

Reactive oxygen species production from smokeless tobacco

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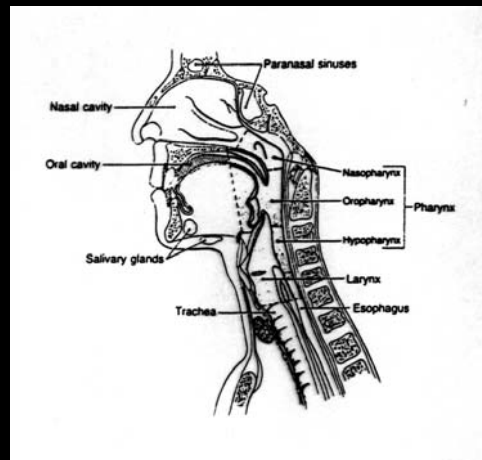
University of Alberta, DPC 2085

Edmonton, AB T6G 2N8



Background

	Incidence	Deaths
Canada ¹	2,926	1,002
U.S.A. ²	28,260 (est.)	7,230 (est.)



¹Canadian Cancer Society, 1998

²United States National Cancer Institute, 2004
From Vokes *et al.*, *NEJM* **328**:184-194; 1993.



■ Free radical

- *Any species capable of independent existence that contains 1 or more unpaired electrons.*

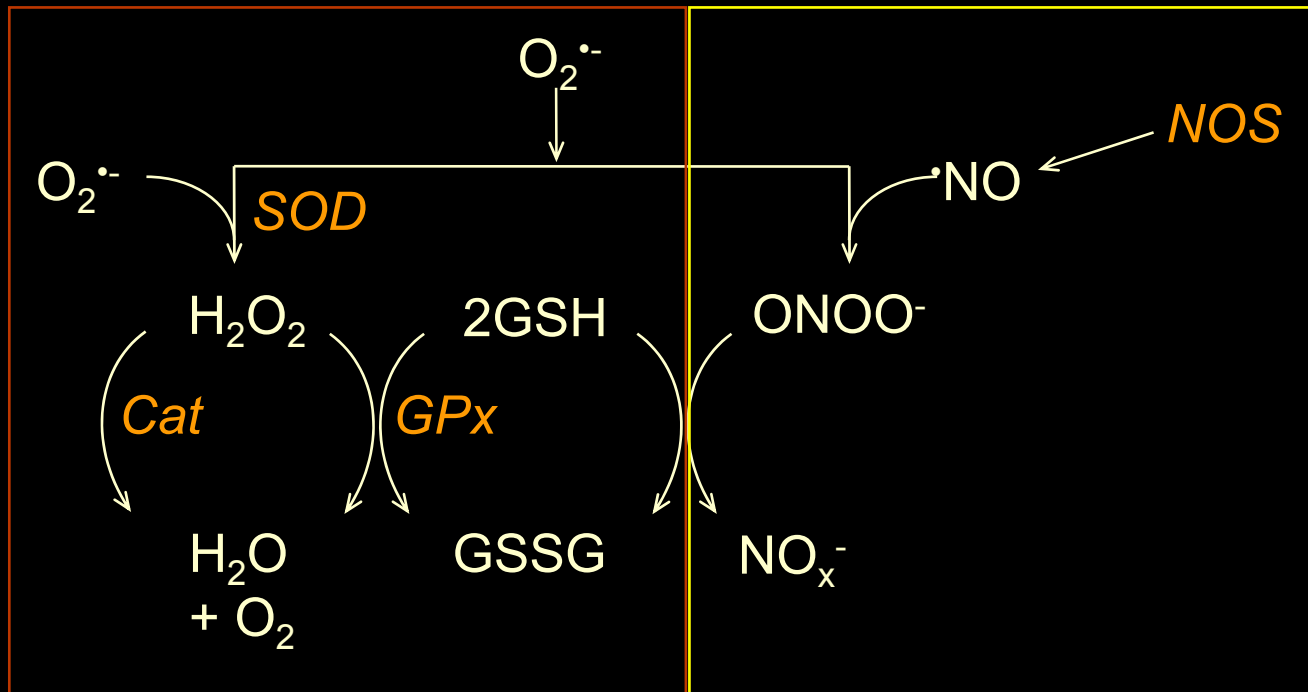


■ Reactive oxygen/nitrogen species

- *Oxygen or nitrogen-containing molecules that have a higher reactivity than ground state molecular oxygen or nitrogen.*



Radicals and antioxidants

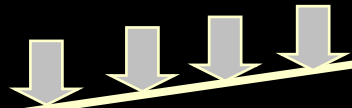


Pro-oxidants

$O_2^{\cdot-}$, HO^{\cdot} , H_2O_2 ,

1O_2 , $\cdot NO$,

$ONOO^-$



Oxidative stress
Nitrosative stress


Antioxidants

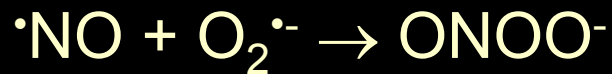
SOD, Cat, GPx,

AscH₂, TOH,

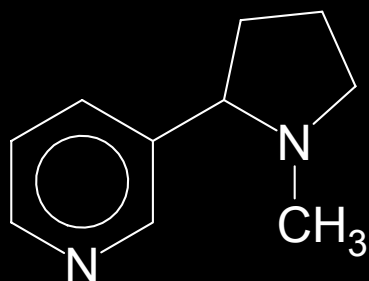
$\cdot NO$, GSH, NAC



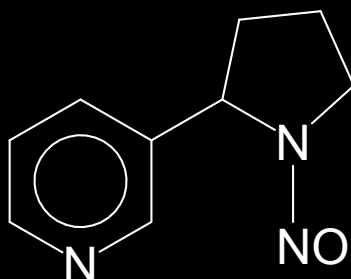
- 
- Cellular damage from supra-physiologic levels of $\cdot\text{NO}$ are mediated by peroxynitrite (ONOO^-), the reaction product of $\cdot\text{NO}$ and $\text{O}_2^{\cdot-}$.



