ANTI PROLIFERATIVE EFFECT OF ENDOGENOUS DOPAMINE REPLICA IN HUMAN LUNG CANCER CELLS (A549) VIA PI3K AND AKT SIGNALLING MOLECULES

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Abstract

Background: Lung cancer is one of the most common primary malignant tumour types in numerous countries. Non-small cell lung cancer (NSCLC) is one of the deadliest cancers which is present in this world. Dopamine, which is secreted in the brain, is a neurotransmitter as it acts as a chemical messenger between neurons. Dopamine receptor D2(DRD2) has multiple roles in clinical progression of NSCLC and is also useful in functional maintenance of cancer cells. It has been proved that regular exercise improves quality of life in cancer patients.The main aim of this study is to assess the anti proliferative effect of endogenous dopamine replicas in human lung cancer cells(A549) through PI3K and AKT signalling pathway..

Materials and Methods: Human lung cancer cell line (A549) was brought from the National Centre for Cell Sciences (NCCS),Pune,India. Cell viability test was done by MTT assay. Gene expression analysis was done by Real Time-PCR. The obtained data were analysed statistically by one-way analysis of variance and Duncan's multiple range test with Graph Pad Prism version 5 to analyse the significance. The significance was considered at $p<0.05$ level in Duncan's test.

Results: The Bcl-2, PI3K and Akt gene expression is reduced on induction of 100 and 200 µg/ml (dosage) of Dopamine with significant difference in comparison with control. Exercise induced Dopamine caused a marked increase in cell death in a dose dependent manner. At the end of 48 hours, maximum inhibition was at 100 and 200 µg/ml. Dopamine reduces the expression of Bcl-2, PI3K and Akt compared to the control cells.

Conclusion: From the obtained results and within the limits of the study, it can be concluded that exercise induced endogenous Dopamine has a massive anti-cancer potential against Lung cancer by interrupting PI3K/Akt signalling molecules as an anti-proliferative agent. Future studies should be conducted on a large scale to make this context evident.

Keywords: Dopamine; Lung cancer; Non Small Cell Lung Cancer; Exercise; Innovative technique

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Introduction:

Lung cancer is one of the most common primary malignant tumour types in numerous countries like North America.(Hoffman, Mauer and Vokes, 2000) Although improvements in treatment of Lung cancer patients have been achieved over the last few years, long term survival rates in this cruel cancer is at a poor level. Non small cell lung cancer(NSCLC) comprises approximately 85% of the Lung cancer cases all over the world(Stock-Martineau *et al.*, 2020). This has been regarded as one of the major intractable diseases. Multicellular organisms comprise a controllable collection of cells. In general, different communication signals regulate various life activities and behaviours. PI3K/AKT signalling pathways consist of 2 parts: Phosphatidylinositol-3-kinase(PI3K) and AKT.(Xu *et al.*, 2020) PI3Ks are a large family of lipid enzymes able to phosphorylase 3-OH group of phosphatidylinositols(PtdIns) on plasma membranes.(Vara *et al.*, 2004)

Dopamine has an important role in renal, hormonal and CNS systems(Finberg, 2019). The Dopaminergic pathway may be of interest in assessing risk of Lung cancer. Dopamine has been used for a long time in treatment of Parkinson's Disease. Dopamine Receptor D2(DRD2) has multiple roles in clinical progression of NSCLC(Lattimer, no date). This is a Protein Coding Gene. Dopamine is a neurotransmitter made in the brain which acts as a chemical messenger between neurons. Regular exercise provides immense health benefits, including reduced rates of many chronic health conditions like obesity, cardiovascular disease. Current

recommendations set forth by WHO include at least 150 minutes of moderate-intensity aerobic physical activity throughout the week for 18 to 64 aged people to promote muscular fitness and to reduce the risk of depression. Exercise and medications thus reduce depression results in an increased level of happiness due to the boosted levels of Dopamine by these regular exercises(Zigmond, Smith and Liou, 2008).

