Antenatal treatment for classic 21-hydroxylase forms of congenital adrenal hyperplasia and the issues

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This paper discusses perinatal issues specific to the classical 21-hydroxylase deficiency forms of congenital adrenal hyperplasia including: prenatal testing, detection, risks, and limitations of such procedures; and prenatal treatment, its potential benefits to the affected female fetus, and risks to both the fetus and the mother. The information may be important for a family to have before and during a pregnancy in order to make an informed decision about what course of action they might want to pursue.

INTRODUCTION

The 21-OH deficiency form of CAH (for this paper, the abbreviation CAH will be used to describe congenital adrenal hyperplasia due to 21-OH deficiency only) affects the development of genetic females by virilization of the external genitalia. The increase in secretion of androgens by the adrenals resulting in the phenotype associated with CAH begins before and/or as normal sexual differentiation takes place; the external genitals can be masculinized by androgens beginning at 6–9 weeks gestation.[1,2] The development of external genitalia is normally controlled by the presence or absence of fetal androgens of gonadal origin. The indifferent gonads appear during the 5th week of development. Testes develop at approximately 43–50 days and descend into the scrotal sac during the 3rd to the 9th month. The ovaries differentiate a few weeks after the testes and descend into the pelvic cavity from the 7th to the 9th month. The adrenal cortex is derived from the coelomic mesothelium, first detected at approximately 7 weeks gestation; steroid production by the fetal zone begins in the latter half of the first trimester.[3] Sexual differentiation normally occurs between the 9th and 13th weeks,[2] and phenotypic sex is established at the end of the first trimester.[4]

PHENOTYPIC FORMS OF CAH

Females with the classical form of CAH, which includes both the salt-wasting (SW-CAH) and simple virilizing (SV-CAH) subtypes, exhibit genital ambiguity ranging in severity through five stages, as described by Prader.[1,5] Females may present at birth with an enlarged clitoris; fusion of labioscrotal folds, and a rugated scrotal sac.[3,6–8] Fetal androgens can also inhibit downgrowth of the vesicovaginal septum in females,[1] and thus severely virilized females may have a single perineal orifice consisting of a fused vagina and urethra (urogenital sinus).[3] This urogenital sinus may appear as perineoscrotal or phallic hypospadias or even as a "normal male" urethra. Such females may require clitoroplasty in infancy, and vaginoplasty and vaginal dilation in young adulthood. Internal female sexual organs develop normally because they are not influenced by androgens.[6,8]

Because the male fetus is normally exposed to high levels of testicular androgens, affected newborn males will not have apparent genital deformities but may present with slight hyperpigmentation of the external genitalia due to excess ACTH and related peptides.[3]

Cortisol deficiency occurs in all infants with classical CAH. It may lead to hypoglycemia, apnea, seizures, vascular instability and collapse, hypotension, hyperpigmentation, and an inability to deal with stress or illness. Approximately 75% of CAH patients have the SW-CAH subtype, wherein the dual defect in cortisol and aldosterone biosynthesis results in the reduction of sodium reabsorption by the renal tubules and therefore an inability to conserve sodium and to excrete potassium and hydrogen ions. This leads to hyponatremia, hyperkalemia, dehydration, hypotension, acidosis, and further hypovolemia.[3,9] Most girls with SW-CAH are noted at birth because of their genital ambiguity. Males affected with SW-CAH may not be detected at birth, but are diagnosed in the newborn period when they present with failure to thrive, vomiting, dehydration, and electrolyte abnormalities. If children are not treated quickly, shock and even death can occur.[6]

In cases with the SV-CAH subtype, sufficient mineralocorticoid is synthesized to prevent salt-losing.[10] These infants will not have neonatal electrolyte disturbances.[3,11] As with the SW-CAH subtype, most girls are diagnosed in the neonatal period because of clitoromegaly. Boys may not be diagnosed until much later, when they present with signs of androgen excess (early pubic hair development, phallic enlargement, acne, and rapid growth).

