

Tau aggregation in the hippocampal formation: an ageing or a pathological process?

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Received 28 May 2002; received in revised form 19 June 2002; accepted 20 June 2002

Abstract

Tauopathy is a concept to describe different genetic or metabolic dysfunctions of tau proteins that generate most of the known dementing disorders. Tauopathy is a degenerating process that also affects the entorhinal formation, and then the hippocampal formation in ageing. In Alzheimer's disease (AD), a disease due to APP dysfunction, a similar tauopathy process is observed in neocortical areas, well correlated to cognitive impairment. One important gap of knowledge is the relationship between tauopathy in the hippocampal formation, ageing, AD, and cognitive impairment. Here we show that the multidisciplinary analysis of numerous brains from non-demented and demented patients suggests the following observations: tauopathy of the hippocampal formation in humans is age-related but not an age-dependent process, also independent of AD, but amplified by APP dysfunctions. Tauopathy in the entorhinal and hippocampal formation could be another type of pathological dysfunction of tau proteins, and a therapeutic target to delay AD. Relevant animal models are desperately needed to address this issue. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Alzheimer's disease; Hippocampal formation; Neurofibrillary degeneration

1. Introduction

Neurofibrillary degeneration (NFD) is a pathological process that has been discovered by Alois Alzheimer at the beginning of the 20th century. Demonstration was made using silver staining of brain tissue sections from a demented patient aged 52 years. Pathological neurofibrils were observed in the cell bodies of neurons, in the neuritic extension and around diffuse deposits to constitute 'senile plaques'

(Alzheimer, 1907). In 1964 Kidd's electron microscopic observations demonstrated that these neurofibrils are constituted of paired helical filaments packed in bundles (Kidd, 1964). Biochemical investigations led to the characterization of the two degenerating processes that characterize Alzheimer's disease (AD), which are amyloidosis and tauopathy. Amyloidosis corresponds to the extracellular aggregation of A β peptides into amyloid plaques (Selkoe, 2000). A β peptides derive from the catabolism of a large transmembrane glycoprotein precursor named APP (amyloid β -precursor protein). Tau pathology, also named tauopathy, corresponds to the intraneuronal

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association of tau proteins into abnormal filaments (Goedert, 1998; Delacourte and Buée, 2000). Tau proteins are a group of six isoforms that stabilize microtubules, the railways of the axonal transport. From genetic studies of familial AD, we know that APP dysfunction and amyloidosis are closely related to AD aetiology, since APP mutations, as well as PS1 that possesses a gamma-secretase activity on APP, are pathological (Selkoe, 2000). On the other hand, tau pathology is strongly correlated to the clinical expression of the disease (Fewster et al., 1991; Bierer et al., 1995; Duyckaerts and Hauw, 1997a; Delacourte et al., 1999; Braak et al., 1999; Mesulam, 1999; Price and Morris, 1999). But little is known on the relationship between APP and tau pathologies, which is the missing link to fully understand AD.

Also, the analysis of brains from aged controls shows that amyloid and tau aggregates are frequently present, but generally at low levels. This has been interpreted as an ageing process and therefore 'sporadic' AD as an accelerating ageing. However, recent findings at the molecular level of amyloidosis and tauopathy, as well as more careful studies on the quantification of brain lesions in non-demented and demented patients are now able to precise the relationship between ageing and AD. This new understanding opens diagnostic and therapeutic perspectives for AD.

2. The difficulty to describe 'normal' brain ageing

In order to analyse and quantify AD changes, the pathological brain tissue is generally compared with the one from aged-matched controls. The ages of the patients are frequently between 70 and 80 years. Patients that are non-demented are generally considered as 'controls'. However we know that the percentage of the population affected by a brain disease increases dramatically after the age of 70 years. Many reports mention that 40% of the population aged 90 years and over are demented. Because brain disorders are extremely frequent at old age, many non-demented patients could be at the infraclinical stage of a disease, and most of the time affected by incipient AD, since this disease is the most frequent age-related brain disorder. Since the pre-clinical stage could take several decades to develop,

and since neuroplasticity and neuronal compensation are able to overcome the pathological process for a long time, it is not surprising to find a huge number of brain lesions in some non-demented patients. This is one important difficulty for the study of 'normal ageing', because it is difficult to delineate the frontier between a putative ageing burden and the first steps of a pathological process.

Furthermore, while AD is frequent in aged populations, other adverse events generally occur with ageing such as a vascular pathology, or other neurodegenerative disorders like synucleopathy, and especially Parkinson's disease. Co-morbidity is almost a rule at old age, which could potentiate the main pathological event and modify its course. So, all lesions found in a brain from a non-demented patient, even with excellent and proven cognitive functions, could be due to a starting pathological process.

