The Analysis of Pyridyloxobutyl RNA Adducts in Rats Treated with 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone

John G. Muzic, Irina Stepanov and Stephen S. Hecht

Masonic Cancer Center, University of Minnesota, Minneapolis, MN 55455

Goal

To identify and chemically characterize pyridyloxobutyl (POB) RNA adducts in rats treated with the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).

Background

- Everyday, there are over 3,000 deaths worldwide due to lung cancer of which ninety percent are due to smoking.[1]
- NNK, formed from the parent compound nicotine, is a potent and specific lung carcinogen consistently found in tobacco and its smoke.[2]
- Previous studies have demonstrated the formation of POB-DNA adducts in rats treated with NNK.[3]
  - There exists no data on the formation of POB-RNA adducts.
- Metabolic activation of NNK by cytochrome P450 enzymes generates the intermediate compound, POB-diazohydroxide.
- POB-diazohydroxide alkylates DNA to produce POB-DNA adducts.[4]
- The formation of POB-DNA adducts is important in carcinogenesis imparted by tobacco-specific nitrosamines.[3]
  - POB-RNA adducts may be important in carcinogenesis.
- POB-DNA adducts may serve as biomarkers of exposure to tobacco-specific nitrosamines.[4]
  - POB-RNA adduct levels are expected to be higher than those of DNA.
  - Adduct levels can be used in utilizing chemopreventative strategies targeted to the nitrosamine activation pathways.[5]
- In this study, we developed a method to isolate and hydrolyze RNA from rat liver treated with NNK, and methods for analysis and quantitation by high performance liquid chromatography and liquid chromatography -tandem mass spectrometry.
- Adducts are currently being synthesized by RNA reactions with NNKOAc to confirm the structures of adducts found in tissue samples.

References

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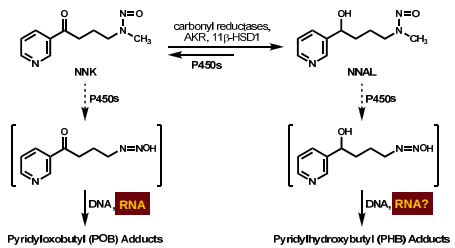
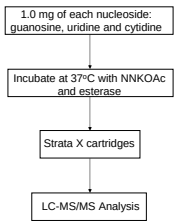
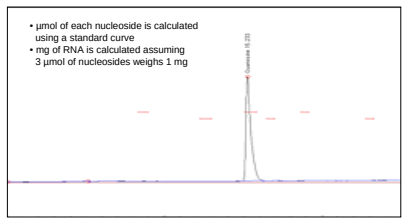
Adduct Formation From NNK[6]



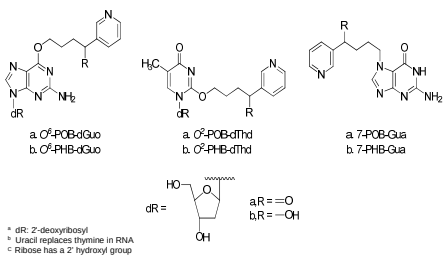
POB-RNA Adduct Synthesis Method



RNA Quantitation

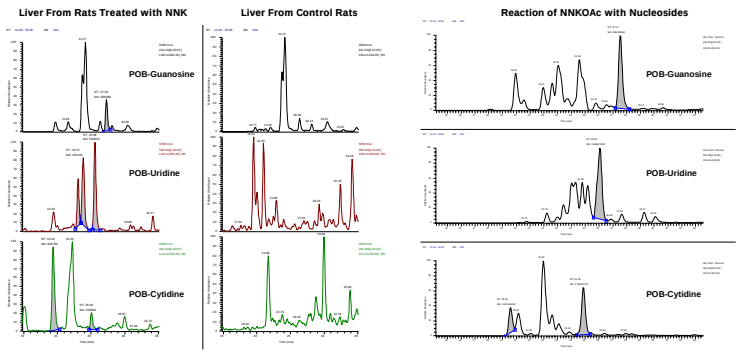


POB-DNA Adducts[6]

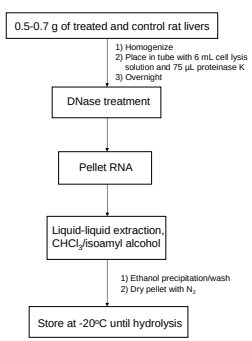


[a] dR: 2'-deoxyribosyl
[b] Uracil replaces thymine in RNA
[c] Ribose has a 2' hydroxyl group

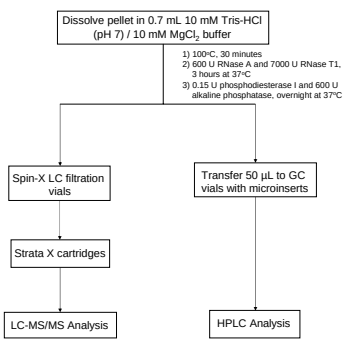
LC-MS/MS Traces of POB-RNA Adducts



RNA Isolation Method



RNA Hydrolysis and POB-Adduct Isolation



Conclusions

- The results of this study demonstrate for the first time that POB-RNA adducts are formed when rats are treated with NNK.
- The reaction of nucleosides with NNKOAc may produce the same adducts found in NNK-treated rat liver.
- In the future, RNA adducts may be used as a reliable biomarker of exposure to tobacco-specific nitrosamines.

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