The influence of recombinant human growth hormone treatment on very small embryonic/epiblast-like stem cells (VSELs)

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Abstract

Background: Present knowledge on the effects of growth hormone (GH) on aging and lifespan are controversial. Clinical data indicate that normal or high levels of GH may accelerate aging and increase the risk of cardio-vascular diseases. Very small embryonic-like stem cells (VSELs) are a population of developmentally early stem cells residing in adult tissues, which could have the potential role in aging and organ rejuvenation.

Objective: The aim of the study was to analyze the effect of GH treatment on VSELs.

Methods: Twenty five patients: GH-deficient (20), Turner Syndrome (3), Prader-Willi Syndrome (2), treated with GH, mean age 9.1±2.7 years were included in the study. The mean GH dose was 0.27 mg/kg/week. Fasting peripheral blood samples were taken before the administration of GH, then two-weeks, one-month, three-months and six-months after it. Subsequently, we evaluated by employing FACS changes in the number of small $CD133^+Lin^-CD45^-$VSELs and $CD34^+Lin^-CD45^-$VSELs - that are precursors of long term repopulating hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs). Statistical analysis was performed ANOVA.

Results: The administration of GH initially stimulated for a month an increase in number of VSELs, and subsequently the number of VSELs decreased. After six months of treatment the number of circulating in PB VSELs was lower as compared to baseline values (p=0.026). The increase in VSELs number paralleled with increase in number of circulating MSCs and EPCs, however two months shift has been observed in case of EPCs. Finally, the number of MSCs and EPCs become lower than before GH treatment.

Conclusions: The treatment with GH modulates the population of VSELs, MSCs and EPCs circulating in PB. Our data suggests that: 1/ VSELs respond to GH treatment, and 2/ since the therapy with GH modulates population of VSELs, therefore it could influence life span and organ rejuvenation.

The authors have NOTHING TO DISCLOSE.

Background

Present knowledge on the effects of recombinant human growth hormone (rhGH) on aging and lifespan are controversial. Clinical data indicate that normal or high levels of GH may accelerate aging and increase the risk of cardio-vascular diseases.

Very small embryonic-like stem cells (VSELs) are a population of developmentally early stem cells residing in adult tissues (*Ratajczak MZ et al. Aging 2012;4:235-246)*. These rare cells, which are slightly smaller than red blood cells,

•become mobilized during stress situations into peripheral blood,
•are enriched in the Sca1+Lin–CD45– cell fraction in mice and the CD133+ Lin–CD45– cell fraction in humans,
•express markers of pluripotent stem cells (*e.g., Oct4, Nanog, and SSEA), and*
•*display a distinct morphology* characterized by a high nuclear/cytoplasmic ratio and undifferentiated chromatin.

The most recent data *in vivo* demonstrated that both murine and human VSELs exhibit some characteristics of long-term repopulating hematopoietic stem cells (LT-HSCs), are at the top of the hierarchy in the mesenchymal lineage, and may differentiate into organ-specific cells (e.g., cardiomyocytes). Moreover, as recently demonstrated the number of these cells positively correlates in several murine models with longevity. VSELs have the potential role in aging and organ rejuvenation and number of these cells decreases in experimental animals after prolonged GH or IGF-1 administration. (*Kucia M et al. Age 2013;35:315-30*).

Objective

The aim of the study was to analyze the effect of rhGH treatment on VSELs in human patient subjects.

Methods

Patients

Twenty five patients after qualification to rhGH treatment, ten with GH-deficiency, three with Turner Syndrome and two with Prader-Willi Syndrome in the age of 5.04-13.37 years, mean 9.1±2.7 years were included in the study. The Local Ethical Committee approved the study. All the parents of the patients gave informed, written consents.

The mean GH dose was 0.27 mg/kg/week.

Material

Fasting blood samples for measurement of blood count and desired parameters were taken before the introduction of GH treatment, and then two weeks, one month, three months and six months after it.

Methods

Subsequently, we evaluated by employing FACS changes in the number of circulating in peripheral blood (PB) small $CD133^+Lin^-CD45^-$ VSELs and $CD34^+Lin^-CD45^-$ VSELs - that are precursors of long term repopulating hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs).

