Regulation and modulation of growth : insights from human and animal studies

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Chapter 7

General discussion

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The aim of this thesis was to investigate the long-term results of two novel treatment modalities in children with ISS (part A), to study the effect of the administration of aromatase inhibitors in the rat (part B), and to assess whether mesenchymal stem cells can differentiate into chondrocytes with a phenotype resembling the epiphyseal growth plate. In this chapter, the results of our findings are discussed in the perspective of current knowledge. Considerations with regard to clinical practice will be presented, the role of model systems in growth studies will be debated, and recommendations for future research will be given.

A. What are the long-term results of two novel treatment modalities in children with ISS?

Despite almost three decades of clinical trials with GH treatment in ISS, many questions have remained with respect to the optimal treatment regimen. The two clinical trials reported in this thesis have contributed to the discussion about optimizing GH treatment strategies in children with ISS. The first trial was designed to study the effect of high dose GH treatment restricted to the prepubertal period in young children with ISS. In the second trial, GH was combined with GnRHa treatment in adolescents with ISS and a relatively early-timed pubertal onset. In both of our studies, subjects were randomly allocated to receive treatment or to participate in a non-treated control group. Non-treated controls did not only serve for comparison with GH-treated subjects, but also illustrated the natural history of ISS. We have reported both interim data (1-3) and adult height data (this thesis) of both trials.

As mentioned in ***Chapter 2***, at the time this study was designed, there were three important unresolved issues with respect to GH treatment of children with ISS. First, it was unknown what the effect would be of a high GH dosage on growth velocity, bone maturation, puberty and adult height. Second, the relative contribution of GH treatment before and during puberty was unclear. Third, there was a need to gain more insight into the factors affecting the growth response, as only a modest part of the inter-individual variation can be explained (4)

Besides these questions on GH monotherapy, there was an additional question that was approached in ***Chapter 3***: what would be the effect of combined treatment with GH and GnRHa on adult height?

Relationship between GH dose and growth response.

Most clinical trials with GH in ISS have used a GH dosage of 40-50 µg/kg/day, or the equivalent dosage corrected for body surface (1.2-1.4 mg/m^2/day). As reviewed in chapter 1, adult height gain on such a dosage is close to 7 cm (5-7). While there is little doubt that lower dosages are less efficacious, leading to an adult height gain of 3-4 cm (8;9), the effect of higher dosages is unclear.

Results of two trials in which high dose GH treatment was applied have been reported. In the first trial (10) there were three study arms: 1) a conventional dose of 40 µg/kg/day; 2) a titration arm in which GH dose was titrated to achieve an IGF-I SDS at the mean level of the normal range; and 3) a titration arm in which GH dose was titrated to achieve an IGF-I SDS level 2 SD above the mean of the normal range. In the last group the growth response was best, but required on average a 2.5 times higher dosage than used in the second group. In the other study (11) two doses were compared: 33 and 67 µg/kg/day. These doses led to a change in height SDS of 1.0 and 1.4, respectively, in comparison to 0.2 SDS in untreated controls. Thus, in both studies a high GH dose had a positive effect on adult height gain.

In contrast, we showed in ***Chapter 2*** that high dose GH treatment restricted to the prepubertal period, and administered to young children, increased height gain during treatment, but also accelerated bone maturation and ultimately did not lead to an increased adult height. Cohen and co-workers did not observe bone age advancement after treatment with a high GH dose (median 98 µg/kg/d, range 20-346 µg/kg/d) titrated on circulating IGF-I levels of 2 SDS (10). However, the dose range in that study was large, and the children who needed high GH doses to reach the aimed IGF-I level may have been more resistant to GH. Also in the study of Albertsson-Wikland no advance of skeletal maturation was observed (11).

We speculate that the difference between our results and those of Cohen *et al.* and Albertsson-Wikland *et al.* can be explained by a difference in age at start of GH therapy. The mean age at start of GH treatment in our study was 8.7 years, considerably younger than the mean age of approximately 11 years in the other studies. The observation in retrospective studies of an inverse association of age at start of GH treatment and height gain (4) is not necessarily in contradiction with our hypothesis, as the average age in those retrospective studies is also around 11 years.

