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o-Aminoazotoluene does induce the enzymes of its own mutagenic activation in mouse liver

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Abstract

The objective of this study was to investigate the CYP1A1 and CYP1A2 mRNAs and enzyme activities in mouse liver during induction with *o*-aminoazotoluene (OAT) as well as the capability of the hepatic S9-fraction from OAT-treated mice to induce its own activation to mutagens in the Ames test using *S. typhymurium* strain TA98. The data obtained indicate that when used at appropriate doses, OAT is a PAH-type inducer of mouse hepatic microsomal monooxygenases, which activity is not less than that of the known inducer 3,4-benzo[α]pyrene. In the absence of S9-fraction enzymes no OAT-mediated mutagenicity was observed in the Ames test. In the presence of the S9-fraction from OAT-pretreated mice, OAT induced as high revertant numbers, as it did in the presence of the S9 fraction from the liver of Aroclor 1254-treated mice. Thus, OAT does induce the enzymes of its own mutagenic activation in mouse liver.

Keywords: *o*-Aminoazotoluene; Cytochrome P450 1A1 and 1A2; CYP1A inducers; Ames test; Mouse liver

1. Introduction

It has been previously shown that the hepatic S9-fraction from rats treated with *o*-aminoazotoluene (OAT) exhibits elevated Cytochrome P4501A1 and 1A2 enzyme activities but does not activate OAT mutagenically in the Ames test (Cheung et al., 1994). However, it was not investigated whether OAT induces the enzymes of its own mutagenic activation in mouse liver, the target organ for OAT-induced carcinogenesis.

Recently, the revival of interest to *o*-aminoazotoluene (OAT), a well known murine hepatocarcinogen which was intensively studied a few decades ago, has been unexpectedly observed: two papers

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devoted to studies of its genotoxicity were published in "Mutation Research" last year (Ohsava et al., 2000; Kohara et al., 2001). OAT has been evaluated by the International Agency for Research on Cancer (IARC) as a possible (Class 2B) human carcinogen (IARC, 1975). It was found to be mutagenic in the Ames test in the presence of a metabolic activation system containing hepatic S9-fractions of phenobarbital-, 3-methylcholanthrene- or polychlorinated biphenyls—treated rats (Ames et al., 1973; Degawa et al., 1982; Joachim et al., 1985; Cheung et al., 1994;). S9-fractions obtained from the liver of hamsters and mice treated with the same inducers were shown to be capable of OAT activation as well (Victorin et al., 1987). Cheung et al. (1994) have shown that OAT is a potent inducer of drug-metabolizing enzymes in rat liver, particularly CYP1A1 and to smaller extent CYP1A2. Among the numerous cytochrome P450s, the CYP1A subfamily is of major interest due to the ability to activate many procarcinogens, including polycyclic aromatic hydrocarbons (PAH) and aryl amines (Pelkonen and Nebert, 1982). However, in the work of Cheung et al. (1994), the S9 fraction from the liver of rats induced with OAT significantly increased the bioactivation of Glu-P-1 (2-amino-6-methyldipyridol[1,2,α:3′2′]dimidazole) to mutagens in the Ames test, but did not stimulate the mutagenic activation of OAT. The authors have drawn a conclusion that OAT being an effective inducer of drug-metabolizing enzymes did not induce the enzymes of its own mutagenic activation. If true, this conclusion, however, is valid for rats only and can hardly be applied to other animal species including mice. In our work we have found that when applied at doses used in carcinogenesis experiments for liver tumor induction, OAT is a potent inducer of drug metabolizing enzymes in the mouse liver, in which it significantly stimulates the activity of enzymes of its own mutagenic activation.

2. Materials and methods

2.1. Experimental animals

Male C57BL/6 mice (aged 12–14 weeks) were supplied by the Institute of Cytology and Genetics (Novosibirsk, Russia). Mice were housed under standard vivarium conditions in a pathogen free environment and had free access to food and water. Induction of microsomal monooxygenases in the mouse liver was performed by i.p. injection of 10 ml/kg body weight of corn oil containing dissolved inducers: Aroclor (50 mg/ml), BP (10 mg/ml) or OAT (5.6–22.5 mg/ml) (Sigma, St. Louis, MO). Control mice received corn oil (25 ml/kg). The animals were decapitated 24–120 h after the injection. The animal protocol was approved by the local animal care committee of Institute of Cytology and Genetics and animal handling followed international guidelines.

2.2. Enzyme assay

After decapitation livers were removed and homogenized in three volumes of the 1.15% KCl solution, and 1 ml portions of the S9 fraction prepared by centrifugation were frozen in liquid nitrogen. The microsomal liver fraction was isolated from freshly excised livers by standard differential centrifugation (Burke et al., 1985). The protein concentrations in microsomes were measured according to Lowry et al. (1951). The cytochrome P450 content in the liver was determined as described by Omura and Sato (1964). The rates of *O*-dealkylation of 7-ethoxy- and 7-methoxyresorufins were assessed according to Burke and Mayer (1974).

2.3. RNA isolation and cDNA synthesis

Twenty-four hours after induction, total RNA was extracted from liver tissue by the SDS/phenol method (Chattopadhyay et al., 1993). Purity of the RNA was assessed by the ratio of the optical densities at 260 and 280 nm, and the integrity examined by electrophoresis on a 1% agarose gel containing of ethidium bromide (0.5 μg/ml). A 1 μg of total RNA was used for synthesis of single-stranded cDNA. Briefly, RNA samples were reverse transcribed at 42 °C for 1 h by incubation with 20 μl of a reverse transcription mixture containing the following constituents: 5 μM of oligo(dT)$_{17}$ primer; 1 × reverse transcriptase buffer; 10 mM 1,4-dithiothreitol; 5 mM $MgCl_2$; 20 units of RNasin; 0.5 mM deoxynucleotide triphosphates; 200 units of Moloney Murine Leukemia Virus reverse transcriptase (Promega, USA). The resultant single-stranded cDNA was used for the PCR procedure.