Serum 2-Hydroxyestradiol 17-Sulphate in Pregnancy

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Abstract. 2-Hydroxyestradiol 17-sulphate (2-OH E₂-17-S) is a catecholized form of sulphated estrogen. *In vitro* studies showed that its antioxidative effect is almost equal to that of free catecholestrogens, such as 2-OH E₂ or 4-OH E₂ and α -tocopherol, but the existence of 2-OH E₂-17-S in human serum has not yet been made clear. 2-OH E₂-17-S strongly antagonizes lipid peroxidation, and so it may play an important role in pregnancy, for example as an anti-oxidant in pregnancy-induced hypertension (PIH). The serum level of 2-OH E₂-17-S was measured during mid to late pregnancy by a direct radioimmunoassay (RIA) without hydrolysis. The serum levels at 28–31 weeks, 32–35 weeks and 36–40 weeks of gestation were 4.68 ± 0.93 (mean $\pm$ SE), 8.38 ± 1.21 and 18.31 ± 3.41 nmol/l, respectively. The serum level in PIH cases at 36–40 weeks (4.64 ± 1.29 nmol/l) was significantly lower than that in normal pregnancy. The 2-OH E₂-17-S level in umbilical arteries was significantly higher than that in maternal peripheral vein. These results suggest that the feto-placental unit plays an important role in catecholizing E₂-17-S to 2-OH E₂-17-S, which may act as an antioxidant in pregnancy.

Key words: 2-Hydroxyestradiol 17-sulphate, Pregnancy, Serum level, Radioimmunoassay, Pregnancy-induced hypertension

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PREVIOUSLY we measured the serum level of estradiol 17-sulphate (E₂-17-S), a sulphated estrogen, during late pregnancy by a direct radioimmunoassay [1]. E₂-17-S is quickly metabolized in the body to 2-hydroxyestradiol 17-sulphate (2-OH E₂-17-S) and/or 4-hydroxyestradiol 17-sulphate (4-OH E₂-17-S) [2, 3]. Both 2-OH E₂-17-S and 4-OH E₂-17-S are more stable forms than the free catechol estrogens (2-OH E₂, 4-OH E₂) [4] and strongly antagonize lipid peroxidation [5]. The serum level of E₂-17-S showed a significant negative regression line with that of lipid peroxides in pregnancy [1]. In the case of pregnancy-induced hypertension (PIH), the serum levels of lipid peroxide was reported as higher than in un-

complicated pregnancy [6]. And the serum level of E₂-17-S was lower in cases of pregnancy-induced hypertension (PIH) than in normal pregnancy [7]. In this study, the serum level of 2-OH E₂-17-S was measured by a direct radioimmunoassay (RIA) in maternal peripheral vein during mid to late pregnancy including cases of PIH, as well as in umbilical vessels at delivery.

Materials and Methods

Reagents and antiserum for 2-OH E₂-17-S

2-OH E₂-17-S [2, 8] and [6, 7-³H] 2-OH E₂-17-S (S.A.47.4 Ci/nmol) [9] were prepared as described previously. Unlabelled conjugated and free steroids were purchased from Sigma Chemical Co. (St Louis, MO). Organic solvents and chemicals used for this experiment were of analytical grade. The antiserum used was obtained from a rabbit immunized with 2-

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OH E₂-17-S-[C-6]-bovine serum albumin conjugate [9]. The antiserum gave a high specificity for 2-OH E₂-17-S, exhibiting no more than 0.1% cross reactivity for any other steroids.

Collection of samples

The peripheral blood samples were collected between 9.00 a.m. and 13.00 a.m. from pregnant women (n=56). These samples were obtained from pregnant women without complication until term or with PIH in order to measure the levels of 2-OH E₂-17-S. The criteria for PIH in this study was as follows: hypertension, systolic blood pressure higher than 160 mmHg and/or diastolic blood pressure higher than 110 mmHg. Lipid peroxide was measured in these women without complication. Samples of umbilical arterial and umbilical venous blood and maternal peripheral venous blood were obtained at each normal vaginal delivery (37~41 W.G.) without any complication. The procedures were done in accordance with the Helsinki Declaration in 1975, as revised in 1983. The aim of this study was explained to the volunteer and informed consent was obtained. Serum was separated after the addition of 2.84 nmol ascorbic acid/0.1 ml Tris-HCl buffer (50mM pH 7.2, 0.05% γ -globulin, 0.06% BSA, 0.01% ascorbic acid) and stored at -20°C until analysis.

