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Single-stranded DNA as an immunohistochemical marker of neuronal damage in human brain: An analysis of autopsy material with regard to the cause of death

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Abstract

Single-stranded DNA (ssDNA) is a marker of apoptosis and programmed cell death, which appears prior to DNA fragmentation during delayed neuronal death. The present study investigated the immunohistochemical distribution of ssDNA in the brain to investigate apoptotic neuronal damage with regard to the cause of death in medicolegal autopsy cases ($n = 305$). Neuronal immunopositivity for ssDNA was globally detected in the brain, independent of the age, gender of subjects and postmortem interval, and depended on the cause of death. Higher positivity was typically found in the pallidum for delayed brain injury death and fatal carbon monoxide intoxication, and in the cerebral cortex, pallidum and substantia nigra for drug intoxication. For mechanical asphyxiation, a high positivity was detected in the cerebral cortex and pallidum, while the positivity was low in the substantia nigra. The neuronal ssDNA increased during the survival period within about 24 h at each site, depending on the type of brain injury, and in the substantia nigra for other blunt injuries. The neuronal positivity was usually lower for drowning and acute ischemic disease. Topographical analysis of ssDNA-positive neurons may contribute to investigating the cause of brain damage and survival period after a fatal insult.

Keywords: Forensic pathology; Immunohistochemistry; Single-stranded DNA; Human brain; Neuronal apoptosis

1. Introduction

Brain dysfunction is involved in the death process not only due to primary brain injury or disease but also due to other traumas and diseases, including asphyxiation, hemorrhagic shock, intoxication, cardiac attack and systemic metabolic disorders. Previous studies mainly investigated brain injury for wound age estimation, and useful findings have been reported [1–4]. Several studies suggested differences in astrocyte injury among acute causes of death [5,6]. However, morphological findings of neuronal damage cannot be distinctly defined in short survival cases. Discrimination of the causes of neuronal damage is also difficult. With respect to this, neuronal apoptosis in the pallidum has been suggested as an early change due to carbon monoxide intoxication [7]. Detection of neuronal apoptosis may be also useful for forensic timing of brain contusions [8].

Neuronal apoptosis is usually detected by *in situ* labeling of DNA fragments, e.g. terminal deoxynucleotidyl-transferase-mediated dUDP nick end-labeling (TUNEL) or *in situ* nick translation (ISNT) [9–12]. However, experimental studies have shown that single-stranded DNA (ssDNA) degradation precedes DNA double-strand breaks (DNAdsb) during a delayed neuronal death process caused by reperfusion after transient brain ischemia or intracerebral hemorrhage, possibly due to oxidative stress [13–18]. Thus, ssDNA can be used as an earlier marker of apoptosis and programmed cell death, which causes neuronal loss [13,14,19–21]. This marker may contribute to investigating neuronal damage in acute death and also timing of brain injury in the early phase [8,13]. The present study

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investigated the immunohistochemical distribution of ssDNA in the brain to examine neuronal damage with regard to the cause of death in medicolegal autopsy cases.

2. Materials and methods

2.1. Materials

Formalin-fixed paraffin-embedded brain tissue specimens from medicolegal autopsy cases ($n = 305$, 226 males and 79 females, postmortem interval <48 h) at our institute were examined. The cases comprised fatalities due to brain injury ($n = 60$), including cases of survival time within 6 h (shorter survival cases, $n = 30$) and over 6 h (longer survival cases, $n = 30$), other blunt injuries ($n = 39$), sharp instrument injury ($n = 28$), acute mechanical asphyxiation ($n = 26$: strangulation, $n = 17$; atypical hanging, $n = 9$), drowning ($n = 15$: freshwater, $n = 10$; saltwater, $n = 5$), fire ($n = 66$), including cases with blood carboxyhemoglobin (COHb) of a lower (<60%) and a higher (>60%) level ($n = 41$ and 25, respectively), carbon monoxide (CO) intoxication without burns ($n = 6$), drug intoxication ($n = 12$: methamphetamine, $n = 7$; sedative-hypnotic drugs, $n = 5$), acute ischemic heart disease (IHD, $n = 29$), cerebral hemorrhage ($n = 12$) and infectious disease ($n = 12$). IHD was used as a control in the present study. Details are shown in Table 1. Fatal brain injury was subdivided into subdural hematoma accompanied by marked brain swelling ($n = 31$), brain contusions ($n = 22$) and brain stem injury ($n = 7$) based on the main lesion. For these groups, clearly representative cases without any significant complication were included. Postmortem interval (estimated time from death to autopsy) and survival time (estimated time from the fatal insult to death), cited from autopsy documents, had been established based on circumstantial and pathological evidence.