PHYSIOLOGY

Both the classical and nonclassical forms of CAH are caused by deficiency of the same 21-OH enzyme. This steroidogenic

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enzyme belongs to the cytochrome P450 group of oxidases.[12] 21-OH is one of a number of enzymes in the steroidogenic pathway, which results in the synthesis of cortisol (the principal glucocorticoid in man) from cholesterol. The cortisol biosynthetic pathway is stimulated first by corticotropin-releasing hormone (CRH) from the hypothalamus and then by adrenocorticotropic hormone (ACTH) from the pituitary. Cortisol from the adrenal glands binds to glucocorticoid receptors in the hypothalamus and pituitary, and exerts a negative feedback on the secretion of CRH and ACTH. When there is a deficiency of the 21-OH enzyme, cortisol secretion is inadequate to turn off CRH and ACTH secretion. 21-OH is also involved in the synthesis of aldosterone (the principal mineralocorticoid) from cholesterol; this pathway is primarily regulated renally by the renin(angiotension mechanism. In CAH, precursor steroids proximal to the blocked step (most notably 17-hydroxyprogesterone, or 17-OHP) accumulate and are shunted into other steroidogenic pathways, particularly androgen biosynthesis; the adrenal androgen androstenedione can be converted peripherally to the much more potent testosterone (Figure 1).[6]

INHERITANCE AND MOLECULAR BASIS

CAH is inherited in an autosomal recessive pattern, having a worldwide incidence of approximately 1:14000.[3,10] Within the Caucasian population, the classical form of CAH is seen at a frequency of 1:5000–15000,[6] for a carrier frequency of 1:60–1:123.[10]

The gene that encodes 21-OH, CYP21, is located at 6p2l.3.[11] This gene is linked to the major histocompatibility complex between HLA-B and HLA-DR, and near HLA class III.[6] Two CYP21 genes flank the 3′ end of C4A and C4B, which encode members of the complement cascade. Each CYP21 gene contains 10 exons[11] that span 3kb of DNA and share 98% homology with each other.[13] The first CYP21 gene encodes an active protein, whereas the second gene is an inactive pseudogene known as CYP21P.[11,14,15]

At least 44 different disease-causing sequence aberrations in 21-OH CAH have been identified.[16] In 95% of cases, inactivation of CYP21 is caused by a transfer of deleterious sequences (a "gene conversion") between CYP21P and CYP21; this results in CYP21 becoming defective or nonfunctional.[1] (Large CYP21 deletions and an intron 2 splice mutation [I2] account for approximately 50% of classical mutations.[3]) The majority of other mutations are point mutations found within the coding region of CYP21, with a 2% prevalence of *de novo* mutations

CAH is not a homogenous disorder; studies report good correlation between genotype and phenotype. In compound heterozygotes, the disease severity corresponds most often to the least deleterious mutation present[1,6]; a mild mutation on one allele allows for 20–50% of normal 21-OH enzyme activity.[1] Differences in phenotypes in patients with identical genotypes are most likely due to environmental factors and constitutional differences in other aspects of steroid metabolism and homeostasis such as androgen sensitivity, metabolic clearance,[2] or other non-CYP21 enzymes with 21–OH-like activity.

