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ORIGINAL ARTICLE

Expression of heat-shock protein 70 (Hsp70) in the respiratory tract and lungs of fire victims

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Abstract Immunohistochemical investigation of the respiratory tract and lungs of 63 fire victims revealed a statistically significant enhanced expression of heat-shock protein 70 (Hsp70) in the epiglottis, the trachea, and the main and the peripheral bronchi compared with a control group. In the fire victims, a strong expression of Hsp70 was discernible not only particularly in the vessels but also in seromucous secretory cells, ciliated epithelial cells, smooth muscle cells, and alveolar cells. The results suggest a vital or supravital reaction due to the inhalation of hot fire fumes.

Keywords Heat-shock protein · Hsp70 · Fire victims · Airways · Lungs

Introduction

When an organism is exposed to stress stimuli, the affected cells quickly respond by expressing heat-shock proteins. Their role is to ensure the survival and function of the cells in manifold ways. They monitor protein synthesis, prevent their aggregation, stabilize various cell structures, are able to repair key enzymes, and modulate the immune response [4, 5, 16, 18, 19, 21, 24, 28, 36]. An important representative of heat-shock proteins is the heat-shock protein 70 (Hsp70) group, which consists of at least five subgroups [22]. Although the functions of Hsp70 are not yet fully understood, there are reliable clues that they are involved in the formation, stabilization, and disaggregation of complex multimer structures and also in protein folding [18, 28]. It is assumed that the intracellular accumulation of Hsp makes the cells resistant to the cytotoxic effects of TNF-α. Finally, TNF-α and IL-1 in turn seem to induce expression of Hsp [23].

Angelidis et al. [1] showed that Hsp70 is directly involved in heat resistance. When heat acts as a stress stimulus, it was observed that Hsp70 is expressed, which controls protein biosynthesis and ensures cell protection and survival of the cell at high temperatures, presumably by binding to ribosomal subunits [4]. Up to now, the role played by heat-shock proteins in burns has been discussed primarily under clinical aspects [15, 20, 26, 30, 37].

In deaths due to fire, examination of the respiratory tract is of crucial importance. A person who was exposed to heat while still alive will often have also inhaled hot gases. The macroscopic and microscopic changes may prove not only that the victim was alive when the fire arose but may also be a hint for the pathomechanisms causative of death, especially in cases with rapid dying [2]. The complex pathophysiological interactions resulting in acute fire-related deaths are still poorly understood today [14, 31, 35]. Although inhalation injury is a frequent consequence of heat exposure, there are only few reports on the expression of heat-shock proteins in the airways and lungs [13, 27, 38, 39] and no systematic investigations on the expression of Hsp70 after traumatization of the lungs. In the forensic literature, the number of studies dealing with heat-shock proteins after burns is also very small [6, 25, 29, 32]. Some authors investigated the heat-induced appearance of other proteins [10, 12]. Furthermore, it is not known to

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Table 1 Frequency of positive staining with Hsp70 antibody in fire victims and controls

Localization	Structures	Fire fatalities (%)	Control group (%)
Respiratory tract	Ciliated epithelium	63	15
Respiratory tract	Seromucous glands	94	8
Respiratory tract	Bronchial muscles	27	5
Tracheal/bronchial vessels	Endothelium	29	2
Pulmonary tissue	Alveocytes	95	19
Pulmonary tissue	Scaled-off alveocytes	96	60
Pulmonary tissue	Alveolar macrophages	98	95
Pulmonary vessels	Endothelium	90	47
Pulmonary vessels	Muscle cells	83	41
Pulmonary vessels	Erythrocytes	92	10
Pulmonary vessels	Leukocytes	86	2

what extent data obtained from patients with burn trauma are applicable to the forensic study material. As the expression of Hsp70 starts very rapidly after the onset of the cell damage, the detection of Hsp70 in fire fatalities may be regarded as a sign of vital or supravital heat exposure.

The purpose of this paper was to investigate whether an enhanced level of Hsp70 is expressed in the respiratory tract and the pulmonary tissue of fire fatalities in comparison with a control group of unselected fatalities.

