

Use of aromatase inhibitors in precocious puberty

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Abstract

During puberty, estrogen causes breast maturation and growth of the uterine lining in girls, and accelerates linear growth and bone maturation in both boys and girls. Decreasing the biosynthesis of estrogen can attenuate these processes. In 12 girls with the McCune-Albright syndrome (MAS), in which precocious puberty is due to production of estrogen from ovarian cysts, testolactone (40 mg/kg per day) decreased the volume of ovarian cysts, the frequency of menses, and the rates of growth and bone maturation, for periods of 1-4 years. In a 6-month pilot study of 12 children (eight boys; four girls) with congenital adrenal hyperplasia, testolactone, in combination with an antiandrogen (flutamide), a mineralocorticoid (fludrocortisone acetate, Florinef), and a reduced glucocorticoid dose, improved the control of growth and bone maturation compared with conventional therapy. In a 6-year study of 10 boys with familial male precocious puberty, testolactone, in combination with an antiandrogen (spironolactone), decreased rates of growth and bone maturation, and increased predicted adult height. All patients who developed evidence for gonadotropin-dependent puberty were also treated with a GnRH analog. Testolactone had no important adverse effects in any group of patients, although the need for a four-times-daily dosing schedule made compliance difficult for many families. We conclude that suppressing of estrogen with testolactone was effective therapy, and that more potent and specific inhibitors of aromatase could further improve the treatment of these disorders.

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Introduction

Although the role of estrogen in human development is conventionally understood to be feminization of the young female - that is, the maturation of breast tissue, formation of female body habitus and, finally, stimulation of the uterine lining and induction of cyclic menses - we now recognize that estrogen plays an equally important role as a powerful regulator of the growth and maturation of bone in both sexes. Clinical evidence for this role of estrogen includes two recent case reports: the first (Smith *et al.* 1994) described a 28-year-old man with unfused epiphyses, tall stature, persistent linear growth into adulthood, and who was found to have complete estrogen resistance as a result of a mutation of the estrogen receptor gene. The second report (Morishima *et al.* 1995) described a 24-year-old man and his 28-year-old sister who presented with tall stature and delayed epiphyseal fusion. Both patients were found to be homozygous for a missense mutation in the aromatase gene (*CYP19*), and were responsive to exogenous estrogen therapy. These

examples illustrate the importance of estrogen in growth and bone maturation, and indicate that suppressing the biosynthesis of estrogens such as estradiol and estrone with the use of aromatase inhibitors could improve our treatment of disorders such as precocious puberty, in which sexual development and the rates of linear growth and maturation of bone are accelerated in early childhood, and final, adult height is decreased as a result of early fusion of the epiphyses.

Precocious puberty

Normal puberty has its onset at age 8-11 years in girls and presents with breast development (thelarche); in boys, puberty begins at 9-12 years, and begins with enlargement of the testes. During puberty, in addition to development of the secondary sexual characteristics, there is a marked increase in the rate of linear growth, followed by a deceleration at the end of puberty (age 14-17 years in girls; 15-18 years in boys) with fusion of the epiphyseal growth plates. These somatic changes are caused by increases in

the gonadal sex steroids (estrogen and testosterone) in concert with increases in the adrenal androgens, and in the rate of secretion of pituitary growth hormone and other hormonal factors. In normal children, puberty is initiated by an increase in the secretion of hypothalamic gonadotropin-releasing hormone (GnRH or LHRH), which acts on the pituitary gland to stimulate the pulsatile secretion of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which, in turn, act on the ovaries and testes to stimulate secretion of the sex steroids.

In precocious puberty (puberty at age <8 years in girls and <9 years in boys), premature secretion of the gonadal sex steroids causes early breast maturation and menses in girls, and development of the testes, penis and somatic virilization in boys. Both sexes experience rapid rates of linear growth and bone maturation, and early fusion of the bony epiphyses causes short adult stature. The increased concentrations of sex steroids may also cause marked emotional lability and behavioral problems, the onset of menses in young girls, and erections and sexual interest in young boys. These behaviors frequently cause severe social difficulties at home and at school.

There are two general categories of precocious puberty. Gonadotropin-dependent ('central') puberty is caused by early maturation of the hypothalamic-pituitary GnRH axis. This category of precocious puberty is now treated with the long-acting GnRH agonist analogs, such as Lupron, histrelin and deslorelin, which suppress the pulsatile secretion of LH and FSH. Gonadotropin-independent ('peripheral') precocious puberty is caused by autonomous secretion of sex steroids from the gonads or adrenal glands, and because the pituitary gonadotropins are frequently suppressed by the increased concentrations of estrogen or testosterone, or are in the prepubertal range, the GnRH analogs are ineffective. Because of the lack of an effective treatment, peripheral precocious puberty has remained a challenge for the pediatric endocrinologist. At the National Institutes of Health, we have used the aromatase inhibitor, testolactone, to treat children with three subcategories of gonadotropin-independent precocious puberty: girls with the McCune-Albright syndrome, boys with familial male precocious puberty, and boys and girls with congenital adrenal hyperplasia. The following sections will outline our experiences with this agent in these conditions.

