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Research report

Cognitive deficit in hippocampal-dependent tasks in Werner syndrome mouse model

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HIGHLIGHTS

- Werner syndrome mice show a reduction in glucose tolerance as observed in aged mice.
- 8 month-old Werner syndrome mice show deficit in hippocampal-dependent memory.
- Reversal learning is impaired in Werner syndrome mice model.

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ABSTRACT

Mammalian aging is often characterized by metabolic disturbances, cognitive declines and DNA repairs deficiency, but the underlying molecular mechanisms are still not well understood. Alterations in DNA repair can significantly exacerbate aging. Mammalian neuronal cells which accumulate unrepaired DNA damage over time could potentially lead to brain functions disorders. Focusing on the ATP-dependent RecQ-type DNA helicase, an enzyme involved in repair of double strand DNA, a mouse model of Werner syndrome (WS) had been proposed as a model of accelerated aging. Until now, no study has investigated the impact of this premature aging syndrome on learning and memory. Spatial memory and cognitive flexibility are particularly affected by the aging process in both men and rodents. Studies have shown that aged mice exhibited similar performance than young adult mice on non-hippocampus dependent memory whereas their performances were decreased in hippocampus-dependent tasks. In this study, we have submitted 3, 5 and 8 month-old WS mice to several behavioral paradigms to evaluate hippocampus-dependent (spatial object location, Morris water maze and fear conditioning) and non hippocampus-dependent (object recognition) memories. No effect on the locomotion activity and anxiety level has been observed in adult WS mice. Interestingly, the 8 month-old WS mice exhibit long-term memory impairment similar to aged mice, suggesting that adult WS mice do develop some aspects of cognitive aging.

1. Introduction

Aging is characterized by a physiological process associated with a progressive loss of functional efficiency of all time-dependent organs. Biological aging is often associated with a decrease of physical performances and with metabolic disturbances [1] and cognitive decline [2]. In the case of physiological aging, clear metabolic changes have been observed including impaired glucose tolerance and type 2 diabetes increase [3]. Spatial learning and memory are cognitive functions most frequently and severely impacted with aging. Indeed spatial memory, which is mediated by the hippocampus, undergoes numerous molecular and physiological changes with aging, including cerebral vascular degeneration, decreased glucose utilization and bioenergetic metabolism [4]. Consistent with cognitive decline, atypical synapse morphology, aberrant protein and neurotransmitter synthesis have been shown with advancing age [5]. Interestingly, studies have shown that aged mice exhibited similar performance than young adult mice on non-hippocampus dependent memory (i.e., object recognition), whereas their performances were decreased in a hippocampus-

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dependant object location task [6]. Moreover, the performance of 18 month-old mice showed reduced motor skills and impaired motor coordination in comparison to young mice [7]. Finally, aged mice spent significantly less time in the open arms of an elevated plus maze than adult mice, suggesting a role of aging in anxiety-related behavior [8]. Overall, we cannot exclude that some of cognitive declines observed during aging in mice could be due to anxiety increase and/or locomotion alteration.

Aging is also known to be associated with DNA damage and altered double-strand break (DSB) repair systems [9,10]. Few studies have established a direct link between defect in DNA repair and cognitive deficit during aging. Using a mouse with a mutation in the gene involved in DNA repair pathway (excision repair cross-complementing group1, *Ercc1*), Borgesius and colleagues have shown that unrepaired DNA damage is correlated with age-dependent cognitive decline and hippocampal synaptic plasticity deficit [11]. More recently, altered DSB repair systems has been proposed as a main factor responsible of learning and memory deficit in an Alzheimer's disease mice model [12]. Moreover, altered DNA repair systems might contribute to cognitive decline by regulating the expression of set of genes involved in learning and memory [13]. Altogether, these studies suggest a relationship between DNA damage, memory deficit and aging.

Most premature aging syndromes, as Werner syndrome (WS), are caused by mutations in genes encoding proteins involved in DNA repair, as DNA helicases [14]. WS is an autosomal recessive disease characterized by early onset of many signs of normal aging [15]. The gene responsible for WS was identified by positional cloning and the gene product contains a domain homologous to the RecQ-type DNA helicases involved in repair of double strand DNA [16]. In WS, telomere dysfunction is causal to the accumulation of DNA damage foci and results in premature senescence. Very few little is known on the possible premature aging of the central nervous system in WS [17]. Normal brain morphology and functions have been described in two patients with WS confirming the believe that segmental premature aging spares the central nervous system [17]. However, Leverenz and collaborators have shown extensive β amyloid deposits in frontal cortex, temporal cortex and hippocampus in a 57 year-old patient with WS [18] but it is difficult to conclude since this patient carries also the apolipoprotein (apo) E ε4, the strong risk factor for AD [19]. In contrast, a deletion mutation of *WRN* gene in a 55 year-old patient showed no association with central nervous system pathology, such as amyloid plaques [19]. However because of the rarity of WS patients, the impact of an accelerated aging in brain functions still needs to be established.