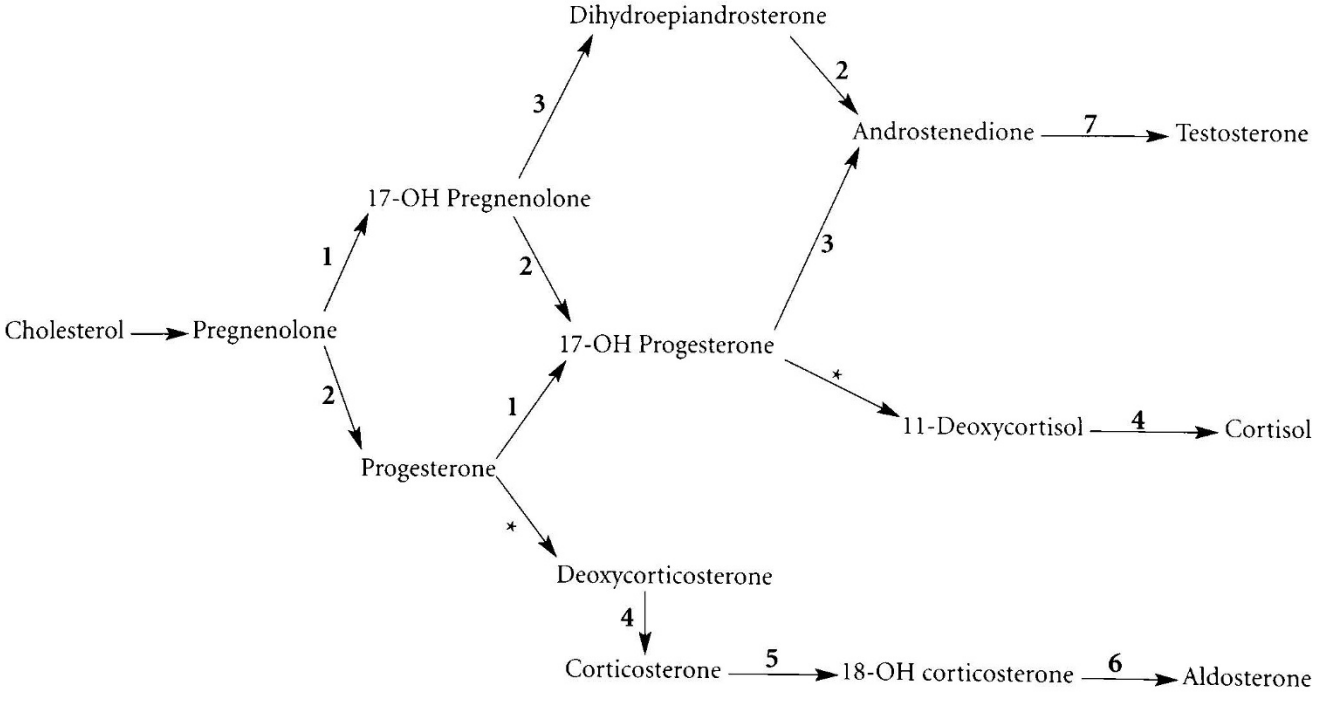

Fig. 1 Adrenal steroidogenesis. *, 21-hydroxylase; 1, 17-hydroxylase; 2, 3β-hydroxysteroid dehydrogenase; 3, 17, 20 Lyase; 4, 11β-hydroxylase; 5, 18-hydroxylase; 6, 18-dydroxylase dehydrogenase; 7, 17β-hydroxylsteroid dehydrogenase.

TREATMENT

Children who are born with either subtype of classic CAH need to be managed by lifelong hormonal replacement therapy to allow normal growth and pubertal development while ensuring good health.[3,7] Treatment for both SW-CAH and SV-CAH includes glucocorticoid therapy (usually hydrocortisone) to suppress ACTH secretion and return adrenal androgen production to normal. Those with SW-CAH are also placed on mineralocorticoid replacement therapy (usually oral fludrocortisone),[6] and they may require sodium chloride replacement, which can take the form of table salt or *ad lib* intake of salty foods in their diet.[3]

Two new therapeutic interventions have been proposed for CAH-affected patients. The first involves a multi-drug regimen consisting of flutamide (an androgen receptor blocker), testolactone (an aromatase inhibitor), and replacement hydrocortisone and fludrocortisone as needed. The second intervention is bilateral adrenalectomy and physiologic replacement of glucocorticoid and mineralocorticoid needs.[17] It is thought that this latter procedure may be justified, because it would completely eradicate all unwanted adrenal androgen production. However, removal of the adrenals may result in a condition known as Nelson syndrome, in which hyperplasia of the ACTH-producing cells of the pituitary leads to autonomous tumor formation.

