Hsp27 and 70 expression in the heart, lung and kidney in SIDS

Elke Doberentz1, Sarah Führing1, Burkhard Madea1,*

Abstract: Purpose. Hyperthermia is one of the known risk factors for sudden infant death syndrome (SIDS). Hyperthermal stress can induce the heat shock response with increased expression of heat-shock proteins (hsps).

Methods. Immunohistochemical staining for hsp27 and hsp70 in the myocardial, pulmonary and renal tissue from 120 SIDS cases and 29 control cases was examined.

Results. Hsp70 immunostaining was negative in all investigated organs of both groups. Hsp27 expression was found in the lungs in a few cases at different intensities in the study and control groups.

Conclusion. The hypothesis of hyperthermia being a pathogenic factor for SIDS was not supported by immunohistochemical visualization of hsp27 or hsp70. However, when the temperature is below 41.8°C, hsp expression is not induced.

Key Words: Hyperthermia, Sudden infant death syndrome (SIDS), heat shock protein, expression in heart, lung, kidney.

In Germany 682,069 infants were born in 2014 [1]. In the same year, 152 infants (0.02%) died from an unknown cause of death, and were classified as sudden infant death syndrome (SIDS). The incidence of SIDS has decreased over time in Germany; only 10 years earlier the incidence was 0.05% and 20 years earlier 0.11%.

According to the triple risk hypothesis, SIDS appears to be the result of an inherent vulnerability with cerebral insufficiency in the critical developmental period, especially in respiratory and cardiac control, with apnea-induced hypoxia in combination with environmental stress factors while sleeping [2, 3]. Many of these risk factors leading to death are known (4-9) (Table 1). The most studied risk factor is the prone sleeping position [2, 10-12]. However, numerous studies have also focused on the risk factor of hyperthermia or increased body core temperature (Table 2).

Hyperthermia and overheating was first detected as a risk factor because high ambient temperatures were found at the place of death or overly warm covering or clothing of the infants was evident. Furthermore, high core body temperatures have been measured in SIDS. As a direct result of hyperthermia, vasodilatation with hyperemia in the tissues and increase of metabolism with consecutive higher need of oxygen is found [19, 20].

Heat shock proteins (hsps) are found in all organisms, including humans [21], in various cellular compartments. In the event of temperature-induced cellular stress, hsps are expressed quickly and at high concentrations [22, 23] and interact with denatured proteins, repairing or degrading them [24]. Pre-death changes of ambient temperatures with an increase or decrease of the core body temperature can be verified by the immunohistochemical visualization of hsp expression [22]. The question therefore arose whether hyperthermia as a potential pre-death risk factor in SIDS can be verified by the expression of hsp27 and hsp70, especially because the increase of the core body temperature in fever also activates the heat shock response with elevated hsp levels [21, 24]. The presented study is a follow up study [25].

MATERIAL AND METHODS

The study group included 120 SIDS cases
During forensic autopsies tissue samples of the heart, lung, and kidney were taken as evidence. The samples were fixed in 8–10% formalin. After fixation, the tissue samples were embedded in paraffin wax, sliced (3–4 μm), and stained with anti-Hsp27 (mouse anti-hsp27 monoclonal antibody; Novo Castra®) and anti-Hsp70 (mouse anti-hsp70 monoclonal antibody, Novo Castra®, Novocastra Laboratories Ltd, Newcastle upon Tyne/United Kingdom) antibodies and hematoxylin-eosin (H&E). In every staining run, tonsil tissue was used as a positive control. One sample of tonsillar tissue was incubated without primary antibody and one without secondary antibody as negative controls. Thirty visual fields in every slide were examined by light microscopy at ×400 magnification. For every analyzed organ structure and case a mean value over all 30 visual fields was calculated and finally graded according the pattern shown in Table 5 and Figs. 1 and 2.

