Serum Concentrations of Cortisone and Cortisol in Premature Infants

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To determine the relationship between biological active cortisol and its inert metabolite cortisone accurately in premature infants, serum cortisone and cortisol concentrations were measured by reversed-phase high-performance liquid chromatography (HPLC) in a group of 232 premature infants and in a control group of 127 children and 88 adults. In the control group, serum cortisone concentrations were greater than serum cortisol levels during the first 2 months after birth; cortisol levels were higher than cortisone levels after 2 months of age. However, in premature infants, serum cortisone concentrations were greater than serum cortisol levels even after the first 2 months, and total concentrations of cortisone and cortisol were equal to those in controls. Results were then analyzed according to the equivalent gestational age of premature infants. Cortisone was predominant in premature infants older than 32 weeks of equivalent gestational age, but cortisol was higher than cortisone from equivalent gestational age 24 to 31 weeks. These findings suggest that the ability of premature infants to secrete glucocorticoids resembled that of normal controls. Also, the fetal zone of the cortex, which is associated with a predominance of cortisone, remained functional in premature infants for a longer time than in control infants. Our findings that in premature infants cortisone was predominant compared with cortisol and the sum of cortisone and cortisol was equal to that in the controls indicate that cortisone cannot be disregarded whenever the cortisol level is estimated, although cortisone itself is recognized to be biologically inactive. Simultaneous measurement of serum cortisone and cortisol concentrations is important when adrenocortical function is being determined, especially in premature infants.

IT IS DIFFICULT to characterize fetal adrenal function, because the two zones of the adrenal cortex have differing functions and rates of growth.[1] The inner fetal zone develops early during intrauterine life and comprises approximately 80% of the weight of the adrenal gland at birth. The outer adult zone exhibits limited growth until the later weeks of gestation.[1] A few studies of steroidogenesis in premature infants have shown that the fetal adrenal cortex can secrete cortisol,[2,3] whereas other reports indicate that cortisol is not excreted, even in stressed premature infants.[4,5] We think this discrepancy in the estimation of fetal adrenal function may be caused both by the unique construction of the fetal adrenal cortex and by the method of measurement used.

There is a rapid interconversion between cortisol and cortisone.[6,7] Cortisone is prominent in fetal serum because the activity of 11β-hydroxysteroid dehydrogenase, which converts cortisol to cortisone, is high in fetal tissues, especially in the inner fetal zone of the adrenal cortex.[6,7] Therefore, serum cortisone levels must be measured simultaneously with serum cortisol levels to accurately assess adrenal function. Serum cortisone concentrations in premature infants have not been extensively studied.

Radioimmunoassay has been used most often in measuring serum concentrations of adrenal steroid. However, it is impossible to determine the correct levels of serum adrenal steroids, including cortisol, by radioimmunoassay because the antibodies of cortisol precursors, cortisone, and cortisol that are used are cross-reactive.[8-10]

We determined serum concentrations of cortisone and cortisol in premature infants using reversed-phase high-performance liquid chromatography (HPLC) to evaluate their capacity to secrete adrenal steroids, especially cortisone and cortisol, and to assess the significance of serum cortisone in such infants.

SUBJECTS AND METHODS

Subjects

We evaluated premature infants with birthweights less than 2,500 g and/or a gestational age less than 38 weeks who were admitted to one of six neonatal intensive care units in Hiroshima between April 1, 1990, and May 31, 1993. These infants, all of whom are available, lacked any severe deformities or asphyxia neonatorum and did not require surgical procedures. Some of them had mild to moderate respiratory distress, but none had a necessity to receive corticosteroids before blood was drawn for study. The mothers had not been given steroids during pregnancy and delivery. It is known that the normal circadian rhythm of adrenocortical function has not yet developed at the beginning of extrauterine life.[11] Therefore, in younger infants (0 to 1 month), samples were obtained at any time of day. In older infants, samples were drawn in the morning (9 AM to 12 noon). The control group included infants (gestational age ≧38 weeks and birthweight ≧2,500 g) and children aged 12 days to 18 years who were seen at the Hiroshima University School of Medicine for minor disorders and had no adrenal diseases, and healthy adults. Blood samples from neonates that were taken before the fourth day of life were excluded because of maternal corticosteroids remaining in the neonate.

