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

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Multi-unit housing

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-pyridinyl)-4-butanal (NNA) is the major TSNA 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-deoxycytodine (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 spectrum, ESI-MS/MS and NMR. These include 8-oxo-2'-deoxyguanosine (8-oxo-dG), N^1-methyl-dG, and O^6-methyl-dG. NNA also forms a major exocyclic dG adduct with m/z 455.17 for $(M+H)^+$ in mass spectrum, which is due to the condensation of NNA and dG with the elimination of H_2O and two hydrogen molecules. In addition, NNA causes novel DNA sugar damage, forming 5',3'-dimethyl-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), including 1-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridinyl)-4-butanal (NNA), 4-(methylnitrosamino)-1-(3-pyridinyl)-1-butanone (NNK) and *N*-nitrosonornicotine (NNN) (Fig. 1, right panel) [2]. NNA is the major TSNA product 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 and reactivity of NNA with DNA.

Using the alkaline Comet assay, we examined the potential of NNA to cause DNA strand breaks in cultured human hepatocellular carcinoma (HepG2) cells. Moreover, the ability of NNA to form DNA adducts with dG and dC *in vitro* was investigated and characterized using HPLC-UV, electrospray ionization mass spectrometry (ESI-MS/MS) and NMR. The measurement of the above DNA damage can be used to assess the biologically effective dose of exposure, understand the mechanism of the biological impacts of tobacco toxins, and serve as biomarkers of exposure [4]. Our results provide evidence, for the first time, that NNA results in DNA strand breaks in exposed cells [5] and forms multiple types of DNA adducts *in vitro*, which may contribute to THS-induced adverse health effects in humans. In addition, the bulky exocyclic NNA-dG adduct identified in this study may be used as a specific biomarker of THS exposure.

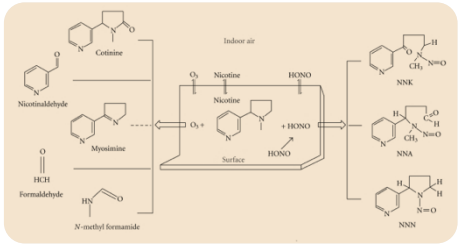

Figure 1

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.

Common THS sites

Inside room | Inside car | Multiunit housing | Public places (casinos, hotels) | Skin, hair, clothing

Results

A. Identification of NNA in lab chamber-generated acute THS, THS+HONO, SHS, and chronic THS [5]

Table I Concentrations of THS constituents in DMEM media (ng/ml) and in cellulose substrates (ng/g)

Sample name		COT	*N*-Formylnornicotine	NNN	NNK	NNA	Nicotelline	2,3'-Bipyridine	PON
Acute THS									
DMEM only		BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Blank		BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
THS	DMEM	27.0	50.9	0.264	0.903	0.43	0.752	189	228
	3MM paper	n.a.	n.a.	BLOQ[a]	15.9[a]	1.6[a]	n.a.	n.a.	n.a.
THS + HONO	DMEM	14.9	27.0	0.145	0.517	1.82	0.312	107	163
	3MM paper	n.a.	n.a.	BLOQ[a]	18.1[a]	5.4[a]	n.a.	n.a	n.a.
SHS		11.3	23.6	0.259	0.149	BLOQ	0.404	90.2	134
Chronic THS									
DMEM only		BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Blank		BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
THS	DMEM	133	585	1.57	7.20	0.39	8.04	147	13.0
	3MM paper	n.a.	n.a.	19.5[a]	78.6[a]	1.4[a]	n.a.	n.a.	n.a.

BLOQ = below the limit of quantitation.
[a]TSNA concentrations were obtained using GC–IT–MS/MS method and are expressed in nanogram per gram of 3MM paper (ng/g).

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

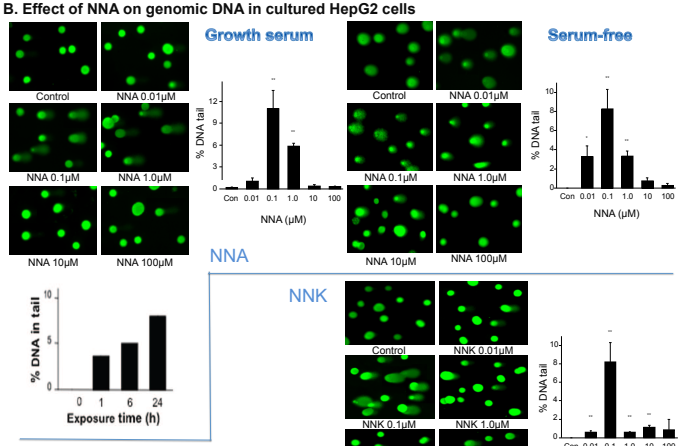

Figure 2. HepG2 cells were exposed to varying doses of NNA dissolved in DMEM medium with/without 10% FBS for 24h. NNK was used as a positive control as previous studies have showed that the comet assay is sufficiently sensitive and specific in measuring NNK-induced DNA damage, including strand breaks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

C. NNA forms both base and sugar adducts *in vitro*

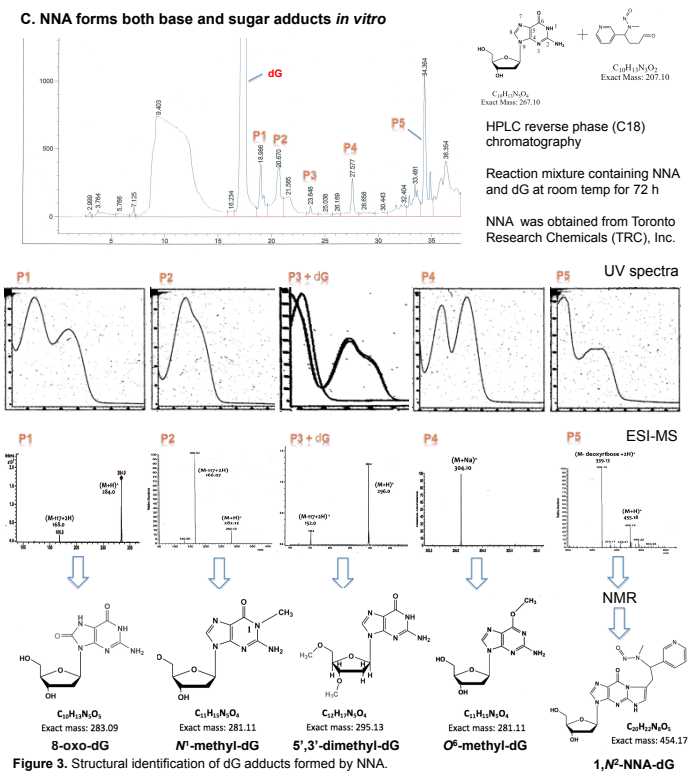

Figure 3. Structural identification of dG adducts formed by NNA.

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^1-methyl-dG, and O^6-methyl-dG. 8-oxo-dG is known to be mutagenic, and is associated with many disease processes.
4. NNA forms an exocyclic dG adduct with a 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.
5. NNA forms a novel sugar damage, 5',3'-dimethyl-dG. If formed in cells, it would lead to the breakage of the DNA backbone.
6. NNA also reacts with dC to form multiple adducts (on-going work).

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Acknowledgments

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