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ORIGINAL ARTICLE

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Heat-induced immunoreactivity of tau protein in neocortical neurons of fire fatalities

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Abstract Tau protein is the main component of neurofibrillary tangles of Alzheimer's disease (AD). Immunohistochemistry of tau protein is useful in the diagnosis of AD but produces diffuse staining of neocortical neurons in fire fatalities. To learn the cause of this phenomenon, we examined the temporal neocortex of 13 fire fatalities and 9 fatalities unrelated to fire. The diffuse tau immunoreactive neurons were observed in 10 fire fatalities with heat coagulation of the cerebrum. Diffuse staining was not found in the three fire fatalities without heat coagulation of the cerebrum or in fatalities unrelated to fire. The immunoreactivity progressively increased as a function of the degree of cerebral heat coagulation. These results demonstrate that diffuse tau immunoreactivity of neocortical neurons is a post-mortem phenomenon caused by prolonged exposure of the head to intense heat. Forensic pathologists should consider this phenomenon when they diagnose AD in fire fatalities.

Keywords Tau protein · Neuron · Immunohistochemistry · Fire fatality · Post-mortem changes

Introduction

Alzheimer's disease (AD) is the leading cause of dementia in the elderly [1, 2]. Patients with AD are at risk of accidents such as falls, traffic mishaps, house fires and hypothermia because of cognitive dysfunction and abnormal behavior such as aimless wandering. In a forensic autopsy of an elderly person, a neuropathological examination may confirm the clinical diagnosis of AD and determine the cause of an accident [3, 4, 5]. The neuropathological diagnosis of AD is based on the histological findings of the cerebral neocortexes in frontal, temporal and parietal lobes. Increased densities of Alzheimer's type of changes, i.e. neurofibrillary tangles (NFTs) and senile plaques (SPs), result in the symptoms of dementia in AD patients and are used as histological hallmarks of AD [6, 7]. Tau protein is the main component of abnormal filaments that form NFTs in neurons [8]. Deposition of beta-amyloid in extracellular spaces forms SP [9]. Silver staining by a modified Bielschowsky method and immunohistochemistry (IHC) of tau protein for NFTs and beta-amyloid for SPs are recommended for use in the diagnosis of AD [4, 5, 7, 10].

We routinely examine the Alzheimer's type of changes in all forensic autopsy cases of older persons involved in fatal accidents, to identify AD and determine the causal relationship to the accidents. We observed that neocortical neurons of fire victims showed diffuse immunoreaction by IHC using anti-tau antibodies. The cause of this phenomenon has not been previously studied. Furthermore, the immunoreactive neurons should be differentiated from NFT-containing neurons for the diagnosis of AD in fire fatalities. To find the cause of the neuronal tau immunoreaction and evaluate its significance for the diagnosis of AD, we analyzed the neuropathological findings of forensic autopsy cases in fire fatalities.

Materials and methods

A total of 13 consecutive forensic autopsy cases involving fire fatalities in our department were studied. All of these subjects were found dead at the scene and the cause of the fire was either accidental house or grass fire, or suicidal blaze with flammable liquids. In 12 cases, the cause of death was certified as immediate fire death based on autopsy findings, such as cherry red skin and soot deposits in the airways and/or high levels of blood carboxyhemoglobin saturation. In one case, the subject died of acute myocardial infarction before the fire started (Table 1). The extent of burn destruction of the body was classified according to the Crow-Glassman scale as level 1 (blisters of the epidermis), 2 (charring of the skin), 3 (missing arms and/or legs), 4 (fragmentation or absence of the skull) and 5 (cremation of the body) [11]. The extent of heat coagulation of the cerebrum was expressed as none – no signs of heat coagulation, mild – yellow to light brown appearance, moder-

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Table 1 Demographics and autopsy findings of fire fatalities

Case	Age (years)	Sex	History of dementia	Cause of fire	Cause of death	CO-Hb (%)	PMI (h)
1	73	F	No	House fire	Fire death	60	15
2	51	F	No	Suicide	Fire death	0	24
3	55	M	No	Suicide	Fire death	0	12
4	80	M	Yes	House fire	Fire death	61	9
5	77	M	No	Grass fire	Fire death	3	21
6	78	M	No	House fire	Fire death	34	22
7	93	M	No	House fire	Fire death	0	14
8	76	F	No	Suicide	Fire death	0	20
9	49	F	No	Suicide	Fire death	4	25
10	75	M	No	House fire	Acute MI	1	18
11	90	F	Yes	Homicide	Fire death	13	13
12	58	M	No	Suicide	Fire death	27	13
13	61	M	No	Suicide	Fire death	3	12

CO-Hb carboxyhemoglobin.
PMI post-mortem interval.
MI myocardial infarction.