Informed consent was obtained from at least one of the parents of all premature infants and of the children in the control group, and from adults who participated in the control group.

Steroid Measurements

Steroid levels were determined by reversed-phase HPLC and direct UV absorbance detection according to the modified method of Weisman et al.[12]

Serum samples of 0.3 mL with 0.7 mL 0.2N sodium hydroxide and

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17-methyltestosterone as an internal standard were extracted with 5.0 mL dichloromethane by being shaken with a shaker for 30 minutes and then centrifuged for 5 minutes at 3,000 rpm. The organic layer was washed with 2 mL 0.001N hydrochloric acid by being shaken with a shaker for 15 minutes and centrifuged for 5 minutes at 3,000 rpm. The organic phase was evaporated to dryness under a vacuum evaporator. The extracts, dissolved in 48 μL of the mixture used as the mobile phase of the HPLC, were subjected to HPLC.

The standard steroids used in this assay (purchased from Sigma Chemical, St Louis, MO) were cortisone, cortisol, 17-hydroxyprogesterone, and 11-deoxycortisol (each 1.0 μg/mL in the mobile-phase mixture).

Reversed-phase HPLC was performed using a composite system consisting of a pump (LC-6AD; Shimadzu, Kyoto, Japan), a detector (SPD-6A; Shimadzu) of 246 nm, a column oven (CTO-6A; Shimadzu) at 40°C, and a chart recorder (C-R4A; Shimadzu). The column used, ODS-M (octadecylated silica-gel column; Shimadzu), was 150 mm in length and had a 6.0-mm internal diameter and 4-μm particle size. The mobile phase was methanol/water/acetonitrile (55:42:3 vol/vol/vol). The flow rate was 1.5 mL/min, and the volume injected was 20 μL.

The mean percent recovery and detection limit of steroids was 76.8% and 1.0 ng/mL, respectively, in 0.3 mL serum.

To clarify the predominance of cortisone or cortisol, we calculated the ratio of the serum concentration of cortisone to the sum of cortisone and cortisol. A ratio greater than 0.5 indicated a predominance of cortisone, and a ratio less than 0.5 indicated a predominance of cortisol.

Statistical Analysis

Data are reported as the mean ± SD. The unpaired *t* test was used in statistical analysis. A level of *P* less than .05 was accepted as statistically significant.

RESULTS

We evaluated blood samples from 232 premature infants, 127 children (aged 12 days to 18 years), and 88 adults (37 men and 51 women).

The mean serum concentrations of cortisone and cortisol, the sum of cortisone and cortisol, and the ratio of cortisone to the sum of cortisone and cortisol in the control group in each age bracket are plotted in Fig 1. Mean concentrations of cortisone in the group less than 1 month of age (39.29 ± 26.64 ng/mL) and the 1- to 2-month group (55.64 ± 26.64 ng/mL) were higher than those of cortisol (28.84 ± 36.53 and 28.60 ± 18.51 ng/mL, respectively). Mean concentrations of cortisol in the groups more than 2 months of age containing the adult groups (52.49 ~ 73.56 ng/mL) were higher than those of cortisone (22.76 ~ 36.39 ng/mL) and significantly higher than the mean concentration of cortisol in the group less than 1 month of age ($P < .025 \sim .0005$). The mean ratio of cortisone to the sum of cortisone and cortisol in the group less than 1 month of age (0.64 ± 0.26) and in the 1- to 2-month group (0.64 ± 0.22) showed the predominance of cortisone. In contrast, the mean ratio of cortisone to the sum of cortisone and cortisol in each group more than 2 months of age (0.25 ~ 0.43) was significantly lower than that in the group less than 1 month of age ($P < .01 \sim .0005$) and showed the predominance of cortisol. The equilibrium of cortisone and cortisol changed at 2 months of age. Cortisone and/or cortisol were detected in all control subjects, whereas neither 17-hydroxyprogesterone nor 11-deoxycortisol were detected.

