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Original article

Ischemic tau protein gene induction as an additional key factor driving development of Alzheimer's phenotype changes in CA1 area of hippocampus in an ischemic model of Alzheimer's disease

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ABSTRACT

Background: Tauopathies are a class of neurodegenerative illnesses associated with the aberrant accumulation of the tau protein in the brain. The best known out of these diseases is Alzheimer's disease, a disorder where the microtubule associated tau protein becomes hyperphosphorylated (which lowers its binding affinity to microtubules) and accumulates inside neurons in the form of tangles. In this study, we attempt to find out whether brain ischemia may play an important role in tau protein gene alterations.
Methods: We have investigated the relationship between hippocampal ischemia and Alzheimer's disease by means of a transient 10-min global brain ischemia in rats and determining the effect on Alzheimer's disease tau protein gene expression during 2, 7 and 30 days post injury.
Results: We found the significant overexpression of tau protein gene on the 2nd day, but on day's 7 and 30 post-ischemia there a significant opposite tendency was observed.
Conclusion: The obtained results offer a novel insight into tau protein gene in regulating delayed neuronal death in the ischemic hippocampus. Finally, these findings further elucidate the long-term impact of brain ischemia on Alzheimer's disease development.

Introduction

The incidence of dementia increases dramatically as a result of transient brain ischemia in humans and animals [1–8]. There is strong evidence that brain ischemia increases the risk of both ischemic dementia with Alzheimer's phenotype and Alzheimer's disease dementia [9]. In fact, the Alzheimer's pathological changes start in hippocampus [10,11] as well as in post-ischemic brain injury dementia [4,6,12,13]. Both, amyloid plaque and neurofibrillary tangles are mainly restricted to hippocampus, the most pronounced brain structure impacted by Alzheimer's disease [10,11]. The aberrant phosphorylation of tau protein inhibits microtubule stability and development, axonal transport and finally compromise cognitive function [14]. In all neurodegenerative diseases, in which tau protein is implicated, it is in a hyperphosphorylated form, which is responsible for its aggregation, leading eventually to neuronal dysfunction and death [15–17].

The reasons for ischemic dementia with Alzheimer's phenotype and Alzheimer's disease dementia have not been clearly identified, but one possible contributor, from several different factors, is the tau protein aberrant phosphorylation response to ischemic brain injury, which compromises microtubule function and development [18], an effect known to lead to apoptosis [17] and contributing significantly to subsequent neurodegeneration in both ischemia [19,20] and Alzheimer's disease [21,22]. Circulating elevated levels of tau protein are associated with Alzheimer's disease neuropathology [23] and ischemic brain injury in humans [24,25]. Additionally, tau protein, elevated in the extracellular

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space, predicted adverse clinical outcome following experimental brain injury [26]. Phosphorylated tau protein has been shown to accumulate inside neurons in brain areas of specific vulnerability like hippocampus after ischemic damage [19,20,27–29] or in Alzheimer's disease [10,14,15]. Tau protein is strong pro-death protein both in ischemic brain injury and Alzheimer's disease and it is likely to contribute to the death of neurons by apoptosis in which it is aggregated after phosphorylation [15,19,20,29]. This was associated with the increased activity of the tau protein phosphorylating enzyme Cdk5, and the consequent development of filaments similar to those present in human tauopathies such as Alzheimer's disease [19]. It has been demonstrated that β-amyloid peptide generation in brain ischemia [30–32] can lead to increased apoptosis [33] but by induction of high level of tau protein phosphorylation it can additionally potentiate amyloid neurotoxicity and finally promote progression of the neurodegenerative diseases [13,20]. There is a strong support for the ischemia-reperfusion theory suggesting that the earliest pathological process in sporadic Alzheimer's disease may be an ischemic episodic damage to hippocampus [12,34,35]. Subsequent brain ischemia generates further production of β-amyloid peptide, a degradation product of amyloid protein precursor, and induction of tau protein phosphorylation, neuronal dysfunction, and finally neuronal death [13,19,20,27,36]. Though, despite the suggested phosphorylated tau protein role in ischemia-induced apoptotic cell death, as yet, there is no definitive data or published work describing the effect of ischemic brain injury on neuronal tau protein gene expression. This study presents time course of the expression of tau protein gene by RT-qPCR in CA1 subfield of rat hippocampus following transient ischemic injury with survival time 2, 7 and 30 days.

Materials and methods

Animals

Global 10-min brain ischemia was induced in female Wistar rats (n = 49, 2 month old, 160–180 g) by cardiac arrest [37]. The animals were housed paired in cages in a room with a controlled temperature of 24 ± 2 °C, humidity of 55 ± 5%, and a 12 h light-dark cycle. They had unlimited access to commercial laboratory chow pellets and tap water. The experiments were carried out in the light phase and the animals were treated according to the NIH Guide for Care and Use of Laboratory Animals and European Communities Council Directive 142. The Local Ethical Committee approved all the scheduled experimental procedures. Following brain ischemia, the animal survival periods were 2 (n = 11), 7 (n = 18) and 30 (n = 20) days. Sham-operated rats (n = 49, survival 2 days n = 11, 7 days n = 18, and 30 days n = 20) underwent the same experimental procedures as these after global brain ischemia and served as respective control groups.

