

Neonatal Serum Deoxycorticosterone Sulfate Levels in Congenital Adrenal Hyperplasia due to 11 β -Hydroxylase Deficiency

Akira Endoh, Akira Kubota, Haruo Ogawa and Yoshio Igarashi

Department of Pediatrics, Hamamatsu University School of Medicine, Shizuoka, Japan

Abstract. Recent studies have demonstrated that deoxycorticosterone sulfate can be formed at extra-adrenal sites, but its actual origin and role are not yet clear. The present study was undertaken to elucidate the origin of deoxycorticosterone sulfate and the usefulness of this steroid for the diagnosis of 11 β -hydroxylase deficiency in the early neonatal period. We measured serum deoxycorticosterone sulfate levels during glucocorticoid therapy in an infant with 11 β -hydroxylase deficiency. Before therapy, the serum deoxycorticosterone sulfate concentration at 6 days of life was higher than normal. Following glucocorticoid therapy, the serum deoxycorticosterone sulfate concentration showed a marked and dose-dependent suppression, as did the serum deoxycorticosterone and 11-deoxycortisol levels. These findings suggest that serum deoxycorticosterone sulfate can be used as another specific indicator for the diagnosis of 11 β -hydroxylase deficiency in neonates, and that the adrenal glands may play an important role in neonatal deoxycorticosterone sulfate production under the control of ACTH.

Key words: 11 β -hydroxylase deficiency (11 β -OHD), deoxycorticosterone (DOC), deoxycorticosterone sulfate (DOC sulfate), neonate

Introduction

Less than 5% of patients with congenital adrenal hyperplasia have 11 β -hydroxylase deficiency (11 β -OHD). Deficiency of this

enzyme blocks the conversion of 11-deoxycortisol to cortisol and 11-deoxycorticosterone (DOC) to corticosterone. The common clinical features are virilization and hypertension. Steroid analysis reveals increased serum levels of 11-deoxycortisol, DOC, and androgens, as well as increased urinary levels of tetrahydro-11-deoxycorticosterone (THDOC), tetrahydro-11-deoxy-cortisol (THS), and 17-ketosteroids. These changes are alleviated by glucocorticoid replacement therapy. However, early diagnosis of 11 β -OHD by the analysis of steroids in neonatal urine is difficult [1], possibly because of dif-

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Correspondence: Dr. Akira Endoh, Department of Pediatrics, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31 Japan

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ferences in the steroid metabolism of newborns and adults. It is therefore necessary to consider the metabolism of DOC and S in the neonatal period. It is known that neonates have high serum levels of DOC sulfate [2-4], but its origin is still controversial, *i.e.* adrenal or extra-adrenal. In this study, we measured the serum levels of DOC sulfate in a neonate with 11 β -OHD during the initiation of glucocorticoid therapy, in order to determine whether this steroid was useful as an indicator of 11 β -OHD and to ascertain its origin.

Materials and Methods

Case report

An infant (birth weight: 3,380 g) was born at 40 weeks of gestation after a normal pregnancy. There was a history of consanguinity, since the maternal great-grandfather was the paternal grandfather's elder brother. The external genitalia were ambiguous and hyperpigmented, and the infant was referred to our hospital for evaluation at the age of 5 days. The clitoris was enlarged and there was a single opening in the perineum. The remainder of the physical examination was normal, including blood pressure and laboratory tests as follows: Na, 136 mmol/L; K, 5.5 mmol/L; and Cl, 103 mmol/L. Chromosomal data revealed a 46,xx karyotype. The detailed endocrinological data are shown in Table 1.

Serum 11-deoxycortisol, DOC, testosterone, and ACTH levels were markedly elevated. Urinary THDOC and THS secretion were also increased. Perineal sinography demonstrated a common urogenital sinus. ¹³¹I-adosterol scintigraphy and abdominal CT showed bilateral adrenal hypertrophy. From these findings the infant was diagnosed as having 11 β -OHD, and hydrocortisone treatment was commenced 39 days after birth.