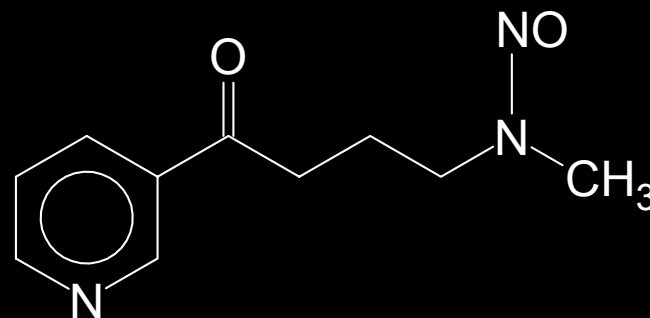
Smokeless tobacco-related compounds



¹Nicotine



²NNN



³NNK

12.0 ± 0.7 mg/g ST

8.73 ± 1.44 mg/g ST

1.89 ± 0.62 mg/g ST

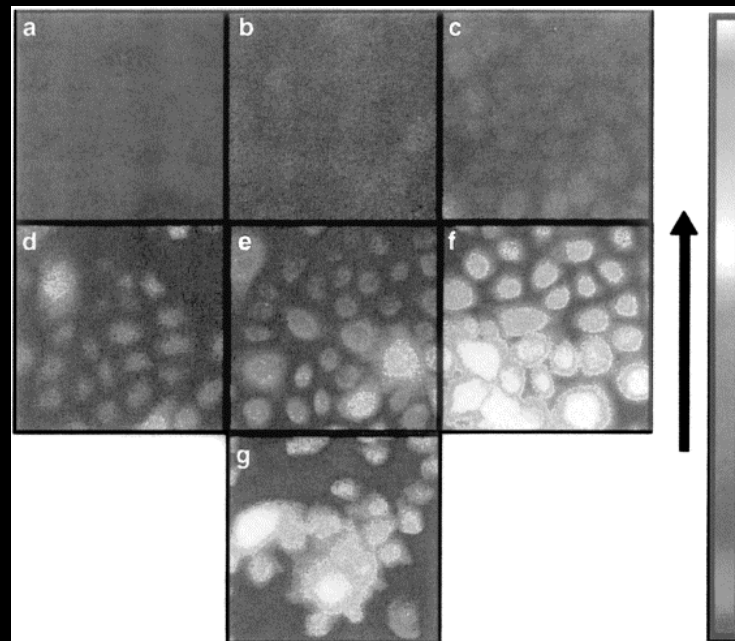
¹Copenhagen brand smokeless tobacco. From Hoffmann D, Djordjevic MV. *Adv Dent Res* 11:322-339; 1997.

²Nitrosonornicotine

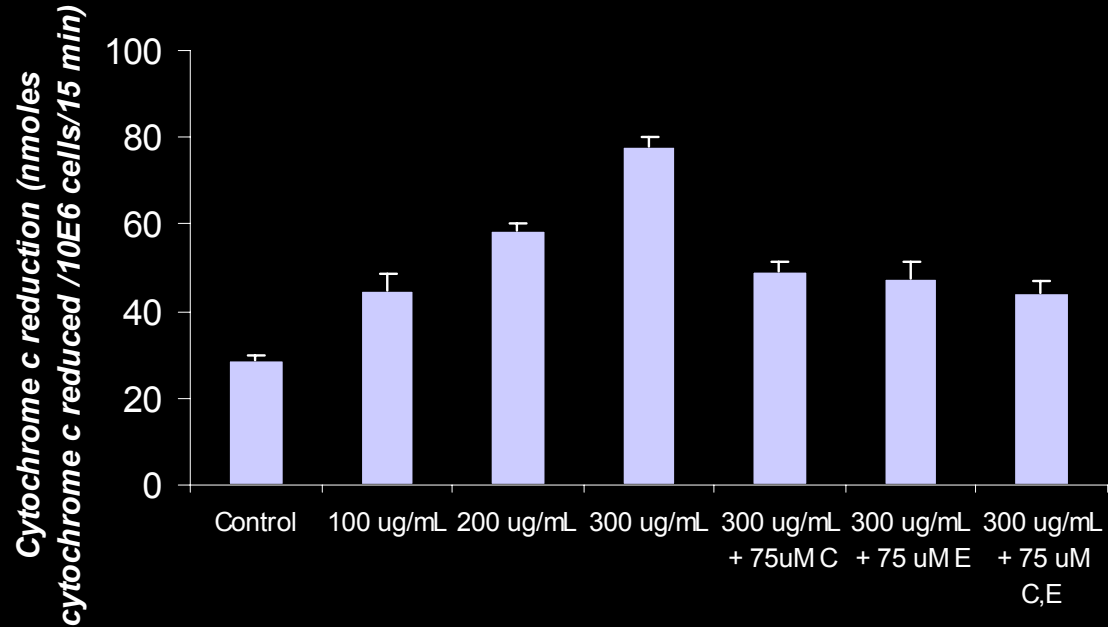
³4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone

Smokeless tobacco

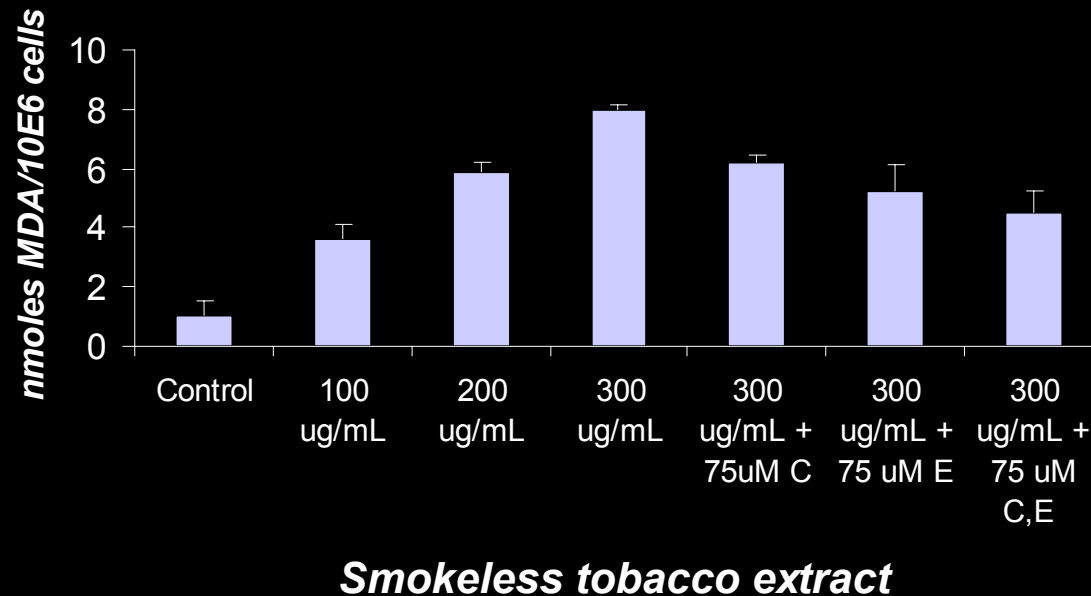
■ 2,7-dichlorofluorescein diacetate fluorescence assay



