

Abstract: Metabolism of carcinogenic tobacco-specific nitrosamines

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3R37CA081301-14S1

Carcinogenic Tobacco-Specific Nitrosamines," we propose to expand upon a remarkable new finding: (*S*)-*N'*-nitrosonornicotine [(*S*)-NNN], a well established constituent of smokeless tobacco products, is a potent oral cavity carcinogen in rats.

Our recent results, which are described in detail in the main body of the proposal, now establish (*S*)-NNN as the only strong oral carcinogen in smokeless tobacco products, known to be a cause of oral cancer in humans. Thus, it is urgent to carry out further research on the presence of (*S*)-NNN in these products and to determine NNN uptake by smokeless tobacco users, as we propose.

Investigation of levels of (*S*)-NNN in tobacco products and human urine, as well as development of an improved urinary biomarker for NNN uptake in humans, the subjects of this Competitive Revision, are not proposed in the current version of R37 CA-81301-14. When that grant was written, the carcinogenicity study described here had not been started, so we did not know that (*S*)-NNN was a powerful oral cavity carcinogen in the rat, although the results of the DNA adduct studies carried out under that grant encouraged us to do the carcinogenicity study.

The specific aims of R37 CA-81301-14 were:

1. Extend our new MS methods for DNA adduct analysis to include the pyridylhydroxybutyl and methyl adducts of NNAL and the 5'-hydroxylation adducts of NNN and improve the sensitivity of these methods in order to apply them to DNA samples from lung tissue of smokers and non-smokers. Develop methods to analyze for selected pyridyloxobutyl-DNA adducts in human urine, thereby leading to a readily available biomarker for individual metabolic activation of NNK and NNN.
2. Improve sensitivity and high throughput for measurement of the NNK metabolite NNAL in human blood and urine, and apply these methods in nested case control studies in which pre-diagnosis samples have been stored from smokers who developed lung cancer and from matched controls. We hypothesize that the NNAL biomarker, together with those developed in aim 1, as well as DNA repair parameters, ultimately will become part of a predictive algorithm to identify those smokers at highest risk for lung cancer, a critical step in lung cancer prevention.
3. Identify the receptor in the rat lung that specifically binds the NNK metabolite (*S*)-NNAL and is arguably critical in the potent lung carcinogenicity of NNK, and extend these studies to human lung.
4. Further develop the toenail biomarkers for NNAL and cotinine by carrying out longitudinal and cessation studies, and by applying these methods to non-smokers exposed to secondhand cigarette smoke.
5. Determine the structure of an inhibitor of carcinogen metabolic activation, which we have identified in human lung microsomes. This cytochrome P450 inhibitor could be a key compound for understanding differences in human response to inhaled carcinogens, such as those present in cigarette smoke.
6. Continue our research on inter-individual differences in human metabolic activation and detoxification of tobacco-specific nitrosamines, and on the endogenous formation of these carcinogens in humans.

Although this Competitive Revision application is clearly related to the Specific Aims of the current grant, the focus on (*S*)-NNN exposure and the development of an improved biomarker for NNN uptake in humans, are not addressed at all in the current grant because we were unaware of the now obvious

importance of (S)- NNN in smokeless tobacco carcinogenesis. We discuss this further under Specific Aim 3 of this competitive revision application. The results of the studies proposed here, with their focus on oral carcinogenesis by smokeless tobacco, will clearly amplify and enhance those of R37 CA-81301.