Serum Total Amylase Activity and its Isoenzyme Fractions in Newborn Pigs with Relation to Growth and Iron Dextran-Treatment

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ABSTRACT. Serum amylase activity was analyzed chromogenically (blue starch method) and its isoenzyme was separated by cellulose acetate membrane electrophoresis in newborn pigs comparing with those in adult pigs. Serum amylase activity in newborn pigs was about one half of adults' and increased with age. Serum amylase isoenzymes were separated into 4 fractions from the cathode. The isoenzymes were also classified in 5 types on the basis of the combination of these 4 fractions. Incidences of the fractions and the types were not significantly different between adults and newborns. After the iron dextran treatment, serum amylase activity as well as levels of erythrocytes, hemoglobin and packed cell volume increased. The rates of increase in hemoglobin and packed cell volume were more significant in the double treatment group than in the single treatment group, while serum amylase activity increased similarly in these 2 groups. The increase in serum amylase activity seemed to indicate that the development of amylase-producing organs was reinforced by the iron compound treatment. It is thought that serum amylase activity may be an indicator of healthy growth in newborn pigs.—**KEY WORDS:** electrophoresis, iron supplement, isoenzyme, pig, serum amylase.

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Serum amylase has been utilized in the routine veterinary laboratory test to diagnose acute pancreatitis, renal insufficiency, salivary duct occlusion or suppuration and intestinal obstruction [5]. In pigs, there are some reports on serum amylase describing its application to the diagnosis of hemorrhagic pancreatitis [9], and as an indicator in the experimental pancreatitis [25], food digestion [15], selenium toxicosis [3] and rejection for pancreatic transplantation [12]. Serum amylase isoenzymes have been studied in pigs [2, 4, 11, 16, 17, 20, 22], and most of the reports are on the genetics using adult pigs. Serum amylase activity in piglets is known to increase with age [13], but there is no report on its isoenzymes in piglets. In the present study, serum amylase activity and its isoenzyme fractions were compared in newborn and adult pigs. Since piglets are treated at birth with iron compound, such as iron dextran, to prevent iron deficiency anemia, the influence of iron dextran to serum amylase activity in newborn pigs was also evaluated in this study.

MATERIALS AND METHODS

Animals: Randomly selected 119 adult and 30 newborn healthy Kagoshima Berkshire pigs without genetic relationship were used for the study. Adult

pigs were from 6 to 10 months of age, including 58 castrated males and 61 females. Thirty newborn pigs, which were 14 males and 16 females, were divided into 3 groups. Nine were untreated control group, 16 were injected once (single treatment group) on the 1st day of age intramuscularly with 1 ml of an iron dextran preparation containing 100 mg of iron (Tonkii-FB 100, Fujita Seiyaku Co., Ltd.), and 5 were injected the same dose twice (double treatment group) on the 1st and 10th day of age.

Hematological and serum amylase analysis: Blood samples were taken between 9 and 11 a.m. from the heart in adults at a slaughter house, and the from cranial vena cava on 1, 10, 20, 30 days of age in newborns at a farm. Hematological parameters evaluated were erythrocyte count (RBC: Coulter counter Model D), hemoglobin concentration (Hb: SLS-hemoglobin method) and packed cell volume (PCV: microhematocrit method).

After the separation of serum by a centrifugation at 4,000 r.p.m. for 15 min, total amylase activity was assayed by the blue starch method (Neo-amylase test, Daiichi Chemical Co., Ltd.). Since serum amylase activity in pigs is higher than that in humans and the substrate concentration in the commercial kit for amylase assay is not sufficient for pigs [14], a less serum amount (20 μ l) than that in man (100 μ l) was applied. A standard curve was made by using an

enzyme solution with known activity (Phadebas ACR serum Sweden, Daiichi Chemical Co., Ltd.).

