Original Paper

HORMONE RESEARCH IN PÆDIATRICS

Horm Res Paediatr 2010;74:428–435
DOI: 10.1159/000315482

Received: November 5, 2009
Accepted: May 10, 2010
Published online: July 14, 2010

A Double-Blind, Placebo-Controlled Comparison of Letrozole to Oxandrolone Effects upon Growth and Puberty of Children with Constitutional Delay of Puberty and Idiopathic Short Stature

Shadab Salehpour[a, b] Parvin Alipour[c] Maryam Razzaghy-Azar[c, d]
Laleh Ardeshirpour[e] Alireza Shamshiri[a] Mahtab Farahmand Monfared[b]
Atoosa Gharib[a]

[a]Mofid Children's Hospital, Shaheed Beheshti University of Medical Sciences, [b]Genomic Research Center, Shaheed Beheshti University of Medical Sciences, [c]H. Aliasghar Hospital, Iran University of Medical Sciences, and [d]Institute of Endocrinology, Iran University of Medical Sciences, Tehran, Iran; [e]Yale University Pediatric Endocrinology, New Haven, Conn., USA

Key Words
Letrozole · Oxandrolone · Constitutional delay of growth and puberty

Abstract
Background/Aims: Constitutional delay of growth and puberty (CDGP) with short stature is one of the most common problems in pediatrics. We compared the effects of letrozole with that of oxandrolone on predicted adult height (PAH), puberty, bone mineral density, serum insulin-like growth factor 1 (IGF-1) and blood lipoproteins. ***Methods:*** In a prospective, double-blind, randomized, placebo-controlled clinical trial, 91 CDGP boys (12.6–14.6 years old) with predicted short stature were treated with letrozole (2.5 mg/day), oxandrolone (2.5 mg/day), or placebo, at the outpatient pediatric endocrine clinic of Mofid Children's Hospital in Tehran for 2 years. ***Results:*** Letrozole differed from oxandrolone and placebo in significantly increasing PAH ($p < 0.05$), and slightly but significantly decreasing HDL-cholesterol. Oxandrolone, and to a lesser degree letrozole, significantly increased the height standard deviation score and bone age compared to placebo. ***Conclusion:*** This first randomized controlled clinical trial in CDGD teenage boys with predicted short stature shows that letrozole increases PAH more than oxandrolone and advances pubertal stage and bone mineralization less.

Introduction

Constitutional delay of growth and puberty (CDGP) is one of the most common problems in pediatrics, affecting 3% of children [1, 2]. Hormonal treatment is an option for those patients experiencing psychological distress because of sexual immaturity, and the attendant short stature [2, 3]. The usual treatment is with physiologic doses of androgen, which accelerate linear growth and onset of pubertal changes [1–3]. However, they do not improve predicted adult height (PAH) [2–4], which is a problem for those with coincidental predicted short stature. Some studies suggest that oxandrolone, an anabolic non-aromatizable androgen, increases PAH [4, 5], while others do not [6, 7]. Recently, the combination of testosterone and letrozole, an aromatase inhibitor, has been reported to increase PAH [8–12] and ultimately near-final height [11]

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1663–2818/10/0746–0428$26.00/0

Accessible online at:
www.karger.com/hrp

Shadab Salehpour, MD
Shaheed Beheshti Medical University, Genomic Research Center
Tabnak Ave, Chamran Express Way, Eveen
PO Box 19395-4719, Tehran (Iran)
Tel. +98 212 242 9610, Fax +98 212 242 9612, E-Mail shadab.salehpour@gmail.com

in CDGP by interfering with estrogen action on epiphyseal maturation and fusion [13].

While most reports indicate that the drug increases PAH and near-final height, a recent Lawson Wilkins Pediatric Endocrine Society review emphasized that more data are needed [14]. In the combined reports, only 61 patients had been treated, and only 2 randomized, double-blinded, placebo-controlled trials had been performed. Three of the 6 original reports derive from the same research group, and 2 of those involve the same patients. Furthermore, there are no available data regarding adult height from the controlled trials [15].

The main objective of this study was to compare the effect of oral letrozole with that of oxandrolone on PAH in CDGP boys with predicted short stature, in a double-blind randomized placebo-controlled trial.

Subjects and Methods

Study Subjects

This was a 5-year controlled randomized clinical trial on 91 consecutively consenting Iranian boys who met our inclusion criteria of CDGP, 12.6–14.6 years of age, and unexplained intrinsic short stature, indicated by a PAH <–1 SD of mean parental height (target height). CDGP was diagnosed when Tanner genital or pubic hair stage was >2 SD delayed for healthy Indo-Iranian boys [16] and testis volumes were ≤3 ml after 12.5 years of age. None of the boys had had a pubertal growth spurt. There was no evidence of underlying disorders to account for the delayed puberty in any of these boys, based on past medical history, physical examination, and laboratory data.

