Serum Growth Hormone and Insulin-Like Growth Factor-1 Concentrations in Japanese Black Cattle with Renal Tubular Dysplasia

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ABSTRACT. To elucidate the cause of growth retardation, 14 cattle with renal tubular dysplasia (RTD) were investigated by determining serum growth hormone (GH) and insulin-like growth factor (IGF)-1 concentrations. Compared with 6 healthy cattle, the baseline, maximum, area under the curve and peak amplitude of serum GH concentrations were higher or tended to be higher. Serum IGF-1 concentrations were lower normal or lower in cattle with RTD than in healthy cattle. Serum IGF-1 concentrations correlated significantly with body weight and chest girth, but not with serum GH and creatinine concentrations. Growth retardation in RTD might be associated partially with GH resistance.

KEY WORDS: growth hormone, growth retardation, IGF-1, Japanese black cattle, renal tubular dysplasia.

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The growth of animals is controlled by hormones and growth factors such as growth hormone (GH), insulin-like growth factor (IGF)-1, thyroid hormones, sex hormones, insulin, and glucocorticoids [22]. Among these physiologically active substances, GH and IGF-1 plays an essential role [4, 10, 14]. Renal tubular dysplasia (RTD) is an autosomal recessive hereditary disease in Japanese Black cattle [18, 19], characterized clinically by growth retardation, overgrowth of hooves, and chronic renal failure (CRF). In human patients with CRF, growth retardation is a serious clinical problem, together with other signs such as metabolic acidosis, malnutrition, renal osteodystrophy and hormone abnormalities [1, 20, 23], although the precise mechanisms are unknown. Some investigators have suggested that the GH and IGF-1 are important factors in growth retardation due to the growth of bones in human patients with CRF [9, 23]. However, serum GH and IGF-1 concentrations are unknown in cattle with RTD. To elucidate the cause of growth retardation, we determined serum GH and IGF-1 concentrations in cattle with RTD, and analyzed GH concentrations using a computer program for analysis of episodic hormone secretion [16].

MATERIALS AND METHODS

Cattle: Fourteen Japanese Black cattle (2 males and 12 females, aged 6 to 28 months old) with RTD diagnosed by detection of a deletion of the *paracellin-1* gene [17] were used (RTD group). All cattle showed growth retardation in spite of a relatively normal appetite. As controls, 6 normal Japanese Black cattle (2 males and 4 females, aged from 5 to

11 months) were used for determinations of serum GH concentrations, and 29 cattle (14 males and 15 females, aged from 6 months to 28 months) for serum IGF-1 concentrations. On the sampling day, cattle were fed only at 16:00 hr, and given water *ad libitum*.

Blood and biochemical analysis: Blood samples collected at 09:00 hr were used. Routine hematology and biochemical values were obtained using an automated blood cell counter (Celltac MEK 5150; Nihon Kohden, Tokyo), a microhematocrit method, and the dry chemistry method (FDC-5500V and FDC-800V, Fuji Film, Tokyo).

Serum GH and IGF-1 concentrations: Blood samples were obtained from the jugular vein from 09:00 hr for 10 hr at 15-min intervals. The sera were stored at -70°C . Serum GH concentration was estimated by radioimmunoassay [15]. Its baseline concentration, amplitude, duration, frequency, and intervals between peaks were analyzed using the PULSAR program for computer analysis of episodic hormone secretion [16]. The area under the curve of serum GH concentrations per hour (AUC_0) and the area under the curve above the baseline concentrations of serum GH per hour (AUC_b) were calculated using a computer program for the analysis of drug pharmacokinetics [25].

Serum IGF-1 concentration was estimated for the first blood sample collected at 09:00 hr. Since the amino acid sequence of bovine and human IGF-1 is identical [10], the estimation was carried out using an immunoradiometric assay used in human diagnostics (Somatomedin C II Bayer, Yuka Medias, Ibaraki, Japan).

Statistical analysis: Data are expressed as the mean and standard deviation. Differences between the values for RTD and control groups were analyzed by the Mann-Whitney test. Correlations between serum GH or IGF-1 concentrations and other test results were determined by Spearman's rank correlation coefficient. $P < 0.05$ was used

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Table 1. Blood and biochemical test results

