In vitro effects of the chemokine receptor 4 antagonist (CTCE-9908) and kynurenic acid on tumour adhesion in melanoma cells

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Introduction

The metastatic behaviour of melanoma has accentuated the need for specific therapeutic targets to inhibit metastasis. Melanoma cells express CXC chemokine receptor 4 (CXCR-4), which is a G-protein coupled receptor.[1] The chemokine ligand 12 (CXCL12) binds to CXCR-4 resulting in tumour cell proliferation, angiogenesis, and adhesion.[2-3] Adhesion is not only implicated in cell survival, as detached cells undergo apoptosis, but is an important parameter of tumour metastasis.[4-5] CTCE-9908, a CXCR-4 antagonist and kynurenic acid (KA) are proposed anti-cancer therapeutic targets through intracellular pathway inhibition. CTCE-9908 competitively binds to and inhibits signalling pathways of the CXCR-4/CXCL12 axis,[6] unlike KA which is non-competitive. These signalling pathways include the mitogen activated protein kinase (MAP-K), phosphoinositide 3-kinase/ protein kinase B (PI3K/AKT), phospholipase C (PLC) and Ras homolog gene member A (RhoA) pathway.[7] Raw 264.7 is a monocyte/macrophage cell line, which also expresses CXCR-4 [8] and was, therefore, used as the control cell line. The positive control cells were exposed to Nocodazole, a microtubule disruptor, that halts cells at the G2/M checkpoint.

Aim

This study aimed to investigate the effects of CTCE-9908 and kynurenic acid (KA) on tumour adhesion in B-16 melanoma cells *in vitro*.

Methods

The cytotoxicity of CTCE-9908 and KA was determined spectrophotometrically by utilizing crystal violet as a DNA stain. The results were used to determine the half maximal inhibitory concentration (IC_{50}) for each compound (using the prism software version 5, 2007). After exposing cells to CTCE-9908 and KA at IC_{50} polarization-optical transmitted light differential interference contrast (PlasDIC) imaging and light microscopy after Haematoxylin and Eosin (H&E) staining were used to assess the morphological changes on the tumour cells.

Results and Discussion

In this study the *in vitro* anti-cancer properties of two compounds (CTCE-9908 and KA), each targeting a variety of cancer-associated signalling pathways, were assessed in B-16 (melanoma) and Raw 264.7 (non-cancerous) cell lines. The cytotoxicity of CTCE-9908 and KA was determined using crystal violet staining. The results were used to determine an IC_{50} of 0.104 mM at 48 hours for CTCE-9908 and 21,52 mM at 48 hours for KA in B-16 cells, which were used to treat B-16 and Raw 264.7 cells in further assays. In B-16 cells, PlasDIC micrographs revealed that CTCE induced compromised density, cell rounding, membrane blebbing and apoptotic bodies, whereas KA induced cell debris, compromised density, cell protrusions and apoptotic bodies (40X magnification). In B-16 cells, H&E stained images revealed that CTCE induced compromised density and cell rounding, whereas KA induced cell swelling, compromised density and apoptotic bodies (100X magnification).

Conclusion

Both CTCE-9908 and kynurenic acid promoted morphological changes in B-16 cells, which are representative of cell death. Future studies on the combination effects may demonstrate viable therapeutic options for melanoma.

References

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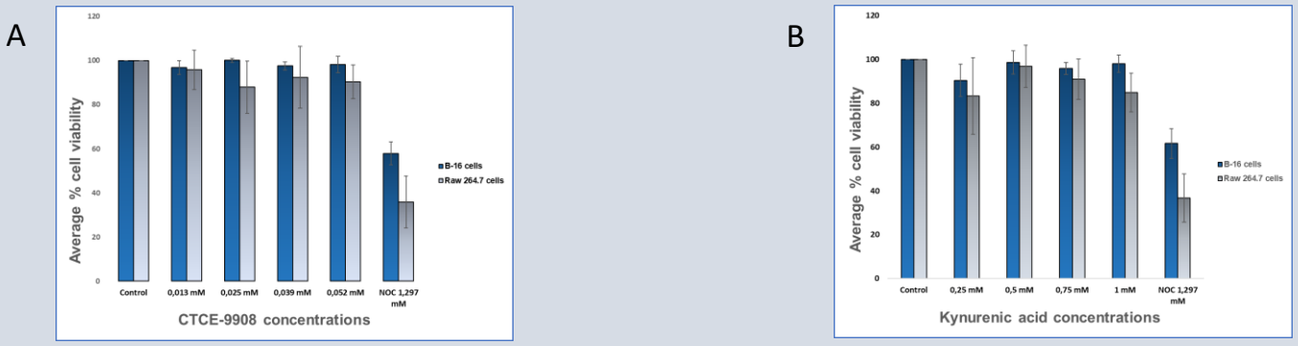

Figure 1: The effect of exposure of serial dilutions for CTCE-9908 and KA on B-16 cells and Raw 264.7 cells at 48 hours (A) the effect of CTCE-9908 on B-16 and Raw 264.7 cells at 48 hours (B) the effect of KA on B-16 cells and Raw 264.7 cells at 48 hours.