PRENATAL TESTING

There are various ways in which prenatal testing for CAH can be undertaken. Assay of amniotic fluid concentration for 17-OHP in mid-trimester reveals a markedly increased level in SW-CAH affected fetuses.[7,18] The normal concentration of 17-OHP in amniotic fluid ranges between 18-270 ng/dl, and the concentration of an affected fetus is >300 ng/dl.[19,20] Amniotic fluid androstenedione and testosterone are also increased in affected female pregnancies, but testosterone is within the upper normal limits with affected males.[18] 17-OHP and androstenedione are less sensitive in detecting CAH subtypes other than SW-CAH.[19] These amniotic fluid levels may measure within the normal range in the SV-CAH and NC-CAH affected.[18,19] Measurement of these hormones does not provide accurate detection of the status of fetuses that are receiving prenatal dexamethasone treatment.

HLA-linkage studies can also be performed to determine if the fetus is affected. Both parents and the proband are HLA-typed before the fetus can be tested to ensure that the HLA linkage is informative. Errors or false results may occur for three reasons: 1) poor hybridization to the target DNA; 2) fetal amniocytes do not express all HLA types[19,20]; and 3) both parents may share the same haplotype, or recombination has occurred.[7,20] A linkage analysis approach using novel, highly informative microsatellite markers from the class III HLA region is now available.[15] Linkage studies with C4 genes may also be performed.[2,7]

Finally, DNA analysis for mutations within the 21-OH gene can be carried out. PCR analysis identifies point mutations, and Southern blot analysis is used for finding large deletions. Potential errors for this type of analysis include misinterpretation of dot blots, maternal contamination of

