Scalp cooling and protection from chemotherapy-induced alopecia: using in vitro human keratinocyte models to study the role of temperature and biological mechanisms of cooling-mediated cytoprotection

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INTRODUCTION

Scalp cooling is an efficient method of preventing chemotherapy-induced alopecia (CIA), with a widely reported success for many anti-cancer drugs [1]; yet some modalities do not respond well to cooling [2,3].

Due to the limited available biology [4], it is essential to establish biological models that will allow the study of chemotherapy-induced cytotoxicity and the effect of cooling in this context in order to provide biological evidence for the role of cooling and a mechanistic basis for the cell responses.

This is expected to permit the improvement of cooling-based CIA-preventative strategies.

EXPERIMENTAL DESIGN

HaCaT cells, which are closely representative of normal human keratinocytes (NHKs), were used to assess cytotoxicity of some commonly used chemotherapy drugs.

HaCaT cells were adapted to culture conditions designed for NHKs (Keratinocyte serum free medium, KSFM) in order to render them even more representative of normal cells (we named this newly adapted cell line “HaCaTa”).

Cells were treated with a range of doses of doxorubicin, docetaxel and the active metabolite of cyclophosphamide (4-hydroxy-cyclophosphamide) (4-OH-CP).

The effects of the TAC combinatorial treatment were investigated (T=Taxotere/Docetaxel, A=Adriamycin/Doxorubicin, C=Cyclophosphamide/Endoxan).

Following treatment at 37°C and in cooled conditions, cell viability was determined 72 hours post-exposure using Cell Titer 96® Aqueous One solution cell proliferation assay (Promega), which measures cell biomass as a marker of cell growth.

RESULTS

1 - The effect of Doxorubicin, 4-OH-CP, and Docetaxel exposure on HaCaT cells at 37°C and 22°C

2 - Phase contrast microscopy of HaCaT cells treated with 4-OH-CP (dose: 10µg/mL)

3 - TAC combinatorial therapy

4 - Establishment of HaCaTa cells

HaCaT cells in DMEM + 10% serum medium

The morphology of adapted HaCaTa cells closely resembled that of NHK cells

5 - Cytotoxicity versus cooling temperature in HaCaT

Cooling protects from drug cytotoxicity and the response is similar to that observed with primary NHK cell cultures (see poster No. P043), and, importantly, further reduction in temperature further increases cytoprotective capacity

6 - TAC combinatorial therapy in HaCaTa cells

The effect of TAC Therapy on HaCaTa cells

1. 4µg/mL Docetaxel
2. 2.5µg/mL Doxorubicin + 10% of Docetaxel
3. 25µg/mL 4-OH-CP + 10% of Doxorubicin
4. 25µg/mL 4-OH-CP

CONCLUSIONS

Cooling efficiently protected HaCaT and adapted HaCaTa cells from toxicity of single drugs over a wide range of concentrations, including the maximal plasma levels clinically reported. Some rescue was observed in response to TAC but significant toxicity remained.

Importantly, our in vitro findings are in close agreement with clinical observations.

Our reductive yet robust culture models will now allow us to better characterise the signalling pathways triggered by chemotherapy drugs in keratinocytes and how cooling influences the signalling circuity in cells to modify cell responses.