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Induction of estradiol-2-hydroxylase and ethoxyresorufin-*O*-deethylase by 3-substituted indole compounds

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Abstract

Estrogen can be hydroxylated at both 2- and 16α-positions. These two reactions are mutually exclusive. The 2-hydroxylated estrogen is relatively inactive compared with the 16α-derivative; hence, one approach in anti-estrogenic therapy is to look for drugs that can induce the 2-hydroxylation pathway. In the present study, using Balb/c and C57B/6 mice as the animal models, the induction effect of several isoprenyl compounds on estradiol-2-hydroxylase and ethoxyresorufin-*O*-deethylase activities was studied. The compounds examined included 2′- and 3′-methylbutadienyl-indoles and their respective acid condensation products, isopropyl indolocarbazole and yuehchukene; positional isomers of indole carbinols and carboxyaldehydes, as well as 3-methylcholanthrene, the prototype inducer of cytochrome P450 1A1. Our results demonstrated that while all of them were capable of inducing cytochrome P450 1A1-mediated ethoxyresorufin-*O*-deethylase activity, only the 3′ isomers could induce estradiol-2-hydroxylase activity. The induction of these two activities did not show any direct correlation, suggesting that cytochrome P450 1A1 was not the same enzyme catalyzing both ethoxyresorufin-*O*-deethylation and estradiol-2-hydroxylation. Nevertheless, both inductions were mediated by the aryl hydrocarbon receptor. Among the compounds tested, yuehchukene showed competitive binding to estrogen receptor. This, together with the induction of estradiol-2-hydroxylase activity, may account for the anti-estrogenic effect of yuehchukene.

Keywords: Anti-estrogenic activity; Aryl hydrocarbon receptor; Ethoxyresorufin-*O*-deethylase; Estradiol-2-hydroxylase; Yuehchukene

1. Introduction

Breast cancer is the major cause of death in hormone-dependent cancer in women. In the United States, about 44,000 women die of breast cancer every year (Davis et al., 1997). The cumulative exposure to estrogen plays a central role in the development and growth of breast cancer. Estrogen, being a lipid-soluble hormone, diffuses freely into the interior of the cell where it binds to the estrogen receptor and induces dimerization. The complex then binds to DNA and induces transcription of genes critical for breast cancer growth.

The basic strategy to diminish the effects of estrogen is by blocking its binding to the estrogen receptor. For example, tamoxifen, the most widely used drug for breast cancer treatment, acts as a competing ligand for the estrogen receptor (Osborne et al., 1996). Recent finding also suggested that tamoxifen has a chemopreventive function against breast cancer (Hong and Sporn, 1997). Unfortunately, long-term administration of the drug may lead to ovarian and endometrial cancer (Gradishar and Jordon, 1997). Thus, tamoxifen itself is a potential carcinogen and long-term use is not advocated.

Another approach to lower the estrogenic effect is by regulating the enzymes involved in the biosynthesis and interconversion of biologically potent estrogens. The key enzymes involved in the formation of estrogen are aromatase, 17β-hydroxysteroid dehydrogenase and estrone sulfatase. Inhibitors of these enzymes provide a means to reduce the circulating concentration of active estrogen (Adlercreutz et al., 1993; Howarth et al., 1994; Penning, 1996). Estrogen can be hydroxylated mainly at two differ-

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ent positions, namely, C-2 and C-16α. The two hydroxylated metabolites differ significantly in their estrogenic potency. The 16α-hydroxy-estrogen forms a long-acting adduct with estrogen receptor and is more liable to cause cell proliferation; on the contrary, the 2-hydroxy metabolite is devoid of estrogenic activity and is also rapidly cleared from the circulation (Schneider et al., 1984; Telang et al., 1992; Bradlow et al., 1996). Since the two hydroxylations are largely mutually exclusive (Fishman et al., 1980), therefore, any drug that increases the 2-hydroxylation reaction can decrease the estrogenic effect; it thus lowers the risk of breast cancer (Bradlow et al., 1995).

