The Effects of Somatostatin Passive Immunization on Milk Yield, Plasma Hormone and Metabolite Concentrations in Rats

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ABSTRACT : The objectives of this study were to measure growth rate and endocrine changes and to improve milk production by somatostatin passive immunization in rat. Experimental animals were 10 weeks old 20 Sprague-Dawley rats. The rats were randomly assigned each 10 in control (normal sheep serum injected: NSS) and treatment (anti-somatostatin injected), and pre-fed for 2 weeks. Anti-somatostatin was purified from serum of 1 year old sheep after somatostatin active immunization, and was injected daily to rats, and growth rate and milk yield were measured for 14 days. Growth rate of litters was 2.15 g/d and 2.32 g/d in NSS and anti-somatostatin injected, respectively. Milk production was increased 6.2% in day 8 and 6.5% in day 12 by anti-somatostatin injection. Plasma growth hormone, insulin, glucose, and urea-N were increased, but non-esterified fatty acid was decreased by anti-somatostatin injection. In summary, passive immunization of somatostatin improved growth rate of litters and milk production in rats.

(**Kew Words**: Somatostatin, Passive Immunization, Milk Yield, Growth, Rat)

INTRODUCTION

Hormonal regulation of animal can be applied by an endocrine and exocrine control methods. Exogenous application could be carried out using natural or synthetic hormones, produced by recombinant DNA technology. Bovine somatotropin (bST) can be applied for animal production (Bauman et al., 1985; Myung, 1990). Growth hormone (GH), secreted from anterior pituitary gland and prevented by somatostatin (Davis et al., 1988), is involved in tissue metabolism, energy repartition, growth rate, and milk production (Aguilar et al., 1988). Several negative concepts such as animal safety, breaking homeostasis, trace in products, are prevent to use this products. The alternative method is an immunological approach to regulate endocrine system.

Somatostatin immunization will prevent somatostatin release and will stimulate GH secretion (Flint et al., 1991; Kuk and Myung, 1993). Also, it abolish somatostatin action of preventing insulin, glucagon, and TSH release. Somatostatin immunization, reported first by Spencer et al. (1983a,b), increased GH and IGF-I concentration in plasma, and improved growth efficiency, feed efficiency, and milk production (Kuk and Myung, 1993). These immunological approach has been performed in many animals. However, the active immunization method varies depending on animal, nutrition, and conjugate. Passive immunization will be an alternative method for improving animal production. This study was performed to test effects of somatostatin passive immunization on milk yield and growth efficiency in rats.

MATERIALS AND METHODS

Animals and Management

Twenty Sprague-Dawley rats (10 weeks old) were assigned randomly in treatment (anti-somatostatin injected) or control (normal sheep serum injected; NSS). Three rats were assigned randomly in shoes-box cage. Diet and water were fed *ad libitum*. Rats were mated at 12 weeks old (250 g BW). Litters were reassigned at groups of eight each after delivery for milk yield and growth performance measurement. Diet formula and composition were shown in table 1. This experiment was conducted in Animal Research Lab, Department of Animal Science, Chonnam National University. Environmental condition was maintained with respect to temperature (21 ± 2℃), humidity (60 ± 5%), and light (07:00-19:00 light; 19:00-07:00 dark).

Anti-somatostatin production

Preparation of the antigen and immunization method

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was achieved by the method previously described by Spencer et al. (1983a). One year old sheep was immunized with somatostatin and then serum was collected. Anti-somatostatin was purified and lyophilized. Somatostatin antibody titers in serum of sheep immunized against somatostatin were assessed by determining the antisera binding capacity for [^{125}I]-somatostatin-Tyr-1 at various dilution (1:100-100,000). The antibody titers were expressed as the dilution of the antibody which achieved 30% binding of [^{125}I]-somatostatin-Tyr-1 after deducting non-specific binding.

