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Research Article

COMPARATIVE STUDY OF ANTI-CANCER ACTIVITY OF APREMILAST AND IMATINIB – *IN VITRO* STUDY OF COLON CANCER

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ABSTRACT

Objective: Among oral phosphodiesterase (PDE) inhibitors, PDE-4 inhibitors such as apremilast display anti-inflammatory effects in numerous in vitro and in vivo studies. Apremilast suppresses the release of various cytokines, interleukins, and other inflammatory mediators, while also reducing the formation of reactive oxygen species. Moreover, it may act as a pro-apoptotic agent in colon and pancreatic cancer cells. Meanwhile, imatinib, a tyrosine kinase inhibitor used to treat gastrointestinal stromal tumors, chronic myeloid leukemia, and other cancers, also shows anti-cancer activity. This study evaluates the effects of both PDE-4 inhibitors and imatinib on HT-29 colon cancer cells using the MTT (methylthiazolyldiphenyl-tetrazolium bromide) assay.

Methods: HT-29 cell lines are incubated at 37°C for 24 h, and drug samples are added at various concentrations. An ultraviolet spectrophotometer was used to add 1 mL of dimethyl sulfoxide. 50% inhibition concentration was measured graphically.

Results: The maximum half of the cell inhibitory 56.09% is at a 31.2 µg/mL concentration of apremilast, and the maximum half of the cell inhibitory 49.17% is at a 62.5 µg/mL concentration of imatinib.

Conclusion: Hence, this study concludes that the percentage of cell inhibition for apremilast is equally potent as imatinib, but at higher concentrations, the percentage of cell viability is less for imatinib than apremilast in colon cancer cell lines. Further studies might be required on humans to prove the effect of the drug on cancer patients.

Keywords: Anti-cancer activity, Apremilast, Imatinib, MTT assay, and HT-29 colorectal cell line.

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INTRODUCTION

Phosphodiesterases (PDEs) are a group of enzymes that regulate intracellular levels of the second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate. cAMP is involved in several cellular effects of hormones and neurotransmitters. PDEs are activated by magnesium ions and are responsible for their actions in various activities of the drug [1]. PDE inhibitors have been approved for the prevention and treatment of several diseases.

PDE3 has high affinity for cAMP among all the isoenzymes [2]. PDE3 has the highest activity in blood vessels and airways; hence, it is considered an effective therapeutic agent in bronchial asthma and cardiovascular disease. PDE3 inhibitors have been effective against airway and vascular smooth muscle relaxation, platelet aggregation inhibition [3]. PDE 3 may induce lipolysis and be effective against the treatment of acute heart failure and intermittent claudication [4].

PDE4, known as cAMP-specific PDE, is found predominantly in most of the inflammatory cells except in platelets, so it is involved in inflammatory airways disease. Recently approved drug known as Rolipram, a cAMP-PDE inhibitor, acts as an ideal drug for the treatment of depression by increasing cAMP levels and thereby leading to enhanced noradrenergic neurotransmission in the central nervous system [5]. PDE inhibitors may have anti-cancer effects on hepatocytes, according to certain research. According to this study, apremilast exhibits anti-cancer efficacy against cell lines that represent colon cancer. There are a number of novel medications for colon cancer cells on the market.

HT-29 is an epithelial-shaped human colorectal cancer cell line. Common therapies for colorectal cancer include the chemotherapy drugs 5-fluorouracil and oxaliplatin, which can affect these cells.

The Food and Drug Administration (FDA) authorized apremilast in March 2014; It belongs to the PDE-4 antagonist, an orally administered drug which is effective in treating psoriasis and several dermatological diseases [6]. Apremilast binds to the PDE-4 enzyme, elevates levels of cAMP, and decreases (Tumor Necrosis Factor [TNF])-α and interleukin (IL)-12 [7]. It has an anti-inflammatory effect on the immune system [8].

Imatinib was approved by the FDA in May 2001. It was recommended as a first-line treatment in chronic myelocytic leukemia in 2002. It has a good therapeutic effect on leukemia, gastric stromal tumors, and gynecological tumors. It directly inhibits the activity of tyrosine kinase, which is involved in the alteration of the function of various genes, such as cell cycle, adhesion, and apoptosis of the cells [9]. It has an anti-proliferation effect through cell cycle inhibition. Therefore, it is used as a positive control.