In contrast to the development of inhibitors for the three key biosynthetic enzymes, the development of inducers for the 2-hydroxylation pathway is still at a very early stage. The most promising compound to date is indole-3-carbinol. It is believed that indole-3-carbinol exhibits its anti-estrogenic effect by stimulating estradiol-2-hydroxylase, via its acid condensation metabolite, indolo[3,2-*b*]carbazole (Liu et al., 1994).

Another indole compound, methylbutadienyl-indole, can also induce the 2-hydroxylase reaction (Ng et al., 1994). Like indole-3-carbinol, the 2′- and 3′-structural isomers of methylbutadienyl-indole can also undergo acid condensation reactions to form isopropyl indolocarbazole and yuehchukene respectively. The latter compound, yuehchukene, is particularly interesting as it exhibits mixed estrogenic and anti-estrogenic effects. However, the mechanism for its anti-estrogenic effect remains to be confirmed (Ng et al., 1994).

In the present study, the structural isomers of methylbutadienyl-indole, together with their acid condensation products, as well as the indole carbinols and indole carboxyaldehydes, were tested on their induction effect of estradiol-2-hydroxylase. The results indicated that only the 3′-isomers could significantly induce the estradiol-2-hydroxylase activity. The necessity of the aryl hydrocarbon receptor in the induction process was also demonstrated by the lack of estradiol-2-hydoxylase induction in aryl hydrocarbon receptor-knockout mice. Besides inducing estradiol-2-hydroxylase activity, yuehchukene also competed with estrogen for binding to the estrogen receptors, both mechanisms might contribute to the anti-estrogenic effect of the drug.

2. Materials and methods

2.1. Animal

Six-week-old female Balb/c (body weight: 19–22 g) and C57B/6 (body weight: 14–17 g) mice were obtained from the Animal House of the Chinese University of Hong Kong. The aryl hydrocarbon receptor-knockout mice (body weight: 15–17 g) are of mixed genetic background (Sv/129/ter × C57B/6) (Fernandez-Salguero et al., 1995). A pair of parental stocks of aryl hydrocarbon receptor-knockout mice was shipped from the National Cancer Institute (National Institutes of Health, Bethesda, MD, USA). They were then bred and reared at the Chinese University of Hong Kong animal rooms on a 12 h light/dark cycle (light: 0600–1800) period. They were used in experiments to determine whether the aryl hydrocarbon receptor was involved in the induction of ethoxyresorufin-*O*-deethylase and estradiol-2-hydroxylase activities.

2.2. Animal treatment

After overnight fasting, the mice were given a single dose of the test compounds per os. The test compounds, including 2′-methylbutadienyl-indole, 3′-methylbutadienyl-indole, isopropyl indolocarbazole, yuehchukene, indole-2-carbinol, indole-3-carbinol, indole-2-carboxyaldehyde, indole-3-carboxyaldehyde and 3-methylcholanthrene, were first dissolved in absolute ethanol before 19 volumes of olive oil were added. For the control group, the mice were given 5% ethanol in olive oil instead. After treatment, the mice were fasted for another 9 h. They were then given free access to food and water for 15 h before being sacrificed.

2.3. Preparation of crude microsomal fraction

The mice were sacrificed by cervical dislocation. Their livers were excised. Livers from two mice were pooled together and treated as one sample. Each sample was immediately homogenized in ice-cold 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol, 10 mM HEPES, pH 7.4 (1:4 w/v) using a Pyrex glass homogenizer. The homogenates were centrifuged at $1200 \times g$ for 30 min at 4°C. The supernatant fractions were further centrifuged at 105,000 $\times g$ for 1 h at 4°C. The pellets were resuspended in 0.1 M potassium phosphate, pH 7.4 containing 1 mM EDTA, 1 mM dithiothreitol, 20% glycerol. The preparations were stored at −70°C before use.

2.4. Protein assay

The protein content was determined by the method of Bradford (1976), using bovine serum albumin as the standard.

