Combusted, but not smokeless, tobacco product preparations cause DNA damage in human oral cavity cells

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ABSTRACT

We examined the effects of reference tobacco preparations on DNA damage in human oral cavity cells. The oral squamous cell carcinoma cell line (101A), normal human gingival epithelial cells (HGEC), and human gingival fibroblasts (HGF) were treated with total particulate matter from 3R4F cigarettes (TPM), 2S3 smokeless tobacco extracted with complete artificial saliva (ST/CAS), or nicotine alone (NIC). Cells were treated for 24 hours with TPM at respective EC50 doses (13.7, 8.6, or 17.2 µg/ml of equi-nicotine units, as determined in previous experiments), or the doses with equi-nicotine units for ST/CAS. Also, cells were exposed to a high dose of ST/CAS (565.3 µg/ml of equi-nicotine units). DNA damage in exposed cells was assessed by alkaline Comet assays and immunofluorescence staining for the damage-specific protein γ-H2AX.

Both assays showed that only TPM caused readily detectable DNA breaks in exposed cells whereas ST/CAS or NIC did not; only the high dose of ST/CAS caused some weakly measurable DNA damage. Intriguingly, the malignant 101A cells were more susceptible to DNA damage than the normal HGEC and HGF cells.

These studies demonstrate that combusted tobacco products can cause substantial DNA damage in normal and malignant oral cavity cells, whereas non-combusted ST/CAS, or NIC alone, exert no detectable or only minimal DNA damage after 24 hour of exposure. The data will assist in evaluating relative genotoxic and other harmful effects of different categories of tobacco products on oral cavity cells. Such knowledge may help to further understand the involvement of combusted versus non-combusted tobacco products in the etiology of oral cancers.

	101A	HGEC	HGF
TPM	160 µg/ml TPM (EC50) 13.7 µg/ml NIC	100 µg/ml TPM (EC25, no EC50 available) 8.6 µg/ml NIC	200 µg/ml TPM (EC22, no EC50 available) 17.2 µg/ml NIC
ST/CAS (low conc.)	0.08% (w/v) 13.7 µg/ml NIC	0.05 (w/v) 8.6 µg/ml NIC	0.10% (w/v) 17.2 µg/ml NIC
NIC (low conc.)	13.7 µg/ml	8.6 µg/ml	17.2 µg/ml
ST/CAS (high conc.)	3.33% (w/v) 565.3 µg/ml NIC	3.33% (w/v) 565.3 µg/ml NIC	3.33% (w/v) 565.3 µg/ml NIC
NIC (high conc.)	565.3 µg/ml	565.3 µg/ml	565.3 µg/ml

Table 1: Doses of TPM, ST/CAS, and NIC, and their NIC equivalents, used for treatment of cells for 16 or 24 h. For each cell, TPM, ST/CAS and NIC low doses contain the same amount of nicotine. High concentration of ST/CAS contains 42-, 66- and 33-fold more nicotine for 101A, HGEC and HGF cells, respectively, compared to TPM; NIC high and ST/CAS high contain the same amount of NIC.

