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MECHANISM OF CHEMOPREVENTION OF CARCINOGENIC HETEROCYCLIC AMINES BY TEA POLYPHENOLS AND COFFEE LIPIDS

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The chemopreventive effect of tea against 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-DNA adduct formation and its mechanism were studied. Rats were exposed to freshly prepared aqueous extracts of green tea (3% w/v) as the sole source of drinking water for 10 days prior to administration with a single dose of PhIP (10 mg/kg body wt) by oral gavage. PhIP-DNA adducts in the liver, colon, heart, and lung were measured using the ^{32}P-postlabelling technique. Rats pre-treated with tea and given PhIP 20 hr before sacrifice had significantly reduced levels of PhIP-DNA adducts as compared with controls given PhIP alone. The possible mechanism of protective effect of tea on PhIP-DNA adduct formation was then examined *in vitro*. It was found that an aqueous extract of green and black tea, mixtures of green and black tea polyphenols, as well as purified polyphenols could strongly inhibit the DNA binding of *N*-acetoxy-PhIP, a putative ultimate carcinogen of PhIP formed *in vivo* via metabolic activation. Among these, epigallocatechin gallate was exceptionally potent. HPLC analyses of these incubation mixtures containing *N*-acetoxy-PhIP and the tea polyphenols each revealed the production of the parent amine, PhIP, indicating the involvement of a redox mechanism. In view of the presence of relatively high levels of tea polyphenols in rat and human plasma after ingestion of tea, this study suggests that direct reduction of ultimate carcinogen, *N*-acetoxy-PhIP, by tea polyphenols is likely to be involved in the mechanism of chemoprotection of tea against this carcinogen.

In a second study in rats, we had observed that a dietary mixture (1:1) of the coffee lipids, kahweol

and cafestol palmitates, exerted a strong inhibitory effect on the formation of colon DNA adducts derived from dosing with PhIP. Since the activation of PhIP to form colon DNA adducts is thought to involve the *O*-acetylation of the putative proximate carcinogenic metabolite, *N*-OH-PhIP, we examined the effect of this treatment on NAT activity as well as on other relevant enzymes. The results showed a dose- and time-dependent decrease (60-70%) in hepatic but not colon NAT activity, as measured by the acetyl CoA-dependent binding of N-OH-PhIP to DNA. The effect was maximal after 5 days of the 10-day treatment period and was found to return to normal activity levels over 10 days when the animals were then placed on a control diet. This effect was closely paralleled by a comparable decrease in NAT mRNA levels as well as a return to control values after treatment cessation. Thus, these data indicated that NATmay be down-regulated in the rat, effectively converting them from a rapid to a slow acetylator phenotype. To test this hypothesis, we subsequently examined the effect of the purified unesterified derivatives, kahweol and cafestol, on NAT activity in cultured primary rat hepatocytes. These experiments showed that NAT activities were indeed strongly decreased (60-90%) by incubation (for 22 hr) with either kahweol or cafestol (at 100 μM). This is the first demonstration of modulation of NAT activity by exogenous agents.