The effects of kynurenine metabolites on cell proliferation and morphology in melanoma and neuroblastoma cell lines

DS Tatchum [1]*, AM Joubert [1], J Serem [2], P Bipath [1], TT Nyakudya [1],Y Hlophe [1]

[1] Department of Physiology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa
[2]Department of Anatomy, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

INTRODUCTION

South Africa has the second highest incidence of skin cancer globally. Elevated alpha-chemokine receptor C-X-C chemokine receptor 4 (CXCR4) expression has been observed in melanoma resulting in disease progression and metastasis [1]. The kynurenine metabolites (L-kynurenine, quinolinic acid and kynurenic acid) inhibit T-cell proliferation resulting in cell growth arrest [2]. It has been shown that chemokines and chemokine receptors play a crucial role in cancer metastasis including melanoma and breast cancer [1]. Despite recent advances in targeted therapies, there are still no treatments or biomarkers to assist with monitoring disease progression.

AIM

In this study the effect of two kynurenine metabolites (quinolinic and L-kynurenine) on cell proliferation and morphology in melanoma and neuroblastoma cell lines will be investigated.

MATERIALS AND METHODS

The melanoma B-16 F10 and neuroblastoma SHSY-5Y cells were treated with L-kynurenine and quinolinic acid for 24 h, 48 h and 72 h. Cell proliferation was assessed using crystal violet and cell morphology was investigated via PlasDIC and haematoxylin and eosin staining (Figure 1).

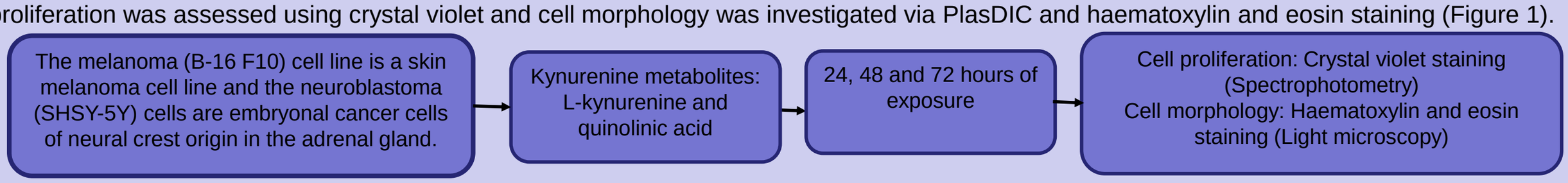

Figure 1: Diagram representing the techniques that were conducted on melanoma B-16 F10 and neuroblastoma SHSY-5Y after 24 h, 48 h and 72 h.

RESULTS

The exposure of B-16 F10 melanoma cells, SHSY-5Y neuroblastoma cells and murine macrophages 264.7 cells to quinolinic acid and to L-kynurenine at concentrations ranging from 1 mM – 4 mM for 24 h, 48 h and 72 h resulted in a decrease in cell density in SHSY-5Y cells, as well as in RAW 264.7 cells (Figure 2). Rounded and shrunken cells were observed after haematoxylin and eosin staining of the melanoma B-16 F10 cells (Figure 3).The half inhibitory concentration (IC50) values of 6.1 mM and 3.1 mM of quinolinic acid and L-kynurenine in RAW 264.7 cells were obtained using GraphPad Prism software.

