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Tau protein as a biomarker for asphyxia: A possible forensic tool?

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ABSTRACT

Asphyxial death has been a problem for forensic investigations due to the absence of a validated biomarker for the diagnosis of this event. Recently, research on brain affection by asphyxia raised hopes on the possible use of CNS markers for asphyxia. The cytoskeletal proteins seem to be attractive targets as they are vulnerable to hypoxia and can be affected in asphyxial deaths. *Tau*, an important cytoskeletal protein, showed affection in many neurodegenerative disorders and recently in some acute incidences like trauma and brain ischemia. In this report we show the affection of the normal pattern of tau and pathological aggregates of tau in the case of brain hypoxia. This may give new clues to asphyxial death investigations.

1. Introduction

Asphyxia diagnosis is still challenging to forensic pathologists as it is difficult to separate hypoxic ischemic disease from agonal hypoxia (Oehmichen et al., 2003). What makes this issue more puzzling is that neuropathological examination shows nonspecific findings (Kühn et al., 2005). It is important to find relevant biological markers to diagnose permanent hypoxic ischemic insult of the brain (Oehmichen et al., 2003). Brain death is the permanent end result of hypoxic ischemia of the brain. It is associated with brain edema, absence of vital reaction, herniation of the brain and softening of the gray matter (Oehmichen, 1994). It is supposed that brain anoxia and subsequent necrosis is a time dependent process although this drastic insult does not involve all neurons at the same time. This hypothesis is supported by the signs of vital reactions within the perivascular tissue of the brain parenchyma (Oehmichen, 1994). Cytoskeletal protein comprises a diverse group of proteins that maintain the structure and function of CNS. Microtubule-associated proteins (MAPs), one of these proteins, appear to be among the most sensitive of the cytoskeletal proteins in response to brain hypoxia/anoxia. Therefore, these proteins, including MAP2, are good sensitive markers for early agonal asphyxia (Hirokawa, 1994; Johnson and Jope, 1992; Tucker, 1990). Experimental studies on rats showed loss of MAP2 immunoreactivity in cases of rat brain with hypoxia–ischemia (Kwei et al., 1993; Malinak and Silverstein, 1996; Ota et al., 1997). More recently, Tau – another cytoskeletal protein – gained more attention due to its involvement in many neurodegenerative disorders. In normal brains, tau is thought to play an important role in microtubule stabilization and assembly. Furthermore, tau may help in signal transduction mechanisms, interactions with the actin cytoskeleton, neurite outgrowth and stabilization during brain development (Kambe et al., 2011). In brains of patients suffering from tauopathic disease, tau is found as aggregated abnormal filaments, such as neurofibrillary tangles localized in the somatodendritic compartments of cells, in contrast to their usual localization in axons. The distribution and ultrastructural morphology of tangles in the brain differ according to specific tauopathies and even to specific disease-causing mutations (Ludolph et al., 2009). Although, tau aggregates have been linked to a group of chronic neurodegenerative disorders, recent research correlated tau aggregates to acute brain insult e.g. ischemia (Villamil-Ortiz and Cardona-Gomez, 2015). The present study aimed to validate tau protein aggregates as a biomarker for asphyxia. Since there are still no solid biomarkers that can be used in forensic studies to assure diagnosis of asphyxia, tau could be a helpful tool in pinpointing asphyxial death.

2. Materials and methods

2.1. Animal model of hypoxia–ischemia (HI)

Twenty mice (C57/bl6) of both genders, 7 days old were used in this study. Animals were purchased from the Medical Experimental Research Center (MERC) of Mansoura University. All research was conducted according to a protocol approved by the Medical Experimental Research Center (MERC) of Mansoura University.

The HI group (10 mice) was exposed to right common carotid artery ligation on PND-7 under isoflurane anesthesia. Two hours after recovery, mice were subjected to 8% O_2 balanced with N_2 for 20 min at 37 °C (Ten et al., 2003). Pups that have formed an experimental group were returned to their dams. Control animals were considered as age- and strain-matched naïve (no HI) mice.

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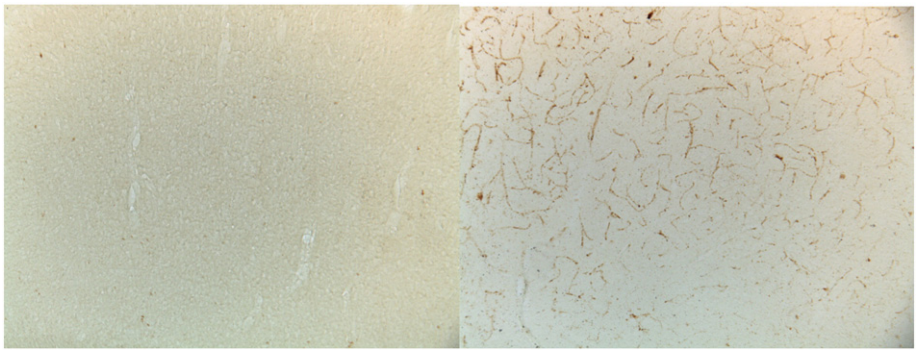

Fig 1. Image shows immunofluorescent staining of the cerebral cortex in the control (left) and HI (right) groups against AT8 as a marker of tau protein.

