INTRODUCTION

In forensic autopsy cases, when there are spilo planta and vesicles on the body surface, those regions are suspected as having thermal trauma or frostbite congelation (1). Although traditionally associated with barbiturate overdose, they can be seen in the setting of coma due to other etiologies. The vesicles observed by Holzer in cases of barbiturate intoxication have been known for a long time (2). The erythematous patches and vesicles that are observed in coma patients, usually from an overdose of medication, are known as coma blisters. However, it is unknown whether the degenerated sweat gland is a necrosis or apoptosis. We immunohistochemically examined such skin lesions to investigate the characteristics and pathogenesis of the coma blister. Skin lesions were obtained from a forensic autopsy case, a woman in her thirties, of caffeine intoxication. Those lesions were observed in the left femoral, the lower left thigh, and the right knee. Histologically, the skin lesions showed that the keratinocytes had necrosed and the epidermis was thin in some areas. Eccrine sweat gland degeneration was observed. Obvious inflammatory cell infiltrations were not detected. Immunohistochemically, we stained each skin lesion against CD3, CD8, CD45RO, cytokeratin, 70 kD heat shock protein, ubiquitin, 150 kD oxygen regulated protein, and caspase-cleaved keratin 18 neo-epitope M30. They were also stained with an in situ apoptosis detection kit. Degenerated sweat glands featured CD45RO and M30 immunoreactivity. Immunohistochemical staining for CD45RO, CK-L, and M30 might be useful to observe sweat gland degeneration in the coma blister. Therefore, the apoptosis might be related to coma blisters and sweat gland degenerations. J. Med. Invest. 60 : 256-261, August, 2013

Keywords: coma blister, sweat gland degeneration, apoptosis, intoxication, forensic autopsy cases

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Immunohistochemical investigation of the coma blister and its pathogenesis

Masayuki Kashiwagi¹, Akiko Ishigami², Kenji Hara¹, Aya Matsusue¹, Brian Waters¹, Mio Takayama¹, Itsuo Tokunaga¹, Akiyoshi Nishimura², and Shin-ichi Kubo¹

¹Department of Forensic Medicine, Faculty of Medicine, Fukuoka University, Fukuoka, Japan, ²Department of Forensic Medicine, Institute of Health Biosciences, the University of Tokushima, Graduate School, Tokushima, Japan

Abstract : The erythematous patches and vesicles that are observed in coma patients, usually from an overdose of medication, are known as coma blisters. However, it is unknown whether the degenerated sweat gland is a necrosis or apoptosis. We immunohistochemically examined such skin lesions to investigate the characteristics and pathogenesis of the coma blister. Skin lesions were obtained from a forensic autopsy case, a woman in her thirties, of caffeine intoxication. Those lesions were observed in the left femoral, the lower left thigh, and the right knee. Histologically, the skin lesions showed that the keratinocytes had necrosed and the epidermis was thin in some areas. Eccrine sweat gland degeneration was observed. Obvious inflammatory cell infiltrations were not detected. Immunohistochemically, we stained each skin lesion against CD3, CD8, CD45RO, cytokeratin, 70 kD heat shock protein, ubiquitin, 150 kD oxygen regulated protein, and caspase-cleaved keratin 18 neo-epitope M30. They were also stained with an in situ apoptosis detection kit. Degenerated sweat glands featured CD45RO and M30 immunoreactivity. Immunohistochemical staining for CD45RO, CK-L, and M30 might be useful to observe sweat gland degeneration in the coma blister. Therefore, the apoptosis might be related to coma blisters and sweat gland degenerations. J. Med. Invest. 60 : 256-261, August, 2013

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Address correspondence and reprint requests to Shin-ichi Kubo, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan and Fax : +81-92-801-4266.

Both Dr. Kashiwagi and Dr. Ishigumi had equal contribution to this manuscript.
MATERIALS AND METHODS

Skin samples

Skin region samples of bullae and/or discoloration from a forensic autopsy case, and normal skin were collected from other autopsy cases, as follows. Case outline: A female in her thirties was caring for her bedridden mother. One day in November, the mother’s care manager visited their house, however, there was no response and none of the doors were locked. The care manager notified the police, and the policeman entered the house and found the daughter dead on her back in her bedroom.