Molecular mechanisms through D2R crosstalk can mediate dephosphorylation of Vascular Endothelial Growth Factor. D2R agonists inhibit tumour growth of Lung cancer models. This Gene expression may be represented as a promising therapeutic alternative for NSCLC patients. Some researchers expressed that D2R agonists have been shown to increase efficacy of Anti cancer drugs in preclinical models of colon cancer(Männel *et al.*, 2017). Scientists reported that NSCLC tumours produce high levels of VEGF. Some observed more angiogenesis, tumour growth, VEGFR-2 phosphorylation in D2R knockout mice(Ljungberg *et al.*, 2003). Lung possesses the highest level of VEGF Gene expression among normal tissues. Dopamine receptors(DRD1-5) mainly reside in the human brain, which play important roles in cancer tumorigenesis and progression. Induction of apoptosis remains uncertain. The understanding of biochemical detail integration modules and nodes of PI3K and AKT pathways. Identification of biomarkers are very difficult, but that can help stratify patients for treatment and follow ups.

Cancer treatment is in the midst of a revolution toward personalised therapy. It has been documented that Dopamine can decrease cell viability and induce apoptosis in leukaemia cells(K562)(Prentice *et al.*, 1983). Dopamine can reduce the frequency of cancer stem cells and induce apoptosis of cancer stem cells. The PI3K pathway is frequently deregulated in lung cancer due to genetic alterations affecting one of its components resulting in increased PI3K signalings. AKT-mediated activation of the PI3K pathway has been associated with chemo and radioresistance in NSCLC(Weng *et al.*, no date). Some even reported the ability of PX-866 to demonstrate anti tumour activity in viva against a variety of cancer cell lines(Ihle *et al.*, 2005).

This research is needed to reduce the mortality which has been currently occurring by lung cancer cells. This reduces the knowledge deficiency among people. Number of researches are low on this topic that the dopamine has a protective role to prevent or inhibit lung cancer cells(A549). Our team has extensive knowledge and research experience that has translate into high quality publications(Dinesh *et al.*, 2013; Krishnan and Lakshmi, 2013; Muthukrishnan and Warnakulasuriya, 2018; Sekar *et al.*, 2019; Gomathi *et al.*, 2020) (Sathivel *et al.*, 2008; Panda *et al.*, 2014; Govindaraju, Neelakantan and Gutmann, 2017; Johnson *et al.*, 2020; Saraswathi *et al.*, 2020).The main aim of this study is to assess the anti proliferative effect of endogenous dopamine replicas in human lung cancer cells(A549) through PI3K and AKT signalling molecules.

Materials and Methods:

The sample collected for this study is lung cancer cells(A549). Human Lung cancer cell lines(A549) were purchased from the National Centre for Cell Sciences(NCCS), Pune, India. Cells were cultivated in a DMEM medium containing 10% Fetal Bovine Serum, 100 microgram/ml Penicillin and 100 microgram/ml streptomycin at 37C with 5% CO2.

Procedure:

Dopamine, dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Pvt Ltd, USA. Trypsin-EDTA, fetal bovine serum (FBS), antibiotics-antimycotics, RPMI 1640 medium and phosphate buffered saline (PBS) were purchased from Gibco, Canada. (5,5,6,6-tetrachloro- 1,1,3,3 -tetraethylbenzimidazolocarbocyanine iodide) and Real Time PCR kit was purchased TAKARA (Meadowvale Blvd, Mississauga, ON L5N 5S2, Canada).

Cell lines and cell culture:

Human lung cancer cell line (A549) was purchased from the National Centre for Cell Sciences (NCCS), Pune, India. Cells were cultured in DMEM medium (Thermo Fisher Scientific, CA, USA) containing 10% fetal bovine serum (Thermo Fisher Scientific, CA, USA), 100 U/ml penicillin and 100 µg/ml streptomycin (Thermo Fisher Scientific, CA, USA) at 37°C with 5% CO_2.

Cell viability by MTT assay:

Cell viability was examined utilizing an altered colorimetric strategy that depends on the capacity of live cells to change over MTT, a tetrazolium compound into purple formazan crystals by mitochondrial reductases (Mosmann, 1983). Briefly, A549 lung cancer cells (1×10^4/well) were exposed to different concentrations of serotonin (100, 200 and 400µM) with A549 cells for 48 h. At the end of the treatment, 100 µl of 0.5 mg/ml MTT solution was added to each well and incubated at 37 ◦C for an hour. Cells were divided into three groups: Group I: Untreated control A549 cells, Group II: A549 Dopamine treated Cells (100µM); Group III: A549 Dopamine treated cells (200µM) . Group IV: A549Dopamine treated cells (400µM)

At the end of the treatment, 100 µl of 0.5 mg/ml MTT solution was added to each well and incubated at 37 ◦C for an hour. The formed crystals were dissolved in dimethyl sulfoxide (100 µl) and incubated in dark for an hour.

