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Title: Hyperoxia mediates barrier permeability dysfunction in 16HBE140- cells

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Body: Background: Reactive oxygen species (ROS) induced by hyperoxia have been postulated to be responsible as mediators of oxidative stress and damage in human and experimental animal lung epithelium. This study aimed to determine the effects of hyperoxia on the tight junction associated protein ZO-1 in the human bronchial epithelial cell 16HBE140-, and the possibility of a protective role of antioxidant vitamins C and E. Methods: 16HBE140- cells were cultured at a density of 2.5×10^5 cell/cm^2 in an air-liquid interface for 6 days where the transepithelial electrical resistance (TER) developed to 230 ± 28 Ω cm^2. The experimental groups were divided into six sets, exposed for 24h to normoxia (21% O_2, 5% CO_2) as a control; hyperoxia (95% O_2, 5% CO_2); hyperoxia with 10^{-7} M vitamin E (α-tocopherol); hyperoxia with 10^{-7} M vitamin C; hyperoxia with 10^{-6} M vitamin C and hyperoxia with a combinations of vitamins E and C (10^{-7}, 10^{-6} M respectively). TER measurement, immunofluorescence staining of ZO-1 and RT-PCR to detect IL8, IL6, TNF-α and ZO-1 expression were used. Results: The reductions of the TER in hyperoxic groups (hyperoxia and hyperoxia with antioxidants) were associated with the reduction in the ZO-1 thickness and downregulation of ZO-1 expression compared with the control. In contrast, the expression of IL-8, IL-6 and TNF-α was upregulated in the hyperoxic groups compared with the control. Conclusion: Hyperoxia induced barrier integrity disruption represented by the decrease in TER and the reduction of ZO-1 levels is associated with an increased in the expression of pro-inflammatory cytokines IL-8, IL-6 and TNF-α. The antioxidant vitamins E and C had only a slight protective effect against hyperoxia damage.