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Research Paper

Regulation of cancer-related genes – *Cyp1a1*, *Cyp1b1*, *Cyp19*, *Nqo1* and *Comt* – expression in β-naphthoflavone-treated mice by miroestrol

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Keywords
cancer risk; estrogenic activity; *Pueraria mirifica*

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Abstract

Objective The effects of miroestrol (MR), an active phytoestrogen from *Pueraria candollei* var. *mirifica*, on expression of cancer-related genes were determined.

Methods Seven-week-old female ICR mice (n = 5 each) were subcutaneously administered estradiol (E2, 0.5 mg/kg/day) or MR (0.5 or 5 mg/kg/day) daily for 7 days. Some were given ER or MR in combination with β-naphthoflavone (BNF, 30 mg/kg/day) for the last 3 days. The expression of cancer-related genes including cytochrome P450 1A (*Cyp1a*), cytochrome P450 1B1 (*Cyp1b1*), aromatase P450 (*Cyp19*), NAD(P)H: quinone oxidoreductase 1 (*Nqo1*) and catechol-*O*-methyltransferase (*Comt*) were evaluated.

Key findings In the presence of BNF, MR suppressed hepatic CYP1A1 activity and CYP1A2 activity, expression of CYP1B1 mRNA and expression of CYP1A1/2 and CYP1B1 protein. E2, by contrast, did not. MR restored expression levels of hepatic NQO1 and uterine COMT in BNF-treated mice. Furthermore, MR increased expression of uterine CYP19 to the same extent as E2.

Conclusion MR may be superior to E2 as it downregulates expression of CYP1. Moreover, MR normalized expression of both NQO1 and COMT, the protective enzymes, in murine liver and uteri. These results support the use of MR as an alternative supplement for menopausal women, MR having the extra benefit of reducing cancer risk.

Introduction

Hormone replacement therapy (HRT) has been reported to increase the risk of breast cancer in postmenopausal women.[1] A metabolite of estrogen, catechol estrogen-3,4- quinone, potentially causes DNA damage, leading to an increase in cancer risk.[2] Several metabolic enzymes are involved in the metabolism of estrogen. Aromatase P450 (CYP19) converts androgens to estrogen.[3] Cytochrome P450 1B1 (CYP1B1) metabolizes estrogen to 4-catechol estrogen and catechol estrogen-3,4-quinone.[3] In addition, cytochrome P450 1A (CYP1A) activates some pro-carcinogens.[4] In humans, there are some protective systems against these carcinogens. NAD(P)H: quinone oxidoreductase 1 (NQO1) enzyme reverts catechol estrogen-3,4-quinone back to catechol estrogen.[3] After that, catechol-*O*-methyltransferase (COMT) enzyme decreases methylation of 4-catechol estrogen.[5] Alteration of these enzymes might therefore increase or decrease the risk of carcinogenesis.

Pueraria candollei var. *mirifica* (PM) is a medicinal Thai plant used for rejuvenation and as an estrogen replacement therapy in menopausal women. Previous studies have shown this traditional medicine to stimulate luteinizing hormone and follicle-stimulating hormone in gonadectomized rats,[6] to inhibit ovulation in monkeys,[7] and to exert an antioxidant effect in ovariectomized mice.[8] Miroestrol (MR; Fig. 1), a compound present in the tuberous root of PM, has been shown to have potent estrogenic-like activity.[9] After exposed for 8 weeks, PM (100 mg/kg/day) had the adverse effects on mating efficiency and reproduction in adult female mice.[10]

However, the information on the effect of MR on expression of cancer-related genes is limited. This study therefore investigated the effects of MR on the expression of the cancer-related genes *Cyp1a1*, *Cyp1b1*, *Cyp19*, *Nqo1* and *Comt* in the livers and uteri of β-naphthoflavone (BNF)-treated mice. Our observations support the use of MR as an estradiol supplement for HRT, with the advantage that it may lower the risk of cancer through improved regulatory expression of cancer-related genes.

Materials and Methods

Chemicals

Miroestrol (MR, Fig. 1) was isolated from the tuberous roots of PM as previously described.[8,11,12] β-naphthoflavone (BNF, Fig. 1) and 17β-estradiol benzoate (E2, Fig. 1) were supplied by Sigma-Aldrich Chemicals (St. Louis, MO, USA). Forward and reverse primers of *Cyp1b1*, *Cyp19*, *Nqo1*, *Comt* and *Gapdh* genes were produced by Bio Basic Inc. (Markham, Ontario, Canada). The primers of each gene are shown in Table 1. ReverTraAce® and Taq DNA polymerase for reverse transcription and polymerase chain reaction were products of Toyobo Co. Ltd. (Osaka, Japan) and Invitrogen® (Life Technologies Corporation, (Carlsbad, CA, USA)), respectively. The antibody of CYP1A1/2 (299124) was a product of Daiichi Pure Chemical Co. Ltd. (Tokyo, Japan). The antibodies of CYP1B1 (H-105, sc-32882), CYP19 (H-300, sc-30086), NQO1 (H-90, sc-25591), COMT (FL-271, sc-25844), rabbit anti-goat IgG-HRP (sc-2768) and the goat anti-rabbit IgG-HRP (sc-2004) were products of Santa Cruz Biotechnology Inc. (Dallas, TX, USA). Vector DAB substrate kit for peroxidase (SK-4100) was supplied by Vector Laboratories (Burlingame, CA, USA). All chemicals were of the highest quality from commercial suppliers.

