Effect of Twice Daily Administration of GH-releasing Peptide-2 for 10 Days on Growth Performance, Plasma GH Responses and Insulin-like Growth Factor-1 Concentrations in Swine

V. Nou, H. Inoue, H.G. Lee, N. Matsunaga, H. Kuwayama and H. Hidari*

Laboratory of Animal Production. Department of Animal Science. Obihiro University of Agriculture and Veterinary Medicine. Obihiro 080-8555, Japan

ABSTRACT : An increase in frequency of administration of exogenous growth hormone (GH) or GH-releasing hormone was reported to be a model to increase blood circulating insulin-like growth factor-1 (IGF-1) and to improve growth performance in animals. We have investigated the effect of twice daily administration of GH-releasing peptide-2 (GHRP-2) on growth performance, GH responsiveness and plasma insulin-like growth factor IGF-1 in swine. We administered to eight swine, 3 control and 5 treatment, a twice daily s.c. injections of GHRP-2 (30 μg/kg BW) for a period of 10 days. Every day blood samples immediately taken before injections of GHRP-2 or saline, at 08:00 h and 16:00 h, were measured for IGF-1 concentrations. Blood samples for GH assay were collected every 20 min on days 1, 6 and 10, from 1 hour before and 3 h after GHRP-2 or saline injections at 08:00 h. GH peak concentrations and GH area under curve (GH AUC) on day 1, 6 and 10 in treatment group of swine were higher than those in control swine ($p<0.05$). Twice daily administration of GHRP-2 caused a significantly attenuation ($p<0.05$) of GH peak concentrations (80.25±13.87, 39.73±5.72 and 27.57±6.06 ng/ml for day 1, 6 and 10, respectively) and GH AUCs (3,536.15±738.35, 1,310.31±203.55 and 934.37±208.99 ng/ml for day 1, 6 and 10, respectively). However, there was no significant difference in GH peak concentration and GH AUC between day 6 and 10. Plasma IGF-1 concentration levels were higher in treatment than control group of swine ($p<0.05$) after 3 days of the treatment, and the levels reached a plateau from day 3 to 10 of experiment. Growth performance did not alter by GHRP-2 administration, even though a numerical increase of body weight gain and feed efficiency was observed. These results indicate that twice daily administration of GHRP-2 for 10 days in swine did not significantly influence on growth performance, caused an overall attenuation of GH response, and that elevation of plasma GH concentrations caused by GHRP-2 administration increased plasma IGF-1 concentrations, even though an attenuation of GH response was observed. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 8 : 1193-1198)***

Key Words : GH, GHRP-2, IGF-1, Growth Performance, Swine

INTRODUCTION

Growth hormone releasing peptide-2 (GHRP-2) is a potent growth hormone (GH) secretagogue and its GH-releasing activity has been generally effective in various animals as well as human (Sawada et al., 1994; Wu et al., 1994; Bowers and Granda-Ayala, 2001). It has been suggested that GHRP-2 stimulates GH release in the following ways: by direct or indirect antagonism of somatostatin (SRIF), by releasing of hypothalamic GHRH, and by direct action on the pituitary *in vivo* (Sawada et al., 1994; Nakagawa et al., 1996; Hashimizume et al., 1998) and *in vitro* (Roh et al., 1997; Shimon et al., 1998). In addition, it has been suggested to act on different receptors of GHRH (GH-releasing hormone) (Wu et al., 1994; Roh et al., 1997), and has synergistic effect with GHRH on the release of endogenous GH (Sawada et al., 1994; Phung et al., 2001), which most of its somatotropic effects were primarily mediated by insulin-like growth factor-1 (IGF-1) (Daughaday et al., 1972). Administration of GHRP-2 increased plasma GH and IGF-1 concentrations in calves and women (Roh et al., 1996; Shah et al., 1999; Lee et al., 2000).

