Abstract

In foetuses at risk of virilising congenital adrenal hyperplasia (CAH), prenatal treatment can be offered by administration of dexamethasone (DEX) via the mother, in order to suppress foetal adrenal androgen oversecretion and prevent genital malformations. The first treated cases were described 20 years ago, and several hundred pregnancies have been reported since. There is a consensus that the treatment effectively prevents or reduces virilisation, but opinions regarding its safety differ. Rare adverse events have been reported in treated children, but no harmful effect has been documented that can be clearly attributed to the treatment. However, few treated foetuses have been followed until adolescence. Animal studies and epidemiological data point to various adverse effects of excess glucocorticoids on the developing foetus. In order to prevent virilisation effectively in females affected with CAH, the prenatal treatment needs to be instituted in the early first trimester, before prenatal diagnosis is possible. Thus, a majority of treated foetuses will receive DEX unnecessarily.

The PREDEX study was initiated in Stockholm in 1999 as an open, controlled, non-randomised, multicentre trial. Participating centres are Stockholm, Bergen, Kuopio, Warsaw, London, Lyon and Barcelona. The study has been approved by the ethics committees in each country. The purpose of PREDEX is to evaluate prospectively the prenatal treatment regarding efficacy in preventing virilisation as well as to study its safety for both mothers and treated children. Children are followed until 18 years of age and a wide range of physiological, metabolic and developmental parameters are considered. In Sweden, treatment is not offered outside the frames of the trial.

Introduction

Ever since the publication 20 years ago describing the first successful case where virilisation of a girl with congenital adrenal hyperplasia (CAH) was prevented by maternal dexamethasone (DEX) medication (1), this treatment has been the subject of intense discussion.

The situation presents us with a unique dilemma. On the one hand, the treatment offers a means to prevent traumatising genital surgery in affected female infants, the benefits of which are clearly tremendous. However, in order to be effective in preventing virilisation in females affected with CAH, the prenatal treatment needs to be instituted in the early first trimester, before prenatal diagnosis is possible. Thus, a majority of treated foetuses will receive DEX without need. No adverse effects have been documented that are clearly attributed to the prenatal treatment, but there are plenty of theoretical harmful effects of foetal glucocorticoid exposure. Therefore, long-term follow-up studies comprising large numbers of treated cases are warranted, addressing a wide variety of somatic and neuropsychological parameters.

Congenital adrenal hyperplasia (CAH)

CAH is the common name for a group of inherited diseases characterised by impaired cortisol synthesis in the adrenal cortex. The major cause of CAH is 21-hydroxylase deficiency (21OHD), which accounts for at least 95% of all cases in most populations. The incidence in Sweden is approximately 1/10 000 live births (2). In CAH due to 21OHD, there is impaired synthesis of cortisol and, in severe cases, aldosterone. Insufficient cortisol production leads to a lack of negative glucocorticoid feedback on the pituitary and hypothalamus, resulting in an increase in corticotropin, hyperplasia of the adrenal cortex, a build-up of cortisol precursors and androgen excess.

The clinical symptoms of CAH due to 21OHD demonstrate a wide spectrum of severity, with the most severe, classical cases diagnosed in the neonatal period due to salt-wasting and prenatal virilisation in affected females, including persistence of a urogenital sinus, labioscrotal fusion and clitoromegaly. Surgery of external genitalia is required to restore a female anatomy in these girls, whereas ovaries are functional and Müllerian-derived internal genital structures are normal. Thus, with proper management, fertility is possible in these patients.

Rationale for prenatal treatment

The idea of prenatal treatment is to treat the foetus with a glucocorticoid via the mother, in order to suppress the foetal adrenal androgen oversecretion and
prevent the genital malformations. The aim is to avoid or substantially reduce the need for difficult and stressful corrective genital surgery in affected females. Hydrocortisone was tried in the first case treated, but due to incomplete prevention of virilisation (hydrocortisone is largely inactivated by the placenta), DEX has subsequently been used.