Isolation of miroestrol

Miroestrol (MR) at 90.2% purity[11] was isolated from the dried tuberous root bark of *Pueraria candollei* var. *mirifica* (PM, collected in Ubon Ratchathani, Thailand, in March 2010). PM was powdered and extracted three times with hexane, and the maceration was extracted three times with ethyl acetate. The ethyl acetate crude extracts were combined, evaporated and fractionated by column chromatography (Silica gel 60 with hexane: ethyl acetate, 3 : 1, 3 : 2, 1 : 1 and 0 : 1). The MR fraction (compared with an authentic standard of MR from Dr. Chaiyo Chaichantipyuth, Chulalongkorn University) was continually purified using a Sephadex LH-20 column (ethyl acetate:methanol, 7 : 3). MR was recrystallized and identified using high-performance liquid chromatography (HPLC) and ^{1}H-NMR spectrum.[8,12]

Animals and treatments

Seven-week-old female ICR mice were supplied by the National Laboratory Animal Center of Mahidol University, Thailand, and housed in the Northeast Laboratory Animal Center at Khon Kaen University, Thailand. The animal handling and treatment protocols were approved by the Animal Ethics Committee for Use and Care of Khon Kaen University (Approval No. AEKKU 12/2555 and 73/2555). Forty mice were divided into eight groups ($n = 5$ per group). First, the control group was subcutaneously given

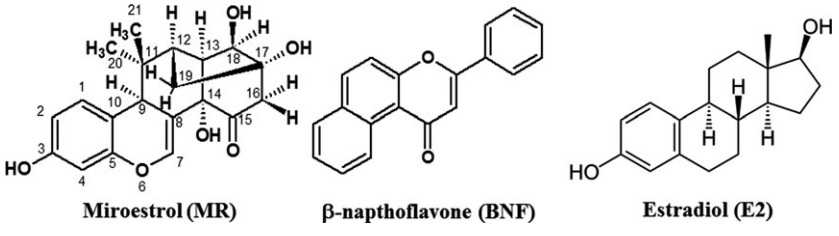

Figure 1 Chemical structures of miroestrol, β-naphthoflavone and estradiol.

Table 1 Primer sequences for semi-quantitative RT-PCR.

Gene	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Size (bp)	Annealing temperature (°C)
Cyp1b1	GAC CCG GAT GTT TTG TGA AT	CAT GGT GAG CAG CAA AAG AA	738	60
Cyp19	TTC TCG TCG CAG AGT ATC CA	CAA GGT GCC TGT CCT CAT TT	766	60
Nqo1	GGA CAT GAA CGT CAT TCT CT	TTC TTC TTC TGC TCC TCT TG	261	55
Comt	TCC ACA ACC TGC T-C ATG GGT	ACA TCG TAC TTC TTC TTC AGC TGG	409	62
Gapdh	TCC ACT CAC GGC AAA TTC AAC G	TAG ACT CCA CGA CAT ACT CAG C	145	64

corn oil (0.1 ml/mouse) once a day for 7 days. The second group was intraperitoneally administered with BNF at a dose of 30 mg/kg/day once a day for the last 3 days. The third group was E2; mice were subcutaneously administered with E2 at a dose of 0.5 mg/kg/day once a day for 7 days. The forth and the fifth, mice were subcutaneously given with MR at doses of 0.5 or 5 mg/kg/day, for 7 days. The 6th to 8th, mice were given E2 at a dose of 0.5 mg/kg/day or MR at doses of 0.5 or 5 mg/kg/day, for 7 days in combination with the intraperitoneal BNF at a dose of 30 mg/kg/day once a day for the last 3 days (the 5th to 7th day of treatments). All mice were sacrificed 24 h after the last treatment.

Preparation of hepatic microsomes

The microsomal fraction was prepared by centrifugation of the liver homogenate in 1.15% KCl at 10 000 ***g*** for 10 min at 4 °C, and the supernatant was subjected to ultracentrifugation at 104 000 ***g*** for 60 min at 4 °C.[13] The concentration of microsomal protein was determined by the Bradford assay using bovine serum albumin (BSA) as a standard.

Assessment of ethoxyresorufin *O*-deethylase and methoxyresorufin *O*-demethylase activity

Assessment of ethoxyresorufin *O*-deethylase (EROD) activity and methoxyresorufin *O*-demethylase (MROD) activity were determined according to the method,[13] with modifications. Briefly, the reaction mixture containing 3 μM Tris–HCl (pH 7.8), 50 μM NADPH, 10 μg of hepatic microsomes and 0.625 μM ethoxyresorufin or methoxyresorufin, in a final volume of 200 μl was incubated at 37 °C throughout the period of spectrofluorometric measurement. The formation of resorufin was analysed after the addition of NADPH using spectrofluorometry with an excitation wavelength of 520 nm and an emission wavelength of 590 nm, compared with the resorufin standard.

Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Mice CYP1B1, CYP19, NQO1, COMT and GAPDH mRNAs were semi-quantified by RT-PCR. Primers were designed using nucleotide data base of NCBI and Primer-Blast online program (available at http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Hepatic and uterine total RNA was reverse transcribed using random primers and ReverTraAce® reverse transcriptase. For the reverse transcription, the reaction mixture was contained 50 ng of total RNA, 2.5 mM of random primers, 1 mM of dNTP, 2 unit of ribonuclease inhibitor and 5 unit of ReverTraAce® and converted to cDNA at the condition: 25 °C for 10 min, 42 °C for 60 min and 95 °C for 5 min. After that, the cDNA was amplified under the PCR conditions shown in Table 1. The reaction mixture of PCR was contained 50 ng cDNA, 0.25 mM of each forward and reverse primers, 1 mM of dNTP, 2 mM $MgCl_2$ and 1.25 unit of Taq DNA

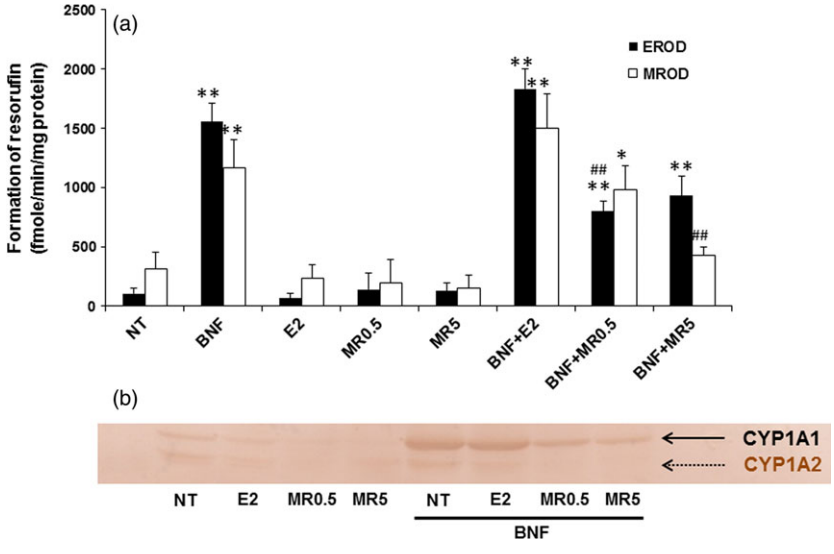

Figure 2 Effect of miroestrol on hepatic EROD activity and MROD activity (a), and CYP1A1 and CYP1A2 protein expression (b) The control group was subcutaneously given corn oil (NT; 0.1 ml/mouse) once a day for 7 days. BNF, mice were daily given β-naphthoflavone (BNF) intraperitoneally at a dose of 30 mg/kg/day for the last 3 days. E2, mice were daily subcutaneously administered estradiol at a dose of 0.5 mg/kg/day. MR, mice were daily subcutaneously administered miroestrol at doses of 0.5 or 5 mg/kg/day, for 7 days. The data are presented as the mean ± SD ($n = 5$). $^{*}P < 0.05$, $^{**}P < 0.001$ versus NT; $^{\#\#}P < 0.001$ versus BNF.

polymerase. Each cycle of PCR condition was started at 95 °C for 30 s, followed by the annealing temperature of each gene (Table 1) for 30 s and the extension at 72 °C for 30 s. The number of cycles (22–32 cycles) for each gene was validated in the linear phrase before running the samples. After separation of the PCR products by 2% agarose gel electrophoresis, the target cDNA bands were detected under ultraviolet light in the presence of Novel Juice (GeneDireX®, (Taipei, Taiwan)). DNA band intensity was determined semi-quantitatively by gel documentation (InGeniusL, InGenius Syngene Bio-imaging) and Gene Tool Match program (Syngene, Lab Focus Co. Ltd., Cambridge, UK). The mRNA levels of the target genes were normalized to that of the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*).

Western blotting

Five to twenty micrograms of microsomal protein or uterine protein was resolved by 10% SDS-PAGE using vertical electrophoresis at 120 V for 2–3 h and then transferred to a Hybond-C membrane at 11V for 40 min. The membrane was blocked with BSA (10 mg/ml) at 4 °C overnight. The specific protein was incubated with the primary rabbit or goat polyclonal antibody of mice CYP1A1/2, CYP1B1, CYP19, NQO1 and COMT (dilution factor = 1 : 2000 to 1 : 5000) at room temperature for 1 h, followed by the second antibody of rabbit anti-goat IgG-HRP or the goat anti-rabbit IgG-HRP (1 : 5000) at room temperature for 1 h. The bands of protein were visualized using 3,3′-diaminobenzidine and hydrogen peroxide as the protocol recommended by the supplier. The absorbance of the protein

