

Neutralizing Effects of Antiserum by Repeated Subcutaneous Administration of Recombinant Human Growth Hormone (rhGH)

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Abstract – Human growth hormone (hGH) forms antibody by repeated administration. The present study investigated to confirm formation of antibody by repeated subcutaneous administration of hGH for two months in rats and dogs. In this result, hGH-injected sera were significantly higher than control sera by 1 : 1,000,000 of dilution factor. After antibody formed sera (anti-hGH sera) and control sera were added to 30 µg/ml hGH, the complex incubated for overnight at 37°C. Anti-hGH sera decreased hGH contents about 90% compared to control sera. Also, body weight gain conducted decreased about 67% compared to control sera in hypophysectomised rat. In conclusion, repeated administration of hGH formed antibody because hGH was foreign protein to rats and dogs. And formed antibody of hGH was blocked and decreased many efficacy of hGH, the antibody was proved to be neutralizing antibody. Thus, because neutralizing antibodies were decreased pharmacological effects of hGH, administration more than two months were no significance.

Keywords □ Human growth hormone (hGH), antibody, neutralizing activity, hypophysectomised rat, body weight gain

Growth hormone (GH) is the major growth-promoting hormone in postnatal life (Henrik *et al.*, 1999). GH plays an important role in metabolism and stimulates many biological activities, including linear growth, lactation, activation of macrophages and muscle protein metabolism (Makower *et al.*, 1989; Chawla *et al.*, 1983; Edwards *et al.*, 1988). GH promotes longitudinal bone growth mainly through activation of insulin-like growth factor I (IGF-I). Human GH (hGH) has been available over 40 years for the treatment of children with GH deficiency. Early treatment and optimization of GH dose are achieved near normal final height (De Muinck Keizer-Schrama and Rikken, 1992, Fraiser, 1983).

Human proteins may be antigenic in animals and cause formation of antibodies. The antibodies decreased circulating IGF-I level, body weight, tibia weight, tibia length and bone width at mid-shaft (Dorchy *et al.*, 1989; Palmer *et al.*, 1994). Antibody against a drug (human proteins) changed the pharmacokinetic parameter (Rehlaender and Cho, 1998). Antibodies blocked the therapeutic activity of drugs (Fraiser, 1983).

The purpose of the present study is to investigate antibody formation against hGH by the repeated administration of hGH in rats and dogs, and to evaluate neutralizing effects of the anti-

hGH sera measuring body weight gain. To neutralizing effects of anti-hGH sera was carried out body weight gain for 4 days in hypophysectomised rats after hGH (30 µg) added in anti-hGH serum and incubated on 37°C for overnight. The hGH anti-hGH serum complex measured hGH contents. If neutralizing antibody proved to decrease efficacy of hGH, administration periods of repeated toxicity will have to terminate.

MATERIALS and METHODS

Materials

Recombinant human growth hormone (hGH, Code no.: DWP412) was obtained from Institute of Bioscience & Biotechnology, Daewoong Pharm. Co. (Yongin, Korea).

Bovine serum albumin (BSA), phosphate buffer saline (PBS), tween 20, anti-dog IgG peroxidase, anti-rat IgG peroxidase, 3,3',5,5'-tetramethylbenzidine (TMB), human serum albumin were purchased from Sigma Chemical Co. (USA). 96 well EIA/RIA plates (High binding) were purchased from Corstar (USA).

Human growth hormone administration

Repeated subcutaneous administration was performed at Korea Research Institute of Chemical Technology.

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Ten Male and ten female Sprague-Dawley (SD) rats were injected hGH subcutaneously (s.c.) at doses of 0.4, 2, 10 IU/kg once daily for two months. And three male and three female beagle dogs were administered once daily for two months as s.c. At doses of hGH were 0.24, 1.2, 6 IU/kg. Control group were injected vehicle in rats and dogs. After administration, blood samples were collected, kept overnight at 4°C, centrifuged at 3000 rpm for 15 min and serum was collected for measurement of antibody titer and neutralizing activity of the antibody.

