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Animal models of smokeless tobacco-induced oral malignancies

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Peroxynitrite (ONOO^-), the reaction product of nitric oxide ($\cdot\text{NO}$) and superoxide ($\text{O}_2^{\cdot-}$) has been implicated as an important modulator of lipid, protein and DNA damage. Several studies have already demonstrated $\text{O}_2^{\cdot-}$ and hydrogen peroxide (H_2O_2) release from tobacco xenobiotics, an effect that can be attenuated *in vitro* by recombinant superoxide dismutase (SOD) and catalase. Peroxynitrite formation is favoured in cells when $\cdot\text{NO}$ and $\text{O}_2^{\cdot-}$ are elevated, or when SOD is low, as is the case in tumour cells. Recently, we have demonstrated that tobacco xenobiotic compounds such as smokeless tobacco, nicotine, nitrosonornicotine (NNN) and 4-(methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) have the capacity to release $\cdot\text{NO}$ in nano- to micromole quantities. We have exposed both immortalized hamster cheek pouch cells (POII) and intact hamster cheek pouch tissues to tobacco xenobiotics to illustrate the nitrosative effects of ONOO^- in these systems. Lipid peroxidation was quantified in POII cells using an ELISA-based 8-isoprostane assay, and DNA damage was assayed by COMET assay. Because lipid peroxidation and DNA damage are not specific markers of ONOO^- damage, we used immunofluorescence techniques to identify a specific marker for ONOO^- -nitrotyrosine. Formation of nitrotyrosine adducts was also identified in hamster cheek pouch tissues chronically exposed to tobacco xenobiotic-derived $\cdot\text{NO}$, and that markers of this damage to lipid, protein, and DNA are quantifiable in both cell cultures and intact animals. This work was supported by grants from CIHR and AHFMR.