パパイン関節内投与により誘発した豚脚弱症の臨床モデル

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Clinical Model of Leg Weakness Induced by Intraarticular Administration of Papain in Pigs

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Abstract : To prepare models of osteochondrosis and osteoarthrosis as etiological factors for leg weakness, which is frequent among locomotory disorders in pigs, we conducted an induction study using 5% papain solution and examined its clinical symptoms and cartilage lesions.

The body temperature and clinical symptoms subsided after 3 days. Mild lameness persisted until Day 14. However, macroscopically observed cartilage lesions serially deteriorated, and the areas of cracking and erosion expanded.

Experimental models prepared by the infusion of papain solution into the knee may play an important role in the establishment of future techniques to diagnose osteochondrosis- or osteoarthrosis-related leg weakness.

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Key words : leg weakness, osteochondrosis, osteoarthritis, papain

Introduction

Recent advances in breeding techniques have facilitated the fattening of pigs in which good meat quality and high meat productivity are maintained. For breeding, pigs in which maturation is accelerated and physical status and litter size are large are commonly selected. However, such rapid development in a short period leads to excessive loading on the limbs, raising the important issue of locomotory disorders in fattening pigs. According to a statistical table published by the Livestock Cooperative Society, in all sows that died or were culled in 2005, the locomotory disorder-related mortality rate was 22.1%, the second highest[1]. In 2006, the incidence of locomotory disorder-related fatal incidents was also 21%[2]. Thus,

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locomotory disorders in sows have resulted in marked financial losses in the pig livestock industry.

Among locomotory disorders, leg weakness mainly causes a primary lesion in the articular cartilage, leading to apparent abnormalities of the limbs. Its main etiological factors include non-infectious osteochondrosis and osteoarthrosis[3]. These disorders may be etiologically associated with heredity, age, and the speed of development[4-7]. Furthermore, osteochondrosis is common in gilts and young sows. The incidence differs among lineages. However, a study reported an incidence of approximately 100% in the terminal stage of fattening[8]. On the other hand, osteoarthrosis is frequent in old sows such as breeding pigs[3]. In any case, osteochondrosis and osteoarthrosis may be fatal for pigs in which rapid weight gain is enforced.

The two disorders both cause leg weakness. However, currently, the presence or absence of cartilage lesions is confirmed by the collection of cartilage specimens after the pigs have been slaughtered. In disorders in which cartilage is destroyed, such as osteochondrosis and osteoarthrosis, matrix metalloprotease III (MMP-3), which decomposes the proteins including proteoglycan that comprise the cartilage matrix, plays a main role in cartilage destruction, facilitating serological diagnosis before culling[9]. However, this method has not been applied to clinical practice. Therefore, if cartilage lesions can be diagnosed based on clinical symptoms before culling by preparing an experimental non-inflammatory cartilage disease model and clarifying the relationship between cartilage lesions and clinical symptoms, it may minimize the financial losses caused by leg weakness.

It is known that the intra-articular administration of the protein-digesting enzyme papain to laboratory animals such as rabbits and guinea pigs induces cartilage lesions related to osteochondrosis and osteoarthrosis[10-12]. However, no study using pigs has been published.

In this study, we experimentally prepared a cartilage disease model by intra-articularly administering the protein-digesting enzyme papain to fattening pigs and macroscopically examined cartilage lesions to investigate their relationship with leg weakness.

Materials and Methods

We used six 120-day-old castrated LW×D fattening pigs weighing 75 to 82 kg, which had been manufactured/bred in the pigpen of Azabu University. In 4 of these pigs, powdery papain (MERCK, Inc.) was dissolved in physiological saline at a concentration of 5% (papain solution), and 3 m*l* of this solution was infused into the articular space at the right distal femur end using a 23G injection needle through an area 3 cm lateral to the patellar ligament on the lateral side of the right knee, as test animals. In 2 other pigs, 3 m*l* of physiological saline were similarly infused, as control animals.

