

Review

Heterozygous inhibition in prion infection

The stone fence model

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Abbreviations: PrP, prion protein; PrP^C, normal cellular isoform of PrP; PrP^{Sc}, abnormal isoform of PrP; CJD, Creutzfeldt-Jakob disease; vCJD, variant CJD; sCJD, sporadic CJD; PRNP, human PrP gene

Key words: prion protein, Creutzfeldt-Jakob disease, polymorphism, knock-in mouse, conversion, heterozygous inhibition, stone fence model

The human PrP gene (*PRNP*) has two major polymorphic codons: 129 for methionine (M) or valine (V) and 219 for glutamate (E) or lysine (K). The *PRNP* heterozygotes appear to be protected from sporadic CJD compared to the *PRNP* homozygotes. The molecular mechanism responsible for these protective effects of *PRNP* heterozygosity has remained elusive. In this review, we describe the inhibition of PrP conversion observed in a series of transmission studies using *PRNP* heterozygous animal models. In vCJD infection, the conversion incompetent human PrP 129V molecules showed an inhibitory effect on the conversion of human PrP 129M molecules in the 129M/V heterozygous mice. Furthermore, though the human PrP 219E and PrP 219K were both conversion competent in vCJD infection, these conversion competent PrP molecules showed an inhibitory effect in the 219E/K heterozygous animals. To explain this heterozygous inhibition, we propose a possible mechanism designated as the stone fence model.

Introduction

Creutzfeldt-Jakob disease (CJD), scrapie, and bovine spongiform encephalopathy are lethal transmissible neurodegenerative diseases caused by an abnormal isoform of prion protein (PrP^{Sc}) that is converted from the normal cellular isoform (PrP^C).¹ The human PrP gene (*PRNP*) has two major polymorphic codons: 129 for methionine (M) or valine (V), and 219 for glutamate (E) or lysine (K).^{2,3} These *PRNP* polymorphisms affect the susceptibility to sporadic (sCJD), variant (vCJD), or iatrogenic

CJD.⁴⁻⁸ In particular, the *PRNP* heterozygotes appear to be protected from sCJD compared to the *PRNP* homozygotes. The frequency of the PRNP 129M/V genotype in sCJD is significantly lower than that in the normal population.⁵ Moreover, the *PRNP* 219E/K genotype is absent in sCJD patients.⁹ The molecular mechanism responsible for these protective effects of *PRNP* heterozygosity has remained elusive.

In this review, we describe the inhibition of PrP conversion observed in a series of transmission studies using *PRNP* heterozygous animal models.^{10,11} To explain this heterozygous inhibition, we propose a possible mechanism designated as the stone fence model.

Two Modes of Heterozygous Inhibition

Inhibition by the conversion incompetent PrP molecules. vCJD prions (genotype: 129M/M and 219E/E) can be transmitted to knock-in mice expressing human PrP with 129M/M (Ki-Hu129M/M) or with 129M/V (Ki-Hu129M/V), but not to those with 129V/V (Ki-Hu129V/V).¹⁰ In transmission experiments using vCJD prions, we found an inhibitory effect of the conversion incompetent PrP 129V molecules in the 129M/V heterozygous animals. The amount of PrP^{Sc} in the spleens of Ki-Hu129M/V mice intraperitoneally inoculated with vCJD prions was much lower than that in the spleens of Ki-Hu129M/M mice (Fig. 1).¹¹ Moreover, the amount of PrP^{Sc} in Ki-Hu129M/V mice was even lower than that in hemizygous knock-in mice expressing human PrP 129M from one allele (Ki-Hu129M/0), which express half the level of PrP 129M compared with Ki-Hu129M/M mice. Thus, we confirmed that the decreased PrP^{Sc} accumulation in Ki-Hu129M/V mice was not due only to the expression level of PrP 129M. These findings clearly showed that the conversion incompetent PrP 129V molecules exerted an inhibitory effect on the conversion of PrP 129M molecules in the 129M/V heterozygous animals.

