Original Article

DOI: 10.7860/JCDR/2015/16015.6979

Evaluation of Anti Cancer Effects of DPP-4 Inhibitors in Colon Cancer- An Invitro Study

Pharmacology Section

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ABSTRACT

Introduction: Among the oral anti-diabetic drugs, Dipeptidyl peptidase - 4(DPP-4) inhibitor is an emerging class of drugs. Inhibitors of DPP-4 enzyme like Sitagliptin and Vildagliptin have shown Anti-oxidant properties in many studies, both invivo and invitro. It has also been characterized as an apoptotic agent on pancreatic cancer cells. In the following study, Anticancer effect of DPP 4 inhibitors on colon cell lines (HT-29) using MTT assay— {3 -4, 5-dimethyl (thiazol – 2 -yl) -3, 5- dimethyl tetrazolium bromide} assay was elucidated.

Aim: To elucidate and compare the anticancer potential of two DPP 4 inhibitors using in-vitro MTT assay on colorectal cell lines (HT-29).

Materials and Methods: We treated HT-29 cell lines with two DPP 4 inhibitors. HT-29 cells were incubated at 37^{0}C and drug samples were added in various concentrations and incubated for 24 hours. MTT dye was added to the sample and it was incubated for 4 hours. One ml of DMSO was added Using an Ultraviolet-Spectrophotometer, measurement of absorbance was done at 570nm following which the half maximal inhibitory concentration was graphically estimated in relation to the percentage of viability of the cell and the sample concentration.

Results: We found that both the drugs have shown anticancer activity starting from low to high concentrations when compared with the control using MTT assay. The IC 50 value of Sitagliptin is 31.2 mcg/ml and Vildagliptin is 125 mcg/ml.

Conclusion: From this study, we found that the drugs have significant Anti-Cancer property, which would probably play a role as cytotoxic agent in tumour cells. Sitagliptin was found to be more potent than Vildagliptin in colon cancer cell lines.

Keywords: Anticancer activity, Colorectal cell lines, MTT assay

INTRODUCTION

Dipeptidyl peptidase (DPP- 4) inhibitors are class of Oral antidiabetic drugs. They are used for the treatment of Type 2 Diabetes mellitus. DPP-4 is an enzyme which puts down the action of hormone, incretin. Incretins belong to the group of hypoglycaemic gastrointestinal hormones. In humans, there are two major incretin hormones. They are glucose dependent insulinotropic peptide-GIP and glucagon - like peptide-1-GLP-1. DPP4 inhibitors inhibit the degradation of GIP and GLP-1 [1-3]. It is proven that GLP-1 arrests cell proliferation and induces death of colon cancer cells, which shows their protective role in colon cancer [4]. The first available DPP-4 inhibitors are Sitagliptin, Vildagliptin. These orally active DPP-4 inhibitors are efficacious and well tolerated.

The need for newer anticancer drugs: Currently, most of the drugs used in the treatment of cancer are cytotoxic. Cytotoxic drugs are not specific only to cancer cells. They also affect normal cells; hence they may be harmful to the body. It is necessary to design newer drugs that are more specific to cancer cells. Many antidiabetic drugs like metformin and Peroxisome proliferator-activated receptor gamma agonists have shown significant anticancer properties in cancer cells. Some studies show that DPP-4 inhibitors causes cancer and some study show that they have anticancer property. This study is done to prove that DPP-4 inhibitors have anticancer activity against colon cancer cell lines.

Sitagliptin: Sitagliptin is an FDA approved anti-diabetic drug in the year 2006 [5]. It is a highly potent DPP4 inhibitor [6]. Sitagliptin is preferred as a second line drug along with combination of other oral antidiabetic drugs, when there is failure of diet or exercise [7]. Studies have shown that when Sitagliptin is given chronically at therapeutic range, it decreases colon cancer in rats [8]. Sitagliptin also has cardio protective effects in mice and it has also shown improvement in Ischemic heart diseases [9,10]. Known adverse effects of these drugs are hypoglycaemia, photosensitivity, nausea and common cold.

