Considerations for Androgen Therapy in Children and Adolescents with Klinefelter Syndrome (47, XXY)

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Introduction

Klinefelter syndrome (KS) (47,XXY) is a relatively common cause of *primary* hypogonadism, occurring in approximately 1:600 males. Pediatric patients with this condition rarely present with simple hypogonadism, for they may also have other phenotypic, developmental, and speech difficulties associated with KS. Timing of adolescent development usually begins within the physiologic range and progresses with penile enlargement and development of pubic hair. The testes only slightly enlarge and the key finding is relatively advanced pubertal development *with* disproportionately small testes. They are not often of firm consistency early in pubertal development. In this report we shall review the physiology of androgen production and activity, discuss issues specific to children and adolescents with KS, and then describe the pharmacologic agents appropriate for androgen replacement therapy.

The goals of androgen therapy for adolescents with hypogonadism are to promote linear growth and secondary sexual characteristics, and to permit the normal accrual of muscle mass, bone mineral content, and the adult regional distribution of body fat. Secondary goals are mainly in the psychosocial sphere, in which pubertally delayed boys may feel that they look too young, are not considered a 'peer' in their age group and have difficulty competing in athletic endeavors. Those with KS may have additional psychosocial factors to consider before starting testosterone therapy or escalating the dose. These considerations are in addition to the effects that testosterone therapy may have on spermatogenesis and the possibility of fertility, even by techniques of assisted reproduction [1, 2].

A variety of androgen preparations are available for adults (oral, injectable with varying intervals between injections, implantable and cutaneous patches and

gels), and most are drug delivery devices that are appropriate for full adult androgen replacement. These doses are most often too large for the induction of puberty and too cumbersome for many young adolescents to use (see below). All preparations deliver testosterone that is readily converted to dihydrotestosterone by 5-a reductase as well as to estrodiol by aromatase. Both effects and side effects are related to these metabolic conversions. Once adequate virilization is induced and virtually full adult height is reached, almost any of the therapies designed for adults can be used and the choice is made mainly on the availability and the subject's preference (see below).

Testosterone biosynthesis at puberty and beyond

At puberty in typical adolescent males (46,XY), circulating levels of testosterone rise exponentially as the hypothalamic-pituitary-gonadal (HPG) axis regains the active state that had been suppressed since fetal life and the "mini puberty" of the first few months of extrauterine life. At first, there are a few small LH pulses that cause the testis to produce small, but measurable, amounts of testosterone. As the negative feedback control system is poised at the (nearly) prepubertal, very sensitive range, these low levels of testosterone are capable of reducing GnRH and, therefore, LH release. As the adolescent matures, the GnRH pulse generator operates increasingly more like that of the adult and the low, but rising, levels of testosterone are no longer able to produce such potent negative feedback control of the hypothalamus and the pituitary. The sum of these processes is an increase in testosterone production, at first only at night (first pulse of LH usually occurs early during the first episode of deep sleep) and then into the day, but with a very distinct day (early morning) – night variation, which may be as high as 10-fold in adolescence. With 'complete' maturation, there are fluctuations in testosterone concentration, up to perhaps 40%, during a period of 24 h, [3, 4]).

In the adult, circulating testosterone regulates its own secretion predominantly by negative feedback inhibition of LH secretion at the hypothalamus predominantly

by affecting GnRH biosynthesis in the anterior pituitary. Conversion to estradiol (E_2) by the aromatase system is important for this physiological response. The physiologic levels of testosterone in the adult male are ~ 10 – 40 nM (~ 290 – 1150 ng/dL) with a daily production rate of 5 – 7.5 mg. As in adolescence, there is a diurnal variation in the circulating levels of testosterone, which is less marked in adulthood compared to adolescence. The levels are higher in the early morning, followed by a progressive decline during the day and evening. The levels of testosterone remain high, although the half-life of testosterone in the circulation is very short. These levels are maintained in the physiological range by the binding of testosterone to sex-hormone binding globulin (SHBG) and other proteins. In total, ~ 43 – 45% is very tightly bound to SHBG (association constant [Ka] = 1×10^9 L/mol), 53 – 55% is loosely bound to albumin and other proteins (Ka = 3.6×10^4 L/mol), and < 2% is unbound (free) [5, 6]. SHBG levels are higher in women than in men (related to circulating E_2 levels), but gradually increase with age in men [3].

Testosterone activates its receptor to produce a myriad of functions known collectively as anabolic (increase in skeletal mass and strength) and masculinizing (androgenic, development of male secondary sex characteristics, including hair growth).The binding of an active androgen stabilizes the androgen receptor augmenting its active half-life. The increase in gene transcription and translation of proteins elicits many changes that enhance muscle hypertrophy, strength, endurance and power. There are some non-genomic actions of androgens in multiple non-muscle tissues such as those of the reproductive tract [7]. Additional activities have been ascribed to both inhibition of glucocorticoid action [8], potentiation of muscle IGF-I action [8] and attenuation of myostatin action and signaling [9].