Twice daily or continuous administration of GHRP-2 has been reported to increase plasma IGF-1 concentrations in cows (Roh et al., 1996; Lee et al., 2000). However, once daily administration of GHRP-2 for 5 consecutive days had no effect on plasma IGF-1 concentrations in healthy young men (Nijland et al. 1998). In humans and rats, increases in frequency of GH administration had been reported to effectively generate an increase in plasma IGF-1 concentrations (Jorgensen et al., 1990, 1991) and promote growth (Jansson et al., 1982). In lambs, an increase in number of GHRH injections caused an increase in the area under curve of GH response (Kensinger et al., 1987). In swine, improved growth performance was positively related to increasing GH responses and IGF-1 concentrations after GH administration (Etherton et al., 1987; Sillence and Etherton, 1987). Moreover, increase in frequency of GHRH administration improved growth performance in swine (Dubreuil et al., 1990).

Recently, the GH-releasing activity of GHRP-2 was examined in swine. Once daily subcutaneous (s.c.) administration of GHRP-2, at a dose of 30 μg/kg BW, which increased GH response similar to a dose of 100

* Corresponding Author: H. Hidari. Tel: +81-155-49-5430, Fax: +81-155-49-5434, E-mail: hdr@obihiro.ac.jp

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μg/kg BW, for 30 days caused a partial attenuation of GH response between day 1 and 10, a tend toward an increase in the response between day 10 and 30, and improved growth performance after at least 20 days but not during 10 days after the administration; however, no information on the IGF-1 generation was reported (Phung et al., 2000). We hypothesized that increase in frequency of GHRP-2 administration would give rise to an early enhancement of growth performance. Therefore, The present study was to conduct to investigate the effect of twice daily s.c. administration of GHRP-2, at the same dose as described in swine above for a period of 10 days, on growth performance, GH responsiveness, and on the release of plasma IGF-1 concentrations in swine.

MATERIALS AND METHODS

Animals

Ten cross-breed (Landrace×Large white×Duroc) castrated male swine of the same littermates (150 days of age), weighing 78.5±1.2 kg, were used in this experiment. The swine were housed in individual pens for the entire experimental period and fed *ad libitum* twice daily at 08:00 h and 16:00 h on a commercial diet for growing swine (16% crude protein, 2.5% crude fat, 5% crude fiber and 7% crude ash). Animals had free access to water at all times. Three days before initiation of treatment, all animals were anesthetized and indwelling catheters were inserted (Phung et al., 2000). Experimental protocol and animal care procedures were approved by the Animal Care and Use Committee of Obihiro University.

GHRP-2 and method of administration

GHRP-2 was generously provided by Kaken Pharmaceutical Co. Ltd. Japan). The peptide was dissolved in 5 ml of saline (0.9% NaCl) the day before treatment and stored at 4°C for s.c. administration via an indwelling catheter. A dose of 30 μg/kg BW of GHRP-2 was used as it has been reported to maximally increase plasma GH response in swine (Phung et al., 2000). All swine were weighted every 5 days, and injecting quantities of GHRP-2 were corrected accordingly by their body weights.

Experimental design

Ten swine were allocated into two groups, control and treatment, of 5 each. Treatment group of swine received twice daily s.c. injections, on their neck, of GHRP-2 at a dose of 30 μg/kg BW at 08:00 h and 16: 00 h for a period of 10 days. Swine of control group were treated with equivalent volume of saline. Two swine of control group were taken from the experiment because of catheter displacement, so there were only 3 swine for control group. Twice daily blood sampling (2 ml) was conducted immediately before injections of saline or GHRP-2 at 08:00 h and 16:00 h for measurement of IGF-1 concentrations. In order to examine GH responsiveness to GHRP-2 with days of treatment, serial blood sampling was conducted, on day 1, 6 and 10 at intervals of 20 min, from one hour before to 3 hours after the injections of GHRP-2 or saline at 08:00 h. All blood samples were taken though indwelling jugular catheters into centrifuge tubes containing heparin (10 IU/ml) and chilled with ice. Individual plasma sample was obtained after centrifugation at 3,000 rpm 4°C and stored at -20°C until assayed for GH concentrations.