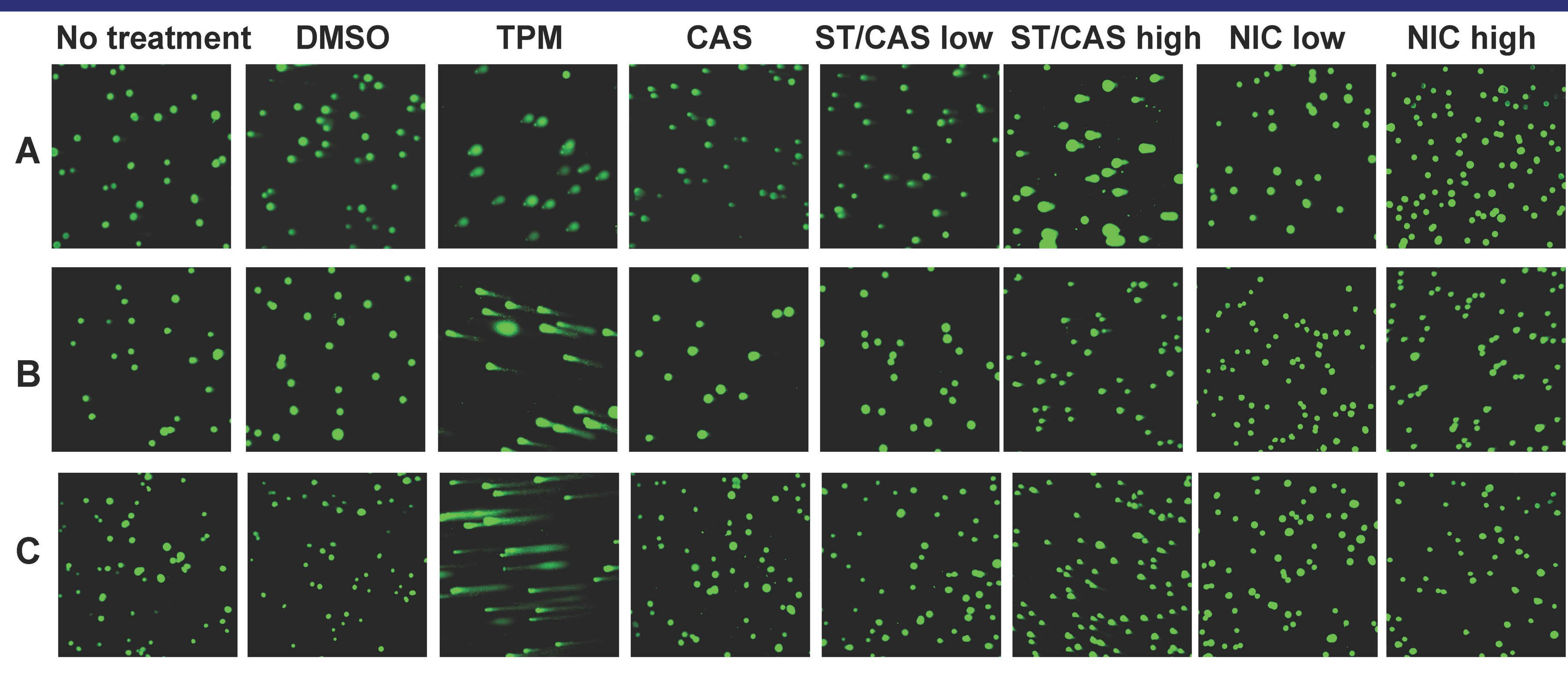

Figure 1: Comet assay images of cells treated by TPM, ST/CAS, or NIC for 24 h. Doses and their NIC equivalents are listed in Table 1. A: 101A cells; B: HGEC cells; C: HGF cells. Solvent controls were DMSO for TPM and NIC, and CAS for ST/CAS. Comet assays for 101A cells were run under condition 1 (20 min alkaline unwinding, electrophoresis at 17 V for 30 min), for HGEC and HGF cells under condition 2 (60 min alkaline unwinding, electrophoresis at 21 V for 45 min). Images for 16 h treatments are not shown here; their quantitations are included in Table 2.

A: Tail moment / condition 1	101A	HGEC	HGF
TPM 16 h	**77 (4)**	0	0
TPM 24 h	**78 (3)**	0	**18 (9)**
ST/CAS low 16 h	0	0	0
ST/CAS low 24 h	0	0	0
NIC low 16 h	0	0	0
NIC low 24 h	0	0	0
ST/CAS high 24 h	**20 (9)**	0	0
NIC high 24 h	0	0	0

B: Tail moment / condition 2	101A	HGEC	HGF
TPM 16 h	N/D	**66 (17)**	**35 (13)**
TPM 24 h	N/D	**65 (21)**	**180 (39)**
ST/CAS low 16 h	N/D	0	0
ST/CAS low 24 h	N/D	0	0
NIC low16 h	N/D	0	0
NIC low 24 h	N/D	0	0
ST/CAS high 24 h	N/D	**2 (1)**	**2 (1)**
NIC high 24 h	N/D	0	0

Table 2: Quantitation of comet tail moments. Cells were treated as shown in Table 1 for 16 h or 24 h, then analyzed under assay condition 1 (A) for three all cell types, or condition 2 (B) for HGEC and HGF cells. Normalized tail moments nTM are listed as mean (SEM) for n = 10 measurements per image field. nTM is defined as: nTM = TL x TI/I, with TL = tail length, TI = tail intensity, and I = total intensity (cell intensity CI + tail intensity TI). 0, no effect observed; N/D, not determined.