Study Protocols

By double-blinded random allocation these boys were divided into three treatment groups: letrozole 2.5 mg daily, oxandrolone 2.5 mg daily, or a placebo tablet identical to letrozole daily. Each case was treated for 2 years. Every 3 months in the pediatric endocrine clinic of Mofid Children's Hospital, a general physical examination was performed by the same physician (S.S.) throughout the study. PAH, height standard deviation score (HSDS), genital and pubic hair stages, and bone age were the primary outcome variables (table 1).

This study was supported by the Genomic Research Center of Shaheed Beheshti University of Medical Sciences and was conducted with approval of the ethical committee of the Center and under the guidelines of the Declaration of Helsinki; written informed consent was obtained from all parents.

Clinical Measurements

Testis volume was measured by Prader orchidometer [17, 18], and genital and pubic hair staging were determined according to Tanner [17, 19]. Height was measured by a standard Secca stadiometer with 0.1 cm precision. We determined target height (mid-parental height, TH) [20] and PAH prior to and after therapy by the Bayley and Pinneau method [21, 22].

Table 1. Pretreatment variables in study groups (mean ± SD)

Study variables	Study groups		
	letrozole	oxandrolone	placebo
Patients, n	31	30	30 ± 22[1]
Age, years	13.6 ± 0.8	13.7 ± 0.9	13.5 ± 0.9
Target height, cm	174.6 ± 4.5	175.0 ± 5.6	176.5 ± 4.0
HSDS	–2.91 ± 0.7	–3.01 ± 0.6	–2.88 ± 0.6
Genital stage	1	1	1
Bone age, years	12.1 ± 1.1	11.8 ± 1.2	11.7 ± 1.2
PAH, cm	167.6 ± 5.0	169.2 ± 4.8	171.9 ± 5.3
IGF-1, ng/ml	317 ± 101	295 ± 83	279 ± 69

[1] In this group, 22 of the 30 patients continued to the endpoint.

Radiological Measurements

Bone age was reported blindly, based on the Greulich and Pyle method [23], by one experienced pediatric radiologist (P.A.). Areal bone mineral density (BMD) was measured by a GE-Lunar dual-energy X-ray absorptiometry (DXA) device (DPX-L) in the standard way, bone mineral content/surface area (g/cm^2) [24, 25], every 12 months. The coefficient of variation of BMD was 0.5% for the lumbar spine and 1% for the femoral neck, according to the manufacturer, and least significant change at 95% confidence was 2 for lumbar spine and 2.8 for femoral neck. We calculated bone mineral apparent volumetric density (BMAD) using published geometric assumptions [26, 27]. We used standard centile curves and tables of Kalkwarf et al. [28] (adjusted for height, age and bone age) to calculate BMD Z-score and reference centile curves and tables for data in healthy children and young adults [29] for BMAD Z-score. We calculated the SDS of each boy's BMAD by the method of Molgaard et al. [30].

Biochemical Measurements

Serum testosterone was measured by RIA after separation of steroid fractions on Lipidex-5000 micro-column (Packard-Becker, BV Chemical Operations, Groningen, The Netherlands) [31]. The sensitivity of the method was 0.0014 mmol/l (0.04 ng/dl) with less than 0.5% cross-reaction with oxandrolone. The intra- and interassay coefficients of variation were 5 and 10%, respectively, at a 19.8-mmol/l (571.6 ng/dl) concentration.

Serum insulin-like growth factor 1 (IGF-1) was determined by an immunometric assay kit (Immunotech Coulter, Marseille, France) [32]. Serum total cholesterol, HDL-cholesterol and triglyceride were determined by enzymatic colorimetric tests (Roche Diagnostic GmbH, Mannheim, Germany) and serum LDL-cholesterol concentrations were calculated by Friedewald's formula [33].

Statistical Analysis

Using SPSS, we analyzed the results by paired t test, ANOVA or Wilcoxon tests, followed respectively by Tukey's or Kruskal-Wallis post hoc tests. We determined the relative attrition and sample size, and performed power calculation, pre- and post-study sensitivity analysis, using Minitab 15 for power and sample size calculation. The study was designed with 90% power for α =

Table 2. Height and bone age SDS differences pre- and post-treatment in study groups (mean ± SD)

Study group	HSDS			Bone age		
	pre	post	difference post-pre (p)	pre	post	difference pre-post (p)
Letrozole	–2.91 ± 0.70	–2.27 ± 0.41	0.64 ± 0.29 (<0.001)	12.1 ± 1.1	13.2 ± 1.0	1.1 ± 0.33[a] (<0.001)
Oxandrolone	–3.01 ± 0.60	–2.37 ± 0.47	0.64 ± 0.33[b] (<0.001)	11.8 ± 1.2	14.1 ± 1.5	2.32 ± 0.53[a] (<0.001)
Placebo	–2.88 ± 0.6	–2.86 ± 0.30	0.02 ± 0.12[c] (<0.001)	11.7 ± 1.2	12.2 ± 1.3	0.48 ± 0.30[c] (<0.001)

[a] $p < 0.01$ letrozole vs. oxandrolone; [b] $p < 0.001$ oxandrolone vs. placebo; [c] $p < 0.001$ letrozole vs. placebo.