GH and IGF-1 Radioimmunoassay (RIA)

Plasma porcine growth hormone (pGH) concentration was measured by the double-antibody RIA procedure. Goat anti-monkey IgG serum (HAC-MKA2-02GTP88) was supplied by Biosignal Research Center Institute for Molecular and Cellular Regulation, Gunma University, Japan. Normal monkey serum was kindly provided by Primate Research Institute, Kyoto University, Japan. pGH antiserum (Lot AFP-10318545) and pGH (Lot AFP-10864) were obtained from Dr. A. F. Parlow (National Hormone and Pituitary Program, Harbor-UCLA Medical Center, Torrance, USA). pGH was used as a reference standard and radioiodinated by the chloramine-T method. The process of measuring plasma pGH concentration was conducted as previously described (Phung et al., 2000). Each plasma sample was run in duplicate. Sensitivity of assay was 0.1 ng/ml, and intra-assay and inter-assay coefficients of variation were 5.74% and 11.13%, respectively.

Plasma IGF-1 concentration was measured by double antibody RIA procedure with anti-rabbit hIGF-1 (NIDDK, lot AFP4892898) obtained from Dr. A. F. Parlow. hIGF-1 (Amersham, code ARM4010, lot 30) was used as standard, and radioiodinated by Chloramine-T method. Plasma sample was first extracted according to the method of Daughaday et al. (1980) and then the process of measurement was conducted as described by Roh et al. (1996). Each plasma sample was run in duplicate. Sensitivity and intra-assay were 0.76 ng/ml and 5.95%, respectively.

Statistical analysis

All data were expressed as the mean±SE. The area under the curve of GH response (GH AUC) was calculated using the trapezoidal method. Peak GH concentration was the highest concentration attained after injection. GH AUC after injections was corrected by minus the sum of GH AUC of samples basically taken at -60, -20 and 0 min. Differences in means of GH peaks and GH AUCs after administration of saline and GHRP-2 within groups of swine were analyzed by one-way analyzed of variance with General Linear Model (GLM) of the SAS program package