Aim and objective

To evaluate and assess the anti-cancer activity in PDE inhibitor – apremilast and tyrosine kinase inhibitor– imatinib, on human cancer of the colon using the *in vitro* MTT test on the HT-29 cell line.

METHODS

Test samples

Apremilast and imatinib.

Reagent
Methylthiazolyldiphenyl-tetrazolium bromide (MTT).

Solvent
Dimethyl sulfoxide (DMSO).

Inclusion and exclusion criteria of the anti-cancer activity of apremilast and imatinib
Established human cancer cell lines that are known to respond to PDE4 and tyrosine kinase inhibitors were included in the investigation. We only chose cell lines with established growth characteristics and published 50% inhibition concentration (IC_{50}) values for imatinib and apremilast. To guarantee active proliferation and dependable medication response, cells in the logarithmic growth phase were included. Cell lines that were free of contaminants such as bacteria, fungus, or mycoplasma were deemed suitable for use in the research. To ensure consistency in the experiment, cells exhibiting aberrant morphology or poor adhesion were eliminated.

Principle of MTT assay
Cell viability and metabolic activity are measured using MTT, a sort of colorimetric assay [10]. Enzymes that are dependent on NAD(P)H transform 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium MTT into formazan, an insoluble form. This is a commonly used method to identify the cytotoxic activity.

The National Centre for Cell Sciences in Pune is the source of the HT-29 cell line. The cells were cultivated at 37°C in DMEM culture fluid.

DMSO and MTT were acquired from Sigma Aldrich in Mumbai and Sisco Research Laboratory Chemicals in Mumbai, respectively.

37°C was used to incubate 24-well plates with 1×10^5 cells per well with 5% CO_2. Different sample concentrations are added as the cell reaches confluence, and the mixture is then incubated for a full day. The sample is cleaned using phosphate-buffered saline. 0.5% MTT at a concentration of 5 mg/mL was followed by a 4-h incubation period. Following incubation, 1 mL of DMSO was applied to each well. Using DMSO as the blank, the amount of absorption was set up at 570 nm using an ultraviolet (UV) spectrophotometer. The IC_{50} was measured [11,12].

Statistical analysis
To compare the anti-cancer effectiveness of imatinib and apremilast in treated cancer cell lines, a statistical analysis was done. Non-linear regression analysis was used to estimate the IC_{50} values, and the MTT test was employed to determine the cell viability percentage. To evaluate significant differences and to distinguish between the treatment and control groups, ANOVA was used.

RESULTS

The cell viability percentages are measured using the *in vitro* MTT assay. The cell viability percentage of apremilast is 67.31%, 58.30%, 56.09%, 49.37%, 42.63%, 32.34%, 29.03%, and 22.50%, respectively, at 7.8, 15.6, 31.2, 62.5, 125, 500, and 1000 µg/mL concentration as shown in Table 1 and Fig. 1. At a concentration of 31.2 µg/mL apremilast, cell inhibition reached a maximum of 54.08%. The different apremilast concentrations in the human colon cancer cell line are shown in Fig. 2. Percentage of viability of the cell in imatinib is 68.31%, 64.40%, 58.08%, 49.17%, 31.73%, 18.34%, 6.75%, and 2.65%, respectively, at 7.8, 15.6, 31.2, 62.5, 125, 500, and 1000 µg/mL concentration as shown in Table 2 and Fig. 3. The highest 50% inhibition for imatinib was observed at 62.5 µg/mL, where cell viability fell to 49.17%. Various concentrations of imatinib in cancer cell line are shown in Fig. 4.

Table 1: Anti-cancer activity of apremilast on the HT-29 colon cancer cell line

S. No.	Concentration (µg/mL)	Dilutions	Absorbance (O.D)	Cell viability (%) mean±SD
1	1000	Neat	0.158	22.50±0.17
2	500	1:1	0.206	29.03±0.33
3	250	1:2	0.244	32.34±0.29
4	125	1:4	0.299	42.63±0.22
5	62.5	1:8	0.347	49.37±0.15
6	31.2	1:16	0.397	56.09±0.20
7	15.6	1:32	0.436	58.30±0.13
8	7.8	1:64	0.486	67.31±0.18
9	Cell control	-	0.734	100

