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# STIMULATION OF DRUG AND CARCINOGEN METABOLISM BY PROLONGED ORAL TOBACCO CONSUMPTION

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**Abstract**—Oral administration of tobacco to rats for 21 days caused remarkable stimulation of the metabolism of phenacetin, aniline and benzo[*a*]pyrene, a carcinogen, by hepatic microsomal mixed function oxidases (MFO). Such treatment for 6 days resulted in a small increase in the activities of phenacetin *O*-dealkylase and aromatic hydrocarbon hydroxylase (AHH) without affecting aniline hydroxylase activity. Nicotine given orally was found to be a relatively weak inducer of phenacetin *O*-dealkylase and aniline hydroxylase, and elicited a maximum increase in their activities within 6 days which remained unchanged even after 21 days of continuous administration. However, these two enzyme systems were not affected following only one or two doses of tobacco and nicotine. Both tobacco and nicotine inhibited these biotransformations *in vitro*.

In many parts of the world including the Indian sub-continent, South Africa and a number of East-Asian countries, tobacco is consumed orally as dried leaves either alone or along with betel nut, betel leaf or slaked lime [1, 2]. Tobacco chewing has been shown to be associated with cancer and other serious ailments [2–4]. We have previously reported differential induction of mixed function oxidases (MFO) responsible for *N*-demethylation of drugs by oral tobacco consumption in rat [5]. The present study evaluates the influence of oral tobacco and nicotine consumption for varying periods on the activities of hepatic microsomal MFO catalysing *O*-dealkylation of phenacetin, aromatic hydroxylation of aniline, a substrate widely used for assessing drug hydroxylases, and hydroxylation of benzo[*a*]pyrene, a carcinogen, in rat. The studies were also extended to characterize the *in vitro* response of the enzyme systems to tobacco and nicotine.

## MATERIALS AND METHODS

Glucose-6-phosphate, nicotinamide adenine dinucleotide phosphate (NADP) and 3,4-benzo[*a*]pyrene were obtained from Sigma Chemical Co. (St. Louis, MO). Triton X–100 and ethylene diamine tetracetic acid (EDTA) were supplied by Rohm and Hass (Philadelphia, PA), and Glaxo Laboratories (BDH), India, respectively. All other chemicals used were of analytical grade and obtained from commercial sources.

Female Albino rats (150–200 g) were administered with a homogenate (in water) of commercially available sun-cured tobacco leaves (Trade name: Ratna Chhap Zafrani Patti No. 64) with the help of a feeding cannula for 1, 6 and 21 days, twice daily (11 a.m. and 4 p.m.) at a dose of 250 mg/kg. Under similar experimental conditions, another set of animals received nicotine base at a dose of 10 mg/kg which was quantitatively equivalent to that present in 250 mg/kg of tobacco [5]. The rats, unless otherwise stated, were killed 18–20 hr after the last dose of tobacco or nicotine to assess the activities of phenacetin *O*-dealkylase, aniline hydroxylase and benzo[*a*]pyrene hydroxylase in the 9000 *g* 15 min supernatant fraction of liver, prepared according to the method described previously [5]. The reaction mixture for phenacetin *O*-dealkylase and aniline hydroxylase was as described for *N*-demethylases [5].

The rate of biotransformation of phenacetin to paracetamol was followed by estimating the product according to the spectrophotometric method of Gwilt *et al.* [6] except that paracetamol was extracted from the reaction mixture with ethylacetate, evaporated to dryness and then extracted with alkali. The activity of phenacetin *O*-dealkylase was 2.50 ± 0.21 nmole of paracetamol formed/mg protein in 20 min. The amount of *p*-aminophenol formed during aniline hydroxylation was estimated spectrophotometrically [7]. The activity of aniline hydroxylase was 3.16 ± 0.40 nmole of *p*-aminophenol formed/mg protein in 20 min.

The hydroxylation of benzo[*a*]pyrene by microsomal aromatic hydrocarbon hydroxylase (AHH) was assessed spectrophotofluorometrically by measuring the fluorescence of hydroxylated metabolites of benzo[*a*]pyrene using 3-hydroxybenzo[*a*]pyrene as the reference standard [8]. The reaction mixture (2 ml) for benzo[*a*]pyrene hydroxylase assay contained phosphate buffer (0.1 M, pH 7.4), 10.6 μmole of glucose-6-phosphate, 0.94 μmole of NADP, 24.6 μmole of $MgCl_2$, 57.3 μmole of nicotinamide, 40 μg of benzo[*a*]pyrene (in 0.04 ml of DMSO) and 9000 *g* supernatant equivalent to 4.4 mg protein. The reaction was run for 10 min and ter-

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Table 1. Stimulation of hepatic microsomal metabolism of phenacetin, aniline and benzo[*a*]pyrene by chronic oral tobacco and nicotine consumption in rat

| Mixed function oxidase | Per cent stimulation | | | |
|---|---|---|---|---|
| | Tobacco | | Nicotine | |
| | 6 days | 21 days | 6 days | 21 days |
| Phenacetin *O*-dealkylase | 57.2 ± 6.7* | 185.5 ± 15.0* | 65.4 ± 4.2* | 57.2 ± 7.5* |
| Aniline hydroxylase | 0 | 160.0 ± 8.6* | 84.6 ± 10.3* | 86.0 ± 6.8* |
| Benzo[*a*]pyrene hydroxylase | 40.7 ± 5.2† | 100.0 ± 15.3* | ND | ND |