Materials and methods

The study group included 44 fire fatalities from the years 1996–2002, on which forensic autopsies had been performed at the Institute of Legal Medicine of Freiburg University. Another 21 cases from the years 1996–2001 were contributed by the Institute of Legal Medicine of the Free University Berlin, so that the total number of fire-related deaths was 65. Of the investigated fire victims, 41 were men and 24 were women, and the average age was 52 years. At the time of death, the mean age of the male victims was 47 years, while that of the female victims was 60 years. The youngest victim was a 6-year-old girl, while the oldest was a old woman aged 92 years. The inclusion criteria were exposure to fire, no survival after recovery from the fire, and absence of late postmortem changes.

For comparison, a group of 55 unselected control cases including natural and nonnatural causes of death from the Institute of Legal Medicine of Freiburg University dating from the years 2000–2001 was investigated. Exclusion criteria were any link with a fire, late postmortem changes, as well as protracted diseases or trauma with intensive medical care immediately preceding death.

Immunohistochemical staining of Hsp70 (Dako, polyclonal rabbit, anti-Hsp70, code no. A500, lot no. 058) was performed with the avidine–biotin method using the Vectastain Universal Elite ABC-Kits of Vector Laboratories (Burlingame, CA, USA). In each case, five slides of the lung tissue, one slide from the trachea, and one slide from the main bronchus of the left lung were examined. The slides were examined under blinded conditions. The stainings were graduated semiquantitatively (negative, slight, moderate, and marked).

For statistical analysis, we used the Wilcoxon test for unpaired samples and the Kruskal–Wallis test with an error probability of $p<0.05$ being considered significant. With the help of logistic regression (Holm-corrected), further differences could be identified between the groups by means of backward and forward elimination.

Results

In a large part of the deaths associated with fire (87.7%), the circumstances and autopsy findings proved that the victims had been exposed to the fire while still alive. In 12.3%, the exposure to the fire occurred perimortem or postmortem. The most common cause of death was peracute burn trauma, followed by poisoning due to the inhalation of fire fumes. In the cases where exposure to the fire occurred perimortem or postmortem, death resulted from either mechanical injuries (traffic accidents with consecutive burning) or from coronary heart disease (followed by a house fire). The majority of fire victims were found in houses (58.5%), another 30.8% burned to death in a vehicle, and 10.7% outdoors.

The control group included 41 men (74.6%) and 14 women (25.5%) deceased, with an average age of 47.3 years. At the time of death, the mean age was 43.9 for the men and 57.3 years for the women deceased. The youngest victim was a male infant aged 4 months at the time of death, while the oldest was a man aged 88 years. In the control group, most of the victims died in accidents (43.6%). There was also a large number of deaths due to a

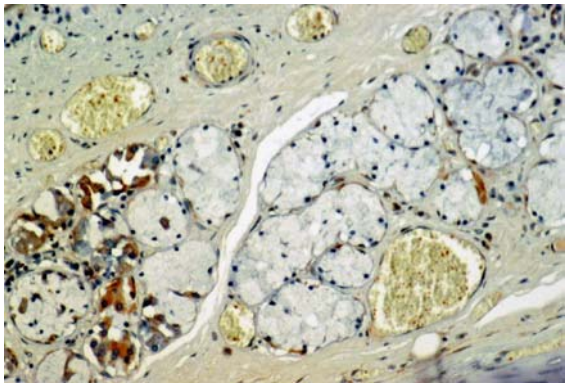

Fig. 1 Trachea of a 69-year-old man with Hsp70 antibody staining of seromucous glands (study group)

