

Evaluation and comparison of anti-cancer activity of dapagliflozin and canagliflozin in oral cancer cell line: an in vitro study

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ABSTRACT

Background: Cancer is rapidly evolving life-threatening ailment in the mankind due to changes in daily food intake and lifestyle changes. Oral carcinoma is 6th major cause of cancer death in the world and it is third major reason of cancer mortality in India. Every cell in the human body requires glucose for its metabolic energy. Besides normal cell, cancer cells also require the glucose for its endurance and multiplication. SGLT2 inhibitors which are aimed at diabetes therapy exhibited anticancer properties also in colon and pancreatic cancer lines. Present study aim is to evaluate the anticancer activity of SGLT2 inhibitors against oral cancer cell by MTT Assay.

Methods: To evaluate the anticancer activity of SGLT2 inhibitors MTT Cytotoxic assay is performed as per standard protocols. Cancer cells were plated in 24-well plates and incubated at 37°C with 5% CO₂ condition. After convergence, samples are added to the plates in various concentrations and allowed to incubate then they are detached from the plates and cleansed with the reagents. The wells are coated with the dye and incubated. Later samples are analysed in UV-spectrophotometer.

Results: Cytotoxic assay showed decrease in cell viability with increasing dose of SGLT2 inhibitors. IC₅₀ values were determined graphically. The IC₅₀ value of dapagliflozin is 400µg/ml and canagliflozin is 250µg/ml respectively after 24 hours of Assessment.

Conclusions: The results of the current study give us an evidence that SGLT2 inhibitors dapagliflozin and canagliflozin exhibits anticancer property in Oral Cancer cell line.

Keywords: Canagliflozin, Dapagliflozin, Oral cancer, MTT Assay, SGLT2 inhibitors

INTRODUCTION

In the current scenario of massive variations in daily regime of humans across globe and owing to these a life-threatening ailment called cancer is evolving swiftly. Cancer is due to loss of cell cycle control associated with abnormal and uncontrollable cell growth.¹ Today cancer is one of the foremost reasons of the deaths. Among all cancers in the world oral carcinoma stands sixth, it is one among leading three causes of cancer in India with low survival rate of mankind.^{2,3} There is increased prevalence of premalignant oral lesions in the diabetes patients leading to oral cancer.⁴ This oral squamous cell carcinoma

classically affects middle age cluster adults who are at consistent acquaintance to tobacco, betel, liquor or micronutrients deficit.⁵ Now-a-days even younger age group patients are affected with oral cancer. The primary onset of oral cancer is sited at base of tongue, tonsils, oropharynx.

Every cell of human body need glucose aimed at its endurance and it is main provider of metabolic energy. Glucose is adopted into the cytosol compartment where it is exposed to glycolysis intended to produce pyruvate that is altered into acetyl Co-A for ATP production in mitochondria. Nevertheless, glucose remains a hydrophilic complex besides in order to passage through the

phospholipid bilayer, precise conveyance proteins are essential, such as sodium glucose co-transporters (SGLT).⁶ Other than normal cells, glucose stands the foremost provider of metabolic energy in tumour cells, which articulated transformed glucose uptake by means of amplified necessity intended for glycolysis or Warburg effect.⁷ With excess production of glucose in diabetic patients, malignant cells accept more glucose besides duplication happens subsequently oral malignancy prevalence is high in these people.

In humans, plasma glucose is filtered inside kidney glomerulus then a reuptake progression stays trailed via sodium glucose co-transporters SGLT1 and SGLT2 ensuing a high reabsorption proportion close to 99%.^{8,9} SGLT2 stands as high capacity transporters expressed in the proximal tubule of the nephron and possibly will be backing near 90% of glucose reabsorption. Hence, SGLT2 inhibitors such as canagliflozin and dapagliflozin lately acknowledged excessive consideration as novel medications intended for the treatment of type II diabetes. Dapagliflozin and canagliflozin SGLT2 inhibitors are chosen for this study since they are metabolically steady, in disparity to further SGLT2 inhibitors such as phlorizin which is inapt for drug development due to its low bioavailability and swift hydrolysis in the kidney.^{8,10}

A recent work showed that SGLT2 transporters are functionally expressed in prostrate and adenocarcinoma of pancreas and that SGLT2 inhibitors may hinder glucose acceptance and, in that way, reduced malignant cancer progress and improved existence in a xenograft model of pancreatic cancer.¹¹ Hence, SGLT2 inhibitors might demonstrate beneficial in tumour healing.

In this work, the effect of SGLT2 inhibitors on KB cells examined using MTT cytotoxic assay and to compare both dapagliflozin and canagliflozin to see which has better activity in this study.

