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High Dose of 3, 7-Dimethyl-1-Propargylxanthine Induces Cell Death in YM-1 and KYSE30 Cancer Cell Lines

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ABSTRACT

Background and objectives: Activation of adenosine A2a receptor has been shown to induce the growth and metastasis of cancer cells. The role of this receptor in esophageal cancer has not yet been determined. The present study aimed to investigate effects of an adenosine A2a receptor antagonist (3, 7-dimethyl-1-propargylxanthine) on growth of esophageal cancer cells.

Methods: Real-time polymerase chain reaction was performed to evaluate mRNA expression of the A2a adenosine receptor in KYSE-30 and YM-1 esophageal cancer cell lines. Effects of the antagonist on viability of the cells were evaluated by MTT assay.

Results: At low concentrations, the antagonist had no effect on cell viability. However, at concentrations ≥200 μM, the antagonist significantly reduced viability of both cell lines (p<0.05).

Conclusion: The results of this study indicate that the adenosine A2a receptor antagonist exerts inhibitory effects on KYSE30 and YM-1 cancer cells in a dose-dependent manner. Therefore, the use of this antagonist can be exploited as a therapeutic target for the treatment of esophageal cancer.

Keywords: Cell Death, 3,7-dimethyl-1-propargylxanthine, Esophageal Neoplasms.

INTRODUCTION

Esophageal cancer is the eighth most common cancer and the sixth leading cause of cancer deaths. The incidence of this disease has increased significantly in recent decades due to its rapid progression and poor prognosis (1). The Golestan Province (northeastern Iran) is one of the hotspots for esophageal cancer (2). Despite recent advances, current anti-cancer therapies are often unable to eradicate cancer (3). In recent years, the discovery of the signaling pathways involved in cancer has led to the identification of molecular targets for cancer therapy. Different experimental studies have shown that targeting the intracellular pathways can eliminate cancer cells (4), and several studies have been performed on purine receptors. There are basically two main families of purine receptors: adenosine receptor or P1-purinergic receptor whose endogenous ligand is adenosine and P2-purinergic receptor which tend to bind uridine diphosphate, uridine triphosphate, adenosine diphosphate, and adenosine triphosphate (5). Adenosine receptors belong to a large family of G-protein-dependent receptors, which are divided into four subgroups: A1, A2a, A2b, and A3. This classification is based on pharmacological findings, biochemical structure, and intracellular signaling (6). Activation of adenosine A1 and A3 receptors inhibits adenylate cyclase and decreases the intracellular level of cyclic adenosine monophosphate, but adenosine A2a and A2b receptors stimulate the adenylate cyclase and increase intracellular cyclic adenosine monophosphate content (7). Up to now, altered expression of the adenosine receptors has been reported in various cancers (8-10).

The development of selective and potent antagonists and agonists of adenosine receptors has been the subject of various pharmacological studies (11). One of the unique effects of adenosine receptors is their involvement in cell growth and cell death, particularly apoptosis (12). The A2a receptor is involved in endothelial cell proliferation. Adenosine has been shown to exert its mitogenic effects by regulating the expression, synthesis, and secretion of growth factors such as fibroblast growth factor and vascular endothelial growth factor. As a result, the migration and proliferation of capillary epithelial cells to a new location and the formation of new blood vessels occur (13).

MATERIALS AND METHODS

The KYSE-30 cell line was purchased from the Pasteur Institute of Iran. The YM-1 esophageal cancer cell line was obtained from the Biochemistry Department of Golestan University of Medical Sciences (Gorgan, Iran). The study received approval from the Ethics Committee of Golestan University of Medical Sciences (ethical code: IR.GOUMS.REC.1398.364). The cells were cultured in Dulbecco's Modified Eagle Medium (Bioidea, Iran) with 10% fetal bovine serum (Gibco, Germany), 100 µg/ml streptomycin (Bioidea, Iran), and 100 U/ml penicillin (Bioidea, Iran). The cells were kept at 37 °C in a humidified atmosphere with 5% CO_2. After trypsinization, the cells were seeded onto T25 flasks (SPL, South Korea) and cultured until reaching >80% confluency.

