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The expression of heat shock protein 70 in kidneys in cases of death due to hypothermia

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Abstract

Background: There exist only a few ''typical'' morphological signs of death due to hypothermia. For forensic practice, the identification of other reliable markers to determine hypothermia as cause of death is important. In the literature hypothermia is discussed as a stress factor for cells. It was the aim of this study to clarify wether an increased HSP 70 expression in the kidneys of fatal hypothermia victims can be observed.
Material and methods: Kidney tissue samples of 100 fatal cases of hypothermia and 50 control cases without hypothermia and burning were investigated. The expression of HSP 70 in both study and control group was graded after immunohistochemical staining using a 4° scale from 0 up to +3.
Results: Altogether, in the study group 89.0% in the tubule epithelium cells and 80.0% in the glomerula presented a HSP 70 expression of different grades. In the control group, 33 out of 50 cases were diagnosed completely without any HSP 70 expression in renal tubules, 17 cases showed a slight (+1) HSP 70 expression in the tubuli. In the glomeruli 42 cases of the control group were completely negative for HSP 70 expression, 8 cases showed a slight (+1) expression in the glomeruli.
Conclusion: Our results show, that hypothermia is a stress factor inducing HSP 70 expression in the renal tubular epithelial cells and in the glomerular podocytes. Although HSP 70 expression was increased in the kidneys in cases of hypothermia, there was no strong correlation to Wischnewski's spots.

Keywords: Fatal hypothermia; Morphological signs; Kidneys; Expression of HSP 70

1. Introduction

The diagnosis of death due to hypothermia may be difficult if the classical signs like Wischnewski's spots and frost erythema are missing. Often, death due to hypothermia can only be diagnosed due to details of the circumstances and the lack of concurrent causes of death [1–3]. Other findings in different organs like bleedings into core muscles are discussed in the literature as signs for death due to hypothermia [4–6]. In recently published investigations, in cases of death due to hypothermia fatty degeneration of renal tubular epithelium of different grades was described, which may be caused by hypoxia due to the hypothermia [7]. Also a fatty degeneration in myocardial cells could be detected, but the differentiation between fatty degeneration caused by hypothermia and pre-existing lipofuscin deposits can be difficult [8]. Furthermore, vacuoles in the pancreatic adenoid cells can be observed in cases of fatal hypothermia but the differentiation between alcoholism and hypothermia as the causes of them can be difficult [9]. Ishikawa et al. reported about fatal hypothermia related vacuoles in the hormone-producing cells of the anterior pituitary [10].

A variety of physical or chemical stress factors, e.g. ischemia and temperature induce the expression of heat shock proteins [11]. HSPs are a specific group of highly conserved molecular chaperones. They are divided into groups according to their molecular weight. The HSP 70 family includes molecular chaperones that are found in most compartments of the eukaryotic cells and are both constitutively expressed and induced by stress [12]. The two major human HSP 70s are HSP 72 and HSP 73. They assist in the correct folding of newly synthesized proteins, in the refolding of partially denatured or misfolded proteins and in the degradation of irreparably

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damaged proteins [13–15]. HSP 70 may also play a role in the inflammatory response after myocardial infarction and is well related to some diseases [16]. Furthermore, HSP 70 prevents apoptosis, thereby increasing the survival of cells exposed to a wide range of otherwise lethal stimuli [17]. In forensic practice the expression of HSP 70 in different internal organs of fire victims for determination between vital and supravital reaction is important [18].

Kita described an induction of the HSP 70 expression in the cerebral cortex in cases of lethal hypothermia in an experimental rat model and autopsy cases [19]. Rada et al. investigated human hepatoma HepG2 cells after hypothermic stress and found a statistically significant increase of HSP 70 expression [20].

In the present investigation, the expression of HSP 70 in the kidneys of fatalities due to hypothermia was investigated. The reason for the choice of investigating the kidneys was, that kidneys obviously are a fast reacting organ, especially in hypothermia [7].

2. Material and methods

The study group contains 100 cases of fatal hypothermia from the time period between 1990 and 2007. The diagnosis ''fatal hypothermia'' was based on circumstances of the case, the kind of discovery of the body, autopsy findings (mainly Wischnewski's spots and frost erythema) and the lack of concurrent causes of death. In the study group 78 cases showed Wischnewski's spots as autopsy finding and 61 cases frost erythema. The study group contained no case without one of these findings.

The study group included 31 females and 69 males in the age between 18 and 93 (average 73.5). The distribution of the relevant pre-existing diseases is arranged in Table 1.

In all cases a complete histological investigation and a toxicological screening, including the determination of blood alcohol concentration (BAC), were performed. Cases with a BAC of more than 2.5 per mille were excluded of the study group.

