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A STUDY ON THE ANTEMORTEM DETECTION OF PSE MUSCLE IN PIGS BY A HALOTHANE TEST, PLASMA CREATINE PHOSPHOKINASE ACTIVITIES AND BLOOD LACTATE VALUES

Hiroko SANO, Shigeo NAMIOKA, Katsumoto KAGOTA, Atsushi YAMADA*, Hajime MIYAZAKI*, Noboru ABE* and Yasunori YONEDA*

Department of Veterinary Internal Medicine
Faculty of Veterinary Medicine
Hokkaido University, Sapporo 060, Japan

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Halothane test, and the determination of plasma CPK activities and blood lactate values before and after the halothane test were carried out on Landrace and Large White pigs at an average of 24 kg live weight. Pigs were slaughtered at 90 kg live weight to examine the muscle quality characteristics in order to determine the appearance of PSE muscle. There was no clear relationship between antemortem parameters employed in this study and incidence of PSE muscle or muscle characteristics. The possibility and value of antemortem detection of PSE muscle by these parameters were discussed.

INTRODUCTION

Recently, the incidence of porcine PSE (pale, soft and exudative) muscle is gradually increasing and has become a serious problem. It is considered that the appearance of PSE muscle may result from an abnormal anaerobic glycolysis after slaughter[21]. However, many factors before and after slaughter might be involved in the onset of abnormal glycolysis and the PSE syndrome[21,22]. This has led to a concentration of effort on the antemortem detection of such abnormal pigs[1,3~6,8~10,13,16~22,24,26,33,35].

It was reported that halothane-susceptible pigs, showing severe leg muscle rigidity and progressive hyperthermia under halothane anesthesia, tend to develop PSE muscle [5,8,9,33]. In addition, these pigs are said to have high levels of creatine phosphokinase (CPK) in the blood and of the production rate of lactate[4,6,8,11,14,28,32,33].

The present experiment was designed to determine the correlations between halothane susceptibility as expressed by leg muscle rigidity, plasma CPK activities or blood lactate values and PSE condition of muscle after slaughter.

* *Takikawa Animal Husbandry Experimen Station, Takikawa 073, Japan*

MATERIALS AND METHODS

Animals A hundred and sixteen purebred Landrace and Large White pigs, kept in Takikawa Animal Husbandry Experiment Station of Hokkaido, were used in this study.

Experimental procedure At an average of 24 kg live weight, pigs were subjected to a modified halothane test of CHRISTIAN[4]. Pigs were exposed to halothane vapor for up to 3 minutes. The gas was administered through a closed circuit system anesthetic machine equipped with vaporizer (Igarashi Medical Industries). Four to six percent vapor was supplied via a face mask with oxygen. Behavior of pigs under halothane anesthesia was carefully observed, particularly for appearance of leg muscle rigidity and erythema in the skin. Rectal temperature was also measured immediately before and after the halothane test.

The ear blood samples were taken before and 2 hours after the test with heparinized sylinder for CPK determination. The blood samples for lactate were taken from the anterior vena cava immediately after the test. The ear blood samples were separated for plasma by centrifuge immediately after the collection and the samples were stored into ice and assayed for CPK activities, according to the methods of OLIVER[23] and HESS et al.[12] with CPK UV Test Kit (Wako Pure Cheimcal Industries) within 2 days. The heparinized blood samples from the anterior vena cava were assayed for lactate within 24 hours after sampling. The samples were stored in ice until analysis. The assay was carried out after the method of PFLEIDERER & DOSE[25] with Lactate UV Test Kit (Boehringer-Manheim, West Germany).

Tested pigs were then reared in pairs. Diets and water were available at all times. At about 90 kg live weight, following an overnight rest without feed but with water *ad lib.*, 110 pigs were slaughtered at Takikawa Animal Husbandry Experiment Station, using electrical stunning, to examine the muscle quality.