Total RNA extraction was performed by using the RNX-Plus kit according to the manufacturer's instructions (Sinaclon, Iran). After treatment with DNase I (Thermo fisher, USA), the extracted RNA (100 ng) was used for cDNA synthesis by using the RevertAid™First Strand cDNA Synthesis kit (Yekta Tajhiz Azma, Iran) based on the manufacturer's instructions. Real-time polymerase chain reaction (PCR) was carried out using the SYBR Green Master Mix (Yekta Tajhiz Azma, Iran). Primer sequences for the A2a adenosine receptor were derived from PrimerBank. Primer sequences for GAPDH were derived from a previous study (8) **(**Table 1). The real-time PCR reactions were done in the StepOnePlus™ System (Applied Biosystem, USA). The cycling conditions were as follows: initial denaturation for 10 minutes at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 seconds, and annealing and extension for 1 min at 60 °C. The obtained cycle thresholds were calculated by using the $2^{-(\Delta CT)}$ method. Relative gene expression levels were calculated by defining a ratio between the amount of gene expression and the endogenous control. An analysis of the melting curve (60 °C to 95 °C increments of 0.3°C) was also performed to determine the melting temperature of specific amplicons and primer dimmers. All tests were performed in triplicate.

Cells (10^4 cells per well) were seeded in 96-well plates. After 24 hours, the cells were treated with 20 µl of 3, 7-dimethyl-1-propargylxanthine (DMPX, Sigma, USA) as

the antagonist of A2A adenosine receptor in various concentrations (0.1, 1, 10, 50, 100, 200, and 500 µM) for 48 hours. Cells treated with 20 µl of dimethyl sulfoxide (DMSO) were considered as negative controls. Next, 20 µl (0.5 mg/ml) of 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) dye (Sigma-Aldrich, USA) were added to each well. After 4 hours, the culture medium was carefully removed, and 150 µl of DMSO solution were added to each well.

Absorbance of each well versus the control was read at 570 nm using an enzyme-linked immunosorbent assay reader. The percent of cells stained by MTT was determined by comparison of the optical density of each sample with that of the control group.

Results are presented as mean ± standard deviation. One-way analysis of variance (ANOVA) with Tukey post-hoc test was used to identify statistically significant differences. All analysis was carried out in SPSS (version 22) at significance of 0.05.

Table 1- Details of the primers used in the real time-PCR reaction

Number	Primer Name	Sequence	Length (bp)
1	Forward primer for A2a adenosine receptor	CGCTCCGGTACAATGGCTT	109
2	Reverse primer for A2a adenosine receptor	TTGTTCCAACCTAGCATGGGA	
3	Forward primer for GAPDH	AAGGTGAAGGTCGGAGTCAA	108
4	Reverse primer for GAPDH	AATGAAGGGGTCATTGATGG	

RESULTS

Expression level of the A2a adenosine receptor in YM-1 cells was slightly higher than in KYSE30 cells (p>0.05) (Figure 1).

The effect of DMPX on the viability of cells was also examined. For this purpose, YM-1 and KYSE30 esophagus cancer cells were treated with various concentrations of DMPX (0.1-500 µM) for 48 hours, and the inhibition of cell proliferation was measured by MTT assay. Based on the results, the antagonist had no effect on cell viability at low concentrations. However, at concentrations ≥200 µM, DMPX significantly inhibited cell proliferation in a dose-dependent manner (Figures 2 and 3). The half maximal inhibitory concentration (IC_{50}) for YM-1 and KYSE-30 cells was 767.5 µM and 682.8 µM, respectively.