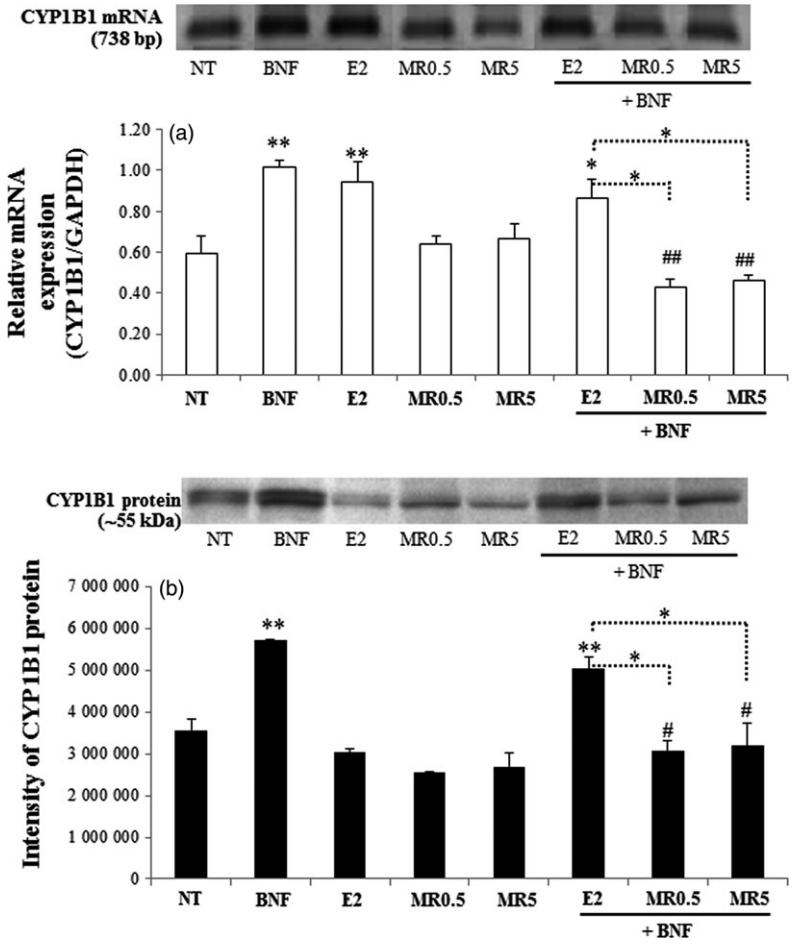

Figure 3 Effect of miroestrol on hepatic CYP1B1 mRNA (a) and CYP1B1 protein (b) expression. The control group was subcutaneously given corn oil (NT; 0.1 ml/mouse) once a day for 7 days. BNF, mice were daily given β-naphthoflavone (BNF) intraperitoneally at a dose of 30 mg/kg/day for the last 3 days. E2, mice were daily subcutaneously administered with estradiol at a dose of 0.5 mg/kg/day. MR, mice were daily subcutaneously administered with miroestrol at doses of 0.5 or 5 mg/kg/day, for 7 days. The data are presented as the mean ± SD (n = 5). $^{*}P < 0.05$, $^{**}P < 0.001$ versus NT; $^{\#}P < 0.05$, $^{\#\#}P < 0.001$ versus BNF.

band was calculated by gel documentation (InGeniusL) and Gene Tool Match program (Syngene). The results of bands' density were calculated from three-independent experiments ($n = 3$ per group).

Statistical analysis

The results were analysed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test (version 11.5; SPSS Inc., (Chicago, Illinois, USA)). *P*-values less than 0.05 and 0.001 were considered statistically significant.

Results

Effect of miroestrol on cancer-related genes in the mouse livers

BNF significantly induced EROD activity and MROD (Fig. 2A) activity in the mouse livers, as well as CYP1A1 and CYP1A2 protein levels (Fig. 2B). E2 (0.5 mg/kg/day) and MR (0.5 and 5 mg/kg/day) did not modify EROD activity or MROD activity, nor did they affect levels of CYP1A1 and CYP1A2 proteins. Interestingly, both doses of MR extensively suppressed the BNF-induced EROD activity and MROD activity, as well as CYP1A1 and CYP1A2 protein in the mouse livers, compared with the BNF group, while E2 did not (Fig. 2). However, both doses of MR could not restore the expressions of mRNA and protein of CYP1A1 and CYP1A2 to the same level as the NT group. Hepatic CYP1B1 mRNA was markedly increased by BNF (Fig. 3A). E2 significantly elevated CYP1B1 mRNA in the mouse livers while that of CYP1B1 protein was unchanged (Fig. 3B). MR itself did not disturb CYP1B1 at either mRNA or protein levels in the normal mice (Fig. 3). MR administration at both 0.5 and 5 mg/kg/day restored the expression of BNF-induced CYP1B1 to comparatively normal levels.

