Aromatase and Estrogen 2-Hydroxylase Activities of Human Placental Microsomes in Pregnancy-Induced Hypertension

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Abstract. 2-Hydroxylation is one of the major metabolic pathways of estrogens and is believed to be catalyzed by a form of cytochrome P450. Recently it has been reported that estrogen 2-hydroxylase activity in human placenta is catalyzed by aromatase. Some investigators suggested the effect of catechol estrogen on human placental steroidogenesis which may be related to pregnancy-induced hypertension (PIH) through the inhibition of catechol-O-methyltransferase (COMT) activity. In order to better understand the interrelationship between placental aromatase and estrogen 2-hydroxylase activities in PIH patients, both activities were evaluated in the PIH placentas. Human placental microsomes obtained from PIH patients were incubated with [1/3-3H]androstenedione or [2-3H]estradiol in the presence of NADPH. Aromatase and estrogen 2-hydroxylase activities were assessed by the tritium water method. The immunosuppression patterns of both activities due to monoclonal anti-aromatase cytochrome P450 antibody (MAb3-2C2) were studied. Estrogen 2-hydroxylase activity was significantly higher in PIH placentas (4.7 ± 0.9 pmol/min/mg protein, n=7) than in normal placentas (3.0 ± 0.7 pmol/min/mg protein, n=7). When the PIH placental microsomes were subjected to immunosuppression by 1 to 100 µg IgG of MAb3-2C2, estrogen 2-hydroxylase activity was suppressed by 94 to 65% whereas aromatase activity was strongly suppressed by 72 to 17%, respectively. From our results of high estrogen 2-hydroxylase activity in PIH placentas, it is assumed that there is a different estrogen catalyzing mechanism in PIH placentas.

Key words: Human placenta, Aromatase, Estrogen 2-hydroxylase, Pregnancy-induced hypertension

HUMAN placental aromatase is a cytochrome P450 enzyme complex which catalyzes an important biotransformation of androgens to estrogens [1]. Microsomal 2-hydroxylation is one of the major metabolic pathways of estrogens [2] and reported to occur in liver [3], brain [4] and placenta [5]. The hydroxylation at C-2 position of the aromatic A ring results in the formation of catechol estrogens [6]. Microsomal 2-hydroxylation of estradiol by human term placenta was first described by Fishman and Dixon in 1967 [5], but the specific cytochrome P450s responsible for this conversion have not been identified. But estrogen 2-hydroxylation by human placental microsomes is believed to also be catalyzed by a distinct cytochrome P450 enzyme [7]. Recently, the capacity of estrogen 2-hydroxylase activity in the human placental aromatase has been described [8]. Osawa et al. [8], with the purified and reconstituted placental aromatase P450, showed evidence that human placental estrogen 2-hydroxylation and aromatization are catalyzed by the same human placental aromatase P450. They also confirmed that both
activities were exhibited in Chinese hamster ovarian cells transfected with human placental aromatase cDNA, pH β-Aro. On the other hand, it has been suggested that the effect of catechol estrogens on human placental steroidogenesis may be related to pregnancy-induced hypertension (PIH) because of evidence indicating that the catechol estrogens can modify the action of catecholestamines by inhibiting of catechol-O-methyltransferase (COMT) as competitive inhibitors [9-11]. However, the metabolism and physiological significance of catechol estrogens have not yet been determined completely. In order to better understanding the relationship between aromatase and estrogen 2-hydroxylase, both activities in placental microsomes obtained from PIH patients were investigated in this study.

Materials and Methods

Chemicals

[1β-3H]Androstenedione (75% at 1β) and [2-3H]estradiol were purchased from Dupont (New England Nuclear, Boston, MA, USA). Trichloroacetic acid (TCA), dithiothreitol (DTT), bovine serum albumin (BSA), and NADPH were purchased from Sigma Chemical Company (St. Louis, MO, USA). The monoclonal anti-aromatase cytochrome P450 antibody, MAb3-2C2 [8], was a generous gift from Dr. Y. Osawa (Hauptman-Woodward Medical Research Institute Inc., Buffalo, NY, USA). All other regents were of analytical grade.

Subjects

Human placentas at term were obtained following delivery from seven subjects of PIH, three subjects of intrauterine growth retardation (IUGR) without PIH, and seven subjects of normal pregnancy as the control at Showa University Hospital. All aspects of this study were approved by the Showa University ethical committee. There were no placentas from women who were known to be smokers during pregnancy. There were no differences in the pregnancy period or placental weight among these groups. Human placental microsomes were prepared according to a modified method of Ryan [12] as previously described [13]. The protein concentration was determined by protein-dye binding method (TONEIN-TP, OZUKA).

