

## **TIME AND DOSE EFFECTS OF CIGARETTE SMOKE AND ACROLEIN ON PROTEIN CARBONYL FORMATION IN HACAT KERATINOCYTES**

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**Objectives:** Cigarette smoke (CS) is an important environmental source of human exposure to a highly toxic and chemically active  $\alpha,\beta$ -unsaturated aldehyde: acrolein. It is capable of causing protein carbonylation and dysfunction, especially in oral tissues of smokers, constantly exposed to CS toxic constituents. The foremost damage is considered to be cumulative, but even a short exposure can be potentially harmful. The objectives of the current study were to examine a short time and dose effects of direct CS and acrolein exposure on intracellular protein carbonylation in epithelial cells.

**Methods:** HaCaT-keratinocytes were exposed to different doses of acrolein and whole phase CS using a unique smoking simulator apparatus that mimics the exposure in smokers. The rate of intracellular protein carbonyl modification was examined 10-60 minutes after the exposure by Western blot. In addition, the effect of pre-incubation with a thiol scavenger N-acetylcysteine (NAC) was also assessed.

**Results:** Intracellular protein carbonyls increased as fast as 10 minutes after CS exposure and their concentration doubled after 20 minutes, with a slight elevation afterwards. Also, carbonyl levels increased gradually as CS and acrolein doses were elevated. Addition of 1 mM NAC neutralized part of the damage.

**Conclusion:** CS and acrolein intracellular protein carbonylation is dose and time dependent. Even a short time exposure to CS and its aldehydic constituents can be potentially harmful.