

He C¹, Jing J¹, Zhang L¹, Zhou X¹, Yang S¹, Xu Y¹, Hu M², Wu M¹

Certain Unknown Effects on the GH Isoform Ratios

National Anti-Doping Laboratory, CHINADA, Beijing, China¹; Department of Endocrinology, Peking Union Medical College Hospital, Peking Union Medical College, Beijing, China²

Abstract

To investigate the effects on human growth hormone (GH) isoform ratios measured by World Anti-Doping Agency (WADA) approved Kits, we analyzed the serum samples collected from volunteers of excretion study, athletes of routine anti-doping tests and patients anonymously provided by the Hospitals. All serums were analyzed with the WADA approved Kits following WADA Guideline. We found that 1) the reactions of RecGH_1 from 53 pregnant women were competitively inhibited by some substances, 2) the situation of human placental lactogen (HPL) spiking test was consistent with the cross reactivity of the antibodies, 3) the analysis of the serum samples from acromegaly patients under the Glucose Tolerance Test indicated that the ratios may be effected by certain pathological conditions, 4) one of the 25 volunteers in our excretion study in 2010 showed that the Ratio_1 was much higher than Ratio_2 about 4 times, but Ratio_1 reanalyzed 2 years later was much lower than before maybe due to the long term storage or the different batches of Kit_1 or something else. Further investigation need to be implemented.

Introduction

The isoform differential immunoassays for the detection of human growth hormone [1,2] have been approved by WADA since 2008. There are several reports about the influences on this method [1,3-8]. Till now we have analyzed thousands of serum samples from Chinese people and found that the GH isoform ratios might be affected by some overt or covert factors. This study was carried out to investigate the effects on GH isoform ratios measured by WADA approved Kits.

Experimental

The serum samples were collected following WADA requirements for GH test from volunteers of excretion study [4] or from routine anti-doping tests, among which serum samples were spiked with NIBSC standard of human placental lactogen (HPL) with the concentration close to 16 to 20 weeks pregnant women. Other serum samples from a pituitary tumor patient, 16 to 20 weeks pregnant women and acromegaly patients under the Glucose Tolerance Test were anonymously provided by the Hospitals. 5 serum samples were collected at 0, 30, 60, 120, and 180 minutes respectively for each acromegaly patient during the Glucose Tolerance Test. All serums were stored in frozen (-20 °C) before analyzed with the WADA approved Kits following WADA Guideline [2]. The WADA approved Kits consist of two parts: Kit_1 and Kit_2. Each kit consists of a test for RecGH (RecGH_1 for Kit_1, RecGH_2 for Kit_2) and a test for PitGH (PitGH_1 for Kit_1, PitGH_2 for Kit_2). The RecGH_1 to PitGH_1 ratios (Ratio_1) and the RecGH_2 to PitGH_2 ratios (Ratio_2) are calculated. Statistics were carried out with SPSS and/or Microsoft Office Excel.

Results and Discussion

1) The concentrations of RecGH₁ were significantly lower than RecGH₂ (P<0.01). Consequently, the isoform ratios of the GH from 53 pregnant women (16 - 20 weeks) measured by Kit₁ were about one tenth of that measured by Kit₂ (Table 1). It indicated that the reactions of RecGH₁ were competitively inhibited by certain substances existing in the sera of pregnant women and might have cross reaction with the antibodies of Kit₁.

	RecGH ₁ (ng/mL)	PitGH ₁ (ng/mL)	RecGH ₂ (ng/mL)	PitGH ₂ (ng/mL)	Ratio ₁	Ratio ₂
Mean	0.206	2.678	1.758	2.348	0.082	0.818
SD	0.175	2.326	1.248	1.913	0.023	0.170
CV%	85.3	86.9	71.0	81.5	28.5	20.7
Max	0.819	10.461	6.597	9.665	0.135	1.355
Min	0.050	0.476	0.483	0.470	0.048	0.578

Table 1: Data measured from the serum samples of 53 pregnant women.

