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Immunohistochemical investigation of a pulmonary surfactant in fatal mechanical asphyxia

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Abstract We evaluated the usefulness of pulmonary surfactant protein A (SP-A) as a practical diagnostic marker of fatal mechanical asphyxia in forensic autopsy cases. A total of 27 cases of asphyxia were examined histologically and immunohistochemically and compared with a control group consisting of 16 cases of poisoning (n = 9) and peracute death (n = 7). Both groups showed histological findings of local atelectasis and local emphysema, congestion, intra-alveolar and interstitial edema in most cases and pulmonary hemorrhages in some cases. The mechanical asphyxia group showed a significantly increased intensity of SP-A staining in the intra-alveolar space accompanied by many massive aggregates in approximately 60% of cases, which was not found in the control group. These structures may be interpreted as aggregates of pulmonary surfactant released from the alveolar wall due to enhanced secretion caused by strong forced breathing or over-excitement of the autonomic nervous system by mechanical asphyxia. The results of our investigation suggest the practical usefulness of the immunohistochemical detection of SP-A in distinguishing mechanical asphyxia from other types of hypoxia.

Key words Surfactant-associated protein A · Immunohistochemistry · Asphyxia

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The article is dedicated to Prof. Michael Staak (Institute of Legal Medicine, University of Cologne) on his 65th birthday, who kindly gave us important advice and great encouragement in this study

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Introduction

Immunohistochemical studies (Du Chesne et al. 1996; Ortmann and Brinkmann 1997; Fineschi et al. 1998) and histological studies (Betz et al. 1997) have been described as useful tools for the forensic evaluation of pulmonary tissue. Immunological (Du Chesne et al. 1996) and biochemical (Osuna et al. 1998) reactions that take place during the survival period have been suggested as promising forensic markers. Morita and co-workers reported an increase of the pulmonary surfactant protein as a result of asphyxia, which led to its forensic application (Morita et al. 1985; Morita and Tabata 1988, 1990), and its importance was discussed (Fujiwara 1988; Kobayashi 1992; Morita 1994). Pulmonary surfactant is the surface active substance that covers the alveolar wall and reduces surface tension to prevent alveolar collapse (Georke 1974; Hagwood and Clements 1990). Phospholipids comprise 85% of pulmonary surfactant and surfactant-associated proteins (SP-A, B, C and D) play a critical role in its activity. SP-A is the major surfactant protein and its deficiency is known to cause infantile respiratory distress syndrome (IRDS) and the acute (adult) respiratory distress syndrome (ARDS); it is known as a useful clinical diagnostic marker (Avery and Mead 1959; Schofield and Cotran 1994; deMello et al. 1993; Lewis and Jobe 1993).

Recently, the possible application of SP-A immunohistochemistry in forensic practice was reported in cases of perinatal respiratory failure (Funayama et al. 1994; Zhu et al. 1996).

The diagnosis of mechanical asphyxia is made by identifying several non-specific signs (Knight 1996a, b). Microscopic pulmonary findings in mechanical asphyxia are characterised by local atelectasis and emphysema, congestion, intra-alveolar and interstitial edema, and hemorrhage, although none of these is specific to the cause of death (Brinkmann and Püschel 1981; Grellner and Madea 1994). In this study, we evaluated the usefulness of SP-A as a practical diagnostic marker of fatal mechanical asphyxia in forensic autopsy cases.

Materials and methods

Forensic autopsy cases

A total of 43 forensic autopsy cases from the Departments of Legal Medicine at Osaka City University Medical School, University of Occupational and Environmental Health (Kita-kyushu) and Wakayama Medical College with a postmortem interval of less than 48 h were evaluated. The mechanical asphyxia group (n = 27, median age 52 years, range 2 months–89 years) included cases of hanging (n = 6), strangulation (n = 12), throttling (n = 2), smothering (n = 2) and choking (n = 5). The survival time in all cases was estimated to have been very short (no more than 30 min). The control group (n = 16, median age 46.5 years, range 21–85 years) consisted of two subgroups of acute poisoning (sedative-hypnotic drug poisoning n = 6, ethanol poisoning n = 3) and peracute death (cerebral crush injury n = 7). The mode of death in the poisoning subgroup was respiratory failure due to the inhibitory effect on the central nervous system, rather than a pulmonary disorder itself. The survival times in these cases were in the range of several hours except in one case (1 day). In the peracute death subgroup, the survival time was presumed to be no more than a few minutes.