Variable	Control group (n=6)	RTD group (n=14)	P<
	Mean $\pm$ SD	Mean $\pm$ SD	
RBC ($10^3/\mu\text{l}$)	8610 $\pm$ 1934	7184 $\pm$ 1799	NS
Hemoglobin (g/l)	113.8 $\pm$ 8.3	100.6 $\pm$ 21.5	NS
Hematocrit (%)	34.3 $\pm$ 3.0	30.3 $\pm$ 5.6	NS
WBC ($10^2/\mu\text{l}$)	80.3 $\pm$ 19.5	94.9 $\pm$ 28.6	NS
Sodium (mmol/l)	142.0 $\pm$ 2.3	137.3 $\pm$ 6.5	NS
Potassium (mmol/l)	3.9 $\pm$ 0.3	4.8 $\pm$ 0.6	0.01
Calcium (mg/dl)	9.9 $\pm$ 0.7	9.9 $\pm$ 1.4	NS
Inorganic phosphorus (mg/dl)	7.4 $\pm$ 1.3	7.7 $\pm$ 1.8	NS
Chloride (mmol/l)	101.3 $\pm$ 2.1	95.7 $\pm$ 5.9	0.05
Urea nitrogen (mg/dl)	13.7 $\pm$ 3.7	63.8 $\pm$ 45.0	0.01
Creatinine (mg/dl)	1.3 $\pm$ 0.3	4.1 $\pm$ 2.4	0.01
Total cholesterol (mg/dl)	94.3 $\pm$ 38.6	162.0 $\pm$ 35.9	0.01
Glucose (mg/dl)	69.0 $\pm$ 9.6	89.5 $\pm$ 19.7	0.05
Total protein (g/dl)	6.4 $\pm$ 3.6	6.8 $\pm$ 0.6	NS
Albumin (g/dl)	2.9 $\pm$ 0.2	3.3 $\pm$ 0.4	0.05
Aspartate aminotransferase (U/l)	67.8 $\pm$ 3.6	113.1 $\pm$ 81.8	NS
Alkaline phosphatase (U/l)	256.8 $\pm$ 73.8	349.1 $\pm$ 150.6	NS

P: Probability of significant difference between the control and RTD groups. NS: Not significant.

as the level of significance.

RESULTS

Routine blood and biochemical parameters: The results are summarized in Table 1. Serum potassium, urea nitrogen (UN), creatinine (CRE), total cholesterol, glucose and albumin concentrations were significantly higher, and serum chloride concentration was significantly lower, in the RTD group than in the control group.

Serum GH concentrations: Figure 1 shows typical patterns of serum GH concentrations in healthy and RTD cattle, and Table 2 shows the results of the PULSAR program analysis. The baseline concentration in cattle of the RTD group tended to be higher than in cattle of the control group. The peak amplitude and maximum concentrations were significantly higher in cattle of the RTD group. The peak frequency was not significantly different between the normal and RTD groups. The AUC₀ and AUC_b were higher in cattle of the RTD group.

Serum IGF-1: Serum IGF-1 concentrations ordered with monthly ages in cattle of the normal and RTD groups are shown in Fig. 2. Serum IGF-1 concentrations were within normal ranges or slightly lower in cattle of the RTD group. Serum IGF-1 concentrations were correlated with monthly ages ($n=14$, $r=0.612$, $P<0.02$), body weight ($n=14$, $r=0.831$, $P<0.01$) and chest girth ($n=14$, $r=0.859$, $P<0.01$), but did not correlate with serum GH concentration ($n=14$, $r=0.123$) and serum creatinine concentration ($n=14$, $r=0.532$) in cattle with RTD (Table 3).

DISCUSSION

Secretion of GH from the pituitary gland is controlled mainly by 2 hormones, growth hormone-releasing hormone

(GHRH), which plays a role in occurrences of peak excretion, and somatotropin release-inhibiting factor (SRIF), which acts to suppress the GH excretion at the trough of GH concentration [2, 10, 24]. Blood GH concentrations were normal or high in humans [8, 9, 21, 22] and rats [7] with CRF, as well as in cattle with RTD of the present study. In human patients with CRF, the baseline concentration of serum GH was increased, but the peak amplitude and maximum concentration were normal. Therefore, AUC₀ was increased, whereas AUC_b was not, and it is considered that decrease in SRIF action was one of the causes of the elevated baseline concentration [13]. In cattle with RTD, however, the baseline concentration, peak amplitude, and maximum concentration of serum GH increased, resulting in increases in AUC₀ and AUC_b. The increase in peak amplitude was observed simultaneously with an increase in the baseline concentration, suggesting a decrease in the action of SRIF and an increase in the action of GHRH. Moreover, the baseline concentration was not significantly correlated with peak amplitude in the RTD group, so separate factors may influence the decrease in the action of SRIF and the increase in the action of GHRH in cattle with RTD.