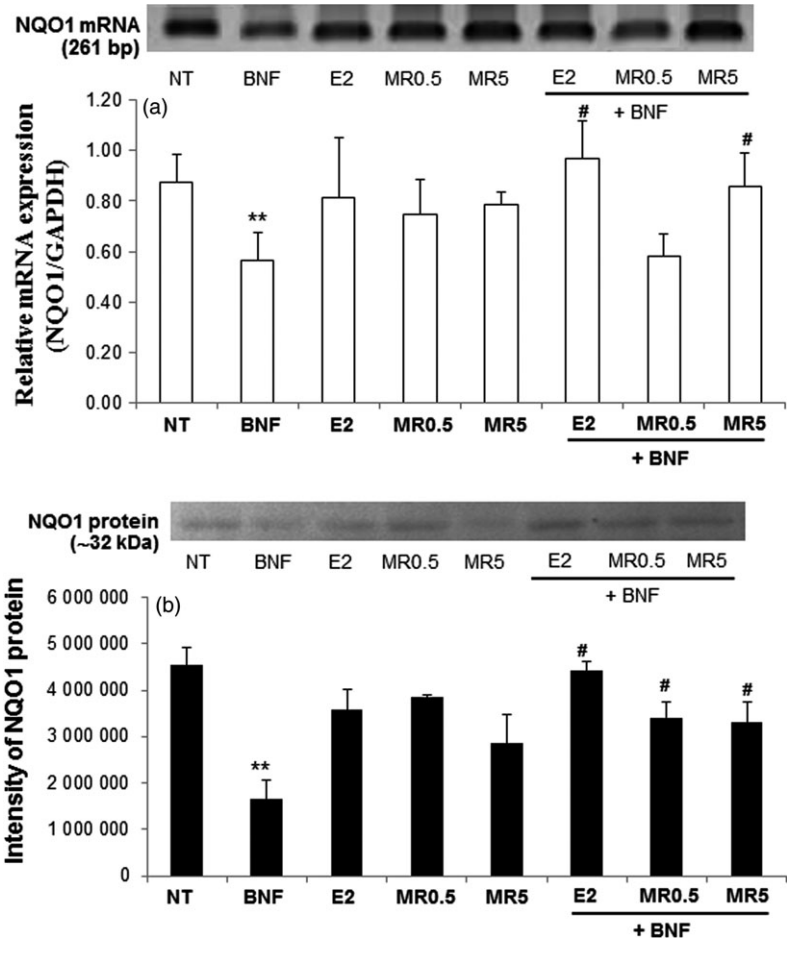

Figure 4 Effect of miroestrol on hepatic NQO1 mRNA (a) and NQO1 protein (b) expression. The control group was subcutaneously given corn oil (NT; 0.1 ml/mouse) once a day for 7 days. BNF, mice were daily given β-naphthoflavone (BNF) intraperitoneally at a dose of 30 mg/kg/day for the last 3 days. E2, mice were daily subcutaneously administered with estradiol at a dose of 0.5 mg/kg/day. MR, mice were daily subcutaneously administered with miroestrol at doses of 0.5 or 5 mg/kg/day, for 7 days. The data are presented as the mean ± SD ($n = 5$). $^{**}P < 0.001$ versus NT; $^{\#}P < 0.05$, versus BNF.

When we examined the effects of MR on expression of the two cancer protective genes, COMT and NQO1, no treatment modified the expression of COMT mRNA or protein (data not shown). The expression of NQO1 was downregulated by BNF at both mRNA and protein levels (Fig. 4), while E2 and MR normalized the expression of NQO1 to the control level.

Effect of miroestrol on cancer-related genes in the mouse uteri

When CYP1B1, CYP19 (aromatase) and COMT expressions were investigated in the mouse uteri, it was found that BNF treatment significantly increased CYP1B1 mRNA and protein expression levels (Fig. 5). This was consistent with the results obtained for the livers (Fig. 3). Again, MR markedly suppressed the BNF-induced CYP1B1 mRNA (Fig. 5A) and protein (Fig. 5B), comparable to the NT group, while E2 did not. Neither BNF nor MR affected CYP19 expression (Fig. 7), but E2 did significantly elevate CYP19 expression. CYP19 mRNA and protein levels were substantially increased by E2 and MR at 5 mg/kg/day in the presence of BNF (Fig. 6). On the other hand, BNF suppressed expression of both COMT mRNA and protein in the uteri (Fig 7). Interestingly, E2 and MR restored COMT expression to levels comparable to normal. NQO1 was not detected in the mouse uteri (data not shown).

Discussion

The primary responsibility of the CYP1 family of enzymes, CYP1A1, CYP1A2 and CYP1B1, is to metabolize polycyclic aromatic hydrocarbons (PAH), aromatic hydrocarbons and hormones. This has led to the suggestion that CYP1 enzymes might play a role in the initiation of some cancers