The anabolic activities of androgens and their androgenic effects on spermatogenesis have been extensively reviewed and are beyond the scope of this report [8]. These depend specifically on the variable number of CAG_n triplet

repeats within the DNA for the androgen receptor. The greater the number of CAG repeats, the lesser the strength of androgen action. Zinn and co-workers evaluated the androgen receptor CAG_n repeat length on the phenotype of boys with Klinefelter syndrome [10]. All had repeat lengths within the normal range (7-35). There was an inverse correlation between penile length, considered to be a biological indicator of early androgen action, and CAGn. However, there was no correlation of the repeat length with testicular volume, degree of hypotonia or height SD score found in the participants of this study. In a group of adults with KS, those with a long CAGn were been shown to have more significant physical features of KS (taller adult height and more gynecomastia), as well as less success in professional employment and long-term relationships [11]. Additional studies are needed to determine if any adjustments in androgen therapy should be considered based on CAGn in males with KS.

Pubertal development in adolescents with KS

The vast majority of boys with KS escape diagnosis before puberty, although many signs (disproportionately long legs with normal arm span for height and speech difficulties) and symptoms (mainly in the developmental and behavioral sphere) have often been present for years. A large population study showed that fewer than 10% of males with KS are identified before pubertal development [12]. With onset of puberty in KS, the testes first enlarge slightly or not at all, and may become subtly more firm than is physiologic for early puberty. The hormonal profile may be quite normal very early on with relatively normal (and increasing) levels of LH and testosterone. FSH and then LH levels most often rise above the normal range, although sometimes remain in the upper range of normal. More recently several investigators have shown low to undetectable levels of anti-Mullerian hormone (AMH) and inhibin B [13]. These levels track the histological degeneration of primary and secondary gonocytes [14-16].

Considerations for androgen therapy in boys with KS

Current practice recommendations in KS include the initiation of androgen therapy in early-to-mid puberty, or at the onset of development of hypogonadism. However, there are 3 physiologic peaks of androgen secretion in males - in the prenatal period, during the “minipuberty” of infancy in the first 4 months of life, and at adolescence. This has raised questions of whether androgen deficiency in KS also occurs prenatally or in early childhood, and whether one should tailor replacement therapy to these distinct epochs? We shall first consider the time of the “mini-pubertal” development. Although the significance of the neonatal HPT activity is unclear, exposure to such levels of hormone may enhance future testicular function, particularly sperm production, and also may contribute to masculinization of the brain related to various cognitive functions and cerebral dominance. It is not yet clear whether this epoch occurs normally in a boy with KS or if there is subnormal androgen production. There are data that note both [17-19]. Anecdotal reports suggest therapy during infancy may lead to improvements in muscle tone, but no definitive or controlled trials have been published. Without controlled trials in infancy, it is not yet clear whether there is any benefit to treatment, and one does not have a dose and timing for administration. Some clinicians have borrowed from the long-standing protocols used in the treatment of micro-phallus—25 mg of one of the long acting esters of testosterone (enanthate or cypionate, see below) administered intramuscularly at monthly intervals for a total of three doses.

For adolescents with KS, the induction of puberty is usually not required as most of the boys begin adolescent development spontaneously. Androgen therapy is often required in mid-to-later puberty given that progression to full pubertal development—both genital and body composition—do not occur spontaneously. That is the more straight forward consideration; however, concerns about possible negative effects on spermatogenesis, gynecomastia, and behavior must also be considered [1, 2]

Androgen therapy

The developmental state of the adolescent and the clinical pharmacology of the testosterone preparation dictate which formulations are appropriate for the adolescent and mature man with KS (see the Endocrine Society, TES clinical practice guideline for complete details [20]). The induction of puberty necessitates much lower doses of T than are appropriate for a late pubertal boy or man. Most of the preparations (see below and guidelines) are metered for the adult man because it is likely that more than 95% of the use of T is in adults. The only preparations for which there are years of clinical data to determine the dose (and ease of use) are the long acting esters, T enanthate or cypionate, and monthly injections of 50 to 75 mg are convenient doses with which to begin in early to mid puberty. Escalation of the dose by 50 mg per month at 4 to 6 month intervals usually works well until the dose is at approximately 150 mg per month. At this time, moving to 100 mg twice monthly may help to smooth out the peaks and valleys, and one can more easily switch to the transdermal gel formulations beginning at 1.25 or 2.5 grams daily. The advantage of the gel is that physiological levels of testosterone, dihydrotestosterone and estradiol may be obtained, the levels of hormones are virtually constant throughout the day and the measured level of testosterone may be used as a guide to dose alteration. It may be appropriate to initiate T therapy with the gel in KS if puberty has begun and progressed to mid-puberty spontaneously. The peaks and troughs of T levels during the interval between injections with the cypionate or enanthate do not permit the level of T to be an accurate guide to dosing amount or interval. Psychological factors of adolescents with KS are also important to consider related to selection of the formulation. If there are high symptoms of anxiety or phobia of needles, the gel is often a more desirable formulation for both the adolescents and their parents. However, the gel requires daily application by the adolescent, and underlying features of learning impairments or attentional deficits

