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Increased expression and localization of cyclooxygenase-2 in astrocytes of scrapie-infected mice

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Abstract

A number of aspects of the pathogenesis of scrapie, the archetype disease of the transmissible spongiform encephalopathies (prion disorders), remain to be elucidated. There is increasing evidence that there are cerebral based inflammatory processes that may contribute to the pathogenesis and to the progression of a number of neurodegenerative disorders, including prion diseases. In peripheral tissues, a key element that controls the generation of proinflammatory mediators is the highly inducible protein cyclooxygenase-2 (COX-2). In this study, in order to examine the possible association of COX-2 with the pathogenesis of scrapie, we analyzed the expression level and the cellular localization of COX-2 in the brains of control and scrapie-infected mice. The COX-2 mRNA and protein levels were increased significantly compared to the control group of mice. By immunohistological analysis, intense immunoreactivity of COX-2 was localized primarily in reactive astrocytes, with virtually no staining in sections from control mice. The staining for COX-2 was co-localized with the pathological form of the prion protein (PrP^{Sc}) and with nuclear factor-kappa B (NF-κB). These results suggest that the upregulation of COX-2 expression in astrocytes may be related to the accumulation of PrP^{Sc}, and that COX-2 may then lead to the progression of scrapie, possibly by propagation of a cerebral inflammatory response.
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Keywords: Scrapie; Prion; Cyclooxygenase-2; Reactive astrocytes; Cerebral inflammatory process

1. Introduction

The spongiform encephalopathies (prion disorders) are a group of transmissible diseases with common pathological changes of the CNS, including neuronal cell loss, vacuolation, astrocytosis and, frequently, the presence of amyloid plaques (Aucouturier et al., 1999; Carp et al., 1994). Creutzfeldt–Jakob disease (CJD) in humans, scrapie in sheep, and bovine spongiform encephalopathy (BSE) in cows are the most extensively studied of prion diseases (Prusiner, 1998). The etiology of these diseases is thought to be associated with the conversion of a normal prion protein, PrP^{C}, into a pathogenic form, PrP^{Sc}, which shows greater protease resistance and accumulates in affected individuals, occasionally in the form of extracellular plaques (Prusiner, 1998). However, the pathogenesis and the molecular basis of neuronal cell death in these diseases remain to be elucidated.

There is increasing evidence that cerebral inflammatory processes may contribute to the pathogenesis and the progression of neurodegenerative disorders including prion diseases and Alzheimer's disease (AD) (Floyd, 1999; Kim et al., 1999; McGeer and McGeer, 1995; Mrak et al., 1995; Williams et al., 1997). In peripheral tissues, a key element that controls the generation of proinflammatory mediators is the highly inducible gene encoding cyclooxygenase-2 (COX-2, EC 1.14.99.1) (Vane et al., 1998). COX is the rate-limiting enzyme in the synthesis of prostaglandins (PGs) from their precursor, arachidonic acid, and

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there are two isoforms of COX, a constitutive type (COX-1) and an inducible type (COX-2) (Vane et al., 1998). Although COX-2 is newly synthesized in many cells and tissues in response to growth factors, tumor inducers, hormones, and various inflammatory agents, it is expressed constitutively in normal brain and plays a role in the regulation of several neuronal functions under physiological conditions (Yamagata et al., 1993). In a number of studies, it has been reported that the expression of COX-2 is increased in neurodegenerative disorders such as brain ischemia and AD (Nogawa et al., 1997; Oka and Takashima, 1997; Pasinetti and Aisen, 1998; Yasojima et al., 1999b). Many epidemiological studies indicate that use of non-steroidal anti-inflammatory drugs (NSAIDs) may delay or slow the clinical expression of AD (McGeer et al., 1996), suggesting that prostaglandins promote the inflammatory processes involved in the pathogenesis of this disease. The mechanism of action for NSAIDs is thought to result from the inhibition of COX (Vane, 1971).

Recently, Walsh et al. (2000) reported the selective upregulation of COX-2 immunoreactivity in glial cells presenting morphology similar to activated microglia in scrapie-infected brain. Although astrocytosis and microglial activation are pronounced features of scrapie pathology (Carp et al., 1993), a number of experimental findings suggest that astrocytes may be involved in the first step in neuropathological changes in this disease (Campbell et al., 1994; Carp et al., 1993; Diedrich et al., 1991a,b; Williams et al., 1997). In clinical animals infected with scrapie (263K or 22L strains), the major cell type containing large accumulation of PrP^{Sc} are known to be glial fibrillary acidic protein (GFAP)-positive astrocytes (Carp et al., 1993; Diedrich et al., 1991a; Williams et al., 1994).