2) The Ratio₁ values of 15 serum samples spiked with HPL samples were also significantly lower than Ratio₂ (Table 2a), while the Ratio₁ of the same samples without spiking HPL were close to the corresponding Ratio₂ (Table 2b). It was consistent with the cross-activity of these antibodies.

a						
	RecGH ₁ (ng/mL)	PitGH ₁ (ng/mL)	RecGH ₂ (ng/mL)	PitGH ₂ (ng/mL)	Ratio ₁	Ratio ₂
Mean	0.927	3.730	2.874	4.124	0.268	0.692
SD	0.897	3.599	2.792	3.740	0.098	0.192
CV%	96.8	96.5	97.2	90.7	36.6	27.8
Max	3.053	10.763	9.074	11.668	0.460	1.115
Min	0.120	0.346	0.340	0.413	0.159	0.443

b						
	RecGH ₁ (ng/mL)	PitGH ₁ (ng/mL)	RecGH ₂ (ng/mL)	PitGH ₂ (ng/mL)	Ratio ₁	Ratio ₂
Mean	2.542	4.543	2.624	4.472	0.566	0.595
SD	2.617	4.366	2.608	4.246	0.164	0.167
CV%	102.9	96.1	99.4	94.9	28.9	28.2
Max	8.044	13.132	8.318	12.974	0.928	0.927
Min	0.241	0.348	0.272	0.345	0.339	0.387

Table 2: Cross-reactivity with HPL. a. Data measured from 15 serum samples spiked with 1.7mg/L HPL. b. Data measured from 15 serum samples without spiking HPL.

3) The analysis of the serum samples from acromegaly patients under the Glucose Tolerance Test showed both the Ratio₁ and Ratio₂ were stable among 4 samples collected during 3 hours of the test, and were higher than the values from the healthy volunteers of the excretion study without rhGH injection (Figure 1). One clinical case showed that the ratios descended and tended to the normal human range after an operation of a pituitary tumors surgery. The ratios measured by the WADA approved Kits may be influenced by certain pathological conditions. But no samples were decided as positives according to WADA Guidelines [2] (Figure 2).

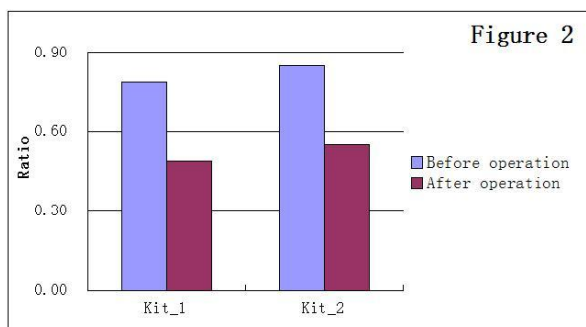
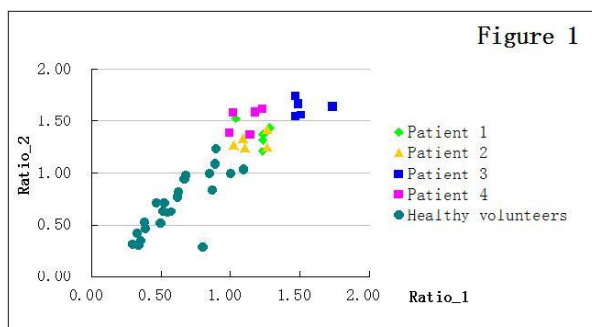


Figure 1: The GH isoform ratios of the serum samples from 4 acromegaly patients each of whom provided 5 samples and from 25 healthy volunteers without rhGH injection. Figure 2: Comparison for the GH isoform ratios of the serum samples from 1 pituitary tumor patient before and after an operation.

4) One of the 25 volunteers in our excretion study in 2010 showed that the ratios of his serum samples measured by Kit_1 were about 4-5 times higher than that measured by Kit_2, because the concentrations of PitGH_1 were significantly lower than PitGH_2. But when the samples were reanalyzed 2 years later, it can be clearly seen that there was no difference between the ratio_2 and significant difference between ratio_1 measured in two years. (Figure 3) It might be due to the long term storage or the different batches of Kit_1 or something else. While there was no difference between both ratio_1 and ratio_2 in two years, when other volunteers' samples were reanalyzed. Two years later, this volunteer was again administered with one dose of rhGH under the same conditions as before. The Ratio_1 were about 2 times of Ratio_2 (Figure 4).