Hippocampal samples

Before taking hippocampal samples, a cold 0.9% NaCl was used for the brain perfusion *via* the left ventricle in order to flush blood vessels. Next, the brains were dissected from the skulls and transferred to an ice chilled Petri dish. The samples taken from ischemic and control hippocampi with a narrow scalpel (CA1 area of bilateral hippocampi of circa 1 mm^3 volume) were immediately placed in RNALater solution (Life Technologies, USA) [33,38–40].

Procedures

Isolation of total cellular RNA was brought about according to the method elaborated by Chomczynski and Sacchi [41]. NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) was used for the assessment of the RNA quality and quantity [33,38–40]. Subsequently, the isolated RNA was stored in 80% ethanol at −20 °C for further analysis [33,38–40]. One microgram of the total RNA was reverse transcribed to cDNA using High-Capacity cDNA Reverse Transcription Kit, according to manufacturer's instructions (Applied Biosystems, USA). Synthesis of the cDNA was carried out on Veriti Dx (Applied Biosystems, USA) under the following conditions: stage I: 25 °C, 10 min, stage II: 37 °C, 120 min, stage III: 85 °C, 5 min, stage IV: 4 °C. The cDNA, obtained by this procedure, was amplified by real-time gene expression analysis (qPCR) on 7900HT Real-Time Fast System (Applied Biosystems, USA) with power SYBR-green PCR master mix reagent, with the use of the manufacturer's SDS software [38]. Amplification protocol comprised the following cycles: initial denaturation: 95 °C, 10 min and 40 cycles, each under two different temperatures: 95 °C, 15 s and 60 °C, 1 min. Monitoring and calculation of the number of copies of DNA molecules was performed on 7900HT Real-Time Fast System (Applied Biosystems, USA) in each amplification cycle. The number of PCR cycles after which the level of fluorescence exceeded the defined threshold cycle (C_T) relative expression Study Software (Applied Biosystems, USA) was used in order to calculate the number of examined DNA molecules present in the mixture at the onset of reaction. Normalization was achieved against endogenous control gene (Rpl13a) [42] and relative quantity (RQ) of gene expression was analyzed based on ΔC_T method and the results were calculated as $RQ = 2^{-\Delta\Delta C}{}_T$ [38,43]. The RQ values were finally analyzed following their logarithmic conversion into logarithm of RQ (LogRQ) [38]. LogRQ = 0 indicates that gene expressions in the calibrated and ischemic samples do not differ. LogRQ < 0 means that there is a reduced gene expression in the ischemic sample, whilst LogRQ > 0 is indicative of an increased gene expression in the ischemic sample in comparison to the calibrated one.

Statistical analysis

Statistical evaluation of the results was performed by Statistica software v. 12 with the help of the non-parametric Kruskal-Wallis test with "z" test – multiple analyzes of differences between groups. Data were presented as means ± SEM. The statistical significance was accepted at $p \leq 0.05$.

Results

The expression of tau protein gene was elevated to a maximum of 3.297-fold change at 2nd day after brain ischemia but the mean expression level was 1.297 ± 0.369. At 7 days after ischemic brain injury, a maximum expression was 0.235 and a minimum −0.492 fold change. The mean expression level was −0.087 ± 0.053. When evaluated at thirty days after brain ischemia, tau protein gene expression was reduced to a minimum of −0.351 and the mean expression level was −0.193 ± 0.022. Fig. 1 shows alterations in the mean expression levels of tau protein gene, statistical significance, following 10-min brain ischemia in rats, being observed between 2 and 7 as well as between 2 and 30 days.

Discussion

The assumed hypothesis of this study was focused on identifying the responsiveness of the tau protein gene in CA1 area of hippocampus to brain ischemia. It has been revealed that hippocampal ischemia significantly up-regulates tau protein gene 2 days after injury which is in accordance with the increase in both total tau protein level in human serum [24,25] and extracellular space of injured brain after global brain ischemia [26] as well as with tau protein phosphorylation levels in animal ischemic brain