Anti-cancer action: The data are presented as mean±SD

Table 2: Anti-cancer study of Imatinib on HT 29 cell line

S. No.	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%) mean±SD
1	1000	Neat	0.158	2.65±0.16
2	500	1:1	0.206	6.75±0.25
3	250	1:2	0.244	18.34±0.23
4	125	1:4	0.299	31.73±0.19
5	62.5	1:8	0.347	49.17±0.21
6	31.2	1:16	0.397	58.08±0.17
7	15.6	1:32	0.436	64.40±0.28
8	7.8	1:64	0.486	68.31±0.19
9	Cell control	-	0.734	100

Anti-cancer action: The data are presented as mean±SD

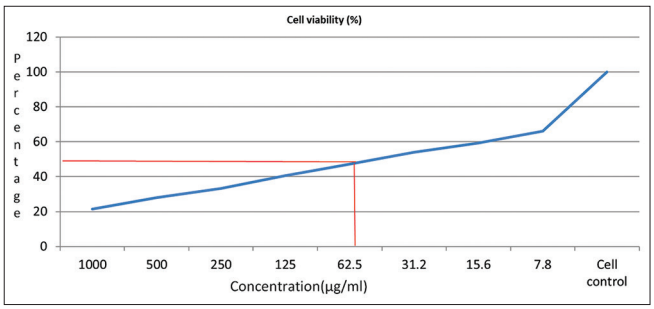

Fig. 1: Anti-cancer activity of apremilast on HT-29 cell line

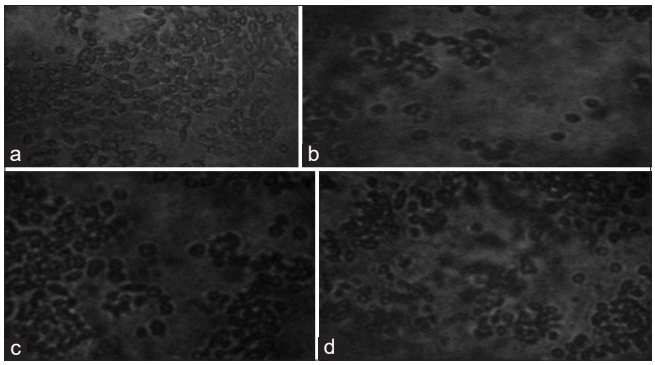

Fig. 2: Anti-cancer effect of apremilast on HT-29 colon cancer cell line. (a) Normal cancer colon cell line, (b) Cancer colon cell line toxicity at the 1000 µg/mL, (c) Cancer colon cell line toxicity at the 62.5 µg/mL, (d) Cancer colon cell line toxicity at the 7.8 µg/mL

DISCUSSION

Several studies with PDE4 inhibitors have shown a significant anti-cancer effect against colon cell lines. PDE4 are the hydrolytic enzymes accountable for cAMP degradation. If the metabolism of cAMP is disturbed, initiated by the altered PDE4 activity, it is responsible for tumorigenesis [13]. PDE4 isoforms might be a target for the novel therapeutic approach for various cancers.

