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Research report

Effects of postmortem delays on protein composition and oxidation

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ABSTRACT

Human autopsy brain tissue is widely used to study neurodegenerative diseases such as Alzheimer's, Parkinson's and other diseases. However, when it comes to an evaluation of data obtained from such tissue, it is essential to consider potential postmortem effects on protein composition, posttranslational modification and proteolysis with increasing postmortem delays. In this study, we analyzed mouse brain tissues with different postmortem delays (pmd) of 0 h, 6 h and 24 h, for changes in protein composition, proteolysis and modifications such as S-nitrosylation, carbonylation and ubiquitination. Proteins involved in Alzheimer's disease (AD) were of special interest, including cytoskeletal and synaptic proteins or proteins involved in inflammation. Several proteins were fairly resistant to degradation during the first 6 h but started to degrade thereafter. S-nitrosylation and carbonylation showed not much variation, except for those proteins that were susceptible to degradation. Brain spectrin was S-nitrosylated at death, and S-nitrosylated degradation fragments were measured at a pmd of 24 h, indicating a susceptibility of brain spectrin to degradation. Furthermore, the physiological role of S-nitrosylation remains to be investigated. When studying human brain tissue, some proteins are more susceptible to degradation than others, while ubiquitination and carbonylation were little affected during the first 24 h after death.

1. Introduction

Human autopsy brain tissue is widely used to study neurodegenerative diseases. However, several factors such as genetic background, differences in gender and age, lifestyle and medication as well as prolonged postmortem delays (pmd) of tissue samples complicate the interpretation of research data. Several transgenic animal models were generated to circumvent pmd problems and to focus on the role of specific proteins such as amyloid precursor protein, presenilins and tau proteins and their implication in the formation of pathological hallmark structures (Spires and Hyman, 2005; Lasagna-Reeves et al., 2011). Alzheimer's disease is the most prominent age-related neurodegenerative disease. Different hypotheses were proposed, but its etiology is still highly debated. To understand mechanisms of this disease, an analysis of human postmortem tissue represents an important source of tissue for various investigations. Proteomic analysis is essential to identify biomarkers, changes in the protein composition and posttranslational modifications (Butterfield et al., 2003; Lovestone et al., 2007). However, by using autopsy tissues results may be affected by events occurring after death (Leuba et al., 2014, 2008; Riederer et al., 2009). Here, we studied mouse brain tissue and effects of various postmortem delays on a set of proteins that are of interest in Alzheimer's disease. The choice of proteins is based on recent studies on human autopsy brain tissue (Leuba et al., 2014, 2008; Riederer et al., 2009) that demonstrated a pathological reorganization of NMDA receptors and PSD-95 protein; and ongoing studies investigating synaptic proteins, effects of oxidative stress and the role of protein oxidation and ubiquitination. Despite differences between mouse and human brain tissue, this study may help in the interpretation of results obtained from autopsy tissue removed with variable postmortem delays.

Increasing evidence suggests that synaptic, cytoskeletal and mitochondrial proteins are affected during Alzheimer's disease (Reddy et al., 2012). Protein modifications induce an activation of astrocytes, a microglia response and exacerbate neuronal damage (Pizza et al., 2011).

Oxidative stress is one of the risk factors of dementia that may influence the progression of the disease (Butterfield et al., 2006a,b); and the result of an imbalance between reactive oxygen species (ROS)/reactive nitrogen species (RNS) production and the antioxidant activity (Korolainen et al., 2006).