3. How to study normal brain ageing?

The royal avenue would be to address this problem in a relevant animal model. But such models do not exist at the present time. Indeed, rodents have neither tau nor APP pathology in ageing. Some old dogs, bear and monkeys have amyloid deposits, but no tau pathology. Transgenic mice with APP and PS1 mutations produce large amounts of amyloid deposits, but without real tau pathology. Also, mice with APP and tau mutations have no cellular and regional profiles observed in human brains. In these mice, tau and APP pathologies interact poorly. Due to the lack of relevant models, the approach must be pragmatic, because perfection cannot be achieved. The best approach would consist of a large prospective and multidisciplinary study of numerous non-demented and demented patients. This would allow studying a population comprising cases with normal ageing, others with preclinical AD as well Alzheimer patients at different stages of the disease. The follow up of controls can generally be performed in geriatrics hospitals, while patients affected by a neurodegenerative disorders can be followed in a neurological department. Also, the prospective study of specific groups of population can be fruitful, such as the Nun's study (Riley et al., 2002).

Pragmatically, normal ageing will correspond to the cases that have normal cognitive functions and the lowest amount of brain lesions. Due to the important co-morbidity in ageing, the so-called normal ageing will be close to the concept of 'successful ageing', and will be more the exception than the rule. However, since tau pathology is systematically present at the age of 75 years, normal ageing will correspond to the minimum levels found at this age. However, this mild tau pathology found in ageing is not necessarily a normal process, and could be a pathological one. To address this question, one should know the pathological process through the examples of tauopathies in AD and other dementing disorders.

4. Tau pathology in ageing and Alzheimer's disease

Tau pathology can be detected with neuropathological or biochemical means. At the neuropathological level, tau pathology is named NFD. Neurofibrils made up of packed paired helical filaments of tau are found in all parts of the degenerating nerve cells. They are found in cell bodies to constitute the neurofibrillary tangles, in the neuritic extensions and in dystrophic neurites around amyloid deposits to form senile plaques. Tauopathy can also be observed in astrocytes (progressive supranuclear palsy, corticobasal degeneration) or in oligodendrocytes (multiple system atrophy). At the last stages of AD, NFD is generally found in all neocortical areas, and in many subcortical nuclei (Buée et al., 2000).

At the biochemical level, tau pathology is well revealed using phospho-dependent tau antibodies, since tau lesions are hyper and abnormally phosphorylated. Western blot analysis demonstrates the presence of three major electrophoretic bands of PHF-tau of 60, 64 and 69 kDa. A fourth minor 74 kDa band is also present. A biochemical quantification with this approach shows that tau pathology is widely distributed at the last AD stages, and affects mainly the limbic and neocortical polymodal association brain areas. The last brain areas to be affected by tau pathology are primary brain areas such as the frontal motor cortex and the visual areas (occipital pole). Tau

pathology is completely absent in the brain of young controls, but frequently present in the entorhinal or in addition in the hippocampal formations of aged people (Buée et al., 2000). Therefore, the question was to determine the fate of tau pathology at the infraclinical stages of AD, for a possible differentiation from normal ageing.

For that purpose, the answer was brought through neuropathological and biochemical analyses of the different brain areas of cases from a large population of non-demented and demented patients. All these studies demonstrate that there is a progressive, sequential, invariable and hierarchical progression of tau pathology in specific brain areas (Delacourte, 2000). The quantification of tau pathology in normal ageing necessitates a sensitive and specific technique. In our hands, the neuropathological approach with phospho-dependent tau antibodies is more sensitive than western blots. The reason is that the histological techniques can detect tangles or 'tombstones' that have a very low turnover, while Western blots detect a more dynamic state of tau aggregation. However, both approaches gave similar results.

Biochemistry on non-demented patients shows that tau pathology is always present in the entorhinal cortex of all people aged over 75 years. Some almost centenarian non-demented patients were found with very mild tau pathology, demonstrating that this degenerating process is not directly related to age. In conclusion, age is a risk factor, but the intensity of tau pathology in aged people is very different among individuals, and can be still at low levels in oldest-old patients.

The neuropathological approach of Braak and Braak on several thousands of autopsied cases, using silver staining on thick brain tissue sections from non-demented and demented people of all ages, defines the distribution of NFD in ageing (Braak and Braak, 1997; Duyckaerts and Hauw, 1997b). NFD is present in the entorhinal region of one case out of four at the age of 25 years, and one case out of two at the age of 50 years (Fig. 1A). This means that this degenerating process which is almost specific to humans is systematically observed in ageing, and that entorhinal and hippocampal formations are very vulnerable areas to tau pathology.

