Individualised vs fixed dose of oral 17β-oestradiol for induction of puberty in girls with Turner syndrome: an open-randomised parallel trial

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Abstract

Context: Oestrogen induction of pubertal changes in Turner girls may reinforce their psychological well-being and may also optimise final height; however, oestrogen type, dose, and route are not well established.

Objective: To induce normal pubertal development in Turner girls and ovarian insufficiency with oral 17β-oestradiol (E2), either as individualised dose (ID) or as fixed dose (FD), and to determine whether growth is affected.

Design: Open-label randomised, parallel groups, multicentre clinical trial in 48 GH-treated Turner girls. Oral E2 was given in tablets, either as an ID of 5–15 mg/kg per day during 2 years or as a FD of 0.2 mg daily during the first year followed by 0.5 mg daily during the second year. Main outcome measures were the event of attaining a Tanner breast staging R4 (primary), FSH, and auxological variables (secondary).

Results: Shorter median time to Tanner staging ≥ B4 in the FD group (733 days) compared with the ID group (818 days) (P=0.046). Higher proportion of girls with Tanner staging ≥ B4 (65%) in the FD group compared with the ID group (42%) (P=0.068). Bone age did not show inadequate acceleration and adult height prediction was maintained in both groups. No oestrogen-related adverse events were reported.

Conclusions: Two-year treatment with oral E2 can progressively induce normal pubertal development in Turner syndrome. Low-dose oral E2 given as a FD produces a satisfactory pubertal development not inferior to ID. Treatment was well tolerated and did not interfere with the growth-promoting effect of GH.

Introduction

Absence of pubertal development is one of the most common clinical features of Turner syndrome (TS); although up to 20% of girls with TS undergo some spontaneous pubertal development, few of them maintain normal ovarian function (1). When oestrogen therapy is required to induce pubertal development, the form, dosing and timing should mimic the process of normal puberty. The induction of pubertal changes in these girls concurrently with their normal peers may reinforce their psychological well-being. The development of secondary sexual characteristics and the maturation of female identity during the induction of puberty are essential for such girls; sufficient uterine growth and development of the endometrium for potential embryo transfer in later life are other goals of exogenous oestrogen therapy (2).

The debate on TS has often focused on the effect of oestrogen therapy on adult height. Delaying oestrogen therapy to optimise height potential as previously recommended (3) tends to undervalue the psychosocial importance of age-appropriate pubertal maturation and may be deleterious for bone and other oestrogen-dependent processes (4, 5). Recent evidence suggests that initiating 17β-oestradiol (E2) at low doses at the age of 12 years or even earlier permits a normal pace of puberty without interfering with the positive effect of rhGH on adult height (6, 7, 8). The combined effect of rhGH and low doses of oestrogens seems to influence height velocity in a favourable way, while too high E2 doses given too early might limit the GH-dependent gain in final height (9, 10).
There is a wide variation in the form, dose, and route of administration of oestrogens used for replacement and induction of puberty (11, 12, 13). Micronised E$_2$, identical to the natural product of the ovary, is available as oral tablets and as transdermal patches or gel and should be considered when replacing young hypogonadal girls because it is the most physiological form of oestrogen available. Previous studies have found no differences in rates of protein synthesis, degradation, lipid oxidation, or whole-body lipolysis between oral and transdermal E$_2$ (14). It has been postulated that oral oestrogen, by passing first through the liver, may directly inhibit hepatic insulin-like growth factor 1 (IGF1) production and promote adipogenesis by increasing fatty acid incorporation into triglycerides (15, 16); in this regard, oral oestrogen presumably acts as a GH antagonist (17). These findings have not been universally confirmed and oral oestrogens are not necessarily deleterious as it has been shown that they prevent the total body fat increase associated with menopause and the shift from peripheral to central fat distribution (18). Furthermore, similar changes in IGF1 have been observed in either oral or transdermal administration of oestrogen without differences in the bioavailable free IGF1 concentrations (14, 19, 20). Oral oestrogens may induce changes in the liver function due to its first-pass hepatic metabolism, but while ethinyl-oestradiol increases hepatic globulins and blood clotting factors, E$_2$ primarily changes lipoproteins in a favourable way (21, 22). Regardless of the route, the ability to monitor plasma E$_2$ levels after several weeks of stable dosing, which has important clinical implications in the follow-up of patients with hypogonadism, is an advantage of using E$_2$ compared with ethinyl-oestradiol.