2.5. Ethoxyresorufin-O-deethylase assay

The assay was performed according to the procedure of Pohl and Fouts (1980). Twenty five micrograms of protein was incubated in 0.5 ml of an assay medium containing 0.1 mM magnesium sulfate, 1.6 mg bovine serum albumin, 1.5 μM ethoxyresorufin, 50 mM potassium phosphate, pH 7.5. The reaction was initiated by the addition of 150 μl NADPH-regenerating system (0.2 unit of glucose-6-phos-

phate dehydrogenase, 0.28 mM β-NADP and 2.5 mM glucose-6-phosphate). After incubation at 37°C for 15 min, the reaction was terminated by precipitating the protein with 1 ml methanol. After centrifugation, the supernatant fluid was taken for fluorescence measurement with an excitation wavelength of 530 nm and an emission wavelength of 590 nm. The amount of resorufin produced was determined with reference to the standard curve.

2.6. Estradiol-2-hydroxylase assay

Estradiol-2-hydroxylase activity was determined by following the formation of $^{3}H_2O$ from [2-^{3}H(N)] estradiol (15 Ci/mmol) according to the procedure of Ng et al. (1994). Fifty micrograms of protein was incubated in 1 ml of 50 mM potassium phosphate, pH 7.4, containing 0.1 mM magnesium sulfate, 1.6 mg bovine serum albumin and 40 nM [2-^{3}H(N)] estradiol. The reaction was initiated by the addition of 150 μl NADPH-regenerating system as described above. The mixture was incubated at 37°C for 10 min before the reaction was stopped by 200 μl of 30% trichloroacetic acid. One milliliter of dextran–charcoal suspension (0.05% dextran T-70 and 0.5% Norit-A) in 50 mM Tris–HCl, pH 7.4, was added to adsorb the unreacted [2-^{3}H(N)] estradiol. At least 99.85% of the substrate was removed by such treatment. The mixture was allowed to stand at 4°C for 15 min before being centrifuged at 1500 × *g* for 15 min. Aliquots of 0.5 ml were transferred to 4-ml scintillant for radioactivity determination.

2.7. Estrogen / anti-estrogen assays

Randomly bred mice of the highly fecund QS strain were ovariectomised when reaching approximately 30 g body weight. Two weeks after ovariectomy they were primed with 1 μg estradiol subcutaneously and if not used within the following 2 weeks received a further priming dose. For receptor assays, mice were pretreated with 0.1 μg estradiol on days 1 and 2 and were killed by cervical dislocation on day 3 while under sodium phenobarbitone anaesthesia. Uteri were dissected onto ice and stored at −196°C until use. Vaginal smear and estrogen receptor assays were carried out as described by Ng et al. (1994). For anti-uterotrophic assays primed ovariectomised animals received, on days 1, 2 and 3, estradiol with or without increasing doses of the test compounds. The dose of estradiol had previously been determined to double control uterine weight. All doses were given per os. Animals were sacrificed as above on day 4 and uterine wet weight, liver weight and body weight measured.

2.8. Statistical analysis

Statistical analysis was performed by either SigmaStat 2.0, Duncan multiple range test/rank test or Student's *t*-test. All the data were presented as mean ± S.D.

2.9. Chemicals

2′-Methylbutadienyl-indole, 3′-methylbutadienyl-indole, isopropyl indolocarbazole, yuehchukene, indole-2-carboxyaldehyde and indole-3-carboxyaldehyde were synthesized in-house. Indole-2-carbinol and indole-3-carbinol were obtained from Aldrich (Dorset, UK). 3-Methylcholanthrene, 7-ethoxyresorufin, resorufin, activated charcoal (Norit-A), glucose-6-phosphate, β-NADP, glucose-6-phosphate dehydrogenase were all purchased from Sigma (St. Louis, MO, USA). The labeled substrate [2-^{3}H(N)] estradiol (15 Ci/mmol) was from New England Nuclear (Boston, MA, USA).

3. Results

3.1. Optimization of condition

To obtain the optimal conditions for the induction of the isoprenyl compounds on ethoxyresorufin-*O*-deethylase and estradiol-2-hydroxylase activities, Balb/c mice received different doses of indole-3-carbinol and 3′-methylbutadienyl-indole per os and were sacrificed at different times to obtain crude microsomal samples for enzyme determination. Two different doses of the drug, 0.80 and 0.26 mmol/kg body weight, were used. The induction of both enzymes was dose dependent (data not shown). Hence, the dose of 0.80 mmol/kg was adopted for studying the effect of the different isoprenyl compounds. After a single dose of the drug, there was a prompt increase in the activity of both enzymes that reached an optimum after 24 h. The activities remained at this maximal level for another 24 h before starting to decline (data not shown). Consequently, 24 h post-treatment was selected as the time to sacrifice animals in subsequent studies.