In all pigs, we investigated body temperature, vitality, appetite, the affected limb's landing condition, and standing condition every day after the infusion and performed palpation to examine any heat sensation/swelling at the injection site. To determine the grade of swelling, we measured articular circumference. To evaluate the inflammatory response, we measured the erythrocyte sedimentation rate (ESR). For ESR measurement, blood was collected from each animal every day from the day of papain infusion until 7 days after infusion. Thereafter, blood was collected at 1-week intervals until 1 month after injection. Immediately after collection, 5 m*l* of blood were placed in an EDTA tube, mixed, and then ESR

was measured within 2 hours.

A pipette for blood sedimentation (Japan Clinical Machine Industry, Co., Ltd.) was filled with blood, and allowed to rest for 1 hour to determine ESR.

In the test animals, 1 pig was autopsied every 7 days for the first 30 days after papain infusion. In the control animals, 1 pig was autopsied at 7 and 30 days after infusion. We macroscopically examined the articular cartilage surface at the distal femur end of the knee into which papain or physiological saline had been intra-articularly infused. For observation, the articular surface was stained using the India ink stamp method.

This study was conducted in accordance with the Azabu University Animal Experiment Protocol No. 0608011.

Results

Clinical signs

In the test animals, standing disturbance and claudication of the affected limb were observed immediately after papain infusion. Some pigs showed anorexia. However, their appetite returned normal state 3 days after the infusion. Mild claudication of the affected limb and landing disturbance persisted until 14 days after infusion. In the control animals, these symptoms were not noted.

Body temperature

The mean body temperature in the control group was 39.0℃. There were no marked changes in temperature until the end of this experiment. In the test animals, slight post-infusion inflammation-related fever (39.6-39.8 ℃) was observed 3 to 4 days after the intra-articular infusion of papain. However, the mean body temperature at Day 5 was 39.1℃, showing no marked difference in comparison with the control animals.

Articular circumference

Using a tape measure, we determined articular circumference, in a standing position, around the knee of the right hind leg into which papain or physiological saline had been intra-articularly infused. In the control group, there was no articular swelling after the infusion of physiological saline, with a mean circumference of 30.3±0.3 cm. In the test animals, the pig with the most marked articular swelling showed a 4 cm increase in its articular circumference 2 days after papain infusion. However, the swelling rapidly subsided. The mean articular circumference in this animal was 30.0±1.1 cm, with no marked difference in comparison to the control. Furthermore, palpation revealed marked arthralgia and heat sensation within 7 Days of the papain infusion, although there were individual differences. Thereafter, palpation did not reveal any abnormalities in any animals in the test.

ESR

The ESR levels on first 7 Days are shown in Fig. 1. In the test segment (n=3), all animals showed an increase in ESR immediately after the infusion of papain solution. In particular, this parameter rapidly increased the day after infusion, but rapidly decreased after 2 days. However, after Day 7, the values were similar between the test and control.

Macroscopic examination of the articular surface

We examined the articular cartilage surface at the distal femur end of the right knee using the India ink stamp method, in which the lesion size and shape can be evaluated based on the amount of India ink penetrating into the lesion site by applying India ink to the articular surface and then wiping it off.

In the test animals, the articular cartilage surfaces of the lateral and medial condyles at the distal femur end 7 days after the infusion

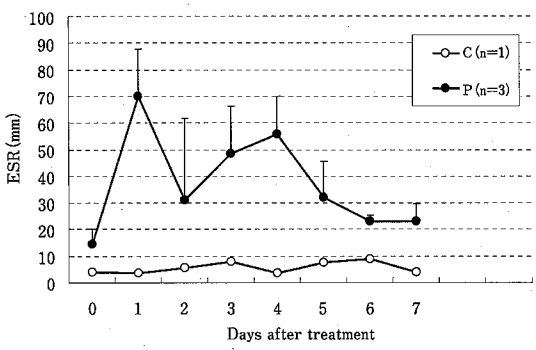

Fig. 1. Changes of ESR levels on first 7days after intraarticular administration with papain in pigs
C indicates ESR levels in control pig that slaughtered at Day 7. (n=1)
P indicates the average of ESR levels after intraarticular administration with papain. (n=3)
(treated animals were slaughtered at Day 7, Day14 and Day 21.)
Control pig that slaughtered at Day 30 and treated pig that slaughtered at Day 30 is not tested.

