HISTOPATHOLOGY OF MUSCLE IN EXPERIMENTAL ACUTE SWINE ERYSIPELAS

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A number of papers have been published to report the histopathological investigation of swine and other animals infected experimentally with *Erysipelothrix insidiosa*. The morphological investigation of acute swine erysipelas has been carried out mainly on visceral organs in acute septicemic cases. On the other hand, no myopathological changes of acute swine erysipelas have been sufficiently studied as yet. Then, the authors tried not only to demonstrate myopathological changes but to know their histopathogenesis of acute swine erysipelas.

The present paper deals with the histopathology of muscle in acute swine erysipelas experimentally induced in animals originated from specific-pathogen-free (SPF) swine in order to obtain accurate findings of the disease.

MATERIALS AND METHODS

Bacterial strain: The freeze-dried high virulent FUJISAWA strain of *Erysipelothrix insidiosa* supplied by Dr. K. ANDO, of the National Institute of Animal Health, Tokyo, was used for the experiments. It was incubated in modified Allen's broth (tryptose broth base with additives: Tween-80, 0.1%; crystal violet, 1:200,000; and NaN_3 , 1:10,000) at 37°C for 18 hours. Then it was suspended in broth at the rate of 6.6×10^8 per 0.1 ml, and 0.1 ml of this suspension was inoculated into animals originated from SPF swine. The spleens were removed and pooled. Then, they were minced, freeze-dried at -80°C, and stored for the subsequent inoculation test. Before animal experiments, the stored preparations were inoculated on the modified Allen's broth.

Swine: Two (four-month-old females) and four (one, six-month-old castrated male, Case 3, and three, six-month-old females, Cases 4, 5, and 6) swine were used in the first and second experiments, respectively, as shown in Table 1. They had been produced at a SPF pigsty of the Chiba Serum Institute and were reared in conventional environments with an ordinary commercial food from the day of inoculation. Cases 5 and 6 of the second experiment were administered orally with swine erysipelas vaccine (live culture-modified) six months before inoculation.

Inoculation: The inoculation site of the abdomen was shaved, washed, and disinfected with alcohol. Then, 0.1 ml of bacterial suspension containing 1.1 or 2.0×10^8 organisms was inoculated intradermally.

Body temperature was taken every day at 10 a.m. and 3 p.m. Clinical signs were observed at the same time.

Histopathological examination: Autopsy was carried out as soon as possible after death. Tissue specimens were collected from skeletal muscles, including musculus capitis (m. masseter), mm. colli, mm. dorsi (m. longissimus), mm. thoracis (m. intercostalis), mm. abdominis, mm. cinguli extremitatum thoracicarum, mm. extremitatis thoracicae liberae,

Table 1. Animals Used in the Experiments

Experiment No.	Case No.	Sex	Age (months)	Days from inoculation to termination	Dose of bacteria inoculated
1	1	♀	4	5	$1.1 \times 10^8 / 0.1$ ml
	2	♀		4	
	3	♂	8	4	
2	4	♀		5	$2.0 \times 10^8 / 0.1$ ml
	5	♀		6	
	6	♀		6	

Remarks: In Cases 5 and 6 swine erysipelas vaccine was injected 6 months before inoculation.

Table 2. Degenerative Changes in Skeletal Muscle

Case No.	1	2	3	4	5	6
Mm. capitis	+	+		—		—
Mm. colli		+	+	—	+	
Mm. cinguli extremitatum thoracicarum	—	+	+	+++	++	+++
Mm. extremitatis thoracicae liberae	+	+++	+++	+++	+++	++
Mm. thoracis	—	+	+	+	+	—
Mm. abdominis	—	+++				+++
Mm. dorsi	+	++	—	+	+	
Mm. femoris	+	+++	++	+++	+++	+++
Diaphragm	+	+	—	+		

Remarks: +, mild; ++, moderate; and +++, severe changes.

mm. femoris, and diaphragm. The liver, spleen, kidney, heart, lung, stomach, small and large intestines, lymph nodes, adrenal gland, thyroid gland, testis, ovary, bone marrow, and skin were also examined. The tissue specimens were fixed in 10% formalin solution, embedded in paraffin, and cut into sections, which were stained with hematoxylin and eosin. Sudan III, periodic acid-Schiff (PAS) stain, Azan stain, and Gram stain were used, if necessary, for staining.