Assays of serum 2-OH E₂-17-S and of lipid peroxide

After the addition of a sodium salicylate solution (315 nmol) in the Tris-HCl buffer (0.1 ml), the 2-OH E₂-17-S in 1 ml serum was extracted with five volumes of ethanol-ethyl ether mixture (4 : 1), and 60% of the supernatant was dried in a stream of nitrogen gas at 40°C to give the sample as a residue. The specimen was dissolved in 2 ml of Tris-HCl buffer and this solution was used for the RIA analysis. The standard sample was prepared from the mixture of "unlabeled 2-OH E₂-17-S" and charcoal-treated serum of a healthy man, and was managed as described above. To each sample and the standard sample were added [6, 7-³H] 2-OH E₂-17-S (2,500 dpm/0.1 ml of Tris-HCl buffer) and the antiserum (0.5 ml, $\times$ 1,000 diluted, by Tris-HCl buffer solution). The solution was then incubated at 4°C overnight. After incubation, the dextran-coated charcoal solution (4°C,

2.5% charcoal+0.25% dextran, 0.25 ml) was added to each reaction mixture, followed by incubation at 4°C for 15 min. The solution obtained was centrifuged at $1,250 \times g$ for 10 min, and the radioactivity was measured in 0.6 ml aliquots of the supernatant count. Serum lipid peroxide was measured by the method of Yagi [10]. The principle of the method was to isolate lipids by precipitating them with serum protein using phosphotungstic acid, followed by fluorometric assay to determine the thiobarbituric acid reaction.

Statistics

Statistical analysis of results was performed by unpaired Student's t-test. A p-value equal or under 0.05 was considered to represent statistical significance.

Results

Extraction of serum 2-OH E₂-17-S

To 1 ml of serum from a healthy man, extraction

Fig. 1. Standard curves $\pm$ SD (N=6) for the RIA of 2-OH E₂-17-S. Please see the CVs of intra- and inter-assay variations in the text.

of serum 2-OH E₂-17-S. [6, 7-³H] 2-OH E₂-17-S, 4,000 dpm was added. After the mixture, the serum sample was then extracted with five volumes of ethanol-ethyl ether solution (4:1). The recovery rate of 2-OH E₂-17-S was $98.9 \pm 9.2\%$ (mean $\pm$ SD).

RIA of 2-OH E₂-17-S

The reaction curve and standard deviation for 2-OH E₂-17-S are shown in Fig. 1. This standard curve was useful over the range 1~7 pmol per tube. The

intra-assay variation was calculated from serum samples at three different points on the standard curve. The coefficient of variance was less than 19.1% at each point. The inter-assay variation was calculated from multiple analysis of three different serum samples. The CV was found to be less than 17.3% for all samples. The recovery experiments were done on serum samples from a healthy man after the addition of three different amounts of 2-OH E₂-17-S. In each case the amount recovered was not significantly different from the amount added. The CV was less than 15.8%.

Fig. 2. Serum 2-OH E₂-17-S levels in peripheral blood of normal pregnant women (○; n=56) and pregnant women with pregnancy-induced hypertension (●; n=9).

Fig. 3. Serum levels of 2-OH E₂-17-S in peripheral venous blood of normal pregnant women (4 cases).

Serum 2-OH E₂-17-S during mid to late pregnancy

Serum 2-OH E₂-17-S levels (mean $\pm$ SE) in peripheral blood of pregnant women at 18 to 40 weeks of gestation are shown in Fig. 2 (56 cases of normal pregnancy and 9 cases of PIH). The serum 2-OH E₂-17-S level increased towards late pregnancy; the level at 28–31 weeks, 32–35 weeks and 36–40 weeks of gestation was 4.68 ± 0.93 nmol/l (mean $\pm$ SE, $n=10$), 8.38 ± 1.21 nmol/l ($n=21$) and 18.31 ± 3.41 nmol/l ($n=17$), respectively. The level in the PIH cases at 36–40 weeks was 4.64 ± 1.29 nmol/l ($n=7$). It was significantly lower ($p \leq 0.05$) than that in normal cases at 36–40 weeks. Fig. 3 shows the

