

measures of liver function in patients with alcoholic hepatitis.

Does nutritional therapy improve survival? This is the most important question, and again the results are suggestive but not conclusive that nutritional therapy is beneficial. Cabre et al. (9) reported 12% 1-mo mortality in malnourished cirrhotic patients treated with enteral nutrition compared with a 47% mortality in patients offered an isocaloric hospital diet. Intravenous hyperalimentation with a "standard" solution improved 1-mo survival in patients with alcoholic hepatitis, whereas oxandrolone and oral hepatic aid treatment of patients with alcoholic hepatitis and moderate malnutrition improved 6-mo survival (1, 10). However, other studies, including the two noted here, have not reported improved survival with nutritional support (4, 7). The reason for the failure to find improved survival could be that nutritional therapy is not effective. However, other possibilities exist. These include suboptimal nutritional intake; insufficient duration of treatment or of follow-up; patients either too ill or not sufficiently malnourished to benefit from therapy; and, last but perhaps most likely, too small a study (i.e., small sample size with a large type 2 error and insufficient power to detect an existing effect).

In summary, malnutrition is present in all patients admitted with alcoholic hepatitis. Supplemental nutrition, either intravenously by nasogastric tube or orally, does not precipitate or exacerbate existing hepatic encephalopathy or fluid retention and may improve both of these. Nutritional therapy overcomes the catabolic state present during the first few weeks of hospitalization and leads to a more rapid increase in muscle and somatic body mass. Current studies suggest that this increase in caloric intake and nitrogen balance results in more rapid improvement in laboratory tests and clinical measures of liver function. This probably translates into prolonged survival, but further studies are needed to answer this question.

Where do we go from here? First, we need to monitor caloric intake during the first few days of hospitalization and be prepared to administer adequate calories (> 35 kcal/kg ideal body weight/day) and protein (> 1 gm/kg ideal body weight/day) to ensure patients are anabolic. Initial supplementation could be with an oral food supplementation followed, if unsuccessful, with tube feeding or peripheral hyperalimentation. In most instances, standard nutritional supplements appear to be as useful as special formulas (1, 2, 6, articles under review). Duration of treatment is unclear but should last at least several weeks or until the anorexia subsides. Second, we need studies to determine which patients will benefit from treatment, optimal duration of treatment and ideal route of administration. Although it is likely that nutritional therapy will improve survival in some patients with alcoholic hepatitis (or cirrhosis alone), in the end it may be just one of the several treatments that, combined together, will yield the optimal results.

The studies reviewed here provide important infor-

mation concerning the safety of nutritional therapy, its reversal of the catabolic state and improvement in laboratory and clinical status. They add additional information along the road to the final clinical question—does nutritional therapy prolong life.

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AFLATOXIN AND HEPATOCELLULAR CARCINOMA: A USEFUL PARADIGM FOR ENVIRONMENTALLY INDUCED CARCINOGENESIS

Lin L, Yang F, Ye Z, Xu E, Yang C, Zhang C, Wu D, et al. Case-control study of cigarette smoking and primary hepatoma in an aflatoxin-endemic region of China: a protective effect. *Pharmacogenetics* 1991;1:79-85.

ABSTRACT

Aflatoxin is believed to be a major causative agent in the high incidence of primary liver cancer seen in certain regions of the world. In Fujian Province, an aflatoxin-endemic region of China, we compared the cigarette smoking habits of 200 primary hepatoma patients with those of 200 matched nonhepatoma controls. We excluded from our study all individuals with evidence of hepatitis B virus serum antigen and/or alcoholic cirrhosis. Interestingly, two groups of hepatoma patients could be discerned. In patients more than 50 years of age, a significantly higher number of cases of primary hepatoma was found among non-smokers than smokers (odds ratio = 2.06; 95% confidence interval = 1.32-3.20). In patients less than 50 years of age, this difference was not seen. Previous studies in the rat, mouse and duck had suggested that agents present in cigarette smoke might induce a cytochrome P450-mediated detoxication pathway, leading to protection against aflatoxin-induced primary liver cancer. Our clinical data in the present study are therefore consistent with the previous laboratory animal experiments.

Ozturk M, et al. p53 mutation in hepatocellular carcinoma after aflatoxin exposure. *Lancet* 1991;338:1356-1359.

ABSTRACT

Mutations of the p53 gene are found in hepatocellular carcinoma (HCC), the most common form of primary liver cancer. Specific mutations might reflect exposure to specific carcinogens and we have screened HCC samples from patients in 14 different countries to determine the frequency of a hotspot mutation at codon 249 of the tumour suppressor p53 gene.

