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Long-term central pathology and cognitive impairment are exacerbated in a mixed model of Alzheimer's disease and type 2 diabetes

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ABSTRACT

Type 2 diabetes (T2D) is a well-characterized risk factor for Alzheimer's disease (AD), the most common cause of dementia. Since both, T2D and dementia are closely related to aging and they chronically coexist in elderly patients, it is of particular relevance to know whether long-term evolution of T2D and dementia interfere with each other years after the onset of the diseases. In order to elucidate this interaction, we have characterized a mixed model of T2D and AD, the APP/PS1xdb/db mouse, at 36 weeks of age, when both diseases have long coexisted and evolved. In aged APP/PS1xdb/db mice we observed dysfunctional metabolic control, when compared with diabetic mice alone, suggesting that AD may also contribute to T2D pathology in the long-term. Learning and memory were severely impaired in APP/PS1xdb/db mice, accompanied by reduced cortical size, neuronal branching simplification and reduction of dendritic spine density. Increased tau phosphorylation was also observed in old APP/PS1xdb/db mice. A shift in amyloid-β (Aβ) pathology was detected, and while insoluble Aβ was reduced, more toxic soluble species were favoured. Microglia burden was significantly increased in the proximity of senile plaques and an overall increase of spontaneous haemorrhages was also observed in APP/PS1xdb/db mice, suggesting a possible disruption of the blood brain barrier in the mixed model. It is therefore feasible that strict metabolic control may slow or delay central complications when T2D and dementia coexist in the long term.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia. Senile plaques (SP) mainly composed by amyloid-beta (Aβ), neurofibrillary tangles with abnormally phosphorylated tau, and neuronal and synaptic loss are the cardinal neuropathological features of AD (Serrano-Pozo et al., 2011). AD pathologies regularly coexist with vascular injury and other co-morbidities in many patients, making the borderlines between AD and other dementias blurred in many cases (Craft, 2009).

The ultimate causes of dementia are not completely understood. Metabolic disorders including type 2 diabetes (T2D), have been identified as important risk factors for developing sporadic AD (Craft, 2009). In support of this idea, epidemiological and clinical studies have revealed a close relationship between T2D and dementia (Ott et al., 1996; Arvanitakis et al., 2004). Moreover, improved metabolic control can significantly ameliorate cognitive dysfunction (Craft et al., 2003; Plastino et al., 2010; Cooray et al., 2011). Also, at a molecular level some relevant links between T2D and AD support the interaction between these diseases. For example: T2D progression correlates with pancreatic amylin deposition, in a similar way to Aβ deposition in AD brain. Since insulin, amylin and Aβ are all degraded by neprilysin and insulin degrading enzyme, these substrates may compete with each other. Also, an imbalance of any of these substrates, as observed in AD or T2D, can affect the degradation rate of other substrates and possibly influence the pathogenesis of related diseases (Gotz et al., 2009). Aβ oligomers may also interfere with insulin signalling in hippocampal neurons (Zhao et al., 2008). On the other hand insulin may regulate Aβ levels by modulation of β and γ secretases (Farris et al., 2003). Central nervous system insulin receptors are highly expressed in regions relevant for cognition, such as cortex and hippocampus. This is consistent with evidence showing that insulin influences memory (Craft, 2009). Since vascular damage has been suggested to reduce Aβ clearance along interstitial fluid drainage pathways

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(Weller et al., 2008; Garcia-Alloza et al., 2011), and insulin participates in neurovascular regulation, it is feasible that metabolic alterations may also underlie the crosstalk between T2D and AD (Correia et al., 2011).

While T2D seems to play a relevant role in developing AD, aging remains the main risk factor for both dementia and T2D. Therefore, examining animal models that harbour both AD and T2D during advanced aging, when both pathologies are fully established, might more closely resemble the natural history of T2D and dementia. Therefore, we have analyzed the relationship between T2D and AD in a complex model resulting from crossing an AD mouse model with amyloid pathology (APPswe/PS1dE9, APP/PS1 from now on) with a classical T2D model, the db/db mouse. To our knowledge, the long-term effect of this relationship has not been studied. At 36 weeks of age, both T2D and AD plaque pathology are fully established and have chronically evolved over time, as observed in patients. We detected a synergistic effect between T2D and AD that worsened with aging. Significant cognitive impairment was observed in APP/PS1xdb/db mice, accompanied by reduced cortical size and tau pathology. Moreover, a significant reduction in spine density was observed in APP/PS1xdb/db mice, similar to the synapse loss observed in AD patients. We also detected a shift in Aβ pathology. Insoluble Aβ levels and SP were reduced, while more toxic soluble species were favoured. Inflammation was highly elevated in APP/PS1xdb/db mice and spontaneous bleeding was worsened in the cortex and hippocampus from our mixed animal model. It is possible that inflammatory process resulting from T2D and AD, and blood brain barrier alterations, might underlie pathological and cognitive features observed in APP/PS1xdb/db mice in the long term.

