The Presence of Disease-Associated Prion Protein in Skeletal Muscle of Cattle Infected with Classical Bovine Spongiform Encephalopathy

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ABSTRACT. The aim of this study was to investigate the presence of disease-associated prion protein (PrPSc) in the skeletal muscle of cattle infected with classical bovine spongiform encephalopathy (C-BSE). The study was carried out systematically in 12 different muscle samples from 43 (3 field and 40 experimental) cases of C-BSE; however, muscle spindles were not available in many of these cases. Therefore, analysis became restricted to a total of 31 muscles in 23 cattle. Even after this restriction, low levels of PrPSc were detected in the muscle spindles of the masseter, intercostal, triceps brachii, psoas major, quadriceps femoris and semitendinosus muscles from 3 field and 6 experimental clinical-stage cases. The present data indicate that small amounts of PrPSc are detectable by immunohistochemistry in the skeletal muscles of animals terminally affected with C-BSE.

KEY WORDS: BSE, muscle spindle, prion, skeletal muscle.

Classical bovine spongiform encephalopathy (C-BSE) in cattle is a fatal neurodegenerative disorder belonging to a group of transmissible spongiform encephalopathies (TSEs). C-BSE was first identified in the United Kingdom in 1986 [18] and subsequently spread to Europe, North America and Japan. The disease is characterized by spongiform changes and the accumulation of a disease-associated abnormal form of prion protein (PrPSc) in the central nervous system. PrPSc is commonly accepted as the pathological agent of TSEs and may be a post-translationally modified form of a normal cellular prion protein (PrPC) [14]. The C-BSE agent is transmissible to various mammalian species. For example, the variant form of Creutzfeldt-Jakob disease (vCJD) in humans likely resulted from consumption of C-BSE-contaminated foodstuff [19]; therefore, issues regarding C-BSE and variant CJD have increased public health concerns regarding the safety of meat products used for food.

A low level of infectivity was identified in the semitendinosus muscle of a clinically C-BSE-affected field case through a mouse bioassay using highly sensitive transgenic mice overexpressing bovine PrPc [3]. Recently, PrPSc deposition in skeletal muscles of cattle experimentally infected with atypical BSE was visualized by immunohistochemistry (IHC) [8, 15]. The detailed topological distribution of PrPSc in the muscular tissues of C-BSE-infected cattle, however, remains unclear [2]. Here, we describe the localization of immunolabeled PrPSc in the skeletal muscles of cattle with natural and induced C-BSE infections.

All animal handling and experimental protocols were approved by the Institutional Animal Use and Care Committee of both the National Institute of Animal Health and Hokkaido Animal Research Center prior to the experiments being carried out.

This study was carried out in 43 C-BSE-infected cattle including 3 naturally-infected fallen stock cases (BSE/JP17, 21 and 22), 28 orally dosed cattle (6 clinically and 22 preclinically) and 12 intracerebrally administered cattle (9 clinically and 3 preclinically) as previously described in detail [4, 12, 13]. As a control, 4 un inoculated Holstein calves were used, 2 of which were sacrificed at the ages of 27 months and 104 months.

