cell viability results in the Caco-2 cell line after 48 hours of incubation with Empagliflozin. Data are expressed as mean ± standard deviation (SD). Different letters indicate statistically significant differences $^{a,b,c}p<0.05$.

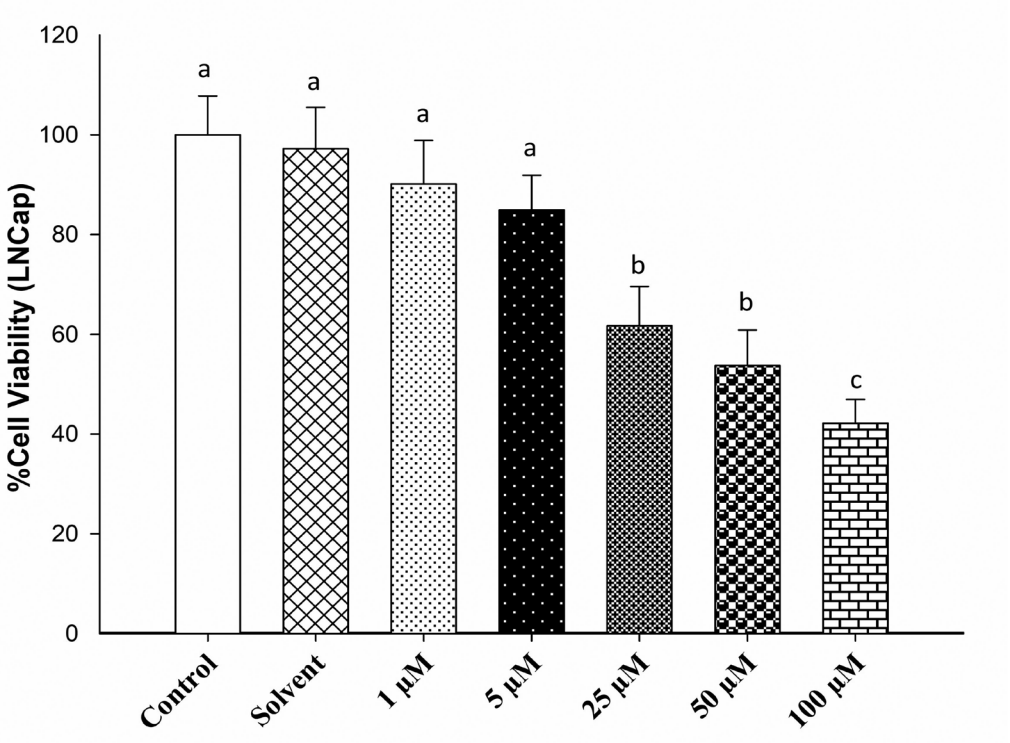

Figure 2: Cell viability results in the LNCaP cell line after 48 hours of incubation with Empagliflozin. Data are expressed as mean ± standard deviation (SD). Different letters indicate statistically significant differences [a,b,c]$p<0.05$.

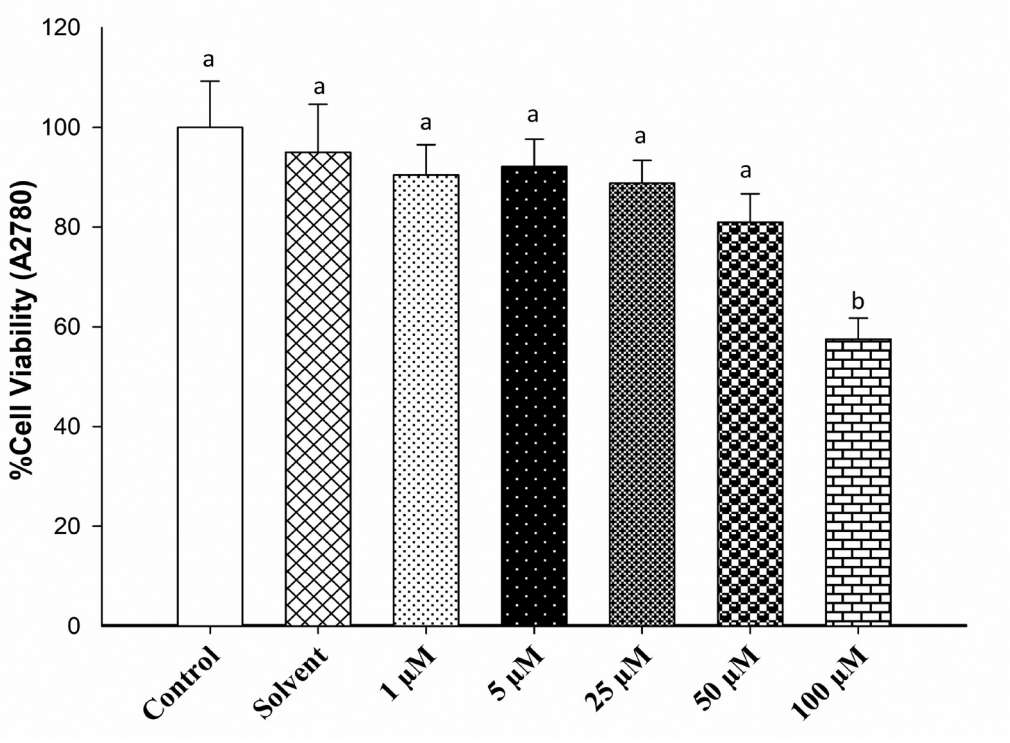

Figure 3: Cell viability results in the A2780 cell line after 48 hours of incubation with Empagliflozin. Data are expressed as mean ± standard deviation (SD). Different letters indicate statistically significant differences [a,b]$p<0.05$.