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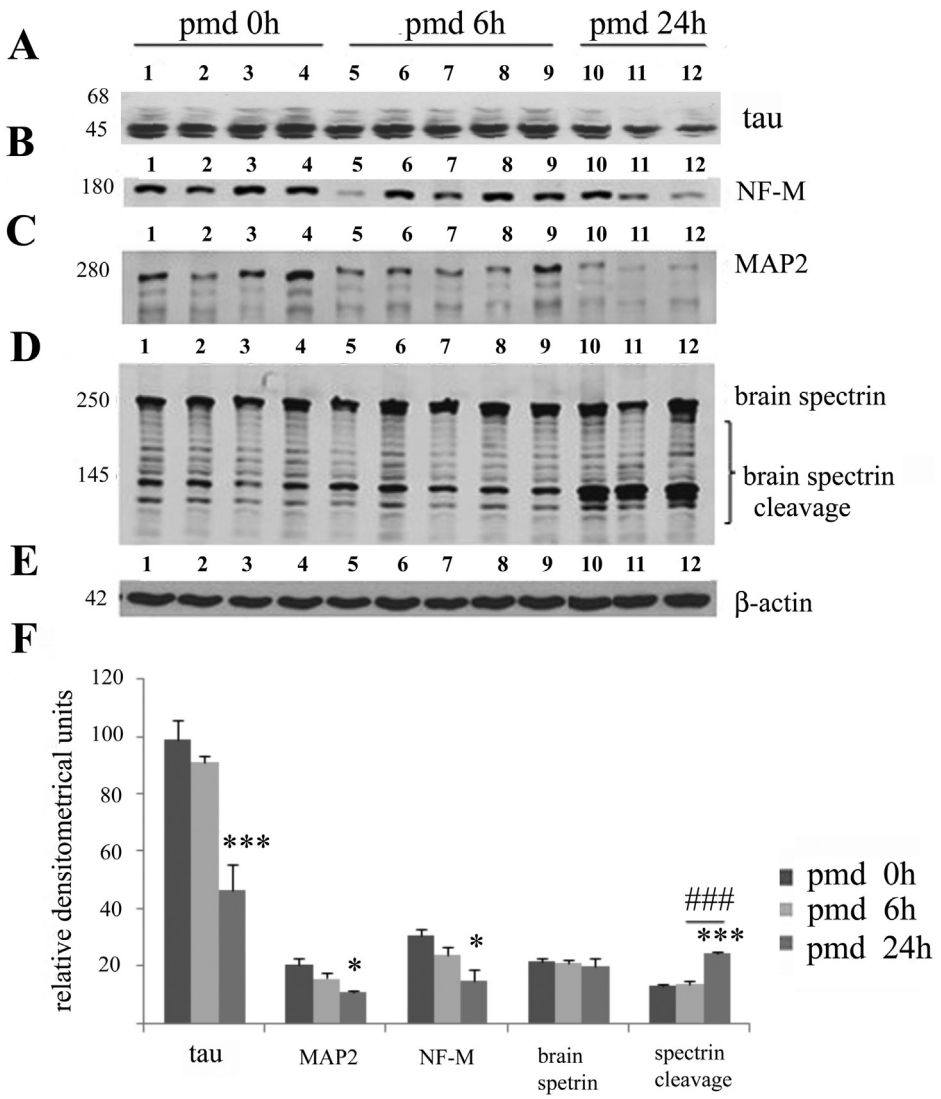

Fig. 1. Effects of post mortem delay on cytoskeletal proteins.
SDS-PAGE 3.6–15% gradient gels with 20 μg of frontal cortex sample per slot. Samples were divided into three groups with pmd of 0 h, 6 h and 24 h. Samples were analyzed by Western blots and immunostained with the following antibodies: Panel A: polyclonal anti-chicken tau protein (Savioz et al., 2003); panel B: monoclonal anti-NF-M; panel C: monoclonal anti-MAP2; and panel D: polyclonal anti-brain spectrin; panel E: β-actin was used to normalize staining intensities. Panel F: normalized staining intensities are represented by mean + SEM. The degradation products of brain spectrin were also quantified. *P*-value are represented by ***<0.001 and *<0.05 when comparing samples to pmd 0 h group and by ### <0.001 when comparing samples with pmd 24 h to pmd 6 h group. Molecular weights are indicated to the left in kDa.

A thorough understanding of these processes is essential to identify triggering factors of the disease and to develop prevention strategies. Therefore, the use of postmortem tissues allows comparative studies between control and Alzheimer brain tissues at different stages of the disease and eventually to find some important targets. However, given that autopsy brain tissue differs in pmd, we set out to use mouse brain tissue and to identify postmortem effects on (i) protein composition, (ii) protein oxidation and (iii) ubiquitination.

2. Material and methods

2.1. Brain samples

C57BL/6 adult female mice ($N = 12$) were sacrificed by cervical dislocation and by decapitation at age of 100 days. Frontal cortex was removed immediately after death (pmd = 0 h, $N = 4$), or heads were kept at room temperature for 3 h and then moved to 4 °C. Brains were removed after 6 h of death (pmd = 6 h, $N = 5$) or 24 h (pmd = 24 h, $N = 3$) and frozen at −80 °C until further use. This procedure was used to simulate what happens once a patient has died and shortly after is moved to the cold room of the pathology department. Human autopsy brain tissue with a postmortem delay of 6–24 h is used for biochemical studies. Past the 24 h, brain tissue is no longer acceptable for proteomic analysis.

Mouse brain tissues were homogenized manually in a ratio of 1:10 (W/V) in phosphate buffer saline (PBS: 5 mM Na_2HPO_4 (Merck, Germany), 155 mM NaCl (Merck,Germany), nanopurified water (Barnstead, USA), pH 7.4), 0.1% Tween 20 (Fluka, United Kingdom), 0.1% Triton X-100 (Chemica, Switzerland) and 1% protease inhibitor cocktail (Sigma, USA). Protein concentration was measured with the Bradford assay. Proteins were diluted by adding SDS-mix (1.23M Trizma-base (Sigma, Germany), 0.15M 2-mercaptoethanol (Sigma, Germany), 346 mM SDS (Sigma, Germany), 1.1 mM Glycerol (Merck, Germany) and Bromophenol Blue (Fluka, Switzerland), pH 6.8) in order to get a final concentration of 2 mg/ml. Finally, these