2. Material and methods

2.1. Animals

APP/PS1xdbdb mice were produced by cross-breeding an AD model:APPswe/PS1dE9 mice (APP/PS1) (Jankowsky et al., 2004) (Jackson Laboratories, ME, USA) with a T2D model: db/db mice purchased from Harlam Laboratories (Netherlands), as previously described (Ramos-Rodriguez et al., 2015b). Both T2D and AD are diseases closely associated with aging and in order to fully characterize these mice, animals were aged up to 36 weeks of age, when both pathologies are well-established.

All experimental procedures were approved by the Animal Care and Use Committee of the University of Cadiz, in accordance with the Guidelines for Care and Use of experimental animals (European Commission Directive 2010/63/UE and Spanish Royal Decree 53/2013).

2.2. Metabolic determinations

Body weight, postprandial blood glucose and plasma insulin levels were determined at 12 and 24 weeks of age, as well as immediately before sacrifice at 36 weeks, as previously described (Ramos-Rodriguez et al., 2013b). Briefly, blood glucose levels were measured using the glucometer Optium Xceed (Abbott, United Kingdom). Plasma insulin levels were measured using ultrasensitive mouse enzyme-linked immunosorbent assay (ALPCO Diagnostics, Salem, NH).

2.3. Actimetry and novel object discrimination (NOD) task

Spontaneous locomotor activity was assessed 10 days prior to sacrifice by measuring the distance travelled by the mice for 30 min in the open field box (22 cm long × 44 cm width × 40 cm high). The next day mice began the NOD test as previously described (Ramos-Rodriguez et al., 2013c). Briefly on day 1 mice were habituated, for 30 min to a transparent rectangular box, where the procedure was carried out. On day 2 animals were exposed to two objects for habituation purposes, which were not used again during the object exploration task on day 3. On day 3 each mouse received two sample trials and a test trial. On the first sample trial, mice were allowed to explore 4 identical novel objects for 5 min (blue balls) arranged in a triangle-shaped spatial configuration. After a 30 min delay, mice received a second sample trial with 4 novel objects (red cones), arranged in a quadratic-shaped spatial configuration, for 5 min. After a delay of 30 min, mice received a test trial with 2 copies of the object from sample trial 2 ("recent" objects) placed at the same position, and two copies of the object from sample trial 1 ("familiar" objects) one of them placed at the same position ("old non displaced" object) and the other in a new position ("familiar displaced" object). Integrated episodic memory for "what", "where" and "when" was analyzed as previously described (Dere et al., 2005): "what" was defined as the difference in time exploring familiar and novel objects, "where" was defined as the difference in time exploring displaced and non-displaced objects and "when" was defined as the difference between time exploring familiar non-displaced and recent non-displaced objects.

2.4. Morris water maze (MWM)

Spatial cognition was analyzed as previously described (Dere et al., 2005; Ramos-Rodriguez et al., 2013c) with minor modifications. Experiments commenced the day after concluding the NOD test. During the acquisition phase animals performed 4 trials/day for 4 days, with the platform submerged. During this phase the platform was located in quadrant 2. The time limit was 60 s/trial, with a 10 min inter-trial interval. If the animal did not find the platform it was placed on it for 10 s. The retention phase started a day after acquisition phase was completed, and consisted in a single trial with the platform removed. A second retention phase took places 72 h after completing the acquisition phase. Time required to locate the platform in the acquisition phase, percentage of time spent in quadrant 2 during the retention phase and swim speed were analyzed using Smart software (Panlab, Spain).

2.5. Tissue processing

At the end of the retention phase in the MWM, animals were overdosed with chloral hydrate (60 mg/kg). All brains were harvested and immediately weighted. Left hemispheres were dissected and cortex and hippocampus were stored at −80 °C until used. Right hemispheres were fixed in 4% PFA for a week before 30 μm coronal brain sections were obtained in a Microm HM450 microtome (ThermoFisher, Spain) and stored in 50% polyethelene glycol at 4 °C until used. A set of 3 brains/group was processed as described below for Golgi-Cox staining.

2.6. Cresyl violet staining

Brain morphology was analyzed in sections selected 1 mm apart (from 1.5 to -3.5 mm from Bregma), mounted and dehydrated in 70% ethanol for 15 min before incubation in cresyl violet as previously described (Ramos-Rodriguez et al., 2013b). Sections were mounted with DPX (Sigma, St. Louis, MO, USA) and images were acquired using an optical Olympus Bx60 microscope with an attached Olympus DP71 camera and Cell F software (Olympus, Hamburg, Germany). Cortex and hippocampus cross-sectional areas were measured using Image J software.