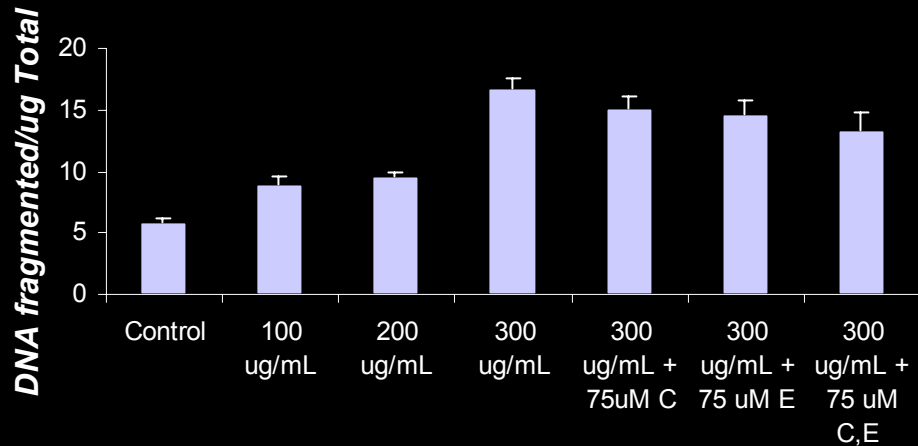
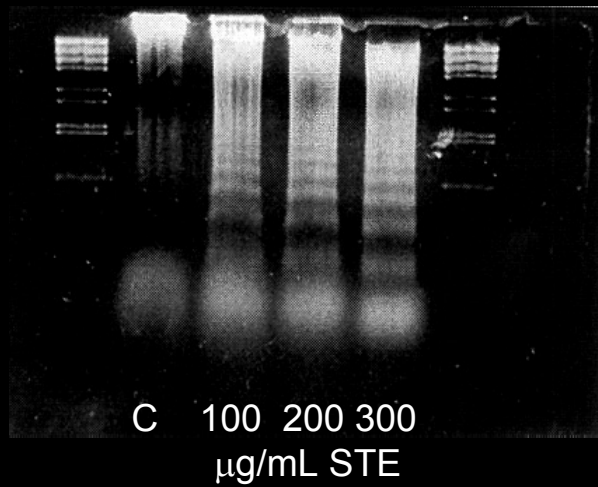
■ Cytochrome c superoxide assay



■ Malondialdehyde (TBARS) lipid peroxidation assay



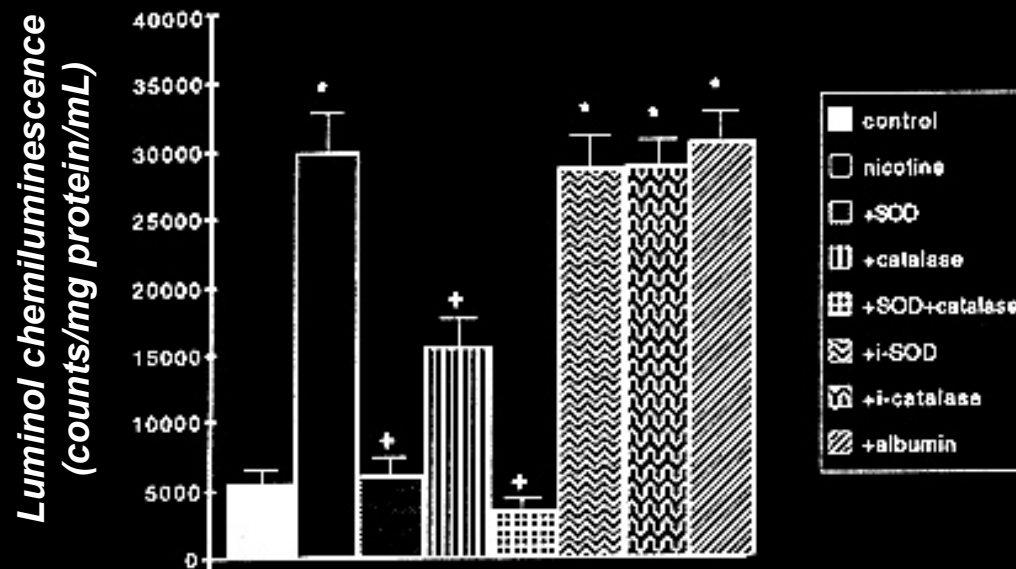
■ DNA fragmentation assay



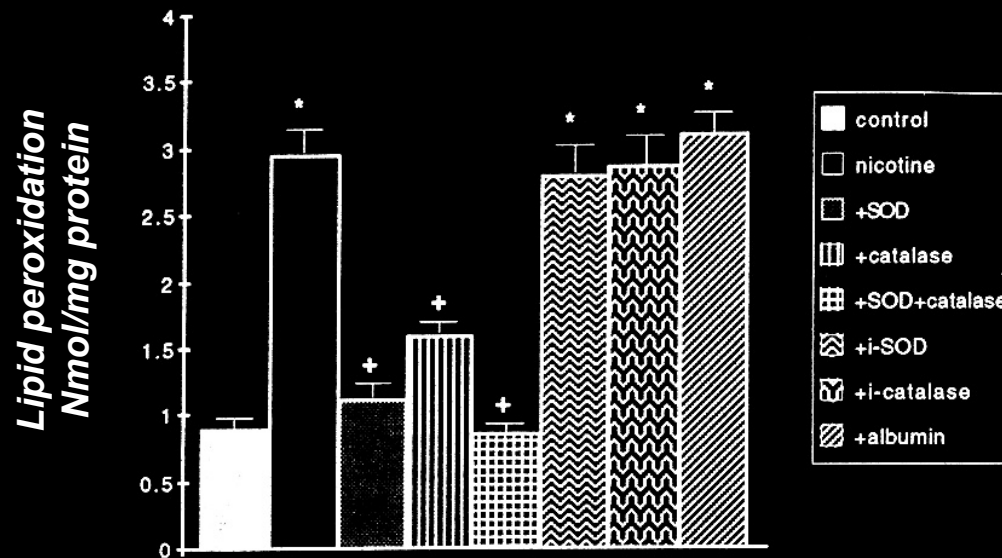
Smokeless tobacco extract

Nicotine

■ Luminol chemiluminescence assay

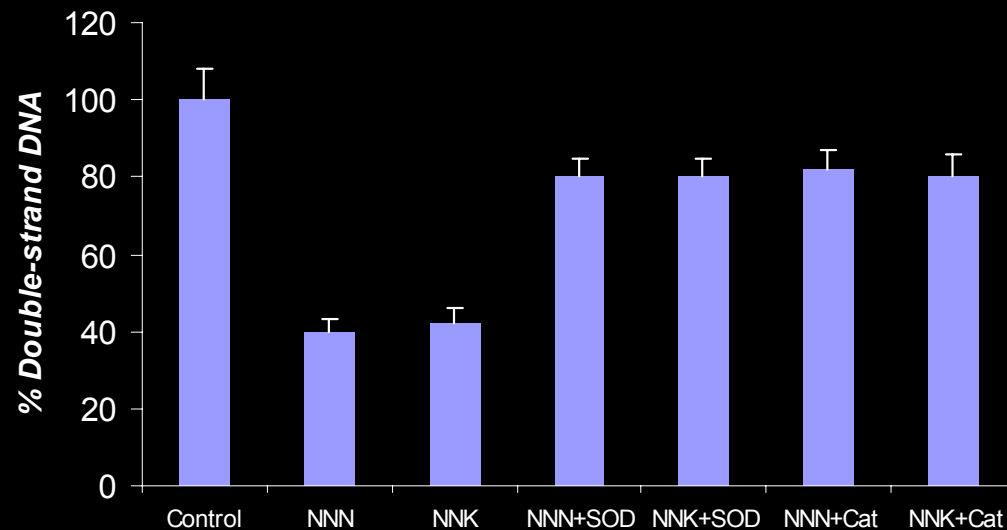


■ Malondialdehyde (TBARS) lipid peroxidation assay



NNN/NNK

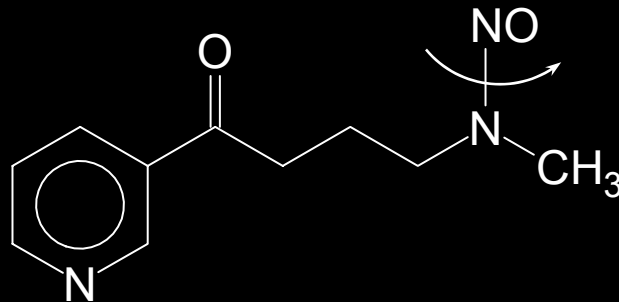
■ Fluorometric DNA unwinding assay



- Can tobacco-related compounds release radicals other than $O_2^{\cdot -}$ or HO^{\cdot} radical?