McCune-Albright syndrome (MAS)

The classical presentation of MAS is the triad of the bone disease polyostotic fibrous dysplasia, *café-au-lait* skin pigmentation, and precocious puberty. Other disorders of endocrine hyperfunction are often seen: hyperthyroidism and nodular goiter are seen in 30-40% of patients,

hypersomatotropinemia and acromegaly may appear in older children and young adults, hypercortisolemia and Cushing's syndrome have been reported in infancy, and rickets may occur as a result of hyperphosphaturic hypophosphatemia. Although seen in both sexes, most reported cases have been females, most of whom have presented with precocious puberty. The underlying mechanism behind this diverse spectrum of manifestations is currently understood to be the presence of an activating mutation in the stimulatory α -subunit of the G-protein-linked cyclic AMP signal transduction system in the affected endocrine glands, bone, skin and other tissues (Weinstein *et al.* 1991). The precocious puberty is due to estrogen secretion from large, unilateral ovarian cysts that enlarge and then involute over periods of days to weeks. Girls commonly present with vaginal bleeding, often before the age of 2 years. Gonadotropin concentrations are usually suppressed or undetectable, both at baseline and after administration of LHRH, and the long-acting GnRH analogs are not an effective treatment.

After a pilot study (Foster *et al.* 1985) of the aromatase inhibitor, testolactone, in a 2-year-old girl with MAS showed an improvement in signs of puberty with no important adverse effects, a total of five patients with MAS (ages 1.2-4.5 years; bone ages 2.0-7.8 years) were enrolled in a 6-month trial of testolactone, 40 mg/kg per day given in divided doses, four times a day (Feuillan *et al.* 1986). Bone maturation was assessed with radiograms of the hand, and ovarian volume and cyst dimensions were measured using pelvic ultrasonography. Serum concentrations of estrone, estradiol and their precursors, testosterone and androstenedione, were measured, as were LH and FSH after administration of 100 μ g GnRH. We found a decrease in concentrations of estradiol, ovarian volume, frequency of menses and rates of linear growth and bone maturation during treatment, with a return to pretreatment values after its discontinuation. In addition, concentrations of GnRH-stimulated LH and FSH increased towards the normal, prepubertal range. The only important adverse events were cramping and diarrhea, which resolved with a temporary reduction in dose. Encouraged by this short-term trial of testolactone, we treated 12 MAS patients (age 1.8-7.8 years; bone ages 5.0-12.0 years) with testolactone for 0.5-5.0 years (Feuillan *et al.* 1993). In this larger group, treated for a longer period, response to therapy was variable: while there were significant decreases in serum estradiol, frequency of menses, and growth rate after 1 year of treatment, three of the seven patients who were treated for 3 years or more exhibited a rebound in serum estradiol and a recurrence of ovarian cysts by 3 years. Only one girl developed LH responses to GnRH that met criteria for central puberty. No girl developed clinically important increases in testosterone or androstenedione, and none

exhibited signs of androgen excess. The predicted adult stature increased in four patients, but decreased in three others as a result of progression of bone disease and recurrent fractures. Compliance with the frequent dosing regimen was apparently good, although many parents acknowledged difficulty with the schedule and it is possible that treatment failures were a result of inadequate dosing. Again, adverse effects were few. One girl with pre-existing increased hepatic enzymes had further increases while on treatment, and discontinued testolactone. There were no other adverse effects of treatment, other than transient cramping and diarrhea in most girls at the beginning of treatment.

Our conclusions were that aromatase inhibitors can be effective therapy for many girls with precocious puberty caused by MAS, and that more potent and long-lasting compounds could improve treatment of this challenging condition.

Familial male precocious puberty (FMPP)

FMPP is an autosomal dominant form of gonadotropin-independent precocious puberty limited to males. Sporadic cases occasionally occur. The mechanism of FMPP has been shown to be an activating mutation of the LH receptor (Shenker *et al.* 1993), and testicular biopsy has shown Leydig cell hyperplasia. Affected boys often enter puberty at 2-3 years of age, with increase in the size of penis and testes, and virilization. Linear growth rate is rapid, but early epiphyseal fusion usually leads to short adult height. Fertility is normal. As in MAS, gonadal activation is initially independent of gonadotropin stimulation, and the GnRH agonist analogs are ineffective in young boys with FMPP. However, prolonged exposure to sex steroids often causes maturation of the hypothalamic GnRH centers, and central puberty frequently supervenes.

Because precocious puberty in boys is caused by the actions of both androgens (which cause virilization and growth) and estrogens (which cause growth and epiphyseal fusion), we are investigating the treatment of FMPP using both a blocker of androgen action (spironolactone) and a blocker of estrogen biosynthesis (testolactone) (Leschek & Cutler 1997).