We detected mutations in 17% of tumours (12/72) from four countries in south Africa and the southeast coast of Asia. There was no codon 249 mutation in 95 specimens of HCC from other geographical locations including North America, Europe, Middle East, and Japan. Worldwide, the presence of the codon 249 mutation in HCCs correlated with high risk of exposure to aflatoxins and the hepatitis B virus (HBV). Further studies were completed in two groups of HBV-infected patients at different risks of exposure to aflatoxins. 53% of patients (8/15) from Mozambique at high risk of aflatoxin exposure had a tumour with a codon 249 mutation, in contrast with 8% of patients from Transkei (1/12) who were at low risk.

HCC is an endemic disease in Mozambique and accounts for up to two thirds of all tumours in men. A codon 249 mutation of the p53 gene identifies an endemic form of HCC strongly associated with dietary aflatoxin intake.

COMMENTS

A renewed interest in aflatoxin as a model procarcinogen can be explained by two recent lines of investigation. One involves the pathways responsible for aflatoxin metabolism, and the other involves the genotoxic mechanisms mediated by aflatoxin metabolites.

Aflatoxin first gained prominence in 1960 when contamination of peanut meal by a metabolite of *Aspergillus flavus* was associated with poultry deaths in southern England (1). This mycotoxin, later purified and designated aflatoxin B1 (AFB1), resulted in extensive hepatic necrosis when administered to animals in large doses or resulted in HCC when dosed chronically (2). Interest in the use of peanut meal by the World Health Organization as a nutritional supplement in developing countries prompted intensive research into AFB1. It was soon recognized that *Aspergillus flavus*, and AFB1, were frequent contaminants of grains and legumes, particularly in Asia and sub-Saharan Africa, where climate and food storage techniques allow for higher quantities of AFB1 in the food supply. The worldwide incidence of HCC was found to closely parallel the prevalence of AFB1-contaminated food products, supporting a strong epidemiological link between AFB1 ingestion and HCC. However, with the advent of viral serological markers in the 1970s, these same regions were also noted to have high rates of hepatitis B, and subsequent population studies clearly defined chronic infection with hepatitis B as a risk factor for subsequent HCC (2). Thus the ability of AFB1 to influence the development of HCC was seriously questioned.

Several lines of evidence support a relationship between AFB1 and HCC. First, rats treated with AFB1 have HCC develop in a dose-dependent fashion (2). Furthermore, AFB1 treatment of transgenic mice with integrated hepatitis B DNA greatly enhanced the development of HCC as compared with mice not treated with AFB1 (3). Second, epidemiological studies from Swaziland, a high endemic region for HCC, show regional differences in the incidence of HCC that correlate more closely with the dietary intake of AFB1 than with the incidence of hepatitis B infection (4). However, the best evidence relating AFB1 ingestion and HCC is provided by Ozturk and colleagues, who noted a specific mutation in the tumor-suppressor gene p53 in hepatoma tissue from patients at high risk for AFB1 ingestion.

Alterations in the p53 tumor-suppressor gene are thought to be the most common genetic alteration in tumors (5). Multiple codons along this gene are susceptible to mutations that result in abnormal cell proliferation, and certain codons are designated as "hot spots" because of their frequent mutation in multiple tumors. Two previous studies have documented a p53 mutation in DNA obtained from tumor tissue at codon 249 in almost 50% of patients studied with HCC (6, 7). This observation was of particular interest because mutations at this codon had not been previously recognized in other tumors. Because hepatitis B and AFB1 were risk factors for most of these patients with HCC, these agents were suspected as mediators of this mutation. AFB1 was a chief suspect because of its bioactivation *in vivo* by P450 enzymes to the highly reactive AFB1-2,3-oxide, which readily forms covalent bonds with guanine bases resulting in DNA adducts (1). Subsequent mitosis results in a G-to-T transversion, which is the observed

p53 mutation at the third base in codon 249 in the DNA prepared from hepatoma samples (8). The study reported by Ozturk and colleagues confirms this suspicion by noting an association between mutations at codon 249 in the p53 tumor-suppressor gene and AFB1 intake.

In four countries where the dietary intake of AFB1 was high, 22% of tumor samples from 49 patients with HCC had this characteristic mutation in codon 249 of p53, compared with only 1 of 114 samples from nine countries where the dietary intake of AFB1 was low. For the most part, however, those countries with the highest rates of AFB1 intake also had the highest frequency of infection with hepatitis B. To further delineate the contribution of these two risk factors, the authors compared patients with HCC from Mozambique with neighboring Transkei. Both countries have similar rates of HCC and infection with hepatitis B, but the estimated AFB1 intake in Mozambique is four-fold higher than in Transkei. Similarly, mutations in codon 249 of p53 were documented in 8 of 15 tumor samples in patients with HCC from Mozambique, compared with only 1 of 12 patients with HCC from neighboring Transkei. Thus it is likely that AFB1 metabolism *in vivo* results in a genotoxic lesion that is associated with HCC. However, it is not clear whether AFB1 exposure alone is sufficient to result in HCC. All of the patients with HCC and p53 mutations at codon 249 had also been infected with hepatitis B. Hepatitis B or other agents of chronic liver disease may well be necessary prerequisites for AFB1-mediated HCC, either through mechanisms of altered gene expression or simply by their ability to promote cell proliferation.