Steroid Analysis

All non-radiolabeled steroids were purchased from Sigma Chemical Co.. Serum levels of testosterone, 17-hydroxyprogesterone, dehydroepiandrosterone, dehydroepiandrosterone sulfate and androstenedione, and plasma ACTH levels, were measured by RIA using kits obtained from Diagnostic Products Corporation (Los Angeles, CA). Urinary steroids were determined as methoxime-trimethylsilyl (MO-TMS) derivatives using glass capillary gas chromatography [5]. Serum DOC, DOC sulfate, and 11-deoxycortisol levels were determined by RIA, as described previously [6]. Briefly, DOC sulfate was measured as follows. After diethyl ether extraction, serum was enzymatically hydrolyzed with sulfatase (Sigma Co.). The DOC derived from DOC sulfate was then extracted and subjected to RIA. The DOC sulfate content was calculated relative to the

Table 1. Endocrinological data of the infant with 11 β -hydroxylase deficiency at 6 days of life.

1) Serum steroids			
progesterone	(nmol/L)	4.3	(1.0 \pm 0.3)
deoxycorticosterone	(nmol/L)	4.2	(2.1 \pm 0.6)
deoxycorticosterone sulfate	(nmol/L)	557.2	(117.9 \pm 30.0)
11-deoxycortisol	(nmol/L)	935.4	(2.1 \pm 0.7)
17-hydroxyprogesterone	(nmol/L)	40.5	(7.3 \pm 3.5)
dehydroepiandrosterone	(nmol/L)	8.0	(1.1 \pm 0.4)
testosterone	(nmol/L)	1.8	(0.7 \pm 0.5)
2) Urinary steroids			
tetrahydro-11-deoxycortisol	(μ mol/24hr)	2.8	(<0.1)
tetrahydro-11-deoxycorticosterone	(μ mol/24hr)	1.8	(<0.1)
17-ks	(mg/24hr)	4.8	
17-OHCS	(mg/24hr)	7.4	
3) Plasma ACTH	(pmol/L)	44.3	

Normal values are given for corresponding age group.

Serum DOC sulfate in an 11-OHD infant

Table 2. Serum steroid and plasma ACTH and PRA concentrations before and after glucocorticoid replacement in the infant with 11 β -hydroxylase deficiency.

Age (days)	S (nmol/L)	DOC (nmol/L)	DOC-S (nmol/L)	17OH-P (nmol/L)	T (nmol/L)	PRA (ng/ml · h)	ACTH (pmol/L)
29				9.1		38.5	19.6
37	330.9	8.5	653.0				
* 39							
51	325.2	1.4	372.1		0.3	3.9	5.3
** 90							
98	7.4	0.5	49.1			3.9	<2.2
129	1.6	0.1	33.9				
normal	2.1 \pm 0.7	2.1 \pm 0.6	117.9 \pm 30	7.3 \pm 3.5	0.7 \pm 0.5	3.2 \pm 1.9	26.7 \pm 1.8

S; 11-deoxycortisol, DOC; deoxycorticosterone, DOC-S; deoxycorticosterone sulfate, 17OH-P; 17-hydroxyprogesterone, T; testosterone

Normal neonatal values (1-2 weeks of age) are given as the mean \pm SD.

* Treatment was started with hydrocortisone at 35 mg/M²/day in 4 divided doses.

** Treatment was changed to hydrocortisone at 45 mg/M²/day in 4 divided doses.

molecular weight of DOC.

Results

The serum concentrations of 11-deoxycortisol (935.4 nmol/L), DOC (4.18 nmol/L) and DOC sulfate (557.2 nmol/L) were higher than normal at six days of life. The patient was placed on 10 mg/day (35 mg/M² body surface area) of hydrocortisone from 39 days after birth. Twelve days of this treatment resulted in suppression of DOC and ACTH; the serum DOC and plasma ACTH fell to normal levels (1.39 nmol/L and 5.3 pmol/L, respectively) (Table 2). However, serum 11-deoxycortisol was not suppressed, and the serum DOC sulfate remained high (372.1 nmol/L). The hydrocortisone was therefore increased to 15 mg/day (45 mg/M²). The new regimen resulted in further reduction of the serum DOC and ACTH, and normalization of serum 11-deoxycortisol and DOC sulfate.

Serum DOC sulfate levels correlated significantly with serum DOC ($r=0.89$; $p<0.05$). The correlation between DOC sulfate and 11-deoxycortisol ($r=0.75$) was not significant.