Vildagliptin: Vildagliptin is another oral antidiabetic drug of the DPP-4 inhibitors family. It inhibits the DPP-4 enzyme competitively and reversibly. It blocks the deactivation of GLP-1 and GIP by DPP-4 enzyme, and allows it to secrete insulin. It also reduces the glucagon release from alpha cells of islets of langerhans [11,12]. Vildagliptin is very effective in type II diabetes mellitus. Many studies have proved that it promotes the function of pancreas and maintains blood glucose levels [13], protects against vascular diseases by promoting endothelial cell network formation and revascularization [14]. It has a protective role in hyperlipidaemia [15] and has anti-inflammatory properties also. It decreases the albumin concentration in diabetic nephropathy and also reduces the atherosclerosis progression in hyperlipidaemic patients. Vildagliptin can cause side effects like hypoglycaemia, pancreatitis, hepatotoxicity, nausea, headache and tremors. In this study, the anticancer activity of Sitagliptin and Vildagliptin is evaluated.

AIM AND OBJECTIVE

To elucidate and compare the Anticancer potential of two DPP-4 inhibitors-Sitagliptin and Vildagliptin using invitro MTT assay on colorectal cell lines (HT-29).

Principle: MTT assay, a colorimetric assay is done to assess the cell viability. Under defined conditions, NAD (P) H-dependent cellular oxidoreductase enzyme reflects the viability of cells present.

NAD (P) H enzymes also reduce the tetrazolium dye MTT 3 - (4, 5 - dimethylthiazol – 2 - yl) - 2, 5 - diphenyltetrazolium bromide to its insoluble formazan, which is purple coloured. This method is safe, easy to use and it also has more reproducibility and commonly used for both cell viability and cytotoxicity tests.

MATERIALS AND METHODS

Test samples: Sitagliptin and Vildagliptin.
Solvent: Dimethyl sulfoxide (DMSO).
Reagent: MTT

HT-29 cell lines were procured from National Centre for Cell Sciences, Pune. The cells were maintained in Minimal Essential Medium enhanced with 10% FBS, streptomycin (100 µg/ml) and penicillin (100 U/ml), in a humidified atmosphere of 50 µg/ml CO_2 at 37°C.

At 37°c, cells were incubated along with 5% CO_2. At different concentrations the samples were added, and incubated for 24 hours. At the end of incubation, samples were transferred from the well following a wash with Phosphate Buffered Saline at a pH 7.4. Thereafter MTT was added and the samples were taken for 4 hours incubation. Subsequent to incubation, 1 ml of dimethyl sulfoxide was totaled in all the wells. With the aid of Ultraviolet Spectrophotometer, the absorbance was measured at 570 nm along with DMSO as blank. Consecutively, measurements for concentration required for 50% (IC 50) inhibition was noted. Cell viability percent was calculated using the formula given below:

$$\text{Percentage (\%) of Cell viability} = \frac{\text{A570 of treated cells}}{\text{A570 of control cells}} \times 100$$

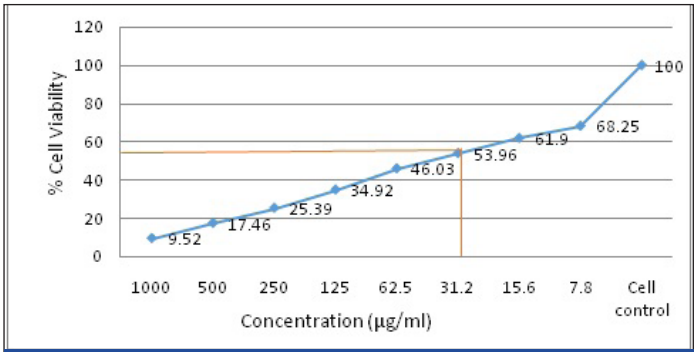
The graph is plotted with the Y-axis showing the percentage of viability of cells and X-axis showing the sample concentration.