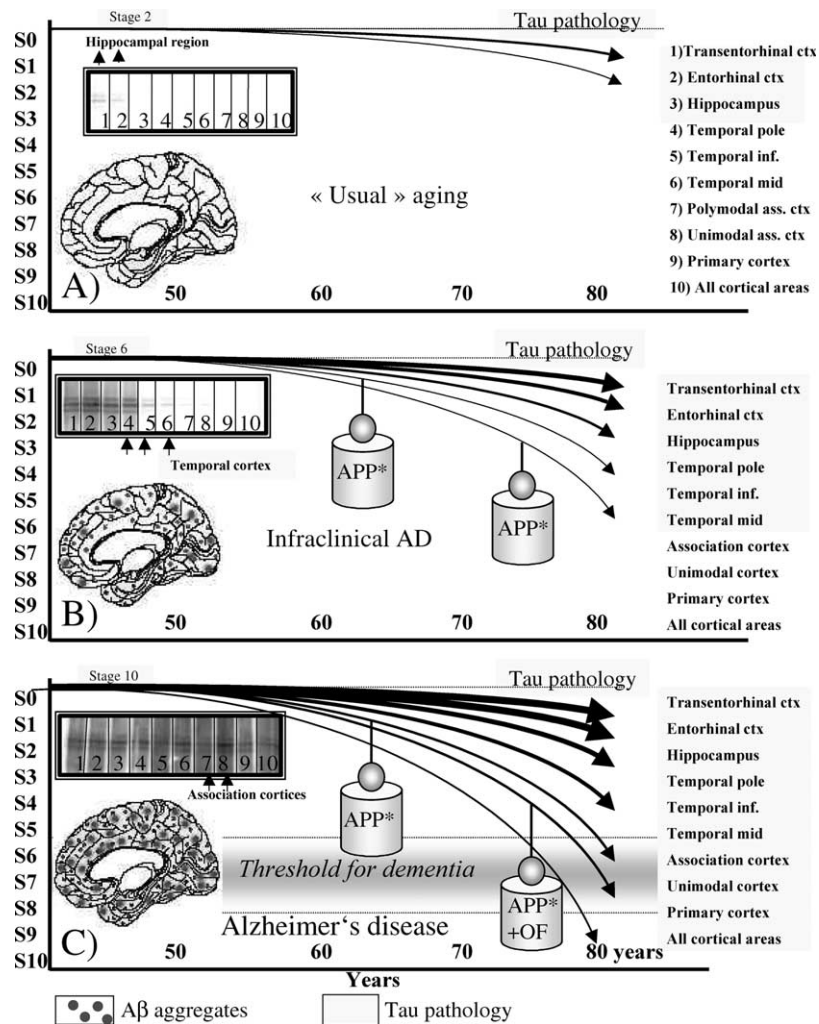


Fig. 1. The pathway of tau pathology and amyloidosis in ageing and Alzheimer's disease. Representation of the dynamics of tau and APP pathologies in ageing and AD. Insert: Western blot analysis of tau pathology in 10 brain areas, mentioned in the right part. Immunodetection with AD2, a monoclonal antibody against phosphorylated tau proteins. Results for tau are represented in yellow. *Facts.* Tau pathology is always detected, but in various amounts, in the entorhinal region of non-demented patients aged over 75 years. Large-scale neuropathological studies have shown that tau pathology is found in 50% of the population aged 50 years. In AD, tau pathology always extends in other brain areas, along a precise pathway, which is sequential and hierarchical. The 10 brains that are successively affected define 10 stages of tau pathology (S1–S10). Tau pathology can be observed without Aβ aggregates, while the opposite is not true. However, extension of tau pathology in association areas is always observed with Aβ deposits. *Hypotheses.* There is a threshold of tau pathology at stages 6 and 7 that separates infraclinical AD from clinical AD. Tau pathology without Aβ deposits demonstrates that this process is independent of AD. Tau pathology is increased in ageing, but not directly linked to age, as demonstrated by mild tau pathology in some oldest-old non-demented patients. Clinical manifestations are always observed at stage 7 or above, when polymodal association brain areas are affected. In AD, APP dysmetabolism (loss of function of APP or neurotoxicity of Aβ peptide) is likely to affect the most vulnerable brain areas and to extend tau pathology. The process of NFD that occurs systematically in ageing is dramatically intensified, due to the burden of APP dysfunctions, and most likely resulting from the loss of APP carboxyterminal fragments. The progression of NFD in other brain areas is also progressively boosted by numerous other factors (OF) such as the lack of trophic factors (TrF) that are no longer supplied by affected neuronal networks, apoptosis that could be programmed following the lack of trophic factor and inflammation due to brain lesions and activation of microglial cells. The progression of NFD that will occur along a precise pathway of cortico-cortical connections could have its own dynamic, relatively independent of the aetiology. Slowing down this dynamic is a pharmacological target for delaying the progression of AD.