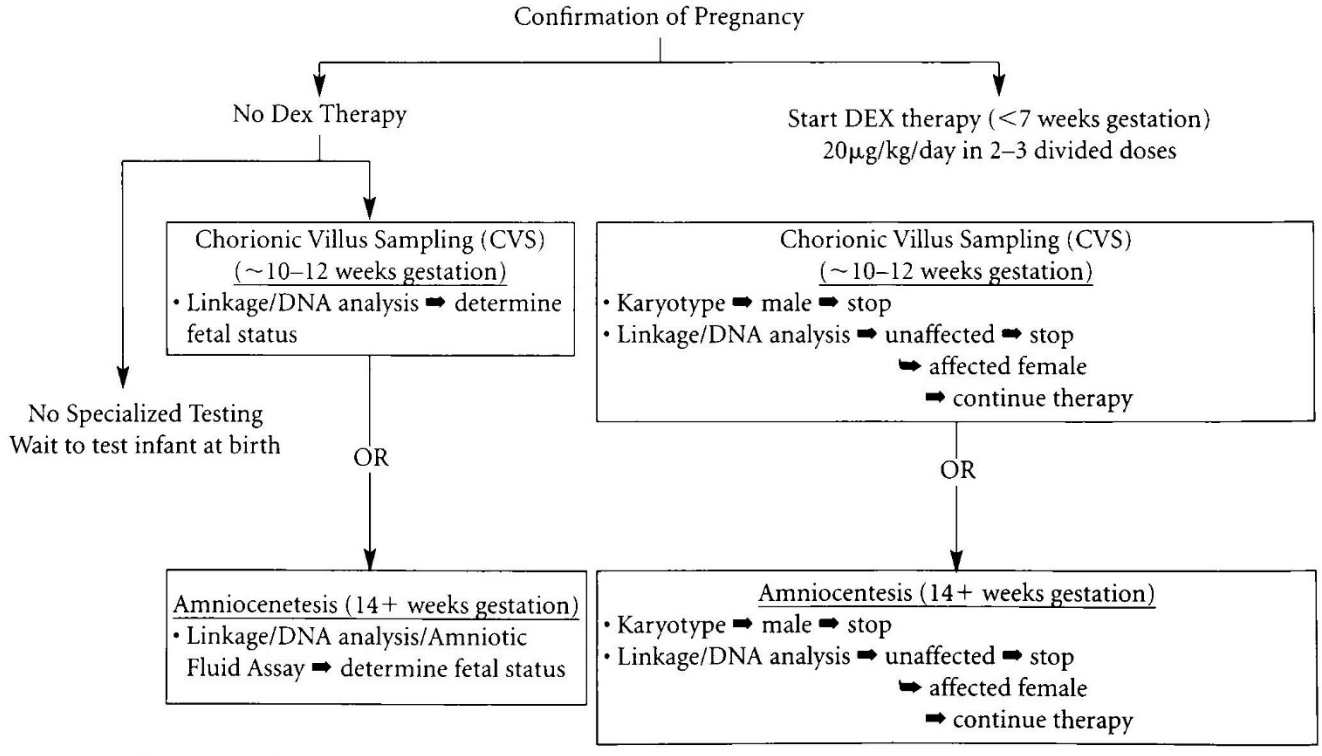

Fig. 2 Procedure for management of CAH at-risk pregnancy.

Table 1

Result summary of prenatal dexamethasone therapy in three study series (five papers).

			# Affected		# Unaffected				
Study group	Study series	Total # pregnancies	# Treated	# Untreated	# Treated	# Untreated	# Maternal complications	# Fetal complications	# Postnatal complications
France	Forest-1989	44	3 males 5 females 2:5 Normal 1:5 Prader 1 1:5 Prader 4 1:5 in progress	0	36	0	3-increase weight gain nervousness irritability	1-IUFD at 8.5 m 1-IUGR 4-SAB	2-dysmature
	Forest-1993	20	1 male 3 females 1:3 Normal 1:3 Prader 3-4 1:3 NC	0	16	0	3-increase weight gain mood swings pedal and leg edema increased blood pressure increased appetite	2-SAB	0
New York, Cornell	Karaviti -1992	86	3 males 4 females 1:4 normal 2:4 Prader 1 1:4 Prader 4	2 males 3 females 1:3 Prader 3 1:3 Prader 4 1:3 NC	20 males 14 females	24 males 15 females	3-increase weight gain mood swings pedal edema increased blood pressure increased appetite	1-stillbirth at 33 wk ga (unaffected, treatment discontinued at 19 wk)	1-cleftlip/palate (untreated, unaffected)
Mercado	176 -1995	9 males	7 males 13 females 4:13 Normal 5:13 Prader 1-2 4:13 Prader 3-4	40 males 8 females 5:8 Prader 3-5 3:8 TAB	22 males 39 females	38 females	3-increase weight gain moderate proteinuria Cushingoid facies increased blood pressure hirsutism	5-SAB (1 untreated, 4 treated)	2-neonatal deaths (unaffected)
Sweden	Lajic -1998	44	9 males 6 females 3:7 Normal 2:7 Prader 1-2 1:7 Prader 2-3 1:7 NC	0	29	0	5-increased weight gain mood fluctuations abdominal striae acne, hirsutism preeclampsia*	4-SAB (unaffected)	2 unaffected males 3 unaffected females 3 affected females

fetal cells, or other technical difficulties.[20] A list of a number of North American laboratories that perform molecular or linkage studies for CAH can be found on the Gene Test™ website (requires registration) at **http://www.genetests.org**. At this time, direct DNA analysis is available for 11 mutations, accounting for up to 90–95% of cases. If no mutation is detected, the residual risk is lowered to approximately 1:1084. More than one mutation can be found on the same gene/chromosome. Therefore, when interpreting results, one must check to see if the mutations found are in the same gene or two separate genes in order to distinguish between a carrier and an affected fetus. Requirements for testing include: blood samples from parents and proband if not previously studied, a pedigree, and a CVS/amniocentesis sample to test the fetus. Prices range between $230 and $1000 for testing of the initial sample, less for additional samples (laboratory contacts, personal communications, and laboratory information packets, 1999).