The premature infants were aged 5 to 167 days and had

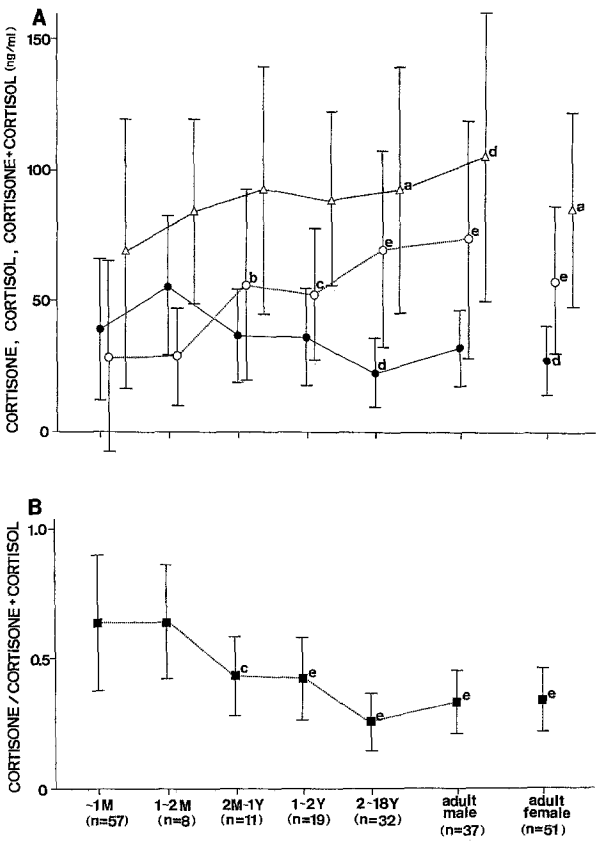

Fig 1. Mean ± SD (A) serum concentrations of cortisone (●), cortisol (○), and cortisone plus cortisol (△) and (B) the ratio of cortisone to cortisone plus cortisol (■) in normal controls by age bracket. $^{a}P < .05$ *v* subjects aged <1 month; $^{b}P < .025$; $^{c}P < .01$; $^{d}P < .005$; $^{e}P < .0005$.

equivalent gestational ages (calculated from the date of conception) of 24 to 62 weeks. Data were examined by age bracket (Table 1) and by equivalent gestational age (Table 2). Cortisone and/or cortisol were detected in all premature infants, whereas neither 17-hydroxyprogesterone nor 11-deoxycortisol were detected as in controls.

The mean concentrations of cortisone were high compared with those of cortisol, and the mean ratio of cortisone to the sum of cortisone and cortisol showed a predominance of cortisone in all age groups (Table 1). In premature infants less than 2 months of age, the mean concentrations of cortisone and cortisol and the ratio of cortisone to the sum of cortisone and cortisol resembled those in the control groups, with no significant differences observed. However, in premature infants over 2 months of age, the mean concentration of cortisone and the ratio of cortisone to the sum of cortisone and cortisol were significantly higher ($P < 0.05$ and .0005) and the mean concentration of cortisol was significantly lower ($P < .05$) than in the control group aged 2 months to 1 year.

The adrenocortical function of neonates is influenced by equivalent gestational age.[13] In the group with an equivalent gestational age from 24 to 31 weeks, the mean concentration of cortisol was higher than that of cortisone (Table 2). The mean ratio of cortisone to the sum of cortisone and cortisol showed a predominance of cortisol and was significantly lower than the mean ratio in groups with an equivalent gestational age of more

Table 1. Serum Concentrations of Cortisone, Cortisol, and Cortisone Plus Cortisol and the Ratio of Cortisone to Cortisone Plus Cortisol in Premature Infants by Age Bracket