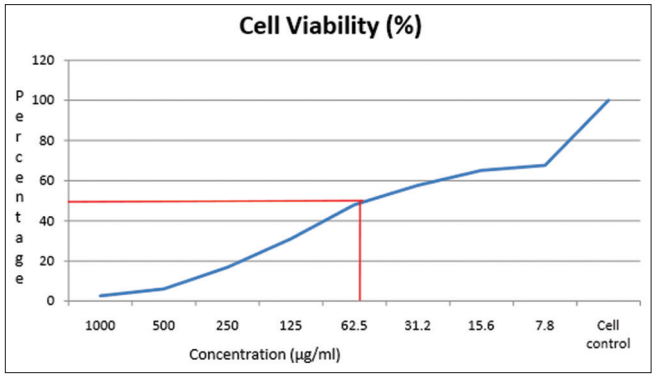

Fig. 3: Anti-cancer study of imatinib on the cell line of HT-29 in colon cancer

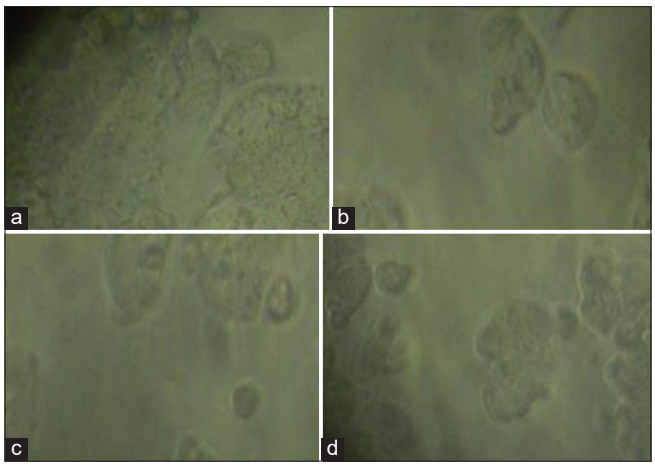

Fig. 4: Anti-cancer effect of imatinib on the HT-29 colon cancer cell line. (a) Normal cancer colon cell line, (b) cancer colon cell line toxicity at the 1000 µg/mL, (c) cancer colon cell line toxicity at the 62.5 µg/mL, (d) cancer colon cell line toxicity at the 7.8 µg/mL

Hence, this study demonstrated the effect of the PDE4 inhibitor apremilast and the control drug imatinib on colon cancer cell lines using the *in vitro* MTT assay. This study proved that apremilast and imatinib have highly significant anti-cancer activity that ranges from low to high doses in comparison to the cell control. The IC_{50} values for imatinib and apremilast are 62.5 µg/mL and 31.2 µg/mL, respectively, as the mean±SD. In colon cancer cell lines HT-29, apremilast is therefore found to be slightly more effective than imatinib.

Apremilast has been shown to be a mild emetic and has less central nervous system effects when compared to other PDE inhibitors, cilomilast [14], and rolipram [15]. Hence it is suggested in the treatment of cancer and chemoprevention.

Several drug interactions for apremilast are rifampicin, phenobarbitone, and phenytoin, which are the most potent enzyme inducers, reducing the levels of apremilast significantly [16]. In accordance with a number of studies conducted on *in vitro* colon cancer cell lines using the MTT assay, tolvaptan's maximum cell viability is 64.77% at 7.9 (µg/mL), whereas its cell viability minimum is 1.49 % at 1000 (µg/mL) [17].

Similar studies have shown that apremilast prompts luminal apoptosis in colon cancer cells by impeding the activity of PDE4B2 [18]. Apremilast modifies the wide range of inflammatory mediators in psoriatic arthritis by reducing the effect of nitric oxide synthase, IL23, and TNF-αin the human synovial cells and increases anti-inflammatory cytokine IL10 [7].

Recent studies show that imatinib inhibits colon cancer by reducing cell proliferation by stimulating P27 cell regulators levels to improve the cell apoptosis rate. P27 is responsible for inhibiting the DNA synthesis [19]. The MTT assay is merely an early test for *in vitro* anti-cancer activity screening. It does not fully support the anti-cancer effect. To determine the mechanism of action of these study medications' anti-cancer efficacy, more research may be conducted using effective methods such as flow cytometry and caspase test.

CONCLUSION

This study concludes the effect of apremilast and imatinib on anti-cancer activity by the MTT assay method. Both medications independently had anti-cancer efficacy, as demonstrated by this experiment. The percentage of cell viability in colon cancer cell lines is lower for imatinib than for apremilast at higher concentrations, but the percentage of cell inhibition for apremilast is just as strong as that of imatinib. PDE-4 inhibitors' strong anti-cancer effects may have lethal effects in a variety of tumor situations. Further studies might be required on humans to prove the effect of the drug on cancer patients.

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CONTRIBUTIONS OF AUTHORS

Dr. Parvathareddy Sowmya designed the study protocol and supervised the experimental work. Mr.Parepalli Suresh performed the *in vitro* experiments and data collection. Dr.C.Balaji analyzed the data and prepared the figures and tables. Dr. Parvathareddy Sowmya drafted the initial version of the manuscript. Dr.Balaji and Dr.Kavitha Ramasamy revised the manuscript critically for important intellectual content. All authors contributed to the manuscript writing, critically reviewed the content, and approved the final version.

CONFLICTS OF INTEREST

Nil.

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Nil

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