# Activity of Cytochrome P450 1A in the Liver of CBA and CC57BR Mice after Prolonged Repeated Induction with *o*-Aminoazotoluene

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Cytochrome P450 1A activity in the liver of CBA and CC57BR mice sensitive and resistant to hepatocarcinogenic effects of *o*-aminoazotoluene, respectively, was measured after prolonged (for several months) repeated administration of this agent. Repeated and single injections of this carcinogen produced similar effects on microsomal monooxygenases in CC57BR mice, but caused different changes in cytochrome P450 1A activity in the liver of CBA mice. Hence, enhanced inducibility of cytochrome P450 1A in CBA mice after prolonged treatment with *o*-aminoazotoluene probably contributes to their sensitivity to hepatocarcinogenic effects of this agent.

**Key Words:** *CBA and CC57BR mice; sensitivity to hepatocarcinoma; repeated induction with o-aminoazotoluene; cytochrome P450 1A*

Hepatocarcinogen *o*-aminoazotoluene (OAT) induces tumors in experimental animals only after prolonged treatment (for several months). Death of differentiated hepatocytes, intense oval-cell proliferation, and activation of extramedullary hemopoiesis in the liver of OAT-treated animals are typical of many mouse strains, including A/He, DD, and SWR mice [2]. These processes result in replacement of hepatocyte population by cells with other metabolic and proliferative characteristics. However, in other mouse strains (BALB/c, CC57BR, and AKR) hepatocyte death and their replacement by other cells were not observed. Hence, in OAT-sensitive mice the metabolic load on survived hepatocytes increases, while in resistant animals it remains unchanged. This probably contributes to the development of hepatocarcinomas in OAT-sensitive, but not in OAT-resistant animals. The only exception is CBA mice resistant to hepatotoxic effects of OAT, but sensitive to its hepatocarcinogenic action. High sensitivity of CBA mice is probably related to peculiarities of OAT metabolism, which involves cytochrome P450 1A (cyt P450 1A) [4]. Here we measured activity of cyt P450 1A in the liver of CBA mice after single or repeated administration of OAT and compared it with respective parameter in CC57BR mice resistant to hepatotoxic and hepatocarcinogenic effects of OAT.

## MATERIALS AND METHODS

Experiments were performed on male CC57BR and CBA mice (Institute of Cytology and Genetics).

Microsomal monooxygenases were induced by single intraperitoneal injection of OAT in a concentration of 200 mg/kg body weight. The animals were killed on day 4 postinjection. Other mice received monthly intraperitoneal injections of 200 mg/kg OAT for 3 months. One month later, these mice were injected with OAT and killed after 4 days. The animals were kept under standard conditions and *ad libitum*

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**TABLE 1.** Content and Activity of Cyt P450 1A in the Liver of CBA and CC57BR Mice after Single and Repeated Induction with OAT ($M \pm m$, $n$=5)

| Series | | | Content of cyt P450 1A, nmol/mg protein | O-dealkylase activity, pmol resorufin/min/mg protein | |
|---|---|---|---|---|---|
| | | | | EROD | MROD |
| **CBA** | single injection | control | 0.60±0.12 | 183±40 | 406±145 |
| | | OAT | 1.11±0.08 | 677±136 | 720±65 |
| | repeated injections | control | 0.56±0.06 | 122±10 | 298±32 |
| | | OAT | 1.30±0.08 | 2725±295 | 2466±412 |
| **CC57BR** | single injection | control | 0.50±0.02 | 222±41 | 448±32 |
| | | OAT | 1.16±0.15 | 2177±153 | 2325±110 |
| | repeated injections | control | 0.45±0.13 | 252±55 | 434±49 |
| | | OAT | 1.18±0.05 | 2019±197 | 2426±453 |

food and water supply and were deprived of food 24 h before killing. Liver microsomal fraction was isolated by differential centrifugation. Protein concentration was measured by the method of Lowry [6]. The total content of cyt P450 1A was determined by the method described elsewhere [7]. The rate of O-dealkylation of 7-ethoxy- and 7-methoxyresorufins were estimated as described [3].

The results were analyzed using Statgraphics software.

## RESULTS

CC57BR and CBA mice have the $Ah^bAh^b$ genotype of sensitivity to polycyclic aromatic carbohydrate induction [8], but differ in their sensitivity to hepatocarcinogenic effects of OAT [1]. Carcinogens binding to Ah receptors and inducing cytochrome P450 1A markedly elevate activities of 7-ethoxyresorufin O-diethylase (EROD) and 7-methoxyresorufin O-demethylase (MROD) of cyt P450 1A. Single injection of OAT increased the total content of cyt P450 1A and specific activities of EROD and MROD in the liver of both mouse strains (Table 1). Test mice had practically similar basal activities of cyt P450 1A. EROD and MROD activities of cyt P450 1A in CC57BR mice increased by 9.8 and 5.2 times after single injection of OAT and by 8 and 5.6 times after repeated injection of OAT, respectively. Single injection of OAT to CBA mice slightly increased the total content of cyt P450 1A (estimated spectrally) in the liver and specific EROD and MROD activities (by 3.7 and 1.8 times, respectively). Prolonged repeated administration of OAT increased specific activities of EROD and MROD in CBA mice by 22 and 8 times, respectively. Thus, OAT displaying no hepatotoxic effects and enhancing (after repeated administration) the inducibility of cyt P450 1A in CBA mice can accelerate OAT N-hydroxylation and induce accumulation of N-hydroxy-OAT in the liver. This reactive metabolite binds to DNA, proteins, and other cell macromolecules [5] and induces the development of hepatocarcinoma. It should be emphasized that many factors, including activity of phase II xenobiotic metabolism enzymes neutralizing reactive metabolites and DNA-repair enzyme systems, affect cell transformation. Our findings demonstrate that the sensitivity to OAT-induced carcinogenesis is determined at the level cyt P450 1A inducibility activating this carcinogen. It can not be excluded that some other factors contribute to animal resistance to OAT-induced carcinogenesis. However, cyt P450 1A inducibility is probably the major determinants of the resistance of CC57BR and CBA mice to OAT.

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