Table 1. Formula and chemical composition of experimental diet (DM basis)

Ingredients (%)	
Corn	55.5
Soybean meal	27.3
Fish meal	2.0
Tallow	12.2
Premix (vitamins & minerals)[1]	3.0
Chemical composition (%)	
Moisture	11.9
Crude protein	22.5
Crude fat	7.0
Nitrogen free extract	41.1
Crude ash	8.0
Calcium	0.7
Phosphorus	0.5
DE (kcal/kg)[2]	3,800

[1] Vitamin & mineral premix contain the following per kg diet. Vit. A, 5,000 IU; Vit. D, 1,000 IU; Vit. E, 36 mg; Vit. K, 0.06 mg; Pantothenate-HCl, 8 mg; Vit. B_{12}, 0.06 mg; Folacin, 1.2 mg; Choline chloride, 1,200 mg; $CaHPO_4$, 22.2 g; NaCl, 1.53 g; K_2SO_4, 6.70 g; MgO, 0.68 g; $FeSO_4 \cdot 7H_2O$, 0.20 g; $CuSO_4 \cdot 5H_2O$, 0.024 g; $MgSO_4$; KI, 0.005 g; $ZnCl_2$, 0.20 g.
[2] Calculated value.

Affinity for somatostatin by radioimmunoassay

The affinity of the anti-somatostatin was estimated by Scatchard plot analysis using the computer software package LIGAND (Munson, 1987). Data for the program were generated by homologous displacement of [^{125}I]-labelled somatostatin from the anti-somatostatin in a liquid-phase radioimmunoassay. In a final volume of 600 ul PBS, triplicate assay tubes contained no unlabelled somatostatin (B_0) or contained exponentially increasing quantities of unlabelled somatostatin (10^{-6}-10^{-12}M), [^{125}I]-labelled somatostatin (approximately 20,000 cpm) and anti-somatostatin stock solution at a final dilution of 1:10,000. Following a 24 hr incubation at 4℃, all tubes received 50 ul of a diluted commercially prepared suspension of bovine IgG. After 24 hr incubation at 4℃, 1.0 ml PBS was added just before separation of bound and free somatostatin by centrifugation at 2,000 g for 20 min. The tubes were then decanted and remaining radioactivity was determined with a gamma counter. Total count and non-specific binding (no anti-somatostatin) tubes were also included in the assay. For calculations, the total cpm added to each tube was adjusted to reflect the maximum binding affinity of the [^{125}I]-labelled somatostatin preparation.

Anti-somatostatin passive immunization

Maternal rats were daily injected anti-somatostatin (700 μg) based on Scatchard plot or normal sheep serum (NSS, 700 μg) for 14 days of lactation in the back or shoulder. The anti-somatostatin was dissolved in ammoniated water (pH 8.5).

Milk yield measurement

Milk yield was measured indirectly using litters (Sampson and Janson, 1984). The number of litters were reassigned each eight after delivery. Litters were separated for 3 hr from maternal rat and then nursed for 3 hr. Milk yield was calculated by the following equation; $Y = 0.0322 + 0.0667X_1 + 0.877X_2$ (Y = milk yield, g/d; X_1 = BW, g; X_2 = average daily gain, g/d).

Plasma hormone, metabolites and statistics

Growth hormone and insulin were analyzed using Coat-A-Count (Diagnostic Products Corporation). Glucose was analyzed using enzymatic GOD method (Glucose-E kit, Inwha Co., Korea). Urea-N was analyzed using Urease-Indophenol method (BUN Kit, Inwha, Korea). NEFA was analyzed using ACS-ACOD method (Enzymatic NEFA Kit, Youngyun Chemical Co., Japan). Statistical analysis of milk yield differences was made by t-test. All data were computed by using the General Linear Models procedure of the Statistical Analysis System (SAS, 1985).