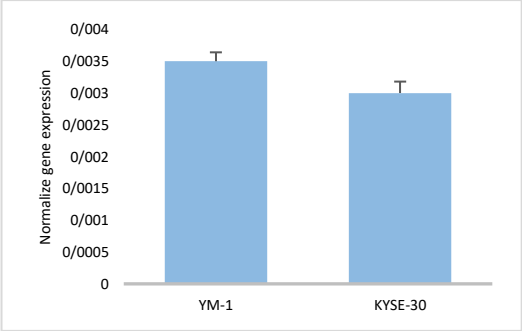

Figure 1- Expression level of A2a adenosine receptor in YM-1 and KYSE30 cells

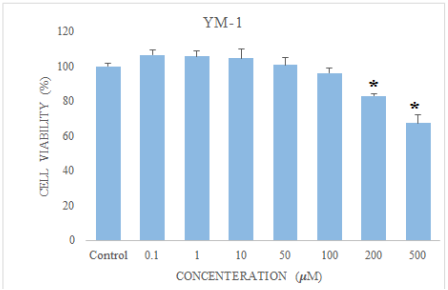

Figure 2-Effect of the adenosine A2a receptor antagonist on the growth of YM-1 cells. * indicates statistically significant difference compared to control cells.

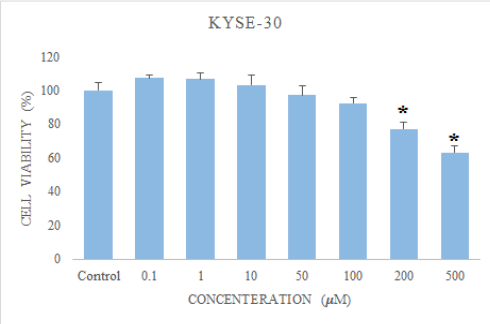

Figure 3- Effect of the adenosine A2a receptor antagonist on the growth of KYSE30 cells. * indicates statistically significant difference compared to control cells

DISCUSSION

Various studies have been performed to identify effective anti-cancer factors, especially through induction of apoptosis. Among different cellular pathways, the role of adenosine receptors in various cancer is well established. In this regard, several types of agonists, antagonists, and allosteric substances for adenosine receptors have been synthesized and used as drug candidates for the treatment of cancers (15, 16).

To our knowledge, our study is the first to evaluate the effects of an A2a receptor antagonist on esophagus cancer cells. Our results indicated the expression of adenosine A2a receptors in KYSE30 and YM-1 cells. Although DMPX had no significant inhibitory effect at low concentrations, at concentrations ≥200 μM, it significantly affected the viability of both cell lines. Mediavilla et al. reported expression of the adenosine A2a receptor on the surface of lung cancer cells as well as inhibition of the cells via the adenosine A2a receptor antagonist. They suggested the denosine A2a receptor as a therapeutic target for lung cancer (17). In line with our findings, Beavis et al. also reported that blocking of the adenosine A2a receptor inhibited CD73 + tumor cells metastasis (18). Nevertheless, adenosine may have different effects on cell proliferation, depending on the involvement of different receptor subtypes in different tumors. For instance, a study by Gessi et al. showed that activation of the A2a receptor stimulated the proliferation of human melanoma (A375) and lung (A549) cancer cells, as well as MRMT1 cancer cells, which was reversed by TP455 as an antagonist (19). In one study, the role of extracellular adenosine in colon cancer cells (Caco-2) was investigated. In contrast to the previous study, this study reported that extracellular adenosine activates caspases 3 and 9 by disrupting mitochondrial membrane potential, thereby inducing apoptosis by A2a receptor activation. According to this study, the adenosine A2a receptor antagonist, DMPX, reverses the effect of adenosine, and the adenosine A2a receptor agonist, CGS21680, was able to mimic the effect of adenosine and reduce cell viability (7).