DISCUSSION

Hyperglycemia is a significant risk factor for cancer, and studies have shown that elevated blood glucose levels can promote tumor volume and growth. Indeed, T2DM is associated with a significantly increased risk of certain cancers, including liver, endometrial, breast, and bladder cancers (20). In patients with T2DM, hyperinsulinemia elevates insulin-like growth factor-1 levels, promoting tumor cell proliferation, survival, and metastasis. Additionally, it exacerbates oxidative stress and inflammation, creating a tumor-promoting microenvironment (21). These pathophysiological processes contribute to DNA damage and cellular dysfunction, facilitating cancer development. However, maintaining glucose levels under control may mitigate oxidative stress and inflammation, thereby preventing DNA damage and inhibiting uncontrolled cell proliferation, ultimately reducing cancer risk (22, 23).

Empagliflozin is a widely used SGLT2 inhibitor in the treatment of T2DM due to its well-tolerated side effect profile. In addition to improving plasma glucose control, it has also been shown to have cardiovascular and renal protective effects (24). Recent studies have suggested that SGLT2 inhibitor drugs may have potential effects not only in the treatment of T2DM but also on cancer cell metabolism and growth (25). Several studies have reported that pancreatic and prostate tumors, as well as glioblastomas, express SGLT2 transporters (10, 26). Furthermore, SGLT2 has been identified as playing a role in glucose uptake in glioma cells, and it has been shown that the SGLT2 inhibitor canagliflozin inhibits glucose uptake in these cells (27). Based on these findings, the present study investigated the effects of empagliflozin on cancer cell viability using A2780, Caco-2, and LNCaP cancer cell lines, and demonstrated notable cytotoxic activity in all tested cell lines. These findings highlight a potential role for SGLT2 inhibition in the regulation of cancer cell survival and proliferation. Consistent with our current findings, previous studies have also demonstrated that empagliflozin exhibits cytotoxic and antiproliferative effects in various cancer cell lines. This agent has been reported to induce significant cytotoxic effects in breast cancer (MCF-7) and lung cancer (A549) cell lines (12). Moreover, nanoparticle formulations have been shown to exhibit stronger cytotoxicity and increase cell death rates in A549 lung cancer cells compared to unencapsulated empagliflozin (28). Additionally, it has been found to exert synergistic effects with chemotherapeutic agents. Its combination with doxorubicin significantly reduced cell viability in TNBC cell lines, leading to a more potent anticancer effect compared to monotherapies (29). Moreover, other SGLT2 inhibitors such as dapagliflozin, canagliflozin, and ipragliflozin have also been reported to exhibit antiproliferative, cytotoxic, and anti-migratory effects in Caco-2, MCF-7, and prostate (Du-145) cancer cell lines, further supporting the consistency of our findings with the existing literature (30). Although the mechanisms underlying these effects are not yet fully understood, they are likely multifactorial in the present study. Empagliflozin has been suggested to interfere with glucose-dependent cancer metabolism by inhibiting glucose uptake via SGLT2 transporters, thereby disrupting metabolic homeostasis. In parallel, activation of pro-apoptotic signaling pathways may further contribute to reduced cancer cell viability (11, 13). Additionally, empagliflozin may modulate the tumor microenvironment by reducing oxidative stress and inflammatory signaling, which are known to facilitate tumor growth and metastasis (31). Also in this study, analysis of IC_{50} values revealed the greatest reduction in cell viability in LNCaP prostate cancer cells, followed by Caco-2 colon and A2780 ovarian cancer cells. These differences in cytotoxicity may reflect variations in SGLT2 expression, which has been reported in ovarian, prostate, and colon adenocarcinomas (32-34).

The present study demonstrates that empagliflozin exerts cytotoxic effects in A2780, LNCaP, and Caco-2 cell lines, suggesting a potential role for SGLT2 inhibition in cancer therapy. However, further studies are required to clarify the underlying mechanisms and the relevance of SGLT2 expression in different tumor types

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Abbreviations List

ATCC: American Type Culture Collection
DMSO: dimethyl sulfoxide
IC50: half-maximal inhibitory concentration
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
SGLT2: sodium-glucose cotransporter 2
T2DM: type 2 diabetes mellitus

Ethical Approval Statement

The study was carried out on human cancer cell lines (A2780, LNCaP, and Caco-2). According to the institutional guidelines, the use of established cell lines does not require ethical approval.

Consent for Publication

Not applicable.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare no competing interests.

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No funding was received for this study.

Author Contributions

Conceptualization: Engin Korkmaz. Methodology: Suat Tekin, Tuba Keskin, Engin Korkmaz. Formal analysis and investigation: Suat Tekin. Writing - original draft preparation: Engin Korkmaz. Writing - review and editing: Suat Tekin. Supervision: Suat Tekin. All authors have read and agreed to the published version of the manuscript.

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