As with all procarcinogens, it is clear that the susceptibility to AFB1-mediated HCC differs among individuals. It is unlikely that differences in p53 expression between individuals account for differences in AFB1 toxicity. With the exception of rare familial syndromes (e.g., Li-Fraumeni syndrome), the p53 tumor-suppressor gene has not been noted to differ appreciably between individuals (5, 8). Interindividual differences in susceptibility to AFB1 will most likely be explained by interpatient differences in the ability to metabolize AFB1.

The metabolism of AFB1 is complex but is largely dependent on oxidative reactions by members of the P450 supergene family. Several different P450 isoenzymes can result in AFB1 metabolites with varying carcinogenic potential (1, 9). The ability of individual P450 isoenzymes to bioactivate AFB1 has been studied by expressing the complementary DNA for each of the human P450s in hepatoma cell lines by means of recombinant vaccinia viruses (9). The formation of DNA adducts [2,3-dihydro-2(N⁷-guanyl)-3hydroxy-AFB1] or mutagenesis (detected by the Ames assay) is a measure of the ability of an individual P450 to bioactivate AFB1. Members of the cytochrome P450III_A subfamily (i.e., P450III_A3 and P450III_A4) appear to be the major (but not only) human P450s that bioactivate AFB1 to a carcinogenic metabolite (9). P450III_A enzymes are found in highest concentration in the liver (10), which

may explain why this organ is selectively targeted in AFB1-associated carcinogenesis (p53, after all, is expressed in every cell). Similarly, other P450 isoenzymes are capable of metabolizing AFB1 to relatively inactive metabolites or to metabolites of limited carcinogenic potential. For example, enzymes belonging to the P450I_A subfamily metabolize AFB1 to aflatoxin M₁, a metabolite with considerably less carcinogenic potential than AFB1 (11).

Interpatient differences in the expression of both P450III_A and P450I_A vary by at least 10-fold, and expression of these enzymes can be altered by exogenous agents (10, 12). For example, P450III_A is inducible by several commonly used medications, including glucocorticoids, antiseizure drugs and rifampin (10). It is also likely that as-of-yet unidentified dietary xenobiotics influence the expression of P450III_A. Similarly, P450I_A enzymes are inducible by the polycyclic hydrocarbons found in cigarette smoke and by dietary "green plant" flavones (12). It is tempting to speculate that environmental agents influence one's susceptibility to AFB1-mediated hepatocarcinogenesis by altering the expression of individual P450 enzymes that bioactivate or detoxify AFB1. The observations by Lin and colleagues support this hypothesis.

In their study, the smoking habits of 200 male hepatoma patients in Fujian Province, a region of high AFB1 intake, were compared with 200 matched controls without hepatoma. Patients with evidence of prior hepatitis B infection or alcohol abuse were excluded. In patients more than 50 years of age, the risk of hepatoma was significantly greater in nonsmokers compared with smokers. The authors attribute this protective benefit to smoking that, by inducing P450I_A enzymes, has increased the metabolism of AFB1 to the less toxic metabolite, AFM₁. As discussed in the article, smoking has previously been shown to be protective against AFB1-induced HCC in several animal models. The authors also discuss several confounding variables that may alter their conclusions. If smoking indeed alters the risk of AFB1-mediated hepatocarcinogenesis, it may be by mechanisms other than the induction of P450I_A enzymes. P450I_A2, which is induced by smoking (12), has also been shown to bioactivate AFB1 to mutagenic metabolites (9). This pathway of bioactivation may be of even greater importance than that observed with P450III_A enzymes at the low concentrations of AFB1 encountered in the diet (13, 14). Altered phase II detoxification pathways or DNA repair mechanisms in individuals who smoke is also a consideration. Nonetheless, this provocative study is the first to suggest a protective effect of smoking by a mechanism that is thought to detoxify AFB1.

Collectively, these two articles have contributed the following to our understanding of AFB1-mediated hepatocarcinogenesis. First, AFB1 is likely to be a procarcinogen that alters cell proliferation by way of a unique mutation in the p53 tumor-suppressor gene. Second, the potential protective effect of smoking on the development of HCC supports the growing hypothesis that

the bioactivation or detoxification of AFB₁ *in vivo* is dependent on the balance of specific P450 isoenzymes that vary among individuals and can be altered by environmental factors such as smoking.

The relationship of aflatoxin to carcinogenesis will serve as a useful paradigm for future studies regarding diet and cancer. In addition to the dose of the suspected carcinogen, interpatient differences in P450 enzymes and the influence of the environment on these enzymes will need to be taken into consideration.

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