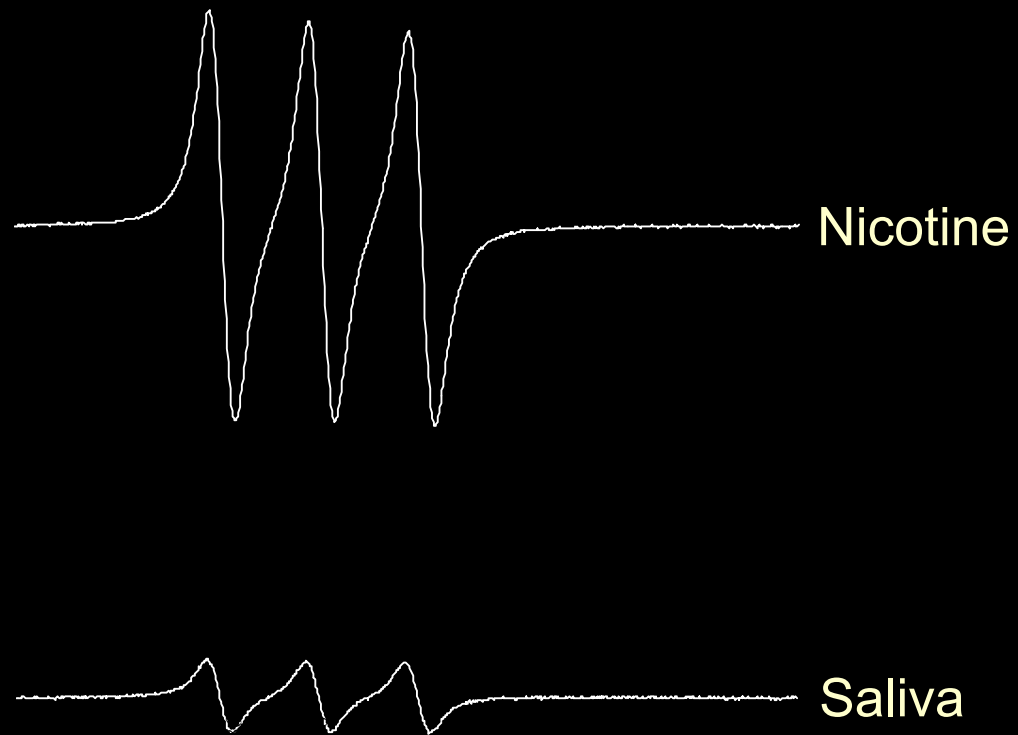
NNN



NNK

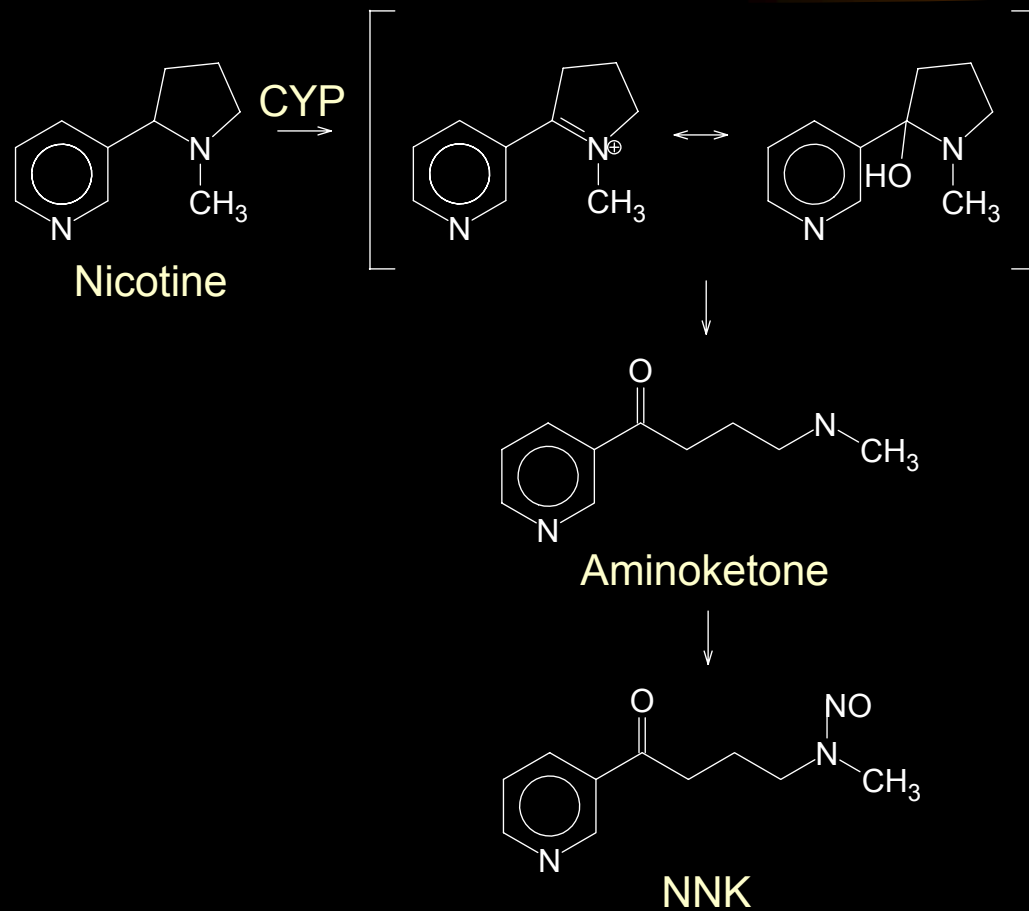
■ Electron paramagnetic resonance spin trapping