Table 1. Risk factors in SIDS (modified according to 4-9)

<table>
<thead>
<tr>
<th>Maternal, prenatal risk factors</th>
<th>Infantile, postnatal risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking during pregnancy</td>
<td>Male</td>
</tr>
<tr>
<td>Alcohol and drug abuse during pregnancy</td>
<td>Premature infant</td>
</tr>
<tr>
<td>Insufficient prenatal care</td>
<td>Nursing &lt; 2 weeks</td>
</tr>
<tr>
<td>Age &lt; 20 years</td>
<td>Prone sleeping position</td>
</tr>
<tr>
<td>Low socio-economic status</td>
<td>Overheating, thermic stress</td>
</tr>
<tr>
<td>Low educational level</td>
<td>Sleeping in bed with parents</td>
</tr>
<tr>
<td>Short interval between pregnancies</td>
<td>Sleeping in own room</td>
</tr>
<tr>
<td>Single mother</td>
<td>Febrile infection</td>
</tr>
<tr>
<td></td>
<td>Soft mattress, soft cover</td>
</tr>
<tr>
<td></td>
<td>Exposed to cigarette smoke</td>
</tr>
</tbody>
</table>

Table 2. Studies about the risk factor hyperthermia in SIDS

<table>
<thead>
<tr>
<th>Authors</th>
<th>Number of cases</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fleming PJ et al., 1990 [13]</td>
<td>67</td>
<td>Hyperthermia is an independent risk factor</td>
</tr>
<tr>
<td>Gilbert R et al., 1992 [12]</td>
<td>95</td>
<td>Combination of viral infection and warm covering increases the risk of SIDS</td>
</tr>
<tr>
<td>Kleemann W et al., 1996 [14]</td>
<td>140</td>
<td>Significant evidence of premortal hyperthermia</td>
</tr>
<tr>
<td>Pfeifer K, 1980 [15]</td>
<td>138</td>
<td>Hyperthermia in 82,6% of the cases</td>
</tr>
<tr>
<td>Ponsonby AL et al., 1992 [16]</td>
<td>41</td>
<td>Hyperthermia is an independent risk factor</td>
</tr>
<tr>
<td>Stanton AN, 1984 [17]</td>
<td>15</td>
<td>40% of the cases with a rectal temperature of above 37 °C (highest 42 °C)</td>
</tr>
</tbody>
</table>

Table 3. Causes of death in the control group

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>7</td>
</tr>
<tr>
<td>Trauma</td>
<td>6</td>
</tr>
<tr>
<td>Malformation</td>
<td>6</td>
</tr>
<tr>
<td>Suffocation</td>
<td>5</td>
</tr>
<tr>
<td>Drowning</td>
<td>2</td>
</tr>
<tr>
<td>Air embolism</td>
<td>1</td>
</tr>
<tr>
<td>Death in childbirth</td>
<td>1</td>
</tr>
<tr>
<td>Cerebral oedema</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 4. Investigated structures in the different organs

<table>
<thead>
<tr>
<th>Organs</th>
<th>Structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>Myocytes, fibrocytes, vessels</td>
</tr>
<tr>
<td>Lung</td>
<td>Peripheral and central bronchial tubes, vessels (endothelium, lumen), inter-alveolar septa, pleura, peribronchial glands, peribronchial connective tissue, ciliated epithelium</td>
</tr>
<tr>
<td>Kidney</td>
<td>Glomerula, tubuli, vessels, connective tissue</td>
</tr>
</tbody>
</table>
RESULTS

Evaluation of hsp27 expression revealed that it was hardly expressed in either the study or the control group. In both groups, there was a low level of hsp27 expression of different intensities in the pulmonary tissue in the respiratory epithelium and vessels (Figs. 3–8).

Hsp70 expression could not be detected in any SIDS or control sample.

DISCUSSION

The most investigated risk factor for SIDS is the prone sleeping position [2, 10–12, 27]. This sleeping position increases the risk of hypercapnia, hypoxia and hyperthermia [3, 28]. However, in addition to the inevitable and continual discussion of the consequent obstruction of respiration and cerebral blood flow, a prone sleeping position per se can lead to disturbance of physiological temperature regulation. Due to the limited mobility in a prone sleeping position and the relatively large contact area of the head and body with the sleeping surface, heat emission can be impeded and heat accumulation can result, which requires physiological mechanisms to maintain the core body temperature at a constant level [29–32].

In the presence of an additional adverse factor, such as warm covering of the infant, decompensation of thermoregulation with an increase of the core body temperature may result. This hyperthermal stress can lead to death in cases of apnea-induced hypoxia [33].

