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| **Mechanisms by which environmentally persistent free radicals induce oxidative stress** |
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| **Introduction/Aim:**  Environmentally persistent free radicals (EPFRs) are oxidant components of air pollution and are present in substantial numbers on particulate matter. EPFRs have half-lives of days to years with long-lasting effects in biological systems. EPFRs participate in a redox cycle in biological systems and produce large quantities of reactive oxygen species. However, the mechanistic links between EPFR exposure and respiratory diseases are unclear. The aim of this study was to understand the mechanisms by which EPFRs induce oxidative stress (OS), the signalling pathways in the response of human respiratory epithelial cells to EPFRs, and to examine whether dietary antioxidants can provide protection.  **Methods:**  Primary human nasal epithelial cells were collected from healthy adults and were grown and differentiated in air-liquid interface culture. The selected wells were pretreated with 20µM astaxanthin, an antioxidant, for 24 hours. The well-differentiated epithelia were subsequently exposed to 1mg/cm2 EPFRs for 4 hours. Samples were collected at 4- and 24-hours post-exposure. Outcomes measured included epithelial membrane integrity (trans epithelial electrical resistance, membrane permeability), cell death (LDH), mitochondrial reactive oxygen species generation (mtROS), mRNA expression (*CYP1A1*, *p21*, *SIRT1*, *FOXO3*, *PINK1*, *MUC5AC*), and protein expression (phosphorylated and total ERK1/2; phosphorylated and total NF-κB).  **Results:**  EPFR exposure caused a decrease in epithelial cell integrity (*p*<0.01), and mtROS production doubled compared with control (*p*<0.05). EPFR exposure increased mRNA expression of *CYP1A1* (xenobiotic metabolism) (*p*<0.05), *SIRT1*-*FOXO3* (antioxidant) (*p*<0.05), *p21* (apoptosis) (*p*<0.01), and *PINK1* (mitophagy) (*p*<0.01); and decreased in *MUC5AC* mRNA expression (mucus production) compared with control (*p*<0.01). ERK1/2 and NF-κB phosphorylation increased following EPFR exposure. Most of these effects were prevented by pre-treatment with astaxanthin (*p*<0.05).  **Conclusion:**  Our results demonstrate that EPFRs cause damage to respiratory epithelium, induce OS and reduce mucus production via ERK1/2-NF-κB pathway. A dietary antioxidant, astaxanthin, protected cells from EPFR-induced OS and negative impacts caused by EPFRs.  **Grant Support:**  This work was funded by a grant from the National Institute of Environmental Health Sciences [3P42 ES013648-08A1S1]; and PDS is funded by the National Health and Medical Research Council, Australia [1193840]. |