■ Chemiluminescent $\cdot\text{NO}$ detection

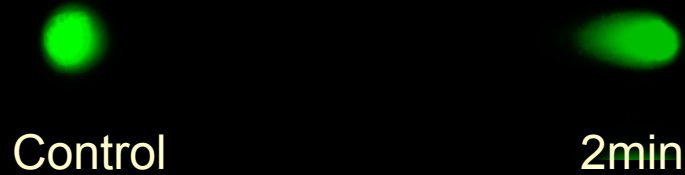
	in PBS (pH 7.4)	in human saliva
PBS	$5 \pm 1 \text{ mM}$	-
Human saliva	-	$38 \pm 17 \text{ mM}$
ST	$1100 \pm 50 \text{ mM}$ $2.53 \pm 0.10 \text{ mmol/g ST}$	$1380 \pm 80 \text{ mM}$ $2.76 \pm 0.16 \text{ mmol/g ST}$
Nicotine	$< 0.02 \text{ mM}$	$150 \pm 12 \text{ mM}$ $2.81 \pm 0.23 \text{ nmol/mg nicotine}$
NNN	$< 0.02 \text{ mM}$	$121 \pm 6 \text{ mM}$ $5.90 \pm 0.30 \text{ nmol/mg NNN}$
NNK	$< 0.02 \text{ mM}$	$113 \pm 5 \text{ mM}$ $5.45 \pm 0.25 \text{ nmol/mg NNK}$



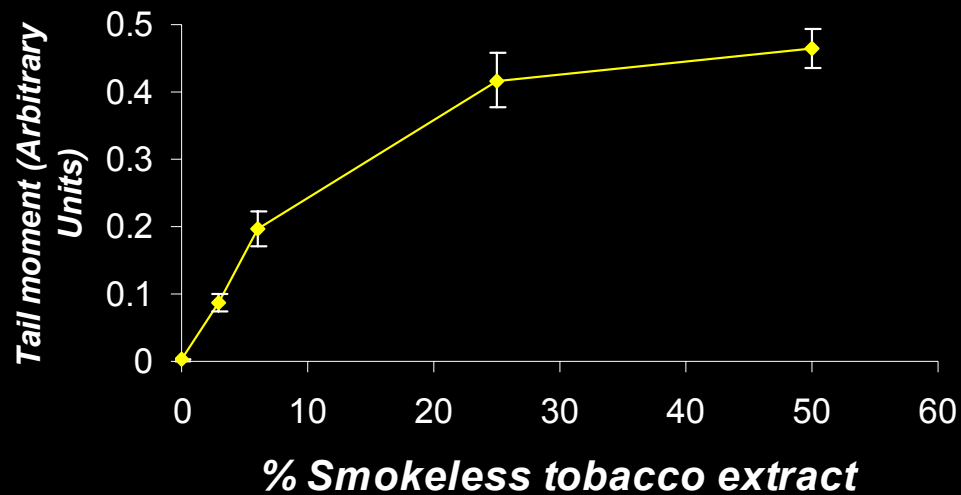
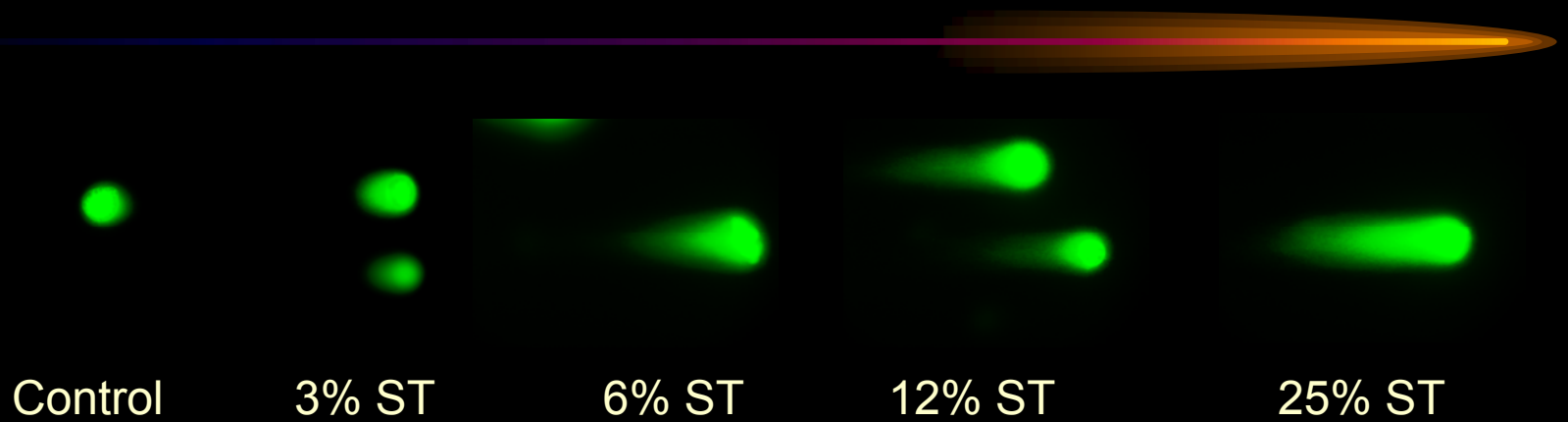


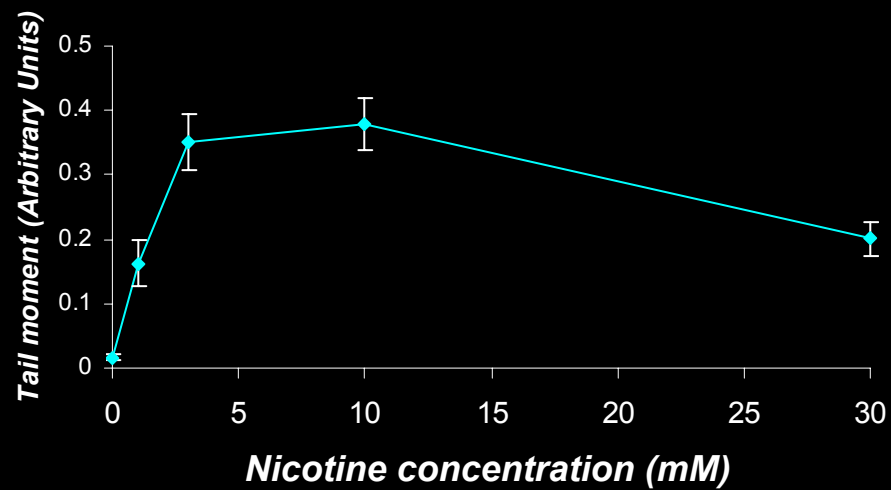
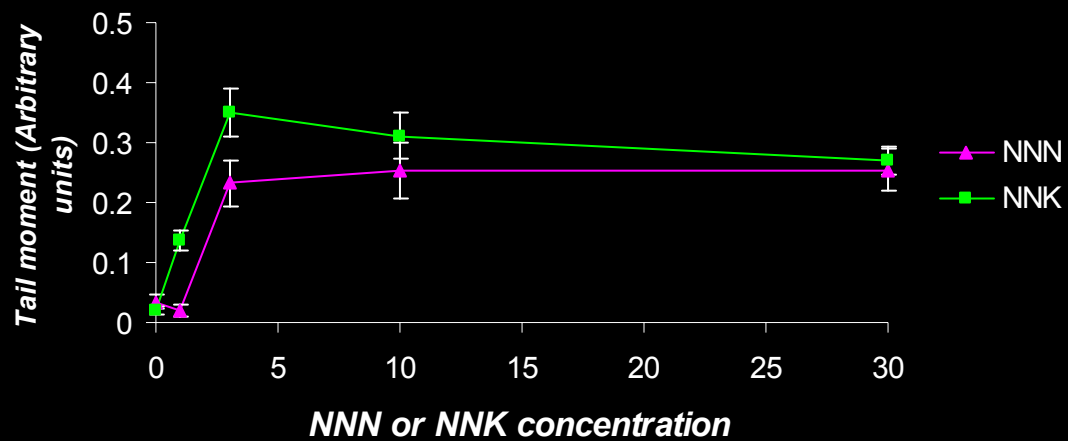
■ Comet assay

