Neuropathological examination of oxidative and alkylation induced DNA damage in the brain of mice chronically exposed to second-hand smoke

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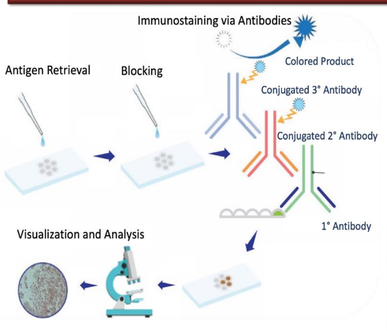
Abstract

Smoke from tobacco products such as cigarettes and cigars contain more than 7,000 chemicals and hundreds of them have been shown to be genotoxic. Smoke exposure in the form of secondhand smoke (SHS) thus can lead to oxidative- and alkylation-induced DNA damage and associated repair of the damage. Our previous studies demonstrated that SHS can induce oxidative DNA damage (i.e., 8-OHdG) in the brain of mice. The goal of this study was to determine if SHS induces different types of oxidative DNA damage, as well as examine the brain for other types of DNA damage such as alkylation and double strand breaks (DSBs). Brain tissue sections of mice that had been chronically exposed to SHS (10 months to ~30mg/m^3) were immunoprobed for *N*-methylpurine-DNA glycosylase (MPG), endonuclease III homolog 1 (NTH1), and γ-H2AX. Both MPG and NTH1 play an important role in repairing alkylation (alkylpurines) or oxidative (thymine glycols) induced DNA damage, respectively. γ-H2AX and p53-binding protein 1 (53BP1) are markers for DSBs that are recruited post-DSBs. All four markers showed increased staining in the pre-frontal cortex of SHS-exposed vs. sham (air) exposed mice. Additionally, γ-H2AX and 53BP1 staining was found to be increased in the dentate gyrus (CA4 region) of SHS-exposed mice. Thus, SHS induces various types of DNA damage and associated repair proteins in brain regions involved in cognitive function and these effects may be responsible for the cognitive dysfunction that we observed in SHS exposed mice.

Background

Exposure to smoke in the form of secondhand smoke (SHS) has been linked to an increased likelihood of the development of neurodegenerative diseases such as stroke[4] and dementia[10]. Our previous study[5] that investigated the mechanism leading to the chronic effects of SHS on the brain demonstrated increased levels of the oxidative DNA damage marker 8-hydroxy-2'-deoxyguanosine (8-OH-dG) in the CA1 region of the hippocampus. AP endonuclease 1 (APE1), a marker of oxidative DNA repair, was also increased in the prefrontal cortex. Both markers indicate that SHS induces oxidative stress induced DNA damage. In this study**,** we continued examining the effects of chronic SHS exposure on the brain by examining an alternative oxidative DNA repair marker (endonuclease III homolog 1 (NTH1))[6], a marker for alkylation DNA repair (*N*-methylpurine-DNA glycosylase (MPG))[3] , and double strand breaks (DSBs) markers (γ-H2AX[2] and p53-binding protein 1 (53BP1)[8]).

Methods



Brain tissue sections (20 µm) were prepared from SHS or Sham (room air) exposed mice. Antigen retrieval was performed with citric acid before the sections was probed with the 1° antibody. Next, we added a biotinylated 2° antibody then detected the markers with NovaRed® stain to visualize the DNA damage or repair proteins. Sections were then imaged on a Leica light microscope.

Antibody stain	Dilution factor
NTH-1	1:50
MPG	1:200
γ-H2AX	1:100
53BP1	1:500

Results

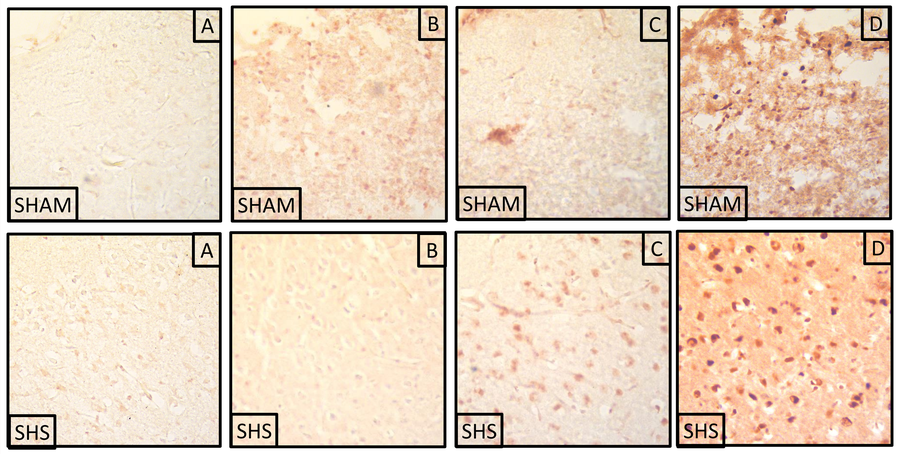


Figure 1. Immunostaining of the pre-frontal cortex with (A) NTH1, (B) MPG, (C) γ-H2AX, and (D) 53BP1 of male mice chronically exposed to SHS. All images were taken at 40x magnification. All three markers showed increased staining in the prefrontal cortex of SHS exposed mice compared to sham mice.

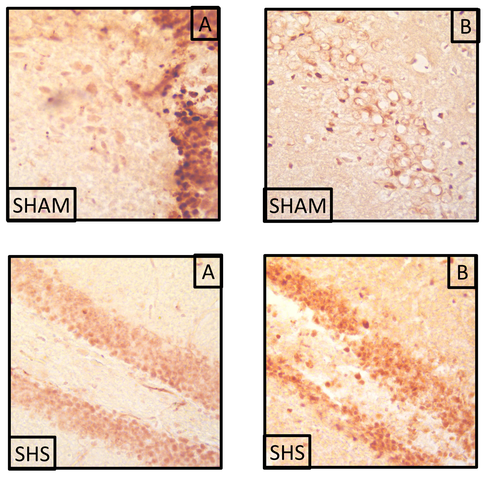


Figure 2. γ-H2AX (A), and 53BP1 (B) staining of the dentate gyrus region (CA4) of the hippocampus in male mice chronically exposed to SHS. Staining was more prominent in SHS than air exposed mice. Staining was comparable for NTH1 and MPG across all hippocampal regions. All images were taken at 40x magnification.

Conclusion

- This study provides additional evidence that SHS induces brain injury through oxidative stress that could explain the behavioral changes and neurocognitive decline in mice that were chronically exposed to cigarette smoke.
- In comparison to air exposed mice, the prefrontal cortex of SHS exposed mice had increased DNA damage (γ-H2AX and 53BP1) and associated increases in oxidative (NTH1) and alkylation (MPG) DNA repair proteins. These results are consistent with our previous study[5] that observed increased markers of oxidative DNA damage and DNA repair in the prefrontal cortex as well as other types of DNA damage (DSBs) and DNA repair (alkylation).
- Prominent staining of the dentate gyrus region of the hippocampus for DSBs (γ-H2AX and 53BP1), a region where adult neurogenesis occurs[1], indicates that these neurons are especially vulnerable to SHS. Since this region contributes to the formation of new memories[9], DNA damage in these neurons could explain the cognitive decline that we observed in our previous studies[5].
- Future studies will determine if SHS also induces nitrative stress or lipid peroxide induced DNA damage in the brain of mice. Quantitative analyses of all these markers will also be required to confirm the findings of this study.

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