The discrepancy between the negative effect of high dose GH treatment in our trial and positive effects on height gain in other studies may rely on an increased sensitivity of the epiphyseal growth plates of younger children in comparison with those of older children to high doses of GH and/or IGF-I. In favor of the hypothesis of age-dependent sensitivity to GH was our finding that a younger age was associated with a lower height gain. Cruickshank and co-workers have

described that GH administration to rats was found to have no effect on GHR expression and to decrease IGF1R expression in the epiphyseal growth plates of prepubertal rats, whereas expression of both receptors was found to be upregulated at the age of sexual maturation, a phase associated with fast growth (12). They hypothesized that the dissociation between GHR expression and exogenous GH at a young age may reflect an inherent mechanism to prevent excessive proliferation and prevent overgrowth of the bone (12).
The combined results of our and other studies in young children imply that at such a young age GH dose may be positively associated with adult height gain in the range of 25-50 µg/kg/d, but that higher doses may decrease adult height gain due to accelerated epiphyseal maturation, while at the same time the effect on growth reaches a plateau after a certain dose.

The relative contribution of GH treatment before and during puberty

An alternative explanation for the attenuated adult height outcome after high dose GH in our study may be the discontinuation of treatment at the onset of puberty, which theoretically may have abolished therapy-induced height gain. Such a catch-down phenomenon has been described in children with persistent short stature born SGA when GH treatment was discontinued 1-3 years before the onset of puberty (13). Those children showed a marked reduction in height velocity and height SDS in the post-treatment follow-up period and a decrease in adult height prediction, suggesting that GH treatment should at least be continued until pubertal onset.
At the time our study was initiated, there were several arguments in favor of the hypothesis that growth enhancing strategies should be designed to improve prepubertal growth. One of these arguments was that height at onset of puberty is positively correlated with adult height in GHD or short SGA children (14-16). Another argument was that prolonged GH treatment during puberty might not be needed, since height gain during the pubertal growth spurt is primarily determined by sex steroid signaling, possibly in combination with an increased secretion of GH and IGF-I. Since children with ISS by definition have a normal GH secretion, one could argue that GH levels should be sufficient at the time of increased sex steroids. A third argument was that Rekers-Mombarg *et al.* demonstrated in previous studies that pubertal height gain was not different between GH-treated and non-treated GHD children (17).
In our study described in ***Chapter 2***, GH treatment was discontinued at the onset of pubertal development. Already at that time, predicted adult height of the GH-treated group was found to be similar to that of the non-treated controls, arguing against the catch-down growth hypothesis as an explanation for the absence of enhanced adult height.

Another topic, that is related to the question whether GH should be continued or not during puberty, is whether GH influences the timing of pubertal onset and the tempo of pubertal development. On regular dosages most studies have not been able to document that GH advances pubertal onset and tempo, but in our study (chapter 2), using a higher dosage, there are at least reasons to suspect such an effect. We believe that this may be related to age as well. In the final analysis on 26 children using a novel technique for expressing pubertal stage in SDS (correcting for age and gender) (18), we found a trend (p=0.2) towards a younger age at Tanner stage 2 in the GH-treated groups compared to controls. While this technique enables appropriate correction for the (statistically significant) age difference between the groups at start of the trial, the inability to reach statistical significance may well be related to the limited number of subjects that could be studied at follow up.
In the larger group of 35 subjects studied 5 years after inclusion, the age difference at start did not reach significance, some patients had not yet entered puberty at the moment of analysis, and another method (cumulative proportions of patients having entered puberty, and calculation of relative risk) was used. In that analysis the relative risk for early puberty, adjusted for age and sex, was 4.7 (1.4-15.8, p=0.012) (2).
There are two other observations that can serve as indirect evidence for an effect on pubertal onset. First, the observation that none of the 12 males in the GH treatment group entered puberty late, compared to 2 out of 8 controls. Second, at follow-up the GH-treated subjects had a significantly shorter leg length than controls and a higher SH/H SDS, suggesting earlier exposure to sex steroids. Unfortunately, the study design during follow-up did not allow for the collection of sufficient data on the progression of puberty.
The higher SH/H ratio may also explain the increase in BMI SDS observed in the GH-treated children, as shown previously in GH deficient children (19). Up to early adolescence, height SDS remained stable at -2.6, but adult height was 0.7 SD higher than height SDS at start, presumably due to a rather delayed and possibly protracted pubertal development. A similar pattern was seen for SH and LL SDS.
In conclusion, high-dose GH treatment limited to the prepubertal period in young children with ISS has no effect on adult height, probably caused by concomitant advance of bone maturation, and may advance pubertal onset.