of papain solution to the knee were not smooth or elastic. Extensive cartilage erosion and rupturing were confirmed (Fig. 2-A). Fourteen days after the infusion of papain solution, a deep groove-like rupture was observed in the articular cartilage of the medial condoyle in addition to extensive erosion of the articular cartilage of the lateral condyle at the distal femur end (Fig. 2-B). Twenty-one days after infusion, the articular cartilage regions of the lateral and medial condyles at the distal femur end were slightly brown. Erosion of the articular cartilage of the lateral condyle had expanded and involved deeper layers (Fig. 2-C). Groove-like rupturing of the articular cartilage of the medial condyle had also slightly expanded. Thirty days after infusion, the articular cartilage regions of the lateral and medial condyles at the distal femur end were slightly dark. As shown in Fig. 2-D, extensive cracks/erosion were observed in the articular cartilages of the lateral and medial condyles, and cartilage thinning was noted.

The articular cartilage surfaces of the lateral and medial condyles of the knee at the distal femur end 7 and 30 days after the infusion of physiological saline into the right knee as a control were smooth and lustrous, with appropriate hardness and elasticity. Neither erosion nor cracking, as observed in the test animals, were noted.

Discussion

It is known that the intra-articular infusion of papain, a protein-digesting enzyme, into rabbits and guinea pigs induces marked erosion and rupturing of the articular cartilage via the decomposition of articular cartilage proteoglycans. Currently, such lesions are used as arthrosis deformans and rheumatoid arthritis models to evaluate the treatment response to these conditions and develop diagnostic techniques for them. In this study, to clarify the pathogenesis of non-infectious osteochondrosis and osteoarthrosis, the main etiologies of leg weakness, which is frequent in pigs, and their clinical findings, we intra-articularly infused papain solution into the hind leg knees of fattening pigs and investigated articular cartilage lesions at the distal femur end, which is a secondary site of osteochondrosis and osteoarthrosis, at 1-week intervals between 7 and 30 days after papain infusion. During this period, extensive erosion and cracking were observed in the articular cartilage at the distal femur end facing the knee. This was consistent with findings previously reported in rabbits and guinea pigs. Thus, it was confirmed that articular cartilage lesions could also be experimentally induced in pigs.

The main etiologies of leg weakness, which

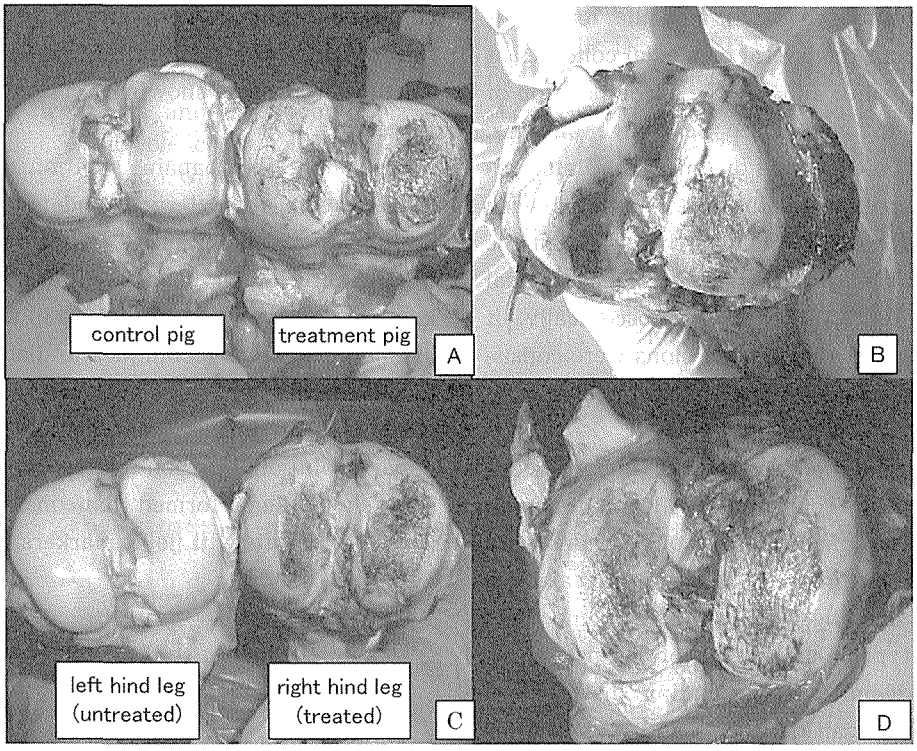

Fig. 2. Comparison of articular cartilage erosion on the distal femur of the right hind leg by the India ink sampling method after intraarticular administration of papain
A. Treatment and control pig at Day 7 after the intraarticular administration of papain.
B. Treated pig at Day 14 after the intraarticular administration of papain.
C. Treated right hind leg and untreated left hind leg at Day 21 after the intraarticular administration of papain.
D. Treated pig at Day 28 after the intraarticular administration of papain.