Autopsy findings: The decedent was 159.7 cm in height and 43.0 kg in weight. Postmortem lividity was intense on the back. Spiloplaxia with excoriation and spiloplaxia with a red halo were observed in the precordial region. The spiloplaxia with the red halo were observed in the left femoral region. Blisters surrounded by rims of erythema were observed in the left femoral region, the lower left thigh, and the right knee (Fig. 1). The blister on the right knee was 1.0 cm in diameter (Fig. 2). Obvious abnormalities, such as hemorrhages were not observed in the subcutaneous region of these wounds. Five hundred and fifty ml of urine had collected in the bladder. There was no fatal trauma or disease observed. Autopsy also revealed that postmortem interval (PMI) was suspected for 4 days.

Toxicological analysis: Caffeine, ibuprofen, bromovalerylurea, bromoisovaleric acid, and ethoxybenzoic acid were detected in the blood. Caffeine, ibuprofen, bromovalerylurea, ethenzamide, ethoxybenzoic acid, and salicylamide were detected in the urine. Quantitative analysis revealed that ibuprofen, bromovalerylurea, and caffeine were 26, 11, and 52 μg/g in the blood, respectively.

Immunohistochemical examinations

Histological and immunohistochemical examinations of the skin lesions were performed. The samples were fixed in 10% phosphate buffered formalin and embedded in paraffin. Sections of 4 μm thickness were stained with Hematoxylin-eosin (HE).

Immunohistochemically, CD3 (Dako, Japan), CD8 (Leica Microsystems, Germany), CD45RO (Dako, Japan), Cytokeratin, Wide Spectrum Screening (CK-W) (Dako, Japan), Cytokeratin, High Molecular Weight (keratin 34βE12) (CK-H) (Dako, Japan), and Cytokeratin 8, Low Molecular Weight (CK-L) (Dako, Japan), 70 kD heat shock protein (HSP70) (Amersham, USA), ubiquitin (Ub) (DAKO, Japan), 150 kD oxygen regulated protein (ORP150) (Abcom, UK) and caspase-cleaved keratin 18 neo-epitope M30 (M30) (PREVIVA AB, Sweden) were observed using a labeled streptavidin biotin (LSAB)/horse radish peroxidase (HRP) technique (HISTOFINE SAB-PO kit, Nichirei, Japan), following the manufacturer’s instructions. Staining specificity was checked using negative control slides omitting the primary antibody. Additionally, tissue specimens other than positive control tissues were used in a negative control study.

To detect the apoptosis, skin samples were stained by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) method with ApopTag® peroxidase in situ Apoptosis Detection Kit (Takara Bio Inc., Japan), also according to the manufacturer’s instructions.

RESULTS

Histological examination of the skin lesions from the right knee showed necrosed keratinocytes and the epidermis was thin in some areas. Subepidermal
vesicles were observed in the blister. In the secretory cells of the eccrine sweat glands, eosinophilic homogenization of the cytoplasm and pyknosis or absence of nuclei were observed (Fig. 3).

Summary of the immunoreactivities in the degenerated sweat glands is shown in Table 1.

The degenerated sweat glands showed good immunoreactivities for CK-W and CK-H just like the control. However, the typical immunoreactivity for CK-L decreased (Figs. 4). Weak CD45RO immunoreactivity was observed (Fig. 5). However, no immunoreactivities for HSP70, Ub, or ORP 150 were observed.

Further, apoptosis was not detected by the TUNEL method. Otherwise, mild degenerated sweat glands showed partial M30 immunoreactivity (Fig. 6).

<table>
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<tr>
<th>Antigens</th>
<th>Lesion</th>
<th>Control</th>
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<tr>
<td><strong>Inflammatory cell markers</strong></td>
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<tr>
<td>CD3</td>
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<td>CD8</td>
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<tr>
<td>CD45RO</td>
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<td><strong>Keratin markers</strong></td>
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<tr>
<td>CK-W</td>
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<td><strong>Stress markers</strong></td>
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<td>HSP70</td>
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<td>M30</td>
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<td>TUNEL</td>
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Fig. 3 In the secretory cells of the eccrine sweat glands, eosinophilic homogenization of the cytoplasm and pyknosis or absence of nuclei were observed (right knee). Length of bar indicates 50 μm.