RESULTS

1. Clinical findings

Loss of appetite, somnolence, tachypnea, and palpitation appeared 2 days after inoculation. Three to 4 days after inoculation, the swine were willing to lie down. Reddish exanthemata appeared on the skin. Then they increased in number and size, and fused with one another, especially in the lower part of the abdomen.

As shown in Fig. 1, body temperature exceeded 40°C on the next day of inoculation. The course of disease up to the termination was 4 to 6 days after inoculation.

2. Macroscopic findings

A cloudy appearance and edematous changes were seen sometimes in mm. femoris and mm. extremitatis thoracicae liberae. Cloudy foci and hemorrhages were present in the mm. femoris of Cases 2 and 4 in the vicinity of the femoral joint. The additional

Fig. 1. Body Temperature of Infected Swine

changes were observed passive congestion of the liver, cloudy swelling, infarction, and hemorrhage of the kidneys, acute hyperemia of the spleen, passive congestion and hemorrhage of the lungs, swelling and focal necrosis of lymph nodes, cloudy heart muscle, and an increase in volume and turbidity of the joint fluid.

3. Microscopic findings

(1) Skeletal muscle: Many foci of degenerative changes were distributed widely in the skeletal muscle, as shown in Table 2. The most remarkable lesions were diffusely present in mm. femoris, mm. extremitatis thoracicae liberae, and mm. abdominis. Focal lesions were predominant in the other portions of skeletal muscle, especially in Case 1.

There were two patterns of degeneration in the skeletal muscle. Some muscle fibers showing hyaline degeneration and swelling were broken into fragments or vacuolated with loss of cross-striation. In some of these fibers, numerous sarcolemmal tubes, which might otherwise be empty, were filled with faintly acidophilic granules or faintly acidophilic to basophilic homogenous substances (Fig. 1). Although the cellular response was generally poor in the lesions, there were accumulations of neutrophils and macrophages and an increase in fibroblastic and histiocytic elements in the interstitial area of degenerated muscle. Mitotic figures were often observed. Calcification was also found in the mm. femoris of Case 4 and the mm. extremitatis thoracicae liberae (Fig. 2) and mm. femoris of Case 5. Fibrosis occurred to the mm. femoris and mm. extremitatis thoracicae liberae of Cases 4 and 5. "Giant muscle cells," containing basophilic sarcoplasm, appeared in the mm. coli of Case 3 and the mm. extremitatis thoracicae liberae of Case 5. Interstitial edema (Cases 4, 5, and 6), hemorrhage (Cases 4 and 6), and serofibrinous exudation (Case 6) were also recognized. Gram staining revealed multiple bacterial emboli. Masses of bacteria were found in proliferated endothelial cells, as well as in the degenerated parenchyma and interstitium (Fig. 9). Fat staining revealed a number of fat droplets in the degenerated areas.

In the other pattern of degeneration, swollen muscle fibers were of irregular shape, showing winding shrinkage, strong eosin affinity, and no cross-striation. Interstitial histiocytic proliferation was of low or moderate degree with few leukocytic elements (Fig. 3). These findings were obtained from the mm. abdominis, mm. femoris, and mm. extremitatis thoracicae liberae. Gram staining revealed bacterial emboli in degenerated areas. Few fat droplets were seen.

In the mm. femoris of Cases 2 and 4, coagulation necrosis was extensive together with severe hemorrhages.

In and around the degenerated areas, there were fibrin and fibrinoid thrombi with

proliferation of endothelial cells, and vacuolar and edematous thickening of vascular walls (Fig. 7), especially in lesions of coagulation necrosis in Case 2. In addition, there were hyaline swelling, loosening with pyknosis, and loss of nuclei in the smooth muscle fibers of vascular walls (Cases 1 to 4 and 6) (Fig. 8), degeneration and loss of peripheral nerve fibers (Cases 2, 4, 5, and 6), and degeneration of muscle fibers with giant cell formation in muscle spindles (Cases 1, 4, 5, and 6) (Fig. 6).