Table 3. Predicted adult height changes pre- and post-treatment in study groups (mean ± SD)

Study group	Pre-PAH	Post-PAH	PAH difference pre- and post-Rx	p	PAH & TH difference post-Rx	p
Letrozole	167.6 ± 5.0	173.7 ± 6.2	6.10 ± 1.9[a]	<0.01	–0.9 ± 1.2[a]	NS
Oxandrolone	169.2 ± 4.8	171.1 ± 5.0	1.9 ± 1.0[a]	NS	–3.9 ± 1.6[a]	<0.01
Placebo	171.9 ± 5.3	173.3 ± 6.0	1.4 ± 0.80[a]	NS	–3.2 ± 1.3[b]	<0.01

[a] $p < 0.001$ letrozole vs. oxandrolone; [b] $p < 0.001$ letrozole vs. placebo.

5% based on the data of Wickman et al. [10]. While the sample size was estimated to be 15 in each group, we started the study with almost twice that number. Thus, in spite of 8 non-completers in the placebo arm, the post-study power remained over 98% for the control group. There was no significant difference between the missing and the remaining subjects in all the primary and secondary outcomes.

Results

Baseline Data

All the letrozole and oxandrolone patients, but 22 of 30 patients in placebo group, completed the study. The loss of these study subjects was due to the parents' belief in lack of treatment efficacy. The three study groups were similar in chronologic age, target height, HSDS, Tanner stages, bone age, PAH, and serum IGF-1 (table 1). Noteworthy is that the letrozole group tended to have a PAH 2–4 cm less than the other groups. They were randomly divided into three groups of equal size, but one third of controls dropped out throughout the study.

HSDS

Both letrozole and oxandrolone significantly increased the HSDS to a similar extent ($p < 0.001$), in contrast to the lack of change in controls (table 2).

Bone Age

Letrozole had a lesser effect on bone age than oxandrolone ($p < 0.01$) (table 2). Bone age increased more on both treatments than in controls ($p < 0.001$).

PAH

Letrozole significantly increased PAH ($p < 0.01$) (table 3). In contrast, oxandrolone ($p = 0.10$) and placebo ($p = 0.51$) treatments did not increase PAH significantly. Nevertheless, post-treatment PAH among groups was similar. The difference between PAH and target height, before (–7.0 cm) and after 2 years of letrozole (–0.9 cm) therapy changed significantly ($p < 0.001$). However, oxandrolone (–5.8 to –3.9 cm) and placebo (–5.4 to –3.2 cm) did not change this difference significantly (table 3).

Puberty

Pubic hair increased more on letrozole and oxandrolone than in controls ($p < 0.01$). It progressed to a similar stage on both letrozole [4 (3–5)] and oxandrolone [4 (4–5)], but remained nearly unchanged in controls [1 (1–2)] after 2 years of therapy (table 4). At the beginning of study, the testicular size of all boys was in the prepubertal range (≤3.0 ml). By the end of the first year of treatment, 22 of 31 (71%) of the letrozole group and the entire oxandrolone group had started testicular enlargement, while

Table 4. Pubertal development pre- and post-treatment in study groups (mean ± SD)

Group	Pre-Rx		Post-Rx		P pre- and post-Rx		Post-Rx IGF-1, ng/ml	Δ IGF-1 for 2 years, ng/ml
	PH	TS, ml	PH	TS, ml	PH	TS		
Letrozole	1 ± 1	2.0 ± 0.7	4 (3–5)[a]	10.1 ± 4.2[b]	<0.01	<0.001	707 ± 100	390
Oxandrolone	1 ± 1	2.1 ± 0.9	5 (4–5)[c]	15.3 ± 5.1[d]	<0.01	<0.001	689 ± 83	412
Placebo	1 ± 1	2.4 ± 0.7	1 (1–2)	3.2 ± 0.9	<0.01	<0.01	301 ± 78	22

Values in parentheses show range. TS = Testicular size (ml); Δ IGF-1 = mean of IGF-1 changes through the study. p values for comparison of Δ IGF-1 relative to Δ TS among groups are non-significant.

[a] $p < 0.01$ letrozole vs. placebo; [b] $p < 0.001$ letrozole vs. placebo; [c] $p < 0.05$ oxandrolone vs. placebo; [d] $p < 0.01$ oxandrolone vs. letrozole.