In 2018, a study reported that adenosine A2a receptor agonists induced cell growth in squamous cell carcinoma, by a mechanism dependent to the ERK1/2 pathway (20). In contrast, some studies reported that activation of the A2a receptor had anti-cancer effects. As it was illustrated by Tamura et al., the adenosine A2a receptor agonist induced apoptosis in liver cancer cells by decreasing Bcl-2 expression and increasing Bad expression (21, 21). These results indicate that the effects of this receptor may vary depending on the type of cancer.

CONCLUSION

The results of this study indicate that the adenosine A2a receptor antagonist exerts inhibitory effects on KYSE30 and YM-1 esophageal cancer cells in a dose-dependent manner. Therefore, the use of this antagonist can be exploited as a therapeutic target for the treatment of esophageal cancer.

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DECLARATIONS

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Ethics approvals and consent to participate

The study received approval from the Ethics Committee of Golestan University of Medical Sciences (ethical code: IR.GOUMS.REC.1398.364).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding publication of this article.

REFERENCES

1. Zhang Y. *Epidemiology of esophageal cancer*. World journal of gastroenterology: WJG. 2013;19(34):5598. [View at Publisher] [DOI:10.3748/wjg.v19.i34.5598] [PubMed] [Google Scholar]

2. Sheikh M, Poustchi H, Pourshams A, Etemadi A, Islami F, Khoshnia M, et al. *Individual and combined effects of environmental risk factors for esophageal cancer based on results from the Golestan Cohort Study*. Gastroenterology. 2019;156(5):1416-27. [View at Publisher] [DOI:10.1053/j.gastro.2018.12.024] [PubMed] [Google Scholar]

3. Masters GA, Krilov L, Bailey HH, Brose MS, Burstein H, Diller LR, et al. *Clinical cancer advances 2015: annual report on progress against cancer from the American Society of Clinical Oncology*. Journal of Clinical Oncology. 2015; 33(7): 786-809. [DOI:10.1200/JCO.2014.59.9746] [PubMed] [Google Scholar]

4. Rowinsky EK. *Signal events: cell signal transduction and its inhibition in cancer*. The Oncologist. 2003; 8(S3): 5-17. [DOI:10.1634/theoncologist.8-suppl_3-5] [PubMed] [Google Scholar]

5. Burnstock G, Verkhratsky A. *Receptors for purines and pyrimidines*. Purinergic Signalling and the Nervous System: Springer. 2012; 119-244. [View at Publisher] [DOI:10.1007/978-3-642-28863-0_5] [Google Scholar]

6. Fredholm BB, IJzerman AP, Jacobson KA, Linden J, Müller CE. *International :union: of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors-an update*. Pharmacological reviews. 2011;63(1):1-34. [DOI:10.1124/pr.110.003285] [PubMed] [Google Scholar]

7. Kazemi MH, Raoofi Mohseni S, Hojjat-Farsangi M, Anvari E, Ghalamfarsa G, Mohammadi H, et al. *Adenosine and adenosine receptors in the immunopathogenesis and treatment of cancer*. Journal of Cellular Physiology. 2018; 233(3): 2032-57. [View at Publisher] [DOI:10.1002/jcp.25873] [PubMed] [Google Scholar]

8. Li Y, Zhu Z, Hou X, Sun Y. *LncRNA AFAP1-AS1 Promotes the Progression of Colorectal Cancer through miR-195-5p and WISP1*. J Oncol. 2021:6242798. [View at Publisher] [DOI:10.1155/2021/6242798] [PubMed] [Google Scholar]

9. Mousavi S, Panjehpour M, Izadpanahi MH, Aghaei M. *Expression of adenosine receptor subclasses in malignant and adjacent normal human prostate tissues*. The Prostate. 2015; 75(7): 735-47. [View at Publisher] [DOI:10.1002/pros.22955] [PubMed] [Google Scholar]

10. Hajizadeh F, Masjedi A, Asl SH, Kiani FK, Peydaveisi M, Ghalamfarsa G, et al. *Adenosine and adenosine receptors in colorectal cancer*. International immunopharmacology. 2020; 87: 106853. [View at Publisher] [DOI:10.1016/j.intimp.2020.106853] [PubMed] [Google Scholar]

