The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) induces mitochondrial and nuclear DNA damage in *Caenorhabditis elegans*

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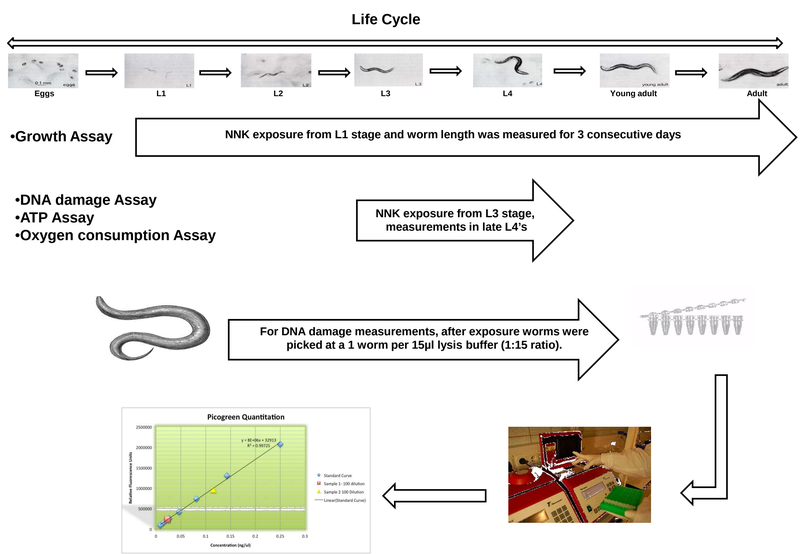
Abstract

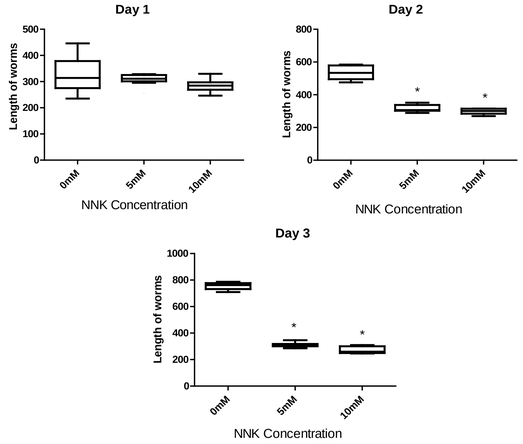
The metabolites of the tobacco-specific carcinogen NNK form DNA adducts in animal models. One report indicates that NNK could cause damage to the mitochondrial as well as nuclear genome in rats (Stepanov and Hecht, 2009 *Chem. Res. Toxicol.* 22: 406–414). Using a different DNA damage detection technology, we tested whether this could be repeated in the nematode *Caenorhabditis elegans*; we also evaluated whether mitochondrial function would be affected. We treated N2 strain (wild-type) nematodes with NNK in liquid culture. Quantitative PCR was applied to analyze NNK-induced nuclear and mitochondria DNA damage. This assay has the advantage of measuring all DNA lesions that inhibit the DNA polymerase, and normalizes results to mitochondrial DNA copy number (Hunter *et al.*, 2010 *Methods* 51:444-451). Our results confirm that NNK causes both nuclear and mitochondrial DNA damage, but surprisingly nuclear DNA damage was greater than mitochondrial DNA damage in *C. elegans*. To test whether the mitochondrial DNA damage was associated with mitochondrial dysfunction, we used a transgenic nematode strain (PE255) that permits *in vivo* measurement of ATP levels and found lower levels of ATP in NNK-exposed animals when compared to the unexposed controls. To test whether the lower levels of ATP were due to the inhibition of respiratory chain components we investigated oxygen consumption in whole *C. elegans* and found reduced oxygen consumption in exposed animals when compared to the unexposed controls. Our data suggest a model in which NNK causes damage to both nuclear and mitochondrial genomes, and support the hypothesis that the mitochondrial damage is functionally important. These results also represent a first step in developing this genetically tractable organism as a model for assessing NNK toxicity.

Materials and Methods

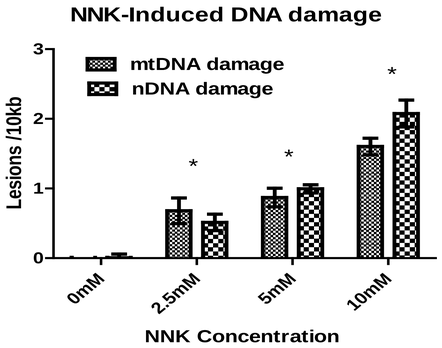


C. elegans develops through four larval stages to adulthood in approximately 3 days. Growth assays were carried out from the first larval stage to adulthood. For DNA damage, ATP level, and oxygen consumption measurements, synchronized third larval stage (L3) N2 (wild-type strain) nematodes were exposed to NNK at different concentrations for 24h and incubated at 20°C. For DNA damage measurements, after 24h, worms were picked at a 1 worm per 15µl lysis buffer (1:15 ratio). Mitochondrial and nuclear DNA damage was determined using a QPCR-based method (Meyer, 2010; Hunter et al., 2010) in which DNA damage is identified via inhibition of the DNA polymerase that carries out the PCR reaction. Mitochondrial and nuclear DNA damage was quantified using the doublestrand DNA-specific dye Picogreen to quantify the QPCR product, which is decreased when polymerase-blocking DNA damage is present.