RESULTS

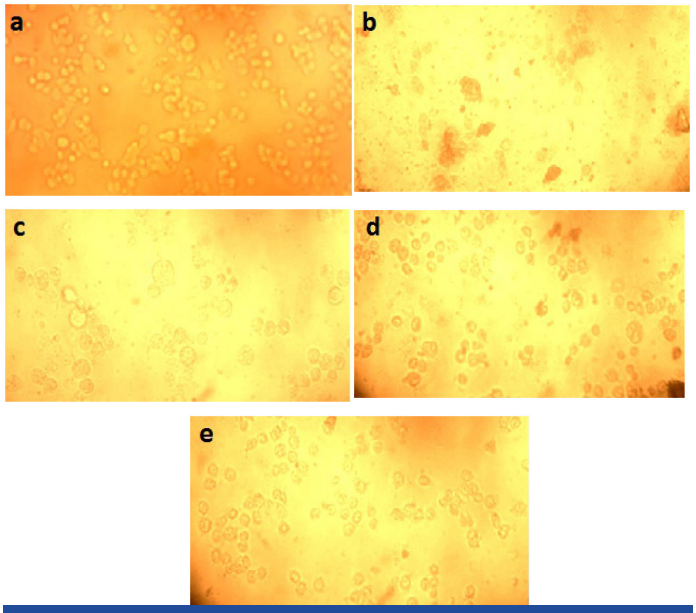
At concentrations of 7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1000 µg/ml, the cell viability of Sitagliptin was 68.25%, 61.90%, 53.96%, 46.03%, 34.92%, 25.39%, 17.46% and 9.52% respectively as shown in [Table/Fig-1,2]. Hence, the half maximal inhibitory concentration of Sitagliptin was at the concentration of 32.1 µg/ml. The anticancer effect of Sitagliptin on colon cancer cell lines at various toxicities is shown in [Table/Fig-3]. At concentrations of 7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1000 µg/ml, the cell viability of Vildagliptin was 74.60%, 69.84%, 65.07%, 57.14%, 49.20%, 39.68%, 28.57% and 15.87% respectively as shown in [Table/Fig-4,5]. Hence, the half maximal inhibitory concentration of Vildagliptin was at the concentration of 125 µg/ml. The anticancer effect of Vildagliptin on colon cancer cell lines at various toxicities is shown in [Table/Fig-6].

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.06	9.52
2	500	1:1	0.11	17.46
3	250	1:2	0.16	25.39
4	125	1:4	0.22	34.92
5	62.5	1:8	0.29	46.03
6	31.2	1:16	0.34	53.96
7	15.6	1:32	0.39	61.90
8	7.8	1:64	0.43	68.25
9	Cell control	-	0.63	100

[Table/Fig-1]: Anticancer effect of Sitagliptin on colon cancer cell line.



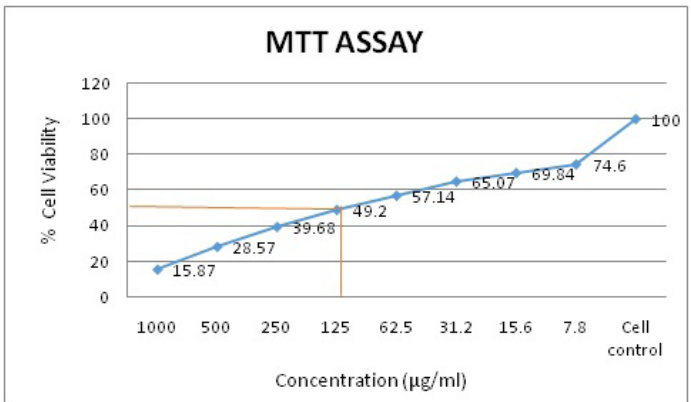
[Table/Fig-2]: Anticancer effect of Sitagliptin on colon cancer cell line graph



[Table/Fig-3a-e]: Anticancer effect of Sitagliptin on colon cancer cell line.
a) Normal Colon Cancer Cell line, b) Cancer cell line at Toxicity- 1000µg/ml,
c) Cancer cell line at Toxicity- 125µg/ml, d) Cancer cell line at Toxicity –62.5µg/ml,
e) Cancer cell line at Toxicity-31.2µg/ml

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.10	15.87
2	500	1:1	0.18	28.57
3	250	1:2	0.25	39.68
4	125	1:4	0.31	49.20
5	62.5	1:8	0.36	57.14
6	31.2	1:16	0.41	65.07
7	15.6	1:32	0.44	69.84
8	7.8	1:64	0.47	74.60
9	Cell control	-	0.63	100