Virilisation of external genitalia by androgens occurs from 6 to 8 weeks of gestation (Fig. 1). Thus, in order to be effective in preventing female genital virilisation, treatment must begin in the early first trimester. The dose is typically 20 mg/kg per day, based on pre-pregnancy maternal weight, to a maximum of 1.5 mg daily in three divided doses, beginning before the seventh week of gestation. This regimen has been shown to normalise amniotic fluid 17-hydroxyprogesterone levels in CAH-affected foetuses (3). Prenatal diagnosis is subsequently performed and treatment is discontinued if the foetus is male or an unaffected female, whereas CAH-affected female foetuses are treated until term.

Treatment is offered to women who have previously given birth to a child with severe CAH. Since CAH is an autosomal recessive disease, the risk of an affected child in these families is one in four in each pregnancy. Since only affected females will suffer from virilising malformations, only one out of eight foetuses will benefit from the treatment. Thus, in seven out of eight cases the foetus will be treated with DEX for a few weeks in early development without any benefit of the treatment.

**Prenatal diagnosis**

Prenatal diagnosis of CAH due to 21OHD was first made by measurements of steroids such as 17-hydroxyprogesterone, Δ4-androstenedione and 21-deoxy-cortisol in amniotic fluid (4). When the gene encoding the 21-hydroxylase enzyme (CYP21) was shown to be located in the HLA class III gene region on chromosome 6p21.3, linkage analysis based on HLA typing of cultured amniocytes became possible (5). Molecular genetic methods for HLA typing were subsequently developed, obviating cell-based assays and enabling HLA typing by DNA-based methods.

The molecular genetics of 21OHD have been extensively studied, and CYP21 genotyping has become a valuable diagnostic tool in CAH. The chromosomal locus has an unusual and complex structure. CYP21 and a highly homologous pseudogene (CYP21P) are located in tandem repeats, predisposing to misalignment during meiosis and interchange of sequences between the genes both through recombination and gene conversion (6, 7). Due to this genomic structure, there is variability in gene copy number in the region, and the majority of all disease-causing mutations in CYP21 (around 95% in most populations) are either gene deletions or belong to a group of ten pseudogene-derived mutations. There are clear genotype–phenotype relationships, enabling prognostic evaluations based on the underlying combination of mutations. Specifically, a group of completely inactivating mutations (Null) together with the common I2 splice and I172N mutations are associated with prenatal virilisation, whereas the P30L, V281L, and P453S mutations are associated with a non-classic phenotype without prenatal genital virilisation (8–12) (Fig. 2). In families who have given birth to a child with CAH, CYP21 genotyping can thus be used to assess the risk of genital virilisation in subsequent pregnancies. This
may otherwise be difficult, especially if the index case is male and the child is diagnosed through neonatal screening, before symptoms have been allowed to appear.

Published follow-up studies

Follow-up studies describing the outcome of several hundred DEX-treated pregnancies at risk of CAH have been published. The largest series include more than 500 cases from North America (13), around 250 pregnancies co-ordinated from France (14), and 44 from Sweden (15). In addition, several small samples or individual cases have been described (16–20).

There is a consensus that the treatment is effective in ameliorating the genital virilisation in affected females and that it completely eliminates it in more than 85%, provided that it is instituted properly in early pregnancy (21). Treatment failures, i.e. affected females requiring genital reconstruction, have generally been attributed to late onset of treatment, cessation of therapy in mid-gestation, non-compliance or sub-optimal dosing, although some cases have remained unexplained. However, in addition to documenting its efficacy, the safety of the treatment for both child and mother has to be established. In this area, opinions still diverge substantially.

Gestational length and rate of foetal wasting have not been different in treated versus untreated pregnancies; birth weight, length and head circumference have also not been significantly different in treated children compared with controls. In general, postnatal growth has also been normal in those cases where it has been followed. However, several adverse events have been reported in children treated during foetal life. These include isolated cases of cardiac septal hypertrophy (22), hydrocephalus (15), hydrometrocolpos (20) and intrauterine growth retardation (19). The Swedish series included more adverse events than other published studies. In this material – in addition to hydrocephalus which was seen in one short-term treated boy – hypospadias, cryptorchidism and slow psycho-motor development was present in one case, whose parents were first cousins, and a short-term-treated girl showed poor growth from 6 months of age. Another short-term-treated child had a several months long episode of vomiting and failure to thrive neonatally. An adenovirus infection was suspected but not confirmed, and she subsequently recovered completely. Among seven full-term-treated Swedish children, pre-eclampsia with intrauterine growth retardation was seen in one girl who had a normal catch-up, whereas another girl displayed severe mood fluctuations. Two full-term-treated sisters showed poor growth and delayed development which were later attributed to a concurrent mitochondrial disease in one of the sisters.