The purpose of this study was to evaluate two different dosing protocols of low-dose oral E$_2$, an individualised dose (ID) depending on weight ($\mu$g/kg per day) and a more simplified fixed dose (FD; mg/day), in rhGH-treated girls with TS and ovarian insufficiency for induction of puberty and to assess whether they may affect growth potential.

Materials and methods

Subjects

The study population met the following inclusion criteria: TS verified by chromosomal analysis, treatment with rhGH, bone age (BA) $\geq$12 and <14 years, signs of ovarian insufficiency (FSH markedly elevated postmenopausal range), well-documented growth rate during the last 12 months before inclusion and signed informed consent given by parents/guardians. Exclusion criteria included signs of spontaneous puberty (Tanner breast stage $\geq$ B2), current or previous treatment with oestrogen, anabolic steroid hormones, known or suspected oestrogen-dependent neoplasia, undiagnosed abnormal genital bleeding, known thyroid diseases not adequately treated, porphyria, cardiac diseases, liver toxicity induced by medication, diabetes mellitus, active or previous deep venous thrombosis or thromboembolic disorders (including family history), end-stage renal disease, hypertension, severe asthma, severe obesity (BMI $>30$ kg/m$^2$), prolonged immobilisation, major elective or post-traumatic surgery, major trauma and known or suspected hypersensitivity to the trial product.

From a total of 50 recruited girls who met the inclusion criteria, 48 were randomised in 18 centres in Spain and were enrolled in the study (two girls refused participation before being randomised). Patients were randomly classified into two groups of 24 patients each to receive either, ID or FD. Two patients from the ID group were withdrawn from the study because of patient’s decision and rhGH treatment interruption, and one patient from the FD group was excluded from the intention to treat population for presenting a baseline BMI $>30$ kg/m$^2$. The number of TS patients with 45,X monosomy was similar in both groups as 50 and 48% of the girls were 45,X in the ID and FD group respectively.

Study design and treatment protocol

It was a multicentre, prospective, randomised, parallel group, open-label clinical trial (registered in www.novonordisk-trials.com). Primary end point was time to staging B4, while proportion of patients in staging B4 at end of trial was a secondary objective. Sample size was based on the statistical power to detect a minimum difference in time to staging B4 of 75 days (s.d., 112) earlier in the FD group. This difference with a 0.05 one-sided $\alpha$ error and a 0.20 $\beta$ error (power of 80%) needed a sample size of 29 patients per group. Patients with standard rhGH treatment were randomly assigned to either an ID regimen of E$_2$, or to a FD regimen of E$_2$, with a 1:1 distribution between both groups during 24 months. The primary criterion for a successful pubertal induction was to achieve staging B4 within a 2-year frame. The process of randomisation was centralised at Novo Nordisk A/S, Bagsvaerd, Denmark.

The ID group consisted of tablets of E$_2$ (Novo Nordisk A/S) with a dosage of 5–15 $\mu$g/kg per day taken orally in the morning for 2 years. Oestrogen treatment was started with a dose of 5 $\mu$g/kg per day and was maintained during the first 6 months of treatment. If pretreatment BA was retarded for more than 2 years, the same dose was maintained during the first treatment year and a dose increase to 10 $\mu$g/kg per day was considered afterwards depending on breast development and BA progression. If pretreatment BA was retarded for <2 years, a dose increase to 10 $\mu$g/kg per day was considered after the first semester of treatment depending on breast development and BA progression. After 18 months, the dose was increased to 15 $\mu$g/kg per day. The dosage was readjusted at each visit and rounded off to 0.05 mg (half a tablet). The mean dose of E$_2$ received at baseline, 6, 12, 18 and
24 months of treatment was 0.23 ± 0.05, 0.26 ± 0.09, 0.45 ± 0.14, 0.57 ± 0.11 and 0.6 ± 0.11 mg/day respectively. The FD group consisted of the administration of E2 0.2 mg/day (2 × 0.1 mg tablets) during the first year followed by the administration of 0.5 mg/day (1 × 0.5 mg tablets; Novo Nordisk A/S) during the second year of the study.

