Neuroscience 330 (2016) 1–11

DECLINE OF MICROTUBULE-ASSOCIATED PROTEIN TAU AFTER EXPERIMENTAL STROKE IN DIFFERENTLY AGED WILD-TYPE AND 3xTg MICE WITH ALZHEIMER-LIKE ALTERATIONS

DOMINIK MICHALSKI, [a*] **HARTMUT PREIßLER,** [b] **SARAH HOFMANN,** [b] **JOHANNES KACZA** [c] **AND WOLFGANG HÄRTIG** [b]

[a] *Department of Neurology, University of Leipzig, Liebigstr. 20, 04103 Leipzig, Germany*

[b] *Paul Flechsig Institute for Brain Research, University of Leipzig, Liebigstr. 19, 04103 Leipzig, Germany*

[c] *Institute of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Leipzig, An den Tierkliniken 43, 04103 Leipzig, Germany*

Abstract—Stroke therapies are still limited to a minority of patients. Considering time-dependent aspects of stroke, the penumbra concept describes the transition from functional to permanent tissue damage. Thereby, the role of cytoskeletal elements, as for instance microtubules with associated tau remains poorly understood and is therefore not yet considered for therapeutic approaches. This study explored the expression of microtubule-associated protein tau related to neuronal damage in stroke-affected brain regions. Wild-type and triple-transgenic mice of 3, 7 and 12 months of age and with an Alzheimer-like background underwent experimental stroke. After 24 h, brain sections were used for immunofluorescence labeling of tau and Neuronal Nuclei (NeuN). Potential functional consequences of cellular alterations were explored by statistical relationships to the general health condition, i.e. neurobehavioral deficits and loss of body weight. Immunoreactivity for whole tau decreased significantly in ischemic areas, while the decline at the border zone was more drastic for tau-immunoreactivity compared with the diminished NeuN labeling. Quantitative analyses confirmed pronounced sensitivity for tau-immunoreactivity in the ischemic border zone. Decline of tau- as well as NeuN-immunoreactivity correlated with body weight loss during the 24-h observation period. In conclusion, microtubule-associated protein tau was robustly identified as a highly sensitive cytoskeletal constitute under ischemic conditions, suggesting a pivotal role during the transition process toward long-lasting tissue damage. Consequently, cytoskeletal elements appear as promising targets for novel therapeutic approaches with the objective to impede ischemia-induced irreversible cellular degradation.

*Corresponding author. Tel: +49-341-9724339; fax: +49-341-9724199.

E-mail address: dominik.michalski@medizin.uni-leipzig.de (D. Michalski).

Abbreviations: Cy, Carbocyanine; NeuN, Neuronal Nuclei; TBS, Tris-buffered saline.

http://dx.doi.org/10.1016/j.neuroscience.2016.05.013

Key words: ischemic stroke, tau, microtubule, cytoskeleton.

INTRODUCTION

Stroke still entails a relevant socio-economic burden and represents a major cause of death worldwide (Donnan et al., 2008; Mozaffarian et al., 2016). Despite numerous efforts concerning the development of novel treatment strategies, today's therapy is limited to intravenous thrombolysis (Hacke et al., 2008), recently added by approaches applying mechanical thrombectomy to achieve recanalization of occluded brain vessels (e.g., Berkhemer et al., 2015). However, due to regulations these interventions are only available for the minority – i.e. about 13% (Dirks et al., 2011) – of patients. Consequently, research has focused on improved understanding of stroke biology aiming at new therapeutic strategies (Meairs et al., 2006; Endres et al., 2008). Considering time-dependent effects, the evolution of the ischemic lesions was summarized by the penumbra concept, describing a transition from a functional – in principle reversible impairment – to permanent tissue damage (Astrup et al., 1981; Dirnagl et al., 1999). While numerous molecular pathologies were discussed underlying the penumbra concept (Sharp et al., 2000), the cellular mechanisms responsible for the shift toward permanent tissue damage remain poorly understood. From a mechanical perspective, elements known to stabilize cellular integrity – i.e. the cytoskeleton including for instance microtubules and associated tau (Butner and Kirschner, 1991; Maccioni and Cambiazo, 1995; Tashiro et al., 1997) – likely play a pivotal role for the development of long-lasting tissue damage after stroke (Zheng et al., 2010).

As previous studies in the field of stroke have addressed the family of microtubule-associated proteins, and have found significant alterations as for instance a reduction of the microtubule-associated protein 2 (MAP2) in ischemic areas (Li et al., 1998; Lian et al., 2015; Michalski et al., 2013; Zhang et al., 1999), data on tau are mainly available from investigations focusing on neurodegenerative disorders (Arendt, 2004; Cairns et al., 2004; Holzer et al., 1994; Lee et al., 2001; Raskin et al., 2015). Thereby, previous reports identified certain tau species – associated with delimited microtubule integrity and impaired neuronal function, overall leading to long-lasting cellular degradation (Gendron and

Petrucelli, 2009; Krüger and Mandelkow, 2015). During the progression of neurodegenerative diseases, hyperphosphorylation of tau was considered as a hallmark of brain pathologies known as tauopathies (Baas and Qiang, 2005; Billingsley and Kincaid, 1997; Gendron and Petrucelli, 2009; Lee et al., 2001; Rudrabhatla, 2014; Wang and Mandelkow, 2016). Studies linking the field of neurodegenerative disorders and cerebral ischemia mainly reported that experimentally induced transient cerebral ischemia significantly alters the phosphorylation status of tau (Dong et al., 2014; Geddes et al., 1994; Koike et al., 2011; Shackelford and Yeh, 1998; Song et al., 2013; Wen et al., 2004). On the other hand, Hesse et al. found a transient increase in total tau, but not phospho-tau in human cerebrospinal fluid after acute stroke (Hesse et al., 2001).

