The *in vitro* effects of CTCE-9908 a chemokine receptor antagonist on melanoma adhesion

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Introduction

Melanoma is one of the most common type of cancers with new cases and deaths that increase yearly.[1] Melanoma cells express specific receptors such as chemokine receptor 4 (CXCR4) that binds to chemokine ligand 12 (CXCL12) thereby leading to cancer survival, proliferation and metastasis. [1] CTCE-9908 is a CXCR-4 antagonist that is used to inhibit the proliferation and metastasis of cancer cells that express the CXCR-4 receptor. [2] It is derived from the CXCL12/ Stromal cell derived factor (SDF-1) ligand and it prevents the CXCL12 ligand from binding to the CXCR4 receptor. The original combination of CXCL12/CXCR4 leads to the activation of Ras-Raf and PI3K which aids the cancer cell in survival, proliferation and chemotaxis. [2]

Aim

To investigate the cytotoxic effects of CTCE-9908 on melanoma B-16 cells.

Methods

Crystal Violet

- CV was used to determine the IC_{50} of CTCE-9908.

H&E and Plas-DIC

- Using the determined IC_{50},
- Haematoxylin and Eosin staining and Plas-DIC (polarization optical differential interference contrast) was used to observe morphology against melanoma B16-F10 cells.

Results and Discussion

A statistically significant decrease in melanoma cell viability was observed and the IC_{50} of CTCE-9908 was 200 µg/mL at 48 hours. H&E and Plas-DIC staining techniques showed membrane blebbing, cell swelling and decreased cell density within the melanoma cell line. CTCE-9908 was also effective at 72 hours albeit not as much as at 48 hours. CTCE at 24 hours was not statistically effective at any concentration.. Nocodazole (NOC) is a potent anti-cancer agent that inhibits microtubule polymerization and in this experiment, it was used as a positive control. The IC_{50} of NOC was found to be at a concentration of 4 µg/mL at 48 hours.

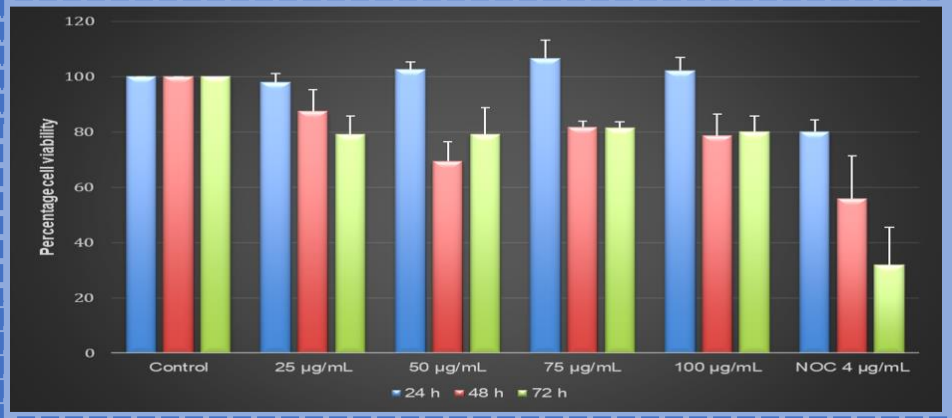

Figure 1: Crystal violet assay assessing cell viability of CTCE-9908 on B16-F10 melanoma cells at 24, 48 and 72 hours. IC_{50} determined at 48 hours.

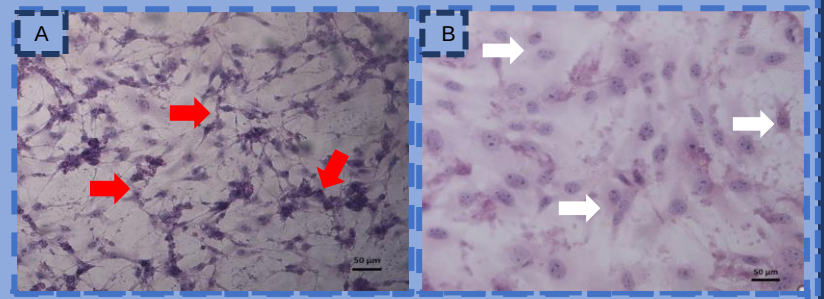

Figure 2: H&E morphology of B16-F10 melanoma cells. A represents control, B represents CTCE-9908 at IC_{50} (200 µg/mL). Scale bar is 50 µm. The red arrows indicate healthy cells that flatten and form networks. The white arrows indicate cell rounding and leakage of cytoplasmic content.

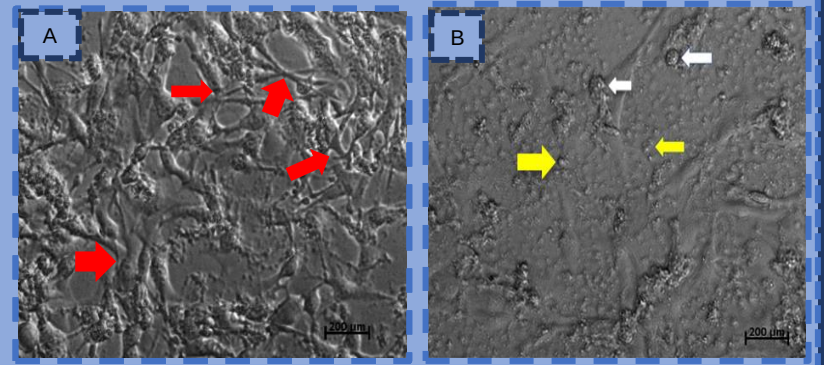

Figure 3: Plas-DIC morphology of B16-F10 melanoma cells. A represents control, B represents CTCE-9908 at IC_{50} (200 µg/mL). Scale bar is 200 µm. The red arrows indicate normal healthy cells that flatten and form a network amongst one another. The white arrows indicate cell rounding and yellow arrows indicate debris.

Conclusion

It is expected that CTCE-9908 will inhibit tumour cell adhesion, while influencing the control cell line less significantly.

References

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[2] Wong D, Kandagatla P, Korz W, Chinni SR. Targeting cxcr4 with ctce-9908 inhibits prostate tumor metastasis. BMC Urol. 2014; 14(1):12. doi:10.1186/1471-2490-14-12

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