3.2. Effects on ethoxyresorufin O-deethylase and estradiol-2-hydroxylase activities

In all experiments, 3-methylcholanthrene, a prototype inducer for cytochrome P450 1A1-catalyzed ethoxyresorufin-*O*-deethylase activity, was used as a positive control. As expected, it gave a more than 10-fold induction in the ethoxyresorufin-*O*-deethylase assay. However, there was no significant effect on the estradiol-2-hydroxylase activity (Table 1).

All the indole carbinols and their respective carboxyaldehydes showed a significant induction in ethoxyresorufin-*O*-deethylase activity. Indole-2-carbinol was the most effective with a 5.4-fold induction. Indole-2-carboxyaldehyde was the least effective one where only a 2.5-fold induction could be observed. The induction effect was different in the estradiol-2-hydroxylase assay. Amongst the four compounds tested, only indole-3-carbinol showed

Table 1
Effects of treatment of different isoprenyl compounds[a] on the activities of ethoxyresorufin-*O*-deethylase (EROD) and estradiol-2-hydroxylase (E_2-2-OHase) in Balb/c mice

Treatment	Dose (mmol/kg)	N[b]	EROD (pmol min^{-1} mg^{-1})	E_2-2-OHase (pmol min^{-1} mg^{-1})
(A) Experiment 1				
Control		3	73 ± 29	1.40 ± 0.19
I-2-C	0.8	3	393 ± 103[c]	1.53 ± 0.29
I-2-CA	0.8	3	186 ± 50[c]	1.01 ± 0.23
I-3-C	0.8	3	297 ± 70[c]	2.79 ± 0.50[c]
I-3-CA	0.8	3	293 ± 74[c]	1.41 ± 0.14
3-MC	1	3	783 ± 118[c]	1.64 ± 0.28
(B) Experiment 2				
Control		3	70 ± 24	1.39 ± 0.23
2′-MBDI	0.8	3	287 ± 37[c]	1.76 ± 0.18
3′-MBDI	0.8	3	336 ± 66[c]	2.45 ± 0.30[c]
3-MC	1	2[d]	959 ± 19	1.56 ± 0.10
(C) Experiment 3				
Control		2	75 ± 16	1.02 ± 0.20
IPP-ICZ	0.08	2[d]	392 ± 72	1.45 ± 0.25
YCK	0.08	2[d]	182 ± 16	2.65 ± 0.13
3-MC	1	2[d]	917 ± 68	1.57 ± 0.19

[a] I-2-C, indole-2-carbinol; I-2-CA, indole-2-carboxyaldehyde; I-3-C, indole-3-carbinol; I-3-CA, indole-3-carboxyaldehyde; 3-MC, 3-methylcholanthrene; 2′-MBDI, 2′-methylbutadienyl-indole; 3′-MBDI, 3′-methylbutadienyl-indole; IPP-ICZ, isopropyl indolocarbazole; YCK, yuehchukene.
[b] Number of samples, each sample contained livers pooled from two mice.
[c] $P < 0.05$, significantly different from the control. Statistical analysis was performed by SigmaStat 2.0, Duncan multiple range tests.
[d] Statistical analysis was not performed because of the limited number of samples.

a statistically significant induction of 2.0-fold (Table 1, experiment 1).

Like the indole carbinols and carboxyaldehydes, both 2′- and 3′-methylbutadienyl-indole could significantly induce ethoxyresorufin-*O*-deethylase by 4- to 5-fold. Both isomers also induced estradiol-2-hydroxylase activity. The 3′-methylbutadienyl-indole was more potent and gave a 1.8-fold induction while only a marginal 1.3-fold induction was observed in the case of 2′-methylbutadienyl-indole (Table 1, experiment 2).