Tissue specimens and immunostaining of SP-A

Specimens of the upper and lower lobes of both lungs were fixed in 10% formalin and routinely processed for paraffin embedding. Serial sections of 4 μm were prepared and used for hematoxylin-eosin (H&E) staining and pulmonary surfactant detection by immunohistochemistry. The anti-human SP-A mouse monoclonal antibody PE-10 (Dako, Kyoto Japan) (Kuroki et al. 1985) was used at a 100-fold dilution with a 30 min incubation at room temperature. a universal streptavidin/biotin immunoperoxidase detection system (OmniTags Kit) and DAB (Shandon/Lipshaw/Immunon, Pittsburgh, Penn.), according to the manufacturer's instructions and counterstained with hematoxylin.

Histological findings such as congestion, intra-alveolar and interstitial edema, and hemorrhages were observed in the H&E stained sections in each case and classified into three groups: – none/very mild, + mild/moderate and ++ severe. The SP-A immunostaining reactivity in the alveolar type II cells and alveolar surface was assessed as follows: – negative, + positive, ++ strongly positive. The findings in the intra-alveolar space were classified into four categories: – negative, -/+ weakly positive, + positive with a few massive aggregates of stained granules, ++ intensely and diffusely positive with many massive aggregates of stained granules (examples shown in Fig. 1 a–c).

Statistical analysis

Statistical analyses by the χ^2 and Mann-Whitney U-tests were performed using StatView 4.5 software (Abacus Concepts Berkeley, Calif.). P values shown for comparisons of histological and immunohistochemical findings between the two groups were calculated by using the positive rate and also by taking the degree of severity/positivity into consideration unless specifically indicated.