The pH of the muscle (*M. longissimus thoracis*) was determined 45 minutes after electrical stunning with pH meter (Horiba D-5). After chilling in cold storage for 24 hours, the right side of each carcass was cross-sectioned between 5th and 6th thoracic vertebrae. The muscle was evaluated subjectively for moisture, color and firmness, and the muscle quality was then classified as normal and slight, moderate and severe PSE muscle. The muscle samples were removed from the same site of the above muscle to estimate physical properties of the muscle (L value, water-holding capacity and spreadability). L value, i. e., brightness by UCS system of color representation, was measured by TC-50 color difference meter (Tokyo Denshoku Company). Water-holding capacity (WHC) and spreadability were measured by the filter paper-press method[28,39]. WHC was expressed as percent and spreadability as spread area (cm^2) per weight (g) of the muscle.

For statistical analysis, the values of CPK activities and ascending rates of CPK after the halothane test were subjected to logarithmic transformation to obtain a normal distribution.

Results

Halothane anesthesia was smoothly introduced in the majority of the pigs employed, which lost their conciousness and relaxed their leg muscle within 1 mintue except in a few cases, and lost their lid reflex within about 1 to 3 minutes. With the introduction of anesthesia, appearances of leg muscle rigidity and congestive erythema in the thoracicoabdominal skin were observed in some of the pigs. Rigidity appeared during 15 seconds to 1 minute and 40 seconds after the start of the halothane administration. Of 49 Landrace pigs, eight (16.3 %) showed positive (+) signs of rigidity and five (10.2 %) were doubtful (±). Of 67 Large White pigs, five (7.5 %) were positive and five (7.5 %) doubtful. Erythema appeared during 10 seconds to 1 minute and 45 seconds. It was observed for 31 (63.3 %) in Landrace and 21 (31.3 %) in Large White pigs. Following the cessation of the halothane administration, pigs became aware quickly from anesthesia and no accident occurred in any pig employed in this study. Rectal temperature during anesthesia did not differ significantly between reacting pigs and non-reacting pigs either for rigidity or erythema.

The CPK levels in plasma increased after the halothane test in most pigs except three pigs. It was remarkable that the range of distribution of the CPK activities was very wide, especially after the halothane test. Landrace pigs exhibited higher ($P<0.01$) CPK levels, both before and after the halothane test, than Large White pigs. The ascending rates of CPK (CPK activities after the test/CPK activities before the test $\times 100$, %) were similar for both breeds. The values of blood lactate examined immediately after the halothane test were distributed from 17.8 to 143.1 mg/dl. There was no significant difference between the breeds.

Differences in the CPK activities and the blood lactate values among each group, classified by the appearance of leg muscle rigidity, are shown in table 1. The blood lactate values were higher ($P<0.01$) in rigidity-positive pigs than rigidity-negative ones of Landrace. No significant relationships were found between the CPK levels and the appearance of leg muscle rigidity. On the other hand, erythema-positive pigs showed higher levels of plasma CPK than erythema-negative pigs (tab. 2). Significant differences in the CPK activities after the halothane test were noted between erythema-positive and erythema-negative pigs in both breeds. Erythema-positive pigs showed significantly higher levels of the ascending rates of CPK in Large White. The blood lactate values were similar for both erythema-positive and negative pigs.

The incidence of PSE muscle (slight, moderate and severe) was 44.2 % of total pigs slaughtered and no significant difference of incidence was seen between Landrace

TABLE 1 *Plasma CPK activities and blood lactate values in leg muscle rigidity-positive (+), doubtful (±) and negative (−) Landrace and Large White pigs*

ITEM	LEG MUSCLE RIGIDITY					
	+		±		−	
	L[a] (8)	W[a] (5)	L (5)	W (5)	L (36)	W (57)
CPK 1[b]						
Mean	2.489	2.042	2.323	2.076	2.303	2.121
SD	0.411	0.147	0.358	0.099	0.358	0.206
CPK 2[c]						
Mean	2.757	2.243	2.885	2.448	2.669	2.477
SD	0.365	0.071	0.279	0.356	0.352	0.311
CPK 3[d]						
Mean	2.343	2.202	2.561	2.430	2.366	2.356
SD	0.216	0.122	0.212	0.342	0.230	0.197
Lactate[e]						
Mean[f]	84.6[1]	64.0	55.3	64.4	51.9[2]	62.5
SD	34.6	36.6	18.8	9.7	24.9	18.9

a L=Landrace, W=Large White; numbers of pigs in parentheses
b Logarithmic values of CPK before the halothane test
c Logarithmic values of CPK after the halothane test
d Logarithmic values of ascending rates of CPK
e Determined on the blood obtained immediately after the halothane test, and results expressed as mg/dl of blood
f Means with different superscripts are significantly different ($P<0.01$).