ANTENATAL DEXAMETHASONE THERAPY

Dexamethasone (DEX) is a potent glucocorticoid that inhibits the adrenal cortex through feedback on the hypothalamus and pituitary.[21] DEX is currently being used for prenatal treatment because, compared with other glucocorticoids, it crosses the placenta more efficiently (approximately 50% reaches the fetal side[19]), has a longer half-life (approximately 4–6 hours), and has a greater suppressive effect on ACTH.[1,7,22]

CAH is the first metabolic disease treated by administering medication to the pregnant mother at risk for carrying an affected fetus. Before DEX, there are no reports of SW-CAH or SV-CAH females born with normal female or Prader Stage 1 genitalia.[3] Early initiation of treatment and good compliance have been shown in various studies to significantly reduce the degree of genital virilization in females compared with their elder affected sister(s).[3,22] Because DEX treatment is used solely to prevent virilization of affected female fetuses, affected male fetuses are not currently being treated routinely.

Because DEX therapy is started very early in gestation and is only continued if the fetus is found to be an affected female, chorionic villus sampling (CVS) at 10–12 weeks is recommended to determine quickly the fetal sex and genotype. If the fetus is not affected, early diagnosis will help in decreasing the length of unnecessary treatment.[3,8] Alternatively, if CVS is not available or is not indicated, amniocentesis at 14+ weeks can be performed.

Most experts feel that DEX therapy should be conducted on a specialized research basis with informed consent, and under the close supervision by those experienced in dealing with this condition. Ideally, the team caring for the mother and fetus should include an endocrinologist, a perinatologist, a geneticist, and a genetic counselor. DEX treatment must be started by 7 weeks gestation,[22,23] before knowing the affected status or the sex of the fetus. Prenatal treatment beginning later than 10 weeks gestation has been shown to have little or no effect on preventing genital virilization.[20] The minimum effective dose should be used. The recommended dosage is 20 μg/kg/day maternal (nonpregnant) weight given in 2–3 divided doses.[3,7,22,24] The maximum dose is 25 μg/kg/day. The mother should not be taken off DEX until it is determined that the fetus is an unaffected female or is male. Treatment should be discontinued as soon as possible (Figure 2).[1] Mothers should be gradually weaned off DEX especially if treated on a long-term basis[3]; it is advised that the dose be decreased by 50% every 4–5 days in three stages over a 2-week period.[1]

The effectiveness of DEX therapy can be evaluated by measuring maternal plasma and/or urinary estriol, which reflects fetal adrenal synthesis of precursor steroids. These should be completely suppressed if the DEX dosage is adequate.[3] Normal maternal serum estriol levels are <0.1 ng/ml up to 9 weeks gestation, 4–5 ng/ml at 16 weeks gestation, and 10–30 ng/ml at term.[8] It may also be helpful to monitor maternal plasma and/or urine cortisol, and plasma dehydroepiandrosterone sulfate levels measured at repeated intervals to evaluate compliance and maternal adrenal suppression.[1,3,22] A maternal 17-OHP level is not a good indicator for maternal compliance, because there is little influence on these levels by treatment, but amniotic fluid 17-OHP level is a good indicator both for fetal suppression and for maternal compliance.[1] High-resolution targeted ultrasound of the perineum can also be helpful in monitoring an affected pregnancy and in planning for neonatal management if the status of the fetus is unknown.[25]

DEX STUDIES

Three major study series have assessed the effects of DEX taken prenatally (Table 1).[8,20,22,23,26] In all of the studies, the frequencies of spontaneous abortions are comparable to that of untreated groups, ranging between 3.8–9.0%. IUGR and/or fetal death occurred in <2% in the treated group, which is not significantly different from that seen in the general population.[3] Infants found to have IUGR later recovered. In two of the study series,[8,20,22,23] no birth defects were found in any of the children treated prenatally. Birth weight, length, and head circumference were normal in these infants, as were physical and neuropsychological development. The majority of affected female treated pregnancies were successful in preventing virilization (i.e. normal or Prader Stage 1 genitalia). In general, in those pregnancies in which treatment was not successful (i.e. virilization resulting in a Prader Stage ≥2), the treatment was started too late, the dosage was either too low or was given in one daily dose, maternal compliance was poor, and/or treatment was discontinued or interrupted at mid-pregnancy, when the clitoris is still responsive to excessive androgen exposure.[22] In the series by Forest et al.,[22,23] when treatment was not completely successful, all girls had two separate openings for the urethra and the vagina, which would make surgical correction easier. Untreated affected female pregnancies resulted in female infants with Prader Stage 2–5 virilization.