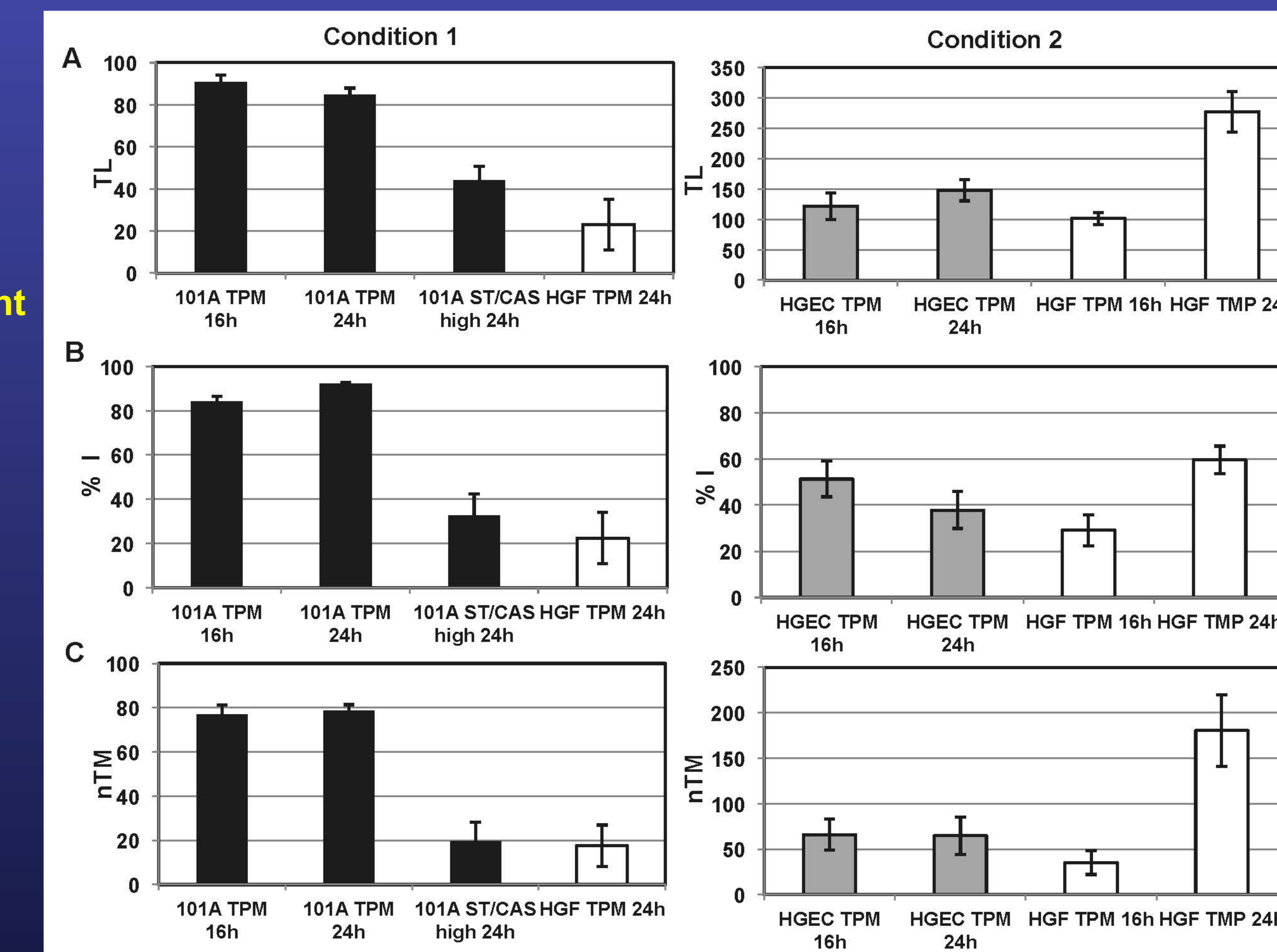

Figure 2: Comparison of parameters contributing to comet tail moments for cells treated with TPM or ST/CAS. A: tail length TL; B: tail intensity as % of total intensity I; C: normalized tail moment nTM. Parameters are defined as above in Table 2. Left panel, condition 1; right panel, condition 2; black bars, 101A; grey bars, HGEC; white bars, HGF.

