British Journal of Pharmacy

https://doi.org/10.5920/bjpharm.598

British Journal of Pharmacy

www.bjpharm.hud.ac.uk

*Proceedings of the APS@FIP Conference 2018*

# The influence of oxygen and pressure on keratinocytes

Kawa A. Obeid[a]*, Adrian C. Williams[b], Keng Wooi Ng[c], Wing Man Lau[c]

[a]School of Pharmacy, University of Sulaimani, Iraq, [b]School of Pharmacy, University of Reading, Berkshire, RG6 6AH, UK, [c]School of Pharmacy, The Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne NE1 7RU, UK.

ARTICLE INFO

Received: 25/05/2018
Accepted: 04/06/2018
Published: 02/04/2019

*Corresponding author.
Tel.: +9647501112412
Fax: +9647501112412
E-mail:
kawa.uobaed@univsul.edu.iq

KEYWORDS: Wounds; hyperbaric oxygen; proliferation; re-epithelialisation

SUMMARY

This study aims to elucidate the respective effects of normobaric hyperoxygenation and hyperbaric pressurisation on key re-epithelialisation processes in wound healing. Cultured human keratinocytes exposed to intermittent normobaric hyperoxygenation exhibited enhanced cellular migration marked by a significant decline in E-cadherin expression. Keratinocyte proliferation, cellular metabolic activity, as well as IL-6 and IL-8 release were also significantly reduced. These changes were not observed with hyperbaric pressurisation alone. Moreover, cellular differentiation was not altered under normobaric hyperoxygenation or hyperbaric pressurisation. Thus, we conclude that hyperoxygenation differentially modulates key cellular processes in re-epithelialisation. Oxygenation, but not pressurisation, appears to be the predominant factor modulating keratinocyte migration and proliferation. These findings argue for an alternative treatment modality to hyperbaric oxygenation for wound healing, focused on enhancing tissue oxygenation without administering hyperbaric pressures.



## INTRODUCTION

Wounds are a serious health problem that costs health organisations billions of pounds each year, and it is also associated with a high mortality rate (Guest et al., 2015). Adequate tissue oxygenation is essential at all stages of wound healing (Davidson and Mustoe, 2001). Wounded tissues have an increased oxygen demand but an undersupply of oxygen due to the disruption of blood vessels (Gajendrareddy et al., 2005). Hyperbaric oxygen (HBO) therapy, which has been used as an adjunct treatment for certain chronic wounds, is the administration of pure oxygen under supra-atmospheric pressures (2-3ATA). HBO increases oxygen tension in plasma and tissues by simple dissolution and diffusion without relying on the oxygen-carrying capacity of haemoglobin, thus facilitating tissue oxygenation despite vascular damage.

Although the role of HBO in the treatment of chronic wounds is well-recognised in clinical practice, there are several limitations in its application including barotrauma. It is not clear whether the clinical benefits of HBO can be decoupled from the effects of the high pressures that give rise to the side effects. The aim of this study is to compare the effects of normobaric hyperoxygenation (NBO; i.e. administration of pure oxygen under atmospheric pressure) and hyperbaric air (HBA; i.e. administration of 95% air/5% $CO_2$ under hyperbaric pressure, 3 ATA) in vitro. Key cellular activities in re-epithelialisation, including keratinocyte migration, proliferation and differentiation, were investigated.

## MATERIALS AND METHODS

HaCaT (immortalised human keratinocyte) cells were grown to a confluent monolayer under standard culture conditions (5% $CO_2$/95% air under

https://doi.org/10.5920/bjpharm.598

normobaric pressure). The cells were then exposed to either NBO or HBA for 2 hours daily over 2 days. Control cells were exposed to neither condition. The scratch assay was performed to determine the rate of keratinocyte migration. The area recovered by the cells was quantified by computer-assisted image analysis using ImageJ software (National Institutes of Health, USA). Meanwhile, IL-6 and IL-8 concentrations in cell culture supernatants were quantified using an enzyme-linked immunosorbent assay (ELISA). Cellular expression of E-cadherin (cell adhesion marker), the proliferating cell nuclear antigen (PCNA; cell proliferation marker) and involucrin (cell differentiation marker) was determined using Western blotting. Additionally, the metabolic activity of the cells was investigated using the tetrazolium (MTT) assay.