As control group, renal samples from 50 autopsy cases were taken. The control group contained 12 females and 38 males in the age between 27 and 82 (average 53.2). Control group includes 25 cases of acute death (period of agony only seconds till few minutes; shot into the head with caliber more than 7.65) and 25 cases of protracted death (period of agony several minutes till few hours, 15× gastrointestinal bleeding, 10× chronic cardiac failure).

Several tissue samples of both kidneys were collected. All tissues were fixed in formaldehyde (8–10%) and afterwards embedded in paraffin. The time period of fixation in both groups varied between few days and several years. In all cases, tissue samples were stained with hemalaun–eosin to exclude severe pre-existing diseases. After that, immunohistochemical staining was performed. For immunohistochemistry, every section needed to be pre-treated by using high temperature antigen unmasking technique (citrate-buffer, pH 6.0). To increase the findings, we applied common swine serum (Dako) in a dilution of 1:5 (in TRIS-buffer, pH 7.5) to block non-specific binding of immunoglobulins. The primary antibody (Novocastra) was used manually in a dilution of 1:30 which needed to be incubated overnight at 4 °C. After incubation, we used the standard ABC-technique for further staining procedures (LSAB-2-System, Dako). As (our) standard substrate–chromogen solution we applied AEC–chromogen. Finally, the slides were immersed in a bath of hematoxylin for about 30 s. The specimens were mounted and coverslipped with an aqueous-based mounting medium (Aquatex, Merck).

Table 1
Relevant pre-existing diseases in the study group

Pre-existing disease	Number of cases
Chronic pulmonary emphysema	45
Fatty liver	72
Arteriosclerosis	52
Hypertrophy of the heart	34
Craniocerebral injury	9
Pneumonia	3

To exclude non-specific reaction of antibodies with human tissue and/or artefacts, controls of immunohistochemistry were performed by omitting the primary or secondary antibodies.

To demonstrate specific binding of HSP 70 antibodies, the antibodies were pre-incubated with recombinant active HSP 70 (Abnova): IgG concentrations of the antibodies were kindly provided on request by Novocastra. The protein concentrations of the recombinant activated HSP were measured by the Bradford assay [21]. A four-fold antigen excess resulted in the absence of immunohistochemical staining.

2.1. Microscopic investigations

In all cases, tubule epithelium cells and glomeruli were evaluated separately. The following modified score according to Remmele and Stegner [22] was used to evaluate the immunohistochemical results in study group and control group. The HSP 70 expression in the tubule epithelium cells were graded in 20 high power fields in mean magnification (200×): (0), no staining; slight (+1), light, but definite colour reaction; moderate (+2), moderate colour reaction; severe (+3), pronounced colour reaction (Fig. 1).

For analysing the glomerular HSP 70 expression, in 20 glomeruli the cells with positive reaction were counted and classified in four grades in a high magnification (400×): 0, no positive cell per glomerulus; +1, 1 up to 5 cells with positive colour reaction per glomerulus; +2, 6 up to 10 cells with positive reaction and +3, more than 10 cells with positive colour reaction per glomerulus (Fig. 2).

Sections were investigated by two independent investigators, but there were only single cases, where a difference in graduation was found concerning grade 2 or grade 3. Finally a decision was possible to contribute the findings to one of three grades. Therefore, interobserver-variability can be regarded as low.

After this, in all cases the arithmetic mean for glomeruli and tubule epithelium cells HSP 70 expression was accumulated separately and then also graded in a 4° scale: arithmetic mean 0 up to 0.49, grade 0; arithmetic mean 0.5 up to 1.49, grade +1; arithmetic mean between 1.5 and 2.49, grade +2 and arithmetic mean more than 2.5, grade +3.

2.2. Macroscopic investigations

After microscopic investigation and grading of the HSP 70 expression in tubule epithelium cells and in glomeruli, the results were compared to gross autopsy findings of hypothermia like Wischnewski's spots and frost erythema.

3. Results

3.1. Tubular HSP 70 immunopositivity

With regard to the study group, there were 11 cases (11.0%) without expression of HSP 70 in the tubular epithelium cells. Forty-five cases (45.0%) showed a HSP 70 expression in tubular epithelium cells grade +1 and 42 (42.0%) with grade +2; 2 cases (2.0%) presented severe expression of HSP 70 in the renal tubule epithelium (grade +3). Table 2 shows an overview. Altogether, 89.0% of the cases in the study group presented signs of HSP 70 expression in the tubule epithelium cells of different grades. The HSP 70 expression in the tubular epithelium cells could be detected both in cytoplasm and nucleus of the cells, but mostly as granular pattern in the cytoplasm.

Regarding the control group, 33 out of these 50 cases were diagnosed without HSP 70 expression in renal tubules, 17 cases