2.2. Histopathology and immunohistochemistry

One day following the HI model, mice were deeply anaesthetized and transcardially perfused with ice-cold 0.1 M phosphate buffered saline (PBS) for 2 min. Thereafter, the brains were quickly removed, post-fixed in 4% paraformaldehyde at 4 °C for 48 h, and washed with saline buffer. The brains were cut into 40-μm sections using a cryostat (Leica, Wetzlar, Germany), collected in 10 regularly spaced series, and stored in 0.1 M PBS containing 0.01% (wt/vol) sodium azide at 4 °C. Free-floating sections were stained with the mouse monoclonal primary antibodies raised against anti-PHF Tau AT8 antibody against pS202/pT205 (Thermo Scientific, Rockford, IL, USA; ratio used was 1:100).

2.3. Image analysis

Stereological cell count was done by a blinded observer to the animals' identity on regularly spaced (1/10) sections (average post-processing thickness was 20 μm) under a 40× objective with the optical fractionator method using the Stereo-Investigator software (MicroBrightField, Inc., Williston, VT, USA) in the frontal and parietal cortex of one hemisphere. The frontal cortex was analyzed between 1.94 and 0.86 mm anterior and 0 to 2.0 mm lateral from bregma, and the parietal cortex between 1.1 and 2.3 mm posterior and 1.0 to 3.0 mm lateral from bregma. Coordinates were based on Paxinos and Franklin (2012).

2.4. Statistical analysis

For statistical analysis we used GraphPad Prism 5.0 (La Jolla, CA, USA). Results were expressed as mean ± SEM. A p-value < 0.05 was assumed to be statistically significant. The experimental groups were compared with a one-way analysis of variance (ANOVA) with the Newman–Keuls post-hoc test, or two-way ANOVA followed by a post-hoc least significant difference (LSD) test, as appropriate.

3. Results

Analysis of tau aggregates 1 day after the HI model showed highly significant elevation compared to the control group as shown in Fig. 1 and Table 1.

Table 1
AT8 IR cell count after 1 day of hypoxia.

Control group	HI group
10,000	$3.5 \times 10^{7*}$

* P value < 0.001 compared to control group.

4. Discussion

The present report shows evidence that abnormal tau aggregates can be increased on exposure to hypoxia. These results build on the known previous studies showing that following stroke 3:4 of patients develop neurodegenerative disorders most commonly dementia and Alzheimer's disease (Wen et al., 2004). Pathological studies after death of these patients show typical AD characterizing pathology in the form of beta-amyloid proteins and tau aggregates (Song et al., 2013). More interesting pathophysiological studies of stroke showed abnormal phosphorylation of tau protein in a pattern similar to chronic disorders characterized by tauopathy (Cespedes-Rubio et al., 2010).

More recently, Villamil-Ortiz and Cardona-Gomez (2015) compared the abnormal phosphorylation and aggregation pattern of tau in transgenic mice compared to the hypoxic–ischemic mice model. Their results showed an acute rise in tau aggregates following ischemia in a pattern comparable to the findings in old transgenic mice. The only difference between the two groups was the rapid recovery of tauopathy in ischemic mice compared to the progressive course in the transgenic animals. Our report confirms the occurrence of abnormal tau aggregates in a hypoxic model of mice. This change, which happens acutely, could be a useful tool for forensic investigation of asphyxial death, as tau protein would represent a unique and new biomarker for such event. Furthermore, from a clinical point of view, results from this study may have considerable implications to other CNS insults e.g. TBI (Traumatic Brain Injury). Tau may be used as a prognostic biomarker following asphyxial incidents (Lv et al., 2015; Takahashi et al., 2014). In the previous works, tau was found to be elevated in CSF samples of neonatal asphyxia cases. This is in contrast to the findings of Liu et al. (2013) who did not find significant elevation in serum levels of tau in similar cases. This can be attributed to the fact that serum levels of cytoskeletal proteins cannot be correlated to CNS injury due to early disappearance by proteases, on the contrary using auto-antibodies would be more reliable method (Abou-Donia et al., 2013). Moreover, the involvement of tau in the present study my extend its importance as a biomarker to other brain injury and its related deaths e.g. heat induced brain damage as in the work of Kibayashi and Shojo (2003). The involvement of tau protein in asphyxial incidents may have great significant implications including: prognostication, therapeutic intervention, and delineating potential treatment windows following such incidents. Overall, our data provides a novel assessment for long term outcomes after Ischemic hypoxic brain insults.

4.1. Limitations

Despite the novelty and implications of this work, this study is not without limitations. First, the current study used a small number of mice of mixed sex without considering sex as a determining factor. Another limitation was that there were no neurobehavioral tests to assess the neurofunctional outcomes in this study that makes it difficult to

know whether biological relationships among tau and other biomarkers, are due to direct effects or simply epiphenomena derived from other causal components of the secondary injury cascade. More important, we need to know whether these findings are active vital reaction or postmortem events. Thus, there is a need for future experimental studies designed to elucidate biochemical interactions between various biomarkers.

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