change in serum levels of 2-OH E₂-17-S during normal pregnancy (4 cases). Each solid line connects samples from the same person. The value increased with the progress of gestation. The serum concentration of 2-OH E₂-17-S in maternal venous blood, umbilical vein and umbilical artery at normal vaginal delivery was 7.97 ± 2.46 (mean $\pm$ SE, $n=8$), 17.62 ± 3.83 ($n=9$) and 25.20 ± 4.29 ($n=6$) nmol/l, respectively (Fig. 4). Each solid line connects samples from the same person. The levels in the latter two groups were higher than the levels of 2-OH E₂-17-S in the former group. The levels in the umbilical arteries were higher ($p \leq 0.05$) than those in the maternal peripheral veins. This suggests that 2-OH E₂-17-S in pregnancy is derived from the fet-

Fig. 4. Serum 2-OH E₂-17-S levels in maternal peripheral vein (M, $n=8$), umbilical vein (UV, $n=9$) and umbilical artery (UA, $n=6$) at delivery without any complication.

Fig. 5. Relationship between serum levels of lipid peroxides and 2-OH E₂-17-S in normal pregnant women (36~40 W.G.).

placental unit, especially from the fetus. The relationship between serum levels of lipid peroxides and 2-OH E₂-17-S in late pregnancy (36~40 W.G.) without PIH are shown in Fig. 5. The high level-group of serum 2-OH E₂-17-S tended to show low levels of lipid peroxides, but not significantly.

Discussion

To the best of our knowledge, this is the first report on measurement of the serum level of 2-OH E₂-17-S during gestation. Yoshizawa *et al.* [9] prepared a specific antiserum to 2-OH E₂-17-S. Watanabe *et al.* [4] determined the plasma level of 2-OH E₂-17-S in rats by a direct RIA by using this antiserum. In the present study, the human serum sample was treated with ethanol-ethyl ether solution and then analyzed by RIA. The extraction of 2-OH E₂-17-S with ethanol-ethyl ether solution was efficient and easy [1].

The serum level of E₂-17-S at 20–23 weeks and 36–39 weeks of gestation was 1.42 ± 0.04 (mean $\pm$ SD) and 3.42 ± 1.09 nmol/l, respectively [1]. In comparison with these levels, the serum level of 2-OH E₂-17-S obtained in the present assay in mid-to late pregnancy was rather high. Takanashi *et al.* [11] reported that the human placental microsome had fairly high 2- and 4-hydroxylase activities of E₂-17-S. In the present study higher levels at 2-OH E₂-17-S were observed in the umbilical arteries and veins.

On the otherhand preeclampsia is closely related to lipid peroxides produced by placenta [6]. This organ has two opposite physiological activities, that is the production of lipid peroxide and the decomposition of the peroxides produced. Normal pregnancy is

generally maintained through the quantitative balance of such oxidants and antioxidants [6]. Previously [1], the serum level of E₂-17-S showed a significant negative regression line for that of lipid peroxides. E₂-17-S itself antagonizes lipid peroxidation, but not very strongly. The addition of various estrogens resulted in antagonizing the NADPH-dependent peroxidation in rat liver microsomes and the ID₅₀ value (concentration to reduce 50% of lipid peroxides in control) for E₂-17-S, 2-OH E₂, 4-OH E₂, 2-OH E₂-17-S and 4-OH E₂-17-S was 49, 1.2, 0.73, 2.2 and 5.3 μ mol/l, respectively [5]. E₂-17-S is easily metabolized to 2-OH E₂-17-S [2], which is a stable form [4] and a strong antagonist of lipid peroxidation. Thus, serum E₂-17-S may therefore be hydroxylated to 2-OH E₂-17-S and may act as a scavenger of peroxides. In the present study the serum level of 2-OH E₂-17-S was lower in the PIH cases. In our previous experiments with the endothelium of human aorta [7] and endothelium of human umbilical vein (unpublished observations), 2-OH E₂-17-S strongly antagonized oxidative stress caused by t-butylhydroperoxide. Further investigations are necessary to clarify the relationship between lipid peroxides and 2-OH E₂-17-S in such complicated pregnancy.

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