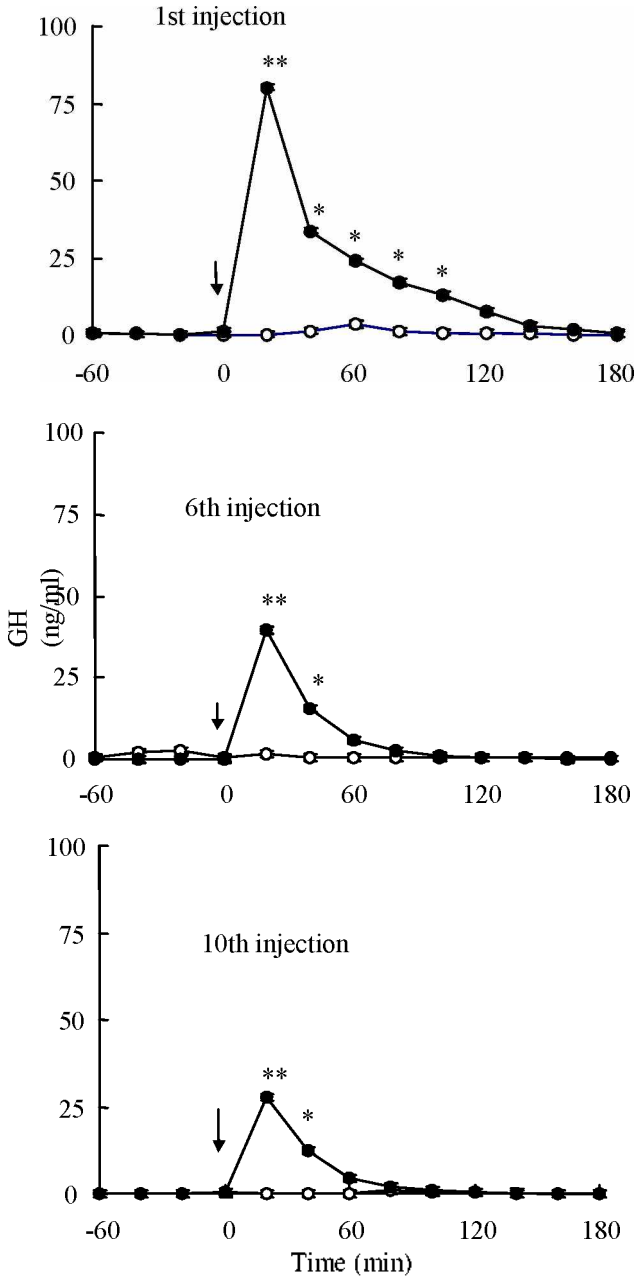

Figure 1. plasma GH concentrations on day 1, 6 and 10 after s.c. injections of saline (o) or 30 μg (•) of GHRP-2 (30 μg/kg BW) at 08:00 h.

Values are expressed as mean±SE of 3 swine for saline-treated and 5 swine GHRP-2-treated group. Arrow indicates time of saline or GHRP-2 injections.

** p<0.01, * p<0.05 significant difference in means between saline and GHRP-2-treated groups.

(SAS institute, Cary, NC) followed by Duncan's multiple range test. Means of plasma IGF-I concentrations, on each day of experiment, were the average of plasma IGF-1 concentrations of plasma samples taken at 8:00 h and 16:00 h. The differences in means of GH peaks, GH AUC responses and IGF-1 concentrations between groups were

Table 1. Effect of twice daily administration of GHRP-2 on Peak GH concentrations and GH area under curve (GH AUC)

Item	Control	Treatment
GH peak concentration (ng/ml)		
Day 1	3.99±2.69[a]	80.25±13.87**[,a]
Day 6	2.35±0.84[a]	39.73±5.72**[,b]
Day 10	3.66±1.45[a]	27.57±6.06**[,b]
GH AUC, ng/ml/min		
Day 1	139.54±97.42[a]	3,536.15±738.35*[,a]
Day 6	83.06±32.39[a]	1,310.31±203.55**[,b]
Day 10	146.34±71.94[a]	934.37±208.99*[,b]

Values are expressed as mean±SE for 3 swine and 5 swine for control and treatment, respectively.

* p<0.05; ** p<0.01 compared with corresponding control.

[a,b] Means in the same column with different superscript are significantly different (p<0.05).

analyzed by the student's t test. Values of p<0.05 were considered statistically significant.

RESULTS

GH peak and GH AUC responses to GHRP-2 injections at 08:00 h on day 1, 6 and 10 were shown in Figure 1 and Table 1. GHRP-2 administration significantly increased plasma GH concentrations throughout the experimental period. Plasma GH concentrations peaked within 20 min and returned to baseline levels between 120 and 150 min. The GH peak concentrations and GH AUCs under 180 min on day 1, 6 and 10 in treatment group were significantly higher than those in control group. In treatment group of swine, an attenuation of GH responses in GH peak concentration (80.25±13.87, 39.73±5.72 and 27.57±6.06 ng/ml for day 1, 6 and 10, respectively) and GH AUC (3,536.15±738.35, 1,310.31±203.55 and 934.37±208.99 ng/ml for day 1, 6 and 10, respectively) were observed with days of treatment. The GH response on day 1 was significantly higher (p<0.05) than that of day 6 and 10. However, there was no statistically difference in GH response between day 6 and 10, even though a numerically lower value of the response only 10 was observed.

Twice daily administration of GHRP-2 administration significantly increased plasma IGF-1 concentrations from day 3, but not on day 6 of treatment, through day 10 over the saline administration (Figure 2). Plasma IGF-1 concentrations started to rise from day 2 and reached a plateau from day 3 to 10 of experiment.

The data of growth rate, daily feed intake and feed efficiency during 10 days administration of saline or GHRP-2 were summarized in Table 2. No significant difference in growth parameters between groups of swine was observed. However, there were numerically higher values of body weight gain and feed efficiency in treatment group than that of control (23% for body weight gain and 25% for feed efficiency over control).