Mice lacking the helicase domain exhibit many features of WS, including a pro-oxidant status and a shorter mean life span [20]. Moreover these WS mice developed severe cardiac interstitial fibrosis in addition to tumors and other symptoms found in patients with WS [21]. As a segmental progeroid syndrome, WS does not exhibit all of the features of normal aging but nevertheless is a very useful model system for the behavioral and molecular study of normal aging. Therefore, to address the question whether accelerated aging in WS affects the central nervous system, we investigated the consequences of *WRN* gene mutation on cognitive characteristics in WS mice. Hence, the first aim of our study was to characterize metabolic and behavioral profiles of WRN deficiency mice focusing on metabolic dysfunction, locomotion, anxiety, and memory.

2. Materials and methods

2.1. Subjects

Mice lacking part of the helicase domain of the *WRN* gene were generated by homologous recombination as previously described [20]. Briefly, 121 amino acid residues of the Wrn protein were deleted in homozygous $Wrn^{\Delta hel/\Delta hel}$ mice. Initially, the genetic background of these mice was both 129/Sv and Black Swiss. $Wrn^{\Delta hel/\Delta hel}$ mice were at least 12-fold backcrossed to an inbred C57Bl/6 background. Only female homozygous $Wrn^{\Delta hel/\Delta hel}$ mice at 3, 5 and 8 month-old of age were used in behavioral studies. The 3 month-old mice were used as control. Both male and female C57Bl/6 and homozygous $Wrn^{\Delta hel/\Delta hel}$ mice were used for the metabolism study. They were housed in a collective cage (5–6 per cage) in a room with controlled temperature (21–23 °C), and a 12-h light/dark cycle. Food and water were provided ad libitum. All experiments were performed in strict accordance with the recommendations of The European Communities Council Directive (86/609/EEC), The French National Committee (87/848) and the guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH publication no. 85–23). In application of the European directive 2010/63/UE and according to the ongoing French legislation at the time of experiments, no specific approval was necessary for this study.

2.2. Metabolic analysis

2.2.1. Glucose tolerance test

Cohorts of mice at different ages were starved for 6 h and then weighed. Baseline blood glucose was measured by tail vein bleeding using a OneTouch glucose meter (Accu-chek®). A single dose of 20% D-glucose in saline (1 g/kg body weight) was administered intraperitoneally (t0), which was followed by sampling blood at different time points (t15–t90) to measure blood glucose.

2.3. Behavioral testings

2.3.1. Locomotor and anxiety-related behaviors

Locomotor activity was recorded in a Plexiglas chamber (25 × 21.5 × 9.5 cm) equipped with infrared photobeams (Apelex, Evry, France). Horizontal and vertical activities were measured as the total number of photobeam breaks per 15 min over a 1 h period, starting at 12:00 h P.M. To measure anxiety-related behavior, mice were submitted to an elevated-plus maze. This maze consisted of a plus-shaped track with 2 closed and open arms (30 × 10 × 20 cm) that extended from a central platform (10 × 10 cm). The apparatus was elevated 50 cm above the floor and was surrounded by a white curtain without any conspicuous cues. Each trial began when the mouse was placed in the central zone and lasted 5 min. The number of entries into closed and open arms and the time spent in each arm were monitored. The maze was cleaned with 70% ethanol between each mouse to remove olfactory cues. Mouse behavior was videotaped and analyzed using ethological software.

2.3.2. Object location and object recognition memory task

Object memory tasks were performed in a circular arena (height: 30 cm; diameter: 40 cm) and placed in a room containing no conspicuous features, illuminated by a white light (60 W) and surmounted by a video camera connected to a video recorder and a monitor. The day before training, mice were submitted to a 10-min session consisting to a context habituation period without objects in the arena. During the training day, mice were placed in the same arena with 2 identical objects, i.e. a green glass Pago® bottle (height: 12.9 cm; diameter: 5.8 cm) and their weight was such that they could not be displaced by animals. 24 h after the 10-min training, one group of mice was submitted to a 10-min testing session in the original training arena in which only one of the two objects was moved to a new location. The other group of mice was submitted to a 10-min testing session in the training arena in which a familiar object and a new object, i.e., a white glass cylinder (height: 7 cm; diameter: 6 cm) inserted on a square plastic base (7 × 7 cm) were