Aflatoxin, Tobacco, Ammonia and the p53 Tumor-Suppressor Gene: Cancer's Missing Link?

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Abstract
Aflatoxin, the fungal carcinogen first identified in 1960, is now recognized as the prototypical laboratory carcinogen. It causes mutations in the p53 tumor-suppressor gene as well as ras mutations, which are involved in the majority of human cancers. Aflatoxin has been shown to contaminate tobacco products. Tobacco-related cancers, including those associated with ETS, often show the same p53 mutations associated with aflatoxin exposure. The role of ammonia in neutralizing aflatoxin contamination is examined, as well as the potential role of the FDA in regulating aflatoxin contamination of tobacco products. [MedGenMed, August 30, 1999. © Medscape, Inc.]

Introduction
The mycotoxin aflatoxin B1 (AFB1), a known contaminant on flue-cured tobacco leaves and likely found in environmental tobacco smoke (ETS), is a profound carcinogen known to mutate the p53 tumor-suppressor gene and to cause ras mutations. Dietary exposure to AFB1 indicates it is a hepatotoxin and hepatocarcinogen, specifically causing p53 mutations at codon 249. Aflatoxin contamination of tobacco is not regulated by the FDA. Aflatoxin has the potential in primary and secondary smoke to be a potent carcinogen, mutating the p53 tumor-suppressor gene that is often associated with smoking- and chewing tobacco-related cancers.

Aflatoxins are toxins produced by fungi that invade agricultural commodities under warm and wet storage conditions after harvesting. Aflatoxin was first identified in 1960 as one of the most potent carcinogens known, and has been recognized as a teratogen, mutagen, carcinogen, immunosuppressant, and potent inhibitor of protein synthesis. The US Food and Drug Administration (FDA) began regulating aflatoxin on agricultural commodities, such as peanuts, corn, and grains, in 1966. Federal and state laws prohibit interstate shipment of contaminated aflatoxin commodities exceeding 20 parts per billion (ppb) (0.5 ppb for milk).

Ignorance with respect to the level of tobacco contamination by aflatoxin and lack of a clear FDA role has resulted in a public health catastrophe. The tobacco industry heavily imports cheaper tobacco from tropical countries such as Brazil and Zimbabwe, in which the level of aflatoxin contamination is also unknown. Presently, in North Carolina alone, the flue-cured tobacco stabilization board has 195 million pounds of tobacco stored for sale, where it may remain for years and become contaminated. Contamination of tobacco may occur during extended storage time as well as during the curing process, yet there is little agricultural literature on this subject. Welty and colleagues of the United States Department of Agriculture (USDA) examined "Fungi Isolated from Flue-cured Tobacco at Time of Sale and After Storage" in 1969, and subsequent research has indicated that many of these species regularly found on tobacco are capable of aflatoxin or other dangerous mycotoxin production. That same year, Harold Pattee of the USDA found, "Under favorable growth conditions, Aspergillus flavus can produce aflatoxins on flue-cured tobacco leaves."

R.J. Reynolds Tobacco Company (RJR) publicly acknowledged the extent of aflatoxin contamination in tobacco in 1997, when the company was granted a patent entitled "Method of Inhibiting Mycotoxin Production." This patent documents the heat stability and toxic effects of aflatoxin, and prescribes a method using a hexenal gas to prevent its formation on many agricultural commodities, including flue-cured tobacco. Since the mid-1950s, the tobacco companies have been aware that ammonium-based compounds neutralize benzpyrene compounds. Upon the discovery of aflatoxin in 1960, and
Chemosynthesis by Phillip Morris in 1966, the tobacco industry was likely aware that ammonia neutralized aflatoxin as well. Aflatoxin is 200 times more carcinogenic than benzo[a]pyrene and decomposes at 269°C, well above the combustion temperature of an idling cigarette.

