**Recent Studies on the Endogenous Formation of *N*-Nitroso-Compounds**

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Endogenous formation of *N*-nitroso- compounds from the reaction of amines or amides with nitrite in the body is a potential source of human carcinogen exposure. In our recent studies, we have investigated the endogenous formation of the tobacco-specific carcinogen *N'*-nitrosonornicotine (NNN) in people using nicotine replacement therapy [1;2]. In separate investigations, we have detected 7-(2-carboxyethyl)guanine in human hepatic DNA [3]. Endogenous nitrosation is one possible source of this DNA adduct. This presentation will summarize data from these projects.

NNN induces oral cavity, nasal cavity and esophageal tumors in rats, and tumors of the respiratory tract in hamsters and mice. NNN is present in all unburned tobacco products such as smokeless tobacco, as well as in cigarette smoke. All tobacco users are exposed to considerable amounts of NNN, considered a human carcinogen by the International Agency for Research on Cancer [4]. NNN is easily and rapidly formed by nitrosation of nornicotine in vitro, and less readily by nitrosation of nicotine [5;6]. Our earlier studies also showed that NNN can be formed in vivo in rats treated with nornicotine and nitrite, and that this endogenous nitrosation can be blocked by ascorbic acid [7;8].

In our recent studies, we have examined the formation of NNN in people who use nicotine replacement products. These products are commonly used by ex-smokers to help maintain a state of smoking cessation. Several types of products are available including nicotine lozenges, nicotine gum, and nicotine patch. In the studies reported here, we investigated urinary levels of NNN in subjects who had stopped smoking with the help of these nicotine replacement therapy products. We observed that several metabolites derived from tobacco smoke constituents such as NNK, pyrene, 1,3-butadiene, acrolein, ethylene oxide, and others decreased smoothly in the days and weeks following smoking cessation [9]. However, this was not the case for NNN. Rather than observing the expected smooth decrease of this tobacco-specific compound after cessation of cigarette smoking, we observed spikes of increased urinary levels of NNN at various times after cessation. The spikes were particularly evident in subjects who used oral nicotine replacement products such as the nicotine lozenge or gum. These results could not be explained by artifactual formation of NNN during collection or storage of the urine samples, or during the analysis for NNN. The results were consistent with endogenous formation of NNN in some subjects, resulting from nitrosation of nornicotine, most likely in the mouth or stomach. In support of this proposal, we have recently examined the nitrosation of [$D_4$]nornicotine in human saliva [10]. [$D_4$]Nornicotine was used in this study to eliminate the possibility of sources of NNN other than endogenous formation. Incubation of [$D_4$]nornicotine with saliva from 10 non-smoking volunteers resulted in the formation of [$D_4$]NNN in samples from 8 of these individuals, without the addition of any other substance to the saliva. We did not observe [$D_4$]NNN in saliva samples incubated with [$D_4$]nicotine. [$D_4$]NNN, identified by mass spectrometry in these experiments, resulted from the reaction of salivary nitrite with [$D_4$]nornicotine. These results are entirely consistent with earlier chemical studies demonstrating the rapid nitrosation of nornicotine. Collectively, these results provide convincing evidence for the endogenous formation of NNN in some people using nicotine replacement products. The source of nornicotine in these studies can be the product itself or metabolism of nicotine to nornicotine. This

potential hazard could be addressed, at least in part, by excluding nornicotine from nicotine replacement products or by formulating them with an inhibitor of nitrosation such as ascorbic acid.

Nicotine replacement therapy demonstrably improves smoking cessation. The benefit of cessation far outweighs the risk of NNN exposure during use of these products, because exposure to multiple carcinogens is decreased. There is presently no evidence that long term users of nicotine replacement products have an increased risk of cancer above and beyond that due to their history of smoking. Nevertheless, it would be prudent to avoid NNN exposure in users of these products.

In the second group of studies to be described here, we have analyzed human liver samples for two DNA adducts – 7-(2′-carboxyethyl)guanine (7-CEG) and 7-carboxymethylguanine (7-CMG) [3]. A total of 24 samples were analyzed by liquid chromatography-mass spectrometry. 7-CEG was detected in all samples, mean 373 fmol/μmol G, but 7-CMG was not detected in any sample. The levels of 7-CEG are comparable to those of $N^2$-ethylidene-dG, formed from acetaldehyde. This adduct has the potential to depurinate spontaneously leading to apurinic sites and mutagenesis.

We considered three possible sources of 7-CEG in human liver DNA. The first would be exposure to 3-(methylnitrosamino)propionic acid (MNPA), which has been detected in some human urine samples. Metabolism of this compound would be expected to produce intermediates that could react with DNA resulting in formation of 7-CEG . We consider this explanation to be fairly unlikely because human exposure to the related nitrosamine *N*-nitrososarcosine is far more common, based on studies of its occurrence in urine, yet we did not detect the corresponding expected DNA adduct 7-CMG in any human liver sample. The second explanation involves reaction of acrylic acid with DNA, which is known to produce 7-CEG, albeit slowly and in low yield. Acrylic acid is a metabolite of acrolein, a common environmental contaminant and endogenous product of lipid peroxidation. All humans appear to have acrolein-DNA adducts in their white blood cells and some other tissues. The third possible explanation involves endogenous nitrosation. 1-Nitroso-5,6-dihydrouracil (NDHU), the nitrosation product of 5,6-dihydrouracil, is a powerful hepatocarcinogen in the rat and reacts readily with DNA to produce 7-CEG. NDHU could be formed endogenously from nitrosation of 5,6-dihydrouracil, a normal pyrimidine metabolite present in human urine and plasma. 5,6-Dihydrouracil is further metabolized to β-ureidopropionic acid and β-alanine, both of which are found in human urine and could also be nitrosated to ultimately produce 7-CEG. We are currently exploring the potential formation of 7-CEG in rats treated with nitrite and 5,6-dihydrouracil or β-ureidopropionic acid. The results of these studies could provide new insights on causes of hepatic DNA damage and possibly hepatocarcinogenesis in humans.

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