In the third and most recent study,[26] 44 pregnancies were followed. Unlike the other studies, some adverse effects were seen in both unaffected, short-term treated infants (two males and two females), and in an unaffected female infant and three affected female infants treated throughout the pregnancy.

These range from hydrocephalus and agenesis of the corpus callosum to developmental delays and growth retardation. It is not known whether the complications resulted directly from DEX treatment.

MATERNAL COMPLICATIONS

In the various prenatal DEX series discussed above, the research groups also looked at the effects of DEX treatment on the mothers. Maternal complications were seen in all of the studies to varying degrees. Complications include: excessive weight gain, which can be controlled by salt restriction in most patients; irritability and nervousness; increase in appetite; mood fluctuations; pedal and leg edema; striae; increased blood pressure; headaches; moderate proteinuria; Cushingoid facies; hirsutism; acne; preeclampsia; and general discomfort. All of these complications are known side-effects associated with glucocorticoid therapy. Increased intolerance has been noted with increased length of treatment. When treatment was discontinued, symptoms disappeared in most mothers.[22] The incidence of these complaints in women treated in the first to early second trimesters range from 10–13%. Mercado et al.[20] found that all mothers would take DEX again, whereas Lajic et al.[26] noted that approximately one-third of mothers would not undergo treatment again.

Other general, well-described side-effects of DEX include: impaired immune defense, glaucoma, subcapsular cataract, gastrointestinal bleeding, pancreatitis, aseptic bone necrosis, osteoporosis, myopathies, impaired glucose tolerance and diabetes, sleep disturbances, psychiatric syndromes, delayed wound healing, atrophy and fragility of the skin, ecchymosis, and pseudotumor cerebri. Contraindications to the use of DEX include peptic ulcers, osteoporosis, psychoses, infectious disease (herpes simplex, keratitis), diabetes, and hypertension. DEX interacts with enzyme inducers such as aminoglutethimide, phenobarbital, phenytoin, rifampin, and ephedrine, and these therefore can reduce the pharmacological effects of DEX.[21]

BEHAVIORAL STUDIES

Androgen exposure during brain development in animals has been shown to cause changes in some brain regions, such as the hypothalamus. Therefore, *in utero* exposure to high levels of androgens is believed to play a role in development.[27] Androgens may also be responsible for brain imprinting of sexual behavior and/or identity. In many species, including rodents, birds, and non-human primates, females exposed *in utero* to high levels of androgens during sensitive periods of fetal brain development express behaviors typical of males, including masculinized or defeminized sexual behavior, rough play, aggression, and male-type maze learning. Progesterone (elevated in CAH) and corticosteroids have also been shown to prevent masculinization in rats.[26] Sexual dimorphism in normal human brain development is thought to be at least partially caused by fetal testicular androgen exposure, but the degree to which the human brain is sexually dimorphic is controversial.[19] CAH females have been found to be masculinized for several, but not all, aspects of sex-typed behaviors (those in which males and females normally differ, such as assertiveness and dominance).[28–30] Additionally, SW-CAH affected females differ significantly from SV-CAH and from unaffected sister controls in having a more masculine orientation and a higher level of activity.[30] The use of DEX during pregnancy may thus protect the brain from the various concerns discussed above by normalizing the amount of fetal adrenal androgens.

