

Null Results in Brief

4-Hydroxy-1-(3-pyridyl)-1-butanone, an Indicator for 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone-Induced DNA Damage, Is not Detected in Human Pancreatic Tissue

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Introduction

Tobacco smoking is the only known etiologic agent that causes pancreatic cancer. The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is a potent carcinogen in laboratory rodents that, independent of the route of administration, induces primarily lung adenocarcinoma (1). When administered in drinking water, NNK and its metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) also induce cancer of the pancreas in rats (2). NNK and NNAL are present in human pancreatic juice (3). Human pancreatic tissue is capable of metabolizing NNK to NNAL, keto acid [4-oxo-4-(3-pyridyl)-1-butanonic acid], and keto alcohol [4-hydroxy-1-(3-pyridyl)-1-butanone (HPB); ref. 4]. These metabolic pathways lead to formation of electrophiles that readily react with cellular macromolecules including DNA. Tobacco-specific nitrosamine-induced DNA adducts identified include *O*⁶-methylguanine, 7-methylguanine, and 4-methylthymidine. There are also NNK adducts resulting from pyridyloxobutylation of DNA, such as *O*⁶-[4-oxo-4-(3-pyridyl)but-1-yl]guanine, 7-[4-oxo-4-(3-pyridyl)but-1-yl]guanine, *N*²-[4-oxo-4-(3-pyridyl)but-1-yl]guanine, *O*²-[4-oxo-4-(3-pyridyl)but-1-yl]cytidine, and *O*²-[4-oxo-4-(3-pyridyl)but-1-yl]thymidine (ref. 5 and references therein). Tobacco smoke induces oxidative stress; oxidative damage markers in biological specimens of smokers were detected at levels significantly higher than those in nonsmokers (reviewed in ref. 1). NNK has also induced oxidative damage in rodent lungs as illustrated by elevated levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) (6). We hypothesized that the presence of NNK in human pancreatic juice may result in the formation of 8-OHdG and HPB-releasing DNA adducts derived from the pyridyloxobutylation pathway in pancreatic tissue; such DNA damage is prerequisite, but not sufficient, for the initiation of carcinogenesis in this organ.

Materials and Methods

Histologically normal pancreatic tissue samples were collected at the University of Ulm in Germany from patients undergoing surgery for pancreatic cancer, Vater's papilla cancer, bile duct cancer, duodenal cancer, or chronic pancreatitis. Approval for human subject research by the Institutional Review Board of the University of Ulm was secured before initiation of this study; all subjects gave written informed consent. Samples were snap-frozen in liquid nitrogen, shipped on dry ice to our laboratory, and stored at -80°C until analyzed. DNA was isolated from 58 samples of pancreatic tissue and analyzed for the HPB-releasing adduct according to Kutzer et al. (level of detection 1 pg/injection; ref. 7). Another 17 samples were used for analysis of 8-OHdG [level of detection 0.5 8-OHdG/10⁶ dG (mol/mol); ref. 6]. The self-reported smoking status of subjects involved in this study was confirmed by determining urinary cotinine and by analyzing hair for cotinine and nicotine.

Statistical Analysis. Continuous dependent variables (cotinine in urine, cotinine and nicotine in hair, and 8-OHdG) were compared between smokers and nonsmokers using the Wilcoxon rank-sum test. To assess any possible linear relationship between continuous variables among smokers and nonsmokers, both separately and combined, Spearman correlation coefficients were computed and tested for statistical significance. The above nonparametric methods were chosen due to the large variability of the data and the relatively small sample size. All tests were two-tailed and considered statistically significant at *P* = 0.05.

In some instances, when measurements of a given parameter were made but compounds were not detected, rather than simply assigning zero, we chose to assign the midpoint between zero and the known minimum detectable level. For example, the least detectable level for cotinine in urine is 5 ng/mL; thus, a value of 2.5 was assigned to all "not detectable levels."

Results

We have analyzed 58 samples of pancreatic DNA by gas chromatography-mass spectroscopy (7). As a positive control, we used DNA isolated from mouse lung treated by a single i.p. injection of NNK (10 μmol/kg body weight). In mouse lung, the levels of HPB were 8.2 ± 1.1 pmol/μmol guanine. HPB was detected at levels twice higher than background in only six of the human pancreatic tissues analyzed (four of these samples were from smokers). In a

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separate study, we have collected 17 samples of pancreatic tissue that were sufficient in size for DNA isolation. These samples were used for the determination of 8-OHdG. This lesion was detected in all samples of pancreatic DNA analyzed. Its levels in smokers' DNA ranged from 4.45 to 8.86 8-OHdG/ 10^6 dG (6.3 ± 2.0 , mean \pm SD). Corresponding levels in nonsmokers' DNA ranged from 0.94 to 18.1 8-OHdG/ 10^6 dG (6.81 ± 5.5 , mean \pm SD). There was no statistically significant difference between these two groups ($P = 0.96$).

Study Limitations. The study has two limitations: (a) the method used for the analyses of HPB-releasing adducts may not be sensitive enough for their quantification in pancreatic tissue although it has been successfully applied for DNA isolated from smokers' lung tissues (1) and (b) the number of pancreatic tissues used for the determination of 8-OHdG was relatively small; however, the selection included heavy smokers with urinary cotinine levels ranging from 1,411 to 3,646 ng/mL.

Discussion

Although we have previously detected NNK in smokers' pancreatic juice at levels of 90.3 ± 165 ng/mL juice (3) and documented that human pancreatic tissue metabolizes NNK by α -hydroxylation (4), this study failed to show the presence of HPB-releasing adducts in smokers' pancreatic DNA. Therefore, it seems that the extent of NNK-induced pyridyloxobutylation in the human pancreas is relatively low or that this adduct is relatively easily repaired. The major metabolite observed in our previous study was NNAL (4). Like NNK, NNAL is also known to induce pancreatic cancer in rodents (2) and can induce DNA adducts resulting from pyridylhydroxybutylation (5) and methylation pathways. Future studies will therefore need to examine the formation of these adducts induced by either NNK and/or NNAL.

The lack of a statistically significant difference between smokers and nonsmokers with regard to 8-OHdG levels is in agreement with a previously reported finding (8).

Despite the outcome of this study, the role of NNK in the induction of human pancreatic cancer cannot be totally disregarded. NNK and its metabolite NNAL are the only environmental carcinogens known to induce pancreatic tumors in laboratory animals and tobacco smoking is unquestionably related to the induction of this malignancy. Future studies need to be aimed at improving sensitivity and selectivity of existing analytic methods to better understand the role of tobacco carcinogens in the etiology of pancreatic cancer.

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