Table 5. Testosterone (T) and HDL-cholesterol levels pre- and post-treatment in study groups (mean ± SD)

Group	T pre-Rx mmol/l	T post-Rx mmol/l	p	HDL pre-Rx mmol/l	HDL post-Rx mmol/l	p
Letrozole	0.25 ± 0.18	15.91 ± 4.88[a]	<0.001	1.57 ± 0.05	1.31 ± 0.13[b]	<0.01
Oxandrolone	0.20 ± 0.14	17.33 ± 5.11[c]	<0.001	1.60 ± 0.10	1.62 ± 0.10	NS
Placebo	0.24 ± 0.20	2.15 ± 1.92	<0.01	1.67 ± 0.10	1.55 ± 0.05	<0.01

[a] $p < 0.001$ letrozole vs. placebo; [b] $p < 0.01$ letrozole vs. oxandrolone and control; [c] $p < 0.001$ oxandrolone vs. placebo.

and none of the control boys had begun. After 2 years of therapy, testicular size increased to >4.0 ml in all of the boys in both the letrozole and oxandrolone groups ($p < 0.001$), significantly more so on oxandrolone ($p < 0.001$), and 6 of 22 (27%) of the boys in the control group ($p = 0.06$) had a testicular size >4 ml (table 4).

Serum Testosterone

The serum testosterone levels were in the prepubertal range in all boys at baseline, appropriate to their prepubertal status. After the first year of therapy, the serum testosterone level in the oxandrolone group (14.56 ± 5.20 mmol/l) was significantly more ($p < 0.001$) than that of the other groups. On the other hand, testosterone increased significantly more on letrozole (5.50 ± 3.71 mmol/l) ($p < 0.01$) than on placebo (2.15 ± 1.92 mmol/l). However, after 2 years of therapy there was no significant difference ($p = 0.08$) between the letrozole and oxandrolone groups in serum testosterone level (table 5) despite much more testicular enlargement on oxandrolone.

Serum IGF-1 Levels

The serum IGF-1 levels at the beginning of study were illustrated in table 1. The means of IGF-1 changes through the study were compared among the groups. During the study, IGF-1 rose similarly on oxandrolone (412 ng/ml) and letrozole (390 ng/ml) treatments, both significantly higher than on placebo (22 ng/ml) ($p < 0.001$). However, these changes were in proportion to the change in pubertal stage ($p = 0.08$) (table 4).

Lipoprotein Profile

Although we did not find any significant difference between the lipid profiles of our patients at the beginning of therapy, a mild but significant ($p < 0.01$) decrease in HDL-cholesterol emerged after 6 months of letrozole therapy and was sustained throughout the study (1.31 ± 0.13 mmol/l). This differed from the unchanged values in the oxandrolone group (1.62 ± 0.10 mmol/l) and the slight fall in the control group (1.55 ± 0.05 mmol/l) (table 5).

BMD

There was no significant difference between the letrozole and the placebo group during treatment (table 6). BMD in the oxandrolone group progressively increased over the 2-year period to 1.2 ± 0.8 in the lumbar spine and 1.1 ± 0.7 in the femoral neck. These both became

Table 6. Bone mineral values pre- and post-treatment in study groups (mean ± SD)

Group	Lumbar spine[1]		Femoral neck[1]		p[1]
	pre	post	pre	post	
Letrozole					
BMD-Z score	–1.5 ± 0.9	–1.5 ± 0.9[a]	–1.5 ± 1.1	–1.2 ± 0.7[a]	NS
BMAD-Z score	–0.0 ± 0.3	0.1 ± 0.2[b]	–0.2 ± 0.0	–0.1 ± 0.0[b]	NS
Oxandrolone					
BMD-Z score	–1.4 ± 1.1	1.2 ± 0.8[a]	–1.3 ± 1.0	1.1 ± 0.7[a]	<0.01
BMAD-Z score	0.2 ± 0.3	1.4 ± 0.4[b]	–0.1 ± 0.1	1.0 ± 0.3[b]	<0.05
Placebo					
BMD-Z score	–1.4 ± 1.0	–1.5 ± 1.1[a]	–1.3 ± 0.9	–1.2 ± 0.6[a]	NS
BMAD-Z score	–0.1 ± 0.3	0.1 ± 0.2[b]	0.3 ± 0.4	0.4 ± 0.3[b]	NS

[a] $p < 0.01$, [b] $p < 0.05$: post-treatment comparisons of the variables between groups.
[1] $p < 0.01$: pre- vs. post-treatment comparisons of the variables within groups.

significantly higher than on ($p < 0.001$) letrozole (–1.5 ± 0.9 and –1.2 ± 0.7, respectively) or placebo (–1.5 ± 1.1 and –1.2 ± 0.6, respectively), which did not differ significantly. Oxandrolone also increased BMAD more than did letrozole and placebo ($p < 0.05$), which had no significant effect. The association between the changes in BMAD and hormonal factors (the means of testosterone and IGF-1) at the beginning and at the end of treatment were assessed by regression analysis, and no significant difference was observed.