Trautman et al.,[31] in a pilot study, looked at 26 children (3 affected) whose mothers had used DEX during pregnancy to determine the effects of therapy on cognitive and behavioral development; they were compared with 14 children from at-risk pregnancies (3 affected) who did not receive DEX. Mothers completed a standard questionnaire. Negative effects on developmental milestones or cognitive development were not seen, nor were gross behavioral disturbances. Of nine temperament categories, DEX-treated children showed a greater trend toward avoidance; they were also found to have increased shyness and withdrawal, less sociability, and higher internalization. There were no significant effects identified related to the dose of DEX, the duration of drug exposure, or the gestational period of exposure.[31]

ARGUMENTS AGAINST THE USE OF DEX

One major argument against the use of DEX is that 7 out of 8 fetuses will be treated unnecessarily, if only temporarily. Therapy must begin very early in the pregnancy to be fully effective; the status of the fetus cannot be determined before initiation of treatment. Some are reluctant to use DEX therapy during pregnancy because of uncertainty about long-term effects on neuropsychological development. These concerns are based primarily on animal toxicology studies. In both rats and monkeys, prenatal DEX treatment has been shown to reduce the size and neuronal content of the hippocampus.[26,32,33] In humans, atypical neuroanatomic features have been associated with learning disabilities, language disorders, and dyslexia.[27] Rats exposed to glucocorticoids in utero have an increased incidence of hypertension later in life. Pups of mice treated with extremely high doses of glucocorticoids also show more aggressive behavior.[26] Monkeys have impaired fetal growth when pharmacological doses of DEX (40–60 μg/kg/day) are used; fetal death occurs at higher doses.[22] As with many animal teratology studies, this dosage is significantly higher than that used for treatment of a human pregnancy.

DEX doses used for treatment are pharmacological, not physiological: 1.0–1.5 mg/day is well beyond that needed to suppress the adult hypothalamic–pituitary–adrenal axis.[32] In addition, fetal adrenal steroidogenesis may not be solely regulated by fetal CRH-ACTH in early gestation, and the negative feedback system in the fetus may not be fully mature. Thus, DEX may have varying degrees of glucocorticoid effect throughout development. Differences in transplacental passage of DEX, in maternal metabolic clearance of exogenous glucocorticoids, and in maternal sensitivity to glucocorticoids may also make the effectiveness of therapy variable.[1,2,23]

OTHER RATIONALES FOR DEX TREATMENT

There are several further reasons why prenatal DEX therapy might be considered. Genital ambiguity may disrupt parent-child bonding. Ambivalence about gender may result, which can cause problems with child rearing and with sense of identity, gender role, and sexual orientation for the child. The need for surgery and the continuous need for special treatment may cause physical and emotional scars.[3,33]

Glucocorticoid therapy can prevent not only genital virilization in affected females, but also adrenal hyperplasia in both sexes.[22] Postnatal therapeutic control of CAH female patients treated prenatally requires lower doses of glucocorticoids, presumably because they did not have hyperplastic adrenals. Therefore, continued DEX therapy for CAH-affected males may also be beneficial.[1] This has not yet been proven, but studies are currently being conducted in this area.

SUPPORT GROUPS

The following support groups can be contacted to get information about CAH or to get in touch with further support groups in a specific area:

- National Adrenal Disease Foundation, 505 Northern Boulevard, Great Neck, New York 11021. (516) 487-4992; http://www.medhelp.org/nadf/nadf5.html; nadf@aol.com
- MAGIC Foundation for Children's Growth, 327 North Harlem Avenue, Oak Park, IL 60302. (708) 383-0808, (800) 362-4423; www.magicfoundation.org/cah.html; mary@magicfoundation.org
- Congenital Adrenal Hyperplasia Support Association (CAHSA), 801 Country Road #3, Wrenshall, MN 55797. (218) 384-3863
- Congenital Adrenal Hyperplasia Message Board; http://www.elevenoclock.com/cah
- Congenital Adrenal Hyperplasia Web Board; http://www.insidetheweb.com/messageboard/mbs.cgi?acct=mb383

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