ELSEVIER

Neurobiology of Aging 27 (2006) 871–879

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

The protective effects of cystamine in the R6/2 Huntington's disease mouse involve mechanisms other than the inhibition of tissue transglutaminase

Craig D.C. Bailey, Gail V.W. Johnson *

Department of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, 1720 7th Avenue South, Sparks Center Room 1061, Birmingham, AL 35294-0017, USA

Received 12 January 2005; received in revised form 5 April 2005; accepted 13 April 2005
Available online 17 May 2005

Abstract

Tissue transglutaminase (tTG) is a multifunctional enzyme that contributes to disease progression in mouse models of Huntington's disease (HD), an inherited neurodegenerative disease that shows an age-related onset. Moreover, administration of the transglutaminase inhibitor cystamine delays the onset of pathology in the R6/2 HD mouse model. However, the contribution of tTG inhibition towards the therapeutic effects of cystamine has not been determined, as this compound likely has multiple mechanisms of action in the R6/2 mouse. In this study, we found that administration of cystamine in drinking water delayed the age of onset for motor dysfunction and extended lifespan to a similar extent in R6/2 mice that had a normal genetic complement of tTG compared with R6/2 mice that did not express tTG. Since the magnitude of cystamine's therapeutic effects was not affected by the genetic deletion of tTG, these results suggest that the mechanism of action for cystamine in this HD mouse model involves targets other than tTG inhibition.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Huntington's disease; Transglutaminase; Cystamine; Mouse; Brain

1. Introduction

Huntington's disease (HD) is a progressive inherited neurodegenerative disorder that inevitably leads to the death of affected individuals. The clinical presentation in HD includes motor dysfunction (chorea), psychiatric disturbances and a general cognitive decline. These clinical symptoms are paralleled by a specific pattern of neuronal dysfunction and degeneration, predominantly in the striatum and at later stages of the disease in the cerebral cortex [47]. The gene that causes HD encodes for a large 350 kDa protein called huntingtin [46], which has been implicated in axonal transport [18,45] and the expression [41,50] and transport [16] of brain-derived neurotrophic factor (BDNF). Mutation of the gene results in a pathological expansion of CAG repeats leading to an abnormally expanded stretch of glutamine residues near the N-terminal of the huntingtin protein [46]. In the non-affected population this CAG domain ranges from 6 to 36 repeats, whereas subjects with more than 39 CAG repeats will develop the disease. Interestingly, there is an inverse relationship between the age of onset for HD and an individual's CAG repeat length, with a longer repeat length conferring an earlier age of onset [7]. A neuropathological hallmark of HD is the presence of insoluble intraneuronal aggregates that contain mutant huntingtin [12,33,43]. The precise role of huntingtin aggregates in HD pathogenesis has not been determined (for a review, see [4]), however considerable evidence now exists to suggest that they are not primary initiators of the disease process [1,25,28,42] and that they may even be protective [1]. Despite the knowledge of the genetic cause of HD, the mechanisms by which mutant huntingtin produces neurodegeneration remain elusive and as such there currently is no known treatment or cure for HD.

Tissue transglutaminase (tTG) is a member of a family of thiol-dependent enzymes that catalyze calcium-dependent

* Corresponding author. Tel.: +1 205 934 2465; fax: +1 205 934 3709.
E-mail address: gvwj@uab.edu (G.V.W. Johnson).

0197-4580/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2005.04.001

peptide crosslinking, polyamination or deamination reactions at glutamine residues within specific protein substrates (for review, see [34]). In addition to its transglutaminase (TG) enzymatic activity, tTG is an established GTPase [30,38] and has recently been reported to act as a novel kinase [37]. A role for tTG in HD aggregate formation was suggested because it can crosslink proteins containing an expanded polyglutamine tract [11] including mutant huntingtin [22,24] in vitro. Moreover, tTG is upregulated in affected areas in HD brain [24,31] and its crosslinked product N^{ε}-(γ-glutamyl)-L-lysine is increased 3-fold in HD cerebrospinal fluid [20] and 10-fold in HD brain [13]. However, the precise role for tTG in aggregate formation remains controversial as mutant huntingtin aggregates can form in vitro in the absence of tTG [9,40] and tTG-catalyzed crosslinking of proteins containing an expanded polyglutamine tract decreases their propensity to aggregate [29]. In addition, tTG has been demonstrated to inhibit huntingtin aggregate formation in the R6/1 [36] and R6/2 [3] HD mouse models. Although the role for tTG in huntingtin aggregate formation is debatable at this point, it is clear that it does contribute to disease progression in mouse models of HD. Genetic deletion of tTG delays the age of onset for motor dysfunction and death in the R6/2 HD mouse model [3], and mitigates body weight loss and brain atrophy in addition to delaying death in the R6/1 HD mouse model [36]. Since tTG contributes to the majority of TG activity in the mouse brain [2,36] these results demonstrate that TG inhibition is a viable therapeutic target to delay the age of onset and/or disease progression in HD.