Then the intensity of the color developed was tested utilizing a Micro ELISA plate reader at 570 nm. The quantity of viable cells was expressed as the percentage of control cells cultured in serum-free medium. Cell viability in the control medium with no treatment was addressed as 100%. The cell viability is calculated using the formula: % cell viability = [A570 nm of treated cells/A570 nm of control cells] × 100

Gene expression analysis by Real Time-PCR:
Samples from each group were submerged in 2 ml Trizol for RNA extraction and stored at −80°C until further processed. cDNA synthesis was performed on 2 µg RNA in a 10 µl sample volume using Superscript II reverse transcriptase (Invitrogen) as recommended by the manufacturer. Real-time PCR array analysis was performed in a total volume of 20 µl including 1 µl cDNA, 10 µl qPCR Master Mix 2x (Takara, USA) and 9 µl ddH2O. Reactions were run on an CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA) using universal thermal cycling parameters (95°C for 5 min, 40 cycles of 15 sec at 95°C, 15 sec at 60°C and 20 sec at 72°C; followed by a melting curve: 5 sec at 95°C, 60 sec at 60°C and continued melting). For quality control purposes, melting curves were obtained for all examples. The particularity of the amplification product was determined by melting curve analysis for every preliminary pair. The data were analyzed by comparative CT method and the fold change is calculated by 2−ΔΔCT method described by Schmittgen and Livak (2008) using CFX Manager Version 2.1.(Bio Rad, USA).

Statistical analysis:
The obtained data were analyzed statistically by one-way analysis of variance (ANOVA) and Duncan's multiple range test with a computer-based software (Graph Pad Prism version 5) to analyze the significance of individual variations among the control and experimental groups. The significance was considered at $p<0.05$ level in Duncan's test.

Results:

Effect of Dopamine on the Cell Viability:
Cell viability of Human lung cancer cells (A549) was determined using MTT assay after administering the different doses of dopamine. It was found to exhibit inhibition of lung cancer cells by decreasing the percentage of viability of cancer cells in a dose dependent manner when compared to control . It was found that maximum inhibition of cell growth was at concentration (100-400µg/ml) used in this study when compared to control (Table1).

Effect of Bcl-2 mRNA expression on the A549 cancer cells (Fold change over control):
The mRNA expression of Bcl-2 was assessed in a dose dependent manner. The cancer cells were significantly inhibited and it was found that there was significant reduction in mRNA expression of Bcl-2 when compared to control at a dose of 100 µg/ml. Further there was significant reduction in mRNA expression of Bcl-2 when compared to control at a dose of 200 µg/ml. Thus the decrease in gene expression was in dose dependent manner (Figure 1).

Effect of PI3K mRNA expression on the A549 cancer cells (Fold change over control):
The mRNA expression of PI3K was assessed in a dose dependent manner. The cancer cells were significantly inhibited and it was found that there was significant reduction in mRNA expression of PI3K when compared to control at a dose of 100 µg/ml. Further there was significant reduction in mRNA expression of PI3K when compared to control at a dose of 200 µg/ml. Thus the decrease in gene expression was in dose dependent manner (Figure 2).

Effect of Akt mRNA expression on the A549 cancer cells (Fold change over control):
The mRNA expression of Akt was assessed in a dose dependent manner. The cancer cells were significantly inhibited and it was found that there was significant reduction in mRNA expression of Akt when compared to control at a dose of 100 µg/ml. Further there was significant reduction in mRNA expression of Akt when compared to control at a dose of 200 µg/ml. Thus the decrease in gene expression was in dose dependent manner (Figure 3).