The amylase isoenzyme was analyzed with electrophoresis on cellulose acetate membrane (Cellogel, Chemetron Co., Ltd.) using discontinuous buffer system [7, 24] immediately after the separation of serum. Electrode solutions were 0.1 M Tris-HCl buffer (pH 8.8) in the anodal tray and 0.025 M borate-HCl buffer (pH 8.2) in the cathodal tray. Two μ l of serum was applied as one sample. Electrophoresis was carried out with a constant current of 1.2 mA/cm width of a membrane for 70 min at room temperature. Enzymes were stained with blue starch polymer as substrate. Three blue starch tablets mixed in 4.5 ml distilled water were used to dye a sheet of membrane (5 $\times$ 6 cm). After electrophoresis the membrane was laid on the surface of the blue starch agar and was incubated for 70 min at 37°C. The reaction was stopped by dipping the membrane in methanol. The isoenzymes appeared as blue bands against a transparent background after the membrane was immersed in a solution consisting of methanol, acetic acid and glycerol (85:14:1, v/v/v). Fractional bands were scanned for quantitation at 600 nm wave length with the densitometer (Ozumor 802, Asuka Kogyo Co., Ltd.). The position of a band mobilizing nearest to

the anode changed with the individual serum and the electrophoretic technique. Contrary to the ordinary nomenclature [10], the amylase isoenzyme bands were numbered in order from cathode to anode according to Otsuki *et al.* [19]. Values were analyzed statistically by a Duncan's multiple range test or Chi-Square test.

RESULTS

There was no significant difference between castrated males and females in serum total amylase activities (Table 1). Activities in adults were significantly ($P < 0.05$) higher than those in newborns (Table 2). Serum amylase isoenzyme was separated by the electrophoresis into 4 fractions designated as Am1 to Am4 from the cathode in both adults and newborns. Figure 1 showed 4 fractions in adults. As shown in Table 2, the incidence of Am4 fraction (1.7 and 0%, in adults and newborns, respectively) was markedly lower than those of the other 3 fractions. There was no significant difference in the incidences of 4 fractions between adults and newborns. Activities of Am1 and Am2 were higher than those of Am3 and Am4 (Table 3).

Serum amylase isoenzyme was classified into 5 types by the combination of above mentioned 4

Fig. 1. Swine serum amylase isoenzyme bands and types. Isoenzyme bands were numbered from cathode to anode; Am1(1), Am2(2), Am3(3) and Am4(4) in order from top to bottom in the figure. The band patterns were classified into 5 types by the combination of above mentioned 4 fractions; Type I, Type II, Type III, Type IV and Type V from left to right.

Table 1. Serum total amylase activities in castrated male and female adult pigs (U/l)

Castrated males	(n=58)	5848.2±2625.6
Females	(n=61)	6100.3±2772.4

Table 2. Serum total amylase activities and incidences of 4 amylase isoenzyme fractions in adult and newborn pigs

	Total amylase activity (U/l) Mean±SD	Incidence (%)			
		Aml ^{b)}	Am2	Am3	Am4
Adults (n=119)	5977.4 ^{a)} ±2693.4	76.4 (91) ^{c)}	100.0 (119)	88.2 (105)	1.7 (2)
Newborns (n=30)	2936.2 ± 947.7	80.0 (24)	100.0 (30)	90.0 (27)	0.0 (0)

a) Value of adults was significantly higher than that of newborns (P<0.05).

b) Incidences of 4 amylase isoenzyme fractions were not different between adults and newborns.

c) Animal numbers detected respective fractions.