Statistical analysis

Statistical analysis was performed ANOVA with post-hoc Turkey test. P< 0.05 was considered as statistically significant.

Results

We found that the administration of rhGH initially stimulated for a month an increase in number of VSELs circulating in PB, and subsequently the number of VSELs decreased [Fig 1-4]. After six months of treatment the number of circulating in PB VSELs was lower as compared to baseline values.

The increase in VSELs number paralleled with increase in number of circulating MSCs and EPCs, however two months shift has been observed [Figure 5 and 6]. The highest level of EPCs was observed after three months of treatment [Figure 4]. Finally, the number of MSCs and EPCs become lower than before GH treatment.

The changes in 34+VSELs/34+HSCs during rhGH treatment were significantly positive [Table 1].

Table 1.
The mean data of VSELs, hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs) and endothelial progenotor cells (EPCs) per micro liter of blood .

	Before GH-therapy	After 2 weeks	After 1 month	After 3 months	After 6 months	P
34+VSELs/ 34+ HSCs	0.152/1.225	0.154/0.986	0.222/1.282	0.174/1.162	0.136/0.831	0.026 /0.237
133+VSELs/ 133+HSCs	0.090/1.043	0.087/0.847	0.102/1.099	0.084/0.914	0.070/0.855	0.722/ 0.502
MSCs	0.389	0.247	0.396	0.307	0.250	0.173
EPCs	0.238	0.282	0.253	0.314	0.103	0.284

Figure 1
Average VSELs 34+ before rhGH treatment (1), 2 weeks (2), one month (3), three months (4), and six months after rhGH treatment introduction (5).

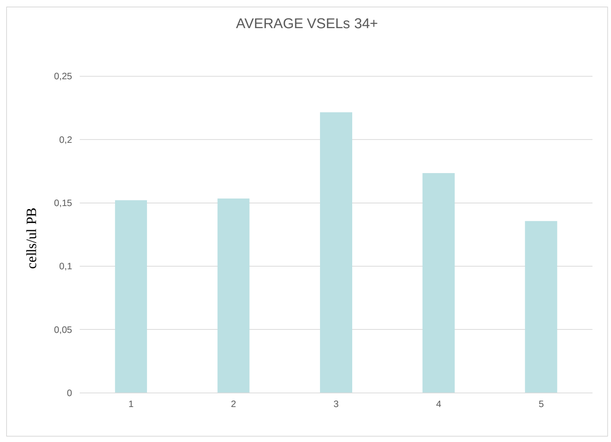
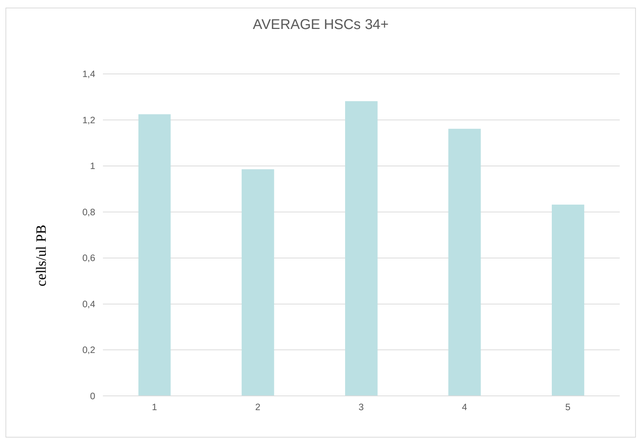


Figure 2
Average HSCs 34+ before rhGH treatment (1), 2 weeks (2), one month (3), three months (4), and six months after rhGH treatment introduction (5).



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Results

Figure 3
Average VSELs 133+ before rhGH treatment (1), 2 weeks (2), one month (3), three month s(4), and six months after rhGH treatment introduction (5).