The initial clinical trials (Laue *et al.* 1989) treated nine boys (ages 3.3-7.0 years; bone ages 6.0-13.5 years) with spironolactone (2.0-5.7 mg/kg per day given in divided doses, twice a day), testolactone (20-40 mg/kg per day given in divided doses, four times a day), and their combination, for periods of 6-12 months. The rates of growth and bone maturation were significantly lower during treatment with combined therapy compared with pretreatment. Importantly, spontaneous penile erections and aggressive behavior disappeared in all boys, and acne

resolved. During treatment, six boys had evidence of pubertal activation of hypothalamic GnRH neurons. Boys who have demonstrated activation of hypothalamic centers are also treated with a long-acting GnRH analog (deslorelin), because later studies showed that, otherwise, the pubertal increase in gonadotropins overrides the blockade of testosterone action and estrogen synthesis (Laue *et al.* 1993). Currently, long-term studies are in progress to determine the effects of this form of therapy on adult height.

Congenital adrenal hyperplasia (CAH)

CAH is a family of inherited (autosomal recessive) disorders characterized by an enzymatic defect in cortisol biosynthesis. The most common cause of CAH is 21-hydroxylase deficiency, which accounts for more than 90% of cases. The incidence of 21-hydroxylase deficiency is approximately 1:14 000 live births worldwide (Pang & Shook 1997). Impaired 21-hydroxylase activity causes deficient production of cortisol and aldosterone, and an accumulation of precursor hormones. The accumulation of precursors occurs because the hypothalamic-pituitary-adrenal axis is normally controlled by negative feedback. With a deficiency of cortisol, corticotropin (ACTH) is oversecreted, and adrenal hyperplasia occurs. The increase in ACTH, in combination with the accumulation of hormone precursors, results in excess androgen production.

Two classic forms of CAH resulting from 21-hydroxylase deficiency exist: salt-losing and non-salt-losing (simple virilizing), reflecting the degree of mineralocorticoid deficiency. Patients with the salt-losing form, if untreated, suffer a life-threatening adrenal crisis, typically between 2 and 3 weeks of age. Boys with the simple virilizing form have sufficient mineralocorticoid production to avoid a neonatal crisis, and are diagnosed between birth and 5 years of age. Both types of CAH result in ambiguous genitalia in girls, who are typically diagnosed in the neonatal period. In both sexes, excess androgens can produce signs of virilization, precocious puberty, premature growth acceleration, early epiphyseal fusion and adult short stature.

The treatment of classic 21-hydroxylase deficiency CAH remains a challenge for the pediatric endocrinologist. Conventional treatment includes administration of a glucocorticoid (usually hydrocortisone (for example as Cortef)) and a mineralocorticoid (usually fludrocortisone (for example as Florinef)) to replace the deficient adrenocortical hormones and suppress the increased hypothalamic and pituitary factors. However, replacement with 'physiologic' doses of these compounds cannot replicate the functioning of a normal hypothalamic-pituitary-adrenal axis; ACTH concentrations remain

increased and hyperandrogenism persists. In contrast, administration of 'supraphysiologic' doses of glucocorticoid can suppress the increased androgens, but often at the expense of iatrogenic hypercortisolism, leading to obesity, poor linear growth and short stature. Other risks of prolonged hyper-cortisolism include hyperglycemia, hypercortisolemia, hypertension and osteoporosis.

Our investigational approach to treatment of children with CAH has been to block the action of adrenal androgens with an antiandrogen (flutamide) and to use testolactone to block the conversion of androgens to estrogen (Merke & Cutler 1997). A reduced dose of hydrocortisone is used, together with fludrocortisone, if needed. A GnRH analog (deslorelin) is added to the regimen when gonadotropin responses to GnRH indicate pubertal activation of the hypothalamic centers. In a randomized, crossover pilot study (Laue et al. 1996) of 12 children with the 21-hydroxylase deficiency form of CAH (eight boys; four girls; ages 1.8-12.2 years, bone ages 2.3-12.5 years), we used flutamide (10 mg/kg per day given in a divided dose, twice a day), testolactone (40 mg/kg per day given in divided doses, three times a day), fludrocortisone (nine children; 100-150 µg/day) and a reduced dose of hydrocortisone (8 mg/m² per day). Growth rate, weight gain and rate of bone maturation all declined during flutamide and testolactone therapy, despite the reduction in hydrocortisone dose and the increase in androgen precursors, both of which would be expected to increase the rate of growth and bone maturation. These observations confirm the powerful effectiveness of these medications in blocking androgens and estrogens. A long-term clinical trial of this investigational approach to treatment of classic CAH is currently under way at the National Institutes of Health and, to date, no adverse effects have been observed.

Conclusions

We found that testolactone is an effective adjunct to antiandrogen and GnRH therapy for the precocious puberty in children with FMPP and CAH, and is partially effective in reducing estrogen concentrations, frequency of menses and rates of bone maturation in girls with MAS. The only important adverse effect has been mild cramping and diarrhea, which may occur early in treatment and resolve with a temporary reduction in dose. The limitations of testolactone include its relatively low potency, and the need for a frequent (every 6-8 h) dosing schedule. Clinical trials using potent and long-lasting third-generation compounds, which are specific to P450 aromatase, are being planned for the treatment of these and other pubertal disorders.

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