- POII cells treated with 12 mM nicotine

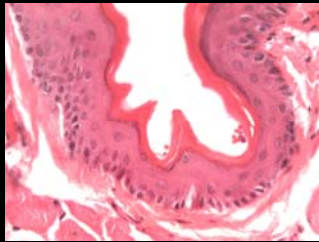


$$\begin{aligned}\text{Tail moment} &= \text{tail length} \times \% \text{ tail DNA} = \\ &= \text{tail length} \times \frac{(\text{total intensity} - \text{head intensity})}{\text{total intensity}}\end{aligned}$$

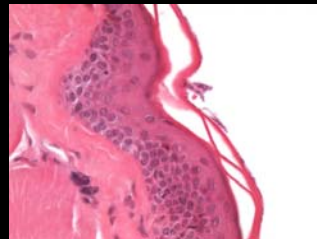




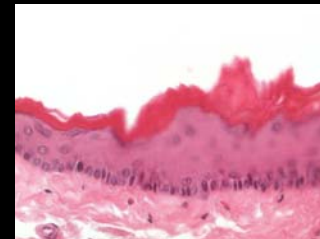
- Hamster cheek pouch tissues treated with smokeless tobacco and smokeless tobacco-related compounds



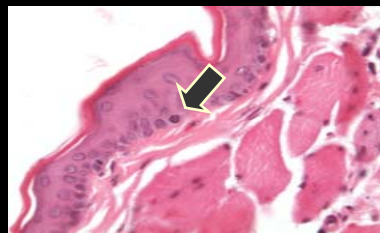
Verruciform
pattern



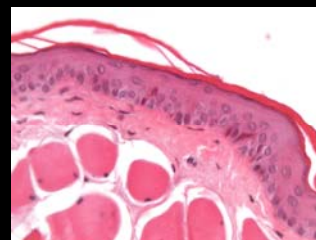
Increased
cellularity




Hyperchromatism



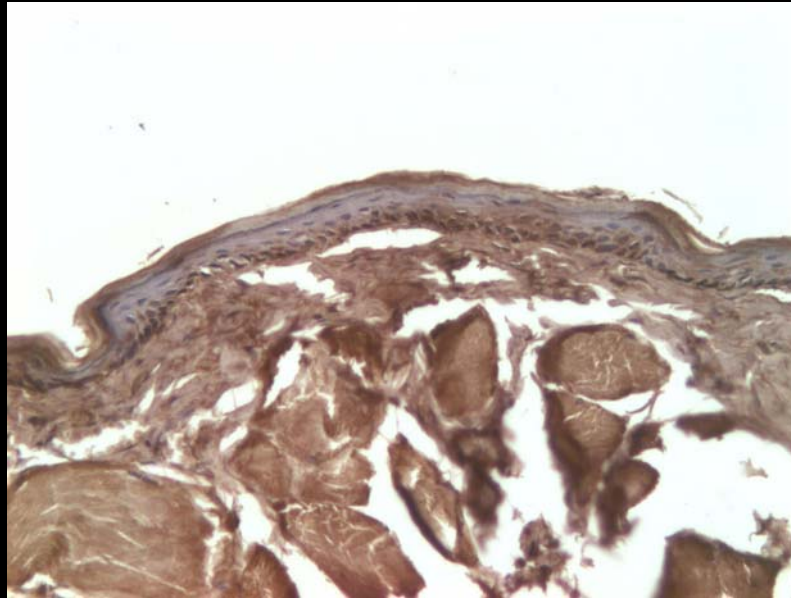
Mitotic
figures



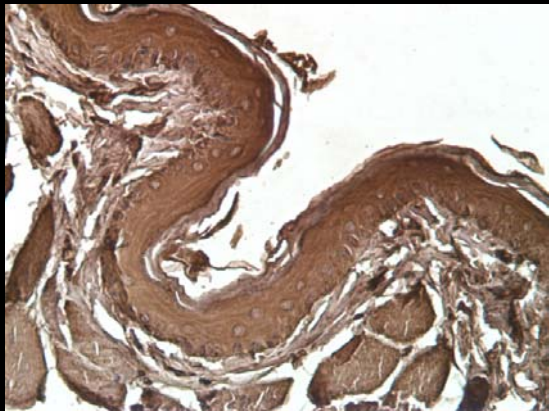
Pleomorphism

- 
- Ischiropoulos (*Archs. Biochem. Biophys.* **356**:1-11; 1998) suggests that of the major putative mechanisms of macromolecular nitrosation, one of the most plausible is the uncatalyzed nitrosation of macromolecules by ONOO^- .

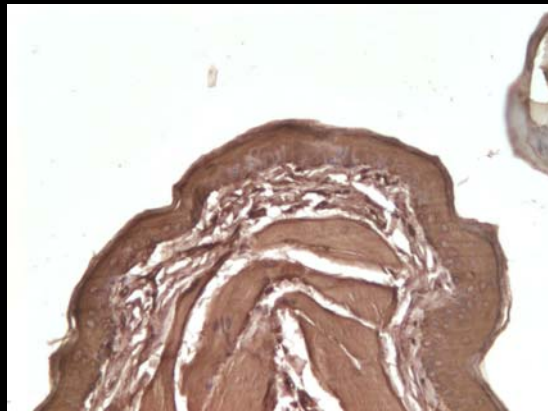
■ 3-Nitrotyrosine (3-NT) immunohistochemistry



