# Studying chemotherapy-induced alopecia in vitro: cellular models accurately mimic the cytoprotective role of cooling against chemotherapy-induced cytotoxicity

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## Introduction

- Chemotherapy-induced alopecia (CIA) is a common and distressing side effect of cancer chemotherapy
- Head cooling represents the only available treatment against CIA and advanced medical devices such as the Paxman scalp cooling system offer a promising solution.

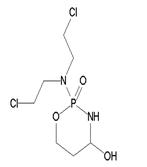
- It is thus necessary to establish biological models that will allow the study of chemotherapy induced cytotoxicity and provide better understanding of the role of cooling

## Aim

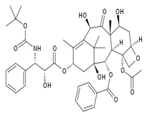
To develop in vitro based cell culture models to test and improve the efficacy of Paxman Coolers' scalp cooling system in hair loss prevention during anticancer chemotherapy.

## Cytotoxicity studies

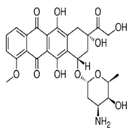
- Normal Human epidermal Keratinocytes (NHK, Invitrogen), were used to assess the cytotoxicity of commonly used chemotherapy compounds, such as taxanes and anthracyclines
- NHK cells were exposed to a wide concentration range of docetaxel, doxorubicin (Sigma) and the active metabolite of cyclophosphamide, 4-hydroxy-cyclophosphamide (4-OH-CP) (Niomech, Germany), and combinations thereof



**Cyclophosphamide active metabolite 4-OH-CP**



**Docetaxel**

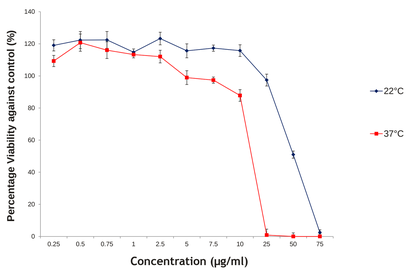


**Doxorubicin**

- To assess the effect of cooling on cytotoxicity, cells treated with the drugs at normal temperature and cooling conditions and cell viability was determined 72 hours after exposure using cell titre Aqueous One Solution (Promega)

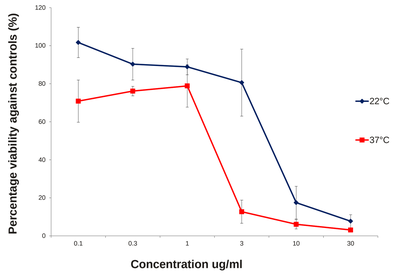
## Results – Cytotoxicity studies

Normal Human Keratinocytes treated with 4-OH-CP (37°C and 22°C)



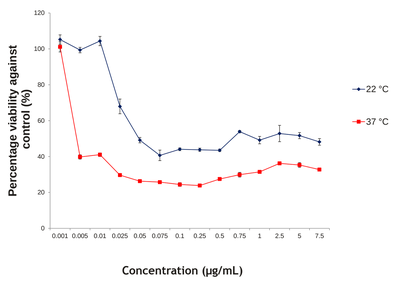

Cooling (22˚C) protects NHK cells when exposed to 4-OH-CP, with greatest rescue shown at 25 and 50 (µg/ml). Experiments were carried out in replicates of 6 (SD ±)

Normal Human Keratinocytes treated with Doxorubicin (37°C and 22°C)



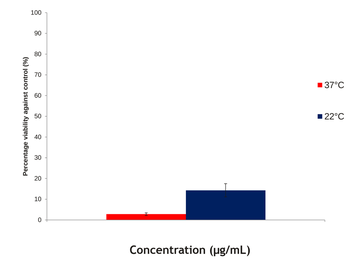

Cooling (22˚C) protects NHK cells when exposed to Doxorubicin, with rescue shown throughout. Experiments were carried out in replicates of 6 (SD ±)

Normal Human Keratinocytes treated with Docetaxel at different temperatures (37°C and 22°C)