Regarding neuropsychological development, a pilot study using maternal surveys suggested that children not affected with CAH who were subjected to prenatal DEX treatment were more shy than unaffected children (23). There was no difference in cognitive abilities. A more recent study from the same group assessing cognitive and motor development by questionnaires to mothers of 487 children (174 DEX treated and 313 untreated) could not document any significant effects of prenatal DEX exposure on developmental outcome (24).

Thus, overall numbers of adverse events are small and the observed abnormalities are diverse, including rare developmental defects, abnormalities of growth and metabolism as well as psychological symptoms. In some cases, alternative explanations for the observed adverse events have existed and in conclusion, no clear untoward effect has been seen that can be attributed to DEX with certainty. However, few treated foetuses have been followed through childhood and adolescence, and long-term prospective studies are lacking.

Maternal side-effects are of the kind that can be expected during treatment with a potent glucocorticoid for an extended period of time; these include weight gain, cutaneous striae, irritability, insomnia, mood swings, oedema, gastrointestinal intolerance, increased blood pressure, headache, proteinuria, Cushingoid facies and general discomfort. Many of these are phenomena that can also be seen during normal pregnancy. These side-effects most often resolve upon discontinuation of treatment.

In the series of treated pregnancies from North America (13), no significant differences in side-effects were seen in the mothers who were treated with DEX compared with untreated mothers – except in weight gain, oedema and striae. There was no difference in the incidence of hypertension or gestational diabetes. All treated mothers stated that they would take the treatment again in the event of a future pregnancy. In the French material, it was concluded that 18% of treated mothers reported some side-effects, whereas more severe complications were seen in 2% (14). In the Swedish women, there were no differences in treated women versus controls regarding blood pressure, glucosuria or proteinuria. However, there was a significant increase in weight gain during the first trimester, and four of 44 treated mothers experienced severe discomfort due to weight gain, mood fluctuations, marked striae, acne and hirsutism. A third of the Swedish women stated that they would not choose to take DEX again in the event of a future pregnancy.

In conclusion, treated mothers experience greater weight gain, oedema and striae than untreated mothers, but DEX treatment during pregnancy did not lead to an increased risk of gestational diabetes or hypertension. Regarding general well-being and side-effects that are difficult to quantitate, results have varied substantially among investigators.

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Basis for concern

The practise of prenatal DEX treatment for CAH has been the subject of intense debate, and the treatment remains controversial (25). The basis for the concern that has been voiced is the lack of sufficient long-term follow-up studies of treated children, in combination with results of animal experiments and epidemiological data in humans.

Animal experiments have revealed adverse effects of supraphysiological glucocorticoid exposure in utero. Treatment of pregnant rats with 20 μg/kg per day DEX throughout pregnancy was associated with decreased birth weight and adult hypertension (26). DEX doses of 100 μg/kg daily caused low birth weight, low kidney weight, decreased numbers of glomeruli, sodium retention and hypertension in rats (27). Last-trimester administration of DEX has been shown to result in hyperglycaemia and hyperinsulinemia in the adult offspring (28), whereas exposure in early pregnancy did not affect glucose homeostasis in adult life. Ultra-high doses of DEX (200 times the dose used in prenatal treatment of CAH) in the gestation of rhesus monkeys depleted hippocampal pyramidal and dentate granular neurons (29); in the rat, a single low dose of DEX in late gestation (50 μg/kg) was shown to alter levels of c-fos and AP-1 in the brain, two transcription factors known to be involved in brain cell differentiation (30).