METHODS

Molecule, cell lines and culture

Dapagliflozin molecule is procured from AstraZeneca India. Canagliflozin molecule is acquired from Manus Akkteva Biopharma LLP, Ahmedabad. From National Centre for Cell Sciences, Pune the KB cell lines procured and conserved in Minimal Essential Medium augmented alongside Penicillin 100U/ml, 10% FBS besides Streptomycin 100µg/ml in 50µg/ml CO₂ at moistened temperature 37°C.

Reagents

MEM was purchased from Hi Media Laboratories. Fetal Bovine Serum was procured from Cistron Laboratories. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT), Trypsin and Dimethyl Sulfoxide (DMSO) were procured from Sisco Research laboratory

situated in Mumbai. All other components and chemicals were obtained from Sigma-Aldrich Inc, Mumbai.

In vitro assay for anticancer activity (MTT Assay)

The cytotoxic activity of dapagliflozin and canagliflozin on KB Cancer Cells was determined by MTT assay.¹² KB Cells 1×10^5 per each well were seeded in 24-well plates and nurtured at 37°C with 5% carbon dioxide condition. Later the cell ranges the convergence, several concentrations of test samples of dapagliflozin, canagliflozin and standard drug 5-Fluorouracil were added from minimum to maximum 1000µg/ml and nurtured for 24 hrs. After nurture, samples were detached from well plates and cleansed by means of saline buffered with phosphate of pH 7.4 or MEM deprived of serum. 100µl per each well (5mg/ml) of 0.5% MTT salt was supplemented and allowed to incubate 4 hours. Later, a volume of 1ml DMSO was supplemented in entire well plate. The absorbance measured at 570nm wavelength by means of UV-Spectrophotometer with DMSO in place of blank. Measurements were detected, and the concentration essential for a 50% dilution (IC₅₀) was plotted on graph paper. The viability of cells percentage is obtained by calculation using equation:

$$\text{Viability of cells percentage} = \frac{A570 \text{ of preserved cells}}{A570 \text{ of control cells}} \times 100$$

Graph plotted with cell viability percentage on Y-axis and concentration of test sample on X-axis. Cell control and sample control is contained within each assay to compare the complete cell viability valuations.

RESULTS

From the results of MTT Assay we perceive that there remained diminution in cell viability by intensification of concentration.

Table 1: Anticancer effect of sample dapagliflozin on KB cell line.

Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1000	Neat	0.183	35.74
500	1:1	0.237	46.28
250	1:2	0.291	56.83
125	1:4	0.339	66.21
62.5	1:8	0.391	76.36
31.2	1:16	0.416	81.25
15.6	1:32	0.441	86.13
7.8	1:64	0.457	89.25
Cell control	-	0.512	100

Dapagliflozin displayed maximum cell viability as 89.25% (minimum cell inhibition as 11.75%) at 7.8µg/ml concentration of dapagliflozin after 24 hours of assay with

KB Cell line and the minimum cell viability is 35.74% (maximum cell inhibition is 64.26%) at 1000µg/ml concentration of dapagliflozin after 24 hours assay (Table 1).

Table 2: Anticancer effect of sample canagliflozin on KB cell line.

Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1000	Neat	0.129	25.19
500	1:1	0.194	37.89
250	1:2	0.275	53.71
125	1:4	0.328	64.06
62.5	1:8	0.375	73.24
31.2	1:16	0.418	81.64
15.6	1:32	0.438	85.54
7.8	1:64	0.452	88.28
Cell control	-	0.512	100

Table 3: Anticancer effect of 5-fluorouracil on KB cell line.

Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1000	Neat	0.011	6.9
500	1:1	0.024	11.34
250	1:2	0.077	18.75
125	1:4	0.114	24.98
62.5	1:8	0.183	48.90
31.2	1:16	0.263	54.78
15.6	1:32	0.368	69.87
7.8	1:64	0.475	78.56
Cell control	-	0.512	100

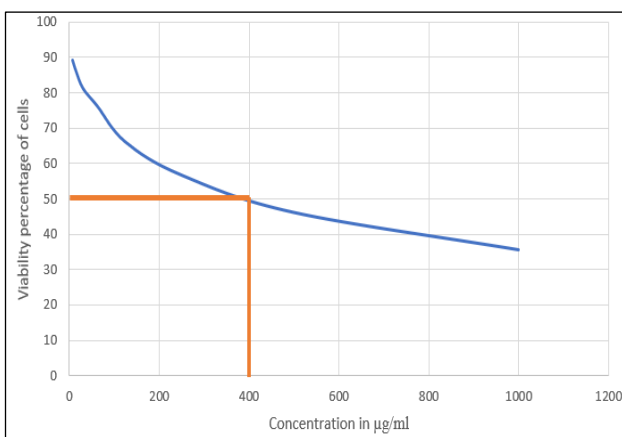


Figure 1: Cell viability assay of dapagliflozin against KB cell line.