Discussion

This randomized controlled blinded clinical trial showed that low-dose oral letrozole treatment for 2 years significantly improved PAH of CDGP boys with predicted short stature by about 6.1 cm. Letrozole also significantly lessened the difference between PAH and target height, from –7.0 cm pre-treatment to –0.9 cm after 2 years of letrozole ($p < 0.001$), which contrasted to the results with both oxandrolone and placebo, neither of which changed this difference significantly.

These findings are consistent with previous studies in which those boys who received letrozole had a greater probability to achieve their target height. Hero et al. [11] found a 5.9-cm increase in PAH after 2 years of therapy with letrozole alone in 31 boys aged 9–14.5 years with idiopathic short stature, and Wickman et al. [10] reported an improvement in PAH of 5.1 cm following the administration of letrozole and testosterone for 18 months in 33 CDGP boys. Later, Hero et al. [8] reported an improvement in the near-final height of 17 boys with CDGP treated with testosterone and letrozole for 12 months. In their study the near-final height of subjects treated with letrozole + testosterone did not differ from their TH, while the near-final height was found to be lower than TH in boys treated with testosterone + placebo.

In our study the bone age increased most on oxandrolone (2.3 years), less on letrozole (1.1 year), and least on placebo (0.5 year), all significantly different from one another. Hero et al. [11] reported a 1.24-year increase in bone age after 2 years of therapy with letrozole alone, and Wickman et al. [10] mentioned only a 0.9-year increase in it after 18 months of therapy with letrozole + testosterone. Thus similar to Wickman et al. and Hero et al., we found a significant reduction but not a complete arrest in bone maturation in the letrozole-treated group. We found that letrozole and oxandrolone significantly increased the HSDS to a similar extent, in contrast to the lack of change in controls. This explains why letrozole increased PAH.

On the other hand, oxandrolone proportionately increased both HSDS and bone age compared to the control group. Therefore as a consequence, it caused an insignificant change in PAH, similar to that of placebo. These results are in agreement with some reports [6, 7], but not with the claims that oxandrolone increases PAH [4, 5].

Testicular volume increased rapidly throughout the study in both the letrozole ($p < 0.001$) and oxandrolone ($p < 0.001$) groups, in contrast to the controls, in whom it increased very little by the first year ($p = 0.06$) but significantly by the end of the second year. Testicular enlargement began during the first year in 22 of 31 (71%)

boys in the letrozole group, all of the oxandrolone, and none of the placebo group. After the second year, all of the boys in the letrozole and oxandrolone and 6 of the 22 (27%) of boys in the control group had a testicular size >4 ml. Testicular size was increased significantly more on oxandrolone than letrozole ($p < 0.01$). On the other hand, letrozole increased pubic hair similar to oxandrolone.

After the first year, the serum testosterone level rose significantly more on letrozole than on placebo and rose even more ($p < 0.001$) on oxandrolone than on both other groups. However after 2 years, the difference between the letrozole and oxandrolone groups was not significant ($p = 0.08$).

Serum IGF-1 rose to similar levels on oxandrolone and letrozole treatments, in proportion to pubertal maturation. In the letrozole group, serum testosterone and IGF-1 levels advanced similarly, yet bone age and testicular size lagged significantly, though they advanced more so than in the placebo group.

These findings differ from those of Wickman et al. [10] and Hero et al. [11] who did not report any increase in bone age and IGF-1 by letrozole treatment. The reasons are unclear. It is possible that aromatase activity may not be as completely suppressed by 2.5 mg daily letrozole in Iranian boys. It also may result from a direct androgenic effect on epiphyseal growth [34] that may be mediated by the androgen receptor and/or changes in the chondrocyte IGF-1 system [35, 36].

There was no significant difference in BMD and BMAD between the letrozole and the control groups during the treatment. These results are similar to those of Wickman et al. [37] and are against a possible osteoporotic effect of letrozole. However, Hero et al. [38] recently reported that letrozole suppresses bone turnover, possibly through an androgen-mediated effect, and stimulates cortical bone growth in pubertal boys with idiopathic short stature through increasing the testosterone-to-estradiol ratio.

Compatible with the results of the study of Zadik et al. [39], we found that oxandrolone increases bone density significantly, compared to the control (placebo) group. Oxandrolone increased both BMD Z-score and BMAD Z-score more than letrozole and placebo, which were similar. These apparent increases in volumetric bone density were in proportion to those in pubertal parameters.

Unfortunately, we did not measure markers of bone turnover in our study. However, the effects of low levels of estrogens on bone mass accrual and on body composition during and beyond puberty need to be carefully followed.

We found that a mild but significant decrease in HDL-cholesterol emerged after 6 months of letrozole therapy which was sustained throughout the study. There was no significant change in serum lipoproteins of the oxandrolone and a slight fall in the control groups, so it seems reasonable to measure the HDL-cholesterol in the follow-up of the patients taking letrozole.