Epidemiological studies have shown that being small for gestational age or underweight at birth correlate with an increased risk of hypertension, hyperlipidaemia, insulin resistance, non-insulin-dependent diabetes mellitus and ischaemic heart disease in adult life (31). Based on these findings, it is hypothesised that the intrauterine environment or specific events in prenatal development are capable of programming later metabolic functions in the adult. Specifically, it has been proposed that exposure of the foetus to maternal glucocorticoids, through a relative deficiency of placental HSD11B2 (the enzyme that normally inactivates maternal cortisol in the placenta) might explain the link between low birth weight and later disease (32).

Newborns who have been treated during foetal life due to a risk of CAH fall into the normal ranges regarding length and weight, but it has been argued that there might be subtle effects on foetal growth that have not been documented (25). The relationship between birth weight and adult blood pressure is continuous and includes infants with birth weights within the low normal range, and the activity of HSD11B2 correlates directly with birth weight within the normal range (33).

In conclusion, theoretical risks of the prenatal CAH treatment include effects on widely varying aspects of human physiology and development. Although the relevance of any of the animal studies for human physiology is not known, and the concerns brought forward by data from epidemiological studies have not been substantiated by follow-up studies so far, most investigators today agree that there is an urgent need for long-term follow-up studies of both short-term and long-term-treated children (21). Study protocols should consider all psychological/behavioural and somatic effects of excess prenatal glucocorticoids that have been observed in animal experiments or in human studies. If possible, these studies should be undertaken as large international collaborations, in order to make possible the collection of sufficient numbers of patients to permit meaningful statistical analyses of the data.

PREDEX: Prenatal treatment of congenital adrenal hyperplasia with dexamethasone – a longitudinal study of outcome measures for mother and child

The PREDEX study was initiated in Stockholm in 1999. The objectives are:

1. To evaluate the effect of maternal/prenatal DEX in preventing/reducing virilisation of external genitalia in female foetuses affected with a severe form of 21OHD.

2. To study the impact of DEX concerning:
   i) The well-being of the mother during treatment.
   ii) Glucose tolerance during pregnancy.
   iii) Blood pressure during pregnancy.
   iv) Bone mineral density (BMD) in treated women.
   v) Foetal growth and duration of gestation.
   vi) Linear growth of treated children until 18 years of age.
   vii) Blood pressure, renal function, bone mineralisation, glucose tolerance, lipid profile, hypothalamic–pituitary–adrenal axis function, motor skills and brain morphology in treated children until 18 years of age.
   viii) Psychosocial development and cognitive development in treated children.

Study design

PREDEX is an open, controlled, non-randomised, multicentre trial. Participating centres are Stockholm, Bergen (Robert Bjerknes), Kuopio (Raimo Voutilainen), Warsaw (Maria Ginalska-Malinowska), London (Peter Hindmarsh/Carolyne Brain), Lyon (Maugelone Forest) and Barcelona (Lourdes Ibanez). The study has been approved by the national ethics committees in each country. Written informed consent is obtained from all participating parents.

Women who have previously given birth to a child with a severe form of 21OHD are informed about the possibility of receiving DEX during their next
pregnancy. Molecular genetic diagnostics are completed before inclusion, to verify the severity of the disease, and to assure that prenatal diagnosis can be rapidly performed without ambiguity. Genotyping is centralised to Stockholm for the Scandinavian countries. Therapy is only offered within the frames of the trial. Exclusion criteria are medical conditions in the mother that could be aggravated by DEX, e.g. diabetes, hypertension, osteoporosis or psychiatric disease.

DEX is introduced before the seventh week of gestation, at a dose of 20 μg/kg pre-pregnancy weight in three divided doses. After 10 completed weeks, a chorionic villous biopsy (CVB) is taken, followed by sex typing and CYP21 genotyping. Sex typing is done by SRY PCR, which is completed within 2–3 days after the biopsy is taken. CYP21 mutation analysis is complemented with linkage analysis, as an extra safety precaution due to the complex nature of the locus and to exclude maternal contamination. CYP21 genotyping is usually completed within 1 week (2 weeks maximum). In the event of a male foetus or an unaffected female, DEX is discontinued by tapering over 1 week. These foetuses are thus subjected to DEX for around 5–6 weeks. Affected female foetuses are treated until term.