Apart from its phosphorylation state, the role of the entire tau protein as a *per se* stabilizing constituent of the cytoskeleton remains poorly understood under acute ischemic conditions, which are usually characterized by an impaired cellular integrity – presumably mediated by a decomposition of cytoskeletal elements. Driven by the hypothesis that tau might represent an ischemia-sensitive component of the cytoskeleton, interventions focusing on the stabilization of tau during the acute phase of stroke are likely to represent a novel, not yet considered therapeutic strategy.

The aim of this study was to investigate the regional expression patterns of microtubule-associated protein tau in relation to the degree of neuronal damage following experimental focal cerebral ischemia. To elucidate potential age- and disease-related effects, analyses comprised wild-type and triple-transgenic (3xTg) mice with age-dependent Alzheimer-like alterations (Oddo et al., 2003; Hawkes et al., 2013) – of different ages up to 12 months. Applying a multiparametric approach, statistical relationships between parameters characterizing the general health condition (i.e. the neurobehavioral deficit and loss of body weight) and altered tau expression were analyzed with the objective to explore potential functional consequences of immunohistochemical alterations.

EXPERIMENTAL PROCEDURES

Study design and content

For the present study a total of 43 mice of both genders (42% males) with a mean body weight of 25.6 ± 4.1 g and three different ages as well as two genetically phenotypes were used. In detail, data emerged from $n = 21$ Sv129/B6-wild-type mice (3 months: $n = 8$; 7 months: $n = 6$; 12 months: $n = 7$) and $n = 22$ 3xTg mice (3 months: $n = 6$; 7 months: $n = 8$; 12 months: $n = 8$), which harbor two mutant human transgenes ($APP_{Swedish\ mutation}$ and tau_{P301L}, driven by neuron-specific Thy1-regulatory elements) added by the homozygous knock-in construct presenilin-1_{M146V}, previously described by Oddo et al. (2003). Animals were bred in the Medizinisch-Experimentelles Zentrum at the University of Leipzig, while breeding pairs were originally provided by Drs. Frank M. LaFerla and Salvatore Oddo (University of California, Irvine, CA, USA). Animal experiments were performed according to the European Communities Council Directive (86/609/EEC) after protocol approval by local authorities (Regierungspräsidium Leipzig; reference number TVV 24/10). Efforts were made to minimize the total number and suffering of animals, which were housed in a humidity-controlled room with 12/12-h light/dark cycle and with free access to food and water.

Primarily, the study was focused on the regional expression pattern of microtubule-associated protein tau (Butner and Kirschner, 1991), with a special focus on the relation to the degree of neuronal damage, i.e. the affection of the neuron-specific nuclear protein (NeuN; Mullen et al., 1992), each assessed by immunofluorescence labeling at 24 h after experimental focal cerebral ischemia. Further, neurobehavioral deficits and changes in body weight were assessed to captured stroke-induced alterations on the general health condition. To ensure sufficient induction of experimental focal cerebral ischemia, mice entering the study had to demonstrate a relevant neurobehavioral deficit in terms of a predefined study inclusion criterion; procedural details are given below.

Experimental focal cerebral ischemia

Ischemic stroke was induced by the filament model as originally described by Longa et al. (1989) with some minor modifications, resulting in right-sided permanent middle cerebral artery occlusion. Briefly, a standardized silicon-coated 6-0 monofilament (Doccol Corporation, Redlands, CA, USA) was inserted into the right external carotid artery and moved forward into the internal carotid artery until bending was observed or resistance was felt. The procedure was done in anesthetized mice using etomidate (33 mg/kg body weight i.p.; Hypnomidate, Janssen-Cilag, Neuss, Germany). Further, local anesthesia of the ventral neck was ensured by subcutaneous injection of lidocaine (Xylocitin 1%, mibe, Brehna, Germany). During surgery, the body temperature was maintained at 37.0 °C using a thermostatically controlled heating pad including rectal probe (Fine Science Tools, Heidelberg, Germany). After surgery, the animals spent time on a commercially available warming pad (37.0 °C) until anesthesia has passed in order to prevent cooling. Post-surgical pain treatment was ensured using metamizole (Novaminsulfon-ratiopharm, ratiopharm, Ulm, Germany) in the glucose-enriched drinking water.

In our hands, the applied model of focal cerebral ischemia in wild-type and genetically altered (3xTg) mice was associated with a quite low intra-procedural mortality (about 2%), while premature death during the observation period occurred in about 20%, stressing the transgenic background (Hawkes et al., 2013).

General health condition

One day after induction of focal cerebral ischemia, neurobehavioral deficits were assessed using a score originally described by Menzies et al. (1992), whereas the score 0 reflects ‘no apparent deficits’, 1 ‘contralateral forelimb flexion’, 2 ‘decrease grip of the contralateral