Effects of Obesity and 7,12-Dimethylbenz(A) Anthracene (DMBA) Treatment on Liver Cytochrome P4501A1 and 1B1 Expression in Ovariectomized Obese Zucker Rats

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Abstract

Obesity is a risk factor for postmenopausal breast cancer development. We have shown that obesity increases DMBA-induced mammary tumor development in intact and ovariectomized Zucker rats. Several data suggest that DMBA requires metabolic activation to exert its carcinogenic effects. The objective of this study was to investigate the effect of obesity on hepatic expression of cytochrome P450 enzymes CYP1A1 and CYP1B1. Several data suggest that DMBA can increase the expression of enzymes that are responsible for DMBA metabolism in the ovariectomized Zucker rat model.

Keywords: Obesity; Zucker rats; CYP 1A1; CYP 1B1

Introduction

For over two decades, the US has experienced a continuing obesity epidemic with a rise in the proportion of overweight and obese adult population. An investigation of the role of overweight and obesity in carcinogenesis documented not only an association between Body Mass Index (BMI) and mortality from various types of cancer but also provided a reliable estimate of the contribution of overweight and obesity to the total mortality from cancer [1]. These authors reported a significant trend demonstrating that individuals with a higher BMI exhibited an increased risk of succumbing to cancers of the breast, uterus, cervix, and ovary [1]. Breast cancer is the most common malignant tumor among women, and of all cancers, it is the second leading cause of mortality in women in the US. Estimates for 2013 predicted that 232,340 women were likely to be diagnosed with invasive breast cancer and that 39,620 women were likely to succumb to this disease [2].

There is a link between obesity and increased risk of breast cancer among postmenopausal women [1]. Recently, we have shown that obesity increases the rate of DMBA-induced mammary tumor development in intact and ovariectomized Zucker rats [3]. DMBA is a model compound that induces mammary carcinogenesis in rodents [4]. Members of the cytochrome P450 family of enzymes are responsible for the metabolic activation of DMBA to form reactive electrophilic intermediates that can form mutagenic adducts with DNA. Formation of these DNA adducts can cause the mutations and genetic damage that are thought to be the initiating steps in carcinogenesis. The actual adduct formation depends, in part, on the activity of phase I enzymes involved in the activation of DMBA. DMBA-induced breast cancer depends on the activity of CYP1A1, CYP1A2, and CYP1B1 enzymes expressed in breast tissue and liver [5]. The main objective of this experiment was to investigate the effect of obesity on hepatic expression of CYP1A1 and CYP1B1 following DMBA treatment in obese and lean ovariectomized Zucker rats.

Several data suggest that DMBA and other polycyclic aromatic hydrocarbons (PAHs) require metabolic activation in order to exert their carcinogenic effects; therefore, DMBA-induced breast cancer is dependent on the activity of CYP1A1, CYP1A2 and CYP1B1 in breast tissue and liver [6]. Since obesity is a major risk factor for breast cancer development, we hypothesize that induced levels of CYP1A1 and/or CYP1B1 will be elevated in obese rats compared to lean Zucker rats. Therefore, the objective of this experiment was to investigate the role of obesity on expression of CYP1A1 and CYP1B1 in the liver using lean and obese Zucker rats.

Materials and Methods

Experimental design

All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences.

Forty-day-old, ovariectomized obese (n=20) and lean (n=20) Zucker rats were purchased from Harlan Industries (Indianapolis, IN, USA). The rats were housed 2 per cage with *ad libitum* access to water.
and a semi-purified diet similar to the AIN-93G diet (Harlan Teklad, Madison, WI, USA) and 10 days later were orally gavaged with either with sesame oil (control) or with 65 mg/kg DMBA (Sigma Chemical Co., St. Louis, MO, USA) in sesame oil as previously reported [7]. All rats were weighed twice weekly. All rats were sacrificed 24 hours post-DMBA treatment. All rats were euthanized, and the liver from each rat was removed and stored at –80°C for biochemical analysis. Liver microsomes were prepared as shown below and CYP1A1 and CYP1B1 expression was measured by Western blotting using goat anti-mouse CYP1A1 and CYP1B1 (Oxford Biochemical Research, Oxford, MI) antibodies.