Cell proliferation

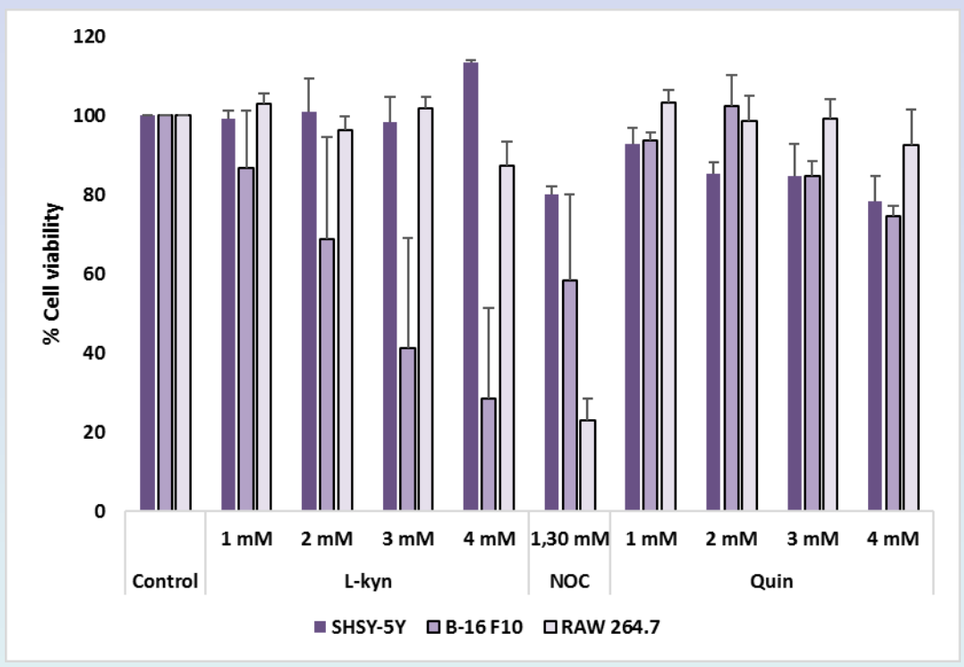

Figure 2: Bar graph representing the percentage cell viability of of B-16 F10 cells (purple), SHSY-5Y cells (dark purple) and RAW 264.7 cells (light purple) after 48 hours of exposure to L-kynurenine and quinolinic acid.. Nocodazole (NOC) was used as a positive control.

Cell morphology

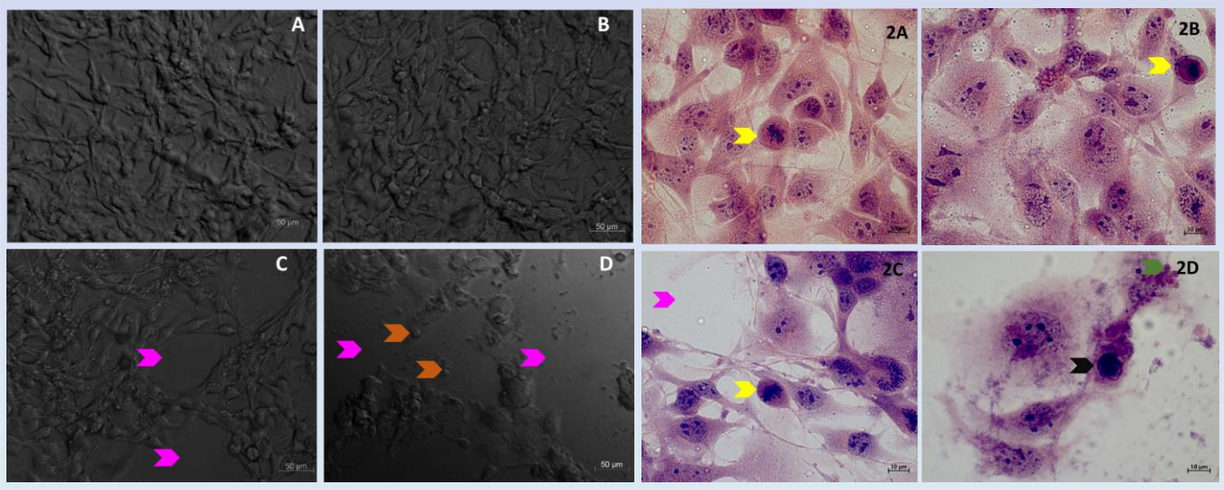

Figure 3: PlasDIC micrographs of RAW 264.7 cells treated with quinolinic acid and L-kynurenine (A and B), B-16 F10 cells () treated with 4 mM Quin and L-kyn C and D) at 20x magnification. H&E images of RAW 264.7 treated with L-kyn (2A) and quin (2B). B-16 F10 exposed to L-kyn (2C) and Quin (2D). Pink arrow heads represent compromised cell density and orange arrow heads represent rounded and shrunken cells. Yellow arrow heads represent cells blocked in metaphase (2B). Pink arrow heads represent compromised cell density, white arrow heads represent cells in anaphase and green arrow heads represent membrane blebbing. (H&E images taken at 100 x magnification).

DISCUSSION AND CONCLUSION

The study revealed that the exposure of B-16 F10 melanoma and neuroblastoma cells to L-kynurenine resulted in a statistically significant decrease in cell density. These findings contribute towards a better understanding of the effect of the L-kynurenine on cell morphology and proliferation in melanoma and neuroblastoma cells.

SELECTED REFERENCES

1. Hassanpour SH, Dehghani M, Review of cancer from perspective of molecular, J Cancer Res Prac 2017; DOI: 10.1016/j.jcrpr.2017.07.001.
2. Tod BM, Kellet PE, Singh E, Visser WI, Lombard CJ, Wright CY. The incidence of melanoma in South Africa: An exploratory analysis of National Cancer Registry data from 2005 to 2013 with a specific focus on melanoma in black Africans. S Afr Mec J. 2019; 109 (4): 245-253.

ACKNOWLEDGEMENTS

This research was supported by grants awarded to Dr Y Hlophe (A1B521) and DR TT Nyakudya (A1C553) from the Research Development Programme (RDP) at the University of Pretoria. And by UCDP Grant awarded to Ms DS Tatchum (A1D783).