RESULTS AND DISCUSSION

The Scatchard plot was shown in figure 1. The linear regression (r=0.937) between bound/free and bound is illustrated. From this plot, the K_d and B_{max} of anti-somatostatin was 0.92 μM and 0.29 μM, respectively. This low K_d value represents that anti-somatostatin has high specific affinity to somatostatin. According to Scatchard analysis, one ml of anti-sera can neutralize 1.64 μg of somatostatin. Total circulating somatostatin is

approximately 600pg in 350 g rat because endogenous somatostatin is approximately 30 pg/ml plasma. Also, half-life of somatostatin is approximately 1 min (personal communication with Dr. Spencer) and then 22.2 ng of somatostatin produced per day. Also tissue somatostatin is about ten times of plasma level. Therefore, total 244 ng of somatostatin should be neutralized in adult rat. Somatostatin production is varying depending on physiological condition of animal. Enough amount of anti-sera injection is required to get effective immunization. Therefore, 200 μl anti-sera is enough to neutralize all endogenous somatostatin in adult rat.

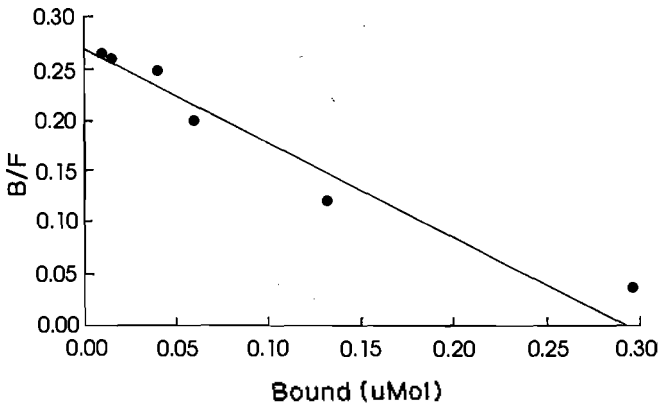

Figure 1. Scatchard analysis of [^{125}I]-somatostatin binding to anti-somatostatin. The ratio of bound/free (B/F) radioactivity is plotted against the total molar concentration of somatostatin bound to anti-somatostatin. Linear regression (r = 0.937) was used to determine the dissociation constant (slope = $-1/K_d$) and maximum density of bindings (X intercept).

Total birth weights of eight litters were 57.2 g in NSS and 58.4 g in anti-somatostatin group (figure 2). Body weight increased gradually during 14 days of lactation. Average daily gain (ADG) was 2.15g in NSS and 2.32g in somatostatin. Anti-somatostatin effect was appeared first day after immunization. Somatostatin immunization increased litter's body weight by 20%. Milk yield was increased by 6.2% at day 8 and 6.5% at day 12, respectively, in somatostatin passive immunization group (figure 3). Daily milk yield was 23.72 g in NSS and 24.84 g in anti-somatostatin injected. Milk yield was highest at day 8 of lactation (figure 3). Milk yield tended to be high throughout 14 days of lactation by somatostatin immunization. However, it was not significantly different except day 8 and 12. Increased growth rate of the young by somatostatin immunization has been reported in young pig (Osborne and Hacker, 1986), growing cattle (Flint et al., 1991), and lambs (Laarveld et al., 1986). This may be due to antibody transfer to litters by passive immunization. According to other reports, adrenocorticotropic hormone (ACTH) and insulin-like growth factor (IGF) may also have effects on growth rate. On the other hand, several reports could not seen these effects (Varner et al., 1980; Du and Hacker, 1992). These variation may relate with genotype, diet, nutrition, and species (Bass et al., 1987).

