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Research Paper

Talin: A potential protein biomarker in postmortem investigations

Zahra K. Campell [a], Insu Kwon [b], Sheree J. Finley [a, c], Youngil Lee [b], Gulnaz T. Javan [a, *]

[a] Forensic Science Program, Physical Sciences Department, Alabama State University, Montgomery, AL, USA
[b] Department of Exercise Science and Community Health, University of West Florida, Pensacola, FL, USA
[c] Ph.D. Program in Microbiology, Department of Biological Sciences, Alabama State University, Montgomery, AL, USA

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ABSTRACT

The determination of the postmortem interval is of utmost importance in medicolegal death investigations. There are a number of ways to estimate the postmortem interval; however, the current established methods are susceptible to numerous biotic and abiotic factors. Previously published studies state that protein concentrations in postmortem brain tissues can detect protein changes via immunoblotting and densitometry techniques.

The objective of the current study was to determine if there is a correlation between protein expression in cadaver tissues and postmortem interval. To this end, 18 brain tissues from cadavers from criminal cases were examined to determine how many hours after death the presence of four proteins (i.e., talin, α-enolase, cofilin-1, and vinculin) are detectable.

Talin protein levels steadily decreased with increasing postmortem interval. Interestingly, the study demonstrated that talin protein levels were statistical significant between postmortem intervals of 24 versus 48 h and 24 versus 72 h by ANOVA. These results provide strong evidence that talin has potential to be used as a unique biomarker for the establishment of an additional method to estimate the time of death.

1. Introduction

Changes in brain biochemistry attributable to death can result in altered concentrations of individual proteins in postmortem brain tissues. A recent study investigated the degradation of thanatophagy proteins and showed that the levels of key proteins involved in postmortem autophagy increased in the brain specimens of actual cadavers from criminal cases.[1] According to the natural calendar of decomposition, the internal organs of the deceased decay in a particular order depending on the cause of death, beginning with the intestines and culminating with the brain due to the fact that medial sections of the brain cool more slowly than other tissues.[2]

The role of key proteins in cerebral tissues such as those involved in the neuronal cell cytoskeleton (e.g. talin-1, vinculin) and nitrated proteins (e.g. α-enolase, cofilin) involved in neurodegenerative diseases have yet to be elucidated in postmortem tissues. Talin-1 is a ubiquitously expressed protein involved in connecting the integrin family of cell adhesion molecules to the actin cytoskeleton of the plasma membrane in antemortem cells.[3–5] These focal adhesions are contingent upon the interface between talin-1 and vinculin, a membrane cytoskeletal protein.[6] Talin and vinculin, with half-lives of 18–21 h.[7] Differences in turnover rates of vinculin and talin caused by viral transformation and cell density. Calpains play major roles in regulating the proteolytic breakdown postmortem muscle proteins.[8] Alpha (α)-enolase has been characterized as a neurotrophic factor, a heat shock protein, and hypoxic stress protein. It has also been reported that its upregulation plays a role in hypoxia tolerance and tumor progression.[9] Cofilin is a member of the actin depolymerizing factor (ADF)/cofilin family, which is a group of actin binding proteins which regulate the assembly and disassembly of actin filaments.[10,11] There are many methods of estimating the postmortem interval (PMI), all of which have varying degrees of accuracy. There is a paucity of studies that correlate the time since death and cytoskeletal and neuronal protein levels. However, a thorough understanding of proteomic degradation in tissues of dead bodies is crucial for the identification of new techniques for criminal investigations.

* Corresponding author.
E-mail address: gjavan@alasu.edu (G.T. Javan).

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Table 1
Demographic information for the cadaver specimens used to study protein levels in postmortem brain tissues.

Case	Age	Sex	Weight (kg)	Height (cm)	Ethnicity	Cause of death	PMI (hrs)
1	55	M	112	198	C	Coronary heart disease	6
2	49	M	117	188	A	Homicide (GSW)	16
3	19	M	80	178	A	Homicide (GSW)	17
4	65	M	96	188	C	Suicide (GSW)	18.5
5	39	F	119	185	C	Car accident	19
6	58	F	75	196	C	Overdose	20
7	50	F	48	170	C	Undetermined	24.5
8	45	M	117	178	A	Homicide (Multiple GSW)	29.5
9	32	M	66	180	A	Homicide (GSW)	32
10	65	M	100	191	C	Coronary Heart Disease	36.5
11	58	M	68	173	C	Suicide	47
12	53	F	62	170	C	Drowning	47.5
13	50	M	64	175	C	Homicide (GSW head)	48
14	48	F	70	160	C	Abused	58
15	67	M	59	175	C	Suicide	66
16	72	F	48	163	C	Blunt Chest Trauma	68
17	31	M	94	178	C	Suicide	70
18	47	F	111	168	A	Stroke	72