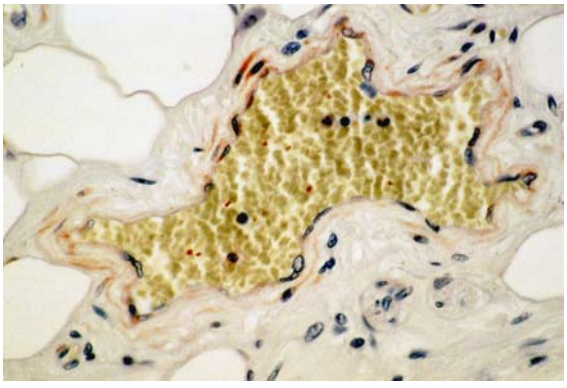

Fig. 3 Peribronchial vessel with anti-Hsp70-positive staining of endothelium, muscle cells, and some erythrocytes (55-year-old man, study group)

natural cause (41.8%); suicides accounted for 9.1% and homicides for 5.5%. The most frequent cause of death in the control group was coronary heart disease (25.5% of the cases). Head injuries and polytrauma accounted for 14.6% each. All other causes of death were <10%.

Staining with anti-heat-shock protein 70 (anti-Hsp70)

Burned bodies

The frequency of cases with positive staining is given in Table 1. In the group of fire-related deaths, an expression of heat-shock protein was observed in the following structures: in the cytoplasm of ciliated epithelial and seromucous secretory cells of trachea and bronchi, in the cytoplasm of alveocytes and alveolar macrophages, and in endothelial cells and smooth muscle cells of vessels both of the respiratory tract and the lung parenchyma as well as in erythrocytes and leukocytes located in the vascular lumen (Figs. 1, 2, 3 and 4).

In the subgroup of victims burned perimortem or postmortem, these structures actually also showed an expression of Hsp70, but the vascular endothelium, the smooth muscle cells of the vascular walls, and the intravasal erythrocytes and leukocytes were stained weaker and less often.

Control group

In the control cases, an expression of Hsp70 was found in the epithelium of the peripheral bronchi, the alveolar macrophages, and the desquamated alveocytes. In vessel walls and in blood cells, Hsp70 could be demonstrated less often than in the study group. The frequency of cases with positive staining is given in Table 1.

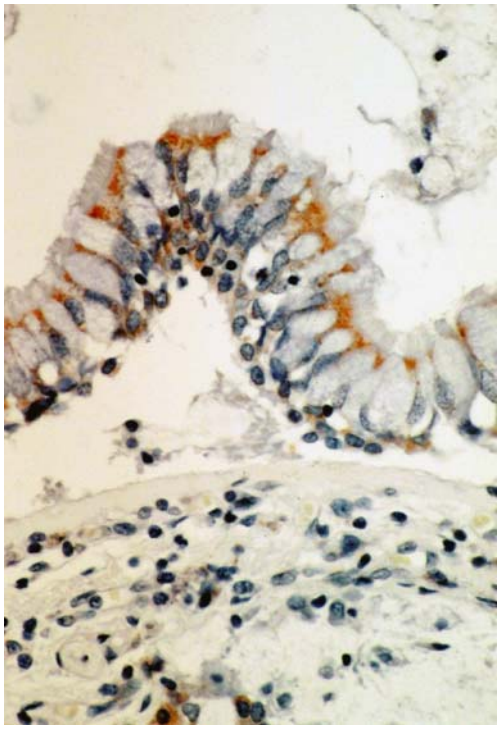

Fig. 2 Vesicular detachment of the tracheal epithelium with pseudo-goblet cells and staining with Hsp70 antibodies (42-year-old woman, study group)

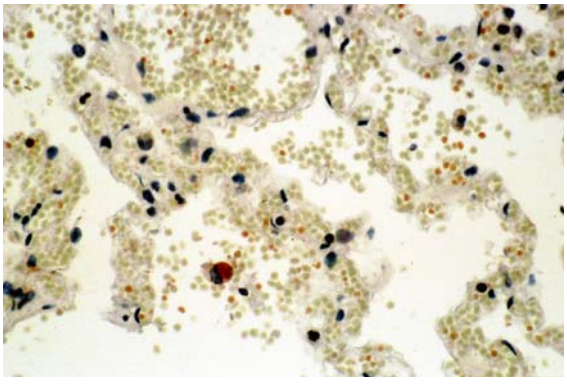

Fig. 4 Alveolar hyperemia and intraalveolar hemorrhage with anti-Hsp70-positive staining of macrophages, granulocytes, and erythrocytes (49-year-old man, study group)

Statistical comparison between fire fatalities and control group

As the number of cases in the second group was very small, no statistical comparison was made between the group of victims clearly exposed to the fire while still alive and the group of perimortem/postmortem fire victims.