Antibody titer in serum

The antibody titer in sera was measured by an ELISA as described previously (Answer *et al.*, 1998; Farrington *et al.*, 1987). The hGH (1 µg/ml) was absorbed on high binding 96 well plates for overnight at 4°C. After washing plates with 0.5% tween 20/PBS (PBS-T), 3% BSA/PBS-T were incubated for 1 hr at room temperature. After washing, serial diluted serum samples were added to the well plates, and incubated for 1 hr at room temperature. The antigen-antibody complex was recognized by anti-dog IgG peroxidase, anti-rat IgG peroxidase. The activity of absorbed peroxidase was measured by adding TMB solution. After 20 min, the absorbance was determined at 595 nm.

Neutralizing activity

Experimental groups were as follow. **Group I:** 0.25% human albumin/saline (diluent), **Group II:** 30µg hGH dissolved in diluent, **Group III:** 30 µg hGH dissolved in 1 : 4 ratio of diluent: control sera (control group in repeated administration) of rats, **Group IV:** 30 µg hGH dissolved in 1 : 4 ratio of diluent: anti-hGH sera(0.4, 2, 10 IU/kg groups of hGH in repeated administration) of rats, **Group V:** 30µg hGH dissolved in 1 : 4 ratio of diluent : control sera (control group in repeated administration) of beagle dogs, **Group VI:** 30 µg hGH dissolved in 1 : 4 ratio of diluent : anti-hGH sera (0.24, 1.2, 6 IU/kg groups of hGH in repeated administration) of beagle dogs.

Group II~Group VI were mixed by 30 µg hGH per ml (diluent, sera mixture). All groups were incubated for overnight at 37°C. And then, hGH contents were measured, and *in vivo* bioassay was conducted in hypophysectomized rats.

Determination of hGH contents

After incubation for overnight, hGH contents of each groups were detected by using hGH ELIZA kit (Medicorp, Canada).

In vivo bioassay

The experimental procedures were followed the *in vivo* bioassay (weight gain) of somatotropin in European Pharmacopoeia (1987).

Male hypophysectomized rats (HYPOX rat; 85-105 g, SLC, Japan) were weighted for 7 days and used in these studies if they changed less than 7 g. HYPOX rats were divided by 6 per group. HYPOX rats were injected subcutaneously into the neck twice daily for 4 days with 0.5 ml of experimental groups (above in Neutralizing activity). Body weights were measured every day, and 16 h after last injection.

RESULTS

Antibody formation

Sera of rat and dog were diluted at the rate tenfold serially to concentration ranging from 1 : 10 (log scale = 1) to 1:

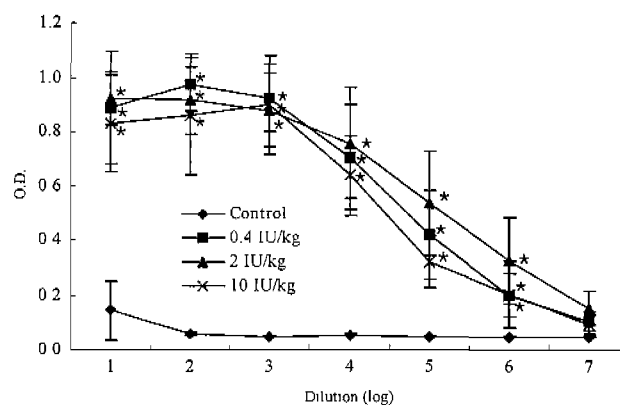


Fig. 1. Anti-hGH sera titer in male rats after s.c. administration of hGH for two months. Each value represents the mean \pm S.D. (n=10). *Significantly different from control group ($p < 0.01$)