## RESULTS AND DISCUSSION

Exposure of cultured keratinocytes to intermittent NBO resulted in increased cellular migration (Fig. 1). This phenomenon was associated with a marked decline in E-cadherin expression (Fig. 2), indicating reduced cellular adhesion. Neither change was observed with HBA exposure.

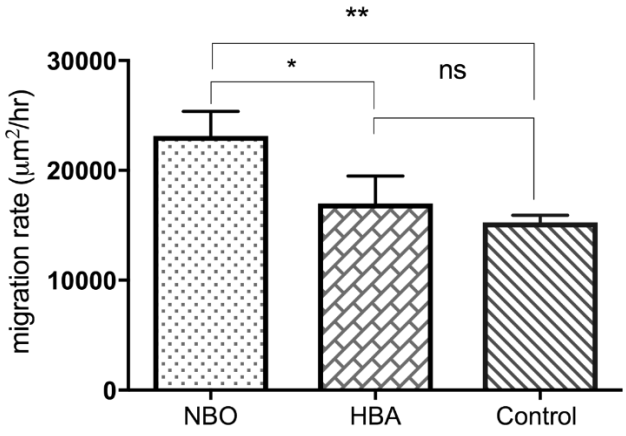


***Fig. 1.*** *The rate of HaCaT cell migration following 2-hour daily exposures to normobaric oxygen (NBO), hyperbaric air (HBA) or neither (control). Data are mean ± standard deviation (n = 3; one-way ANOVA with Tukey's HSD test: *p ≤ 0.05, **p < 0.01, $^{ns}$p > 0.05).*

Exposure to NBO, but not HBA, also resulted in a marked reduction in PCNA expression, indicating reduced cell proliferation, and cellular metabolic activity. Moreover, a marked reduction in the release of the pro-inflammatory markers, IL-6 and IL-8, was observed with NBO only. Neither NBO nor HBA altered involucrin expression.

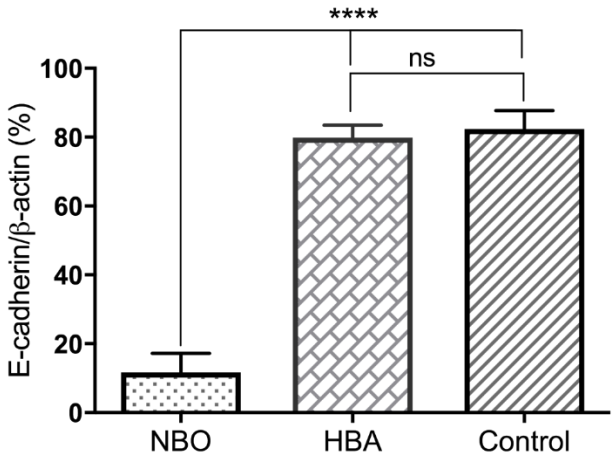


***Fig. 2.*** *E-cadherin expression by keratinocytes cells following 2-hour daily exposures to NBO, HBA or neither (control). Data are mean ± standard deviation (n = 3; one-way ANOVA with Tukey's HSD test: ****p < 0.0001; $^{ns}$p > 0.05).*

## CONCLUSIONS

Hyperoxygenation, but not hyperbaric pressurisation, differentially modulates keratinocyte migration and proliferation. Normobaric hyperoxygenation may be an alternative treatment modality for chronic wounds that avoids some adverse effects of HBO, such as barotrauma.

## ACKNOWLEDGEMENTS

The authors thank the Kurdistan Regional Government for sponsoring this project.

## REFERENCES

Davidson, J.D., and Mustoe, T.A., 2001. Oxygen in wound healing: more than a nutrient. Wound Repair Regen. Off. Publ. Wound Heal. Soc. Eur. Tissue Repair Soc. *9*, 175–177.

Gajendrareddy, P.K., Sen, C.K., Horan, M.P., and Marucha, P.T., 2005. Hyperbaric oxygen therapy ameliorates stress-impaired dermal wound healing. Brain. Behav. Immun. *19*, 217–222.

Guest, J.F., Ayoub, N., McIlwraith, T., Uchegbu, I., Gerrish, A., Weidlich, D., Vowden, K., and Vowden, P., 2015. Health economic burden that wounds impose on the National Health Service in the UK. BMJ Open *5*, e009283.