Variable measurements

Efficacy of treatment was assessed by Tanner puberty staging and serum FSH levels, both performed every 3 months. Other secondary efficacy variables included BA determination with X-ray of the left hand and wrist every 12 months, according to the method of Greulich & Pyle (23) and performed by the same investigator, and with central reading, height and weight every 3 months and adult height prediction calculated according to the method of Bayley & Pinneau (24). Safety was assessed by the effects of treatment on blood pressure, HbA1c levels, glycaemia, haemoglobin, creatinine, alanine amino transferase/aspartate amino transferase (ALT/AST), alkaline phosphatase and the incidence of adverse events.

All analytical determinations were performed in the laboratory of each participating centre by automated procedures. Auxological data were normalised according to age and gender and expressed in SDS according to the Spanish reference standards (25).

Statistical analysis and ethics

Baseline quantitative variables were compared using a Student’s t-test for independent groups (normal distribution) or by a Mann–Whitney U test (nonparametric distribution). Categorical variables were compared by the Fisher’s exact test or the χ² test. Efficacy variables were analysed by the χ² test. Multivariate logistic regression models were used for categorical variables and log-rank test with Kaplan–Meier estimates and Cox proportional hazard models for the analysis of the time to Tanner puberty staging. Repeated ANOVA and ANCOVA analyses were used for other quantitative analysis, with the treatment group as the main factor and baseline values as covariables. P values in multiple comparisons were adjusted by Bonferroni correction procedure. A two-sided α-error of 5% was considered for all comparison analysis. The study agreed with the Declaration of Helsinki and was approved by the Ethics Committee of Clinical Investigation of each participating centre. An informed consent form was signed by the parents or guardians and by patients older than 12 years of age.

Results

Baseline characteristics

Table 1 summarises baseline characteristics of all girls compared by treatment groups. The ID group showed statistically higher chronological age (CA) than the FD group. BA was similar in both groups. The BA/CA quotient was significantly lower in the ID group compared with the FD group expressing a higher delay of BA. Height SDS for CA was similar in both groups (−2.61 ± 1.09 vs −2.73 ± 1.2 in the ID and FD group respectively). Adult height prediction SDS was not significantly different between the groups (−1.17 ± 1.30 vs −1.83 ± 1.16 in the ID and FD group respectively). At baseline, all girls were at Tanner staging B1 in both groups. Pubarche was similar in both groups as the proportion of girls with Tanner staging ≥ P2 was 96 and 86% in the ID and FD group respectively, without significant differences.

Tanner puberty staging and FSH levels

Puberty advanced gradually along the study with increasing oestrogen doses in both groups. However, the median time needed to achieve Tanner staging ≥ B4 was statistically lower in the ID group (733 days) than in the ID group (818 days) (log-rank test, P = 0.046; Cox regression model, P = 0.042; Fig. 1). The probability of reaching a puberty staging ≥ B4 after a determined period was 3.12 times higher in the FD group than in the ID group (Cox proportional hazard models; Fig. 1). After 12 months of treatment, the proportion of girls with B2 were 63 and 52% and with B3 were 25 and 26% in the ID and FD groups respectively. The proportion of girls ≥ B4 after 24 months was higher, though not significantly, in the FD group (65.0%; 95% confidence interval (95% CI) 46.0–85.0%) compared with the ID group (42.0%; 95% CI 22.0–63.0%) (logistic regression adjusted for baseline differences, P = 0.068; Fig. 2A).

Pubarche advanced in both groups. The proportion of girls with Tanner staging ≥ P4 after 24 months was significantly higher in the FD group (87.0%; 95% CI 66.0–97.0%) than in the ID group (71.0%; 95% CI 49.0–87.0%) (logistic regression adjusted for baseline differences, P = 0.043; Fig. 2B).