TABLE 2 *Plasma CPK activities and blood lactate values in erythema-positive (+) and negative (−) Landrace and Large White pigs*

ITEM	ERYTHEMA			
	+		−	
	L[a] (31)	W[a] (21)	L (18)	W (46)
CPK 1[b]				
Mean	2.375	2.160	2.261	2.085
SD	0.402	0.263	0.287	0.161
CPK 2[c]				
Mean[d]	2.779[1]	2.617[2]	2.577[3]	2.378[4]
SD	0.356	0.414	0.300	0.212

ITEM	ERYTHEMA			
	+		−	
	L[a] (31)	W[a] (21)	L (18)	W (46)
CPK 3[e]				
Mean[f]	2.423	2.477[5]	2.316	2.293[6]
SD	0.243	0.221	0.196	0.176
Lactate[g]				
Mean	61.6	64.7	50.8	60.5
SD	28.1	15.3	28.2	19.8

a L=Landrace, W=Large White; numbers of pigs in parentheses
b Logarithmic values of CPK before the halothane test
c Logarithmic values of CPK after the holothane test
d 1 *vs* 3, $P<0.05$; 2 *vs* 4, $P<0.01$
e Logarithmic values of ascending rates of CPK
f 5 *vs* 6, $P<0.001$
g Determined on the blood obtained immediately after the halothane test, and results expressed as mg/dl of blood

TABLE 3 *Incidence of PSE muscle and muscle quality traits in leg muscle rigidity-positive (+), doubtful (±) and negative (−) Landrace and Large White pigs*

ITEM	LEG MUSCLE RIGIDITY					
	+		±		−	
	L[a] (8)	W[a] (5)	L (5)	W (4)	L (34)	W (54)
Incidence of PSE muscle						
Severe	1	0	0	0	0	0
Moderate	2	2	1	0	3	4
Slight	0	0	2	1	11	19
Total	3	2	3	1	14	23
%	37.5	40.0	60.0	25.0	41.2	42.6
Muscle quality						
pH						
Mean	6.17		6.11		6.07	
SD	0.35		0.47		0.37	
L value						
Mean	49.3		50.0		49.4	
SD	4.5		2.8		3.7	
WHC (%)[b]						
Mean	73.16		73.24		74.63	
SD	5.17		6.41		5.18	
Spreadability (cm^2/g)						
Mean	30.44		30.81		31.45	
SD	5.38		5.69		3.57	

a L=Landrace, W=Large White; numbers of pigs in parentheses
b Water-holding capacity

TABLE 4 *Incidence of PSE muscle and muscle quality traits in erythema-positive (+) and negative (−) Landrace and Large White pigs*

ITEM	ERYTHEMA			
	+		−	
	L[a] (26)	W[a] (19)	L (21)	W (44)
Incidence of PSE muscle				
Severe	1	0	0	0
Moderate	2	3	4	1
Slight	9	8	4	14
Total	12	11	8	15
%	46.2	55.0	38.1	34.1
Muscle quality				
pH				
Mean	6.07		5.96	
SD	0.39		0.96	
L value				
Mean	49.8		49.2	
SD	3.7		3.7	
WHC (%)[b]				
Mean	75.02		73.96	
SD	5.76		5.09	
Spreadability (cm^2/g)				
Mean	31.09		31.41	
SD	4.52		4.91	

a L=Landrace, W=Large White; numbers of pigs in parentheses
b Water-holding capacity

and Large White pigs. The incidence of PSE muscle scored 70 % in the former half of whole slaugher period (from May to August of the year) and 29 % in the latter half (from September to December of the year).

Each group classified by the appearance of leg muscle rigidity showed similar results for the incidence of PSE muscle and muscle physical properties (tab. 3). Erythema-positive group exhibited higher incidence of PSE muscle than erythema-negative group, but these differences were not significant statistically. The values of physical properties did not differ between the both groups (tab. 4).