(2) Heart muscle: The hyaline fragmentation and swelling of muscle fibers were observed in the mm. papillares of Cases 3 to 6. These changes were frequently associated with infiltration of neutrophils, swelling and proliferation of reticuloendothelial cells, hyperplasia of fibroblasts, formation of thrombi and emboli, edema, and hemorrhage. Furthermore, the hyaline degeneration and interstitial histiocytic proliferation were present in some other regions of Cases 4 and 6. In addition, hyaline swelling and fragmentation of vascular smooth muscle were seen in Cases 3 and 4 (Fig. 5). Gram staining revealed the presence of multiple bacterial masses in blood vessels. Some bacteria were phagocytosed by endothelial cells in inflammatory regions.

(3) Smooth muscle: Remarkable degeneration was recognized in the muscle layers of the small intestine of Case 4. The swollen smooth muscle fibers were stained strongly with eosin, showing fragmentation accompanied by karyorrhexis and karyopyknosis. Cellular response was poor. Fibrin and fibrinoid thrombi were observed in intermuscular connective tissues and submucosa (Fig. 4). In Gram staining, no bacterial masses were recognized in any region. No remarkable changes of smooth muscle were seen in Case 4 or any other case.

(4) In other organs, the following changes were observed: Focal degeneration of striated muscle fibers in the tongue and esophagus, infarction and granular and hyaline droplet degeneration of the kidneys, focal necrosis of uriniferous tubules with hyaline casts, an increase in glomerular cells with thrombus in the kidneys, passive congestion and intralobular focal necrosis in the liver, stagnant edema and hemorrhage in the lungs, infectious spleen, acute lymphadenitis, catarrhal and diphtheroid enteritis, and activation of reticuloendothelial cells.

DISCUSSION

The present experiment has revealed that degenerative changes in muscle fibers are distributed widely in the skeletal, cardiac, and smooth muscle, as well as the striated muscle of the tongue and esophagus. The changes were the most remarkable in skeletal muscle, especially in mm. femoris, mm. extremitatis thoracicae liberae, and mm. abdominis.

In lesions of striated and smooth muscle, muscle fibers showed hyalinosis, fragmentation, vacuolarization, and loss of cross-striation. In the skeletal muscle, the primary changes were such waxy degeneration as encountered in other muscle diseases of domestic animals, hemoglobinemia paralytica equorum, bluetongue, and muscular rheumatism in lambs, and subsequent fibrosis, calcification, and a slight regeneration of muscle fibers. The morphological changes of the heart and smooth muscle were essentially the same as those of the skeletal muscle. The changes of the skeletal muscle, however, were higher in severity and wider in distribution than those of the other muscles. Furthermore, interstitial cellular response was scarcely observed in the smooth muscle. Parenchymal degeneration was mainly recognized together with changes of myocarditis in the heart muscle.

The histopathogenesis of muscular changes in acute bacterial infection remains obscure. JUBB and KENNEDY³⁾ described that some toxic effect or metabolic disturbance

might be related to the myopathy occurring in bacterial diseases. In experimental swine erysipelas, ROONEY⁴⁾ found in the skeletal muscle segmental hyaline and granular necrosis of muscle fibers with capillaries plugged by masses of mononuclear cells and bacteria. He emphasized that the capillary lesions played an important role in the pathogenesis of muscular changes. From the present experiment, it was presumed that the vascular alterations might be important in the formation of muscular lesions in the case of acute swine erysipelas, since many fibrin and fibrinoid thrombi were found in and around the degenerated areas of muscular tissues, especially skeletal muscle. In these areas, bacterial embolism, phagocytosis of bacteria, and swelling and proliferation of endothelial cells were recognized. Furthermore, hyalinization, vacuolarization, edema formation, and cellular proliferation in blood vessels of small and medium caliber, and hyaline swelling and loosening of the smooth muscle of blood vessels of large caliber were present. COLLINS and GOLDIE¹⁾, and GODGLÜCK and WELLMANN²⁾ reported such vascular lesions in the case of experimental swine erysipelas. SATOH et al.⁵⁾ observed the same lesions in spontaneous acute cases of this disease. In peracute enzootic muscle dystrophy of foals, YAMAGIWA et al.⁶⁾ stated that the vesicle formation of arterial walls in fresh muscle lesions might be very significant for the establishment of this disease. Also in the present experiment, it was speculated that the changes of vascular walls caused by the direct and/or indirect effect of bacteria might be related intimately to the formation of muscular lesions. In the authors' experiment with rats, intramuscular inoculation with the culture filtrate of *Erysipelothrix insidiosa* caused changes of the vascular wall together with muscular lesions (unpublished data).