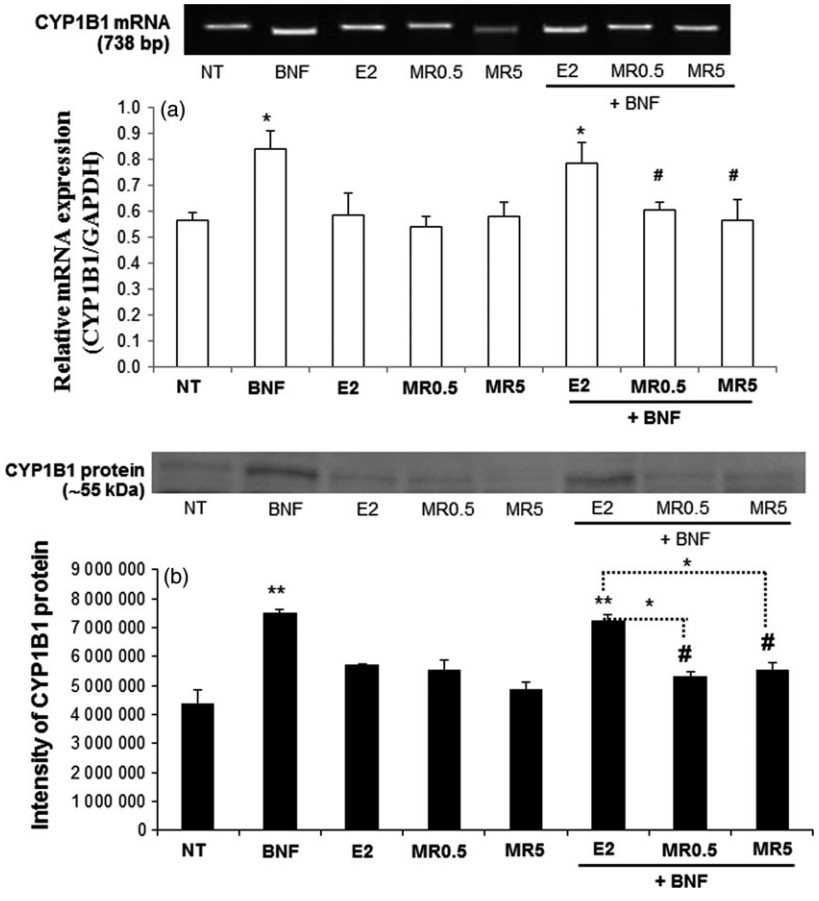

Figure 5 Effect of miroestrol on uterine CYP1B1 mRNA (a) and CYP1B1 protein (b) expression. The control group was subcutaneously given corn oil (NT; 0.1 ml/mouse) once a day for 7 days. BNF, mice were daily given β-naphthoflavone (BNF) intraperitoneally at a dose of 30 mg/kg/day for the last 3 days. E2, mice were daily subcutaneously administered with estradiol at a dose of 0.5 mg/kg/day. MR, mice were daily subcutaneously administered with miroestrol at doses of 0.5 or 5 mg/kg/day, for 7 days. The data are presented as the mean ± SD (n = 5). *P < 0.05, **P < 0.001 versus NT; $^{\#}P$ < 0.05, versus BNF.

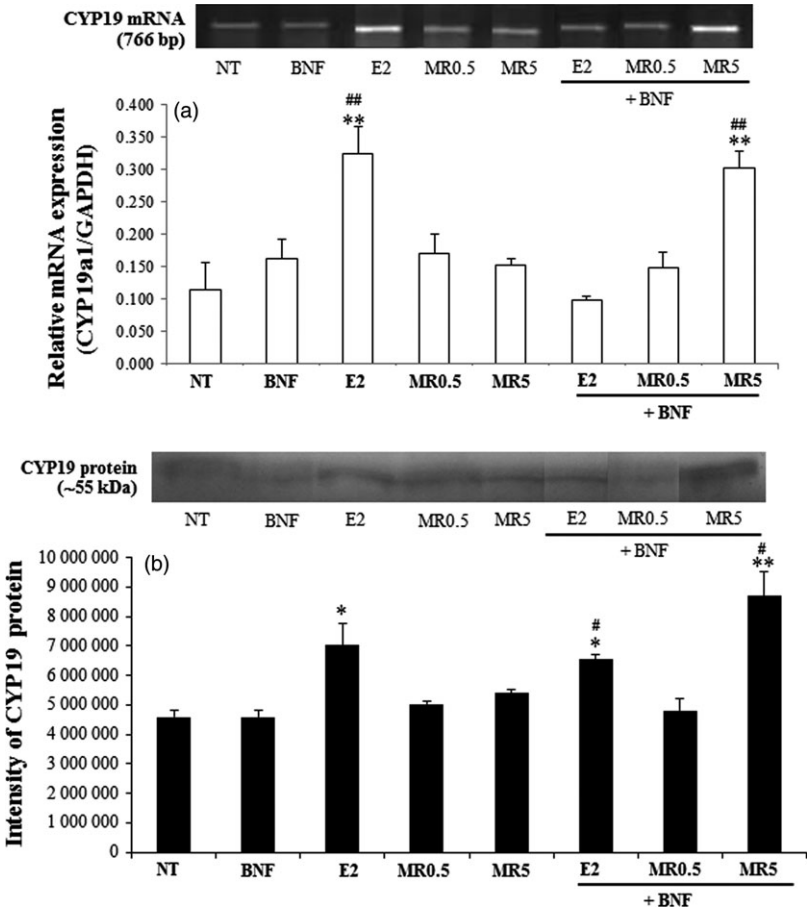

Figure 6 Effect of miroestrol on uterine CYP19 mRNA (a) and CYP19 protein (b) expression. The control group was subcutaneously given corn oil (NT; 0.1 ml/mouse) once a day for 7 days. BNF, mice were daily given β-naphthoflavone (BNF) intraperitoneally at a dose of 30 mg/kg/day for the last 3 days. E2, mice were daily subcutaneously administered with estradiol at a dose of 0.5 mg/kg/day. MR, mice were daily subcutaneously administered with miroestrol at doses of 0.5 or 5 mg/kg/day, for 7 days. The data are presented as the mean ± SD ($n = 5$). $^{*}P < 0.05$, $^{**}P < 0.001$ versus NT; $^{\#}P < 0.05$, $^{\#\#}P < 0.001$ versus BNF.

