Identification of 16 α ,19-Dihydroxyandrostenedione in the Serum of Pregnant Women by Gas Chromatography-Mass Spectrometry

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16 α ,19-Dihydroxyandrostenedione [16 α ,19-(OH)₂ AD], a potential precursor of estriol, was identified in the serum of pregnant women by gas chromatography-mass spectrometry (GC-MS). Preliminary study indicated that the average serum concentration of 16 α ,19-(OH)₂ AD in 10 normal pregnant women was 154.2 $\pm$ 20 pg/ml. In contrast, the serum concentration of 16 α ,19-dihydroxydehydroepiandrosterone was less than the detection limit (30 pg/ml) of the GC-MS method.

Key words 16 α ,19-dihydroxy androgen; serum concentration; pregnant woman; estriol formation; feto-placental unit

19-Hydroxyandrostenedione (19-OH AD) has been reported to be an amplifier of aldosterone action and is itself a hypertensive steroid.^{1–5} The concentration of 19-OH AD in plasma of patients with hypertensive disease of pregnancy is significantly higher than that of normotensive pregnant women at the same time of gestation.^{6–8} Serum level of 19-OH AD increases during pregnancy. This is mainly due to the increase of not only placental aromatase which catalyzes the first hydroxylation at C-19 of androstenedione (AD), producing the 19-hydroxy steroid, during aromatization sequence, but also AD in placenta available for aromatase reaction.⁹

Estriol, the major estrogen in pregnancy, essentially originates in the placenta; 16 α -hydroxylated C₁₉ steroid precursors, 16 α -OH AD and 16 α -hydroxydehydroepiandrosterone (16 α -OH DHEA), which are produced in fetus, are converted into estrogen by placental aromatase.^{9,10} The production of estriol proceeds through their 19-hydroxy intermediates, similar to the AD aromatization.^{11,12} Based on these facts, it is suggested that the 16 α ,19-dihydroxy steroids, 16 α ,19-(OH)₂ AD and 16 α ,19-(OH)₂ DHEA, may be found as steroid metabolites in the serum of pregnant women and may be involved in the regulation of blood pressure in pregnant subjects.

Taken together, we were interested in the identification and quantitative analysis of 16 α ,19-dihydroxy steroids in human. Thus, we report here their determination in the serum of the third trimester pregnant women with a normal blood pressure using gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

16 α ,19-(OH)₂ AD and 16 α ,19-(OH)₂ DHEA¹³ and their deuterated compounds,¹⁴ [7,7,16 β -²H₃]-16 α ,19-(OH)₂ AD and [2 β ,7,7,16 β -²H₄]-16 α ,19-(OH)₂ DHEA, were synthesized according to the methods previously reported.

Extraction of the 16 α ,19-Dihydroxy Steroids from the Serum of Pregnant Women The deuterated 16 α ,19-(OH)₂ AD and 16 α ,19-(OH)₂ DHEA (2000 pg each), internal standards, were added to the serum (2 ml) and then lipid substance was removed by extraction with hexane (2 ml), thereafter, the steroids were extracted with ethyl acetate contain-

ing 10% methanol (2 ml $\times$ 3). After evaporation of the solvent, the residue was dissolved in 250 μ l of methanol followed by dilution with water (5 ml) and then placed in a Sep-PaK C₁₈ cartridge (Waters, Milford, MA, U.S.A.). After a wash with water (5 ml), followed by 5% (v/v) methanol (5 ml), the steroid fraction was eluted with 50% (v/v) methanol (2.5 ml). After evaporation of the solvent, the dihydroxy steroids were converted into the corresponding 16 α ,19-bis(trimethylsilyl) (bis-TMS) derivative according to the method previously reported,¹⁴ and then analyzed by GC-MS. The recovery rates for 2000 pg of 16 α ,19-(OH)₂ AD and 16 α ,19-(OH)₂ DHEA added to the standard serum were 92.2 $\pm$ 4.3 and 91.7 $\pm$ 2.1% ($n=4$), respectively.

Calibration curves of the two steroids added to the standard serum were linear between 100 and 5000 pg using 2000 pg of the corresponding deuterated internal standards.