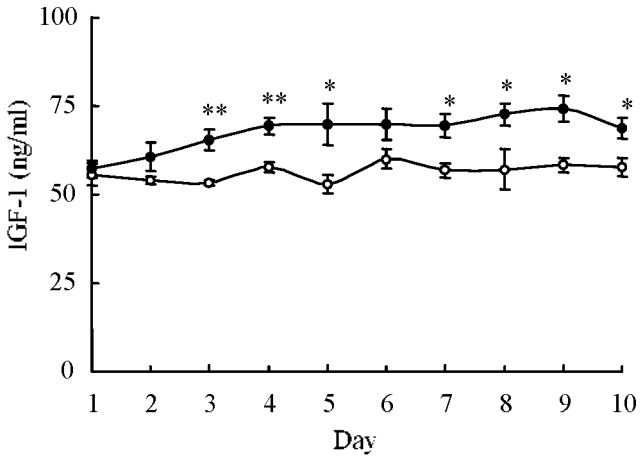

Figure 2. Daily profile of plasma IGF-1 concentrations after twice daily injections of saline (o) or 30 μg (•)/kg BW of GHRP-2. Values are mean±SE of 3 swine and 5 swine for control and treatment groups of swine, respectively.
**p<0.01, *p<0.05 significant difference in means between control and treatment groups.

DISCUSSION

It has been previously reported that increasing in frequency of GH or GHRH administration was a model to increase in GH response, blood circulating IGF-1 and in turn enhanced growth performance (Jansson et al., 1982; Kensinger et al., 1987; Jorgensen et al., 1990, 1991). Our present study is the first report in swine the effect of twice daily administration of GHRP-2 for a period of 10 days on the above described growth parameters.

In our present results, a partial attenuation of GH response to twice daily administration of GHRP-2 occurred, which was most pronounced between the day 1 and 10. Between day 6 and day 10, the decline in response was only small and not significant. This is similar to the report in healthy young men in which once daily administration of GHRP-2 for five days caused response attenuation between day 1 and 5, but not between day 3 and 5 (Nijland et al., 1998). However, in previous report from our laboratory, once daily administration of GHRP-2, at the same dose as in our study, for 30 days caused a partial attenuation of GH response between day 1 and 10, but a trend toward an increase in the response between day 10 and 30 was observed (Phung et al., 2000). This could be explained that after having reached a certain level, desensitization will not increase or that responsiveness to GHRP-2 is restored after long-term administration of GHRP-2. Therefore, the discrepancy might be depended on period or frequency of GHRP-2 administration. In contrast, in swine, infusion of porcine GHRH at 15 ng/kg/min BW for six days, or twice daily intravenous (iv) injections of porcine GHRH for 85

Table 2. Effect of twice daily administration of GHRP-2 for 10 days on growth performance in swine

Item	Control	Treatment
Body weight (kg)		
D1	77.73±3.78	78.72±0.60
D10	85.47±4.12	88.44±1.83
Body weight gain (kg/day)	0.77±0.18	1.00±0.09
Feed intake (kg/day)	3.65±0.27	3.52±0.10
Feed efficiency (gain/intake)	0.21±0.04	0.28±0.03

Values are expressed as mean±SE of 3 swine for control and 5 swine for treatment group. There is no significant difference in means between control and treatment groups.

days did not attenuate GH responsiveness (Dubreuil et al., 1990; 1991). GHRH is essential for the somatotrope proliferation (Ling et al., 1993), and absence of GHRH secretion by neonatal administration of monosodium glutamate, a model to specifically destroy hypothalamus GHRH neurons, decreased pituitary weights in rats (Kovacs et al., 2000). Even though GHRP-2 has a concomitant action on hypothalamus to release GHRH and on pituitary on the release of GH, whether which site is predominant on the release of GH is still controversial (Sawada et al., 1994; Nakagawa et al., 1996; Gondo et al., 2001).