may then make daily compliance difficult for the adolescent without close monitoring by their parents/caretakers.

While there are various other formulations including oral preparations (including the undecanoate), the transdermal patch, the buccal, bioadhesive tablets, and the injectable undecanoate in oil, these are not often used for the induction of puberty in KS, nor are there published reports for the use of these preparations for this purpose.

Treatment-emergent adverse events with some caveats concerning adolescents with Klinefelter syndrome

The most common adverse events are actually due to the pharmacodynamic properties of testosterone activity. These include acne, oily skin, a modest increase in red cell mass and hematocrit (rarely outside of the normal range), a worsening of gynecomastia, and detrimental effects on spematogenesis [14-16]. These occur with virtually any of the preparations and are seemingly not dose dependent (however, see above for the issue of CAG_n repeats within the androgen receptor).

Since a subset of adolescent males with KS can have underlying behavioral or emotional difficulties, concerns about possible adverse behavioral alterations also often arise, although these may be difficult to differentiate from the underlying behavioral state and the physiological alterations at puberty in any male [20]. Although there are no studies of the psychological or behavioral effects of testosterone therapy in adolescents with KS, one study in adults reported improvements in mood, attention, and social relationships following testosterone treatment. [21] For adolescents with KS who have behavioral difficulties, it is recommended that these are evaluated and stabilized as much as possible prior to initiating testosterone therapy. Therapy should be initiated at conservative doses, and transition to the gel or injections at more frequent

intervals to minimize peaks and troughs should be considered. It is also important to note that testosterone at supraphysiologic levels can lead to irritability and behavioral difficulties, thus testosterone levels, dosage, and administration should be reviewed with patients if there is a significant change in behavior during treatment.

Specific preparations may have some idiosyncratic adverse events. For example, the enanthate or cypionate are injected and also lead to significant peaks and valleys in T concentrations that may lead to behavioral alterations or agitation at different times after the injection and fatigue just before the subsequent injection. Alterations in the schedule of timing and dose (smaller doses, at weekly or biweekly intervals) may smooth out these effects. The newest long-acting injectable, the undecanoate maintains a steady T concentration without peaks and troughs, however it is not yet available in the United States. It may prove cumbersome in the induction of pubertal development due to an approximately three month span of action. However, in adult men it may very well be the treatment of choice for the infrequency of injections and the quite stable circulating levels of testosterone during the interval between injections [22].

Personal experience (ADR)

I have evaluated a large number of boys with Klinefelter syndrome, including those diagnosed *in utero*, infants and children with developmental delays for whom the diagnosis is made following genetic testing for fragile X or other genetic syndromes, and those first considered at adolescence. My experience falls into several categories.

For those with a diagnosis made *in utero* (most often because of advanced maternal age and not because of some difficulty with the present pregnancy) the most important issues are to review the spectrum of the clinical course (see articles by C. Samango-Sprouse and N. Tartaglia in this issue). Some parents

may have received out-of-date information about the behavioral phenotype, and referral to national advocacy organizations and genetic centers are recommended for updated information and support. Although there are some important developmental and behavioral risks to watch for, it is important to emphasize that the majority of boys do not have significant problems given that only 10% of boys with KS are identified in childhood. Recommendations for developmental screenings and learning assessments are included in the articles by C. Samango-Sprouse and N. Tartaglia, and my advice to these parents is to avail themselves of appropriate therapies sooner rather than later if needed.

As an endocrinologist, I receive many inquiries and requests for androgen "therapy" to substitute for the missing "mini-puberty". There is a lot of lore about this spread mostly through the internet, and many parents strongly desire this therapy in hopes of 'normalizing' development, especially if their child's pediatrician will not administer it. As noted above, there remains controversy whether "mini-puberty" occurs or is attenuated in boys with Klinefelter syndrome compared to 46,XY infants. The data simply currently do not exist concerning the effects of the "minipuberty" on physical or cognitive development in typical 46,XY males, or whether there is any benefit in supplementing testosterone in infants with KS. This question begs double-blind, controlled trials. A second point is that the penile length in boys with KS is often less than in 46, XY boys, but not in the range of micro-penis. Borrowing from experience with boys with the latter, it is likely safe to administer testosterone enanthate or cypionate, 25 mg per dose for 3 doses given one month apart, in an attempt to increase penile length. The penis will sometimes grow and there are seemingly few side effects, including transient (but minimal) pubic hair and an increased frequency of erections.