Serum IGF-1 concentrations and serum GH concentrations have a significant correlation in normal cattle [11]. Cattle in the RTD group, however, had normal or lower serum IGF-1 concentrations in spite of normal or higher serum GH concentrations, indicating GH resistance. Causes of GH resistance have been shown to be malnutrition [3, 5], parasitic diseases [6], liver diseases, and molecular defects of GH and GH receptors [11], as well as renal failure [9, 21]. In human patients with CRF, it is considered that GH resistance may be due to reduced numbers of GH receptors in the liver (decrease in GH sensitivity) [23], and an increase in plasma concentrations of IGF binding protein (IGFBP). This indicates that CRF is associated with GH resistance in

Fig. 1 Serum GH concentrations in a control animal (No. 4549) and an animal with RTD (No. 4746).

Table 2. Serum growth hormone parameters analysed by the PULSAR program

Group	Cattle number	Baseline concentration (ng/ml)	Peak amplitude (ng/ml)	Peak duration (min/1 peak)	Peak frequency (peak/min)	Peak interval (min)	Maximum concentration (ng/ml)	AUC ₀ (ng•min/ml)	AUC _b (ng•min/ml)	Classification
RTD (n=14)	4665	6.27 ± 2.80	24.86 ± 22.57	36.0 ± 39.1	0.0167	67.5 ± 31.2	67.4	891	569	B
	4666	3.95 ± 2.56	31.54 ± 56.19	25.7 ± 11.3	0.0233	50.0 ± 18.2	160.0	938	716	B
	4711	5.55 ± 1.33	7.50 ± 2.54	27.0 ± 26.8	0.0167	75.0 ± 71.4	14.7	478	175	C
	4712	3.83 ± 0.75	11.93 ± 0	60.0 ± 0.0	0.0033	0.0 ± 0.0	16.8	313	121	B
	4713	5.32 ± 2.59	4.55 ± 1.51	30.0 ± 15.0	0.0100	120.0 ± 42.4	14.6	388	99	C
	4714	3.57 ± 1.05	2.86 ± 1.95	15.0 ± 0.0	0.0067	150.0 ± 0	8.1	219	31	C
	4718	6.10 ± 1.09	4.51 ± 1.89	20.0 ± 8.7	0.0100	150.0 ± 84.9	12.7	396	70	C
	4745	5.92 ± 1.55	22.73 ± 26.33	72.5 ± 39.6	0.0100	117.0 ± 30.7	76.2	660	319	B
	4746	8.08 ± 3.47	10.47 ± 6.34	53.6 ± 43.2	0.0117	97.5 ± 40.0	28.7	733	279	A
	4853	5.68 ± 3.13	16.76 ± 5.22	93.8 ± 14.4	0.0067	190.0 ± 95.3	31.1	627	327	B
	4861	13.80 ± 8.08	20.63 ± 18.76	39.0 ± 22.8	0.0083	116.3 ± 61.7	63.6	1067	413	A
	4866	13.12 ± 9.33	13.05 ± 5.04	80.0 ± 31.2	0.0050	120.0 ± 84.9	39.3	841	233	A
	4867	12.83 ± 4.38	25.66 ± 5.45	100.0 ± 22.9	0.0050	232.5 ± 137.9	47.4	1111	462	A
	5088	2.25 ± 2.07	5.86 ± 4.29	55.0 ± 58.2	0.0100	120.0 ± 64.5	16.9	271	168	C
Mean ± standard deviation		6.88 ± 3.74	14.49 ± 9.26**	50.5 ± 27.6	0.0103 ± 0.0057	114.3 ± 60.2	42.7 ± 40.5**	638 ± 307*	284 ± 200**	
Control	(n=6)	4.21 ± 2.80	3.79 ± 0.89	43.4 ± 9.5	0.0133 ± 0.0020	89.8 ± 21.3	10.4 ± 3.6	294 ± 105	126 ± 40	

Data are presented as mean ± standard deviation. AUC₀: Area under the curve per hr of serum GH concentration. AUC_b: area under the curve per hr of serum GH concentrations above the baseline concentration. A: Cattle with high baseline concentration and peak amplitude of serum GH. B: Cattle with normal baseline concentration and high peak amplitude of serum GH. C: Cattle with normal baseline concentration and peak amplitude of serum GH. * and **: Significantly different from values in the control group. Single and double asterisks indicate P<0.05 and P<0.01, respectively.

cattle with RTD, but the specific mechanisms are unknown.

Serum IGF-1 concentration correlated significantly with body weight and chest girth. These significant correlations suggest that IGF-1 might correlate with the growth of cattle with RTD. Growth retardation in cattle with RTD may be associated with GH resistance (abnormalities from GH receptors to IGF-1 synthesis), but it may not be a primary cause.

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Fig. 2. Serum IGF-1 concentrations in control cattle and cattle with RTD. ●: Female with RTD, ▲: male with RTD, ○: female control, △: male control. —: Regression line of male controls ($n=14$, $y=16.80x+190.26$, $P<0.01$). ----: Regression line of female controls ($n=15$, $y=7.19x+124.68$, $P<0.01$).

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