Canagliflozin maximum cell viability is 88.28% (minimum cell inhibition is 11.72%) at 7.8µg/ml concentration of canagliflozin after 24 hours and the minimum cell viability

is 25.21% (maximum cell inhibition is 74.79%) at 1000µg/ml concentration of canagliflozin after 24 hours of assay (Table 2). The maximum and minimum cell viability for 5-fluorouracil is 72.85% at 7.8µg/ml concentration and 4.9% at 1000µg/ml concentration respectively (Table 3). The inhibitory concentration (IC₅₀) at half maximum value of dapagliflozin is ascertained at 400µg/ml and IC₅₀ Value of canagliflozin is ascertained at 250µg/ml after 24 hours of assessment. The IC₅₀ value of 5-Fluorouracil is 58µg/ml. The IC₅₀ values are depicted in the figures (Figure 1 and 2).

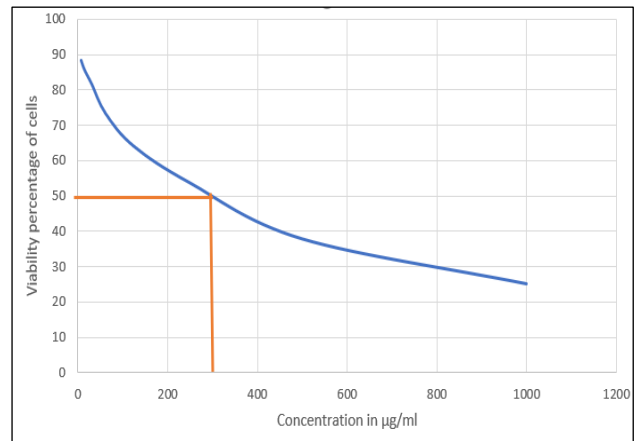


Figure 2: Cell viability assay of canagliflozin against KB cell line.

DISCUSSION

Apart from modulating sodium glucose transport inhibition in diabetic patients, SGLT2 inhibitors exhibited anticancer activity in colon and pancreatic cancer cells in a recent study by Scafoglio et al, done on dapagliflozin and canagliflozin, this phenomenon might be due to inhibition of SGLT2 intermediated glucose uptake.¹¹ However, standalone restriction of glucose uptake is not enough to reduce cell viability/progression.¹³ Hence it is not clear if this is the chief mechanism by which SGLT2 inhibitors restrict growth. A recent study by Villani et al, stated canagliflozin triggered AMPK by constraining mitochondrial respiration in human embryonic kidney cells. Canagliflozin prevents both glucose acceptance and complex - I reinforced respiration concurrently, this twin mechanism of action may be significant for facilitating the inhibitory properties of propagation.¹⁴ The encounter of a novel mechanism of glucose transportation in cancer rises the interrogation of the importance of these SGLT equated by the previously renowned GLUT- dependent glucose acceptance.^{15,16} The key change amongst GLUTs besides SGLTs is that while GLUTs passage glucose down the concentration slope, SGLTs connect the sodium rise crosswise the plasma membrane to initiate glucose acceptance, namely sodium-glucose cotransport. Consequently, the dynamism for glucose cotransport is gained indirectly from ATP pouring the Na/K pump used to uphold the sodium incline over plasma membrane.^{17,18} In epithelial cells, GLUTs and SGLTs are conveyed in

distinct apical, basolateral and plasma membranes, nevertheless in tumour cells the dispersal and comparative status of the two forms of glucose transporters are not until now identified. It is likely that in tumour cells, GLUT1 and SGLT2 canal glucose in the direction of diverse cellular partitions and metabolic paths, together with mitochondrial oxidation, the pentose phosphate pathway, aerobic glycolysis, and the hexosamine path.^{19,20}

In a recent study by Saito et al, it has been reported that dapagliflozin was inhibiting the glucose uptake of cancer cells and causing cytotoxicity.¹³ Correspondingly in our study canagliflozin and dapagliflozin also have shown significant loss of cell viability in oral squamous cell carcinoma KB cell lines at concentrations of 250µg/ml and 400µg/ml in comparison with standard drug 5-fluorouracil, also we have detected the cells underwent reduction and decline in number exhibiting positive cytotoxic activity and the probable mechanism would be because of the restriction of glucose uptake and curbing Mitochondrial intracellular ATP synthesis thereby limiting the cell cycle of cancer cells.²¹ In present study canagliflozin was more cytotoxic than dapagliflozin in KB cell line as established by IC₅₀ values.

CONCLUSION

This study establishes that SGLT2 inhibitors has encouraging cytotoxic action against oral squamous cancer KB cell line with canagliflozin unveiling more cytotoxicity than dapagliflozin and both drugs have potential to be used as anticancer agents in cancer therapy nevertheless further in vivo studies are required.

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Ethical approval: Not required

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