via pro-carcinogen activation.[4] CYP1A1 and CYP1B1 enzymes have been detected in human breast tumours at higher than normal levels.[14,15] CYP1A2 metabolizes several drugs and many environmental aromatic amines. PAHs induce human CYP1A2 gene expression in the liver, gastrointestinal tract, nasal epithelium and brain.[16] Hence, the expression of CYP1 has been used to study the regulatory mechanism of cancer development. BNF is a synthetic derivative of a naturally occurring flavonoid that strongly induces CYP1 enzymes via activation of an aryl hydrocarbon receptor (AhR).[17] BNF exhibits hepatocellular tumour-promoting activity by increasing both the number and size of preneoplastic foci for positive glutathione S-transferase placental form (GST-P) in rats initiated with N-diethylnitrosamine.[18] BNF was employed in the present study as a representative environmental toxin that humans might be exposed to in daily life. BNF significantly increased the expression of CYP1A1, CYP1A2 and CYP1B1 mRNA in primary mouse hepatocytes, confirming previous observations by Chatuphonprasert *et al.*[19]

Estrogen is a well-known risk factor for breast cancer progression.[1] The level of 2- and 4-hydroxyestradiol formation in neoplastic mammary tissue is a marker of breast cancer and may imply a mechanistic tumour growth.[1] Metabolites of estrogen are usually converted to catechol estrogen-3,4-quinone by CYP1B1.[3] Environmental contaminants such as chlorinated hydrocarbons can elevate CYP1A1, CYP1A2 and CYP1B1 expression in the liver, kidney and mammary glands of rats. Consequently, greater quantities of E2 metabolites are formed due to increase catalysis of 2- and 4-hydroxylation of E2 by CYP1 enzymes.[20]

EROD and MROD reactions can be used as a proxy measure of CYP1A1, CYP1A2 and CYP1B1 enzyme activity.[4] In the livers, MR suppressed EROD activity and MROD activity, CYP1A1, CYP1A2 and CYP1B1 proteins, as well as

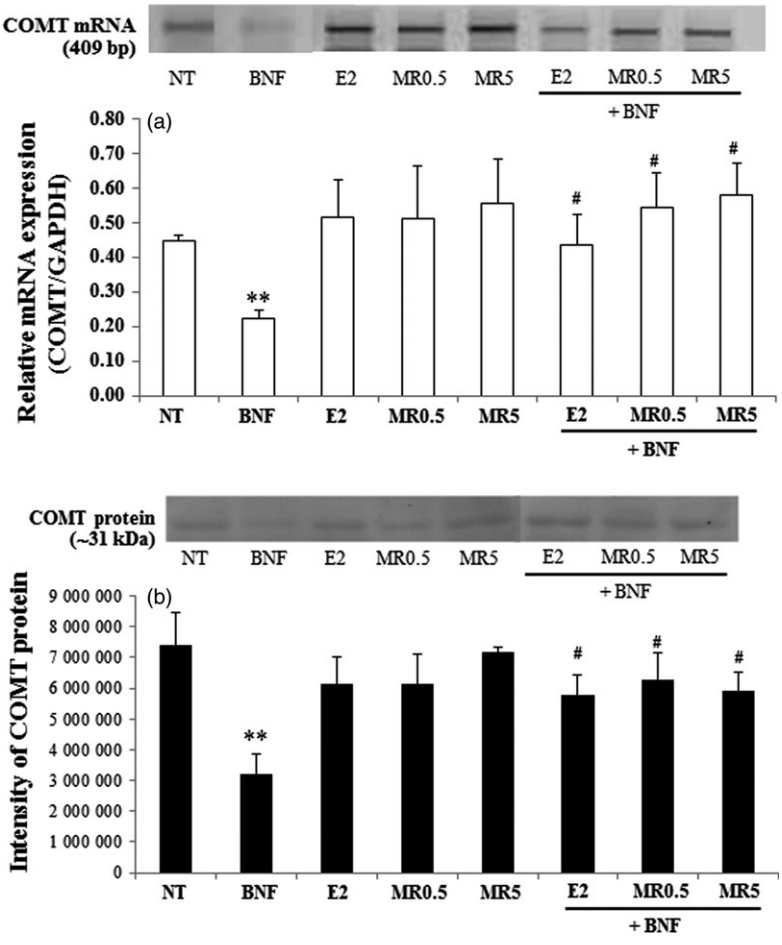

Figure 7 Effect of miroestrol on uterine COMT mRNA (a) and COMT protein (b) expression. The control group was subcutaneously given corn oil (NT; 0.1 ml/mouse) once a day for 7 days. BNF, mice were daily given β-naphthoflavone (BNF) intraperitoneally at a dose of 30 mg/kg/day for the last 3 days. E2, mice were daily subcutaneously administered with estradiol at a dose of 0.5 mg/kg/day. MR, mice were daily subcutaneously administered with miroestrol at doses of 0.5 or 5 mg/kg/day, for 7 days. The data are presented as the mean ± SD (n = 5). $^{**}P < 0.001$ versus NT; $^{\#}P < 0.05$ versus BNF.