Table 3. Incidence and activity of serum amylase isoenzyme fractions within each type in adult and newborn pigs

Type	Incidence of types (%)		Activity of amylase isoenzyme fractions (U/l)								Serum total amylase activity (U/l)	
			Am1		Am2		Am3		Am4			
	A	N	A	N	A	N	A	N	A	N	A	N
I	1.7 (n=2)	0.0 (n=0)	2235.9 ^{a)} ± 499.0 (47.0) ^{b)}	—	1753.7 ± 313.0 (36.8)	—	594.6 ± 161.2 (12.5)	—	175.1 ± 186.4 (3.7)	—	4759.3 ± 1159.6	—
II	9.2 (n=11)	10.0 (n=3)	2315.0 ± 771.9 (61.2)	680.3 ± 106.1 (54.0)	1467.8 ± 599.3 (38.8)	579.0 ± 779.3 (46.0)	—	—	—	—	3782.7 ± 1266.9	1259.0 ± 662.0
III	65.5 (n=78)	70.0 (n=21)	2058.7 ± 975.6 (33.4)	934.8 ± 595.1 (31.8)	3610.8 ± 1882.5 (58.6)	1805.0 ± 888.4 (61.5)	492.1 ± 429.3 (8.0)	198.7 ± 158.5 (6.7)	—	—	6161.5 ± 2703.6	2936.6 ± 947.7
IV	21.0 (n=25)	20.0 (n=6)	—	—	6075.7 ± 2260.2 (88.7)	2078.0 ± 811.0 (93.8)	777.7 ± 576.5 (11.3)	138.2 ± 62.3 (6.2)	—	—	6853.4 ± 2604.4	2216.1 ± 765.0
V	2.5 (n=3)	0.0 (n=0)	—	—	2751.3 ± 1409.5 (100.0)	—	—	—	—	—	2751.3 ± 1409.5	—

A; Adult pigs, N; Newborn pigs, a) Mean and standard deviation of the isoenzyme activity (U/l). b) Percentage of activity within a type.

Table 4. Change of serum amylase activity and hematological parameters with age and by iron dextran treatment in newborn pigs

Day	Control group (n=9)				Single treatment group ^{a)} (n=16)				Double treatment group ^{b)} (n=5)			
	RBC ×10 ⁶ /μl	Hb mg/dl	PCV %	Serum amylase U/l	RBC ×10 ⁶ /μl	Hb mg/dl	PCV %	Serum amylase U/l	RBC ×10 ⁶ /μl	Hb mg/dl	PCV %	Serum amylase U/l
1d	4.90 ^{c)} ±1.42	7.4 ±2.5	30.3 ±7.3	2128.3 ± 957.9	5.17 ±0.73	8.3 ±1.2	31.3 ±7.0	2739.4 ±1123.1	4.96 ±1.01	6.6 ±1.0	27.8 ±5.3	3146.4 ± 733.9
10d	4.93 ±1.59	6.6 ±1.4	24.9 ±5.4	2832.1 ±1130.7	6.28 ^{d)} ±0.99	8.9 ±1.6	36.3 ±4.9	3197.2 ±1415.7	6.17 ^{d)} ±0.49	8.2 ^{d)} ±1.3	36.6 ^{d)} ±2.2	3654.3 ± 989.3
20d	4.05 ±1.42 ^{c)}	5.7 ±1.4	21.1 ±4.8	2773.5 ±1600.2	6.48 ^{d)} ±1.00	10.2 ^{d)} ±0.8	34.8 ±3.0	4081.3 ^{d)} ±1894.0	6.07 ^{d)} ±0.78	10.0 ^{d)} ±0.8	36.7 ^{d)} ±3.7	4040.1 ±1320.6
30d	3.92 ^{c)} ±1.91	5.4 ±1.5	18.6 ±6.8	2845.1 ±2346.4	6.66 ^{d)} ±1.06	9.9 ^{d)} ±1.1	31.6 ±2.8	4838.0 ^{d)} ±2695.9	6.59 ^{d)} ±0.95	10.0 ^{d)} ±0.7	34.8 ^{d)} ±3.3	5031.3 ^{d)} ±1290.8

1d; Before treatment. a) Single injection of 1 ml/iron dextran preparation on the 1st day of age. b) Double injection of the same dose on the 1st and 10th day of age. c) Means and standard deviations. d) Significantly different from the value of 1d (P<0.05)

fractions (Table 3), which were Type I; Am1, Am2, Am3 and Am4, Type II; Am1 and Am2, Type III; Am1, Am2 and Am3, Type IV; Am2 and Am3, and Type V; Am2. The incidence of Type III (65.5 and 70.0%) was highest among these 5 types. Total activities of Type III and IV were similar and much higher than those of the other 2 types. The incidences and the activities of these 5 types showed the same tendency in adults and newborns. Type I and V were detected in a few adult pigs and none in newborn pigs.