Statistical analysis of the total burn group and the control group using the Wilcoxon test for unpaired samples showed that the strongest difference between the groups was the expression of Hsp70 in the cytoplasm of alveocytes and erythrocytes with an χ^2 value of 75.14 and 58.42, respectively, and an error probability of p=0.00. In the staining of alveolar macrophages, no statistically significant difference was found.

The results were checked with the help of Holm-corrected logistic regression by means of backward and forward elimination, which confirmed the sequence of the Wilcoxon test. Thus, the presence of immunohistochemically demonstrated Hsp70 in erythrocytes was found to be the most important difference between the groups with an odd ratio of 26.793.

Discussions

The results of our study suggest that a higher level of Hsp70 is expressed in the respiratory tract and the lungs after exposure to fire. This applied not only to tissues in which a direct contact with the (hot) fire fumes can be assumed (ciliated epithelium of the airways, seromucous glands of the airways, alveocytes, and alveolar macrophages) on the one hand, but also to cells in the blood vessels (endothelium, smooth muscle cells of the vascular walls) suggesting a vascularly mediated response.

That Hsp70 could be demonstrated in vascular structures in cases of vital burns supports the hypothesis that for the development of inhalation trauma, the blood flow in the tracheal wall, the bronchial wall, and the lungs is of crucial importance [17, 33, 34]. After the inhalation of hot gases, local blood flow increases dramatically. As animal experiments were able to demonstrate, thermal lesion to the respiratory tract causes damage to the lung tissue even if the latter is not ventilated, i.e., if no aerogenous, direct effect of the heat or smoke on the pulmonary tissue was possible. It is assumed that via bronchopulmonary shunts toxically acting mediators from the damaged sections of the trachea and large bronchi spread along the vessels [9, 17, 33, 34]. In that case, the enhanced expression of heat-shock proteins would have to be regarded as a kind of systemic reaction to a local noxious effect.

As the statistical evaluation showed, the presence of Hsp70 in the erythrocytes of the bronchial vessels proved to be the parameter best suited to differentiate between fire fatalities and controls. Increased stainability of the erythrocytes with anti-Hsp70 antibodies could also be observed in the vessels of the epiglottis, the trachea, and the alveolar walls. In the controls, this feature was either absent or only discrete. In those few cases in which burning occurred perimortem or postmortem, the expression of Hsp70 was less pronounced in the blood cells and endothelium than in the vital burns. However, as the number of cases was small, this difference could not be proved statistically.

In addition to the stainings associated with vessels, the cytoplasm of alveocytes also showed an accumulation of Hsp70. This may be interpreted as a reaction to the inhalation of hot gases or smoke particles. Moreover, desquamated cells also showed an expression of Hsp70. Shedding of alveocytes as a consequence of vital heat exposure has been repeatedly described [3, 7, 8, 11].

Vital exposure to fire may cause extensive damage to the respiratory tract and the lungs. The enhanced expression of Hsp70 in the group of fire-related deaths can be regarded as an expression of that damage. Similar results were reported by Quan et al. [29], who found an enhanced expression of ubiquitin in the brain stem of fire victims as against control cases, and by Fineschi et al. [6], who found heat-shock proteins in the skin in a case of a fatal heat stroke. In this context, it has to be taken into consideration, however, that an enhanced expression of Hsp70 in the respiratory tract was not only found after vital heat exposure but also after perimortem or postmortem exposure to the effects of heat. However, in the latter cases, death occurred shortly before exposure to the fire, so that the Hsp70 expression may be a local supravital reaction. The ability to express Hsp70 seems to be maintained for a short time even after respiratory and circulatory arrest. Thus, an enhanced expression of Hsp70 alone cannot be regarded as a clear vital sign. This aspect requires further investigation.

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