Previous studies have demonstrated that the conversion incompetent PrP molecules exhibit inhibitory effects on the

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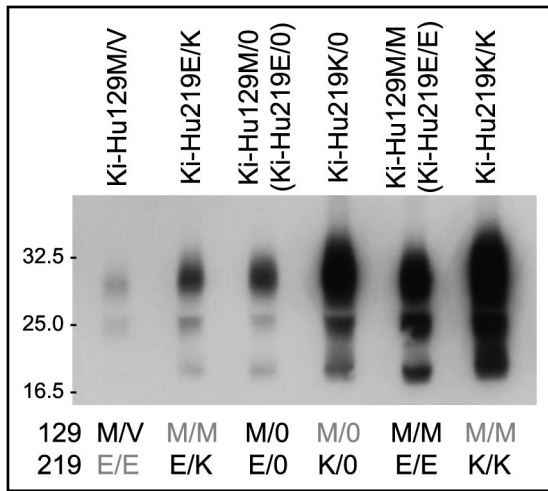


Figure 1. Heterozygous inhibition in vCJD infection. Western blot analysis of PrP^{Sc} in the spleens of knock-in mice intraperitoneally inoculated with vCJD prions. The amount of PrP^{Sc} in the 129M/V heterozygous mice was even lower than that in the 129M/O hemizygous mice. Furthermore, the amount of PrP^{Sc} was the highest in the 219K/K mice, whereas the PrP^{Sc} accumulation in the 219E/K heterozygous mice was even lower than that in the 219E/O hemizygous mice or 219K/O hemizygous mice. Therefore, we found that both the conversion incompetent PrP and the conversion competent PrP showed inhibitory effects in the heterozygous animals.

conversion of the co-existing conversion competent PrP. This type of inhibition has been referred to as a dominant negative effect. When the endogenous mouse PrP gene was ablated, transgenic mice expressing exogenous human PrP or hamster PrP became more susceptible to human prions or hamster prions, respectively.^{12,13} In a cell-free conversion system using mouse and hamster PrP, the conversion but not the binding to PrP^{Sc} was inhibited by the conversion incompetent PrP in a dose-dependent manner.¹⁴ Furthermore, dominant negative mutations in mouse PrP have been studied intensively due to their potential for therapeutic applications.¹⁵⁻²² In accord with these reports, the conversion incompetent human PrP 129V molecules in our study showed an inhibitory effect on the conversion of the human PrP 129M molecules.

Inhibition by the conversion competent PrP molecules. When we performed intraperitoneal inoculation of vCJD prions into knock-in mice expressing human PrP with 129M/M and 219E/E (Ki-Hu219E/E, a synonym of Ki-Hu129M/M), 129M/M and 219K/K (Ki-Hu219K/K), or 129M/M and 219E/K (Ki-Hu219E/K), we made two important findings. (1) Ki-Hu219K/K mice showed high susceptibility to vCJD prions. (2) Nevertheless, the heterozygous Ki-Hu219E/K mice showed the lowest susceptibility among the knock-in mice with polymorphism at codon 219.¹¹ The amount of PrP^{Sc} and the number of PrP-positive follicular dendritic cells in the spleens of Ki-Hu219K/K mice were higher than those of Ki-Hu219E/E mice (Fig. 1). By contrast, the amount of PrP^{Sc} accumulation in Ki-Hu219E/K mice was even lower than that in the hemizygous Ki-Hu219E/O mice or

Ki-Hu219K/O mice. Thus, though the human PrP 219E and PrP 219K were both conversion competent in vCJD infection, these conversion competent PrP molecules showed an inhibitory effect in the 219E/K heterozygous animals.

