9-(2-Phosphonyl-methoxyethyl)-adenine promotes erythrocytic differentiation and disrupts cell replication in chronic myelogenous leukemia K562 cells

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Introduction

Background

Hematopoiesis involves the formation of new blood cells from hematopoietic stem cells (HSCs) that are present in bone marrow (1,2). If replication is disrupted while HSCs are differentiating, it can lead to uncontrolled cellular proliferation and the development of cancer (2).

Chronic myelogenous leukemia (CML) forms as a result of a translocation mutation between the 9th and 22nd chromosomes of HSCs (2). This mutation causes the excision and joining of the *bcr* and *c-abl* genes, and the expression of the tyrosine kinase oncoprotein Bcr-Abl, which disrupts cell replication and promotes unregulated cell proliferation (2).

K562 cells are a human CML cell line that can be induced to differentiate into erythrocytic-like cells (2,3,4). These cells can then express fetal hemoglobin (HbF), and its constituent subunits, which can be measured and used as markers of differentiation (2,3,4).

The current drug used to treat CML (imatinib mesylate) functions by specifically targeting and inhibiting tyrosine kinases such as the Bcr-Abl oncoprotein found in K562 cells (2). Though this drug has been effective as a therapeutic agent, multidrug resistance has become an issue since it reduces the effectiveness of this method of treatment (2). This makes finding inducing agents that can safely and reliably be used for differentiation therapies an important avenue to explore more in CML cancer research.

My Experiment

The objective of this project was to test the effectiveness of the inducing agent PMEA (9-(2-Phosphonyl methoxyethyl)adenine) on cellular differentiation in the human K562 leukemia cell line. This was done to obtain additional information about its effectiveness as a therapeutic agent for CML treatment.

PMEA influences the erythrocytic differentiation of K562 cells by promoting hemoglobin expression, particularly fetal hemoglobin (HbF), and by increasing the rate of cell death by stalling cellular replication (3,4). The expression of the gamma-globin protein subunit from fetal hemoglobin (HbF) was measured as a marker of cellular differentiation to confirm whether or not PMEA was successful as an inducing agent on K562 cells (2,5).

In this study, we used a cell growth curve to measure cell proliferation, tested for hemoglobin expression via benzidine staining, and then looked specifically at gamma-globin expression via both RT-qPCR and Western Blotting procedures.

Methods and Results

Cell Culturing and Harvesing

- $1x10^5$ K562 cells were cultured for 7 days in RPMI 1640 media either in the presence or absence of PMEA. A concentration of 45mM PMEA was used. Cell counts were taken with a TC10 cell counter daily to measure the rate of proliferation, with passaging occurring on day three of culturing (Fig 1).

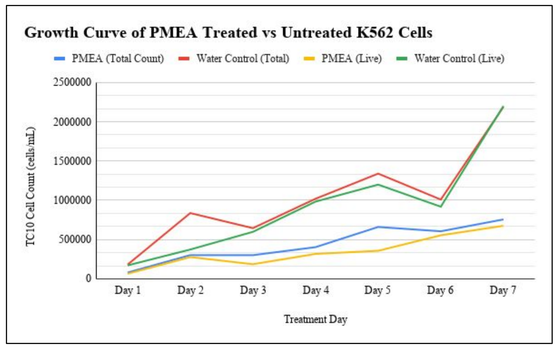

***Fig 1**. K562 cells treated with PMEA exhibit lowered rates of cellular proliferation.* Lower TC10 cell counts for both total and live cells were observed in PMEA treated cultures, compared to a water control culture for each day of culturing.

Benzidine Stain

- 1mL of culture from both the treated and untreated K562 cell samples was mixed with 0.1mL of benzidine stain. Cells were counted in triplicate using a hemocytometer, with the average percent benzidine positive cells calculated (Fig 2).

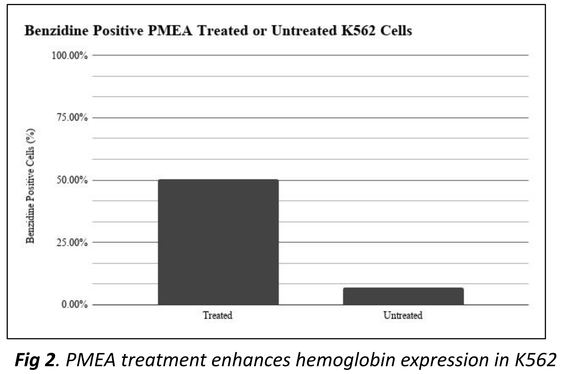

***Fig 2**. PMEA treatment enhances hemoglobin expression in K562 cells.* A benzidine stain procedure revealed that K562 cells treated with PMEA exhibited increased expression of hemoglobin compared to untreated cells following the incubation period.

RT-qPCR

- Total RNA was extracted from PMEA treated and untreated K562 cells. Reverse transcription was performed, followed by two rounds of qPCR with ɣ-globin specific primers. The data was normalized using 18S mRNA amplified with 18S specific primers (Tbl 1).

***Tbl 1**. RT-qPCR C(t) values and relative fold change in gene expression for gamma-globin in PMEA treated and untreated K562 cell lines*

Round 1	Round 2	Mean
Relative fold change in gene expression: 11.70	Relative Fold change in gene expression: 11.88	Average fold change in gene expression: 11.79

- K562 cells treated with PMEA exhibited an average relative fold increase in gene expression for gamma globin mRNA of 11.79 compared to untreated cells, following 2 rounds of qPCR.

Western Blotting

- Total protein was extracted from PMEA treated and untreated K562 cells. Protein concentration was calculated using a BioRad Protein Assay and BSA standard curve. Ponceau staining ensured consistent loading between lanes (Fig 3A). Samples were loaded in triplicate for SDS-PAGE, and expression was analyzed via immunoblotting using anti-Hbɣ, anti-Hbα, and anti-GAPDH primary antibodies (Fig 3B).

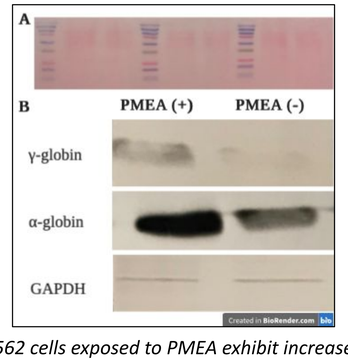

***Fig 3**. K562 cells exposed to PMEA exhibit increased expression gamma and alpha globin proteins.* K562 cells treated with PMEA showed increased expression of both gamma and alpha globin proteins following western blotting, suggesting PMEA enhances globin expression in general.

Conclusions

- PMEA disrupts cell replication in K562 CML cells
- Erythrocytic differentiation can be induced in K562 cells via exposure to PMEA
- PMEA has the potential to be a viable inducing agent used in differentiation therapies

Future Directions

- What needs to be confirmed still: the specific stage of replication that PMEA disrupts, and if PMEA is responsible for increasing the expression of globin proteins in general (not just gamma-globin).
- Additionally, further experiments involving PMEA and other types of cancer cells, or normally functioning cells, would be necessary to perform in order to measure any toxic or unexpected effects caused by PMEA before it could be considered as an appropriate inducing agent for cancer treatments with humans

References

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Further Information: My final research paper can be found in the MUSe MacEwan student journal: https://journals.macewan.ca/muse.