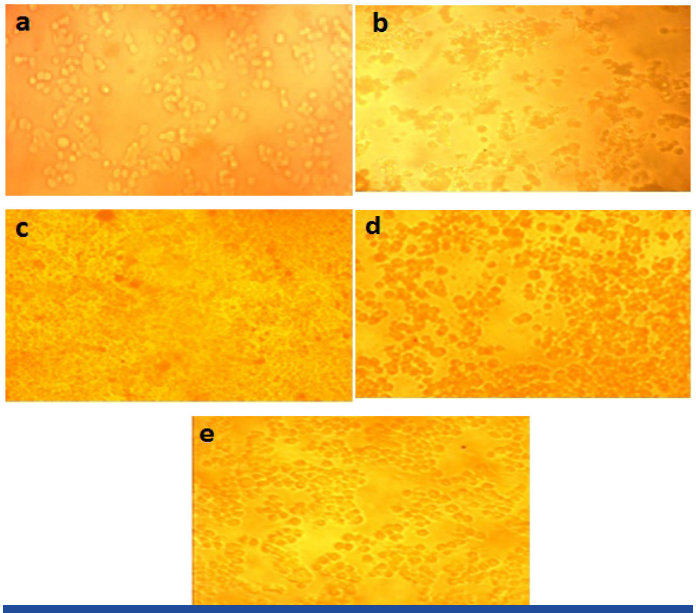
[Table/Fig-4]: Anticancer effect of Vildagliptin on colon cancer cell line.



[Table/Fig-5]: Anticancer effect of Vildagliptin on colon cancer cell line graph

DISCUSSION

Previous studies done with DPP-4 inhibitors have shown their significant anticancer activity against pancreatic cell lines. Hence, this study was aimed to elucidate its effects on colon cancer cell lines. It is found that both the drugs have shown anticancer activity starting from low to high concentrations when compared with the control using MTT assay. The IC 50 value of Sitagliptin is 32.1 µg/ml and Vildagliptin is 125 µg/ml. Hence, Sitagliptin was found to be more potent than Vildagliptin on HT-29 tumour cell lines. The change in colour of the cell lines highlights the presence of anticancer activity which was further quantified using spectrophotometer.



[Table/Fig-6a-e]: Anticancer effects of Vildagliptin on colon cancer cell line.
a) Normal Colon Cancer Cell line, b) Cancer cell line at Toxicity- 1000µg/ml
c) Cancer cell line at Toxicity- 250µg/ml, d) ancer cell line at Toxicity –125µg/ml
e) Cancer cell line at Toxicity-62.5µg/ml

These DPP4 inhibitors can play a beneficial and protective role especially in Type 2 Diabetic patients with cancer, since it has both actions in reducing blood sugar levels and anticancer activity.

Similar studies have shown that Sitagliptin also reduces colon carcinogenesis and blood ROS levels in rats when administered at human therapeutic dosage. Precancerous lesions in Dimethylhydrazine induced rats were measured to obtain the results for its chemo preventive activity [16]. And this was reported to be the first study showing the protective role of DPP-4 inhibitors in intestinal carcinogenesis. The carcinogenic effect was found to be independent of the variation of proliferation and morphometric parameters of small intestine and colon. In addition studies have reported that Sitagliptin also has effect on preneoplastic cells. Recent studies show GLP-1 play a beneficial and protective role in transplanted tumours and colon cancer cell lines [4].

Since DPP-4 is highly associated with the Fibroblast activation protein (FAP) expressed by the tumour associated fibroblasts [17], few studies have proved that Vildagliptin, an inhibitor of FAP inhibits lung tumourigenesis [18]. However, there is no clear study with evidence for the role of Vildagliptin in colon cancer. FAP is also over expressed in colon carcinogenesis which can justify the protective effect of DPP-4 inhibitors in colon cancer. Compared to the previous conflicting results, our study clearly consolidates the chemo preventive activity of DPP4 inhibitors, both Sitagliptin and Vildagliptin, invitro. It can also justify intervention trials in colon cancer patients or patients operated for polyps. Sitagliptin is indicated to non diabetic patients as well since it has no risk for hypoglycaemia.

LIMITATIONS

MTT assay is only a preliminary test done for screening. It does not confirm the anticancer activity completely. Further researches should be done with evaluation techniques like flow cytometry and invitro bioassays to confirm the anticancer effects of DPP4 inhibitors.

CONCLUSION

From our study, the anticancer activity as measured by the MTT method showed that both the drugs had anticancer activity individually. Percentage inhibition of Sitagliptin is more than Vildagliptin, in colon cancer cell lines. This significant anticancer activity of DPP-4 inhibitors could play a role as a cytotoxic agent in many tumour conditions.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jul 31, 2015**
Date of Peer Review: **Sep 21, 2015**
Date of Acceptance: **Oct 20, 2015**
Date of Publishing: **Dec 01, 2015**