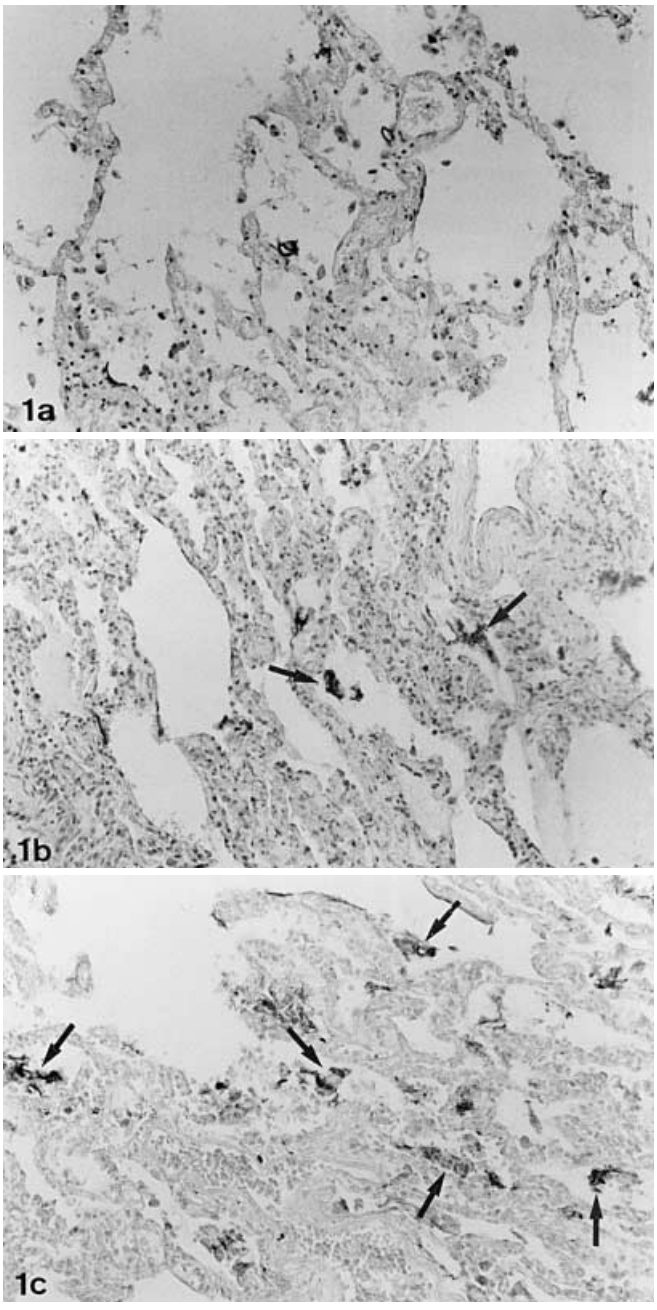

Fig. 1 Immunohistochemical staining of SP-A in the intra-alveolar space: **a** weakly positive; **b** positive with a few massive aggregates of granular stains; **c** intensely and diffusely positive with many massive aggregates of granular stains. Massive aggregates are shown in **b** and **c** *(arrows)*. Original magnification × 200

Results

The histological findings of H&E-stained sections were characterised in both groups by local atelectasis and/or local emphysema, congestion, edema and hemorrhages. Local atelectasis and/or local emphysema and congestion were found in all cases in both groups and there were no significant differences in the degree of severity (P > 0.05). In the mechanical asphyxia group intra- and interstitial alveolar edema were more common (24 out of 27 or 88.9%) than those in the control group (9 out of 16 or 56.3% and 8 out of 16 or 50.0%, P = 0.011 and 0.005, respectively). Hemorrhages were seen in 59.3% (16/27), 77.8% (9/7) and 0% (0/7) of the mechanical asphyxia, poisoning, and peracute death groups (or subgroups), respectively.

Immunostaining of SP-A at the alveolar surface demonstrated a membranous or linear pattern of staining

Table 1 Immunohistochemical distribution of intra-alveolar SP-A in mechanical asphyxia

Cause of death	Score				Total
	–	–/+	+	++	
Hanging	0	2	0	4	6
Strangulation	0	3	2	7	12
Throttling	0	0	1	1	2
Smothering	0	0	0	2	2
Choking	0	3	0	2	5
Total	0	8	3	16	27

Table 2 Immunohistochemical distribution of intra-alveolar SP-A in acute poisoning resulting in central respiratory depression

Cause of death	Score				Total
	–	–/+	+	++	
Sedative-hypnotic drug	0	6	0	0	6
Ethanol	0	3	0	0	3
Total	0	9	0	0	9

Table 3 Immunohistochemical distribution of intra-alveolar SP-A in peracute death from brain injury

Cause of death	Score				Total
	–	–/+	+	++	
Cerebral laceration	0	4	1	0	5
Brain stem contusion	0	1	1	0	2
Total	0	5	2	0	7

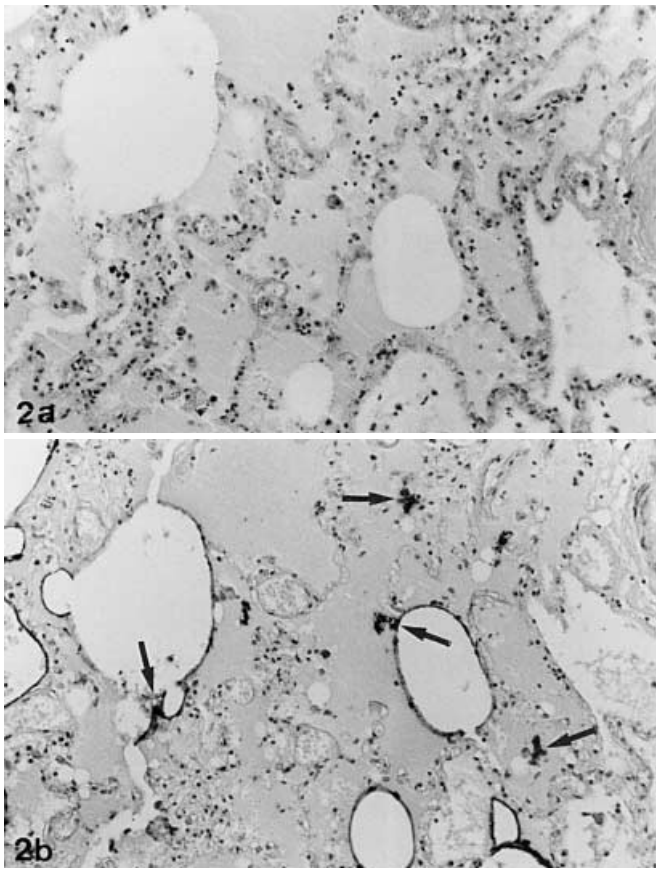

Fig. 2 Detection of the massive aggregates of SP-A granular stains by **a** H&E staining and **b** SP-A immunostaining of the serial section. Massive aggregates are indicated by the *arrows*. Original magnification × 200