Under acid conditions, 2′-methylbutadienyl-indole could condense to form isopropyl indolocarbazole (Lee et al., 1996) and a similar reaction occurs for 3′-methylbutadienyl-indole to give yuehchukene (Cheng et al., 1985). The yield of these acid condensation reactions was about 10%; hence, in studying their effects, a dose of only one-tenth of that used for the methylbutadienyl-indoles, i.e., 0.08 mmol/kg body weight, was used. The results showed that isopropyl indolocarbazole was more potent (5.2-fold) in the induction of ethoxyresorufin-*O*-deethylase; by contrast, yuehchukene gave a higher increase (2.6-fold) in the induction of estradiol-2-hydroxylase activity (Table 1, experiment 3).

3.3. Effects on body weight, liver weight and total microsomal protein content

Treatment with the various isoprenyl compounds did not seem to have any significant effect on body weight, liver weight and total microsomal protein content of the animals, demonstrating no obvious acute toxicity. The decrease was less than 4% in all the three parameters. In contrast, a larger decrease of 7 to 8% in the body and liver weight was observed in the 3-methylcholanthrene treated mice (Table 2).

3.4. Effects on the aryl hydrocarbon receptor-knockout mice

All the drugs failed to give any significant induction of either enzyme activity in the aryl hydrocarbon receptor-knockout mice (Table 3). However, for the wild-type C57B/6 mice, the effects of treatment with indole-3-carbinol and 3′-methylbutadienyl-indole were similar to those for the Balb/c mice. Both compounds could induce ethoxyresorufin-*O*-deethylase (4.8- to 6.2-fold) and estradiol-2-hydroxylase (1.9- to 2.1-fold) activities.

3.5. Vaginal smear assays

None of the compounds tested, at doses up to 100 times that of estradiol, showed evidence of either estrogenic or anti-estrogenic activity (data not shown).

Table 2
Effects of treatment with different isoprenyl compounds[a] and 3-MC on the body weight, liver weight and total microsomal protein content in Balb/c mice

Treatment	N[b]	Body weight (g)	Liver weight (g)	Total microsomal protein content (mg)
Control	8	40.5 ± 3.0	2.63 ± 0.18	16.7 ± 1.2
Isoprenyl compounds	22	39.6 ± 2.2	2.59 ± 0.21	16.1 ± 2.2
3-MC	7	37.2 ± 2.6[c]	2.45 ± 0.20	16.6 ± 2.0

[a] Isoprenyl compounds included indole-2-carbinol, indole-2-carboxyaldehyde, indole-3-carbinol, indole-3-carboxyaldehyde, 2′-methylbutadienyl-indole, 3′-methylbutadienyl-indole, isopropyl indolocarbazole and yuehchukene.
[b] Number of group of animal or tissue. The number in each group is two.
[c] $P < 0.05$, significantly different from the control. Statistical analysis was performed by Student's *t*-test.

Table 3
Effects of treatment with I-3-C, 3′-MBDI and 3-MC on the activities of EROD and E_2-2-OHase in C57B/6 mice and aryl hydrocarbon receptor-knockout mice

Treatment	Dose (mmol/kg)	N[a]	EROD (pmol min^{-1} mg^{-1})	E_2-2-OHase (pmol min^{-1} mg^{-1})
(A) C57B/6 mice				
Control		3	68 ± 26	0.76 ± 0.33
I-3-C	0.8	3	325 ± 82[b]	1.61 ± 0.47
3′-MBDI	0.8	3	419 ± 15[b]	1.46 ± 0.10[b]
3-MC	1	3	1172 ± 540[b]	1.56 ± 0.29[b]
(B) Aryl hydrocarbon receptor-knockout mice				
Control		3	16 ± 3	0.51 ± 0.09
I-3-C	0.8	2[c]	7 ± 6	0.40 ± 0.22
3′-MBDI	0.8	3	14 ± 11	0.69 ± 0.58
3-MC	1	3	15 ± 5	0.63 ± 0.26

See Table 1 for the abbreviations.
[a]Number of samples, each contained livers pooled from two mice.
[b]$P < 0.05$, significantly different from the control. Statistical analysis was performed by SigmaStat 2.0, rank test.
[c]Statistical analysis was not performed because of the limited number of samples.

