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

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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 intramusculary at day1
Low dose GnRHa group(5) : 25mcg of GnRHa injected intramusculary at day1
High dose GnRHa group(5) : 50mcg of GnRHa injected intramusculary at day1

2. Experimental procedure

1) Day1 : Body weight and head to tail length measured before injection of GnRHa
2) Day 13: 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) Day 14 : 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, celluar 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.

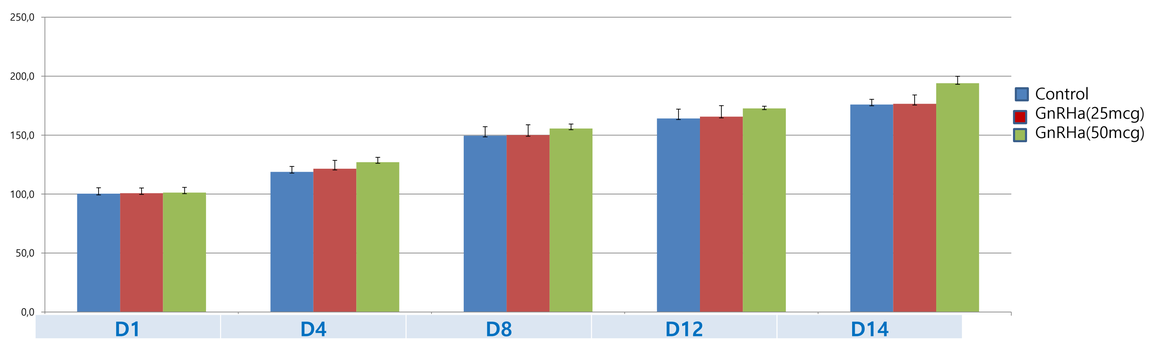
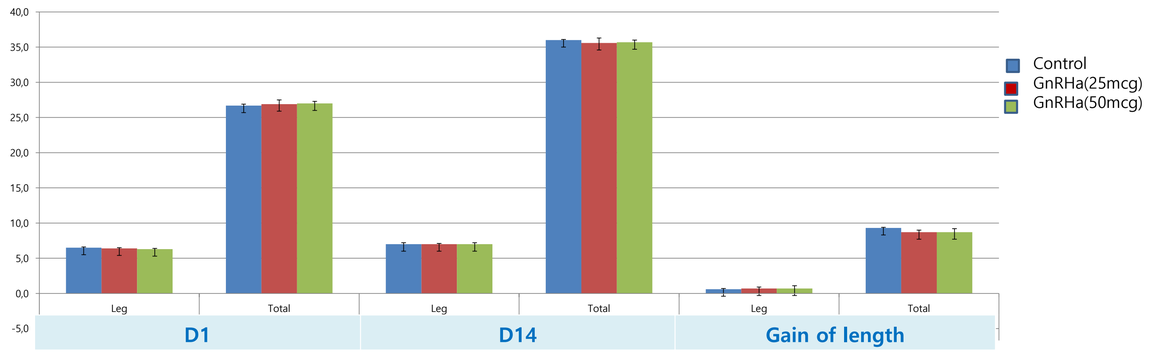
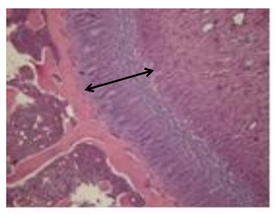


Fig. 1-2. Leg and head to tail length of rat.



2. Tibial Growth plate thickness(H-E stain)

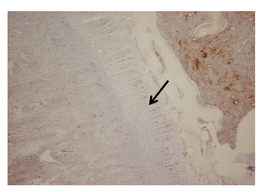
To account for natural size variation across the epiphysis, measurements were taken at three equidistant points spanning the width of the growth plate (10× objective) and the mean thickness was recorded.



Group	mean thickness(um)
Control	8.86
GnRHa(25mcg)	8.18
GnRHa(50mcg)	9.6

3. BrdU

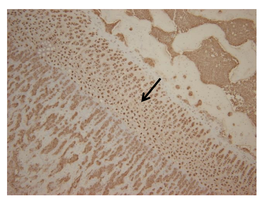
To count the number of BrdU positive-staining cells, three areas of each slide were selected randomly, and the number of total cells and the number of stained cells in a rectangle of 150×250 μm in size were assessed. The ratio of positive cells in each zone of the growth plate was calculated as the number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



Group	mean Brdu (%)
control	26
GnRHa(25mcg)	19
GnRHa(50mcg)	20

4. Expression of VEGF

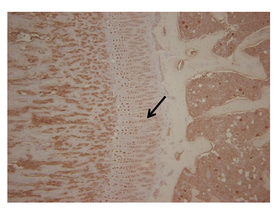
To count the number of VEGF positive-staining cells, three areas of each slide were selected randomly, and the number of total cells and the number of stained cells in a rectangle of 150×250 μm in size were assessed. The ratio of positive cells in each zone of the growth plate was calculated as the number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



Group	mean VEGF (%)
control	32
GnRHa(25mcg)	29
GnRHa(50mcg)	31

5. Expression of IGF-1R

Tibial growth plates were stained using a rabbit polyclonal antibody against the IGF-IRα (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)	27
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 exprerimental peroid. Also enough number of animal should be considered.

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