The rats were administered tobacco (250 mg/kg) or nicotine (10 mg/kg) twice daily (11 a.m. and 4 p.m.) orally and killed after 18–20 hr from the last dose. Values represent means ± S.E. obtained from 6–8 animals.
ND, not done.
* $P < 0.01$, † $P < 0.05$.

minated by adding 1 ml of Triton X–100 reagent (10% Triton X–100 (v/v), 1% EDTA (w/v) in 1 N NaOH). The fluorescence was read in an Aminco Bowman spectrophotofluorometer using excitation and emission wavelengths as 465 and 520 nm, respectively. The activity of AHH was 0.0294 ± 0.0045 nmole of hydroxy metabolites of benzo[*a*]pyrene formed/mg protein in 10 min.

The reactions were run in an atmosphere of oxygen. Protein was estimated by the method of Lowry *et al.* [9]. The rate of all the reactions was linear with respect to protein and time.

## RESULTS AND DISCUSSION

Our findings revealed that chronic oral administration of tobacco to rats markedly enhanced the rate of phenacetin *O*-dealkylation, aniline hydroxylation and benzo[*a*]pyrene hydroxylation by hepatic microsomal MFO (Table 1). The increase in the rate of metabolism of phenacetin, aniline and benzo[*a*]pyrene was 185.5, 160.0 and 100.0%, respectively. The kinetics of MFO stimulation with respect to time was also evaluated by investigating their activities after 1 and 6 days of tobacco treatment. The animals treated for 1 day exhibited 60.0% reduction in the biotransformation of benzo[*a*]pyrene by AHH to its hydroxylated metabolites. A comparable decrease of 64.0% in the hepatic AHH activity was observed in animals given a single oral dose of tobacco (500 mg/kg) and killed after 2 hr. The inhibition of AHH was selective since none of the other MFO including *N*-demethylases [5], *O*-dealkylase and aromatic hydroxylase was affected under similar experimental conditions. This finding is contrary to that reported for AHH response to cigarette smoke in rat where a single exposure to smoke was adequate for several fold induction of the enzyme system [10], possibly due to the presence of high concentrations of polycyclic aromatic hydrocarbons [11], the potent inducers of MFO [12]. Tobacco treatment for 6 days was able to evoke moderate augmentation of phenacetin *O*-dealkylase and AHH activities. Under these conditions the activity of aniline hydroxylase like *N*-demethylases [5], was not affected. It may be interpreted from the results of the current study that the process of enzyme stimulation is slow and, therefore, repeated tobacco dosage for longer duration is required to produce a 2–3 fold increase in the hepatic biotransformation of phenacetin, aniline, and benzo[*a*]pyrene presumably as a result of delayed *de novo* synthesis of enzyme protein. Earlier studies carried out in our laboratory have shown approximately 2–3 fold induction of hepatic microsomal *N*-demethylases for amidopyrine, morphine and pethidine in rat following oral tobacco administration (2 doses of 250 mg/kg/day) for 28 days while short-term treatment for 2 and 7 days was devoid of any effect [5].

In the current study the induction kinetics of MFO catalysing phenacetin and aniline biotransformations in nicotine treated rats was different from that observed during tobacco treatment in that oral treatment of animals with 10 mg/kg nicotine base, a dose equivalent to 250 mg/kg tobacco, for 6 days resulted in 65.4 and 84.6% increase in the metabolism of phenacetin and aniline respectively, which was not significantly changed until 21 days of continuous administration (Table 1). In this respect, these enzyme systems differ from *N*-demethylases which were not affected until 7 days under similar experimental conditions [5]. The two enzyme systems, in agreement with earlier observations [5, 13, 14] were not influenced by either one or two doses of nicotine. The increase in drug biotransformations due to nicotine is attributed to the *de novo* synthesis of the microsomal enzyme protein [14].

Addition of tobacco into the reaction mixture at a final concentration of 4.2 mg/ml reduced microsomal phenacetin *O*-dealkylation, aniline hydroxylation and benzo[*a*]pyrene hydroxylation 25.3, 15.6 and 100%, respectively. Under these *in vitro* conditions, nicotine at a final concentration of 1 mM, which was equivalent to 0.16 mg nicotine or 4.2 mg tobacco with respect to nicotine content per ml of the incubation mixture, caused 30.0 and 22.8% inhibition of phenacetin and aniline metabolism, respectively. Nicotine has been shown previously to inhibit microsomal oxidases *in vitro* [13, 15]. Moreover, nicotine (8.3 mg/kg) was also found to inhibit AHH activity 24 hr after a single intraperitoneal dose in saline [15]. Kinetic analysis of rat liver microsomal amidopyrine *N*-demethylase inhibition by tobacco and nicotine revealed the non-competitive and competitive nature of enzyme inhibition, respectively [5].

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