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Morphological analysis of astrocytes in the hippocampus in mechanical asphyxiation

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ABSTRACT

The present study investigated the morphology of astrocytes in the hippocampus and serum S100B levels in cases of mechanical asphyxia due to neck compression ($n = 23$: atypical hanging, $n = 7$; ligature/manual strangulation, $n = 16$) with regard to the classical autopsy findings, compared with those of other types of asphyxiation ($n = 9$) and acute myocardial infarction/ischemia (AMI, $n = 20$). The decrease in intact astrocyte number, as shown by S100 and GFAP-immunostaining, was larger for asphyxiation due to neck compression compared with that for other asphyxiation and AMI, showing a correlation with the increase in the serum S100B levels. The decrease in intact astrocyte number and increase in serum S100B were closely related to the severity of conjunctival petechial hemorrhage and fracture(s) of the hyoid bone and/or thyroid cartilage in asphyxia due to neck compression. These findings suggest that hippocampal astrocyte injury is caused by cerebral hypoxia accompanied by congestion, especially in mechanical asphyxia due to neck compression.

1. Introduction

Mechanical asphyxiation is often involved with homicide, and is thus of special forensic significance [1]. Fatal mechanisms are different depending on the cause of asphyxiation, which involves hypoxia and hypercapnea due to airway obstruction, and cerebral ischemia or congestion and/or possible vagus reflex due to neck compression [2]. Pathological evidence for the cause of asphyxia, and classical signs of fatal asphyxiation involving facial congestion and petechial hemorrhages are important for determining the cause of death [1–3]. Immunohistochemical and biochemical methods have been reported to investigate the pressure to the neck [4–8]. However, it is difficult to evaluate the severity of cerebral ischemia or hypoxia in relation to the pressure to the neck. In the central nervous system (CNS) impairment of astrocytes, which provide structural, trophic, and metabolic support to neurons, is involved in the death process or posttraumatic CNS disorders [9,10]. Previous studies showed that astrocytes are more rapidly and severely injured in the cerebral cortex than neurons during fatal brain damage, accompanied by an elevation in the serum S100B level [7,8]. Furthermore, cerebral damage in asphyxiation may be typically seen in the hippocampus because this part is the most vulnerable to hypoxia/ischemia [11,12].

In the present study, we investigated the morphology of astrocytes in the hippocampus and serum S100B levels in cases of mechanical asphyxia due to neck compression with regard to the classical autopsy findings compared with those of other types of asphyxiation.

2. Materials and methods

2.1. Materials

Formalin-fixed paraffin-embedded hippocampus tissue specimens from forensic autopsy cases within 48 h postmortem at our institute were examined: Total, $n = 52$, 35 males and 17 females, 14–93 (median, 56) years of age, postmortem interval, 7.2–48 h (median, 21.3 h).

The cases comprised asphyxiation by neck compression ($n = 23$: atypical hanging, $n = 7$; ligature/manual strangulation, $n = 16$), other asphyxiation ($n = 9$: aspiration of vomit, $n = 4$; choking on a foreign body, $n = 3$; smothering, $n = 2$) and acute myocardial infarction/ischemia (AMI, $n = 20$) (Table 1). Cases were without medical intervention or complications including brain infarction and hemorrhage. The above-mentioned causes of death were classified on

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Table 1
Case profiles ($n = 52$).

Cause of death	n	Male/Female	Age (years) range median		Survival time (h) range median		PMI (h) range median	
Atypical hanging	7	6/1	35-68	(58)	0.5	0.5	12.5-32	(22.7)
strangulation	16	7/9	14-93	(46)	0.5	0.5	7.5-42	(21.3)
Others*	9	7/2	53-80	(60)	0.5	0.5	7.2-48	(17.9)
AMI	20	15/5	42-79	(58)	0.5-3	0.5	8.0-41	(20.5)

PMI, postmortem interval; AMI, acute myocardial infarction/ischemia.
* Aspiration of vomit, n = 4; choking on a foreign body, n = 3; smothering, n = 2.

pathological and toxicological bases, and clearly accountable cases were included. The AMI group consisted of cases of sudden death, which showed macro- and microscopical findings of acute ischemic heart disease without any evidence of cause of death other than heart attack. The aspiration cases were acute deaths, showing complete obstruction of airways by vomit without any other evidence of cause of death [13].

2.2. Tissue specimens

Ten percent formalin-fixed, paraffin-embedded hippocampus tissue specimens were used. Serial horizontal sections 5 μm thick were prepared and used for hematoxylin–eosin (HE) and immunostaining.