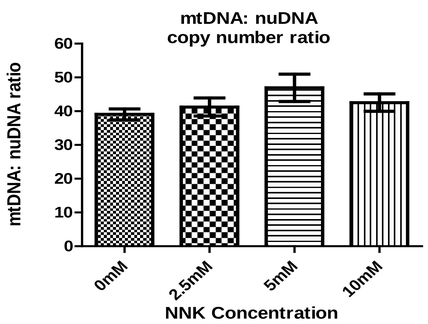
Results



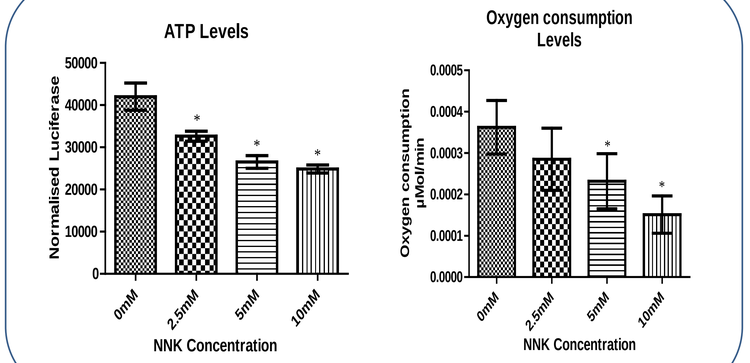
Inhibition of larval growth induced by NNK was quantified by measuring the worm length at different concentrations of NNK for 3 consecutive days from the first larval (L1) stage. On day 2 and 3 the nematodes exposed to NNK were smaller than the controls. The box plots depict 5th, 25th, 50th, 75th, and 95th percentiles. Asterisks indicate a significant treatment effect compared with untreated control (P<0.05).



Exposure to NNK resulted in both nDNA and mtDNA damage in *C. elegans*. There was a dose-dependent increase in damage ($P < 0.0001$ for main effect of exposure, 2-factor ANOVA)), but nDNA damage was indistinguishable from mtDNA damage ($P > 0.05$ for main effect of genome and interaction, 2-factor ANOVA) . Bars indicate mean values ± standard error of the mean; n = 6 in two separate experiments). Asterisks indicate a significant treatment effect compared with untreated control (P<0.05).



The exposures did not result in a marked change in mtDNA:nDNA ratio ($p > 0.05$, 1-factor ANOVA) as measured by real-time PCR. The mtDNA:nDNA ratio changes if mitochondrial copy number per cell changes. Bars indicate mean values ± standard error of the mean; n = 6 in two separate experiments.



L3 staged PE255 worms were exposed to NNK on a 6 well plate for 24hrs. (A) Steady state ATP levels were significantly lower at higher concentrations of NNK. Asterisks indicate a significant treatment effect compared with untreated control (1-factor ANOVA, P<0.05). (B) Animals exposed to 2.5mM, 5mM and 10mM NNK had lower O_2 consumption compared with untreated nematodes. Statistically significant differences from untreated controls were observed at 5mM and 10mM NNK (1-factor ANOVA, P< 0.05).

Conclusion

Our data suggest that NNK causes:

- Damage to both nuclear and mitochondrial genomes in *C. elegans*,
- Larval arrest,
- Lower ATP content and
- Reduced oxygen consumption

This supports the hypothesis that the mitochondrial damage is functionally important. These results also represent a first step in developing this genetically tractable organism as a model for assessing NNK toxicity.

References

•Stepanov, I. and S. S. Hecht (2009). "Mitochondrial DNA Adducts in the Lung and Liver of F344 Rats Chronically Treated with 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone and (S)-4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol." Chemical Research in Toxicology 22(2): 406-414.

•Meyer, J. N. (2010). "QPCR: a tool for analysis of mitochondrial and nuclear DNA damage in ecotoxicology." Ecotoxicology 19(4): 804-811.

•Hunter, S. E., D. Jung, R. T. Di Giulio and J. N. Meyer (2010). "The QPCR assay for analysis of mitochondrial DNA damage, repair, and relative copy number." Methods 51(4): 444-451.

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