is as an important issue in the pig-farming field, osteochondrosis and osteoarthrosis, mainly developed in the articular cartilage of the distal-femur-end, the articular cartilage medial condyle of the knee, and in the medial condyle of the distal humeral end of the elbow. Osteochondrosis lesions expand via the necrosis of chondrocytes and degeneration of the cartilage matrix localized in the deep layer of the articular cartilage, leading to articular cartilage rupture, ulcers, exfoliation, and erosion as serious findings. Osteoarthrosis lesions also expand via similar changes in the superficial layer of the articular cartilage. In this study involving the infusion of papain solution, extensive erosion and rupturing were observed in the articular cartilage of the lateral and medial condyles at the distal femur end a short period after infusion. No lesions were localized in the medial condyle at the distal femur end. As direct intra-articular infusion of papain solution was performed, the solution may have diffused with synovial fluid in the articular space, resulting in lesion enlargement involv-

ing the total area of the articular cartilage, regardless of the lateral or medial condyles at the distal femur end, via the decomposition of the proteoglycan-based cartilage matrix. Whether papain solution infusion-related articular cartilage lesions are associated with osteochondrosis or osteoarthrosis must be evaluated based on the results of histological examination. However, macroscopically, it was confirmed that these lesions were consistent with serious lesions related to osteochondrosis or osteoarthrosis.

The articular circumference markedly increased within 7 days of the infusion of papain solution into the knee, although there were individual differences. Palpation revealed arthralgia and heat sensation. Some pigs showed typical symptoms of leg weakness such as claudication, standing disturbance, and unstable gait between 7 and 14 days after the infusion of papain solution. The ESR value rapidly increased immediately after the infusion, but then rapidly decreased. ESR is mainly employed as a simple diagnostic method to evaluate abnormalities such as inflammation and allergy in human clinical practice. However, the phenomenon observed in this study after the infusion of papain solution into the knee may have been based on the response of the articular cartilage facing the articular space and its peripheral tissue to the proteolytic actions of papain.

In previous studies involving the infusion of papain solution, rabbits and guinea pigs were used. However, these animals are highly sensitive to papain (at concentrations ranging from 0.2 to 4.0%, marked articular lesions were observed)[10-12]. However, no study involving the infusion of papain solution into pigs has been conducted. As the sensitivity of pigs to papain is unclear, we infused 5% papain solution in this study. As a result, acute symptoms including slight fever were noted early after infusion. However, the clinical symptoms subsided after 1 week, showing a chronic course thereafter.

Based on the papain solution infusion-related acute clinical signs and macroscopic findings of the joint, animal models prepared by the infusion of papain solution into the knee may play an important role in the establishment of future techniques to diagnose osteochondrosis- or osteoarthrosis-related leg weakness. In future, an induction study of papain solution infusion-related articular lesions should be performed that considers serological diagnosis with joint markers.

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パパイン関節内投与により誘発した豚脚弱症の臨床モデル

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要　約　豚の運動器疾患で多く見られる脚弱症の原因である骨軟骨症と骨関節症のモデル作成として，5%パパイン液を用いた発生試験と，その臨床症状や軟骨病変の観察を行った。体温や臨床症状は3日ほどで改善したが，軽度の跛行は14日まで観察された。しかし，軟骨の肉眼的な病変は日を追うごとに重篤化し，亀裂や糜爛などの病変の領域は広範囲になった。膝関節へのパパイン液注入の試みは，今後の骨軟骨症や骨関節症による脚弱の診断技術を確立する上で，大きな役割を果たすモデルになりえる可能性が考えられた。

キーワード：脚弱症，骨軟骨症，骨関節症，パパイン