Discussion

It has been reported that the diagnosis of 11 β -OHD is difficult in the early neonatal

period [7]. Steroid metabolism in this period is different from that in later life, because of the fetal nature of the adrenals and the presence of maternal and placental steroids. Neonatal plasma ACTH and serum progesterone, DOC and 11-deoxycortisol are known to be higher than in later life. In the first few days of life, THS, the major metabolite of 11-deoxycortisol, was not detectable in the urine. Furthermore, neonatal adrenal glands show exceedingly high production of 3 β -hydroxy-5-ene steroids (sulfates), which interfered with the accurate determination of the relatively minor amounts of steroid-glucuronide in the early neonatal urine. However, we considered that more specific metabolites of DOC and 11-deoxycortisol may exist in the early neonatal period. We reported that DOC showed a significant correlation with DOC sulfate in umbilical arterial cord blood [8]. The present study is the first assessment of serum DOC sulfate levels during glucocorticoid therapy for a neonate with 11 β -OHD. The serum DOC sulfate concentration was markedly raised at 6 days of age, and declined dose-dependently together with the DOC and 11-deoxycortisol levels after glucocorticoid therapy was started. Furthermore, a significant correlation was found between serum DOC sulfate and serum DOC. Thus, the serum DOC sulfate levels were helpful for the diagnosis and management of 11 β -OHD in the neonatal period. Another study

has demonstrated that urinary 6α -hydroxytetrahydro-11-deoxycortisol is also a useful and specific marker for 11β -OHD in neonates [9].

The observations on the changes of the serum DOC sulfate concentrations during glucocorticoid therapy in infancy were also useful for evaluating its origin. Recent studies have demonstrated 21-hydroxylase and 21-sulfotrans activity in various fetal tissues, including the liver, kidney, aorta and thymus [10-12]. DOC sulfate could thus be formed by these enzymes from circulating progesterone at extra-adrenal sites in the fetus. However, its actual origin still remains unclear. Furthermore, it has been shown in adults that extra-adrenal 21-hydroxylase activity is independent of ACTH [13], but this has remained unclear in neonates. If serum DOC sulfate levels were decreased by glucocorticoid therapy, it would suggest that the DOC sulfate in serum might be related to adrenal glands which are dependent on ACTH. Adrenal glands have the highest 21-sulfotrans activity of human fetal tissues [14]. Klein *et al.* found in a sulfation study that 20% of DOC was converted to DOC sulfate by human newborn adrenals [15]. However, the influence of extra-adrenal production of DOC sulfate cannot be excluded. DOC is a precursor of DOC sulfate, so that glucocorticoid administration will reduce production of DOC sulfate at extra-adrenal sites by decreasing circulating DOC levels. Although it has been demonstrated in adults that DOC sulfate formed in the liver, which is considered the main site of extra-adrenal production, did not enter the circulation [16], there is no evidence that the situation in the other tissues is the same as in the liver. In view of these findings, the decrement of serum DOC sulfate with the significant correlation to DOC in response to glucocorticoid therapy in our case of 11β -OHD suggests that serum DOC sulfate is not formed from circulation progesterone at extra-adrenal sites, but is produced from sulfurylation of DOC in the adrenal glands and/or extra-adrenal sites.

There is a broad spectrum of clinical and biochemical manifestations in patients with 11β -OHD [17]. Hypertension, hypokalemia and virilization are not correlated with the 11-deoxycorticosterone level [18], and several patients with untreated 11β -OHD have developed salt wasting in the early neonatal period [19-21]. Although the present case showed no evidence of salt wasting, and had normal serum electrolytes, the plasma renin activity was increased. One explanation could be that neonates with congenital adrenal hyperplasia show a variable degree of insensitivity to mineralocorticoids [22]. Another explanation could be the presence of anti-mineralocorticoid steroids (*e.g.* progesterone or 17-hydroxyprogesterone) or a change in the metabolic clearance of DOC during this period. DOC sulfate has no mineralocorticoid activity and it is considered that formation of the 21-sulfate of DOC represents its main route of deactivation. This study showed that the level of DOC sulfate, as well as that of DOC, was much higher than normal. We therefore speculate that sulphylation of DOC could be an important regulator of its mineralocorticoid activity.

In conclusion, we found that the high serum DOC sulfate level in an infant with 11β -OHD was decreased by glucocorticoid therapy. Serum DOC sulfate appears to be a useful indicator for the diagnosis of 11β -OHD in the early neonatal period and may be produced by sulfurylation of DOC in the adrenal glands and/or extra-adrenal sites.

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