

hGH isoform profiles in Japanese male subject –Reference population, rhGH administration and influence of GH secretagogue–

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Abstract

Administration of exogenous 22-kDa recombinant human growth hormone (rhGH) suppresses the pituitary growth hormone (GH) secretion by negative feedback; then, the elevated 22-kDa GH to non-22 kDa GH ratio (Rec/Pit ratio) can be utilized to detect doping with rhGH (isoform differential immunoassay) and the method is currently approved by the WADA. A synthetic hexapeptide GHRP-2 (pralmorelin) representing growth hormone secretagogues has been used for diagnostic tests of GH deficiency in Japan. Several dietary supplements are available on the Internet, and GHRP-2 has been identified in the supplements. The influence of intravenous administration of growth hormone releasing peptide GHRP-2 on the isoform differential immunoassay for detecting rhGH doping has been investigated. In this study, Japanese reference population (n=100) was used, with 0.04 mg/kg rhGH subcutaneous administration (n=5), 100 µg of GHRP-2 intravenous administration (n=10) and 0.04mg/kg rhGH combined with 100 µg of GHRP-2 (n=10) in Japanese male subjects. All the subjects are different groups. The results indicated that the low dose (0.04mg/kg) of rhGH led to significantly increased Rec/Pit ratio compared with the Japanese reference limit ($P<0.001$). Because GHRP-2 dose led to increases in concentrations of the 22-kDa GH form and all other pituitary GH forms, no significant change in the Rec/Pit ratio was observed ($P>0.05$). In a combined administration study, after GHRP-2 dose the Rec/Pit ratios decreased to 39.9–43.9% compared with the elevated ratio caused by the rhGH dose. On the basis of these findings, we concluded that the isoform differential immunoassay is a highly sensitive and effective method for detection of rhGH doping in Japanese subjects; however, GHRP-2 administration cannot only be detected by the isoform differential immunoassay but also masks rhGH doping. The analysis of GHRP-2 was found to be

suitable for compensating for the disadvantages of the isoform differential immunoassay because GHRP-2 and its metabolite D-Ala-D-(β -naphthyl)-Ala-Ala-OH (AA-3) in urine could be detected during the periods of masking of the Rec/Pit ratio by means of liquid chromatography/tandem mass spectrometry.

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