The predominantly negative results of the present study, with no proof of increased hsp-expression in SIDS cases, lead to the discussion whether hyperthermia can really be counted as a risk factor or even influences death at all, as an increase of the core body temperature is not associated with verified hsp expression.
Studies have shown that the core body temperatures in infants which were designated as SIDS after autopsy is 38–40°C [17, 18]. Indeed, hsp expression is dependent on the level of the core temperature or the temperatures to which cells are directly exposed. The physiological core body temperature in humans is 37°C and can vary by up to 1°C [34]. At 37°C there is a physiological low hsp expression in all cells [21, 35, 36], which can vary in level between the different hsp families. When there is fever accompanying infections, the balance between heat generation and elimination is disturbed and the heat shock response is induced, with expression of hsps when there is an increase of more than 4°C in core temperature above baseline [21, 24]. Nevertheless, some tissues, such as the brain and kidneys, are more sensitive than others and may even react at lower core temperatures [37]. Thus, fever could be verified by the postmortem demonstration of hsp expression.

However, induction of hsp expression occurs above 40°C, whereby 41.8 to 42.5°C is the optimal stimulus for hsp expression [35, 36]. Furthermore, a study by Becker et al. has shown that at temperatures below 41.8°C, hsps are not markedly expressed by healthy cells [38]. Above 41.8°C hsp expression increases twentyfold [36, 39]. Other studies revealed that around temperatures of 41°C cell death occurs [40, 41]. In fire victims with a rapid elevation of the body’s core temperature it could be demonstrated that hsp27 is expressed fast (within minutes in various organs, especially pulmonary tissue). With increasing survival time hsp70 expression predominates hsp27 expression [23]. Moreover, in hypothermia, marked hsp expression could be demonstrated, especially in the kidney [26]. Therefore, regarding the present results and the death circumstances of the infants, no Hsp expression would be expected at body core temperatures between 38 and 40°C, as no cell stress occurs.

The results of the present study are congruent with other published studies. Hsp27 expression was demonstrated, because hsp27 is always present at a certain level in all human tissues [42]. In contrast, Hsp70 is mostly expressed under stressful conditions [42]. This could explain the negative results in the study and control groups for Hsp70. Notably, while healthy cells are protected by hsps and their repair mechanisms, heating above 45°C leads directly to cytotoxic effects [35].

In contrast to our results, Rhode et al. published a study demonstrating higher expression of hsp70 encoding genes in the SIDS group compared with a control group in cases of thermal stress of approximately 40°C and with different incubation times over several hours [28]. They investigated cultured fibroblasts taken from Achilles tendons at forensic autopsy without studying the corresponding gene products, the hsps. In contrast, we investigated the hsp expression directly in the organs of infants.

When cells are exposed to moderate sublethal temperatures, they can subsequently tolerate fatal temperatures without damage to the cell structures.
[41-43]. This is the phenomenon of thermotolerance - the second way for hsps to react to heat stress (heat shock response). The higher and longer the sublethal temperature, the more pronounced and long-lasting the thermotolerance. Thermotolerance or adaption to heat is developed more slowly than a direct heat shock response [39, 44-46]. In an animal-based study, 16 to 24 hours after heat exposure thermotolerance occurred in cells and within approximately 24 hours [45]. There is no agreement in the literature as to whether this thermotolerance is associated with increased hsp expression or not. Some studies revealed heat exposure without heat shock protein expression [47, 48]. Alternatively, possibly only select hsps are involved in thermotolerance [49]. Therefore, possibly cells develop thermotolerance from a slow rising core body temperature (formation of fever) and can thus tolerate high fever without relevant cell damage. Interestingly, even cross-tolerance has been reported, in which exposure to stress factors can result in tolerance to other stimuli [50].

Furthermore, insufficient hsp expression has been discussed in SIDS infants, where the cells are exposed to noxious influences without protection. Elevated temperatures would damage cell structures without the protection of hsps, leading to cell death. Various gene polymorphisms are hypothesized, with the result that ineffective hsps are formed, which are insufficient to protect the cell structures [51, 52].

CONCLUSIONS

Pre-death hyperthermic effects with the expression of hsp27 and hsp70 could not be verified by immunohistochemistry in SIDS. A lack of expression of hsp27 and 70, however, does not exclude pre-death hyperthermia and its influence on SIDS. The physiological increase in body core temperature, in accordance with the results of other studies, causes no relevant cell stress associated with an increase in hsps. However, hyperthermia is not likely to have a significant effect on the death rate of infants in SIDS when hsp expression is absent. In the event of decompensation of the tolerance period, an increase in hsp expression, which would be detectable, would be expected.

Conflict of interest. The authors declare that they have no conflict of interest concerning this article.

Ethical approval. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References