Expression of Brdu, VEGF, IGF-1R and change of the growth plates from sex hormone-inhibited adolescents rats - Pilot study

Background
GnRH analogue is a well-established therapeutic approach in the management of precocious puberty in children. But GnRHa inhibits growth spurt during early puberty too. So it has limitation of final height gain. It is needed to study about ideal suppression level of sex steroid and what factor associated with growth decrement during GnRHa treatment.

The purpose of this study is to evaluation of VEGF, IGF-1 receptor and 5-bromo-2'-deoxyuridin(BrdU) expression, and change of cartilage layers in growth plate of rat treated with GnRhA.

Method
1. Experimental design
Animals: Female SD(Sprague-Dawley) rats of 3 weeks of age (total 15) Normal control group(5) : normal saline injected intramuscularly at day1 Low dose GnRhA group(5) : 25mcg of GnRhA injected intramuscularly at day1 High dose GnRhA group(5) : 50mcg of GnRhA injected intramuscularly at day1

2. Experimental procedure
1) Day1: Body weight and head to tail length measured before injection of GnRhA
2) Day13: BrdU was thawed in phosphate buffered saline (PBS) and injected peritoneally(100 mg/kg) into experimental animals twice, at 25 hours and 1 hour prior to their sacrifice.
3) Day14: Body weight and head to tail length measured repeatedly. Then their proximal tibial growth plate was harvested and stained with hematoxylin and eosin (H&E). The thickness, cellular change of cartilage layers were evaluated.
4) BrdU, VEGF antibody(DAKO), IGF-1 receptor were stained by method of immunohistochemistry.

Results
1. Body weight, leg and head to tail length of rat.
Mean body weight of GnRhA(50mcg) was increased than other gourps 2 weeks later. However length gain of GnRhA groups was shorter than control(Fig 1-1 and 1-2).

Fig. 1-1. Body weight of rat.

Fig. 1-2. Leg and head to tail length of rat.

2. Tibial Growth plate thickness(H-E stain)
To account for natural size variation across the epiphysis, measurements were taken at three equidistant points spanning the width of the growth plate (10× objective) and the mean thickness was recorded.

Group | mean thickness(μm) |  
--- | --- |  
control | 8.86 |  
GnRhA(25mcg) | 8.18 |  
GnRhA(50mcg) | 9.6 |

3. BrdU
To count the number of BrdU positive-staining cells, three areas of each slide were selected randomly, and the number of total cells and the number of stained cells in a rectangle of 150×250 μm in size were assessed. The ratio of positive cells in each zone of the growth plate was calculated as the number of positive cells to the total number of cells.

Group | mean BrdU (%) |  
--- | --- |  
control | 26 |  
GnRhA(25mcg) | 19 |  
GnRhA(50mcg) | 20 |

4. Expression of VEGF
To count the number of VEGF positive-staining cells, three areas of each slide were selected randomly, and the number of total cells and the number of stained cells in a rectangle of 150×250 μm in size were assessed. The ratio of positive cells in each zone of the growth plate was calculated as the number of positive cells to the total number of cells.

Group | mean VEGF (%) |  
--- | --- |  
control | 12 |  
GnRhA(25mcg) | 29 |  
GnRhA(50mcg) | 31 |

5. Expression of IGF-1R
Tibial growth plates were stained using a rabbit polyclonal antibody against the IGF-1R(Santa Cruz Biotechnology, sc-712). Positively stained chondrocytes, indicated in brown, were detected in various regions of the growth plate (arrow). The percentage of positively stained chondrocytes are calculated for each group of the growth plate.

Group | mean IGF (%) |  
--- | --- |  
control | 28 |  
GnRhA(25mcg) | 37 |  
GnRhA(50mcg) | 28 |

Summary of pilot study
In the GnRhA treated groups, dose dependently body weight increased. Less shorter length gain, dose dependently growth plate thickness increased, and decreased cellular proliferation than control group (by BrdU). But no differences in VEGF, IGF-1R expressions.

Future directions
This study has just pilot study to check GnRhA effect to growth plate. In proper study, it is needed to GnRhA receptor measurement and more longer experimental period. Also enough number of animal should be considered.