There are studies suggesting that HDL-cholesterol is regulated via an androgen-mediated mechanism in pubertal boys. Androgen treatment in boys with delayed puberty decreased HDL-cholesterol concentration [40–42]. Long-term, low-dose (2.5 mg) oxandrolone, as is used for growth purposes, was reported to have no significant effect on the lipoprotein profile [43]. The reported effects of high-dose oxandrolone on lipid levels are conflicting [44–46]. Wickman et al. [47] found that testosterone alone did not change the high-density lipoprotein cholesterol concentration, but the co-administration of letrozole with testosterone decreased it; the concentrations of LDL-cholesterol and triglycerides were not changed either by letrozole or testosterone therapy.

None of our patients in the oxandrolone group developed either gynecomastia which is reported to be the most common or hepatic complications which are thought to be the potentially worrisome side effects of the therapy [48]. Other less serious side effects of letrozole and oxandrolone (e.g. facial acne) were the same between the two groups, compared to controls and were well tolerated by the patients.

Letrozole causes a decrease in serum estrogen level which may suppress the maturation of germ cells, Sertoli cells and seminiferous tubules [49, 50]. There is strong evidence of a positive correlation between fully developed spermatogenesis and strong immunoreactivity for both cytochrome P450 aromatase and estrogen receptor β not only in Sertoli cells but also in pachytene spermatocytes and round spermatids. These data suggest a role for estrogen in the hormonal regulation of spermatogenesis [51]. A few patients have been described suffering from aromatase deficiency due to inactivating mutations in the CYP19 gene. One patient had macroorchidism [52], one had a normal sperm count with decreased motility [53], and another had microorchidism and was infertile due to sperm immotility [54]. However, letrozole has been reported to induce spermatogenesis in a 31-year-old infertile man [55]. Thus, the long-term effects of letrozole on sperm maturation are unclear.

Although this study is unique in comparing letrozole alone to oxandrolone and placebo, there are limitations

to this study. PAH was similar among the groups at the end of the trial in spite of letrozole significantly having increased PAH. This may be explained by the tendency of the letrozole group to be slightly shorter than the other groups at the beginning and the high dropout rate of many shorter controls during the study in spite of adequate initial randomization. Follow-up to adult height will be necessary to provide definitive evaluation of the efficacy and safety of these drugs.

Conclusion

This is the first randomized controlled clinical trial in CDGP teenage boys comparing letrozole with oxandrolone and placebo. Letrozole caused a significantly greater increase in PAH than oxandrolone or placebo, while advancing virilization similarly to oxandrolone, and no adverse events were encountered in the 2-year study period.

Acknowledgments

The authors are grateful to Prof. Robert L. Rosenfield of the University of Chicago for his helpful critical comments and also the Lawson Wilkins Pediatric Endocrine Society and Dr. Vahid R. Yassaee, President of Genomic Research Center, for the grants provided to support this study. We also thank the nurses and other staffs of the Pediatric Endocrinology Section of Mofid Children's Hospital and the Institute of Endocrinology and Metabolism who helped us with the study.