Age	Cortisone (ng/mL)	Cortisol (ng/mL)	Cortisone Plus Cortisol (ng/mL)	Cortisone/Cortisone + Cortisol
<1 mo (n = 160)				
Mean ± SD	46.81 ± 38.67	26.12 ± 31.76	72.90 ± 56.36	0.68 ± 0.25
Range	0.00~222.30	0.00~158.79	3.90~356.37	0.00~1.00
≧1 mo < 2 mo (n = 42)				
Mean ± SD	45.98 ± 25.43	21.61 ± 21.97	67.59 ± 39.71	0.74 ± 0.20
Range	12.36~125.20	0.00~64.40	12.36~183.38	0.40~1.00
≧2 mo (n = 11)				
Mean ± SD	53.30 ± 25.35*	25.34 ± 35.20*	78.64 ± 56.43	0.75 ± 0.16†
Range	16.72~107.49	0.00~130.59	20.17~238.08	0.45~1.00

*$P < .05$, †$P < .0005$: statistically significantly different *v* controls in the same age bracket.

than 32 weeks. In contrast, in groups with an equivalent gestational age of more than 32 weeks, the mean concentration of cortisone was higher than that of cortisol and the mean ratio of cortisone to the sum of cortisone and cortisol indicated the predominance of cortisone (Table 2).

DISCUSSION

Previous studies showed that concentrations of adrenal steroid precursors, especially 17-hydroxyprogesterone or 11-deoxycortisol, are higher than cortisol concentrations in premature infants.[1,5] The activities of 21-hydroxylase and/or 11β-hydroxylase are assumed to be decreased in premature infants compared with mature infants based on these studies. However, these findings lack reliability because the radioimmunoassay method used in the studies involves antibody cross-reactivities. Serum concentrations of adrenal steroids can be measured with accuracy using HPLC. In this study, total concentrations of cortisone and cortisol in premature infants less than 1 month of age were equal to those in the control group, and neither 17-hydroxyprogesterone nor 11-deoxycortisol were detected in our premature infants. Our finding indicates that the adrenal cortex of the fetus had the same capacity to secrete glucocorticoid as the mature adrenal cortex, and that the activities of 21-hydroxylase and 11β-hydroxylase were not decreased in premature infants versus the controls. However, our study did not show whether the production of glucocorticoid in the fetal adrenal cortex is sufficient for stressful conditions. The low cortisol concentrations reported previously in premature infants[4,5] may not indicate insufficient production of glucocorticoid, since cortisol is readily converted to cortisone in the fetal zone of the adrenal cortex.[6,7] In our premature infants, none had concentrations of cortisone and cortisol that were decreased concurrently, and total concentrations of cortisone and cortisol in premature infants were equal to those in controls. In some cases with low cortisol concentrations, measurement of cortisone concentrations was useful for estimation of the ability to secrete glucocorticoid.

We know of no other data on serum cortisone and cortisol concentrations measured by HPLC concurrently in subjects of various ages from neonates including premature babies to adults. Sippell et al[14] examined plasma cortisone and cortisol concentrations in children younger than 15 years of age and in adults by radioimmunoassay. They showed that cortisone concentrations were higher than cortisol levels in infants less than 7 days of age; after 2 weeks of age, cortisol was greater than cortisone. They supposed that the cause of their result was adaptation of the adrenal neocortex to extrauterine life after the disruption of the fetoplacental unit.[14] However, the radioimmunoassay method is unable to determine whether cortisone or cortisol is predominant, because of the antibody cross-reactivity between cortisone and cortisol.[10] In our study, cortisone predominated in the serum of infants younger than 2 months, whereas

Table 2. Serum Concentrations of Cortisone, Cortisol, and Cortisone Plus Cortisol and the Ratio of Cortisone to Cortisone Plus Cortisol in Premature Infants by Equivalent Gestational Age