Moreover, it was considered that some toxic or mechanical effects of bacteria might play a considerable part in the formation of muscular lesions. In rats inoculated intramuscularly with the culture filtrate of *Erysipelothrix insidiosa*, waxy degeneration, fibrosis, calcification, and regeneration of muscle fibers were observed at the site of injection of skeletal muscle (Fig. 10). In the present experiment, necrotic and degenerative changes were also produced in the liver and kidney, and bacterial masses were present within degenerated muscle. It is difficult, however, to determine a toxic factor acting directly either upon muscular tissues or upon blood vessels and followed by secondary muscular degeneration.

ROONEY⁴⁾ induced the degeneration of peripheral nerve fibers experimentally in acute swine erysipelas. Also in the present experiment, degeneration and loss of some peripheral nerve fibers were observed in the skeletal muscle of four cases, although the changes of peripheral nerve fibers were rather mild and detectable also in unaffected areas.

SUMMARY

Histopathological investigation was carried out on the muscles of six swines originated from specific-pathogen-free (SPF) swine and inoculated intradermally with *Erysipelothrix insidiosa*. Degenerative changes were demonstrated in most skeletal muscles of all the cases, in the heart muscle of four cases, and in the smooth muscle of the small intestine of one case. The changes were the severest in skeletal muscle, especially in mm. femoris, mm. extremitatis thoracicae liberae, and mm. abdominis. Waxy changes were sometimes followed by fibrosis, calcification, and regeneration of muscle fibers. Vascular alterations and some toxic or mechanical effects of bacteria would be important in the histopathogenesis of muscular degeneration.

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実験的急性豚丹毒における筋肉の病理組織学について

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実験的に豚丹毒菌を感染させた SPF 由来豚、6 例の筋組織について、病理組織学的に検索が行なわれた。筋線維の蠟様変性は、全例のほとんどすべての骨格筋、4 例の心筋および 1 例の小腸の平滑筋に認められた。病変は骨格筋、特に大腿筋、

自由前肢筋および腹筋において、最も高度であった。時に、線維化、石灰変性および筋線維の再生のような二次的变化も認められた。筋変性の病理組織発生において、血管変化および菌体の毒性的または機械的影響が重要であろうと考えられた。

EXPLANATION OF PLATES

PLATE I

All the specimens were stained with hematoxylin and eosin, except that of Fig. 9.

- Fig. 1. Mm femoris showing remarkable fragmentation and vacuolarization with poor cellular response. Case 4. $\times 60$.
- Fig. 2. Mm. extremitatis thoracicae liberae. Degeneration, massive histiocytic and fibroblastic hyperplasia, and calcification are seen. So-called muscle giant cells are present. Case 5. $\times 170$.
- Fig. 3. Mm. abdominis. Irregularly swollen muscle fibers show winding and shrinkage with a slight interstitial cellular response. Case 2. $\times 470$.
- Fig. 4. Degeneration of smooth muscle of the small intestine. Case 4. $\times 680$.

PLATE II

- Fig. 5. Heart muscle showing hyaline degeneration and interstitial histiocytic proliferation. Smooth muscle of the vascular wall also shows hyaline swelling and fragmentation. Case 4. $\times 600$.
- Fig. 6. Degeneration of muscle fibers with increase of cellular elements in muscle spindle in mm. extremitatis thoracicae liberae. Case 4. $\times 680$.
- Fig. 7. Edematous and vacuolar thickening in the arterial wall with cellular proliferation in mm. femoris. Case 6. $\times 680$.
- Fig. 8. Mm. cinguli extremitatum thoracicarum. Smooth muscle of the vascular wall shows hyaline swelling and loosening with pyknotic changes and loss of nuclei. Case 6. $\times 680$.
- Fig. 9. Bacterial masses and embolism within degenerated mm. femoris. Case 4. Gram staining. $\times 480$.
- Fig. 10. Waxy degeneration with calcification, histiocytic and fibroblastic hyperplasia in mm. femoris of a rat inoculated intramuscularly with culture filtrate of *Erysipelothrix insidiosa*. $\times 340$.