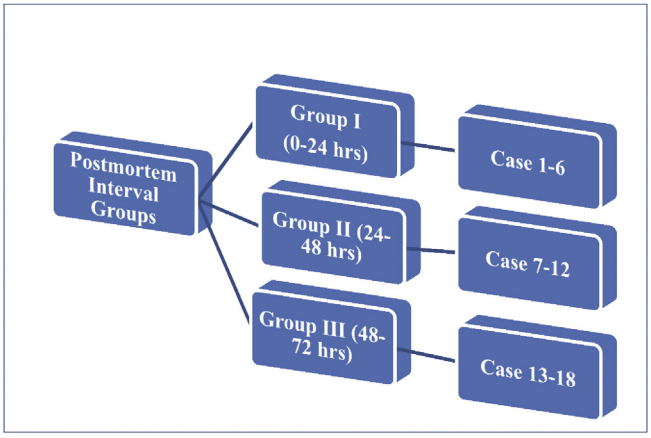

Fig. 1. Samples were binned based on the PMIs in Group 1 (0–24 h), Group II (24–48 h), and Group III (48–72 h).

2. Materials and methods

Cadaver cases Adult cadavers were kept at 1 °C in the morgue of the Alabama Department of Forensic Sciences Medical Laboratory Montgomery, AL. Brain samples were collected from 18 corpses of various manners of death (homicide, overdose, and suicide). The cohort data reported for each case were the cadaver age at death, sex, weight, height, cause of death, and PMI (Table 1). All procedures were in accordance with institutional guidelines and were approved by the Alabama State University Institutional Review Board (IRB) number 2016011.

2.1. Tissue collection and homogenization

The forensic autopsies were carried out in a morgue at 20 °C. Dissection of brain tissues was performed via sterile scalpels, placed in labeled polyethylene bags, and stored at −80 °C until the time of analysis. A glass tissue grinder (Fisher Scientific, Pittsburgh, PA) was used to homogenize the cerebral tissues, and the cells were lysed in ice-cold tissue lysis buffer (pH 7.4) containing 50 mM Tris-HCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100 with complete protease inhibitor (Roche Applied Science, Indianapolis, IN), and phosphatase inhibitor (Roche Applied Science, Indianapolis, IN). The tissue homogenates were incubated on ice for 30 min, and then the tissue lysates were collected by centrifugation at 20,000 g for 20 min.

2.2. Immunoblotting

The proteins in the lysed brain tissue were separated using SDS-PAGE precast gels (Life Technology, Carlsbad, CA) under reducing conditions and transferred to nitrocellulose membrane. The membranes were blocked with 5% nonfat milk for 90 min at 21 °C then incubated overnight with antibodies at 4 °C. The primary antibodies used in the study were vinculin and talin-1 (Abcam, Cambridge, UK) and cofilin and α-enolase (Santa Cruz Biotechnology Inc., Santa Cruz, CA). After washing primary antibodies, membranes were incubated with designated secondary antibodies (goat anti-mouse or rabbit HRP conjugated) (Life Technology, Carlsbad, CA). Blots were analyzed with a QuantityOne (Bio-Rad, Hercules, CA) and quantified with LI-COR Image Studio (LI-COR Biosciences, Lincoln, NE. Protein expression was normalized glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Immunoblotting was performed in triplicate.

2.3. Statistical analysis

All values were expressed as means of ±SEM. Statistical analysis was performed using ANOVA to identify statistical significance between groups by Graph Pad Prism 6. Statistical significance was set at $p < 0.05$.

3. Results

Of the 18 cadaver cases, two were excluded due to low protein concentrations (<0.120 g) as determined by a Molecular Devices Spectra Max M3 spectrophotometer. The remaining 16 cases were binned into three postmortem interval time groups, 0–24 h, 24–48 h, and 48–72 h (Fig. 1). The results of densitometry analysis demonstrated PMI-dependent changes in the postmortem cerebral tissues. Talin-1 bands steadily decreased with increasing post mortem interval (Fig. 2). There was a statistically significant diminished levels in talin-1 between the 0–24 h and 24–48 h, as well as between 0-24 h and 48–72 h. Vinculin shows consistent expression across all PMIs. Interestingly, α-enolase demonstrated the least expression in the first 24 h compared to the other proteins. Cofilin-1 showed a decrease in intensities. There were no statistically significant changes observed for vinculin, α-enolase, and cofilin.