Table1: Assessment of Cell Viability

Dopamine Concentration (µM)	Cell viability (%)
0	100
100	78

200	50
400	40

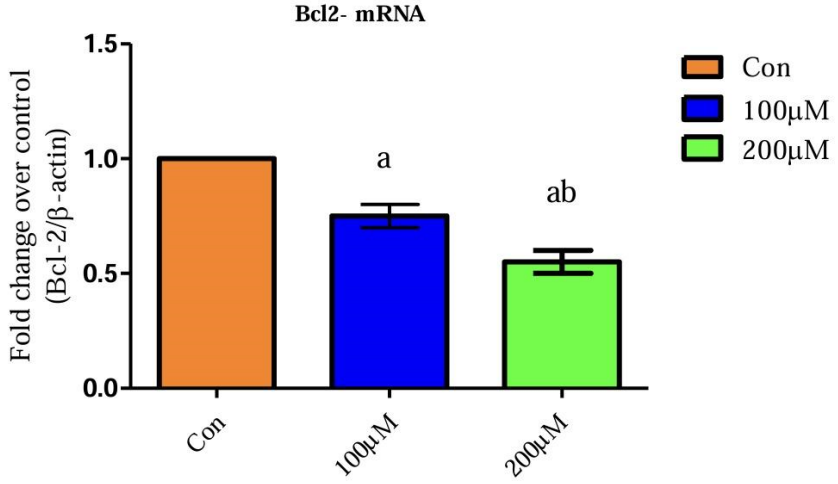

Fig 1 : Effect of dopamine on Bcl-2 mRNA expression in A549 cells.X axis represents dopamine concentration, Y axis represents fold change over control.Orange colour represents control, blue colour represents 2mM, green colour represents 4mM. a-compared with untreated control cells, b-compared with 100mM treated A549 cells. There is a significant difference between the test and control groups with p value < 0.05.

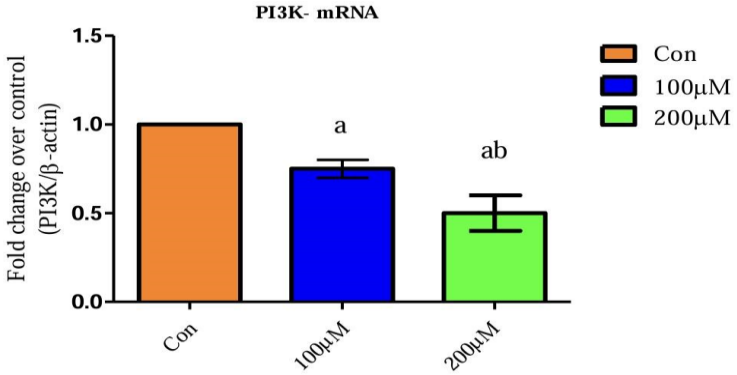

Fig 2 : Effect of dopamine on PI3K mRNA expression in A549 cells.X axis represents dopamine concentration, Y axis represents fold change over control.Orange colour represents control, blue colour represents 2mM, green colour represents 4mM. a-compared with untreated control cells, b-compared with 100mM treated A549 cells. There is a significant difference between the test and control groups with p value < 0.05.

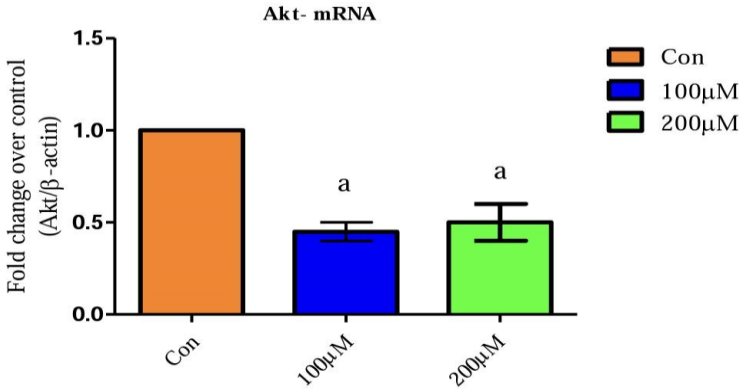

Fig 3 : Effect of dopamine on Akt mRNA expression in A549 cells. X axis represents dopamine concentration, Y axis represents fold change over control.Orange colour represents control, blue colour represents 2mM, green colour represents 4mM. a-compared with untreated control cells. There is a significant difference between the test and control groups with p value < 0.05.

Discussion:

The results of the present study suggest that exercise-induced endogenous dopamine secretion may act against cancer cell proliferation by modulating the PI3K/Akt signaling pathway. In this report, we demonstrate that exercise induced Dopamine D2R agonists inhibit the growth and development of A549 human tumour cells of lung cancer by inducing an effect in PI3K and Akt signalling pathways. Catherine L Granger et al in the year 2017 expressed that the significance of actual exercise in different clinical conditions, the proof is not in any case changed into clinical practice because of the presence of a few boundaries. The author tended to the patient-level variables like sedentary way of life, natural components to be considered to distinguish individualized exercise prescriptions that can aid in the prognosis of lung cancer (Granger *et al.*, 2017).