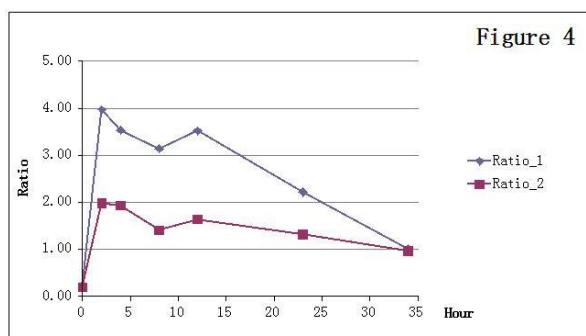
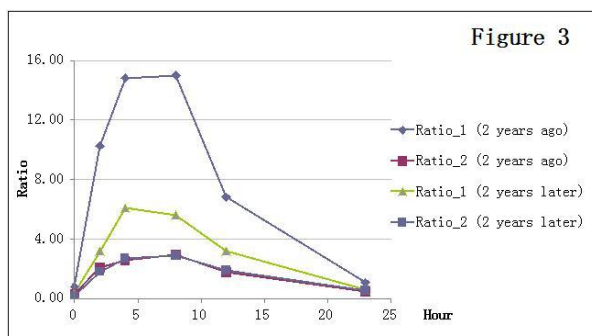


Figure 3: The changing of the GH isoform ratios in 2 years. Figure 4: The kinetic curve of the GH isoform ratios after one dose injection.

Conclusions

Several factors observed in this study may influence the isoform ratios of the GH, but they have never led to false positives using two Kits with two different antibodies. However, there are still some unknown effects on the ratio, and further investigation need to be implemented.

References

[1] Bidlingmaier M, Suhr J, Ernst A, Wu Z, Keller A, Strasburger C, Bergmann A. (2009) High-sensitivity chemiluminescence immunoassays for detection of growth hormone doping in sports. *Clin Chem.* **55**(3):445-453.

- [2] World Anti-Doping Agency. Guidelines for hGH isoform differential immunoassays, June 2010, Version 1.0 (2010); http://www.wada-ama.org/Documents/Resources/Guidelines/WADA_Guidelines_hGH_Differential_Immunoassays_EN_June10.pdf
- [3] Yang S, He C, Dong Y, Wang S, Xu Y, Wu M, Jin Z, Wu Q, Zhu H, Pan H, Gong F, Gu F. (2009) The use of "Kit A" for the Detection of rhGH Doping. In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) *Recent Advances in Doping Analysis* **17**, Köln, pp 117-125.
- [4] Jing J, Yang S, Zhou X, He C, Zhang L, Xu Y, Xie M, Yan Y, Su H, Wu M. (2011) Detection of Doping with rhGH - Excretion Study with WADA Approved Kits. *Drug Test Anal* **11-12**, 784-790.
- [5] Kohler M, Gotzmann A, Görgens C, Flenker U, Thevis M, Schänzer W. (2010) Comparison of hGH serum concentrations of Caucasian and African top athletes: an ethnicity study. In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) *Recent Advances in Doping Analysis* **18**, Köln, pp 196-199
- [6] M. Okano, Y. Nishitani, M. Sato, A. Ikekita, S. Kageyama. (2010) Influence of intravenous administration of growth hormone releasing peptide-2 (GHRP-2) on detection of growth hormone doping: growth hormone isoform profiles in Japanese male subjects *Drug Test Anal* **2**(11-12): 548-556.
- [7] P. Kaliszewski, D. Michalak, A. Pokrywka, D. Kwiatkowska. (2009) Validation of Differential Immunoassays of hGH Isoforms; KIT 1 and 2. In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) *Recent Advances in Doping Analysis* **17**, Köln, pp 297-300.
- [8] M. Okano, Y. Nishitani, M. Sato, S. Kageyama. (2012) Effectiveness of GH isoform differential immunoassay for detecting rhGH doping on application of various growth factors. *Drug Test Anal* **4**(9): 692-700.

Acknowledgements

We give special thanks to Prof. Dr. Jin Zimeng at the department of endocrinology of Peking Union Hospital for his valuable consultation, to Shanghai United Cell Biotechnology CO.Ltd for the donation of rhGH preparations.