Journal of Molecular and Genetic Medicine

Hakkak et al., J Mol Genet Med 2013, S1
DOI: 10.4172/1747-0862.S1-003

Research Article Open Access

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

Reza Hakkak[1*,2,3], Stewart Macleod[2,3] and Soheila Korourian[4]

[1]Departments of Dietetics and Nutrition, University of Arkansas for Medical Sciences, 4301 W. Markham St., Little Rock, AR 72205, USA
[2]Departments of Pediatrics, University of Arkansas for Medical Sciences, 4301 W. Markham St., Little Rock, AR 72205, USA
[3]Arkansas Children's Hospital Research Institute, 13 Children's Way. Little Rock, AR 72202, USA
[4]Department of Pathology, University of Arkansas for Medical Sciences, 4301 W. Markham St., Little Rock, AR 72205, USA

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 CYP1A1 and CYP1B1 following DMBA treatment in obese and lean ovariectomized Zucker rats. Forty day-old, ovariectomized obese (n=20) and lean (n=20) Zucker rats were placed on AIN-93 G diet and 10 days later were orally gavaged with either with sesame oil (control) or with 65 mg/kg DMBA in sesame oil. All rats were sacrificed 24 hours post-DMBA treatment. Liver microsomes were prepared, and CYP1A1 and CYP1B1 expression was measured by Western blotting using goat anti-mouse CYP1A1 and CYP1B1 antibodies. DMBA treatment significantly ($p<0.001$) increased expression of CYP1A1 in lean and obese ovariectomized rats compared to the control group. CYP1B1 expression was not affected by obesity or DMBA. These 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

***Corresponding author:** Dr. Reza Hakkak, Department of Dietetics and Nutrition, 4301 W. Markham St., Mail Slot 627, Little Rock, AR 72205, USA, Tel: 501-686-6166; Fax: 501-686-5716; E-mail: HakkakReza@uams.edu

Received October 07, 2013; **Accepted** October 30, 2013; **Published** November 06, 2013

Citation: Hakkak R, Macleod S, Korourian S (2013) Effects of Obesity and 7,12-Dimethylbenz(*A*) Anthracene (DMBA) Treatment on Liver Cytochrome P4501A1 and 1B1 Expression in Ovariectomized Obese Zucker Rats. J Mol Genet Med S1: 003. doi: 10.4172/1747-0862.S1-003

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 CYP1 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, 10mM Tris pH 7.4, 0.13 M NaCl, 2.7mM KCl containing 0.005% Tween-20) and 50 g/L powdered non-fat dried milk. Blocked membranes were incubated for 16hours 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 1hour 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 Biophotonics 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 were 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 (dihydrodiolepoxides) 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

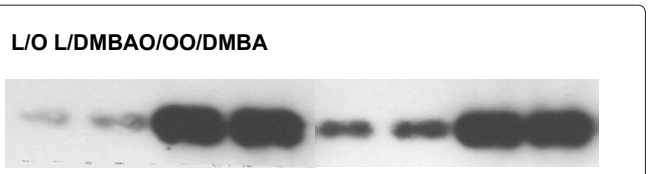

Figure 1: The Liver CYP1A1 expression from Pool of samples (8-10/group). L/O; lean oil treated; L/DMBA; Lean and DMBA treat; Ob/O; Obese oil treated and Ob/DMBA; obese and DMBA treated rats.
Liver CYP1A1 expression from Pool of samples (8-10/group).

	L/O	L/DMBA	Ob/O	Ob/DMBA
CYP1A1	0.76 ± 0.09	2.66 ± 0.19[1**]	0.89+0.20	2.39 ± 0.10[2**]
CYP1B1	0.74 ± 0.10	0.84 ± 0.07	0.78+0.06	0.72 ± 0.05

**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
[1]L/O vs. L/DMBA; [2]Ob/O vs Ob/DMBA

Table 1: Liver CYP 1A1 and CYP 1B1 Expressions Following DMBA (Mean+SE).