Table 2 Demographics and autopsy findings of controls unrelated to fire

Case	Age (years)	Sex	History of dementia	Cause of death	CO-Hb (%)	PMI (h)
1	84	F	Yes	Drowning	n.e.	36
2	84	M	No	Asphyxia by chest compression	n.e.	36
3	75	F	Yes	Laceration of liver (traffic accident)	n.e.	7
4	89	F	Yes	Hypothermia	n.e.	36
5	76	M	Yes	Hypothermia	n.e.	72
6	80	F	Yes	Epidural hematoma	n.e.	24
7	25	M	No	Acute CO poisoning	72	30
8	57	M	No	Acute CO poisoning	74	36
9	77	F	Yes	Traumatic shock	n.e.	18

CO-Hb carboxyhemoglobin.
PMI post-mortem interval.
n.e. not examined.
CO carbon monoxide.

Table 3 Crow-Glassman scale (*CGS*) and neuropathological findings of fire fatalities

Case	CGS	Heat coagulation of the brain	IHC of tau protein		IHC of beta-amyloid SP	Gallyas method NFT	Modified Bielschowsky method	
			Diffuse staining	NFT			SP	NFT
1	1	None	None	0	7	0	5	0
2	1	None	None	0	0	0	0	0
3	2	None	None	0	0	0	0	0
4	2	Mild	Slight	0	3	0	4	0
5	2	Mild	Slight	2	4	0	3	0
6	3	Mild	Slight	0	0	1	0	0
7	3	Moderate	Moderate	2	7	0	8	0
8	4	Severe	Moderate	0	0	0	0	0
9	3	Severe	Intense	0	0	0	0	0
10	4	Severe	Intense	0	6	0	6	0
11	4	Extensive	Intense	0	12	0	10	0
12	4	Extensive	Intense	0	0	0	0	0
13	4	Extensive	Intense	0	0	0	0	0

The numbers of NFT and SP are counted at ×200 magnification.
IHC immunohistochemistry.
NFT neurofibrillary tangle.
SP senile plaque.

ate – shrunken and firm, severe – partially destroyed, and extensive – charred and crumbled. The control group consisted of nine fatalities unrelated to fire including two cases of acute carbon monoxide poisoning (Table 2). Carboxyhemoglobin saturation levels in heart blood were analyzed by spectrophotometry. A possible history of dementia in each fatality was evaluated by reviewing autopsy reports, police investigation reports for information about interviews with family members, friends or witnesses and we also reviewed clinical charts if available. In two fire fatalities and six control fatalities, symptoms of dementia were recognized by family members or clinicians (Tables 1, 2).

The brains were fixed in 10% formalin for 2 weeks. Since our previous study showed that the density of Alzheimer's type changes was higher in temporal than in frontal and parietal neocortexes [5], a temporal lobe specimen that included the superior, middle and inferior temporal gyri was taken from a coronal section of the cerebrum at the level of the lateral geniculate bodies according to the guidelines for the diagnosis of AD [6, 7]. The specimens were processed and embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin-eosin. The serial sections were silver stained by Gallyas and modified Bielschowsky methods; the former has a high sensitivity for NFTs and the latter stains both NFTs and SPs [12, 13]. IHC was performed on the sections with monoclonal anti-tau antibodies that recognize phosphate-independent epitopes (clone T14, Zymed, USA, ready-to-use; clone Tau 2, Novocastra, UK, dilution 1:50), polyclonal anti-tau antibody that reacts with phos-

phorylated tau protein (pS202, Biosource, USA, dilution 1:100) and monoclonal anti-beta-amyloid (clone 6F/3D, Novocastra, UK, dilution 1:20) using a labeled streptavidin-biotin method as described previously [3, 14]. Histology sections were examined without knowledge of the medical history and autopsy findings in each case. The maximum numbers of NFTs and SPs were counted manually at ×200 magnification.

Results

In 10 out of 13 fatalities exposed to fire, neocortical neurons of the temporal lobe showed diffuse or fine granular cytoplasmic and perinuclear staining using IHC with 3 different anti-tau antibodies. The presence of immunoreactive neurons was unrelated to the carboxyhemoglobin saturation levels. All of the 13 fatalities had mild to extensive heat coagulation of the cerebrum. The density of immunoreactive neurons and their immunoreactivity increased relative to the Crow-Glassman Scale classification of the body and the degree of heat coagulation of the cerebrum. For example, a few neurons in superficial neocortical layers showed weak immunoreactivity in fatalities with mild cerebral heat coagulation. Numerous neurons in all neocortical layers showed strong immunoreactivity in fatalities with severe or extensive cerebral heat coagulation. The adjacent sections stained by the Gallyas method did not show the diffuse or fine granular cytoplasmic and perinuclear staining in neurons. Similar densities of NFT and SP were observed by IHC and silver staining (Table 3, Fig. 1).