References

1 Rosenfield RL: Diagnosis and management of delayed puberty. J Clin Endocrinol Metab 1990;70:559–562.
2 Ambler GR: Androgen therapy for delayed male puberty. Curr Opin Endocrinol Diabetes Obes 2009;16:232–239.
3 Richmond EJ, Rogol AD: Male pubertal development and the role of androgen therapy. Nat Clin Pract Endocrinol Metab 2007;3:338–344.
4 Lampit M, Hochberg Z: Androgen therapy in constitutional delay of growth. Horm Res 2003;59:270–275.
5 Papadimitriou A, Wacharasindhu S, Pearl K, Preece MA, Stanhope R: Treatment of constitutional growth delay in prepubertal boys with a prolonged course of low-dose oxandrolone. Arch Dis Child 1991;66:841–843.
6 Wilson DM, McCauley E, Brown DR, Dudley R: Oxandrolone therapy in constitutionally delayed growth and puberty. Bio-Technology General Corporation Cooperative Study Group. Pediatrics 1995;96:1095–1100.
7 Zadik Z, Sinai T, Zung A, Reifen R: Vitamin A and iron supplementation is as efficient as hormonal therapy in constitutionally delayed children. Clin Endocrinol (Oxf) 2004;60:682–687.
8 Hero M, Wickman S, Dunkel L: Treatment with the aromatase inhibitor letrozole during adolescence increases near-final height in boys with constitutional delay of puberty. Clin Endocrinol (Oxf) 2006;64:510–513.
9 Dunkel L: Update on the role of aromatase inhibitors in growth disorders. Horm Res 2009;1:57–63.
10 Wickman S, Sipila I, Ankarberg-Lidgren C, NorjavaaraE, Dunkel L: A specific aromatase inhibitor and potential increase in adult height in boys with delayed puberty: a randomised controlled trial. Lancet 2001;357:1743–1748.
11 Hero M, Norjavaara E, Dunkel L: Inhibition of estrogen biosynthesis with a potent aromatase inhibitor increases predicted adult height in boys with idiopathic short stature: a randomized controlled trial. J Clin Endocrinol Metab 2005;90:6396–6402.
12 Dunkel L, Wickman S: Treatment of delayed male puberty: efficacy of aromatase inhibition. J Pediatr Endocrinol Metab 2001;14:1541–1546.
13 Grumbach MM: Estrogen, bone, growth and sex: a sea change in conventional wisdom. J Pediatr Endocrinol Metab 2000;13:1439–1455.
14 Cernich J, Jacobson JD, Moore WV, Popovic J: Use of aromatase inhibitors in children with short stature. Pediatr Endocrinol Rev 2004;2:2–7.
15 Shulman DI, Francis GL, Palmert MR, Eugster EA; Lawson Wilkins Pediatric Endocrine Society Drug and Therapeutics Committee: Use of aromatase inhibitors in children and adolescents with disorders of growth and adolescent development. Pediatrics 2008;121:975–983.
16 Traggiai C, Stanhope R: Disorders of pubertal development. Best Pract Res Clin Obstet Gynaecol 2003;17:41–56.
17 Semiz S, Kurt F, Kurt DT, Zencir M, Sevinç O: Pubertal development of Turkish children. J Pediatr Endocrinol Metab 2008;21:951–961.
18 Prader A: Testicular size: assessment and clinical importance. Triangle 1966;7:240–243.
19 Tanner J: Growth at Adolescence, ed 2. Oxford, Blackwell, 1962.
20 Sobradillo B, Zachmann M, Frank M, Frisch H, Prader A: Bayley-Pinneau, Roche and Tanner height predictions in various conditions. Pediatr Res 1978;12:151.
21 Bayley N, Pinneau SR: Tables for predicting adult height from skeletal age: revised for use with the Greulich-Pyle hand standards. J Pediatr 1952;40:423–441.
22 Sperlich M, Butenandt O, Schwarz PH: Final height and predicted height in boys with untreated constitutional growth delay. Eur J Pediatr 1995;154:627–632.
23 Greulich WW, Pyle Si: Radiographic Atlas of Skeletal Development of Hand and Wrist, ed 2. Stanford, Stanford University Press, 1959.
24 Wang J, Thornton JC, Horlick M, Formica C, Wang W, Pierson RN Jr: Dual X-ray absorptiometry in pediatric studies: changing scan modes alters bone and body composition measurements. J Clin Densitom 1999;2:135–141.
25 Yilmaz D, Ersoy B, Bilgin E, Gümüşer G, Onur E, Pinar ED: Bone mineral density in girls and boys at different pubertal stages: relation with gonadal steroids, bone formation markers, and growth parameters. J Bone Miner Metab 2005;23:476–482.
26 Carter DR, Bouxsein ML, Marcus R: New approaches for interpreting projected bone densitometry data. J Bone Miner Res 1992;7:137–145.