Cooling (22˚C) protects NHK cells when exposed to Docetaxel, with rescue shown throughout. Experiments were carried out in replicates of 6 (SD ±)

Normal Human Epidermal Keratinocytes treated with TAC (37°C and 22°C)




Cooling has minimal effect on NHEK cells when exposed to TAC. Experiments were carried out in replicates of 6 (SD ±)

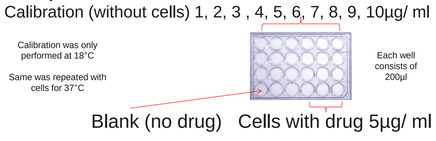
We show that cooling is extremely efficient at protecting NHK cells from drug-induced toxicity against all individual drugs. Notably, cooling showed maximal capacity to protect from cytotoxicity caused by drug concentrations representative of maximal plasma drug levels clinically reported. In contrast to results with individual compounds, combination of these drugs (also referred to as TAC regimen) caused cytotoxicity that was not rescued by cooling.

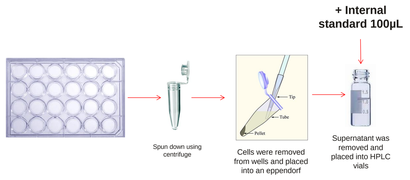
## Drug uptake studies

**CELL CULTURE**

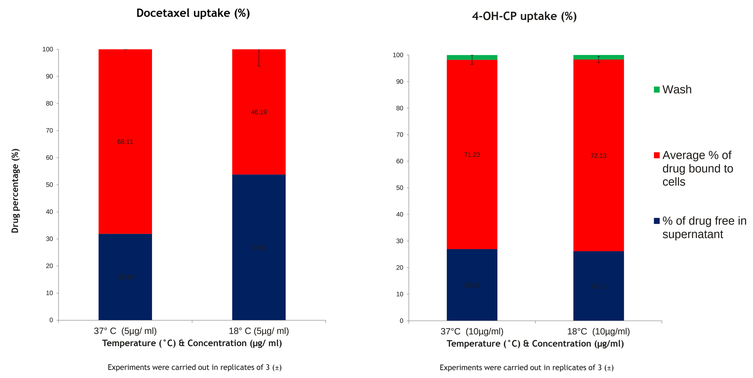
- **1 x $10^6$** cells were incubated with each drug
- These were placed in eppendorfs and spun down using a centrifuge and then the supernatant was removed and the drug of interest was added
- A calibration was also made respective to the drug concentration used
- The plates were then placed in an incubator shaker for 1hour at an rpm of 180
- This was set at a temperature of 37°C for none cooling and 22°C for cooling

**Example Docetaxel**
Calibration (without cells) 1, 2, 3 , 4, 5, 6, 7, 8, 9, 10µg/ ml




Method was established using a HPLC Shimadzu Prominence (SIL-20A HT autosampler), data was analysed using LC Solutions (software) with an X Bridge 4.6x250mm (5µm) column for all 3 drugs




Experiments were carried out in replicates of 3 (±)

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Drug uptake is shown to be either an active or a passive process. Docetaxel experiment shows a reduction in drug uptake when cells are cooled at 18°C however when compared to 4-OH-CP experiment there is no distinct difference between cooling and non-cooling the cells.

## Conclusion

- Results show that cooling at 22°C rescues cells from drug cytotoxicity for all 3 drugs when compared to non-cooling (37°C)
- Cell rescue is observed for a range of concentrations including clinical relevance drug doses
- Drug uptake data shows how docetaxel drug uptake is affected by cooling and 4-(OH)-CP is not
- This shows how there are possibly 2 different mechanisms involved (active or passive) for drug uptake into cells by these two anti-cancer drugs
- We provide for the first time evidence that, despite their reductive nature, our in vitro models are robust and biologically relevant and will help us understand the role of cooling in rescuing from keratinocyte cytotoxicity. This will permit the design of scalp cooling-based protocols with improved efficiency.
- Our in vitro findings are in agreement with available clinical observations

## Acknowledgements

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## References

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