Factors affecting the growth response

The study described in ***Chapter 2*** has not contributed to the identification of factors that can predict growth response to GH therapy. In our study only age was a (positive) predictor of treatment effect. In the first part of the study, it was shown that plasma IGF-I during treatment

was positively associated with growth response in the first year (2), but in the final analysis we have demonstrated that short-term growth is not necessarily predictive for long-term success.
In the last decades several other studies have investigated the possible predictors of growth response, either on first year height velocity or adult height gain. These were recently reviewed (20). Data from the large KIGS cohort have shown that positive predictors for adult height after GH treatment include midparental height and the first year growth response (expressed as studentized residual), while age is a negative predictor (4).

The effect of a combined treatment with GH and GnRHa on adult height

In ***Chapter 3*** we have shown that 3 years of treatment with GH and a GnRH agonist in short children with a relatively early puberty increases adult height, with a mean gain of 5 cm. This result confirms the results of previous less well controlled clinical trials. However, this positive effect apparently has a price. Postponing puberty in adolescents has some negative impact on psychosocial status (21), and in boys there are indications that it decreases bone mineral density of the lumbar spine.

Conclusions

We conclude that a regimen of high dose GH started at an early age in children with ISS, and restricted to the prepubertal phase, is not effective, and therefore not to be advised. The combination of GH and GnRHa in young teenagers with short stature and relatively early puberty can be considered in selected cases, but its effect is modest and bone mineral density may be at risk in males.

B. The effect of the administration of aromatase inhibitors in the rat

The main reason to perform the studies described in ***Chapters 4 and 5*** was that aromatase inhibitors (AIs) would be a logical class of drugs for growth enhancement in boys who are short at the onset of puberty. Clinical trials had already been planned, whereas at the same time there was uncertainty about the short- and long-term adverse effects of these drugs, as locally produced estrogens are important in many tissues. With our studies we hoped to gain more insight into the effect of aromatase inhibition on growth, as well as on potential adverse results of such treatment in several tissues.

An unexpected outcome of our studies was that the effect on growth was strongly dependent on gender. Female rats became larger after treatment, while male rats became shorter. The growth attenuation observed in male rats was not consistent with the growth phenotype of male aromatase-deficient or estrogen-resistant patients. However, the negative effects of exemestane treatment on growth and BMD in male rats are in line with similar findings in male 6 weeks old rats treated with the nonsteroidal aromatase inhibitor vorozole (22) and in 4 weeks old male rats treated with the selective estrogen receptor modulator tamoxifen (22;23). Growth attenuation was also observed in male aromatase knockout (ArKO) mice and estrogen receptor alpha (ERα) and ER$\alpha\beta$ knockout mice (24-26). We must therefore conclude that the male rat is not a suitable model to study the effect of AIs on growth.

With respect to side effects, in both genders a negative effect on bone quality was observed, which was not unexpected based on the pivotal role of estrogens in bone metabolism (27;28). In female rats there was a negative effect on the ovaries, leading to abnormalities similar to those observed in girls and women with the polycystic ovary syndrome (PCO). Such a phenotype has also been reported in female aromatase-deficient patients. These adverse effects argue against the use of aromatase inhibitors in girls with ISS, at least until additional research has proven beyond doubt that the observed ovarian changes are reversible and do not negatively affect fertility later in life. The development of the male genital tract was not impaired by aromatase inhibitor treatment, and no adverse effects were seen on brain morphology in both genders.