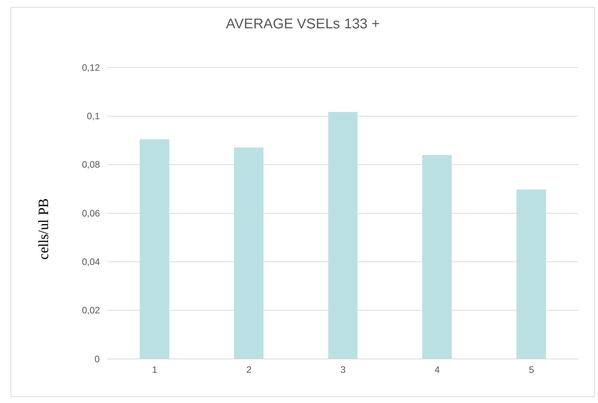


Figure 4
Average HSCs 133+ before rhGH treatment (1), 2 weeks (2), one month (3), three months (4), and six months after rhGH treatment introduction (5).

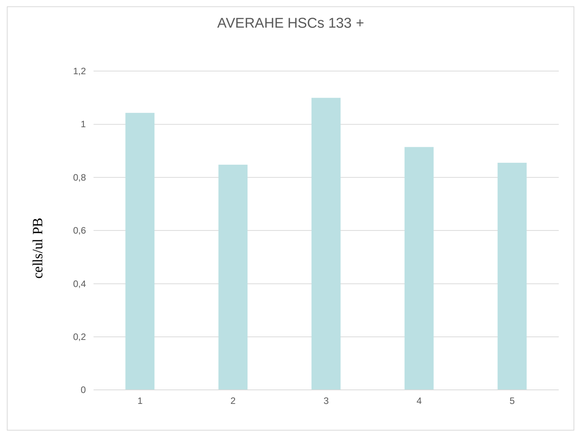


Figure 5
Average EPCs before rhGH treatment (1), 2 weeks (2), one month (3), three months (4), and 6sixmonths after rhGH treatment introduction (5).

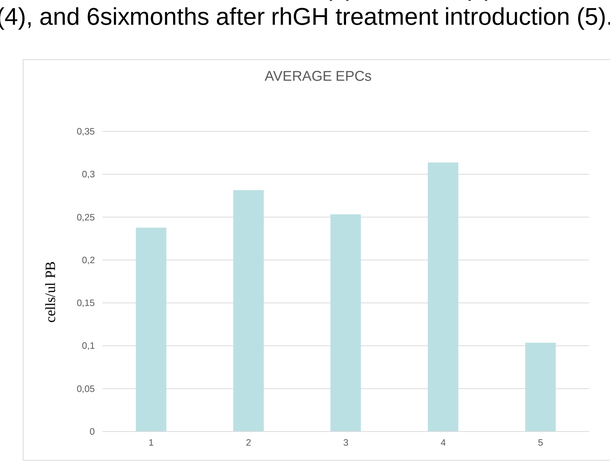
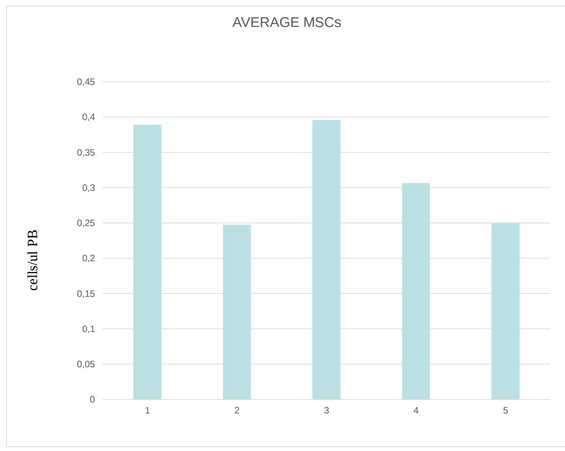


Figure 6
Average MSCs before rhGH treatment (1), 2 weeks (2), one month (3), three months (4), and six months after rhGH treatment introduction (5).



Conclusions

The prolonged treatment with rhGH modulates the population of VSELs, MSCs and EPCs circulating in PB.
Our data suggests that:
1/ VSELs respond to GH treatment, and
2/ since the therapy with rhGH modulates population of VSELs, therefore it could similarly as in experimental animals influence life span and organ rejuvenation in patients.

Acknowledgements

This study was supported by a grant „harmonia" nr UMO-2014/14/M/NZ3/00475, from National Science Centre.

55ESPE 600--P1
Growth
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DOI: 10.3252/pso.eu.55ESPE.2016
Poster presented at: ESPE 2016 PARIS