3.6. Estrogen receptor assay

Yuehchukene, as expected, showed some competition with estradiol for binding to the estrogen receptor. All other compounds showed no dose-dependent competition (Fig. 1).

3.7. Anti-uterotrophic assay

No compound showed anti-estrogenic activity in this assay but 3′-methylbutadienyl-indole showed a potentiation of the effects of estradiol. No significant effects on body or liver weight were observed (data not shown).

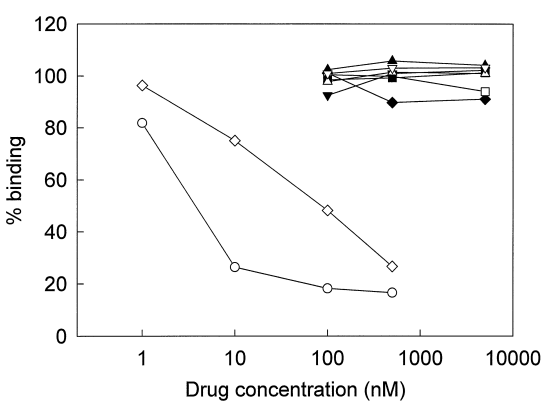

Fig. 1. Uterine cytosolic estrogen receptor binding. Uterine homogenate (2 mg/ml) was incubated with 3 nM [^{3}H]-estradiol in the presence of different concentrations of test compounds. (○) Estradiol; (▲) indole-2-carbinol; (▼) indole-2-carboxyaldehyde; (△) indole-3-carbinol; (▽) indole-3-carboxyaldehyde; (■) 2′-methylbutadienyl-indole; (□) 3′-methylbutadienyl-indole; (♦) isopropyl indolocarbazole; (◇) yuehchukene.

4. Discussion

Estrogen can be oxidized, reduced or hydroxylated at several positions to yield a number of metabolites with differing estrogenic activities. Presently interest is turned to the induction of the 2-hydroxylation reaction to form inactive catechol estrogen at the expense of the formation of 16α-hydroxylated estrogen.

Cruciferous vegetables are rich in glucosinolate and it will be transformed into indole-3-carbinol and then further to indolo[3,2-*b*]carbazole, under acidic conditions. Indole-3-carbinol has been shown to induce the estrogen 2-hydroxylation pathway in both cell culture (Tiwari et al., 1994) and animal studies (Michnovicz and Bradlow, 1990). Its beneficial effect in reducing the risk of human breast cancer has also been discussed (Michnovicz and Bradlow, 1990). Its acid-derived condensation product, indolo[3,2-*b*]carbazole, has been shown to possess both estrogenic and anti-estrogenic activity (Liu et al., 1994). Similar mixed estrogenic and anti-estrogenic activities have been observed in our study of yuehchukene, a *bis*-indole alkaloid isolated from *Murraya paniculata* (Rutaceae) (Ng et al., 1994). The mixed estrogenic and anti-estrogenic activities of these compounds suggest a possible treatment of human breast cancer by modulating the level of estrogen to maintain a lower level of action so that it is sufficient to protect against osteoporosis and heart disease in menopausal women while lowering the risk of breast cancer in this group.

The present study investigated the effects of some isomers/derivatives of indole-3-carbinol and yuehchukene in an attempt to understand the structural requirement for the induction of estradiol-2-hydroxylase. We also tried to examine whether such induction is related to the induction of ethoxyresorufin-*O*-deethylase, as suggested previously