In the meantime, more data have become available about the long-term effect of AIs in adolescent boys with short stature. In children with GH deficiency, the addition of AIs (anastrozole) to GH treatment had a modest positive effect on predicted adult height (29). In children with ISS, AIs alone (letrozole) for 2 years increased adult height by 5.9 cm (30). Initial worries that letrozole would lead to abnormalities of the thoracic vertebrae (31), have recently been confirmed (32). MRI-analysis of the vertebral spine demonstrated that letrozole-treated boys with ISS developed vertebral deformities, whereas no such findings were reported in the non-treated control group (32).

Although the clinical trial with letrozole in boys with ISS showed a modest positive result on predicted adult height (30), the Finnish investigators emphasized that aromatase inhibition should be considered an experimental form of treatment, and that more studies had to be performed before AIs could be applied in clinical practice on a routine basis (33). The recent identification of vertebral body deformities potentially induced by estrogen depletion further justifies a reluctant attitude towards this type of treatment.

C. Development of an *in vitro* model for growth plate physiology: do mesenchymal stem cells differentiating into chondrocytes show a phenotype resembling the epiphyseal growth plate?

In ***Chapter 6*** we have shown that human fetal MSCs can indeed differentiate into chondrocytes, and that the gene expression profile resembles more the profile of epiphyseal chondrocytes than that of articular chondrocytes. We have therefore concluded that this model is suitable to further study the role of the numerous genes that are associated with the transition from immature chondrocytes in the resting zone, towards actively replicating chondrocytes in the proliferative zone, and finally to hypertrophic chondrocytes.
We believe that this model can serve for various purposes. First, the model can be used to study the effect of various growth factors and hormones on chondrocyte differentiation and may assist in increasing the understanding of the interplay between those various factors. This may result in the identification of new drug targets and the development of new drugs. Second, the model can be used to assess whether genes that seem to be associated with growth, for example the genes encountered in genome-wide association studies (GWAS) on height (34-36) are indeed expressed during chondrocyte differentiation. Similarly, SNP-array studies in children with short or tall stature have shown deletions and duplications in various chromosomal regions, disabling various genes associated with growth regulation. The search for candidate genes, as detected in these studies, can be facilitated by checking the role of the essential genes that orchestrate chondrocyte differentiation.

D. Conclusion and suggestions for future studies

The results of the two clinical trials included in this thesis have shown that a high GH dosage in young children with ISS restricted to the prepubertal phase is not efficacious, and we believe that even if GH had been continued during puberty, the result would not have been better. The treatment course of 3 years of GH plus GnRHa is more effective (5 cm height gain), but may have undesirable side effects. This indicates that for the treatment of ISS, in countries where GH is registered for this indication, a dosage of 40-50 µg/kg/day or 1.2-1.4 mg/m2/day may still be the best option. However, the pros and cons of GH treatment in ISS are still heavily debated among pediatric endocrinologists and legislators, since the modest gain in adult height is counterbalanced by a very long and intensive treatment (daily injections for 4-8 years), high costs, and little effect on psychosocial status. Future studies in this area could focus on better

tools to assess psychosocial consequences of short stature, and on developing psychological counselling programs for short children who seem to suffer from their shortness.

The results of the animal studies on AIs show that for the analysis of drugs that intervene with sex steroid signaling, rodents are not an optimal model. The role of sex steroids in rodent growth appears very different from that in the human. Still, such studies can serve to collect information about possible side effects of steroids and their antagonists. As it is uncertain which other animal models are better suited, well-controlled clinical trials on AIs in children and adolescents are needed before their use in clinical practice can be propagated.

The *in vitro* model of MSCs differentiating into epiphyseal chondrocytes can be used in future studies aimed at a better understanding of the physiology of the epiphysis, and to assess the influence of various growth factors and growth-modifying drugs.

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