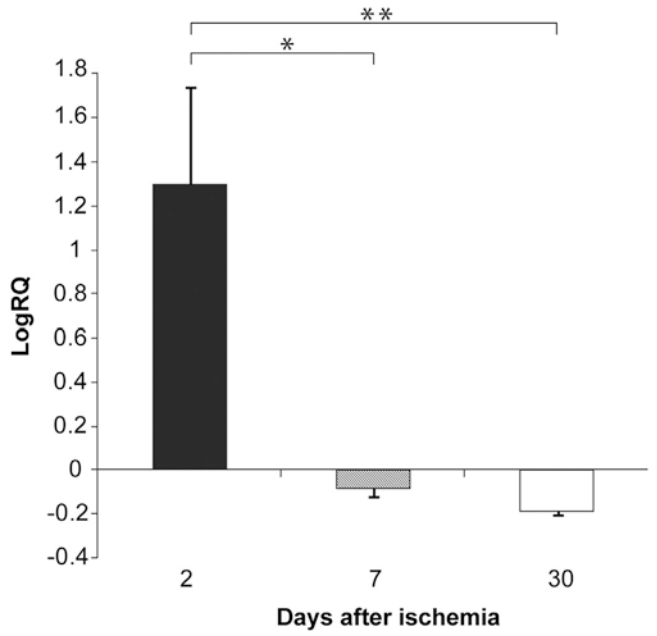

Fig 1. The mean expression level of tau protein gene in the hippocampal CA1 area in rats 2 (n = 11), 7 (n = 18), and 30 (n = 20) days after 10-min global brain ischemia. Marked SEM-standard error of the mean. Indicated statistically significant differences in levels of gene expression between 2 and 7 ($z = 3.253$, $p = 0.0034$) and between 2 and 30 days ($z = 4.862$, $p = 0.00003$) after 10-min brain ischemia (Kruskal-Wallis test). $^{*}p < 0.01$, $^{**}p < 0.001$.

[19]. The early overexpression of the tau protein gene was accompanied by late downregulation of this gene during 7-30 days. It has been documented that tau protein gene is significantly upregulated 2 days post-ischemia when delayed neuronal death is induced [12,13,35]. At that time, tau protein gene overexpression appears to be a critical trigger for the hippocampal CA1 subfield neuronal death being completed on 7–30 days after ischemia [12,13,35].

The observations from this study have clearly illustrated that ischemic hippocampus indeed has an influence on the tau protein gene induction, reflected in its huge overexpression, beginning within a stage-specific neuronal CA1 death window (2 days after ischemia) and then followed by downregulation when neuronal death is completed (7–30 days after ischemia) [12,13,35]. As already mentioned, the overexpression of the tau protein gene was associated with early histological signs of acute neuronal death in the CA1 hippocampal area within 2 days after ischemia [13,35]. Within this particular time, the substrate for β-amyloid peptide, amyloid protein precursor, was downregulated [38], being to some extent delayed for 7 days, and this is in accordance with observations by Magnoni et al. [26]. They have noted that an increase in tau protein level in extracellular space of the injured brain correlates with the reduced β-amyloid peptide level, predicting worse clinical outcomes. It can be also noted that ischemia induces the expression of tau protein gene during onset of acute neuronal death in the CA1 hippocampal area, this effect being parallel with the apoptotic caspase 3 gene upregulation involved in neuronal death [44]. The precise mechanism, by which caspase 3 and tau protein together execute CA1 hippocampal neuronal death, is not fully understood. Caspase can use tau protein as a substrate and finally can cut it [45–48]. The existing data clearly point to a correlation between the elevated level of activated caspase and increased concentrations of truncated tau protein and formation of neurofibrillary tangles [21,49]. Also, the cognitive decline was significantly negatively correlated with the increased caspase activity and tau protein truncated by caspase 3 [48].

The overexpression of tau protein gene after ischemia correlated with the same pattern of the total blood tau protein level in patients after global brain ischemia [24,25] and extracellular space concentration in experimental brain injury [26] and finally follows the hyperphosphorylated tau protein level [19]. The total tau protein mRNA level illustrates that the upregulation of transcription results in excessive protein translation and subsequent phosphorylation and the transcriptional levels are similar in pattern to the protein levels [19,29]. The above observations are in agreement with tau protein rephosphorylation level during reperfusion in the hippocampus after global cerebral ischemia due to four-vessel occlusion model in rats [50] and in the frontal cerebral cortex after global cerebral ischemia due to cardiac arrest in beagles [51]. Additionally, hyperphosphorylation of tau protein in the hippocampus has been reported in response to reperfusion after transient global brain ischemia due to bilateral common carotid artery occlusion in Mongolian gerbils [52]. Additional data showed consistent elevated concentrations of Cdk5 in rats exposed to focal brain ischemia compared to controls [19]. Hyperphosphorylation of tau protein may alter its degradation (through the proteasome or autophagy) and its truncation by proteases [53].

Conclusions

Taken together, the data from this study provide evidence that the exposure to ischemia causes a significant increase in the hippocampal expression of tau protein gene during neuronal death in the CA1 sector [54]. Noteworthy, this might create a novel insight into tau protein gene in regulating delayed neuronal death in the ischemic hippocampus. As a consequence of the above effect, there was observed an elevated concentration of tau protein in blood and extracellular space of brain with aberrant phosphorylation [26,29]. These results support the earlier data by Wen et al. [19] who demonstrated that Cdk5 was involved in neurofibrillary tangle-like tauopathy induced by transient brain ischemia in female rats.

Conflict of interests

S.J. Czuczwar declares financial support from UCB, GlaxoSmithKline, Bayer, Novartis, Sanofi-Aventis, Janssen for lecturing. He has also received an unrestricted grant from GlaxoSmithKline. Other authors report no conflict of interests.

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