GC-MS Conditions A Hewlett Packard 5970B GC-MS (Palo Alto, CA, U.S.A.) and a Finnigan MAT SSQ GC-MS (San Jose, CA, U.S.A.) instruments were used. GC and MS conditions for the HP system (electron impact, EI) and for the SSQ system (chemical ionization, CI) were essentially similar to those previously reported, respectively.^{14,15}

The quantitative analysis of the bis-TMS derivatives of the 16 α ,19-dihydroxy steroids was conducted with a selected ion

Fig. 1. Structures of 16 α - and/or 19-Hydroxy Steroids

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Fig. 2. EI-Mass Spectra of the Bis-TMS Derivative of $16\alpha,19-(\text{OH})_2$ AD in the Serum of Pregnant Women (A) and That of the Authentic Sample (B)

Fig. 3. CI-Mass Spectra of the Bis-TMS Derivative of $16\alpha,19-(\text{OH})_2$ AD in the Serum of Pregnant Women (C) and That of the Authentic Sample (D)

monitoring method (EI mode) with a fragment ion, m/z 432 and 436 ($M^+ - 30$) for $16\alpha,19-(\text{OH})_2$ AD and its deuterated internal standard, respectively, and with a base ion, m/z 356 and 359 ($M^+ - 18$, a base peak ion) for the DHEA derivative and its deuterated internal standard, respectively.

The detection limits of the dihydroxy steroids were 20 pg for the AD analogs and 10 pg for the other, respectively, at a S/N ratio of 3.

RESULTS AND DISCUSSION

Identification of $16\alpha,19-(\text{OH})_2$ AD and $16\alpha,19-(\text{OH})_2$ DHEA in the serum of the normotensive pregnant women was first carried out. Mass spectra (EI mode) and retention time ($t_R = 15.1$ min) of the $16\alpha,19$ -bis-TMS derivative of $16\alpha,19-(\text{OH})_2$ AD were identical with those of the authentic sample,¹⁴⁾ respectively (Fig. 2). The CI-MS spectrum showed a protonated molecular ion ($[M+H]^+$, m/z 447) and was also

identical with the authentic sample (Fig. 3).

In contrast, another dihydroxy steroid, $16\alpha,19-(\text{OH})_2$ DHEA, could not be detected in the serum at a level as low as 30 pg/ml, the detection limit ($S/N=3$) of the assay used in this study. A 4-en-3-one structure is suitable for a substrate for aromatase but a steroid having a 3β -hydroxy-5-ene structure such as DHEA is known to be a poor substrate for aromatase. This can explain the present results.

The two $16\alpha,19$ -dihydroxy steroids could not be detected in the serum of non-pregnant women (both follicular and luteal phases).

The concentration of $16\alpha,19-(\text{OH})_2$ AD in the serum (37 to 40 weeks, $n=10$) was initially determined using the GC-MS method (Table 1). The concentration (154 pg/ml) was lower than that (11.2 ng/ml,¹⁶ 1.52 ng/ml,⁸) or 345 pg/ml⁶) of 19-OH AD in the normal third trimester pregnant women with a normal blood pressure. Since estriol is the major estrogen in the serum of the pregnant women, it is supposed that $16\alpha,19-(\text{OH})_2$ AD, a potential intermediate of estriol formation in a feto-placental unit, may be observed at a concentration in the material peripheral plasma, being comparable with that of 19-OH AD, a potential intermediate of estrone/estradiol formation. However, the observed lower concentration of $16\alpha,19-(\text{OH})_2$ AD in the maternal plasma than that of 19-OH AD suggests that the $16\alpha,19$ -dihydroxy steroid may not be released from the active site of aromatase during 16α -OH AD aromatization so much as 19-OH AD during AD aromatization; in other words, aromatization of 16α -OH AD may proceed more efficiently than that of AD in placenta.

Preliminary study shows that $16\alpha,19-(\text{OH})_2$ AD significantly affects the blood pressure of rat (private communication from Dr. H. Sekihara); thus, further studies concerning the concentrations in pregnancy as well as the correlation with the gestation progress and hypertensive disease of pregnancy are required.

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Table 1. The Serum Concentrations of the $16\alpha,19$ -Dihydroxy Steroids in the Serum of the Third Trimester Pregnant (37 to 40 Weeks) Women

Steroid	Serum concentration pg/ml ^{a)} (range)
$16\alpha,19-(\text{OH})_2$ AD	154.2 ± 15 (120—185)
$16\alpha,19-(\text{OH})_2$ DHEA	ND ^{b)}

a) Mean $\pm$ S.D. ($n=10$). b) Not detected.

versity) for valuable discussion.

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