| Table 1 Baseline variables in both groups of patients. Values are given as mean ± S.D. |
|---------------------------------|---------------------------------|---------------------------------|
| ID group (n=24) | FD group (n=23) | P value |
| Mother’s height (cm) | 161.9 ± 8.6 | 160.0 ± 7.4 | NS |
| Father’s height (cm) | 167.7 ± 8.0 | 171.1 ± 6.2 | NS |
| CA (years) | 14.3 ± 1.4 | 13.4 ± 1.0 | 0.01 |
| Height SDS for CA | −2.61 ± 1.09 | −2.73 ± 1.2 | NS |
| BA (years) | 12.4 ± 0.5 | 12.5 ± 0.6 | NS |
| BA/CA quotient | 0.87 ± 0.08 | 0.94 ± 0.07 | 0.01 |
| BMI (kg/m²) SDS | 0.97 ± 0.21 | 1.64 ± 0.22 | 0.04 |
| Adult height prediction SDS | −1.17 ± 1.30 | −1.83 ± 1.16 | NS |
| 45, X karyotype | n=12 (50%) | n=11 (48%) | NS |
Baseline FSH serum levels were equally elevated in both groups (84.5 ± 10.2 and 81.3 ± 10.3 mIU/ml in the ID and FD groups respectively) and decreased during the study. FSH levels were 65.9 ± 9.6 and 67.5 ± 9.7 mIU/ml after 6 months of treatment, 68.8 ± 10.2 and 67.6 ± 10.3 mIU/ml after 12 months of treatment, 61.0 ± 10.0 and 57.0 ± 10.1 mIU/ml after 15 months of treatment, 61.1 ± 8.3 and 59.7 ± 8.4 mIU/ml after 18 months of treatment and 54.7 ± 8.5 and 61.0 ± 8.6 mIU/ml at the end of the study, in the ID and FD groups respectively. FSH levels at the end of the study were significantly lower than at baseline in both groups (P < 0.05). There were no significant differences between the groups. When analysing both groups together, there were statistically significant differences compared with baseline at the 6th and 15th months of treatment and thereafter until the end of the study (P < 0.05).

**Auxological results**

After 2 years of treatment, adult height prediction was very similar for both treatment groups (−1.3 ± 0.2 vs −1.5 ± 0.2 SDS in the ID and FD groups respectively) without significant differences. BA did not show inadequate acceleration as BA/CA ratio after 1 year of treatment was 0.85 and 0.90 and after 2 years of treatment 0.86 and 0.90 in the ID and FD groups respectively. BMI SDS progressed along the study as expected without showing significant differences; at baseline and after 2 years of treatment, BMI SDS were 0.97 ± 0.21 and 1.27 ± 0.24 in the ID group and 1.64 ± 0.22 and 1.38 ± 0.26 in the FD group.

**Figure 1** Time to Tanner staging B4 (days). I, Individualised dose group; II, fixed dose group. Y-axis refers to cumulative probability of persistence in the study without Tanner staging B4.

**Figure 2** Pubertal development. (A) Percentage of study patients achieving the designated stage of breast development at each time point since initiation of treatment with 17ß-oestradiol. (B) Percentage of study patients achieving the designated stage of pubarche development at each time point since initiation of treatment with 17ß-oestradiol.
**Safety**

Adverse events were similar between treatment groups with an overall incidence of 38.0 and 33.0% in the ID and FD groups respectively (P=0.76), with none of the adverse events being of severe intensity. There were only two adverse events reported to be of moderate intensity within each treatment group (Crohn’s disease and acute appendicitis in the ID group and lymphedema and iron deficiency anaemia in the FD group), all of them with an improbable relationship to E2 and they did not request any treatment interruption. There was only one serious adverse event in the ID group (acute appendicitis), which required hospitalisation for surgical treatment, but it was assessed as improbably related to E2 and it did not require treatment interruption. There were no significant changes in safety analytical parameters or vital signs throughout the study. Liver function tests remained normal and did not change after the 2 years of treatment. ALT (UI/l) changed from 25.9 ± 3.5 at baseline to 29.4 ± 4.5 at the end of the study in the ID group and from 27.3 ± 3.2 to 26.1 ± 4.2 in the FD group. Similarly, AST changed from 25.5 ± 2.7 to 24.8 ± 2.5 in the ID group and from 27.3 ± 2.5 to 25.5 ± 2.4 in the FD group.

**Discussion**

This study presents the results of puberty induction with low-dose oral E2 in two different dosage protocols (ID of μg/kg per day vs a FD of mg/day) for 2 years. We hypothesise that when inducing puberty with oral E2 in girls satisfactorily treated with rhGH, a simplified FD could be equally optimal, therefore avoiding the need to adjust the dose at each visit. In this study, low-dose oral E2 given as a FD produced satisfactory pubertal development which was not inferior to ID without interfering the growth-promoting effect of GH and without adverse effects.