In three fire fatalities without heat coagulation of the cerebrum and all control fatalities unrelated to fire, neocortical neurons of the temporal lobe did not show the diffuse or fine granular cytoplasmic and perinuclear staining

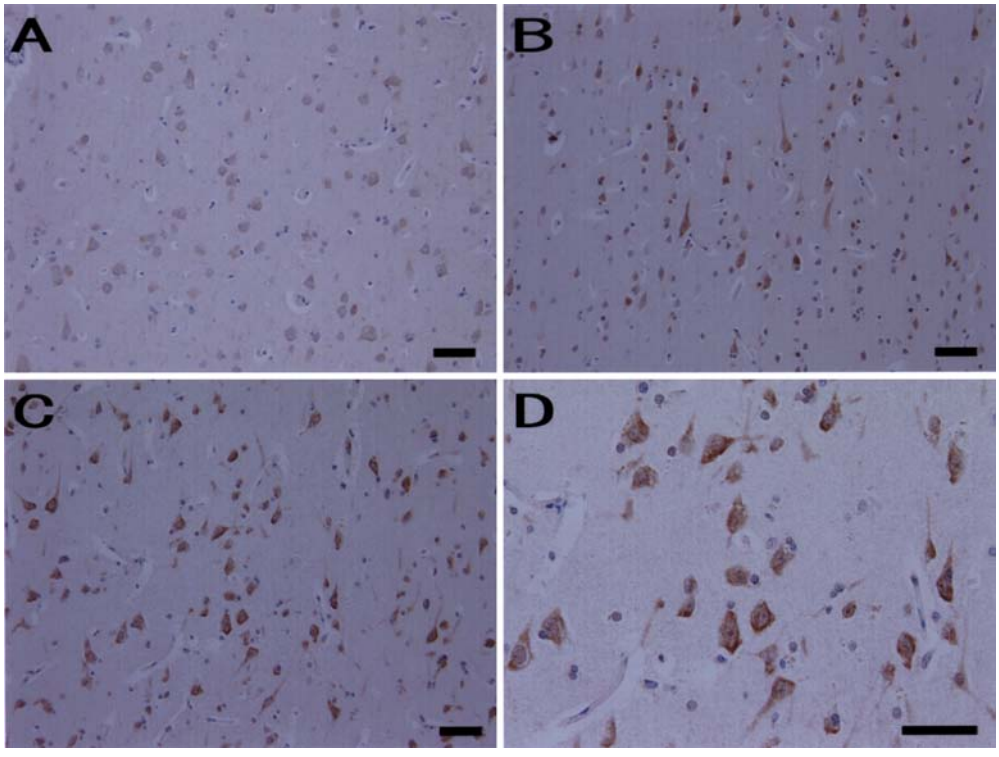

Fig. 1A–D Immunohistochemistry using anti-tau antibody (clone T14) in temporal neocortex of fire fatalities (*bar* 50 µ). **A** Slight staining of neurons in the cerebrum with mild heat coagulation. **B** Moderate staining of neurons in the cerebrum with moderate heat coagulation. **C** Intense staining of neurons in the cerebrum with severe heat coagulation. **D** High power view of immunoreactive neurons

Table 4 Neuropathological findings of controls unrelated to fire

Case	IHC of tau protein		IHC of beta-amyloid SP	Gallyas method NFT	Modified Bielschowsky method	
	Diffuse staining	NFT			SP	NFT
1	None	6	6	3	6	0
2	None	0	1	0	3	0
3	None	0	12	0	16	0
4	None	0	3	2	9	0
5	None	11	12	10	18	10
6	None	7	10	13	20	8
7	None	0	0	0	0	0
8	None	0	0	0	0	0
9	None	1	0	0	0	0

The numbers of NFTs and SPs are counted at ×200 magnification.
IHC immunohistochemistry.
NFT neurofibrillary tangle.
SP senile plaque.

using IHC with anti-tau antibodies. Similar densities of NFT and SP were observed using IHC and silver staining (Tables 3 and 4).

In 3 out of the 8 fatalities with a history of dementia (cases 3, 5, 6 of the controls) and none of the 14 fatalities without a history of dementia, the density of SPs met the pathological criteria for defining AD [6, 7].