Intense immunoreactivities of prostaglandin E_2 (PGE_2) and prostaglandin F2α (PGF2α), the metabolites produced in the COX pathway, have been reported in the brains of scrapie-infected mice (Williams et al., 1997, 1994). PGs are likely to contribute to neuroinflammatory processes that can augment neurodegeneration (O'Banion et al., 1996). During the course of scrapie, PGE_2 immunoreactivity was observed after PrP^{Sc} deposition and was predominantly associated with astrocytes (Williams et al., 1997). From these observations, it is suggested that the alteration in COX-2 expression in scrapie may be related to the accumulation of PrP^{Sc} in astrocytes, which may, in turn, participate in the progression of the disease. In the present study, we describe results on the expression level and cellular localization of COX-2 in the brains of scrapie-infected mice, correlate our findings with the occurrence of PrP^{Sc} and discuss the possible relationship of COX-2 to the disease process. The findings provide insights into the mechanism of host response to scrapie infection and to possible vulnerable sites for therapeutic intervention.

2. Materials and methods

2.1. Mice and scrapie strain

Male C57BL/6 mice 4–6 weeks of age were obtained from the Experimental Animal Center of Hallym University. The original stock of the ME7 scrapie strain, kindly provided by Dr. Alan Dickinson of the Neuropathogenesis Unit (Edinburgh, Scotland), was maintained by serial intracerebral passage of brain homogenate from terminally affected mice. Inocula were prepared from brain tissue homogenized in sterile phosphate-buffered saline (PBS) at 1% (w/v). Mice were inoculated intracerebrally with either 30 μl of scrapie or normal brain homogenate (control group).

2.2. Antibodies

Polyclonal antibodies to COX-2 and nuclear factor-kappa B (NF-κB) were purchased from SantaCruz Biotechnology (USA). Rabbit polyclonal antibody to ME7 PrP was kindly provided by Dr. Richard J. Kascsak (New York State Institute for Basic Research in Developmental Disabilities, New York, USA) (Kascsak et al., 1993). Rabbit polyclonal anti-glial fibrillary acidic protein (GFAP) and rat monoclonal anti-mouse F4/80 antibodies were purchased from Jackson Laboratories (USA) and Serotec (UK), respectively. Unless otherwise described, all other chemicals were from Sigma (USA).

2.3. Preparation of tissue

After the scrapie incubation periods of 150 days, 3 mice per group were sacrificed for immunohistocheimstry. Animals were anesthetized with 16.5% urethane and then perfused transcardially with cold PBS followed by cold 4% paraformaldehyde in PBS. The brains were immediately removed, cut into blocks, postfixed in the same fixative for 1 h at 4 °C, rinsed with PBS, dehydrated with ethanol, and embedded in polyester wax (Polyscience, USA). Coronal sections of the brain were cut with a microtome (Leica, Germany). For competitive RT-PCR, Western immunoblot, and PGE_2 determination, unfixed brains were immediately removed form anesthetized mice and stored at −70 °C until analysis.

2.4. Competitive RT-PCR

To determine the magnitude of mRNA induction of COX-2, we carried out competitive RT-PCR analysis by using non-homologous competitor of COX-2. First, a DNA competitor was synthesized for the construction of COX-2 RNA competitor using Competitive DNA Construction Kit (Takara, Japan) according to the manufacturer's instructions. Nucleotide sequences of the primers used were: sense (68 mer), 5′-*ATT-TAG-GTG-ACA-CTA-TAG-AAT-AC*A-CTC-ACT-CAG-TTT-GTT-GAG-TCA-TTC-**GTA-CGG-TCA-TCA-TCT-GAC-AC**-3′; antisense (54 mer), 5′-TTT-GAT-TAG-TAC-TGT-AGG-GTT-AAT-G**GC-GTG-AGT-ATT-ACG-AAG-GTG**-3′: where the underlined sequences indicate the position which is recognized by a pair of COX-2 specific primers (Ohara et al., 1998); where the bold sequences indicate the nucleotides which bind to the template DNA for the amplification of DNA competitor; where the italic sequences indicate the SP6 promoter which is recognized by SP6 RNA transcriptase. One μl of the DNA competitor was used for the synthesis of RNA competitor, which was done by *in vitro* transcription of DNA competitor using Competitive RNA

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