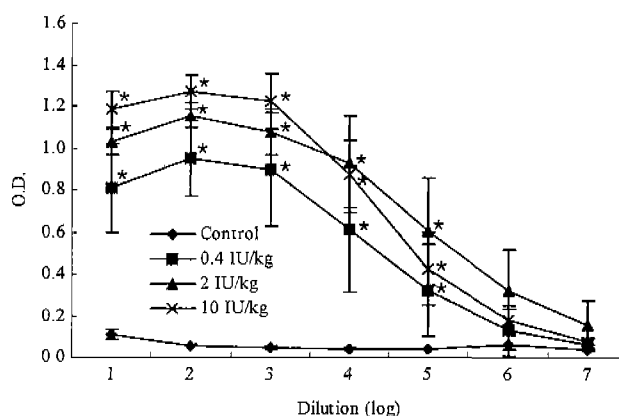


Fig. 2. Anti-hGH sera titer in female rats after s.c. administration of hGH for two months. Each value represents the mean \pm S.D. (n=10). *Significantly different from Control group ($p < 0.01$).

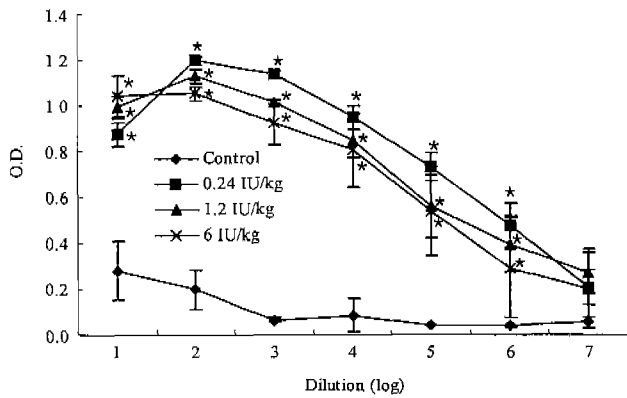


Fig. 3. Anti-hGH sera titer in male dogs after s.c. administration of hGH for two months. Each value represents the mean \pm S.D. (n=3). *Significantly different from Control group ($p < 0.01$).

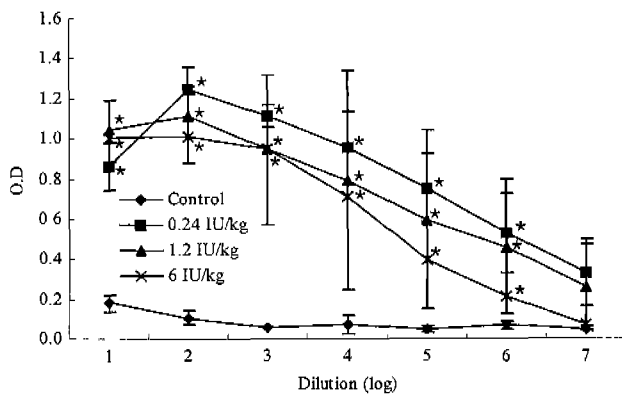


Fig. 4. Anti-hGH sera titer in female dogs after s.c. administration of hGH for two months. Each value represents the mean \pm S.D. (n=3). *Significantly different from Control group ($p < 0.01$).

10,000,000 (log scale = 7) in 3% BSA/PBS-T.

In male and female rats, during treatment for 2 months of doses (control, 0.4, 2, 10 IU/kg), the anti-hGH sera titer significantly increased compared to control sera at 0.4, 2, 10 IU/kg. And positive ratio was significantly formatted to 1 : 1,000,000 of dilution factor in male rats (Fig. 1), to 1 : 100,000 of dilution factor in female rats (Fig. 2). But dose-response relationship was not observed for either the incidence or the magnitudes of responses.

In male and female dogs, during injection for two months of doses (control, 0.24, 1.2, 6 IU/kg), the anti-hGH sera titer significantly increased compared to control sera at all treatment of hGH. And positive ratio was significantly formed to 1 : 1000,000 of dilution factor in male and female dogs (Fig. 3, 4). And dose-response relationships were not observed.