Aromatase assay

Aromatase activity was determined by the tritiated water method with [1β-3H] androstenedione as previously described [14]. Briefly, human placental microsomes (50 μg protein) and BSA (5 mg) were preincubated with [1β-3H] androstenedione (3.09 × 10³ dpm ³H/ng, 0.1 μM) as substrate at 37 °C in a total volume of 1.0 ml of 0.1 M phosphate buffer (pH 7.6). After 2 min, NADPH (final concentration 0.75 mM) in 0.1 ml of the phosphate buffer was added (time zero). The reaction was terminated after 5 min by the addition of 0.25 ml of 30% TCA and 0.5 ml of 5% activated charcoal suspension. The mixture was then shaken for 30 min and filtered through a cotton-plugged Pasteur pipette. A 1.0 ml aliquot of the filtrate was mixed with 10 ml of Aquasol 2 (Dupont, New England Nuclear, Boston, MA, USA) and counted in an Aloka LSC-651 liquid scintillator. Aromatase activity was assessed by the tritium released into water from the 1β-position of the substrate (75% release into water) [15]. The results were expressed as the mean ± SD and the comparison between the groups was performed by applying Student’s t-test.

Estrogen 2-hydroxylase assay

A modification of the procedure of Dannan et al. [7] based on the tritiated water method was used to determine estrogen 2-hydroxylase activity as previously reported [8]. Human placental microsomes (0.5 mg protein) were incubated for 20 min at 37 °C with [2-3H]estradiol (1.5 × 10⁵ dpm ³H/μg, 5 μM) as substrate in the presence of NADPH (0.75 mM) in a total volume of 1.0 ml of 0.1 M phosphate buffer (pH 7.6). The incubation was terminated by the addition of 0.25 ml of 30% TCA and 0.5 ml of 5% activated charcoal suspension. The sample was further processed in the same manner as in the aromatase assay. Estrogen 2-hydroxylase activity was assessed by tritium release into water from the 2-position of the substrate.
Monoclonal antibody suppression of human placental aromatase and estrogen 2-hydroxylase activities

To determine monoclonal anti-aromatase P450 antibody (MAb3-2C2) suppression, human placental microsomes which were obtained from PIH were preincubated with [1β-3H] androstenedione or [2-3H]estradiol as substrates and 1–100 μg IgG of MAb3-2C2 in the same manner under our standard conditions.

Results

The results of the initial kinetic analysis of placental estrogen 2-hydroxylase are shown in Fig. 1. The Km value of 0.45 μM for estradiol with a Vmax of 12.9 pmol/min/mg protein is shown.

As shown in Table 1, aromatase and estrogen 2-hydroxylase activities in normal placentas were 76.2 ± 16.9 and 3.0 ± 0.7 pmol/min/mg protein (mean ± SD, n=7) with the Km of 22 nM and 0.45 μM, respectively. On the other hand, aromatase and estrogen 2-hydroxylase activities in PIH placentas were 81.0 ± 13.5 and 4.7 ± 0.9 pmol/min/mg protein, respectively. Although there are no significant differences between estrogen 2-hydroxylase activities of IUGR without PIH and normal placentas, estrogen 2-hydroxylase activity in PIH is significantly higher than that in normal placentas.

Considering that there are differences in the aromatase P450 concentration per mg protein in each placenta, the ratio of aromatase to estrogen 2-hydroxylase activity is estimated. As shown in Fig. 2, the figure obtained by dividing estrogen 2-hydroxylation by aromatization in PIH placentas (0.06 ± 0.01, n=7) is significantly higher (P<0.02) than that in normal placentas (0.04 ± 0.01, n=7).

When the PIH placental microsomes were subjected to immunosuppression by 1 to 100 μg IgG of MAb3-2C2, estrogen 2-hydroxylase activity was suppressed by 94 to 65%, but aromatase activity was strongly suppressed by 72 to 17%. The 65% of estrogen 2-hydroxylase activity was maintained by adding 100 μg IgG of MAb3-2C2, as shown in Fig. 3.

Discussion

Previous investigators [16-18] have reported a decrease in human placental aromatase activity in placentas obtained from smokers. It was also reported that human placentas from smokers exhibited enhanced catechol estrogen formation [19]. A study [18] has shown that aromatase activity in placentas of severe toxemia with IUGR was lower than that in normal placentas, while aromatase activity in placentas of IUGR without any

| Table 1. Gestational age, placental weight and placental microsomal aromatase and estradiol (E2) 2-hydroxylase activities |
|-----------------|-----------------|-----------------|
| weeks           | placental weight (g) | aromatase activity (pmol/min/mg) | E2 2-hydroxylase activity (pmol/min/mg) |
| normal (n=7)    | 38.5 ± 2.5        | 425.7 ± 76.5    | 76.2 ± 16.9      |
| PIH (n=7)       | 38.9 ± 1.8        | 415.7 ± 106.9   | 81.0 ± 13.5      |
| IUGR (n=3)      | 39.3 ± 0.1        | 390 ± 149.3     | 77.9 ± 23.1      |

