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Research Paper

Experience-dependent reduction of soluble β-amyloid oligomers and rescue of cognitive abilities in middle-age Ts65Dn mice, a model of Down syndrome

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ABSTRACT

Down syndrome (DS) is the most diffused genetic cause of intellectual disability and, after the age of forty, is invariantly associated with Alzheimer's disease (AD). In the last years, the prolongation of life expectancy in people with DS renders the need for intervention paradigms aimed at improving mental disability and counteracting AD pathology particularly urgent. At present, however, there are no effective therapeutic strategies for DS and concomitant AD in mid-life people. The most intensively studied mouse model of DS is the Ts65Dn line, which summarizes the main hallmarks of the DS phenotype, included severe learning and memory deficits and age-dependent AD-like pathology. Here we report for the first time that middle-age Ts65Dn mice display a marked increase in soluble Aβ oligomer levels in their hippocampus. Moreover, we found that long-term exposure to environmental enrichment (EE), a widely used paradigm that increases sensory-motor stimulation, reduces Aβ oligomers and rescues spatial memory abilities in trisomic mice. Our findings underscore the potential of EE procedures as a non-invasive paradigm for counteracting brain aging processes in DS subjects.

1. Introduction

Down syndrome (DS), a condition due to chromosome 21 trisomy, is the most common genetic cause of intellectual disability, with an incidence ranging from 1 in 700 to 1 in 1000 live births (Dierssen, 2012; Roizen and Patterson, 2003). People with DS display a plethora of severe disabilities (Nadel, 2003; Pennington et al., 2003), encompassing impairments in language, cognitive performance and adaptive behaviour (see (Bartesaghi et al., 2011) for a recent review), and develop early-onset Alzheimer's disease (AD) pathology if they live into their fourth decade of life, including deposition of the Aβ peptide as insoluble β-amyloid plaques in the brain parenchyma (Choi et al., 2009). Since the β-amyloid (Aβ) precursor protein (App) gene is located on human chromosome 21 (HSA21), App triplication is thought to play a key role in the early-onset AD phenotype in DS patients (Gyure et al., 2001). Recent findings have shown that soluble forms of Aβ (called Aβ oligomers), rather than insoluble forms (fibrils and plaques), are associated with memory impairments in early stages of AD, and are considered highly dangerous forms of Aβ aggregates disrupting synaptic plasticity in AD-like dementia (Ferreira et al., 2015; Shankar et al., 2008). Hence, these molecules might also be involved in learning and memory dysfunctions displayed by adult DS subjects. This attractive hypothesis, however, has never been tested in DS mouse models.

The most extensively used and best characterized mouse model of DS is the Ts(17^{16})65Dn line (hereafter Ts65Dn) (Davisson et al., 1993; Reeves et al., 1995). Even if Ts65Dn mice contain an extra copy of a number of genes that are not orthologues of human chromosome 21, they summarize the main hallmarks of the DS phenotype (for a recent review, see (Bartesaghi et al., 2011)), including a serious cognitive impairment in paradigms requiring the integrity of the hippocampal system, such as contextual (Bianchi et al., 2010; Costa et al., 2008) and spatial memory (e.g. (Costa et al., 2009; Demas et al., 1996; Escorihuela et al., 1995; Reeves et al., 1995)). Moreover, Ts65Dn mice display typical signs of AD neuropathology, with significant degeneration of basal forebrain cholinergic neurons (BFCNs) and failure in NGF retrograde transport, together with age-dependent dysregulation of APP and increased Aβ expression (Choi et al., 2009; Netzer et al., 2010). Behavioural deficits in working and reference memory displayed by Ts65Dn mice increase in severity with aging (Hunter et al., 2003), a phenotypic worsening that has been suggested to be related to an age-dependent dysregulation of various triplicated gene transcripts,

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including App (Choi et al., 2009). Strikingly, pharmacological lowering of Aβ levels has been shown to rescue learning and memory abilities in juvenile Ts65Dn mice (Netzer et al., 2010), providing compelling support to the general validity of this transgenic line as a model for AD-like pathology in DS subjects.

One promising strategy successfully used to induce functional compensation in mouse models of DS and other developmental disorders is exposure to environmental enrichment (EE) conditions, providing the animals with the opportunity for high levels of sensory, cognitive, social and motor stimulation (Nithianantharajah and Hannan, 2006; Sale et al., 2009; Sale et al., 2014). The therapeutic value of this non-invasive approach has been previously shown in juvenile and in young adult trisomic mice, resulting in a compensation of their spatial memory abilities (Begenisic et al., 2015; Begenisic et al., 2011; Martinez-Cue et al., 2002). However, to date the impact of EE has never been investigated at older ages in DS mouse models. Moreover, despite the interest attracted by Aβ oligomers in the pathophysiology of AD and brain aging (Lesne et al., 2013), their possible involvement in the pathogenesis of AD-like dysfunctions has never been tested in Ts65Dn mice.

Here we focused on middle-aged Ts65Dn mice and investigated, for the first time in this model, levels of hippocampal soluble Aβ oligomers. We report that 12-month old Ts65Dn mice exhibit a marked increase in soluble Aβ oligomer levels compared to age-matched euploid animals. Moreover, we found that long-term exposure to EE reduced Aβ oligomers and rescued hippocampal-dependent spatial memory abilities in aged trisomic mice.