Control



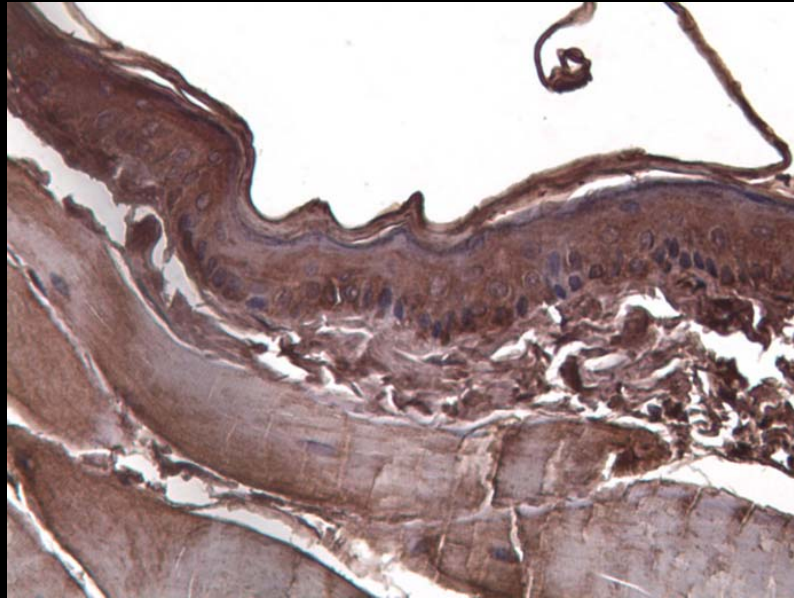
Smokeless tobacco



NNN

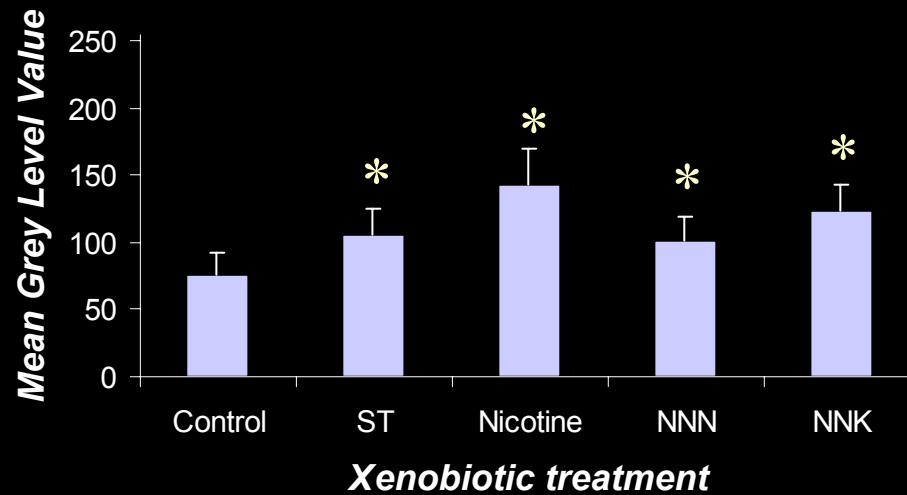


NNK

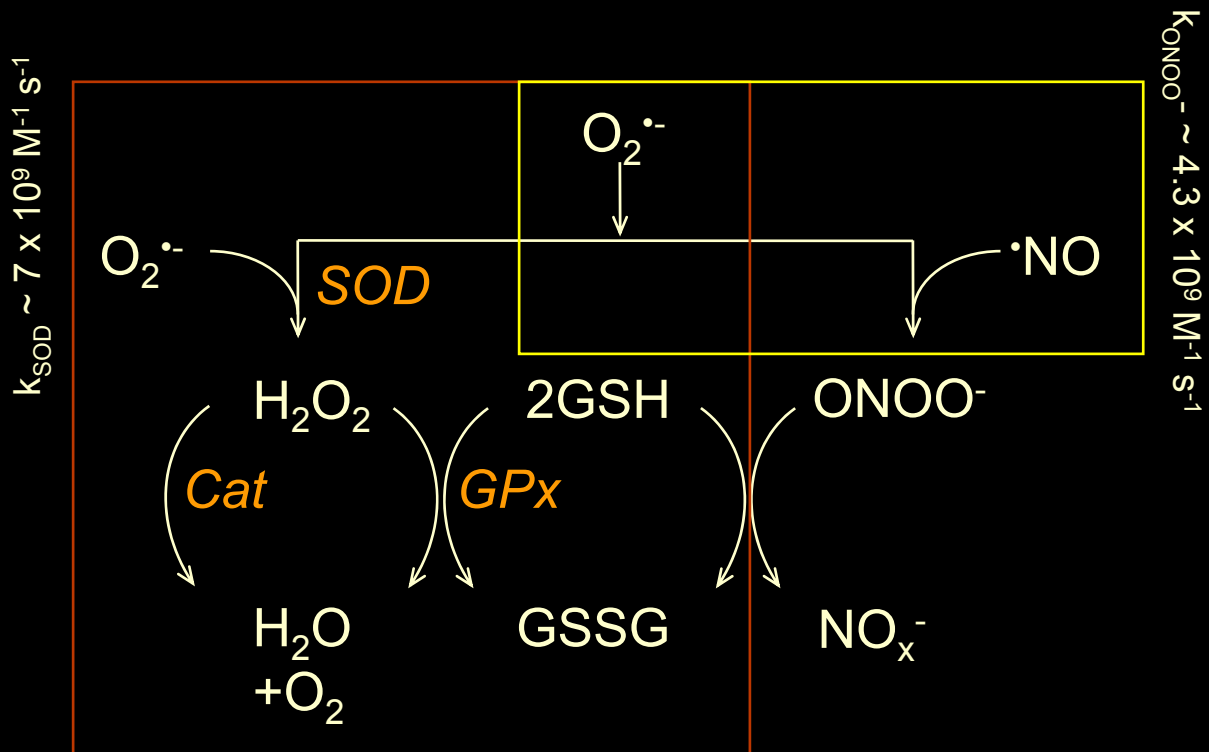


Nicotine

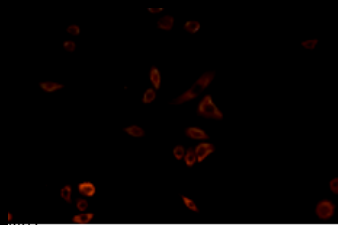
■ Semi-quantitative 3-NT immunohistochemistry



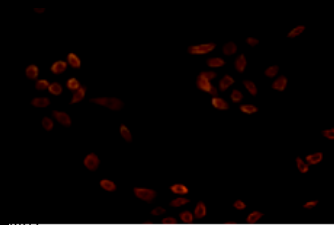
* $p < 0.005$



■ *SOD1* transgene overexpression



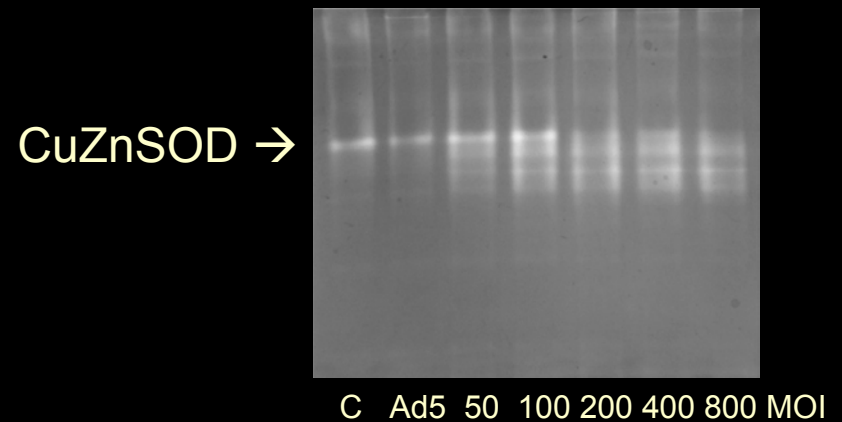
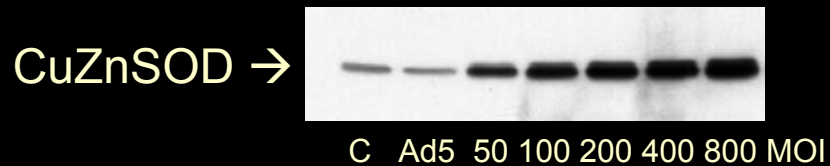
HBEC-1



