NNA, a Thirdhand Smoke Constituent, Induces DNA Damage in Vitro and in Human Cells

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ABSTRACT

Thirdhand smoke (THS) exposure is a newly identified health risk. Recent indoor chemistry studies have revealed that sorbed nicotine reacts with the common indoor pollutant nitrous acid (HONO) to form mutagenic/carcinogenic tobacco-specific nitrosamines (TSNAs). 1-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-4-butanal (NNA) is the major TNSA product that was identified from THS, and is absent in freshly emitted secondhand smoke (SHS). We recently examined the genotoxicity of NNA in human HepG2 cells as well as its ability to modify both 2-deoxyguanosine (dG) and 2-deoxyerythroidine (dC) in vitro. In an alkaline Comet assay, it caused concentration-dependent DNA strand breaks in HepG2 cells at non-cytotoxic concentrations ranging from 0.01 μM to 100 μM for 24 hours. In the reaction of NNA with dG, several adducts were identified with HPLC-UV, ESI-MS/MS and NMR. These include 8-oxo-2'-deoxyguanosine (8-oxo-dG), N\textsuperscript{1}-methyl-dG, and O\textsuperscript6-methyl-dG. NNA also forms a major exocyclic adduct with m/z 455.17 for (M+H)\textsuperscript+ in mass spectrum, which is due to the condensation of NNA and dG with the elimination of H\textsubscript{2}O and two hydrogen molecules. In addition, NNA causes novel DNA sugar modifications, including the previously characterized adducts 8-oxo-dG, N\textsuperscript{6}-methyl-dG, and O\textsuperscript2-methyl-dG. Taken together, these results provide evidence for the DNA damaging potential of NNA, which, in part, may contribute to THS-induced adverse health effects in humans. In addition, the NNA-specific DNA adducts identified can be used as specific biomarkers of THS exposure.

Introduction

One important feature of THS is that it undergoes a chemical transformation during its aging process [1]. An example is that recent indoor chemistry studies have revealed that sorbed nicotine reacts with the common indoor pollutant nitrous acid (HONO) to form tobacco-specific nitrosamines (TSNAs). NNA is the major TNSA product that was identified from THS, and is absent in freshly emitted SHS. Although NNK and NNN are human carcinogens [3] that have been extensively studied, there is little information about the genotoxicity of NNA. In an alkaline Comet assay, it caused concentration-dependent DNA strand breaks in HepG2 cells at non-cytotoxic concentrations ranging from 0.01 μM to 100 μM for 24 hours. In the reaction of NNA with dG, several adducts were identified with HPLC-UV, ESI-MS/MS and NMR. These include 8-oxo-2'-deoxyguanosine (8-oxo-dG), N\textsuperscript{1}-methyl-dG, and O\textsuperscript6-methyl-dG. NNA also forms an exocyclic adduct with m/z 455.17 for (M+H)\textsuperscript+ in mass spectrum, which is due to the condensation of NNA and dG with the elimination of H\textsubscript{2}O and two hydrogen molecules. In addition, NNA causes novel DNA sugar modifications, including the previously characterized adducts 8-oxo-dG, N\textsuperscript{6}-methyl-dG, and O\textsuperscript2-methyl-dG. Taken together, these results provide evidence for the DNA damaging potential of NNA, which, in part, may contribute to THS-induced adverse health effects in humans. In addition, the NNA-specific DNA adducts identified can be used as specific biomarkers of THS exposure.

What is thirdhand smoke (THS)?

THS consists of residual tobacco smoke pollutants that remain on surfaces and in dust after tobacco has been smoked, or are re-emitted back into the gas phase, or react with oxidants and other compounds in the environment to yield secondary pollutants.

A. Identification of NNA in lab chamber-generated acute THS, THS+HONO, SHS, and chronic THS [6]

B. Effect of NNA on genomic DNA in cultured HepG2 cells

C. NNA forms both base and sugar adducts in vitro

Findings

1. NNA was identified in both acute and chronic THS samples.
2. NNA, similar to NNK, causes significant DNA damage at nM concentrations in human cells as measured using the Comet assay.
3. Multiple products are detected and identified from the reaction of NNA with dG in vitro, including the previously characterized adducts 8-oxo-dG, N\textsuperscript{6}-methyl-dG, and O\textsuperscript2-methyl-dG.
4. 8-oxo-dG is known to be mutagenic, and is associated with many disease processes.
5. NNA forms an exocyclic dG adduct with m/z 454.17 in mass spectrum, which has a potential to serve as a biomarker of THS exposure in addition to its biological implications.
6. NNA forms a novel sugar damage, 5,3'-dimethyl-dG. If formed in cells, it would lead to the breakage of the DNA backbone.
7. NNA also reacts with dC to form multiple adducts (on-going work).

References


Acknowledgments

Supported by the Grants R01-HL120797 and 1R01-ES018078-B01 from the Tobacco-Related Disease Research Program (TRDRP) of the State of California. The Related Disease Research Program (TRDRP) of the State of California.

Figure 1. Structural identification of dG adducts formed by NNA.

Figure 2. HepG2 cells were exposed to varying doses of NNA dissolved in DMEM medium with/without 10% FBS for 24h. NNA was used as a positive control as previous studies have showed that the comet assay is sufficiently sensitive and specific in measuring NNA-induced DNA damage, including strand breaks and alkali-labile sites [6]. This study shows that NNA, similar to NNK, induces DNA damage at low nanomolar concentrations [5].