Equivalent Gestational Age (wk)	Cortisone (ng/mL)	Cortisol (ng/mL)	Cortisone Plus Cortisol (ng/mL)	Cortisone/Cortisone + Cortisol
24-31 (n = 8)				
Mean ± SD	63.78 ± 45.60	96.94 ± 127.02	160.72 ± 157.02	0.43 ± 0.25
Range	0.00~133.63	16.79~423.51	20.73~553.23	0.00~0.89
32-34 (n = 40)				
Mean ± SD	49.15 ± 29.24	36.43 ± 91.07	85.58 ± 86.02	0.69 ± 0.22‡
Range	2.53~132.11	0.00~589.72	13.34~709.15	0.14~1.00
35-37 (n = 74)				
Mean ± SD	40.21 ± 25.79*	21.56 ± 32.86§	61.77 ± 49.81§	0.74 ± 0.22§
Range	3.90~112.34	0.00~158.79	3.90~238.63	0.15~1.00
38-62 (n = 101)				
Mean ± SD	52.43 ± 43.82	29.95 ± 32.18§	82.38 ± 60.49‡	0.66 ± 0.25†
Range	0.00~323.29	0.00~141.56	9.23~356.37	0.00~1.00

*$P < .025$, †$P < .01$, ‡$P < .005$, §$P < .0005$: statistically significantly different *v* subjects with equivalent gestational age 24 to 31 weeks.

cortisol was greater than cortisone in infants more than 2 months of age in the control group. The predominance of cortisone continued after 2 months from birth in premature infants. Morphologically, the size of the inner fetal zone of the adrenal cortex begins to diminish after birth, transforming into the outer adult zone. The fetal zone disappears approximately 6 weeks after birth.[15] Functionally, the fetal zone declines about 2 months after birth, as implied by the change in the ratio of cortisone to cortisol at that time. The continuing predominance of serum cortisone over cortisol more than 2 months from birth in premature infants is probably caused by the persistence of the inner fetal zone of the cortex morphologically and functionally.

In premature infants of 24 to 31 weeks equivalent gestational age, serum cortisol predominated over cortisone, whereas in groups of more than 32 weeks equivalent gestational age, cortisone was greater than cortisol. Previous studies reported that the activity of the fetal zone of the adrenal cortex was closely connected with the equivalent gestational age.[13] Premature infants have the capacity to secrete glucocorticoid even in the group less than 31 weeks of equivalent gestational age, and the activity of 11β-hydroxysteroid dehydrogenase in this group seems to be more immature than in other groups older than 32 weeks of equivalent gestational age. The increased activity of 11β-hydroxysteroid dehydrogenase and the development of fetal zone activity in premature infants older than 32 weeks of equivalent gestational age may cause a prominence of cortisone in the serum. The mean ratio of cortisone to the sum of cortisone and cortisol from 35 to 37 weeks of equivalent gestational age was highest in our premature infants classified by equivalent gestational age. The activity of 11β-hydroxysteroid dehydrogenase in the fetal zone and the other organs may be higher in the group from 35 to 37 weeks of equivalent gestational age than in other groups. Serum cortisone concentrations may be important to the study of the capacity of glucocorticoid secretion over 32 weeks, and especially over 35 weeks, of equivalent gestational age in premature infants.

The ratio of cortisone to the sum of cortisone and cortisol was significant to decide the predominance of cortisone or cortisol. Hillman and Giroud[16] reported the predominance of cortisone compared with cortisol in umbilical cord plasma by the ratio of cortisone to cortisol. Beitins et al[17] reported that the ratio of plasma cortisol to cortisone in neonates was lower than in the mothers. The ratios of cortisone to cortisol or cortisol to cortisone are undoubtedly useful to interpret the predominance of cortisone or cortisol, but when serum cortisone or cortisol are not detected, these ratios cannot be calculated. The ratio of cortisone to the sum of cortisone and cortisol that we used was also beneficial to understand the pattern for the predominance of cortisone and cortisol in such cases.

Since cortisone itself is recognized to be biologically inactive, its importance has been obscure. Although it is not known what regulates the interconversion of cortisone and cortisol, from our observation of higher levels of serum cortisone versus cortisol in normal control neonates and premature infants, except those younger than 31 weeks of equivalent gestational age, investigation of serum cortisone and cortisol seems useful for the estimation of adrenocortical function in neonates and premature infants.

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