The Stone Fence Model of Heterozygous Inhibition

The molecular mechanisms responsible for the inhibitory effect of the conversion incompetent PrP have been studied previously.^{14,23,24} Briefly, though the conversion incompetent PrP molecules are not converted into PrP^{Sc}, they bind to PrP^{Sc} and are incorporated into amyloid fibrils with the conversion competent PrP molecules. Based on these previous findings, we propose a possible mechanism to explain the inhibitory effect of the conversion competent PrP as well as the conversion incompetent PrP. We designated the mechanism as the stone fence model (Fig. 2).

PrP^C is converted to PrP^{Sc} and then piled up into amyloid fibrils according to the nucleated polymerization hypothesis.²⁵ In homozygous (219E/E or 219K/K) or hemizygous (219E/O or K/O) animals, the conversion results in a single PrP^{Sc} population (Fig. 2). This means that the same blocks (the same PrP^{Sc}) would be piled up into the amyloid fibrils without any delay. By contrast, in the heterozygous 219E/K animals, the conversion results in at least two distinct PrP^{Sc} populations (219E PrP^{Sc} or 219K PrP^{Sc}) with different structures. These two PrP^{Sc} blocks would be piled up into the same fibril just like a stone fence composed of heterologous blocks. However, the fibril elongation would be delayed because the two types of PrP^{Sc} blocks interfere with each other due to their incompatible structures. Thus, the two PrP^{Sc} populations with different structures can act as decelerators of each other in the process of stacking.

The decelerator hypothesis based on the stone fence model is compatible with the possible mechanism for the inhibitory effect

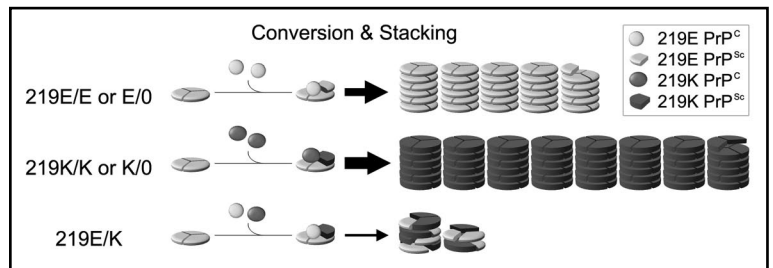


Figure 2. The stone fence model: a possible mechanism of the heterozygous inhibition in vCJD infection. PrP^C is converted to PrP^{Sc} and then piled up into amyloid fibrils according to the nucleated polymerization hypothesis and the trimeric models.²⁵⁻²⁸ In the homozygous (219E/E or K/K) or the hemizygous (219E/O or K/O) animals, the PrP^{Sc} blocks are piled up into the amyloid fibrils without delay because only a uniform PrP^{Sc} population exists. Though the initial seed is 219E PrP^{Sc} also in the 219K/K or 219K/O animals, the resulting 219K PrP^{Sc} acts as a new seed in the subsequent steps and are efficiently piled up. Therefore, the inhibitory effect of the initial 219E PrP^{Sc} seed is negligible in these animals. By contrast, in the heterozygous (219E/K) animals, at least two PrP^{Sc} populations are generated. These two PrP^{Sc} blocks are piled up into the same fibril just like a stone fence composed of heterologous blocks. The two types of PrP^{Sc} blocks interfere with each other due to their incompatible structures and delay the fibril elongation. Thus, the distinct PrP^{Sc} populations act as decelerators of each other in the heterozygous animals.

of the conversion incompetent PrP molecules.^{14,23} The conversion incompetent PrP is incorporated into amyloid fibrils with the conversion competent PrP, but acts as a decelerator at the conversion step. Therefore, the heterozygous inhibition can be caused both by the conversion incompetent PrP and the conversion competent PrP, and can occur both at the conversion step and the stacking step. Since it remains to be determined whether the human PrP 219E is efficiently converted by 219K PrP^{Sc}, we cannot rule out the possibility that the inhibition in the 219E/K heterozygous animals also occurs at the conversion step in addition to the stacking step.