The correlations between the blood parameters and the muscle physical properties are shown in table 5. It was revealed that several significant relationships existed between the blood parameters and the muscle physical properties. Correlation coefficients between the ascending rates of CPK and L value were positively significant in both

TABLE 5 *Correlation coefficients between blood parameters and muscle physical properties in Landrace and Large White pigs*

MUSCLE	BLOOD			
	CPK 1[a]	CPK 2[b]	CPK 3[c]	Lactate
		Landrace		
pH	0.2077	0.1135	−0.1637	−0.1203
L value	−0.2807	−0.1369	0.3021*	0.3960**
WHC[d]	0.2483	0.2312	−0.1213	−0.4489***
Spreadability	0.0129	−0.0697	−0.2245	−0.3255**
		Large White		
pH	0.0091	−0.0565	−0.1063	−0.1038
L value	0.0193	0.1845	0.3014*	0.1732
WHC[d]	0.0067	−0.0622	−0.1378	−0.1198
Spreadability	−0.0468	−0.0541	−0.0675	−0.1115

a Determined on plasma before the halothane test
b Determined on plasma after the halothane test
c Ascending rates of CPK
d Water-holding capacity
* $P<0.05$, ** $P<0.01$, *** $P<0.001$

Landrace and Large White pigs. In Landrace, significant correlations were found between the blood lactate vlaues and muscle physical properties, i. e., L value, WHC and spreadability, respectively, while no significant correlation existed in Large White. Other correlations shown in table 5 were not significant.

Discussion

There are many reports of an abnormal response of pigs to halothane anesthetics [2,11,15,34]. This response is characterized by muscle rigidity as an earlier sign, fulminating hyperthermia and high levels of lactate production and of blood creatine phosphokinase. CHRISTIAN[4] and EIKELENBOOM & MINKEMA[8] reported that the halothane-susceptible pigs showed the leg muscle rigidity by the administration of relatively highly concentrated halothane vapor with oxygen for short time. These halothane-susceptible animals exhibit higher levels of blood CPK even at rest as reported by ALLEN et al.[2]. The present study, however, indicates that the CPK levels at rest do not differ significantly between halothane-susceptible and resistant pigs, as suggested by CHRISTIAN[6] and other researchers[8,33].

The increased levels of plasma CPK after the halothane test could be caused by the anesthesia or by blood collecting procedure. Such a collecting manner caused the

elevation of about 10 % of the CPK levels[17]. One hundred and ten pigs used in the present study exhibited more than 110 % of the ascending rates of CPK. Therefore, almost all of these pigs would be affected by the attack of halothane vapor rather than by the stress through the handling for the blood collection.

Both the CPK levels after the halothane test and the ascending rates of CPK were not significantly different among each group classified by the appearance of leg muscle rigidity. These results did not agree with the other previous reports[6,34] that the CPK levels of halothane susceptible (i. e., rigidity-positive) pigs elevated higher than resistant pigs after the halothane test. It is interesting that erythema-positive pigs exhibited higher levels of CPK than erythema-negative ones. There is no previous report revealing the relationships between erythema during the halothane test and CPK values in the blood, although the appearance of erythema during the halothane anesthesia has been reported by MATSUBARA[18]. The present study finds that no rigidity-positive pigs show the symptom of malignant hyperthermia syndrome and an exclusively high elevation of plasma CPK activities after the halothane anesthesia. Pointing out the appearance of erythema during the halothane test might be important, in relation to the CPK levels after the halothane test. However, the evaluation of this finding would remain obscure in some points that 1) the relationship between the rigidity and the erythema was not clear, 2) the detection of erythema would be difficult in colored breeds such as Hampshire, and 3) erythema may also appear by the handling for blood collection.