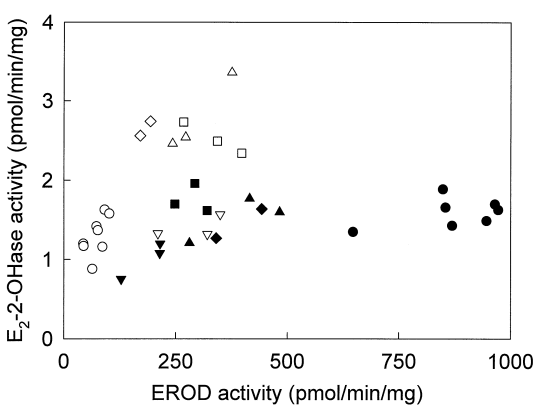

Fig. 2. Correlation between the activities of ethoxyresorufin-*O*-deethylase (EROD) and estradiol-2-hydroxylase (E_2-2-OHase) in Balb/c mice after treatment with different compounds. (○) Control; (▲) indole-2-carbinol; (▼) indole-2-carboxyaldehyde; (△) indole-3-carbinol; (▽) indole-3-carboxyaldehyde; (■) 2′-methylbutadienyl-indole; (□) 3′-methylbutadienyl-indole; (♦) isopropyl indolocarbazole; (◇) yuehchukene; (●) 3-methylcholanthrene.

(Tiwari et al., 1994; Leighton et al., 1995). There appeared to be no direct relationship between the two enzyme activities (Fig. 2). The 3′-substituted compounds, except indole-3-carboxyaldehyde, were relatively potent in inducing estradiol-2-hydroxylase. On the other hand, 3-methylcholanthrene was potent only in inducing ethoxyresorufin-*O*-deethylase. In the 3-methylcholanthrene treated mice, the 10-fold induction in ethoxyresorufin-*O*-deethylase contrasts dramatically with the negligible increase in estradiol-2-hydroxylase activity. 3-Methylcholanthrene is well known as an inducer of cytochrome P450 1A1 (Riddick et al., 1994) and the ethoxyresorufin-*O*-deethylase is also a standard assay for such isoform (Pohl and Fouts, 1980). Thus, the negligible effect of 3-methylcholanthrene on the estradiol-2-hydroxylase activity suggested that the lowering of estrogenic activity was not related to cytochrome P450 1A1 activity. The specific isoform involved in 2-hydroxylation has yet to be determined. In rats, a wide variety of cytochrome P450s from families 1A, 2B, 2C and 3A have been implicated in such reactions (Ball et al., 1990). In human, cytochrome P450 1A2 (Aoyama et al., 1990), 3A4/3A5 (Kerlan et al., 1992) and 1B1 (Hayes et al., 1996) have also been reported to exhibit such activity. Nevertheless, using knockout mice with a default aryl hydrocarbon receptor, the present study confirmed the previous demonstration by direct receptor binding studies (Jellinck et al., 1993) that the induced estradiol-2-hydroxylase was mediated by a mechanism involving the aryl hydrocarbon receptor.

These studies provide some clues to the mechanism of the anti-estrogenic effect of yuehchukene. Treatment with 3′-methylbutadienyl-indole, the precursor of yuehchukene, resulted in a significant induction of estradiol-2-hydroxylase. Under the acid condition of the stomach, it is believed that 3′-methylbutadienyl-indole will undergo an acidic condensation reaction that leads to the formation of yuehchukene-like compound which then induces estradiol-2-hydroxylase. Treatment with yuehchukene directly showed an induction of 2.6-fold in the activity of this enzyme. This result is different from our previous study in rats in which the induction of estradiol-2-hydroxylase was only minimal (Ng et al., 1994). The most likely explanation for the discrepancy may be the different doses of yuehchukene used. In the previous study (Ng et al., 1994), a dose equivalent to only 2.7 μmol/kg body weight, which is about 1/30 of that employed in the present study, was used. At such a low dose, it also failed to show any induction on the ethoxyresorufin-*O*-deethylase activity (Ng et al., 1994). Besides the induction of estradiol-2-hydroxylase, the present study also demonstrated that yuehchukene competed with estrogen for binding to the estrogen receptor. This might also contribute to its anti-estrogenic activity at high estrogen level.

In conclusion, the present study provides insights into the biochemical basis for the anti-estrogenic effect of yuehchukene. Yuehchukene through induction of estradiol-2-hydroxylase diminished the supply of potent estrogenic species. Yuehchukene also competed with estrogen for receptor binding. Such mixed estrogenic and anti-estrogenic properties (Ng et al., 1994), make yuehchukene appear as another natural compound which could serve as a promising prototype in the design of drugs for the management of breast cancer.

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