**Controls**

Three different control groups are used: (i) pregnancies that resulted in older, not prenatally treated siblings with CAH; (ii) pregnancies in families with no history of CAH, chosen from maternity health care centres in the close vicinity to the co-ordinating centres; (iii) pregnancies where prenatal therapy was declined.

**Practical procedures**

Treatment is initiated by a local paediatrician after contact with the co-ordinating centre in each country. The women are followed during pregnancy at the local maternity health care centre. The children are followed by the local paediatrician; at 5, 12 and 18 years, the children are sent to the co-ordinating centre for an extended evaluation.

Only the non-invasive tests are performed in the controls. Reference values obtained from population studies will be used for the other parameters.

For each pregnancy, there is a booklet containing study forms and questionnaires. This booklet follows the mother at the maternity health care centre and the child at the visits to the paediatrician. Results of tests are continually entered into this booklet.

A database is currently being established, which will be accessible for participating physicians through the internet. When finalised, all data will be entered directly into this database at www.predexstudy.com. For construction of the database, PREDEX contracted a Swedish IT company called SoftIT. The database will follow the layout of the old study forms and will meet standards and security measures that are required by Swedish authorities. Only authorised persons who have received a personal user identification and password will be able to enter the database. There will be several levels of authorisation. Patient data will be coded and all communication will be encrypted. Security copies will be stored safely and log files will be created whenever the database is used. The database will have a module for searching information of interest in order to facilitate statistical analysis of the data.

**Progress of PREDEX**

The initiative to launch a collaborative, prospective, follow-up study of children treated prenatally with DEX was taken by Professor Martin Ritzén in 1997. Colleagues from seven additional countries agreed to participate (Denmark has subsequently withdrawn). In March 2001, a meeting was held in Stockholm, with the purpose of discussing the study protocol and other practical matters.

PREDEX is a large and challenging undertaking, and the many different parameters that should be investigated in each case are demanding, both for the patients and the health care providers supporting them. With the debate that has been present since the mid-1990s, doctors and patients have also become more cautious in initiating therapy. There has also been substantial administrative delay in several countries, with varying local regulations regarding ethical approval etc. For all these reasons, the number of included patients is still small.

As of early spring 2004, Sweden has 11 entered cases (seven short term, two full term, two controls). Poland has nine cases (seven short term, two controls). Norway has two (both short term) and Finland has two (one short term, one control). From France, UK and Spain, no cases have been entered to date. Thus, the total number of cases amounts to 24, with 17 short-term treated, two full-term treated and five controls from CAH families declining DEX. No child has yet reached the age of 5 years, when the first extended evaluation is planned.

**Extended retrospective analysis of prenatally treated children in Sweden**

In addition to the prospective study described above (PREDEX), an extended retrospective study is currently under way, analysing children who were treated with DEX during foetal life between the years 1985 and 1995 in Sweden. This is an extension of the follow-up study of 44 pregnancies that was published 6 years ago (15). Growth, glucose, insulin, HbA1C, lipid profile, blood pressure and renal function are measured. In addition, the children’s behaviour and cognitive development are assessed using a battery of standardised tests are continually entered into this booklet.
neuropsychological tests, interviews and questionnaires (parental ratings as well as self-evaluations by the children). The same licensed clinical psychologist is performing all tests, and the children are between 7 and 18 years of age at testing. These neuropsychological tests take around 4 h to complete and are performed at the Karolinska Hospital or at a paediatric clinic close to the family’s home. As of spring 2004, 14 children have been tested. No statistically analysed results are available yet.

Concluding remarks

An extensive long-term, prospective, follow-up study of prenatally DEX-treated children and mothers is under way, in an attempt to establish the efficacy and safety of prenatal CAH treatment. This is a huge undertaking, and the study has been delayed for practical reasons in several of the participating countries. The number of entered cases is small and results that can be analysed statistically will obviously not be available for many years to come. Still, we strongly feel that the efforts must continue to collect as much data as possible, and despite the long-term commitment and lack of immediate feedback, we maintain our standpoint and do not offer treatment outside the frames of the follow-up study.

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