The neurotransmitter dopamine is found to downregulate the mRNA gene expression of BCL2 in a dose-dependent manner. Kirsteen J Camphell in his investigation in 2018 revealed that mitochondrial apoptosis is constrained by the reasonable articulation supportive of apoptotic and against apoptotic quality articulation regulation. This ensures the equilibrium of programmed cell death and modulates the cell division in moderation(Campbell and Tait, 2018).

Akt helps to aid cell survival, inhibits pro apoptosis, when its level is increased in gene expression, it favors cancer cell proliferation. PI3K aids cell growth and proliferation, at the time of its increased level, favors cancer cell proliferation. Bcl-2, an anti apoptotic gene, when increased in gene expression, prevents programmed cell death. MTT, used to test cell viability, decreases in percentage of cells which denotes protective role of test compounds against cancer cells. Since Dopamine decreases AKT, PI3K, Bcl-2 levels in gene expression due to which cell cancer is prevented, it has an anti-cancer effect against Lung cancer cells.

The author reported targeting the BCL-2 gene expression can aid in the prevention of the occurrence of cancer and also can have a good prognostic effect in early-stage tumors. Deregulation of BCL2 is becoming a target activity in various types of cancer and targeted cancer therapies can aid in maintaining the quality of life of cancer survivors.

Ma Ming et al in their study attempted to identify the role of miRNA-125a-5p regulation in controlling hepatocellular carcinoma by targeting BCL2 gene expression. The study concluded the miRNA-125a-5p expression is downregulated in patients with hepatocellular carcinoma. Its upregulation is observed to inhibit the proliferation of hepatocellular cancer cells and its metastasis by targeting BCL2 gene expression (Campbell and Tait, 2018; Ming, Ying and Ling, 2019). The results of the present study demonstrated the effect of dopamine in controlling the cell proliferation in cancer cells in a dose-dependent manner by targeting BCL2 mRNA gene expression. (Li *et al.*, 2019)

Whereas Danielle Haack et al in their study reported the role of exercise in neuroprotection against the impact of chronic stress, which is not in agreement with the results of the current study(Persico *et al.*, 1998). The results suggest the voluntary exercise with chronic stress upregulates the PI-3 kinase activity that aids in cell survival and promotes the upregulation of Bcl 2 expression. The upregulation of PI-3 kinase activity also Induces the phosphorylation of Akt.

In this study, AKT level in gene expression is reduced due to the effect of Dopamine. S6 Kinase, target of mTORCI, inhibits adaptor molecule insulin receptor substrate which prevents activation of PI3K by Insulin Growth Factor. Active extract(Liquiritin), ISL-inhibits cell proliferation and induces cell cycle arrest and apoptosis of A549 cells. There are no opposing findings found against this result.

Here, PI3K level in gene expression is reduced due to the effect of Dopamine. On recent preclinical and clinical research data on PI3K pathway by either monotherapy or by combination with chemotherapy, Lung cancer prevention method was effective. Some came to the conclusion that genetic alterations are useful to define a complex genomic network, PI3K acts as a protective role in response to targeted treatments(Shanmugam, Mathan and Selvaraj, 2018). But others stated that Tumor cells with the PI3K pathway show increased levels of sensitivity of PI3K inhibitors(Murali *et al.*, 2021). Bcl-2 level is rapidly reduced in gene expression in this study by the effect of Dopamine. Ca+2 released and cell death induction can be done by this case.

This study has low or small study size due to which effect change possibility occurs. Further studies can be conducted on a large scale which are necessary to determine Dopamine's efficacy in NSCLC patients in combination with standard chemotherapy regimens.

Conclusion:

From the obtained results and within the limits of the study, it can be concluded that exercise induced endogenous Dopamine has a massive anti-cancer potential against Lung cancer by interrupting PI3K/Akt signalling molecules as an anti-proliferative agent. Daily cardiovascular exercise appears to regulate the growth of lung

adenocarcinoma tumors, possibly by modulation of the anti proliferative pathway. Future studies should be conducted on a large scale to make this context evident.

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Conflict of interest:
The author declares that there was no conflict of interest in the present study

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