with a positive rate of 92.6% (25/27) and 93.8% (15/16), in the mechanical asphyxia and control groups, respectively. However, when only the strongly positive cases were considered, these were found more often in the mechanical asphyxia group (40.7%, 11/27) than in the control group (2/16, 12.5%, $P = 0.02$). Alveolar type II cells were positively stained in nearly all cases in the control group (15/16, 93.8%), while in only 59.3% (16/27) of the mechanical asphyxia group. In the mechanical asphyxia group, however, positive cases were more strongly positive (22.2%, 6/27) than those in the control group (12.5%, 2/16). The SP-A immunostaining pattern of the intra-alveolar space differed significantly between the two groups. Although SP-A was positively stained in a granular pattern in all cases of both groups, massive aggregates of stained granules (Fig. 1 b, c) were detected in 70.4% (19/27) of the mechanical asphyxia group, while there was only two cases showing only very few massive aggregates in the control group (peracute death subgroup) as shown in Tables 1, 2 and 3 ($P < 0.0001$). Furthermore, intense and diffuse positive staining with many massive aggregates of granular stains was only found in the mechanical asphyxia group (16/27, 59.3%, $P < 0.0001$). These massive aggregates were detectable only by SP-A immunostaining and not by H&E (Fig. 2 a, b). There was a significant correlation between positivity for massive aggregates and histological findings such as congestion, intra- and interstitial edema and hemorrhage ($P > 0.05$). There were no significant differences in the immunohistochemical findings between the different mechanisms of mechanical asphyxia (e.g. hanging, strangulation, throttling, smothering and choking).

Discussion

The ability to distinguish between mechanical asphyxia and other causes of death is an important problem in forensic practice. For instance, from the autopsy findings it is occasionally difficult to determine whether a food bolus in the larynx is the cause of the death (Knight 1996 a). Microscopic findings of mechanical asphyxia as described previously (Brinkmann and Püschel 1981) are useful but non-specific and are found not only in mechanical asphyxia but also in hypoxia from other causes. Although there was a tendency towards more severe pulmonary oedema in mechanical asphyxia compared to the controls, this may not help to conclusively distinguish between these two pathological states.

Recently, an experimental study using rats to diagnose mechanical asphyxia by analysing pulmonary surfactant

phospholipids was reported (Hirvonen 1997). Although there was a significant difference in serum concentrations of the phospholipids between mechanical asphyxia and the control hypoxia group, the possibility of its practical use was not indicated. Furthermore, the concentrations of phospholipids in the lungs were similar in both groups.

In our analysis of pulmonary surfactant using SP-A histochemistry, there was no significant difference between mechanical asphyxia and control groups in the staining pattern of the alveolar surface and alveolar type II cells. However, when we examined the staining pattern of the intra-alveolar space, many prominent massive aggregates of granular SP-A positive staining were found exclusively in the mechanical asphyxia group (approximately 60% of all cases in this group). This may be a result of enhanced secretion of the pulmonary surfactant. Although different mechanisms occur in different types of asphyxia, there were no significant differences in the immunohistochemical findings among these types.

One of the common phenomena in mechanical asphyxia is assumed to be strong forced breathing that often takes place in the course of the fatal process, which may be a major difference from the process occurring in the control group. It was probably absent in the poisoning subgroup due to inhibition of the central nervous system and also in the peracute death subgroup because of the very short survival time. Another possible reason for over secretion of SP-A may be excitement of both sympathetic and parasympathetic nerves during mechanical asphyxia (Kobayashi 1992) (probably suppressed in cases of fatal sedative-hypnotic drug or alcohol poisoning), since both autonomic nerves stimulate surfactant secretion (Brown and Longmore 1981).

Although more cases must be examined before a final conclusion can be reached and the mechanism of the production of massive aggregates remains to be determined, the immunohistochemistry of SP-A may be a useful tool to distinguish mechanical asphyxia from other hypoxic cases in the forensic practice.

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