2.2.1. Immunohistochemistry

Monoclonal mouse anti-human glial fibrillary acidic protein (GFAP) (Dako A/S, Glostrup, 100-fold dilution) and Polyclonal rabbit anti-cow S100 (Dako A/S, Glostrup, 400-fold dilution) were used, with 12 h incubation at 24 °C, in a universal streptavidin/biotin immunoperoxidase detection system (Omni Tags kit) and color development with 3,3′-diamino benzidine tetrahydrochloride (DAB, Shandon/Lipshaw/Immunon, Pittsburgh, Penn.) according to the manufacturer's instructions (counterstaining with hematoxylin). Endogenous peroxidase was inactivated by incubation with 0.3% hydrogen peroxide for 15 min. For the control study to confirm the specificity of immunostaining, phosphate-buffered saline was substituted for the primary antibody.

2.2.2. Quantitative analysis of GFAP-immunopositivity and S100-immunopositivity

In the CA4 central region of the hippocampus, the total numbers of neurons and glial cells, including astrocytes, oligodendrocytes and microglias, and the number of astrocytes in which GFAP-immunopositivity and S100-immunopositivity was detected, respectively, were counted in two fields at 400× magnification, and the percent positivity was estimated as follows: The percentage of positive astrocytes = number of positive astrocytes/total number of glial cells × 100.

2.2.3. Analysis of serum S100B protein

Serum concentrations of S100B were analyzed by enzyme-linked immunosorbent assay (ELISA) using two monoclonal antibodies, clone 8B10 and clone 6G1, for which the detection limit was 0.05 ng/ml (established by SRL Inc., Tokyo) [7]. Hemoglobin contamination of <0.08 g/dl did not interfere with the measurements [14].

2.2.4. Conjunctival petechiae

The numbers of petechiae in the left (upper and lower) and right (upper and lower) conjunctivae were counted, respectively, and the total number was evaluated as follows: (a) score 1 (p1), <5; (b) score 2 (p2), 5–10; (c) score 3 (p3), >10.

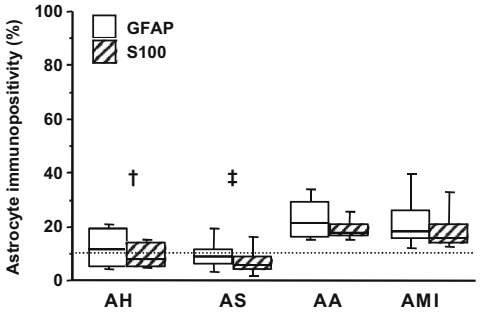

Fig. 1. Astrocyte immunopositivity for GFAP and S100-immunostaining in the CA4 region of the hippocampus in relation to the cause of death. AH, atypical hanging; AS, ligature/manual strangulation; AA, other types of asphyxiation; AMI, acute myocardial infarction/ischemia. GFAP-immunopositivity: †AS vs. AA and AMI ($p < 0.01$); ‡AH vs. AMI ($p < 0.05$). S100-immunopositivity: †AS vs. AA and AMI ($p < 0.001$); ‡AH vs. AA and AMI ($p < 0.05$). Performed by Scheffe test.

2.2.5. Facial congestion

The severity of the facial congestion was scored as follows: (a) score 1 (f1), pallor or slightly congested; (b) score 2 (f2), moderately congested; (c) score 3 (f3), markedly congested, partly with petechiae.

2.2.6. Statistical analyses

The Pearson product–moment correlation coefficient was used to compare two parameters. Comparisons between groups were performed by nonparametric Mann–Whitney *U*-test, and the Scheffe test was used for analysis involving multiple comparisons. These analyses were performed using the statistic software/Statview (version 5.0, SAS Institute Inc., SAS Campus Drive Cary). A *p*-value of less than 0.05 was considered significant. In Figs. 1 and 4–6, the results of the data analyses are shown as box-plots, for which 50% of the data is summarized in the box. The line in each box represents the median, and the lines outside of each box represent the 90% confidence intervals.

3. Results

3.1. Number of neurons with regard to the cause of death

The total number of neurons did not show age dependency, and no significant difference was detected among the causes of death.

3.2. Astrocyte immunoreactivity with regard to cause of death

The total number of glial cells did not show age dependency, and no significant difference was found among the causes of death. A very strong correlation was observed between astrocyte S100 and astrocyte GFAP-immunopositivities ($r = 0.85$ $p < 0.001$).

The total number of astrocytes, as shown by S100 and GFAP-immunopositivities, did not show age dependency either. The astrocyte immunopositivities for GFAP and S100 were significantly low in asphyxiation by neck compression (1.5–22.7%, median