27 Lu PW, Cowell CT, Lloyd-Jones SA, Briody JN, Howman-Giles R: Volumetric bone mineral density in normal subjects, aged 5–27 years. J Clin Endocrinol Metab 1996;81:1586–1590.
28 Kalkwarf HJ, Zemel BS, Gilsanz V, Lappe JM, Horlick M, Oberfield S, Mahboubi S, Fan B, Frederick MM, Winer K, Shepherd JA: Whole-body bone mineral content in healthy children and adolescents. J Clin Endocrinol Metab 2007;92:2087–2099.
29 Ward KA, Ashby RL, Roberts SA, Adams JE, Zulf Mughal M: UK reference data for the Hologic QDR Discovery dual-energy X-ray absorptiometry scanner in healthy children and young adults aged 6–17 years. Arch Dis Child 2007;92:53–59.
30 Molgaard C, Thomsen BL, Prentice A, Cole TJ, Michaelsen KF: Whole-body bone mineral content in healthy children and adolescents. Arch Dis Child 1997;76:9–15.
31 Apter D, Jánne O, Karvonen P, Vihko R: Simultaneous determination of five sex hormones in human serum by radioimmunoassay after chromatography on Lipidex-5000. Clin Chem 1976;22:32–38.
32 Brugts MP, Ranke MB, Hofland LJ, van der Wansem K, Weber K, Frystyk J, Lamberts SW, Janssen JA: Normal values of circulating insulin-like growth factor-I bioactivity in the healthy population: comparison with five widely used IGF-I immunoassays. J Clin Endocrinol Metab 2008;93:2539–2545.
33 Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
34 Keenan BS, Richards GE, Ponder SW, Dallas JS, Nagamani M, Smith ER: Androgen-stimulated pubertal growth: the effects of testosterone and dihydrotestosterone on growth hormone and insulin-like growth factor-I in the treatment of short stature and delayed puberty. J Clin Endocrinol Metab 1993;76:996–1001.
35 Van der Eerden BCJ, Van Til NP, Brinkmann AO, Lowik CWGM, Wit JM, Karperian M: Sex differences in the expression of the androgen receptor in the tibial growth plate and metaphyseal bone of the rat. Bone 2002;30:891–896.
36 Eshet R, Maor G, Ben Ari T, Ben Eliezer M, Gat-Yablonski G, Philip M: The aromatase inhibitor letrozole increases epiphyseal growth plate height and tibial length in prepubertal male mice. J Endocrinol 2004;182:165–172.
37 Wickman S, Kajantie E, Dunkel L: Effects of suppression of estrogen action by the P450 aromatase inhibitor letrozole on bone mineral density and bone turnover in pubertal boys. J Clin Endocrinol Metab 2003;88:3785–3793.
38 Hero M, Mäkitie O, Kröger H, Nousiainen E, Toiviainen-Salo S, Dunkel L: Impact of aromatase inhibitor therapy on bone turnover, cortical bone growth and vertebral morphology in pre- and peripubertal boys with idiopathic short stature. Horm Res 2009;71:290–297.
39 Zadik Z, Sinai T, Borondukov E, Zung A, Yaniv I, Reifen R: Longitudinal monitoring of bone accretion measured by quantitative multi-site ultrasound (QUS) of bones in patients with delayed puberty (a pilot study). Osteoporos Int 2005;16:1036–1041.
40 Morrison JA, Sprecher DL, Biro FM, Apperson-Hansen C, Lucky AW, DiPaola LM: Estradiol and testosterone effects on lipids in black and white boys aged 10 to 15 years. Metabolism 2000;49:1124–1129.
41 Kirkland RT, Keenan BS, Probstfield JL, Patsch W, Lin TL, Clayton GW, Insull W Jr: Decrease in plasma high-density lipoprotein cholesterol levels at puberty in boys with delayed adolescence. Correlation with plasma testosterone levels. JAMA 1987;257:502–507.
42 Arslanian S, Suprasongsin C: Testosterone treatment in adolescents with delayed puberty: changes in body composition, protein, fat, and glucose metabolism. J Clin Endocrinol Metab 1997;82:3213–3220.
43 Querfeld U, Dopper S, Gradehand A, Kiencke P, Wahn F, Zeisel HJ: Long-term treatment with growth hormone has no persisting effect on lipoprotein(a) in patients with Turner's syndrome. J Clin Endocrinol Metab 1999;84:967–970.
44 Halstead LS, Groah SL, Libin A, Hamm LF, Priestly L: The effects of an anabolic agent on body composition and pulmonary function in tetraplegia: a pilot study. Spinal Cord 2010;48:55–59.
45 Malmendier CL, van den Bergen CJ, Emplit G, Delcroix C: A long-term study of the efficacy of oxandrolone in hyperlipoproteinemias. J Clin Pharmacol 1978;18:42–53.
46 Hazzard WR, Wahl PW, Gagne C, Applebaum-Bowden D, Warnick GR, Albers JJ: Plasma and lipoprotein lipid responses to four hypolipid drugs. Lipids 1984;19:73–78.
47 Wickman S, Saukkonen T, Dunkel L: The role of sex steroids in the regulation of insulin sensitivity and serum lipid concentrations during male puberty: a prospective study with a P450-aromatase inhibitor. Eur J Endocrinol 2002;146:339–346.
48 Kennedy MC: Anabolic steroid abuse and toxicity. Aust NZ J Med 1992;22:374–381.
49 Albrecht ED, Lane MV, Marshall GR, Merchenthaler I, Simorangkir DR, Pohl CR, Plant TM, Pepe GJ: Estrogen promotes germ cell and seminiferous tubule development in the baboon fetal testis. Biol Reprod 2009;81:406–414.
50 McCarthy MJ, At-Taras EE, Pearl CA, Nitta-Oda BS, Roser JF, Conley AJ, Berger T: Suppression of endogenous estrogen during development affects porcine epididymal sperm maturation. Mol Reprod Dev 2006;73:1122–1128.
51 Carreau S, Bourguiba S, Lambard S, Galeraud-Denis I, Genissel C, Levallet J: Reproductive system: aromatase and estrogens. Mol Cell Endocrinol 2002;193:137–143.
52 Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K: Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab 1995;80:3689–3698.
53 Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS: Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 1994;331:1056–1061.
54 Carani C, Qin K, Simoni M, Faustini-Fustini M, Serpente S, Boyd J, Korach KS, Simpson ER: Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med 1997;337:91–95.
55 Patry G, Jarvi K, Grober ED, Lo KC: Use of the aromatase inhibitor letrozole to treat male infertility. Fertil Steril 2009;92:829.