5. Tau pathology in other neurodegenerative disorders

Tau pathology is not exclusively found in AD. Indeed, this disease is observed in a majority of dementing disorders, such as Parkinsonian syndromes and frontotemporal dementias. These tauopathies are due to different dysfunctions of tau such as mutations, abnormal splicing, loss of normal tau proteins, aggregation of specific isoforms of tau proteins and abnormal phosphorylation (Delacourte and Buée, 2000; Buée et al., 2000; Sergeant et al., 2001). This demonstrates that different abnormalities on tau can produce a degenerating and dementing process, and that tau defects are frequent in the human brain.

6. Amyloid deposition in ageing and Alzheimer's disease

Neuropathological and biochemical approaches converge to describe amyloid deposition as a heterogeneous process, affecting preferentially the neocortex, but in a diffuse and not constant way (Braak et al., 1999). In early stages of AD, A β deposition can be found first in the frontal or the parietal cortex. However, the occipital cortex seems to be the predilection area for A β deposition. Our biochemical analyses show that A β 42 species are the first deposits found in the brain of non-demented patients. Their concentrations increase progressively, in parallel to tau pathology, and increase dramatically at the last stages of AD. At the opposite, A β 40 aggregates are found later in the course of AD, and sometimes are not detected. They are a late and unspecific marker of AD (Delacourte et al., 2001).

7. Amyloid and tau aggregation in ageing and Alzheimer's disease: which comes first?

The relationship between amyloidosis and tau pathology was studied on 60 non-demented and 70 demented patients. The correlation between amyloid deposits and tau pathology is weak at the level of their spatial distribution in brain areas, but strong if we compare the stages of tau pathology versus

the average amount of A β 42 aggregates in the neocortical areas. Indeed, we demonstrated with our biochemical approach, and in excellent agreement with numerous neuropathological studies (Fewster et al., 1991; Bierer et al., 1995; Duyckaerts and Hauw, 1997a; Braak et al., 1999; Mesulam, 1999; Price and Morris, 1999), that the pathway of tau pathology is precise, sequential, predictable, stereotypical and hierarchical. Ten stages of tau pathology were defined, according to the 10 brain areas that are successively affected (Delacourte et al., 1999). Here, our precise typing and quantification of amyloid deposits shows that the A β burden is diffuse, widespread and extremely heterogeneous, also in good agreement with neuropathological studies (Braak et al., 1999). It is interesting to note that each time we detected A β 42 cortical aggregates, we found a tau pathology, at least in the entorhinal region. But the opposite was not true, since a few rare cases had no trace of amyloid but tau pathology up to stage 6.

Our results demonstrate that tau pathology is upstream of A β deposition, but that the spreading of tau pathology in brain areas, along cortico-cortical connections is fuelled by APP dysfunction. Indeed, the progression of tau from the hippocampal formation to neocortical areas in AD is always and exclusively observed in the presence of amyloid deposits (Delacourte et al., 2001). The synergy for tau pathology spreading is likely to be driven by APP dysfunction, and more precisely by APP carboxy-terminal fragments, whose levels are decreasing in AD (Sergeant et al., 2002). These fragments have likely a trophic activity, and their decrease could magnify and expand tau pathology.

In summary, we observe a synergy of two degenerating processes, a tauopathy and an APPathy. Their convergence provokes AD, and entorhinal and hippocampus tauopathy is the springboard of AD (Fig. 1).

8. Conclusion: tau aggregation in the hippocampal formation: an ageing or a pathological process?

Together, one can see that tau pathology is a main feature of AD. But finding tau pathology at low levels does not mean that AD is beginning to develop. In fact, following our observations in the human brain,

we state that only A β deposits reflect AD. Indeed, tauopathy observed in ageing is likely one of the different tauopathies, which are either the consequence of mutations on tau gene, direct or indirect abnormal tau splicing, abnormal phosphorylation or abnormal tau levels (Delacourte and Buée, 2000). Because of the so many dementing disorders linked to a tau defect, there are good reasons to believe that tau aggregates in the entorhinal and hippocampal areas found without A β deposits reflect a pure pathological process that develops in a very vulnerable brain region. We demonstrated that this process is age-related because age is a risk factor, but age-independent in that some centenarians have only mild tau pathology.

But tau pathology can be exacerbated by numerous other factors, and especially APP dysfunction, to spread in other brain areas and to provoke a progressive loss of cognitive functions and AD. Therefore, our observations suggest that the tauopathy of the hippocampal formation in humans is a pathological process independent of AD, but that can be amplified by APP dysfunctions.

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