Outside of the first 4-6 months of life, there does not appear a great need for androgen therapy during childhood, for the "mini-puberty" has been completed and adolescent development is in the future. Whether androgen deficiency exists at this time (and during mini-puberty) remains controversial and unanswered.

Proper clinical trials are important and at least one is ongoing, using oxandrolone as the androgen in prepubertal males with XXY.

The endocrinological reasons for administering testosterone at puberty are quite straightforward given that one can monitor pubertal development, the hormones of the hypothalamic-pituitary-testicular axis and adolescent “behaviors”. A secondary reason is for appropriate body composition and muscle mass, especially in the upper body and shoulder area. Boys with KS have apparently less muscle mass than their counterparts (at this age) with constitutional delay of growth and adolescence. The overarching principle is to advance pubertal development while doing no harm. Therein lie the major difficulties. Behavioral difficulties can sometimes relate to testosterone levels, although most would agree that those are only one aspect and are not a common side effect. It seems prudent to keep the levels as steady as is possible and in the normal adolescent range for stage of sexual development, so that the salutary androgen effects occur with as few of the untoward effects as possible.. As noted above, the most common preparation used early in puberty is the injectable T ester (enanthate or cypionate). The pharmacokinetics are such that the bigger the dose at longer intervals leads to supraphysiologic levels just after injection and days and weeks of sub-physiologic levels before the next dose. This is usually less of a problem at the low doses (50 to 75 mg per month) but of greater significance with 100 mg per month or even per two weeks. Individually “titrated” doses usually work best with approximately 100 to 200 mg administered every 7-14 days.

For the later stages of puberty, one can consider the gel at the 1.25 or the 2.5 gram doses, and allow for increasing to the 2.5-10 gram doses usually required in early adulthood. As noted above advantages are one can normalize the circulating levels of the hormones of the HPG axis and that these levels are physiologic for virtually the entire 24 hour period following daily administration. The side effects are those of any androgen, but with the additional caveat of transfer to women and children from direct contact or through the use of a

common towel. For these reasons the FDA has mandated a “black box” warning. Case reports and reviews of transfer to children of both sexes have been published [23, 24]. As described above, there is great variability in practice among endocrinologists, and some practitioners start with the 1.25 gram dose when initiating testosterone treatment. I feel strongly that 1.25 gram of gel is too high to start if no pubertal development has started. However, in most boys with KS puberty does start naturally and then fails to progress, and for this circumstance starting with the 1.25 gram pump of gel is a reasonable choice.

Transdermal patches with skin irritation at the application site and buccal bioadhesive T tablets (gum-related adverse events) and T pellets (surgical incision required) are rarely used in adolescents in the US and I have no experience with them. Orally administered testosterone undecanoate is erratically absorbed and requires multiple daily administrations. It is not approved in the US. I believe that the injectable form of testosterone undecanoate will have a major impact on the use of testosterone once it becomes available in the US. It has been used for a number of years in Europe and elsewhere around the world with general satisfaction with the formulation administered every 10-14 weeks and has the benefit of relatively stable testosterone (and the metabolite) levels within the physiological range. I suspect that this form will become standard for late adolescent and young adult men with any form of permanent hypogonadism. The major disadvantage is the large injection volume (4 mL), but it is administered quite infrequently. Despite that, in a single cross-over study comparing T undecanoate with implantable depot testosterone for the maintenance of testosterone replacement therapy in androgen deficient men, a large majority of subjects preferred the injectable form [22]

Conclusion

Boys with the Klinefelter syndrome may be sub-sufficient in androgen activity and require replacement therapy. That is controversial for the “mini”-puberty during

the first few months of life. Whether androgen therapy will be helpful to boys between “mini” puberty and adolescence is being studied with the weak androgen, oxandrolone. Replacement starting in mid-puberty is required for most males with KS and important for the developmental of secondary sexual characteristics, and to permit the normal accrual of muscle mass, bone mineral content, adult regional distribution of body fat. Secondary goals of psychosocial development and both positive or negative behavioral effects of testosterone in KS need further study. Although not available in the US at present, I suspect that the undecanoate ester with a 2 ½ to 3 ½ month interval between injections may very well become an important addition to the US pharmacopeia as it has in Europe, and its use will become prominent in late adolescents and adults with KS.

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