Hematological parameters of newborn pigs in the control group tended to decrease with time. The administration of iron dextran increased their Hb and PCV far more significantly in the double treatment group than in the single treatment group (Table 4). While serum total amylase activity showed a considerably constant level by 30th day in the control group, the activities in both of the treated groups increased significantly in the same inclination as hematological parameters.

DISCUSSION

In this study no statistical difference were seen between castrated male and female adult pigs on serum amylase activity. This agreed with the result of Morizono *et al.* [16]. Considering that there is no sex difference in dogs and cats [5], it seems that no sex related difference is present in serum amylase in pigs.

The chromogenic assay for serum amylase activity and the cellulose acetate membrane electrophoresis for its isoenzyme separation are popular methods in the routine laboratory test [7, 14]. The ratio of serum amylase activity of adults (5977.4 U/l) to newborns (2936.2 U/l) in the present experiment was 2:1. This ratio was slightly lower than those in Polish Landrace pigs obtained by enzyme immunoassay (4.4:1), radioimmunoassay (5.2:1) and blue starch method (3:1) [23]. Difference in the ratio was thought to indicate that the level of serum amylase activity might be different with the breed of pigs as well as with the method of determination.

The increase of serum amylase activity with age is thought to be associated with the growth of amylase-producing organs in piglets [6]. It is presumed in healthy humans that in addition to the pancreas and salivary gland, the liver, striated muscle, fallopian tubes or testes, uterus, adipose tissue, and gallbladder contribute to the serum total amylase [28].

Serum amylase activity increased with age, but the incidence of isoenzyme fractions as well as types did not change in this study. This finding may suggest that unchanged incidences and types are associated with the parallel increase of individual isoenzyme activity with growth. Amylase isoenzyme in humans was indicated to show autosomal dominant inheritance [27]. The type of amylase isoenzyme in pigs was thought also to be inherited in the similar manner.

Pigs are born with only a limited store of iron. Milk of pigs is low in iron. Unless pigs have an access to exogenous sources of iron, anemia develops in 2–3 weeks. Infection, malnutrition, hemorrhage and parasitosis increase iron requirements [26]. Iron deficiency with low hemoglobin levels may leave piglets more susceptible to infections such as navel ill or diarrheal diseases [21]. For the prevention of anemia in newborn pigs, iron compounds have been injected intramuscularly on the 2nd or 3rd day of life. Iron dextran is the first choice of the iron supplements in newborn pigs [1]. Hematological parameters increased after the treatment of iron dextran and its effect was enhanced by the repeated administration. Serum amylase activity also increased with the iron treatment. There was not marked difference, however, between the single and the double treatment groups. This finding also seemed to suggest the increase of serum amylase activity might depend indirectly on the development of amylase-producing organs by the iron treatment.

Salivary flow rate and amylase activity are not reduced in patients with iron deficiency anemia [18]. Pancreatic amylase output even increases in patients with pernicious anemia [8]. These reports suggest that anemic condition may not be a cause of decreased amylase secretion. Significant increase of amylase activity in the iron-treated newborns in the study allowed us to conclude that the repair of anemia might increase the blood perfusion within amylase-producing organs. As the result, promoted growth and function of the gland seemed to induce the high serum level of amylase. Low serum amylase activity in newborn pigs is thought probably to denote the immature state of the amylase-producing organs. Therefore, serum amylase activity seemed to be an indicator of the healthy growth in newborn pigs.

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