11. Dhalla AK, Shryock JC, Shreeniwas R, Belardinelli L. *Pharmacology and therapeutic applications of A1 adenosine receptor ligands*. Current topics in medicinal chemistry. 2003; 3(4): 369-85. [View at Publisher] [DOI:10.2174/1568026033392246] [PubMed] [Google Scholar]

12. Klotz K-N, Hessling J, Hegler J, Owman C, Kull B, Fredholm B, et al. *Comparative pharmacology of human adenosine receptor subtypes-characterization of stably transfected receptors in CHO cells*. Naunyn-Schmiedeberg's archives of pharmacology. 1997;357(1):1-9. [View at Publisher] [DOI:10.1007/PL00005131] [PubMed] [Google Scholar]

13. Auchampach JA. *Adenosine receptors and angiogenesis*. Circulation research. 2007;101(11):1075-7. [View at Publisher] [DOI:10.1161/CIRCRESAHA.107.165761] [PubMed]

14. Liu Z, Yan S, Wang J, Xu Y, Wang Y, Zhang S, et al. *Endothelial adenosine A2a receptor-mediated glycolysis is essential for pathological retinal angiogenesis*. Nature communications. 2017; 8(1): 1-18. [View at Publisher] [DOI:10.1038/s41467-017-00551-2] [PubMed] [Google Scholar]

15. Di Iorio P, Caciagli F, Giuliani P, Ballerini P, Ciccarelli R, Sperling O, et al. *Purine nucleosides protect injured neurons and stimulate neuronal regeneration by intracellular and membrane receptor-mediated mechanisms*. Drug development research. 2001; 52(1-2): 303-15. [View at Publisher] [DOI:10.1002/ddr.1128] [Google Scholar]

16. Effendi WI, Nagano T, Kobayashi K, Nishimura Y. *Focusing on adenosine receptors as a potential targeted therapy in human diseases*. Cells. 2020;9(3):785. [View at Publisher] [DOI:10.3390/cells9030785] [PubMed] [Google Scholar]

17. Mediavilla-Varela M, Luddy K, Noyes D, Khalil FK, Neuger AM, Soliman H, et al. *Antagonism of adenosine A2A receptor expressed by lung adenocarcinoma tumor cells and cancer associated fibroblasts inhibits their growth*. Cancer biology & therapy. 2013;14(9):860-8. [View at Publisher] [DOI:10.4161/cbt.25643] [PubMed] [Google Scholar]

18. Beavis PA, Divisekera U, Paget C, Chow MT, John LB, Devaud C, et al. *Blockade of A2A receptors potently suppresses the metastasis of CD73+ tumors*. Proceedings of the National Academy of Sciences. 2013; 110(36): 14711-6. [View at Publisher] [DOI:10.1073/pnas.1308209110] [PubMed] [Google Scholar]

19. Gessi S, Bencivenni S, Battistello E, Vincenzi F, Colotta V, Catarzi D, et al. *Inhibition of A2A adenosine receptor signaling in cancer cells proliferation by the novel antagonist TP455*. Frontiers in pharmacology. 2017;8:888. [View at Publisher] [DOI:10.3389/fphar.2017.00888] [PubMed] [Google Scholar]

20. Palani CD, Ramanathapuram L, Lam-Ubol A, Kurago ZB. *Toll-like receptor 2 induces adenosine receptor A2a and promotes human squamous carcinoma cell growth via extracellular signal regulated kinases ½.* Oncotarget. 2018;9(6):6814. [DOI:10.18632/oncotarget.23784]

21. Tamura K, Kanno T, Fujita Y, Gotoh A, Nakano T, Nishizaki T. *A2a adenosine receptor mediates HepG2 cell apoptosis by downregulating Bcl-XL expression and upregulating bid expression*. Journal of cellular biochemistry. 2012;113(5):1766-75. [DOI:10.1002/jcb.24048]

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