The incidence of PSE muscle determined by the present method was observed similarly both in rigidity-positive and negative pigs. This result is not in agreement with other previous reports[8,18,20,33] suggesting that the incidence of PSE muscle was significantly related to the rigidity-positive pigs. These previous reports were almost about Pietrain, Landrace and Hampshire pigs, but scarcely about Large White pigs. The frequencies of halothane susceptibility scored 30 to 100 % for Pietrain, 5 to 85 % for Landrace and 0 to 3 % for Large White[9,16,19,20,24,33]. In the present study, the frequency of the susceptibility in Large White was only half of that in Landrace. However, the incidence of PSE muscle for both breeds was similar in number, which coincided with the results of previous reports[7,32]. The incidence of PSE muscle in pigs is not particularly related to halothane susceptibility by the field halothane test, which may indicate that there would be other important factors related to the incidence of PSE muscle. Therefore, our results seem to show that there is no high correlation between halothane susceptibility and antemortem detection for PSE muscle.

In the present study, significant correlation was detected between the ascending rates of CPK and L value of the muscle physical property. On the other hand, there was no significant correlation between other CPK parameters and muscle physical properties. The relationships between the blood lactate values of Landrace and the muscle physical properties were detected in this study, as reported by BICKHARDT[3] and YAMASHITA

et al.[35]. This indicates that the blood lactate values under various physical stress, e. g., haltohane anesthesia as in this study and severe exercise[3], were significantly related to muscle quality after slaughter. In the present study, however, these significant relationships were not detected for Large White pigs. Blood lactate may be changeable through various factors and also unstable in storage[29]. Therefore, further studies should be required to apply the blood lactate values to the antemortem diagnosis of PSE muscle.

There are some reports[1,3,10,26] concerning a significant correlation between the CPK activities in the blood and the muscle property, though these findings are not identical to those of this study. One of the reasons for this might be that the experimental procedures used in this study were different from those of other previous workers as following: 1) Stress condition at the blood sampling for CPK measurement is various [1,3,10,26]. Severe exercise, transportation and heat stress were given to the pigs prior to the blood sampling. The majority of CPK enzyme exists in the skeletal muscle cell[27], and, what kind of mechanisms cause the escaping of CPK enzyme from skeletal muscle in the case of physical stresses and halothane anesthesia is still unknown[31]. In man, consistant elevation of serum CPK level after severe physical exertion in normal subjects was reported by VEJJAJIVE & TEASDALE[31], and halothane-suxamethonium combination caused an increase in serum CPK activity[14]. 2) Other workers[3,11] carried out the blood sampling for CPK measurement between 8 and 24 hours after giving stress. BICKHARDT[3] and ELIZONDO et al.[10] revealed that the change of CPK levels in plasma can be more accurately determined 8 to 24 hours after standard exertion, considering the delay in CPK efflux after stress. CHRISTIAN[5] reported, however, that the peaking of CPK levels occurs between 4 and 8 hours after the halothane exposure but it is elevated to predictive levels by 2 hours. 3) In other experiments, the determination of the CPK levels in blood was performed later and nearer to the slaughter time[1,10] than in the present study. In the present study, the blood samples were taken at an average of 24 kg live weight of pigs. ELIZONODO et al.[10] and ADDIS et al.[1] carried out the sampling at 60 kg live weight and immediately before the slaughter, respectively. They indicated the significant correlation between the CPK levels in serum and postmortem muscle quality. On the other hand, HWANG et al.[13] reported no significant correlation between the blood CPK levels at 50 kg and postmortem muscle quality. 4) ADDIS et al.[1] and ELIZONDO et al.[10] found significantly high correlations between postmortem muscle quality and the serum CPK levels after giving physical exertion immediately before the slaughter. Stresses immediately before the slaughter such as physical exercise, transportation and others might be considered to cause the PSE condition in muscle property.

The present study revealed that halothane-susceptible (rigidity or erythema-positive) pigs with high plasma CPK both at rest and after the halothane test did not necessarily have the PSE condition of the muscle. From these results, it is considered that halothane susceptibility and the CPK releasing factors from muscle cell may not be highly

related to the postmortem muscle quality. Therefore, other factors in the later fattening period may affect essentially the occurrence of PSE muscle.

Concerning our results, the relationships among halothane susceptibility, the CPK values in plasma and the blood lactate values remained obscure. Further studies would be needed to clarify the mechanism of porcine halothane susceptibility expressed by the halothane test for the antemortem detection of the pigs with PSE condition of the muscle.

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