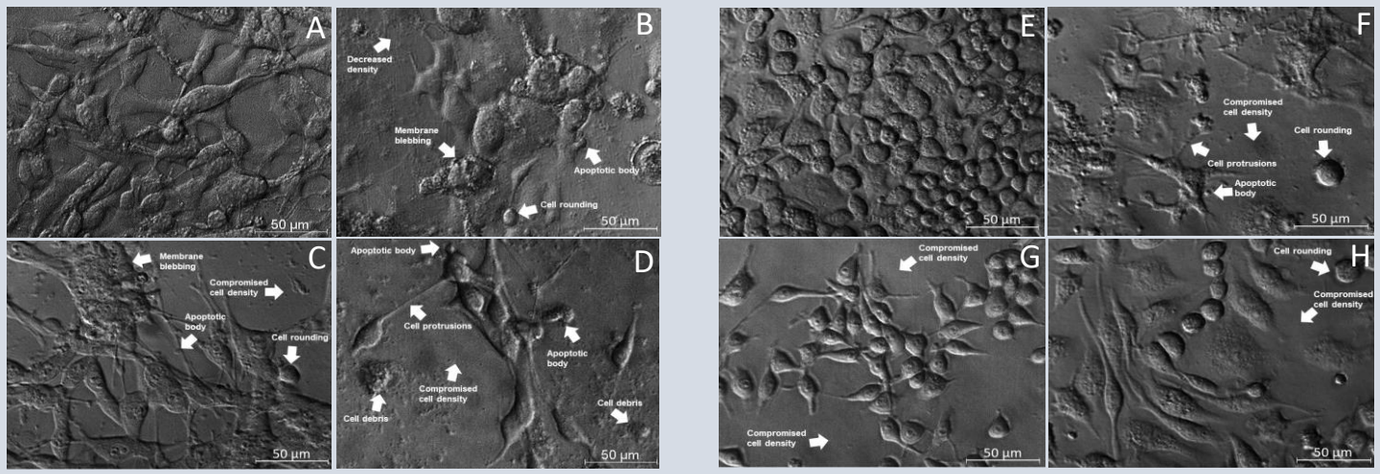

Figure 2: PlasDIC micrographs of CTCE-9908 and KA-treated B-16 and Raw 264.7 cells (A) B-16 control cells (B) NOC-treated B-16 cells at 1,297 mM (C) CTCE-9908-treated B-16 cells at an IC_{50} of 0.104 mM (D) KA-treated B-16 cells at an IC_{50} of 21,52 mM (E) Raw 264.7 control cells (F) NOC-treated Raw 264.7 cells at 1,297 mM (G) CTCE-9908-treated Raw 264.7 cells at an IC_{50} of 0.104 mM (H) KA-treated B-16 cells at an IC_{50} of 21,52 mM. Cells were exposed to treatments for 48 hours and micrographs were taken at 40X magnification.

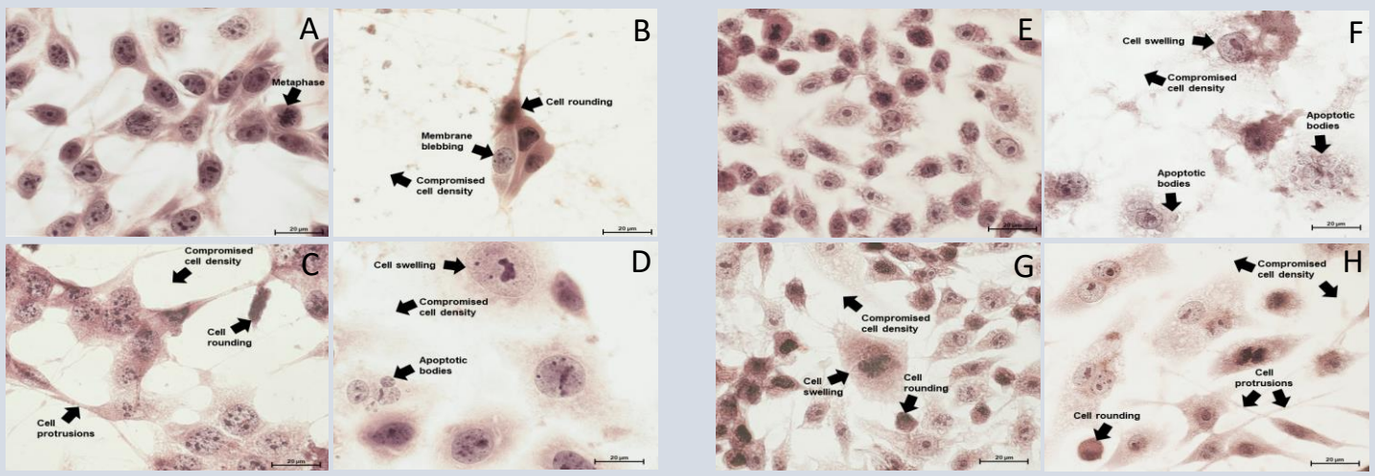

Figure 3: H&E stained images of CTCE-9908 and KA-treated B-16 and Raw 264.7 cells (A) B-16 control cells (B) NOC-treated B-16 cells at 1,297 mM (C) CTCE-9908-treated B-16 cells at an IC_{50} of 0.104 mM (D) KA-treated B-16 cells at an IC_{50} of 21,52 mM (E) Raw 264.7 control cells (F) NOC-treated Raw 264.7 cells at 1,297 mM (G) CTCE-9908-treated Raw 264.7 cells at an IC_{50} of 0.104 mM (H) KA-treated Raw 264.7 cells at an IC_{50} of 21,52 mM. Cells were exposed to treatments for 48 hours and images were taken at 100X magnification.

Acknowledgements: This study was supported by the RDP of Dr Hlophe and Dr Serem by the University of Pretoria..