RJR documents indicate that as early as 1968, collaborating researchers at the Wisconsin Alumni Research Foundation found a 100% carryover of aflatoxin from combusted tobacco. The heat stability of aflatoxin may explain studies that have shown polychlorinated dibenzofurans in ETS at levels two to ten times higher than those in mainstream smoke, which is combusted at higher temperatures. Aflatoxins are chemically classified as dibenzofurofurans, which are highly oxygenated heterocyclic compounds, and as such easily would be amenable to deactivation by a catalyst such as palladium. The Liggett Group developed a less hazardous cigarette in 1977 using a palladium catalyst that essentially reduced the biological activity of the smoke condensate to zero, but it was never marketed.

Aflatoxin is metabolized to the active carcinogen — the epoxide — by benzo[a]pyrene, a product of combustion. Use of smokeless tobacco products often leads to oral cancers in a few years, indicating that benzo[a]pyrene is not the responsible compound in these cases. Uncombusted aflatoxin may be a causal agent or promoter of the early onset of oral malignancies, as p53 mutations have been found in tumors in proximity to the oral cavity.

Aflatoxin has been shown to cause cancer in every animal model and cellular system studied, and to form adducts in the p53 tumor-suppressor gene that mutates in approximately half of all human cancers. Cherpillod and Amstad showed that AFB1 binds to the middle and third positions of p53 codon 248, inducing G-T transversions associated with lung cancer, and binds strongly to the third base pair of codon 249, generating a G-T transversion in a liver cancer mutational hotspot. Benzopyrene has been shown to bind to positions in codon 248, but has not been shown to target codon 249. These codons are adjacent, and carcinogenic targeting is presently not well-understood; it has been suggested that targeting is affected by the positions of different nucleotides in short sequence.

"G:C to T:A transversions are the most frequent substitutions observed in cancers of the lung, breast, esophagus and liver," states Dr. Curtis C. Harris of the National Cancer Institute (NCI). "G to T transversion is more common in lung cancers from smokers when compared to never smokers." Donnelly and coworkers state, "In addition to being a potent hepatocarcinogen, aflatoxin B1 (AFB1) is a pulmonary carcinogen in experimental animals, and epidemiological studies have shown an association between AFB1 exposure and lung cancer in humans. In their study, lung tumor samples collected from 76 mice treated with doses of AFB1 showed 100% K-ras mutations.

Lasky and Silbergeld suggest, through study of p53 mutations, that environmental carcinogens are a cause of breast, esophageal, lung, ovarian, pancreatic, prostate, and skin cancers. They state, "In lung cancer p53 mutations have been found in 56% of tissue samples, and in colorectal, esophageal, ovarian, pancreatic, and skin cancers, prevalences of 44-50% have been reported." They suggest that G-T transversions in breast and lung cancers are caused similarly by exogenous mutagenic chemicals. Aflatoxin from primary smoke and ETS is the likely carcinogen, as these cancers are more often associated with tobacco use.

Most recently, p53 mutations have been found in ETS lung cancer patients. A study done by Husgafvel-Pursiainen and associates investigated the presence of mutations in the p53 gene in samples of lung cancer patients who had never smoked, but who had reported a detailed history of exposure to ETS. "Although based on a relatively small number of mutated lung cancer cases among non-smokers, our findings are consistent with a carcinogenic effect of ETS on the human lung," the authors state.

As with all other susceptible agricultural commodities, levels of mycotoxin and aflatoxin contamination of tobacco should be regulated by the FDA. The technology to prevent, remediate, and terminally test for these toxins is currently available for a fraction of the cost of the morbidity and mortality it will prevent.

**Conclusion**

These studies strongly suggest that the genetic mutations known to be associated with aflatoxin are the same mutations often associated with the use of tobacco products. Aflatoxin is more than likely a causal agent or promoter of tobacco-associated cancers. The advancing technology of molecular epidemiology will presumably confirm this theory in the near future, with significant repercussions for public health and the tobacco industry.
References

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