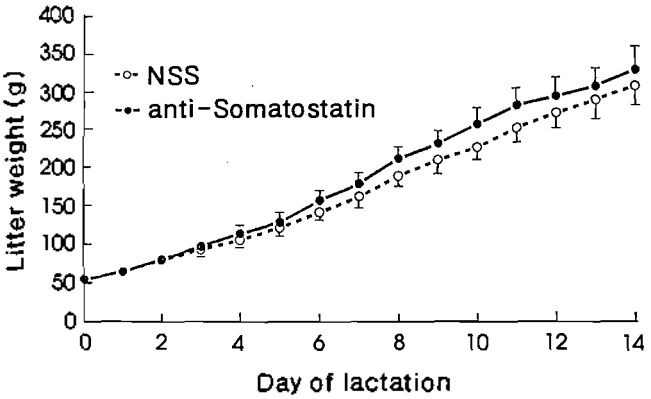

Figure 2. Litter (8 litters/group) weight of rats injected normal sheep serum (NSS) or anti-somatostatin injected for 14 days of lactation. Differences between treatments are significant ($p < 0.05$).

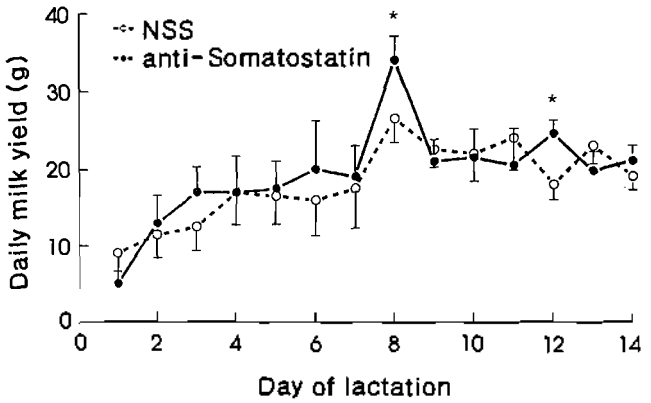

Figure 3. Daily milk yield of rats injected normal sheep serum (NSS) or anti-somatostatin injected for 14 days of lactation. Differences between treatments are significant ($p < 0.05$).

Plasma hormones and metabolites were increased by somatostatin immunization except NEFA concentration (table 2). GH and insulin were increased by 35% and 55%, respectively, in somatostatin immunization group. Plasma urea-N was increased by 20% in anti-somatostatin treatment. However, NEFA was decreased 17% in somatostatin immunization group. Plasma glucose level

was increased ($p<0.05$) by anti-somatostatin treatment. We assumed that increased growth rate more likely related with high GH and insulin secretion, enhance mammary development, and milk yield (Kuk and Myung, 1993). It does not appear to act through changes in plasma insulin and glucose concentrations and in glucose tolerance, which is in agreement with Spencer et al. (1983a). The other possibility is that the growth promoting effect of immunization against somatostatin may be mediated by differences in voluntary feed intake. Increased GH and other metabolites will improve growth performance and milk yield for animal production (Kerr et al., 1990; Holder et al., 1991).

Table 2. Plasma concentration of hormones and metabolites from rats injected normal sheep serum (NSS) or anti-somatostatin for 14 days of lactation

Item	NSS	anti-somatostatin
GH (ng/ml)	3.57 ± 0.38	4.83 ± 0.53
Insulin (μIU/ml)	20.1 ± 4.72	31.1 ± 5.64
Glucose (mg/ml)	0.88 ± 0.06	1.11 ± 0.10*
NEFA (mmole/ml)	0.77 ± 0.08	0.64 ± 0.10
Urea-N (mg/ml)	7.91 ± 1.24	9.36 ± 1.77

[1] Mean ± SD (n = 10).
* Significance of t-test for equality of two experimental groups ($p < 0.05$).

In conclusion, somatostatin immunization increased milk yield in early lactation and then enhanced growth rate of the young. Anti-somatostatin neutralizes endogenous somatostatin, increases reciprocally GH action and enhances growth and milk production (Ferland et al., 1976; Terry and Martin, 1981). Somatostatin immunization activates GH and IGF-1 in the local tissue (Spencer et al., 1983a,b; Varner et al., 1980). However, the mechanisms responsible for the increased growth rate is not clear.

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