Twelve skeletal muscle samples, including the masseter muscle, pectoral muscle, intercostal muscle, triceps brachii muscle, longissimus thoracis muscle, psoas major muscle, gluteus medius muscle, quadriceps femoris muscle, semitendinosus muscle, diaphragm and tongue (apex and dorsum), were chosen for sampling as these are most frequently consumed by people in Japan. Each muscle sample was collected from each animal at necropsy and cut into 2 pieces, one of which was fixed in 10% buffered formalin solution (pH 7.4) for hematoxylin and eosin (H&E) staining and IHC, while the other was frozen at −80°C for western blotting (WB). IHC for PrPSc was performed using a mouse monoclonal antibody (mAb) F99/97.6.1 (VMRD, Pullman, WA, U.S.A.) by the 2-step polymer method (Histofine Simple Stain MAX-PO; Nichirei, Tokyo, Japan) or tyramine signal amplification (TSA-biotin system; PerkinElmer, Boston, MA, U.S.A.)
The biochemical properties of PK-resistant PrPSc were analyzed by WB using mAb T2 as described in detail elsewhere [10]. PrPSc was only detected in muscle spindles, while no other structures of the muscle samples showed reactivity by IHC. Specifically, PrPSc immunolabeling was not detected in myofibrils, intramuscular nerve fascicles or in most ganglia of tongue muscular tissues for all muscle samples examined. The PrPSc immunolabeling intensity was weak using the conventional 2-step polymer method, and the TSA method gave the best results (Fig. 1). The morphological appearance of PrPSc presented as granular or dot-like deposits. Although the study was carried out systematically in 12 skeletal muscles including tongues and diaphragm of 43 C-BSE-infected cattle, muscle spindles were not available in many of these samples. Therefore, the study was restricted to analysis on H&E-stained sections of 23 animals (Table 1). The total number of muscle sampled owing to usable spindles was...
No muscle spindles were detected in the gluteus medius muscle, diaphragm or tongue. Even after this restriction, the global frequency of immunolabeled PrPSc detection was 9 (3 natural and 6 experimental) of 23 cases. Positive immunolabeling was detected in 16 of the 31 muscle samples containing spindles, including the masseter muscle (4/9; number of positive samples/number of detected samples), intercostal muscle (2/5), triceps brachii muscle (4/4), psoas major muscle (1/2), quadriceps femoris muscle (1/1) and semitendinosus muscle (3/3). In experimentally challenged animals, PrPSc detection was associated with clinical symptoms, but not with preclinical status. No positive spindles were observed in the 14 preclinical and 4 control animals. By WB analysis, a very weak signal for PrPSc was detected in 2 different muscle samples from naturally (masseter muscle from BSE/JP17) and experimentally (intercostal muscle from ID#5413) C-BSE-infected animals (Fig. 2). However, a detectable PrPSc signal was obtained from only 1 of 3 tissue pieces; adjacent locations were used for PrPSc immunohistochemistry within the same tissue in each case.

The results of this study indicate that low amounts of PrPSc are deposited in the muscle spindles of C-BSE-infected cattle. To the best of our knowledge, this is the first, or at least the most comprehensive, report on the localization of PrPSc by IHC in skeletal muscles of C-BSE affected cattle. Localization of PrPSc in the muscle spindles of skeletal muscles in the study was consistent with that of atypical BSE in cattle [8] and natural scrapie in sheep [1]. In addition, immunolabeled PrPSc localized at the terminal nerve endings of myofibrils has been reported in hamsters with scrapie and [16] in rodent and primate models with C-BSE and CJD, respectively [6, 9, 17].

PrPC is primarily expressed in neural tissues, but is also distributed in non-neural organs and tissues, such as the spleen, lymph node, heart, skeletal muscle, kidney, uterus, adrenal gland, intestine and mammary gland [7]. In muscle tissues, PrPC is located primarily at the neuromuscular junction [5]. Somatic motor neurons known as efferent nerve fibers arise from the ventral horn of the spinal cord and innervate skeletal muscle tissues at the neuromuscular junction via alpha motor neurons or intrafusal muscle fibers of the muscle spindles via gamma motor neurons. In addition, type Ia afferent sensory fibers connect to the muscle spindles. However, there are no precedents for a primary role for sensory neural pathways in the pathogenesis of BSE [10]. PrPSc accumulation in the peripheral nervous tissues may be attributed to a high degree of neurotropism in C-BSE [3, 10]. Our results indicate the centrifugal spread of the infectious agent from central nervous tissues through the somatic motor and/or sensory pathways to the muscle spindles of various muscle tissues during the clinical stage of the disease in both naturally occurring and experimentally-induced C-BSE.
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