Figs. 4 The degenerated sweat glands showed good immunoreactivities for CK-W and CK-H just like the control, but decreased the typical immunoreactivity for CK-L.

Fig. 5 The degenerated eccrine sweat glands showed weak CD45RO immunoreactivity (right knee). Length of bar indicates 50 μm.
DISCUSSION

Skin blisters with underlying sweat gland necrosis were first reported by Larrey in 1812 occurring in comatose patients after carbon monoxide intoxication (8). Since then, most previously reported cases with characteristic bullous skin lesions and sweat gland necrosis were comatose secondary to an overdosage of drugs (3-5, 9), carbon monoxide poisoning (10), alcohol toxicity (11), or central nervous system disorders (12) such as hypoglycemia (13) or diabetic ketoacidosis (14). Similar clinical and histopathologic features can also be observed in cases with non-drug-induced coma (6, 15), and in such cases can be interpreted as a drug side effect in the absence of coma (7). Therefore, local pressure, hypoxia, and/or drug toxicity are contributing factors to the formation of coma blisters and sweat gland necrosis (10, 16-20). However, the underlying pathogenesis remains unclear.

Histochemical analysis revealed features similar to those reported in the literature (6, 7, 15, 18). However, inflammatory infiltration, often reported to be observed around the vessels in drug-induced coma (18), was not observed in this case.

In this study, immunohistochemical analysis revealed that sweat gland degeneration was CK-W positive. This result agrees with Setterfield’s report (21). CK-L is known to be immunopositive in secretory cells, such as eccrine gland (22-24). Degenerated sweat gland was shown the decreased immunoreactivity for CK-L.

In addition, CD45RO immunoreactivity might suggested a connection between sweat gland degeneration and the inflammatory infiltration participation of monocytes, macrophages and/or granulocytes (25). CD3 and CD8 immunoreactivities were not observed in sweat gland regions, suggesting less relation to T-cell filtration (26, 27).

Degenerated epidermis and sweat gland degeneration observed in coma blisters were described as necrosis in almost all previous literature (4, 5, 7, 11) and it was the same even after the apoptosis was defined (28). However, a coma blister was observed in a case of DNA fragmentation in one report (29).

During epithelial cell apoptosis, intermediate filaments are reorganized and keratin 18 is cleaved by caspases to liberate M30, one of the most specific and earliest detected indications of apoptosis (30-32). The TUNEL method detects single-stranded (33) and double-stranded breaks associated with apoptosis. Drug-induced DNA damage is not identified by the TUNEL assay unless it is coupled to the apoptotic response (34). In addition, this technique can detect early-stage apoptosis in systems where chromatin condensation has begun and strand breaks are fewer, even before the nucleus undergoes major morphological changes (34, 35).

In this study, the apoptosis was detected by M30 but not by the TUNEL method. This result was not contradicted, because the nucleus had undergone severe morphological changes in the degenerated sweat gland, and the skin lesions were obtained from drug-intoxication cases. Therefore, the apoptosis might be involved in the sweat gland degenerations of coma blisters.

In this study, no sweat gland degenerations were observed except the regions of coma blister. And sweat gland degeneration was not observed in other cases which were suspected in same PMI. So, the sweat gland degeneration was considered as specific for coma blister.

Immunohistochemical staining for CD45RO, CK-L, and M30 might be useful in observing the sweat gland degeneration in coma blisters. There are not so many forensic autopsy cases of the coma blister. Before this study, we found only one case of coma blisters in a case of a female in her twenties with barbiturate intoxication. Prospective studies would be useful to further elucidate the underlying pathogenesis.

CONCLUSIONS

We investigated the characteristic bullous skin lesions without local pressure after overdosage of...
drugs. Microscopically, sweat gland degeneration was observed. Immunohistochemical examination of CD45RO, CK-L, and M30 might be useful for the diagnosis of coma blisters. Further, apoptosis might be involved in coma blisters and sweat gland degenerations.

CONFLICTS OF INTEREST

We declare that the presenting authors have no conflicts of interest.

REFERENCES