2. Materials and methods

All the procedures employed in this study were approved by the Italian Ministry of Public Health (Authorization n. 160/2013 — B, 25/06/2013). In all experiments, researchers were blinded to experimental conditions. Three independent groups of animals were used for: i) MWM, ii) Y-maze, and iii) Western blot analyses.

2.1. Animals and rearing conditions

Adult segmental trisomy 16 Ts65Dn (Stock Number: 005252) and WT mice (from the same genetic background) of postnatal age >60 days (P60) were purchased from Jax Laboratories (Jax West Laboratories, Davis, CA) and housed in plexiglas cages, kept on a 12 h/12 h light-dark cycle (light on: 7:00 A.M.; light off: 7:00 P.M.) and acclimated to these controlled housing conditions for at least one week before inclusion in the study. Since trisomic and WT animals were purchased independently, no littermates were included in this study.

After acclimatizing, mice were either maintained in standard rearing conditions or placed in environmental enrichment, until they reached the age of 12 months, when all the analyses were performed.

Previous work demonstrated that rearing Ts65Dn male mice in EE conditions might actually result in a deterioration of their cognitive abilities in the acquisition trials of the Morris water maze, while a beneficial effect was evident in enriched females (Martinez-Cue et al., 2002). Successive work pointed out that Ts65Dn males have a tendency toward social subordination; thus, an excess of social stimulation in enriched conditions can disturb their behavioural and learning skills (Martinez-Cue et al., 2005). In the present study, only female mice were included, to avoid possible confounding effects due to different amount of social stress experienced by enriched vs. standard reared males.

2.2. Rearing conditions

Environmental enrichment (EE) consisted of a large cage (44 × 62 × 28 cm) with a wire mesh lid containing several food hoppers, running wheels, and differently shaped objects (e.g. tunnels, shelters, stairs) that were repositioned twice per week and completely substituted with others once per week (Begenisic et al., 2011; Sale et al., 2007). Every EE cage housed at least five females; we used a protocol of EE in which trisomic and euploid mice were reared in separate enriched cages.

Standard conditions (non-EE) consisted of a standard laboratory cage (26 × 42 × 18 cm) housing a maximum of three females of the same genotype.

Litter and food were the same in both experimental conditions; food and water were available ad libitum.

2.3. Behavioural tasks

All behavioural analyses were performed during the light phase of the daily cycle, starting at 4:00 P.M.

2.4. Morris water maze

Twelve month old mice (n = 14 WT-non-EE, n = 13 WT-EE, n = 8 Ts65Dn-non-EE, n = 8 Ts65Dn-EE) were trained for four trials per day and for a total of 5 days in a circular water tank, made from grey polypropylene (diameter, 100 cm; height, 40 cm), filled to a depth of 25 cm with water (23 °C) rendered opaque by the addition of a small amount of atoxic white paint. Four positions around the edge of the tank were arbitrarily designated North (N), South (S), East (E), and West (W), which provided four alternative start positions and also defined the division of the tank into four quadrants: NE, SE, SW, and NW. To avoid possible confounding effects due to reduced visual acuity in Ts65Dn mice (Begenisic et al., 2011), the tank was surrounded by a set of extra-maze cues in the visual discrimination range fully detectable by all the three groups (i.e., spatial frequency of visual stimuli not more than 0.15 cycles per degree). A circular clear Perspex escape platform (diameter, 10 cm; height, 2 cm) was submerged 0.5 cm below the water surface and placed at the midpoint of one of the four quadrants. The hidden platform remained in the same quadrant during training, while the start positions (N, S, E, or W) were randomized across trials. Mice were allowed up to 60 s to locate the escape platform, and their escape latency was automatically recorded by the Noldus Ethovision system. On the last trial of the last training day, mice received a single probe trial, during which the escape platform was removed from the tank and the swimming paths were recorded over 60 s while mice searched for the missing platform; the swimming paths were recorded and analyzed with the Noldus Ethovision system.

2.5. Spontaneous alternation

Spontaneous alternation was measured using the Y-maze, as described in (Begenisic et al., 2014). We used a Y-shaped maze which was constructed with three symmetrical grey solid plastic arms at a 120-degree angle (26 cm length, 10 cm width, and 15 cm height). Mice (n = 9 WT-non-EE, n = 9 WT-EE, n = 9 Ts65Dn-non-EE, n = 9 Ts65Dn-EE) were individually placed in the center of the maze. Each mouse was allowed to freely explore the three arms for 8 min. Arm entry was defined as all four limbs within the arm. A triad was defined as a set of three arm entries, when each entry was to a different arm of the maze. The maze was cleaned with 10% ethanol between sessions to eliminate odor traces. All sessions were video-recorded for offline analysis. The number of arm entries and the number of triads were recorded in order to calculate the total number of entries and the alternation percentage (generated by dividing the number of triads by the number of possible alternations and then multiplying by 100).

2.6. Western blotting

After chloral hydrate anaesthesia, the brain (n = 8 WT-non-EE, n = 8 WT-EE, n = 8 Ts65Dn-non-EE, n = 8 Ts65Dn-EE) was removed from the skull and the hippocampi were dissected out, frozen in dry ice and stored at −80 °C. Samples were homogenated and soluble proteins