* mean ± SD. * P<0.01.
complication was not significantly lower, but little is known concerning estrogen 2-hydroxylase activity in placentas obtained from PIH. And there has been no comparison of aromatase and estrogen 2-hydroxylase activities in the same PIH placenta. This study firstly demonstrated that estrogen 2-hydroxylase activity in PIH placentas is significantly higher than that in placentas of normal subjects and IUGR without PIH. The ratio of aromatase to estrogen 2-hydroxylase activity in PIH placentas was also higher than that in normal placentas. It has been reported that the 2-hydroxyestrogens can modify the action of catecholamines by inhibiting COMT and a relationship between catechol estrogen metabolism and PIH [9-11] has been suggested. Gelbke et al. [6] showed that the administration of 20 mg of estrone increased systolic and diastolic blood pressure 1 h after the injection at which time the maximal increase in 2-methoxyestrone levels in serum was observed. The affinity of COMT to catechol estrogen is 100-fold higher than to catecholamines [20]. It has been demonstrated that COMT activity in erythrocytes was not significantly different in toxemia and normal pregnant women [21], but there are no data showing that COMT activity in the placenta of PIH patients is lower than that in normal pregnant women. Kono [9] measured human plasma unconjugated 2-hydroxyestrone and 2-hydroxyestradiol in pregnancies by specific radioimmunoassay, and reported that the levels of catechol estrogens increased tremendously as pregnancy progressed. The human placental catechol estrogen biosynthesis during pregnancy therefore seems to be an important factor in hypertension through the inhibition of COMT activity by catechol estrogens. Although it was recognized that the limitations of in vitro experimental techniques would preclude direct extrapolation of these results to in vivo situations, human placental catechol estrogens may contribute to the mechanism of hypertension during pregnancy. Osawa et al. [8] recently found evidence that human placental estrogen 2-hydroxylase is catalyzed by aromatase P450 through purification and reconstitution in normal placentas, and also evidence in a stable aromatase cDNA expression system. Taking the results obtained in the present study of high estrogen 2-hydroxylase activity and normal aromatase activity in PIH into consideration, several possible explanations can be postulated. For instance, the change in enzyme conformation due to some factors may be related to high estrogen 2-hydroxylase activity in PIH placentas even though aromatase and estrogen 2-hydroxylase are catalyzed at the same active site. One of the possibilities is that estrogen 2-hydroxylase activity in PIH placentas may be catalyzed not only at the same site of aromatase P450 activity but also by a different monoxygenase P450 activity. Detailed analysis with immunosuppression by MAb3-2C2 was therefore carried out. In the study

Fig. 2. Comparison of the ratio of aromatase to estrogen 2-hydroxylase activity between normal and PIH placentas. Results are expressed as the mean ± SD of assays. * P<0.02.

Fig. 3. Monoclonal antibody suppression of microsomal enzyme activities. Human placental microsomes were incubated with appropriate substrates and NADPH to assess aromatase and estradiol 2-hydroxylase activities after preincubation with MAb3-2C2 as described in Materials and Methods. Aromatase activity of human placental microsomes (●), estrogen 2-hydroxylase activity of human placental microsomes (○).
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by Osawa et al., when the purified and reconstituted aromatase was subjected to immunosuppression by MAβ3-2C2, the inhibition pattern of estradiol 2-hydroxylase activity was almost identical to that of aromatase activity, but rat liver microsomal 2-hydroxylase activity was not suppressed by MAβ3-2C2 [8]. In our results with placental microsomes obtained from PIH, estrogen 2-hydroxylase activity was not completely by MAβ3-2C2, but aromatase activity was strongly immunosuppressed by the same dose of MAβ3-2C2. The discrepancy between the results obtained from normal and PIH placentas may be partially explained by the second possibility mentioned above. Although aromatase is the predominating cytochrome P450, Gough et al. [22] reported the presence of another enzyme which catalyzes the hydroxylation of xenobiotic substances. Numerous studies on the development of the drug-metabolizing system in fetuses and placentas have been published [22–25]. For example, it has been reported that a placental cytochrome P450 is responsible for the hydroxylation of benzo[α]pyrene via aryl hydrocarbon hydroxylase (AHH). Previous studies on AHH activity have demonstrated higher levels of this activity in placentas of smokers but not in those of nonsmokers [19, 25]. Pasanen et al. [26] indicated that P450 1A1 is probably responsible for cigarette-smoke induced activity. Furthermore, human placentas obtained from smokers exhibited enhanced catechol estrogen formation and this activity appeared to be highly correlated with placenta AHH activity [19]. Kitawaki et al. [18] reported results showing that the ratio of aromatase to AHH activity was much higher in smoker placentas than in normal placentas. Moreover, Spink et al. [27] reported that P450 1A1 catalyzed the hydroxylation of estradiol at the C-2, C-15α, and C-6α in incubations with microsomes from 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin-treated MCF-7 cells, whereas the expression of P450 1A2 in human liver is known to catalyze microsomal hydroxylation predominantly at C-2. These reports could be one of the explanations of our results that the elevated estrogen 2-hydroxylase activity in PIH placentas might be catalyzed by aromatase P450 and another inducible cytochrome P450.

The present results demonstrating high 2-hydroxylase activity in PIH placentas are of clinically interest as it may be related to COMT activities and high concentrations of catechol estrogens in PIH patients. However, further studies to clarify this mechanism have to be undertaken in the future.

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References