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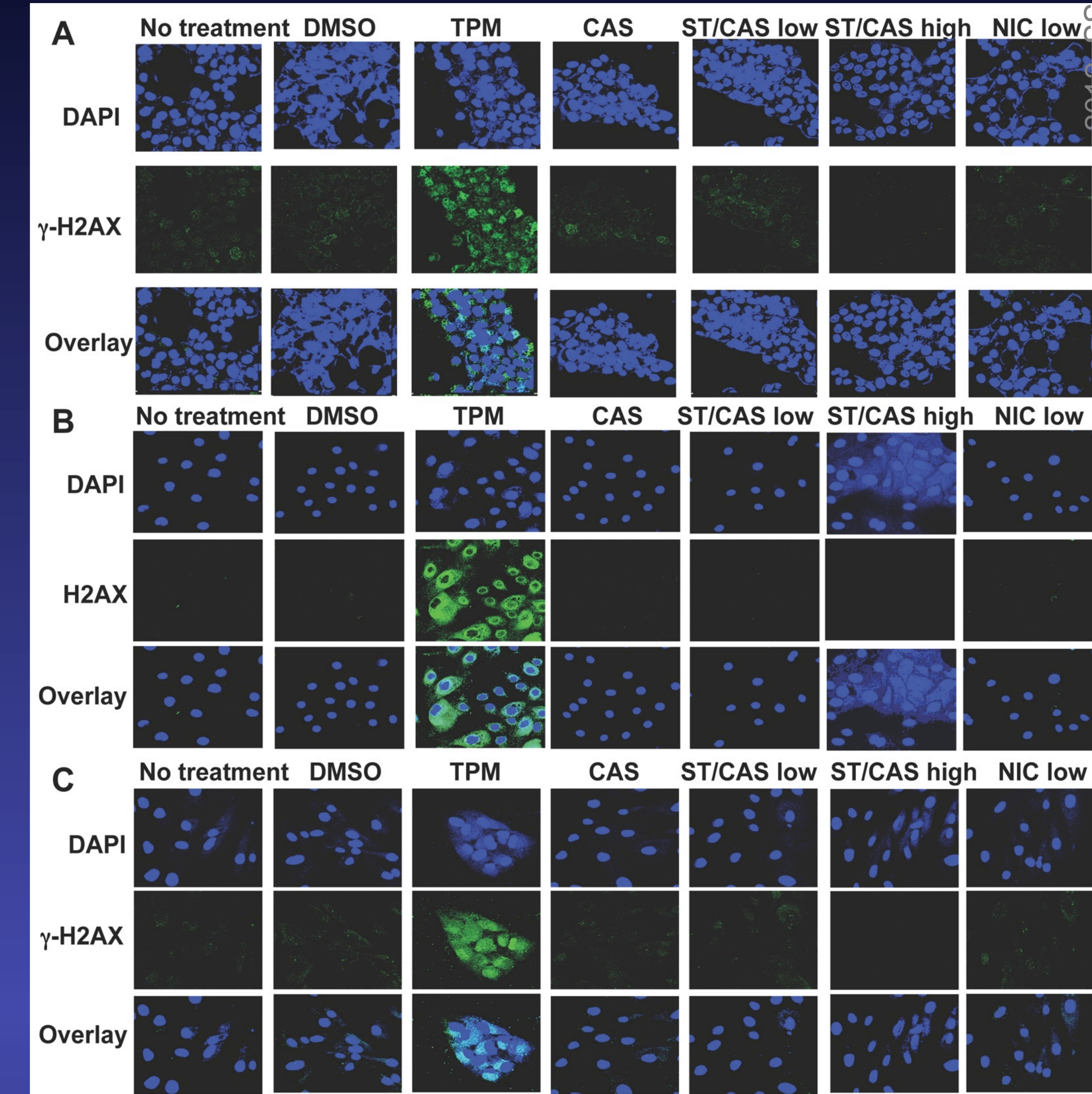

Figure 3: Immunostaining for DNA damage-specific protein γ-H2AX in cells treated by different tobacco agents for 24 h. Doses and their NIC equivalents are listed in Table 1. A: 101A cells; B: HGEC cells; C: HGF cells. Solvent controls were DMSO for TPM and NIC, and CAS for ST/CAS. Nuclei were stained with DAPI; magnification 60 X.

CONCLUSIONS: These preliminary investigations showed:

- Combusted tobacco products can cause substantial DNA damage in normal and malignant oral cavity cells
- Non-combusted ST/CAS or NIC alone exert no detectable or only minimal DNA damage after 24 hour of exposure.
- The malignant 101A cells were more susceptible to DNA damage than the normal HGEC and HGF cells.
- These initial results are directionally concordant with epidemiology regarding risks for oral cancer from smoking versus use of smokeless tobacco products
- The data will assist in evaluating relative genotoxic and other harmful effects of different categories of tobacco products on oral cavity cells.
- Such knowledge helps to further understand the involvement of combusted versus non-combusted tobacco products in the etiology of oral cancers.

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