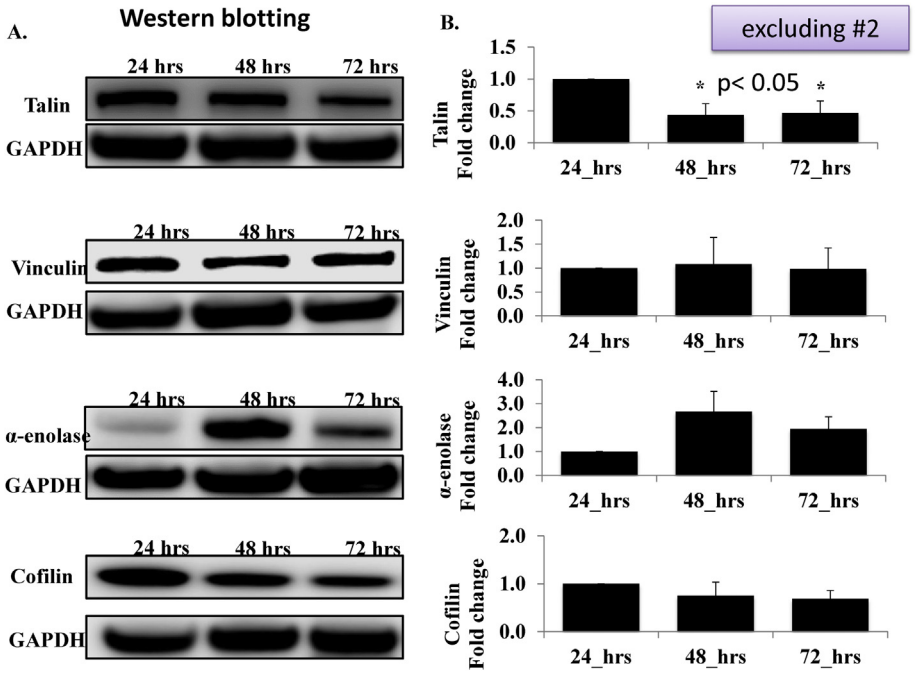

Fig. 2. Talin-1 levels decreased in a PMI-dependent manner in postmortem brain tissues. (A) Western blot analysis of talin-1, vinculin, a-enolase, and cofilin in postmortem brains at various PMI. (B) Quantitation of talin-1/GAPDH, vinculin/GAPDH, a-enolase/GAPDH, and cofilin/GAPDH.

4. Discussion

Death leads to several sequential events after a person dies. For example, the heart stop beating and the lack of oxygen causes hypoxia within the cells.[12] Hypoxia and ischemia triggers several factors that lead to the degradation of proteins via proteolytic and autolytic enzymez.[13] Axonal processes of neurons remained immunoreactive. In the current study, western blot analysis showed distinctive degradation patterns in the four selected proteins. A particularly interesting finding from this study was that talin-1 significantly decreased as the PMIs increased. According to Tan et al. (2015), talin-1 is essential for axonal regeneration in an animal model study in dorsal root ganglion (DRG) neuron culture from Sprague-Dawley rats.[14] Finehout et al. (2006) demonstrated that α-enolase levels increase after death in antemortem samples collection by lumbar puncture compared to postmortem samples collected from the ventricular cerebrospinal fluid (CSF).[15] In the current study, the greater than three-fold increase observed for α-enolase within 48 h confirmed the results of the Finehout et al. (2006) study. Of note, another interesting finding of the current study is that the GAPDH protein, which was used as an internal loading control for immunoblotting analysis, remained strongly detectable even at the longest PMIs up to 72 h.

5. Conclusion

In this study, using brain tissue samples from actual criminal casework, immunoblotting techniques were demonstrated a substantial correlation between protein integrity and PMI. The current confirms that proteins are detectable in cadaver brain tissue even up to 72 h after death. Remarkably, talin-1 exhibited statistically significant decreases until the longest PMI time (Group 3). In conclusion, this study showed that talin-1 has a statistically significant correlation that provides strong evidence for its potential use as a biomarker to establish precise PMI estimations. Future studies would involve mechanistic animal models (i.e., mice, pig) to investigate PMI-mediated protein degradation to provide new insight into formulating a new forensic method to determine the time of death.

Conflict of interest

None.

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