obesity in breast carcinogenesis. Exposure to PAH present in cigarette smoke and in meats cooked at high temperature has been implicated in the development of breast cancer [9]. We have reported previously that intact obese Zucker rats had increased susceptibility to DMBA-induced mammary tumors compared to lean Zucker rats [7]. We used the obese Zucker rat model to investigate if high adipose tissue could promote mammary tumor induction in the absence of ovarian estrogen [3]. We found that 36% of the obese ovariectomized (O/O) rats developed mammary tumors while lean ovariectomized (L/O) rats developed no mammary tumors (P<0.001) and that 59% of the obese sham (O/S) rats developed mammary tumors compared to 30% of the lean/sham (L/S) rats (P<0.05). We concluded that obesity increases the susceptibility of ovariectomized Zucker rats to DMBA-induced mammary tumors, suggesting that adipose tissue-derived estrogen in obese animals may be sufficient to promote DMBA-induced tumors in this model [3]. Although the DMBA-induced mammary tumor model has been used with Sprague-Dawley rats extensively [8,10-13], there are no published reports that used DMBA for the induction of CYP1A1 and CYP1B1 in Zucker (lean and obese) rats. Therefore, we used ovarectomized obese Zucker rats as a model for obesity and breast cancer in postmenopausal women to investigate the effects of obesity on DMBA metabolism. In this present study, we found that DMBA treatment increased both CYP1A1 in lean and obese rats. While obesity numerically increased the baseline (oil-treated rats), it was not significant. Also, hepatic expression of CYP1B1 was not affected by DMBA or obesity. Christou et al. [5] determined that expression of CYP1A1 and CYP1B1 are induced by PAH such as DMBA in a cell-specific manner in cultured rat mammary cells. Rat mammary epithelial cells expressed CYP1A1 only after treatment with PAH or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCCD). Very low levels of CYP1B1 were detected in these cells, but selective antibody inhibition studies showed this enzyme accounted for 40% of DMBA metabolism. In mammary stromal fibroblasts, only CYP1B1 activity was detected. In rat mammary fibroblasts, low levels of CYP1B1 were detected that were induced to higher level by PAH treatment. Treatment of rat mammary fibroblasts or mammary epithelial cells with a mixture of hormones [17β-estradiol (0.2 μM) progesterone (1.5 μM) cortisol (1.5 μM) and prolactin (5 μg/ml)] resulted in a 75% decrease in both the constitutive and induced expression of CYP1A1 and CYP1B1. Treatment of rat mammary fibroblasts with 17β-estradiol increased constitutive expression of CYP1B1 at the level of mRNA and protein but did not affect levels in PAH-induced cells. These experiments

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 ovatiectomized and obese ovatiectomized 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.

Acknowledgements

This study was supported through awards by the Susan G. Komen Foundation and by the Arkansas Biosciences Institute through the Arkansas Children's Hospital Research Institute to RH. The authors would like to thank John Gregan at Arkansas Children Hospital Research Institute for valuable assistance with preparation of this manuscript.

References

1. Calle EE, Thun MJ (2004) Obesity and cancer. Oncogene 23: 6365-6378.
2. Society AC (2013).
3. Hakkak R, MacLeod S, Shaaf S, Holley AW, Simpson P, et al. (2007) Obesity increases the incidence of 7,12-dimethylbenz(a)anthracene-induced mammary tumors in an ovariectomized Zucker rat model. Int J Oncol 30: 557-563.
4. HUGGINS C, GRAND LC, BRILLANTES FP (1961) Mammary cancer induced by a single feeding of polymucular hydrocarbons, and its suppression. Nature 189: 204-207.
5. Christou M, Savas U, Spink DC, Gierthy JF, Jefcoate CR (1994) Co-expression of human CYP1A1 and a human analog of cytochrome P450-EF in response to 2,3,7,8-tetrachloro-dibenzo-p-dioxin in the human mammary carcinoma-derived MCF-7 cells. Carcinogenesis 15: 725-732.
6. Rundle A, Tang D, Hibshoosh H, Estabrook A, Schnabel F, et al. (2000) The relationship between genetic damage from polycyclic aromatic hydrocarbons in breast tissue and breast cancer. Carcinogenesis 21: 1281-1289.
7. Hakkak R, Ronis MJ, Badger TM (1993) Effects of enteral nutrition and ethanol on cytochrome P450 distribution in small intestine of male rats. Gastroenterology 104: 1611-1618.
8. Rowlands JC, He L, Hakkak R, Ronis MJ, Badger TM (2001) Soy and whey proteins downregulate DMBA-induced liver and mammary gland CYP1 expression in female rats. J Nutr 131: 3281-3287.
9. Hakkak R, Holley AW, Macleod SL, Simpson PM, Fuchs GJ, et al. (2005) Obesity promotes 7:12-dimethylbenz(a)anthracene-induced mammary tumor development in female zucker rats. Breast Cancer Res 7: R627-633.
10. Hakkak R, Korourian S, Shelnutt SR, Lensing S, Ronis MJ, et al. (2000) Diets containing whey proteins or soy protein isolate protect against 7,12-dimethylbenz(a)anthracene-induced mammary tumors in female rats. Cancer Epidemiol Biomarkers Prev 9: 113-117.
11. Yang SH, Suh JH, Lee MG, Kim SH (2013) Pharmacokinetic drug interactions between ondansetron and tamoxifen in female Sprague-Dawley rats with DMBA-induced mammary tumor. Anticancer Res 33: 521-528.
12. Yang SH, Choi HG, Lim SJ, Lee MG, Kim SH (2013) Effects of morin on the pharmacokinetics of etoposide in 7,12-dimethylbenz[a]anthracene-induced mammary tumors in female Sprague-Dawley rats. Oncol Rep 29: 1215-1223.
13. Rengarajan T, Nandakumar N, Balasubramanian MP (2012) D-Pinitol attenuates 7, 12 dimethylbenz [a] anthracene induced hazards through modulating protein bound carbohydrates, adenosine triphosphatases and lysosomal enzymes during experimental mammary carcinogenesis. J Exp Ther Oncol 10: 39-49.

This article was originally published in a special issue, **Molecular & Cellular Aspects in Obesity & Diabetes** handled by Editor(s). Dr. Masayoshi Yamaguchi, Emory University School of Medicine, USA