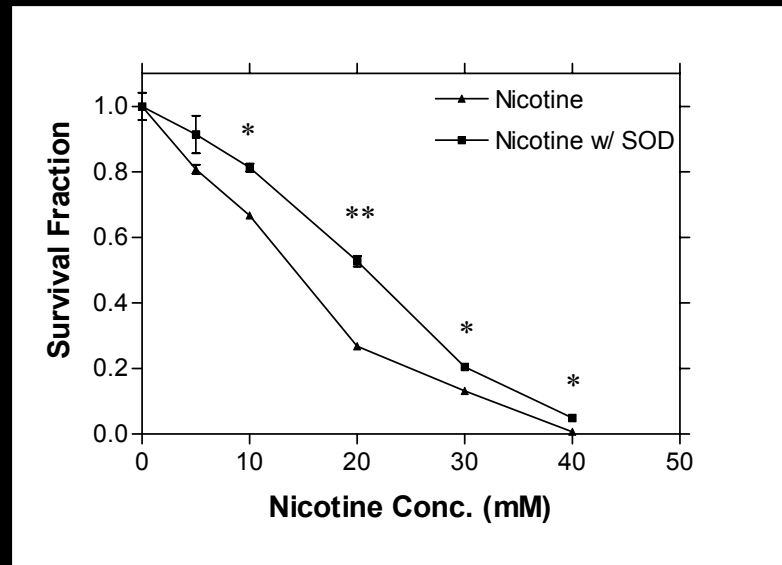
200 MOI Ad5



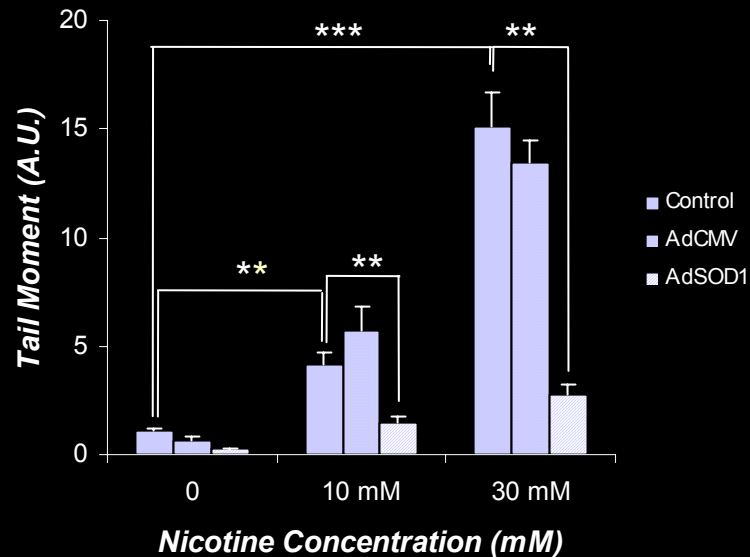
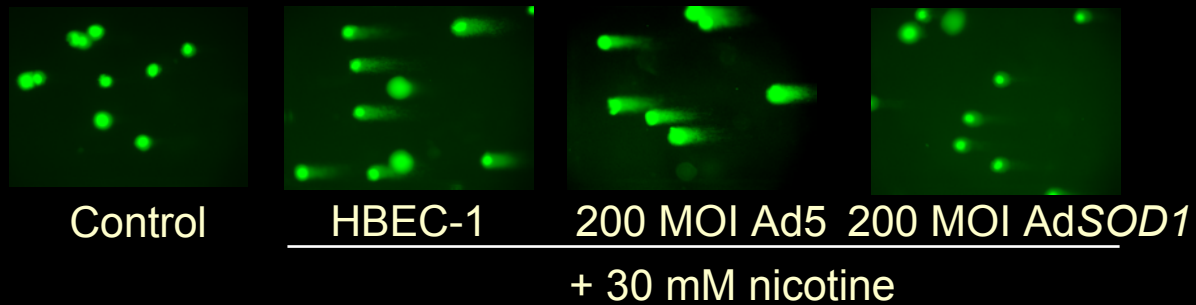
200 MOI AdSOD1



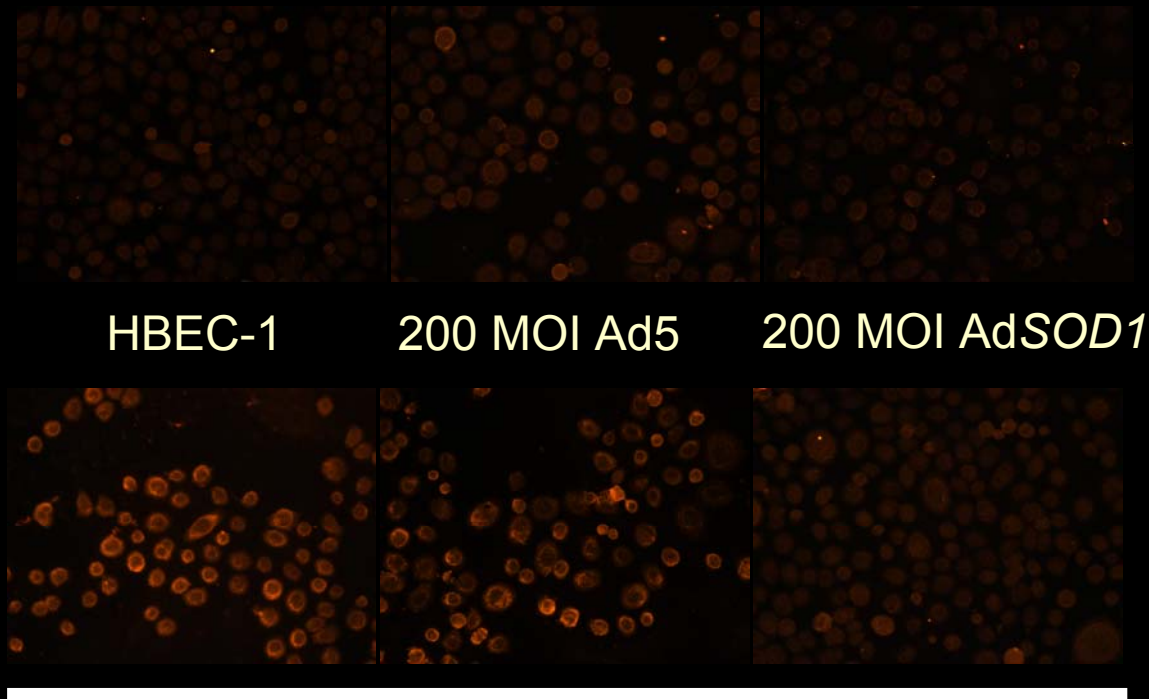
■ *SOD1* protects against nicotine toxicity



■ *SOD1 protects against DNA SSBs from nicotine*



- *SOD1* inhibits 3-NT immunoreactivity



+ 10 mM nicotine

Acknowledgements



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