Treatment of R6/2 mice with the in vitro TG inhibitor cystamine delays body weight loss, behavioral deficits and mortality [13,23]. Early cystamine treatment appears to decrease huntingtin aggregate number, as one study observed a decreased number of huntingtin protein aggregates within the striatum of R6/2 mice when cystamine treatment began at 3 weeks of age [13] while another study found no effect of cystamine on huntingtin aggregates when treatment began at 7 weeks of age [23]. In addition to inhibiting the TG reaction, it has been demonstrated that cystamine inhibits caspase 3 in vitro and in cell culture [32], although one study suggests that cystamine does not inhibit caspase 3 in vitro when a high concentration of a reducing reagent is used in the assay [23]. Cystamine administration also results in increased glutathione content in cell culture [15,21,32], and increased L-cysteine content both in cell culture and in R6/2 mouse brain [15]. Although cystamine does not increase glutathione content in the R6/2 mouse brain [15] and has not yet been demonstrated to inhibit caspases in brain, these additional roles for cystamine are directly relevant to its therapeutic potential in HD. Caspases not only play central roles in mediating apoptotic cell death but caspase cleavage of mutant huntingtin is proposed to facilitate pathogenesis in HD [27,48,49]. Moreover, glutathione and L-cysteine are members of the cell's antioxidant machinery and oxidative stress likely plays a major role in mutant huntingtin-induced neurodegeneration [6,8]. Cystamine administration to R6/2 mice results in beneficial effects that are either different from, or in addition to the beneficial effects of genetic deletion of tTG, including decreased aggregate formation, decreased clasping and abnormal movements and the lessening of both body and brain weight loss. Thus, there is good evidence to hypothesize that the therapeutic effects of cystamine in the R6/2 HD mouse model may not be due to tTG inhibition, but rather a result of its actions at other targets.

The objective of this study was to determine the contribution of tTG inhibition towards the mechanism of action of cystamine in the R6/2 HD mouse model. This objective was met by comparing the therapeutic effects of chronic oral administration of cystamine to R6/2 mice that either had a normal genetic complement of tTG (R6/2:tTG+/+) or that had tTG genetically deleted (R6/2:tTG−/−). The rationale for this experimental design is that beneficial effects of cystamine in R6/2:tTG−/− mice would be the result of modes of action other than tTG inhibition, since tTG is not expressed in these mice. We show here that the ability of cystamine to delay the age of onset for clasping, rotarod dysfunction and death in the R6/2 mouse is not affected by the genetic deletion of tTG. These results suggest for the first time that the mechanism of action for cystamine in this HD mouse model involves targets other than tTG inhibition.

2. Materials and methods

2.1. Experimental animals and cystamine treatment

R6/2 ovarian transplant female mice [35] were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and tTG knockout mice have been described previously [39]. Mice were genotyped as previously described [35,39]. Each mouse line was maintained on a mixed-strain background (CBA × C57BL/6 for R6/2 mice; 129SVJ × C57BL/6 for tTG knockout mice). Two successive matings were performed in order to obtain R6/2 mice that were either R6/2:tTG+/+ or R6/2:tTG−/− from the same litter: first, R6/2 ovarian transplant females were bred with tTG knockout males. Male offspring from this cross that carried the R6/2 transgene (all offspring were tTG heterozygous knockouts) were then bred with female tTG heterozygous knockout mice. This second mating produced littermates that carried the R6/2 transgene at a 50% frequency, and were wildtype for tTG, heterozygous knockouts for tTG or homozygous knockouts for tTG. In order to examine the contribution of tTG inhibition towards cystamine's mechanism of action in R6/2 mice, data are shown only for R6/2:tTG+/+ and R6/2:tTG−/− mice that received either cystamine or water treatment. It should be noted that neither cystamine treatment nor genetic deletion of tTG affected any phenotype measured in this study in the absence of the R6/2 transgene (data not shown).

Cystamine treatment was provided in ad libitum in drinking water during breeding and continued postnatally. Cystamine (Sigma–Aldrich, St. Louis, MO, USA) was diluted in

Download English Version:

https://daneshyari.com/en/article/330626

Download Persian Version:

https://daneshyari.com/article/330626

Daneshyari.com