CYP1B1 mRNA. In the uteri, MR increased the expression of CYP1B1 mRNA and protein in the BNF-treated mice. E2 did not positively modify BNF-induced CYP1 expression in either the liver or the uteri. These observations demonstrate an extra benefit of MR over E2. Our previous study revealed that administration of MR (0.5 mg/kg/day, for 7 days) downregulated expression of hepatic CYP1A2 mRNA in both male and female mice.[21] *In vitro* and *in silico*, MR potentially inhibited CYP1A1 activity and CYP1A2 activity.[8] These findings confirmed the inhibitory effect of MR on CYP1, an inhibition that might block the pathway responsible for pro-carcinogen activation, and lower the quantity of toxic E2 metabolites generated by CYP1 enzymes.

NQO1 plays an important role in the protective mechanism against reactive oxygen species and inhibition of neoplasia.[22] NQO1 converts E2-quinones back to E2-catechols, reducing the number of E2-quinone molecules available to react with DNA, the first step of carcinogenesis.[23] Singh *et al.*[24] reported that the level of NQO1 protein increases in female rats after treatment with E2 for 4 months, but it decreases after chronic exposure to E2 for 8 months. Furthermore, treatment with vitamin C and butylated hydroxyanisole for 120 days increased the level of NQO1 due to their antioxidant properties via nuclear factor erythroid 2-related factor 2 (Nrf2).[24] We did not observe any modification of NQO1 by E2 or MR. This difference in results might be explained by the different duration of treatments. Short-term treatment with E2 and MR prevented a BNF-induced reduction in NQO1 expression. Although the expression of both CYP1A1 and NQO1 has been proposed to be regulated via AhR, NQO1 was not induced by BNF, whereas CYP1A1 was.[25] Brauze *et al.*[25] found that BNF regulated rat liver NQO1 in a time-dependent manner. NQO1 levels significantly increased within

8 h of BNF treatment (80 mg/kg) but gradually decreased during the next 24–48 h. By contrast, BNF induced a very high level of CYP1A1 expression from 8 to 24 h, this high-level expression being maintained for a period of 144 h.[25] The findings of Brauze *et al.*[25] might explain why BNF induced CYP1A1 but not NQO1 in the present study, because we collected samples 24 h after the last treatment.

Besides NQO1, there is another enzyme, COMT, that participates in detoxification by reducing levels of active carcinogens. Methylation is the principal pathway by which COMT inactivates catecholamines and catecholestrogens.[26] BNF is reported to have a limited effect on COMT expression. Polychlorinated naphthalenes (Halowax 1051), which are inducers of the CYP1 family of enzymes, induced CYP1A1 expression but suppressed COMT expression in cocultures of granulosa and theca interna cells.[27] No significant modification of hepatic COMT levels was detected in the present study, but expression of COMT mRNA and protein was markedly suppressed by BNF treatment in the mouse uteri. E2 and MR notably restored COMT expression levels to normal. Therefore, MR might reduce toxin levels through a positive improvement of COMT expression in the BNF-treated mice.

Estrogen is catalysed by a specific form of cytochrome P450, namely aromatase cytochrome P450 (CYP19). Transcripts of the human *CYP19* gene are expressed in the ovary, placenta, testes, adipose tissue and brain.[28] The CYP19 enzyme converts androstenedione to estrone (E1) and metabolizes testosterone to estrogen (E2) in several tissues.[29] The association between CYP19 and breast cancer is still unclear.[30] BNF did not affect the expression of testicular CYP19 mRNA in male mice.[31] This finding is supported by results from the present study, which showed that BNF did not modify CYP19 expression in murine uteri. Our study also found that E2 and MR (5 mg/kg/day) upregulated CYP19 expression in murine uteri, confirming the estrogenic activity of E2 and MR. MR did not affect CYP19 expression in the normal state, and interestingly, it induced the expression level of CYP19 in the presence of BNF. This phenomenon might be explained by the cross-talk between estrogen receptor (ER) and AhR.[32] PAHs such as BNF and dioxins induce CYP1 expression via the complex aryl hydrocarbon receptor (AhR)-nuclear translocator (Arnt) pathway, and dioxin responsive elements.[32] Heterodimerization of AhR and Arnt has been proposed to affect many processes such as inhibition of E2-induced genes, a decrease in E2 level, limitation of E2 co-activators and ERα downregulation.[32,33] Therefore, ER ligands such as E2 and MR might be regulated by several pathways. Further studies will be required to clarify the effect of MR on the regulatory mechanism of ER-AhR cross-talk.

Conclusion

The present study showed that MR has several potential benefits including suppression of CYP1 expression, the pro-carcinogen activating enzymes, and normalization of NQO and COMT expression, the protective enzymes, in the livers and uteri of BNF-treated mice. MR also upregulated the expression of CYP19, as did E2. These results support the use of MR as an alternative HRT supplement for menopausal women due to its protective effect against cancinogenesis.

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Conflict of interest

The authors report no conflict of interest.

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