Short Communication

Time Course of Expression of 7,12-Dimethylbenz[a]anthracene-induced CYP1A1 and CYP1B1 mRNA and Protein in Rat Liver

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Abstract: 7,12-Dimethylbenz[a]anthracene (DMBA) is an indirect carcinogen that enlists the host metabolism to produce its ultimate carcinogenic form, and it is known that this metabolism is conducted by cytochrome P450 1A1 and/or 1B1(CYP1). However, the time course of expression of rat liver CYP1 following DMBA administration has been unclear. After DMBA administration (100 mg/kg b.w.) to SD rats, expression of liver CYP1mRNA was observed at 6 hr and gradually increased with time to peak on days 1–2, but then disappeared on day 5. Expression of CYP1A1 protein was first observed at 12 hr, peaked on day 2 and decreased on day 5, while expression of CYP1B1 protein was first observed on day 2 and decreased on day 5. Expression levels of CYP1A1mRNA and protein were higher than those of CYP1B1. The present study suggests that high CYP1 enzyme production and DMBA metabolism might occur in rat liver substantially on day 2 following DMBA administration. (J Toxicol Pathol 2003; 16: 287–290)

Key words: 7,12-dimethylbenz[a]anthracene, rat, liver, CYP1A1mRNA, CYP1A1, CYP1B1mRNA, CYP1B1

The liver has a primary role in the metabolism of 7,12-dimethylbenz[a]anthracene (DMBA), a member of the polycyclic aromatic hydrocarbons (PAHs), although both its proximate and ultimate metabolites are transported to other organs. The mutagenic and carcinogenic activities of DMBA are dependent upon its metabolism by the phase I enzymes, especially the cytochrome P450 1A1- and/or 1B1(CYP1)-dependent mono-oxygenase system, to its ultimate reactive metabolite, i.e., 3,4-dihydrodiol-1,2-epoxide. Moreover, it has been shown that the administration of PAHs including DMBA induces CYP1, which metabolized the PAHs.

Previously we observed the temporal depletion of rat hepatocyte glycogen granules with high proliferation of the smooth endoplasmic reticulum (sER) on day 2 following DMBA (100 mg/kg b.w.) administration, and suggested that the highest metabolism of DMBA might occur on day 2, while the induction of CYP1mRNA was observed within 6 hr of DMBA administration. However, no investigation concerning the time course of expression of liver CYP1 mRNA and proteins following DMBA administration has been reported. Thus, to clarify the initial liver changes due to DMBA administration, we investigated the serial time course of expression of CYP1mRNA and proteins following DMBA administration.

Sixty 42-day-old female Sprague-Dawley slc (SD) rats (Japan SLC) were housed, three per plastic cage, on hardwood-chip bedding in an environment-controlled room on a 12 hr light/12 hr dark cycle at 22 ± 2°C and 55% ± 5% relative humidity, with a conventional diet (MF, Oriental Yeast Co.). All experimental procedures were conducted with the approval of the Animal Care and Use Committee of the Kyorin University School of Medicine. Guidelines set by the National Institute of Heath and Public Health Service Policy on the Humane Use and Care of Laboratory Animals were followed at all times. After one week of acclimatization, thirty 50-day-old female rats received 100 mg/kg body weight DMBA (Tokyo Chemical Industry Co., Ltd.) in corn oil (i.g.), and 30 received an equivalent volume of corn oil (~0.5 ml/animal, i.g.). In this study, the dose of DMBA was settled following the previous study of Huggins et al. (1961).
anesthesia. Liver samples were obtained from the rats, and representative blocks of each liver were fixed in 10% phosphate-buffered formalin and routinely processed for hematoxylin-eosin staining.

Immunohistochemical expression of CYP1A1 was analyzed by the avidin-biotin complex (ABC) method. After deparaffinization, 4-µm thick sections were treated sequentially with 0.3% H₂O₂ for 10 min, then blocked with 10% goat serum or horse serum in PBS for 20 min. Sections were rinsed in PBS and treated with primary antibodies of rabbit anti-rat-CYP1A1 (Affiniti Res. Inc., diluted 1:1000). Bound IgG was detected with biotinylated goat anti-rabbit IgG (Vector Lab., diluted 1:100) followed by avidin-biotin complex (ABC)-peroxidase (Vector Lab.) and diamino-benzidine (Sigma). Sections were then counterstained with hematoxylin. As the negative control, non-immunized rabbit serum was substituted for the primary antibody.

Measurements of RNA levels were performed using RT-PCR. RNAs were prepared for the semi-quantitation of CYP1A1, CYP1B1 and β-actin. PCR primer pairs used to amplify these specific RNAs are identified elsewhere¹¹,¹². Total RNA was isolated from frozen liver samples using an acid-guanidinium-phenol-chloroform method¹¹. Total liver RNA (1 µg) was reverse-transcribed using Superscript (Life Technologies, Inc.) in a total volume of 20 µl following the manufacturer’s protocol. PCR reactions were performed by first-strand cDNA synthesis (2 µl) of each sample with 10X PCR buffer minus Mg, 50 mM MgCl₂, 10 mM dNTP mix and Platinum Taq DNA Polymerase 5 units/µl (Life Technologies, Inc.) including CYP1A1, CYP1B1, and β-actin with 10 µM each sense and antisense primers. Amplifications were 94°C – 30 sec, 57°C – 30 sec and 72°C – 30 sec for 30 cycles using a Gene Amp PCR system 9700 (PE Biosystems). PCR products were separated by 2% agarose gel electrophoresis in 1 X TBE buffer, stained with 1 µg/ml ethidium bromide and exposed to film.