The brain tissue specimens were taken from cerebral cortex in the posterior part of superior frontal gyrus of the frontal lobe, pallidum (globus pallidus) in the basal ganglia and substantia nigra of the midbrain in consideration of neuroanatomical characteristics and cerebrovascular system. In brain injury cases, specimens involved in the primary injury were excluded in the present study. For these specimens, neurons were clearly distinguished from other cell populations.

2.2. Methods

2.2.1. Tissue sections

Serial sections (4 μm thick) were prepared from formalin-fixed paraffin-embedded brain tissue specimens of the cerebral cortex of the frontal lobe, pallidum (coronal section) and midbrain (horizontal section). The tissue sections were used for hematoxylin–eosin (HE) and immunostaining.

2.2.2. Immunostaining

Polyclonal rabbit anti-ssDNA serum (Dako, Kyoto) was used (dilution, 1:400), with incubation for 20 h at room temperature, with a Vectastain Universal Elite ABC kit (Vector Laboratories, Burlingame, CA.) according to the manufacturer's instructions (counterstaining with hematoxylin). Endogenous peroxidase was inactivated by incubation with 0.3% hydrogen peroxide for 30 min. For the control study to confirm the specificity of immunostaining, phosphate buffered saline or normal rabbit serum was substituted for the primary antibody.

Table 1
Case profiles

Cause of death	*N*	Male/female	Age (years)		Survival time (h)[a]		CPR[b]	PMI (h)[c]	
			Range	Median	Range	Median		Range	Median
Brain injury	60	52/8	2–88	54.0	<0.5–168	6.5	15	3–37	15.8
Acute death	30	28/2	18–88	51.5	<0.5–6	0.5	14	4–31	17.0
Delayed death	30	24/6	2–82	58.0	7–168	24.0	1	3–37	12.7
Blunt injury	39	28/11	16–91	55.0	<0.5–6	0.5	22	6–37	16.2
Sharp instrument injury	28	22/6	19–81	47.5	<0.5–6	0.5	18	6–30	14.2
Asphyxiation	26	14/12	2–71	46.0	<0.5–1.5	<0.5	4	7–48	22.5
Strangulation	17	8/9	2–69	44.0	<0.5–1.5	<0.5	2	21–47	20.3
Atypical hanging	9	6/3	35–67	53.0	<0.5	<0.5	2	7–47	21.9
Drowning	15	10/5	45–76	53.0	<0.5–6	<0.5	3	14–47	28.5
Fresh water	10	6/4	45–75	54.5	<0.5–1.5	<0.5	2	14–47	29.0
Salt water	5	4/1	48–76	52.0	<0.5–1.5	<0.5	1	14–37	17.0
Fire fatality	66	47/19	35–93	71.5	<0.5–3	<0.5	11	7–42	13.2
COHb < 60%	41	32/9	37–93	74.0	<0.5–3	<0.5	9	7–37	13.3
COHb > 60%	25	15/10	35–87	70.0	<0.5–3	<0.5	2	9–42	13.2
CO intoxication	6	4/2	27–59	48.5	<0.5–1.5	0.5	1	7–11	25.0
Drug intoxication	12	10/2	28–71	38.0	1.5–36	4.0	2	6–38	26.0
Methamphetamine	7	7/0	30–71	38.0	1.5–36	4.0	1	6–28	25.8
Sedative-hypnotics	5	3/2	28–56	34.0	1.5–4	1.5	1	10–38	30.0
IHD	29	23/6	51–63	62.0	<0.5–2	<0.5	21	8–35	17.8
Cerebral hemorrhage	12	6/6	39–72	58.5	<0.5–36	2.0	3	5–34	24.9
Infectious disease[d]	12	10/2	12–84	52.0	4–240	100	3	17–24	21.5
Total	305	226/79	2–93	55.0	<0.5–240	<0.5	103	3–48	17.1

[a] Estimated time from fatal insult to death.
[b] The number of cases with CPR (cardiopulmonary resuscitation).
[c] Estimated time from death to autopsy (PMI, postomortem interval).
[d] Pneumonia ($n = 5$), sepsis ($n = 7$) from peritonitis ($n = 5$) and other origin ($n = 2$). COHb, carboxyhemoglobin; CO, carbon monoxide; IHD, acute ischemic heart disease.