International Journal of Mycobacteriology xxx (2016) xxx–xxx

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/IJMYCO

In vitro effects of water-pipe smoke condensate on the endocytic activity of Type II alveolar epithelial cells (A549) with bacillus Calmette–Guérin

Ian M. Adcock [a,*], *Esmaeil Mortaz* [b], *Shamila D. Alipoor* [b], *Johan Garssen* [c,d], *Ali Akbar Velayati* [e]

[a] *Cell and Molecular Biology Group, Airways Disease Section, National Heart and Lung Institute, Imperial College London, London, UK*
[b] *Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[c] *Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands*
[d] *Nutricia Research Centre for Specialized Nutrition, Utrecht, The Netherlands*
[e] *Mycobacteriology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

ARTICLE INFO

Article history:
Received 18 September 2016
Accepted 20 September 2016
Available online xxxx

Keywords:
Endocytosis activity
Type II alveolar epithelial cells (A549)
Water pipe

ABSTRACT

Objective/Background: Tuberculosis (TB) is a major global health problem and poses immense threats to many populations. The association between tobacco smoke and TB has already been studied. Water-pipe smoking has become an increasing problem not only in Middle Eastern countries but also globally as it is considered by users as being safer than cigarettes. The presence of high levels of toxic substances in water-pipe smoke may be predisposing factors that enhance the incidence of pulmonary disorders in water-pipe smokers. For example, uncontrolled macropinocytosis occurs in alveolar epithelial cells following exposure to water-pipe smoke, which may predispose individuals to pulmonary infection. In this work, we studied the effects of water-pipe condense (WPC) on the internalization of *Mycobacterium bovis* (bacillus Calmette–Guérin [BCG]) by macropinocytosis in Type II alveolar epithelial cells (A549).

Methods: A549 cells were treated by WPC (4 mg/mL) for 24 h, 48 h, 72 h, and 96 h, respectively. The effect on cell proliferation was studied using a methylthiazolyldiphenyl-tetrazolium bromide (MTT) reduction assay. Cells were exposed to fluorescein isothiocyanate (FITC)–dextran (1 mg/mL; control) and FITC–BCG (multiplicity of infection, 10) for 20 min at 37 °C before their collection and the uptake of BCG–FITC was determined by flow cytometry. Similar experiments were performed at 4 °C as a control.

Results: WPC (4 mg/mL) after 72 h (1.4 ± 0.2-fold, $p < 0.05$) and 96 h (1.6 ± 0.2-fold, $p < 0.05$) hours increased the uptake of BCG–FITC. No effect on BCG–FITC uptake was observed at 24 h or 48 h. WPC also significantly increased the uptake of FITC–dextran (2.9 ± 0.3-fold, $p < 0.05$) after 96 h. WPC also significantly decreased cell proliferation after 24 h (84 ± 2%), 48 h (78 ± 3%), 72 h (64 ± 2%, $p < 0.05$), and 96 h (45 ± 2%, $p < 0.05$).

* Corresponding author at: Imperial College London, South Kensington Campus, London SW7 2AZ, UK. Tel.: +44 (0)20 7589 511.
E-mail address: ianmadcock@hotmail.com (I.M. Adcock).

Peer review under responsibility of Asian African Society for Mycobacteriology.
http://dx.doi.org/10.1016/j.ijmyco.2016.09.036

Conclusion: WPC exposure increased epithelial cells' permeability and death and enhanced their capacity for macropinocytosis. Our *in vitro* data suggest possible harmful effects of WPC on the ability of lung epithelial cells to phagocytose mycobacteria. Further studies will be conducted to understand the mechanism of action of WPC.

Conflicts of interest

The authors have no conflicts of interest to declare.

Please cite this article in press as: IM Adcock et al. In vitro effects of water-pipe smoke condensate on the endocytic activity of Type II alveolar epithelial cells (A549) with bacillus Calmette–Guérin. Int. J. Mycobacteriol. (2016), http://dx.doi.org/10.1016/j.ijmyco.2016.09.036