Neutralizing activity

hGH content

hGH content of experimental groups (above Neutralizing

activity) were measured after incubation for overnight at 37°C. In Group II, III and IV, hGH content of Group IV was decreased about 95% compared to Group II and III. In Group II, V and VI, hGH contents of Group VI was decreased about 90% compared to contents of Group II and V (Fig. 5).

Weight gain assay

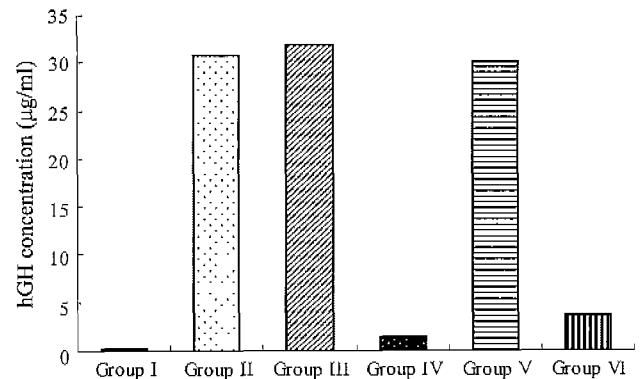


Fig. 5. Mean of hGH contents measured after all experimental group incubated for overnight (30 μ g hGH / ml all experimental group). Group I: Only diluent, Group II: 30 μ g hGH / diluent, Group III: 30 μ g hGH / diluent: control sera (4:1) of rats, Group IV: 30 μ g hGH / diluent: anti-hGH sera (4:1) of rats, Group V: 30 μ g hGH / diluent: control sera (4:1) of dogs, Group VI: 30 μ g hGH / diluent: anti-hGH sera (4:1) of dogs.

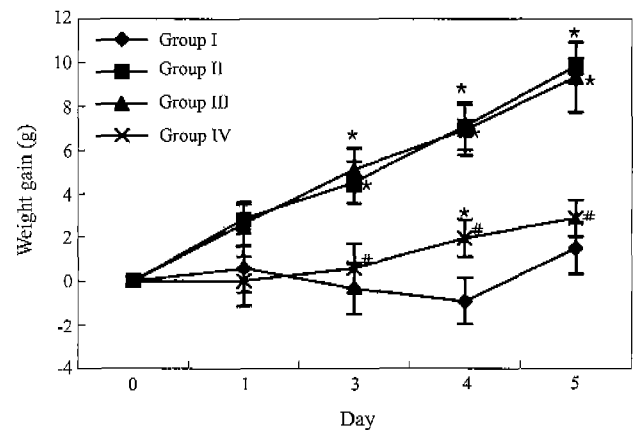


Fig. 6. Effects of experimental group (group I, II, III, IV) treatment on body weight gain. HYPOX rats were injected twice daily experimental groups for 4 days. Group I: Only diluent, Group II: 30 μ g hGH / diluent, Group III: 30 μ g hGH / 1:4 ratio of diluent: control sera of rats, Group IV: 30 μ g hGH / 1:4 ratio of diluent: anti-hGH sera of rats, *Group II, III, IV were significantly increased different from Group I ($p < 0.01$), #Group IV was significantly decreased different from Group III ($p < 0.01$).

After incubation of all experimental groups, body weight changes of HYPOX rats during the study were expressed Fig. 6, 7. The body weight gain tended to decrease with Group IV

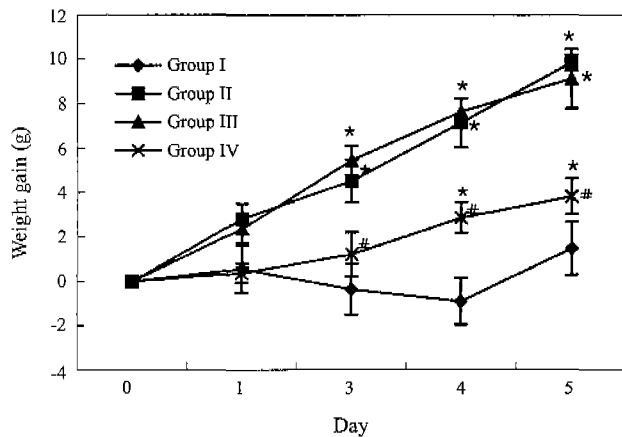


Fig. 7. Effects of experimental group (group I, II, V, VI) treatment on body weight gain. HYPOX rats were injected twice daily experimental groups for 4 days.