Twice daily administration of GHRP-2 for 10 consecutive days increased plasma IGF-1concentrations, which started to significantly rise over saline group from day 3 of treatment. This present result is the first report on the effect of GHRP-2 on blood circulating IGF-1 in swine. The increase could be explained by the action of GHRP-2 in stimulating endogenous GH and in turn the secretion of IGF-1, and by the evidence of up-regulation of both hepatic GH-binding and mRNA abundance of IGF-1 and GH receptors after chronic GH administration in rats (Bick et al., 1992) and swine (Coleman et al., 1994; Brameld et al., 1996). It is similar to the reports, in which once daily injections for 6 days or infusion for 14 days of GHRP-2 increased plasma IGF-1 concentrations in cows (Roh et al., 1996; Lee et al., 2000). In contrast, once daily s.c. administration of GHRP-2 (100 μg/kg BW) for 5 days did not increase plasma IGF-1 concentrations, in spite of the persistent GH-releasing effect (Nijland et al., 1998).

Noticeably, in our results, the increase of plasma IGF-1 concentrations reached a plateau from day 3 to day 10, even though GH response attenuation was observed. These results could be explained that after GH-induced IGF-1 concentrations having reached a maximum level, it will not increase anymore by continuous administration of GHRP-2, and that the abundance of plasma IGF-1 concentrations cause feed-back mechanism down-regulating GH receptors, GH secretagogue activity and pituitary GHRH receptor gene expression (Min et al., 1996; Ghigo et al., 1999; Korytko and Cuttler, 2001). On the other hand, although we did not measure plasma cortisol and ACTH, an increase of

the two hormones in blood circulation by chronic administration of GHRP-2, as reported by Raun et al. (1998), would possibly reduce the effectiveness of GHRP-2-induced GH to stimulate IGF-1 production. Similar to this, Lee et al. (2000) reported that administration of GHRP-2 for 6 days significantly increased plasma IGF-1 concentrations from day 1 to day 3 and it returned to baseline control from day 4 to day 6 of treatment, although the GH response to GHRP-2 was constantly observed (Lee et al., 2000). Moreover, in the report of Vance et al. (1989), infusion of GHRH (10 ng/kg/min) for 14 days increases plasma IGF-1 concentrations from day 3 and it seems plateau until day 14 of treatment in GH-deficient boys. In contrast, continuous administration of GHRH increased the concentration of IGF-1 during period of treatment in swine and cows (Dubreuil et al., 1991; Vanderkool et al., 1995). In normal rats, infusion of GHRP-6 (100 μg/kg BW) for 14 days decreased plasma IGF-1 concentrations (Thomas et al., 2000). This discrepancy might be partly due to differences in GH secretagogues or animal species.

Increases in GH response by frequent administration of GHRH or GH was reported to improve growth performance in swine and hypophysectomized rats (Jansson et al., 1982; Dubreuil et al., 1990). Phung et al. (2000) reported that one daily s.c administration of GHRP-2, at the same dose used in our experiment, for 30 days increased significantly body weight gain, feed efficiency and average daily gain, even though there was no any information on the effect on IGF-1 generation. The same author also reported that no difference in growth performance of swine after 10 days in GHRP-2-treated over the saline-treated group was observed. In our present results, the significantly improve growth performance was not seen after twice daily administration of GHRP-2. However, the increase in IGF-1 concentrations might indicate a trend toward improvement of growth performance since we observed numerically higher of body weigh gain and feed efficiency over the control group. The more increase in frequency of GHRP-2 administration would significantly improve growth performance could not be excluded from our present study.

In summary, twice daily s.c. administration of GHRP-2 at dose of 30 μg/kg BW for a period of 10 days and caused a state of decreasing GH responsiveness in swine. The increase in endogenous GH secretion by GHRP-2 administration increased plasma IGF-1 concentrations. Plasma IGF-1 concentration started to significantly increase over the control swine from day 3 and it plateau till day 10 of treatment. However, growth performance was not significantly improved.

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