**Western immunoblot analysis**

The expression of hepatic CYP proteins was measured from microsomes as described previously [8]. Briefly, proteins were fractionated on 10% polyacrylamide gels and transferred to Hybond-P membrane (Amersham Pharmacia Biotech, Arlington Heights, IL). Membranes were blocked for 8 hours in Tris-buffered saline (TBST, 10 mM Tris pH 7.4, 0.13 M NaCl, 2.7 mM KCl containing 0.005% Tween-20) and 50 g/L powdered non-fat dried milk. Blocked membranes were incubated with 16 hours with primary antibody diluted in TBST containing 50 g/L powdered non-fat dried milk at the following dilutions: AhR, 5 mg/ml; ARNT, 1:500; CYP1B1, 1:4,000; monoclonal CYP1A1; and CYP1A2, 1:200. Membranes were washed three times with TBST and incubated for 1 hour in TBST containing 50 g/L powdered non-fat dried milk containing horseradish peroxidase-conjugated secondary IgG diluted 1:5,000. After washing the membranes three times in TBST, proteins were visualized by the use of the ECL Plus system (Amersham Pharmacia Biotech) and subsequent autoradiography on X-ray film. Autoradiographs were quantitated by using a Biophotons digital imager and the Image Quant image analysis program.

**Statistical analysis**

We used two-way ANOVA and the Tukey test using SigmaStat Software. We used P values for assessment of the level of significance and a value less than 0.05 were considered significant.

**Results**

All rats gained weight during this course of experiment. Also, obese rats gained significantly more weight (P<0.001) than lean rats.

Lean rats treated with DMBA had significantly higher (P<0.001) hepatic expression of CYP1A1 compared to oil control-treated lean rats. Also, DMBA treatment increased (P<0.001) the hepatic expression of CYP1B1 compared to oil control-treated obese rats (Table 1). The Western immunoblot analysis of pool (8-10) of liver samples is shown in Figure 1 and clearly shows the increased expression of CYP1A1 by DMBA. The hepatic expression of CYP1B1 was not affected by obesity or DMBA treatment (Table 1).

**Discussion**

DMBA is a procarcinogen that requires metabolic activation by cytochrome P450 enzymes to reactive metabolites (dihydrodiol epoxides) that can form mutagenic DNA adducts. DMBA is a well characterized procarcinogen which requires metabolic activation and has been used extensively to induce mammary tumors in Sprague-Dawley rats. Humans are exposed to DMBA and other polycyclic aromatic hydrocarbons (PAH) through environmental or dietary sources, which may function in a synergistic manner with

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**Table 1:** Liver CYP1A1 and CYP1B1 Expressions Following DMBA (Mean±SE).

<table>
<thead>
<tr>
<th></th>
<th>L/O</th>
<th>L/DMBA</th>
<th>Ob/O</th>
<th>Ob/DMBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>0.76 ± 0.09</td>
<td>2.66 ± 0.19**</td>
<td>0.89 ± 0.20</td>
<td>2.39 ± 0.10**</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>0.74 ± 0.10</td>
<td>0.84 ± 0.07</td>
<td>0.78 ± 0.06</td>
<td>0.72 ± 0.05</td>
</tr>
</tbody>
</table>

**P<0.001**

L/O: lean oil treated; L/DMBA: Lean and DMBA treat; Ob/O: Obese oil treated and Ob/DMBA: obese and DMBA treated rats

L/O vs. L/DMBA; **Ob/O vs Ob/DMBA**

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**Figure 1:** The Liver CYP1A1 expression from Pool of samples (8-10/group).
demonstrate that the metabolism of PAHs such as DMBA is regulated by constitutive and induced levels of CYP1A1 and CYP1B1 and that these levels can be modulated by hormones, including estradiol and progesterone. Differences in estradiol levels in our obese Zucker rat model and between lean ovariectomized and obese ovariectomized rats are presumably due to the contribution of estrogen from adipose tissue and may increase constitutive expression of CYP1B1. One limitation of this study is that the rats were killed at 51 days of age which represents the early stage of obesity in this model. Future experiments will be extended for an additional 50 days to observe the effects of prolonged obesity and to potentially see the effects of modulation of CYPs in this model system.

In summary, our preliminary data suggest that DMBA treatment increased expression of CYP1A1 that is responsible for DMBA metabolism in the ovariectomized Zucker rat. Also, obesity resulted in higher basal CYP1A1 expression in untreated Zucker rats. Future experiments will be needed to investigate the effects of obesity over a longer period of time on the expression of CYP1A1 and CYP1B1.

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References