Group I: Only diluent, Group II: 30 μ g hGH / diluent, Group V: 30 μ g hGH / 1:4 ratio of diluent : control sera of beagle dogs, Group VI: 30 μ g hGH / 1:4 ratio of diluent : anti-hGH sera of beagle dogs, *Group II, V, VI were significantly increased different from Group I ($p < 0.01$), #Group VI was significantly decreased different from Group V ($p < 0.01$)

and VI compared to Group II, III and V. The mean body weight gain per day of Group II, III and V was increased about 3 ± 1 g. And Group IV and VI were increased about 1 ± 0.5 g. The mean weight gain during 4 days for Group IV and VI were suppressed about 67% compared to Group II, III and V.

DISCUSSIONS

This present studies described antibody formation and neutralizing effects of the antibody after repeated subcutaneous administration of hGH in rats and dogs. And then, to reduce administration period of repeated toxicity have a purpose of this studies

In male and female rats, antibody titer in anti- hGH sera were significantly higher than control sera by 1:1,000,000, 1:100,000 of dilution factor. Also, antibody titer of male and female dogs was significantly increased by 1:1,000,000 of dilution factor. This finding was predictable because hGH was foreign protein to rats and dogs.

In study, to see neutralizing activity of anti-hGH sera, the hGH contents of complex (above in Neutralizing activity) were decreased in anti-hGH sera compared to control sera. Anti-hGH sera of rat and dog decreased about 95% or 90% compared to control sera. Weight gain assay conducted in with the complex (above in Neutralizing activity) in hypophysectomised rats suppressed the weight gain about 60% compared to control sera in rats and dogs. This result was to prove that the antibod-

ies have neutralizing activity.

Hayashida and Contopoulos (1967) reported that biological activity of GH in the tibia assay could be neutralized by its specific antiserum, or by anti-pituitary serum (Duquesnoy and Good, 1970). Palmer *et al.* (1994) reported that anti-hGH significantly decreased tibial weight, length and width at mid-shaft. And A. Schurmann *et al.* (1995) reported that GH treatment increased spleen and thymus growth, but anti-hGH serum decreased. And long-term administration of anti-hGH serum reduced circulating IGF-I (Flint and Gardner 1989; Palmer *et al.* 1994; Schlemmer *et al.*, 1991; Mullis and Brickell, 1992), in other anti-hGH serum significantly reduced muscle weight and protein synthesis (Palmer *et al.* 1993). Thus it is possible that antiserum treatment reduce hGH effects. Gause *et al.* (1983) studied that antiserum had high capacity to neutralized GH *in vitro*. Thus antiserum neutralized the insulin-like effect of GH. The administration of antiserum *in vivo* would have the theoretical capacity of neutralizing GH. Antiserum treatment is effectively immunoneutralised endogenous GH, and then weight gain, organ growth and another function by GH was decreased. Fraiser (1983) reported that a small percentage of patients developed antibodies that blocked the therapeutic effective of the hGH.

In result of our studies, repeated injection of hGH in rats and dogs formed high concentration antibody, and this anti-GH serum decreased pharmacological effects of hGH. Therefore the antibodies in this anti-hGH serum were proved to be neutralizing antibody. Thus, repeated administration of hGH in rats and dogs were no significance to conduct more than two months.

In conclusion, neutralizing antibody was considered to be basis to terminate repeated administration of foreign protein including hGH in rats and dogs.

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