Mysterious Phenomena in Prion Diseases Revisited With The Stone Fence Model

The stone fence model can explain the prion strain interference.^{29,30} The preceding infection with a prion strain prior to the superinfection with another prion strain interferes with the replication of the superinfected strain.³¹ According to the stone fence model (Fig. 2), the pre-existing PrP^{Sc} and the superinfected PrP^{Sc} might be piled up into the same fibril, but act as decelerators of each other due to their incompatible structures. To pile up the two distinct PrP^{Sc} populations into the same fibril, still unidentified interactions between the PrP^{Sc} molecules such as inter-oligomer interaction³² or protofibril stacking³³ might underlie the stacking step. Thus, if the pre-existing PrP^{Sc} dominates enough, the elongation of the amyloid fibrils seeded by the superinfected PrP^{Sc} is decelerated by the pre-existing PrP^{Sc}. Meanwhile, the efficacy of interference depends on the combination of the prion strains co-infected. Scrapie 22L strain but not Chandler strain interfered with the replication of the superinfected Gerstmann-Sträussler-Scheinker disease Fukuoka-1 strain.³⁴ The compatibility between the two types of PrP^{Sc} blocks might determine the efficacy of interference.

The stone fence model can also account for the absence of the *PRNP* 219E/K genotype in sCJD patients.⁹ In our experiments using vCJD prions, the human PrP 219E and PrP 219K were both conversion competent, whereas the susceptibility of the 219E/K heterozygous animals was even lower than that of the hemizygous animals. Furthermore, intracerebral transmission experiments using sCJD prions revealed that the human PrP 219E and PrP 219K were both conversion competent also in sCJD infection.^{11,35} Though the susceptibility of the 219E/K heterozygous animals to sCJD prions remains to be determined, the absence of the *PRNP* 219E/K genotype in sCJD patients is probably due to the heterozygous inhibition. Even though the human PrP 219E or PrP 219K spontaneously converts into PrP^{Sc}, subsequent fibril formation and elongation would be decelerated because the heterologous PrP^{Sc} blocks act as decelerators of each other.

Lessons From the Heterozygous Inhibition Experiments

Mouse PrP 218K (corresponding to human PrP 219K) molecules are conversion incompetent in mouse scrapie infection and show dominant negative effects both in vitro and in vivo.¹⁵⁻²⁰ Therefore, the absence of the *PRNP* 219E/K genotype in sCJD patients was formerly explained by the dominant negative effect.

However, our study revealed that the human PrP 219K molecules are conversion competent in sCJD infection as well as vCJD infection. This discrepancy suggested that the mutations in mouse PrP exhibit different effects from those of the corresponding mutations in human PrP as regards the conversion competence. This could have great significance for transgenic models expressing mouse PrP with mutations corresponding to the human pathogenic mutations. Otherwise, the distinct prion strains used in the experiments might underlie the discrepancy.¹⁹

To compare precisely the susceptibility of the experimental animals with different PrP genotypes, knock-in mice including heterozygous mice have an advantage over transgenic mice because they have identical genetic backgrounds, identical PrP expression levels, and equivalent expression from the heterozygous genes.¹⁰ Furthermore, the hemizygous knock-in mice express exactly half the level of PrP compared to the homozygous mice.¹¹ Since the expression level of PrP affects the length of the incubation period regardless of the PrP genotype, the heterozygous and the hemizygous knock-in mice are both indispensable to analyze the heterozygous inhibition.

Conclusion

The present study, together with evidence from other groups, suggests that heterozygous inhibition is a universal phenomenon that can be caused by both conversion incompetent PrP and conversion competent PrP, or by both PrP^C-heterozygosity and PrP^{Sc}-heterozygosity. The decelerator hypothesis based on the stone fence model paves the way for the solution of this phenomenon. To determine whether the efficacy of heterozygous inhibition is affected by the infected prion strain or the host PrP genotype, other heterozygous models need to be examined.

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