Oral E2 has been given classically as an ID starting with 5 μg/kg per day and increasing progressively up to 10–15 μg/kg per day (26). This dosing protocol may be laborious as patients have to be frequently weighed to adjust the dose; however, a fixed dosing protocol should be easier and more acceptable. The median time needed to reach a Tanner staging ≥ B4 was significantly shorter in the FD group vs the ID group and the probability of a TS girl reaching a Tanner staging ≥ B4 was 3.12 higher when using a FD compared with an ID. Furthermore, at the end of the study, the number of patients that achieved Tanner staging ≥ B4 or ≥ B3 was higher, though not statistically different, in the FD group compared with the ID group. It should be considered that a limitation of the study was that it could not enrol the pre-specified sample size in each of the groups. With the sample size finally included and the difference observed in time to staging B4, the study had a power of 73%. The variability observed in both groups reflects the marked individual differences in target organ sensitivity. In our study, the majority of TS girls from the FD group reached Tanner stage B4 after 2 years of treatment, which is similar to the time interval between B2 and B4 in the normal population (25). The same results have been observed previously with a 2-year interval between B2 and B4 by other authors (8, 27). Oral preparations remain the preferred method for inducing puberty in patients with TS, despite the theoretical reasons for favouring transdermal routes of administration (11), as acceptance of medication by the oral route is excellent and easy to use. In our study, compliance was controlled in both groups appropriately.

The mean CA at start of treatment was 14.3 years in the ID group and 13.4 years in the FD group. This is a relatively late age for starting puberty but it is in concordance with the current prescribing practice published in recent questionnaires (12, 13) and with other studies (28, 29). Pubic hair appeared before oestrogen was initiated in nearly all girls from both groups and progressed similar to other studies (30). Before initiation of treatment, only 54 and 21% of TS girls presented with Tanner staging ≥ P3 in the ID and FD groups respectively. This is considered late compared with Spanish reference standards where P3 is acquired at 11.9 years and P4 at 13.4 years (25). Our observations are in agreement with the previous studies where primary gonadal failure in TS has been associated with late onset and slow progression of pubarche (31).

The degree of suppression of circulating gonadotropins has been used as a surrogate marker of the efficacy of oestrogen replacement in TS. Baseline FSH levels were markedly elevated in both groups and showed a progressive and significant decline but did not normalise after 2 years of treatment despite the satisfactory feminisation acquired. Other studies using similar doses of oestrogens in adolescent TS girls have showed similar results (26). This is probably due to the low dose used, the short duration of the study or to a different sensitivity of the gonadostat due to the lack of inhibin observed in patients with primary gonadal dysfunction (30, 32). In our study, the higher FSH suppression was observed in both groups during the first 6 months of the treatment, which coincided with the lowest doses of E2 used and subsequent increments of E2 were not followed by a more intense FSH suppression. This may imply that early low doses of E2 are more FSH suppressive than higher doses employed later as the girls aged, which may be related to the documented decrease in sensitivity of the gonadostat to the negative oestrogen feedback with advancing age and neuroendocrine puberty (27, 33). The discrepancy observed between the adequate feminisation reached and the lack of normalisation of FSH concentrations suggests that the degree of suppression of gonadotropins cannot be used for measuring oestrogen action and
potency in TS. Both FSH concentrations and clinical assessment should be considered before tailoring individual pubertal induction with oral E$_2$. Our protocol of E$_2$ did not have a negative effect on growth. BA progressed as expected in both groups without inadequate acceleration, thus preserving adult height potential. Our patients were already receiving rhGH and most of the catch-up growth had already occurred before oestrogen treatment was initiated. A limitation of the study was that other measurements of oestrogen action such as body composition, uterine length or bone metabolism were not recorded, although the effect of oral E$_2$ in these variables in TS girls had already been studied by different authors (14, 26, 34). The safety profile of the study is in agreement with the previous study results with oral or transdermal administration of E$_2$ (8, 34, 35, 36). No adverse events detected were considered oestrogen related and treatment was safe and well tolerated.

In conclusion, low-dose oral E$_2$ given as a FD produced satisfactory pubertal development which was not inferior to ID and without interfering with the growth-promoting effect of GH and therefore might be preferable as it is easier. Oral E$_2$ is safe and well tolerated. Therefore, in rhGH-treated TS girls aged 13 years and with satisfactory adult height prediction, we propose the use of a FD of oral E$_2$ of 0.2 mg/day for the first year and 0.5 mg/day for the second year in order to induce puberty adequately.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
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