Discussion

In this study, diffuse tau immunoreactive neocortical neurons were observed in fire fatalities with heat coagulation of the cerebrum but were not found in fire fatalities without heat coagulation of the cerebrum or in fatalities unrelated to fire. The immunoreactivity progressively increased as a function of the degree of cerebral heat coagulation. These observations demonstrate that the diffuse tau immunoreaction of neocortical neurons is specific to fire fatalities and is associated with heat coagulation of the cerebrum. Because heat coagulation of the cerebrum is a result of prolonged exposure of the dead bodies to fire, our findings indicate that diffuse tau immunoreaction of neocortical neurons is a post-mortem phenomenon peculiar to prolonged exposure of the head to intense heat by fire. Although post-mortem heat hematoma and heat brain herniation in the epidural space of burned bodies have been described in the literature [15, 16], the observed phenomenon of this study has not been reported previously.

IHC with anti-tau antibodies has been used to identify tau protein incorporated into NFT that exists in different conformations from those of normal neurons. Standard immunohistochemical methods do not assist in the visualization of normal tau protein in neurons of formalin-fixed brain sections [17, 18, 19]. Heat is known to denature protein and may change the immunoreaction [20]. Therefore, positive tau immunostaining of neurons in fire fatalities indicates that heat induces a conformational change of tau protein resulting in increased immunoreactivity. Similar phenomena have been observed when a microwave oven is used for fixation of fresh brain tissues, which produces intense tau immunostaining in normal neurons. It is suggested that microwaves alter protein metabolism in fresh tissues and denature tau proteins, resulting in increased immunoreactivity [17].

Abnormal phosphorylation of tau protein is a critical event linked to generation of NFTs in AD [21, 22]. The immunoreaction of neocortical neurons in our fire fatalities against phosphorylation dependent anti-tau antibody (pS202) indicates that heat induces phosphorylation of tau protein. It is suggested that physically stressful stimuli play a role in the pathogenesis of AD by inducing changes in tau protein. An in vivo study with rats showed that heat shock caused by raising the body temperature to 42°C for 45–60 min alters the phosphorylation of tau protein in the cerebrum [23]. An in vitro study with cultured neuron-like cells showed that heat shock caused by placing them in a 45°C incubator for 1 h produces aggregation of tau immunoreactive proteins [24]. Although we did not observe NFT formation in heat-induced tau immunoreactive neurons using the Gallyas staining method, our findings strongly suggest that heat and other stressful stimuli may promote the genesis of NFTs in AD.

Both tau protein and ubiquitin are components of NFTs [21, 25]. Quan et al. [26, 27] examined the midbrain of forensic autopsy cases and observed an increased ubiquitin immunoreactivity in neurons of the substantia nigra in fire fatalities as well as in fatalities from acute asphyxiation and drowning. Ubiquitin is an element required for an adenosine triphosphate (ATP)-dependent proteolytic system that is probably responsible for a highly selective breakdown of abnormal intracellular proteins. Ubiquitin activated by ATP is covalently linked to the target protein. Once proteins are ubiquitinated, they are rapidly degraded by an ATP-dependent protease [28]. An immunohistochemical study showed that ubiquitinated granular structures increase at the initial stage of neurofibrillary degeneration [29]. Ubiquitin activated by heat may contribute to the conformational change of tau protein in fire fatalities.

Two pathological diagnostic criteria by Khachaturian [6] and Mirra et al. [7] based on neocortical SP counts have been used for the post-mortem diagnosis of AD. However, these criteria are not always suitable for diagnosing AD in forensic autopsies of the elderly [5]: only three out of eight fatalities with a history of dementia met the criteria for defining AD in this study. Furthermore, increased numbers of neocortical SPs have been observed in aged cognitively normal persons [30, 31]. Therefore, it is important to consider both pathological findings and clinical history of dementia for the post-mortem diagnosis of AD in forensic autopsies. The examination of neocortical NFTs is also essential to enhance confidence in the diagnosis of AD [30, 32]. In the diagnosis of AD in fire fatalities, heat-induced tau immunoreactive neurons should be differentiated from Alzheimer's type of changes. A lack of fibrillary bundles distinguishes heat-induced tau immunoreactive neurons from Alzheimer's NFT-containing neurons. However, tau immunoreactive neurons without fibrillary bundles represent a premature stage of NFT formation, termed pretangle neurons in AD [21, 33, 34]. Because heat-induced tau immunoreactive neurons and pretangle neurons have an almost identical microscopic appearances, differentiation of the two types of neurons is difficult in fire fatalities with heat coagulation of the cerebrum. Forensic pathologists should consider this phenomenon when they diagnose AD using IHC in fire fatalities. In this study, IHC for beta-amyloid assisted in distinct visualization of SPs even in the cerebrum with extensive heat coagulation, indicating that the diagnosis of AD based on SP counts should be attempted even in severely charred bodies.

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