For western blot analysis, rat livers were homogenized in 50 mM Tris-HCl, 150 mM KCl (pH 7.4), 1% Triton X-100, and 0.25 mM phenylmethylsulfonyl fluoride (PMSF) and centrifuged at 9000 g for 30 min at 4°C. The supernatant obtained was centrifuged at 105,000 g for 90 min at 4°C. The pellet was suspended in 50 mM Tris-HCl (pH 7.4), 1% Triton X-100, and 1 mM PMSF, and the protein concentrations were determined with a bicinchoninic acid protein assay reagent kit (Pierce) with bovine serum albumin (BSA) as a standard. Microsomal samples were subjected to electrophoresis on a 10% SDS polyacrylamide gel using 1 µg/ml ethidium bromide and exposed to film. The proteins were transferred to a nitrocellulose membrane and blocked in 1 µg/ml polyvinyl alcohol, 25 mM Tris (pH 7.4), and 150 mM NaCl for 1 min. Western blot analysis was performed using anti-rat-CYP1A1 (Affiniti Res. Inc.) or anti-rat-CYP1B1 (Gentest) antibodies. CYP1A1 and CYP1B1 antibodies were diluted 1:10000 and 1:5000, respectively, in phosphate-buffered saline with Tween-20 and incubated overnight at 4°C, followed by incubation with peroxidase-labeled anti-chicken IgG and anti-rabbit IgG, respectively, for 1 hr. The immunoreactive proteins were detected using an ECL detection kit (Amer sham Life Sciences).

Several previous studies have described the expression of rat liver CYP1 mRNA and/or protein within one day of intragastric DMBA administration¹¹,¹³–¹⁷, but their expression after that was unclear. In the present study, following DMBA administration, the expression of liver CYP1A1mRNA was observed at 6 hr, and it gradually increased with time to peak on days 1–2 but then disappeared on day 5 (Fig. 1, Table 1). By Western immunoblot and immunohistochemical analysis, the expression of CYP1A1 protein, however, was first observed at 12 hr, was revealed to peak on day 2 and decreased on day 5 (Figs. 2, 3). On the other hand, the expression of CYP1B1mRNA was also observed at 6 hr following DMBA administration, and showed a similar time course to CYP1A1mRNA (Fig. 1, Table 1), while the expression of CYP1B1 protein was first observed by Western immunoblot analysis on day 2 and decreased on day 5 (Fig. 2).
Although the expression levels of CYP1A1 mRNA and protein were higher than that of CYP1B1, the expression of CYP1B1 protein was clearly revealed on day 2 after DMBA administration (Figs. 1, 2). This is particularly important in light of recent evidence concerning the activity of CYP1B1 in several biochemical processes important to carcinogenesis. Indeed, it has been reported that CYP1B1 possesses a greater capacity to bioactivate a number of PAH procarcinogens including DMBA than CYP1A1.

In the present study, it should be noted that the expression of CYP1 mRNA did not correlate with observed protein levels for the same CYP1 protein. Induction of CYP1 mRNA usually occurs rapidly and in the absence of similar rates of protein synthesis. This differential response between CYP1 enzymes and the corresponding mRNA transcripts was also apparent in the liver of rats in response to DMBA administration. Although the delay of translation from mRNA to proteins is frequently observed in several cells, the mechanisms underlying the discrepancies between constitutive mRNA and protein levels are unclear. The present study revealed the rapid transcription of CYP1 mRNA following DMBA administration, but translation proceeded at 12 hr for CYP1A1 and on day 2 for CYP1B1 (Figs. 2, 3). The rapid transcription of CYP1 mRNA is thought to be conducted by the aryl hydrocarbon receptor (AhR) and AhR-nuclear translocator in hepatocytes, but factors conducting their translation are unclear. Further analysis of the time course induction of AhR and ARNT following DMBA administration is required.

Meanwhile, the results of the our previous study indicated that exposure of rats to DMBA induced temporary glycogen storage in the hepatocytes on day 1 with decreased levels of glycogen phosphorylase $a$, while on day 2...
following DMBA administration, the marked proliferation of sER in hepatocytes was seen with enhancement of hepatic glycogenolysis. The sER membranes contain enzymes, and a proliferation of the membranes may be associated with increased quantities of metabolic enzymes, including CYP1. Moreover, the metabolic processing of PAHs including DMBA requires glycogenolysis and glucolysis. The present study suggests that high CYP1 enzyme production and DMBA metabolism might be promoted in rat liver on Day 2 following DMBA administration.

References


