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RESEARCH ARTICLE

Multiorgan morphological changes caused by hyperthermia: Case study on experimental model

Emina Dervišević[1*], Nina Čamdžić[2], Suada Kuskunović-Vlahovljak[2], Muamer Dervišević[3]

1. Department of Forensic Medicine, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina
2. Department of Pathology, Faculty of Medicine, University of Sarajevo,Sarajevo, Bosnia and Herzegovina
3. Clinic for Lung Diseases "Podhrastovi", University Clinical Centre of Sarajevo, Sarajevo, Bosnia and Herzegovina

Morphologic changes in organs vary from nonspecific to specific ones, depending on causes of sudden death, e.i whether it is an acute, subacute or chronic event. The aim of this pilot study was to observe the appearance and occurrence of morphological characteristics on organs that were exposed to long-term effects of hyperthermia. A sample of 7 rats was exposed to a water temperature of 41 °C, which is defined in the literature as "heat stroke temperature", both sexes, weighing 250 to 300 g were used. Tissue samples, obtained by dissection of rats, were fixed in 10% buffered neutral formalin, at room temperature, then incorporated into paraffin blocks, cut at 4-5 microns, mounted and stained with standard hematoxylin-eosin (HE) method. In order to prove/exclude lipid and glycogen accumulation in hepatocytes we did additional histochemical staining, using Sudan black and Periodic Acid Shiff (PAS) method, respectively. We obtained samples from kidney, liver, pancreas, spleen, lung and brain. Analyzing tissue samples of different organs obtained from seven Wistar rats, we gained insight into morphological changes caused by induced hyperthermia. All sampled organs showed congestion and some degree of oedema. The most prominent changes were observed in liver and lung samples. Tissue samples of the lung of all seven rats showed signs of acute bronchitis and bronchiolitis, together with signs of initial bronchopneumonia. We also noticed signs of focal acute emphysema as well as focal accumulations of foamy macrophages. Our study suggests that changes in the vascular bed occur soon after hyperthermia and while some organs are more tolerant to heat stroke than others, most organs show similar changes consisting of capillary dilation, congestion and interstitial extravasation, observed after 30 minutes at a temperature of 40.5 °C, with the most significant changes observed in liver and lung samples.

Keywords: hyperthermia, heat stroke, experimental

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Introduction

Hyperthermia is a condition of elevated body temperature, above the upper physiological range [1,2]. In the 19th century, an animal model of pigs was developed to study disorders caused by hyperthermia, which was the originator of a later studies that proved the role of hyperthermia in diseases such as haemorrhagic shock and encephalopathy syndrome, as well as some cases of sudden infant death syndrome [3-6]. Several studies related to heat stroke in rats have been performed as experimental models [7-9]. The models were based on exposure of rats to high temperatures, dry air or water, until the core temperature reached a predetermined temperature (40.5 °C).

A body temperature value of 40.5°C on exposure for 15 minutes was accepted as a reference for the diagnosis of heat stroke. No direct conditioned-consequential association between hyperthermia and mortality (less than 10% death) was found in rats exposed to lower temperatures during the experiment [10]. The first model of hyperthermia was developed on a dog in 1973 and on a rat in 1976 [11]. Following the historical sequence, more models of hyperthermia have been developed, but most of them cause heat stroke with high-temperature dry air. In the animal model of hyperthermia, the study of Suzuki et al. [12] point to hyperthermia as a cause of death during bathing and the association between high water temperature and survival time. Animal studies suggest that changes in the vascular bed occur soon after hyperthermia and while some organs are more tolerant to heat stroke than others, most organs show similar changes consisting of capillary dilation, vascular pathway and interstitial extravasation, observed after 30 minutes at a temperature of 40.5°C [13]. Morphologic changes in organs vary from nonspecific to specific ones, depending on causes of sudden death, e.i whether it is an acute, subacute or chronic event.

There are a numerous pathophysiological mechanisms related to harmful effects of hyperthermia, which are reflected in direct cell damage, local effects, e.g. cytokine stimulation and inflammatory response, and systemic effects, e.g. translocation of intestinal bacteria. The state of hyperthermia can be the result of two processes. According to the available literature, with increasing body temperature, cardiac output and blood pressure drop drastically and are associated with myocardial oxygen consumption. Hypoxia causes numerous heart muscle injuries), from subendocardial haemorrhage, myocardial necrosis and rupture between fibrin fibers. The effect of hyperthermia on the cardiovascular system in rat embryos has been studied. An increase in internal temperature in rats from 37˚C to 42˚C also causes tachycardia and increases mean blood flow and vascular resistance by 13%. In the state of heat stroke, large amounts of calcium are released from the sarcoplasmic reticulum of the heart muscle, causing a hypermetabolic state [14].

* Correspondence to: Emina Dervišević
E-mail: eemina.dervisevic@mf.unsa.ba

Studies on biopsy material show dilatation of glomerular capillaries, bleeding into the interstitium and vascular path, in small and large vessels [15]. Stimulation of the renin-angiotensin system in hyperthermia reduces renal blood flow. Direct thermal injury, renal hypoperfusion and rhabdomyolysis are also likely to contribute to acute renal injury (AKI). Hyperthermia is accompanied by liver dysfunction. At temperatures above 40 °C, an increase in plasma aspartate transaminase (AST) and alanine transaminase (ALT) was observed, and damage to hepatocellular tissue was sufficient to require transplantation in some cases; however, the results of transplantation are disappointing, with only a minority surviving in the long term [16].

The aim of the pilot study was to observe the morphological changes of different organs that were exposed to long-term effects of hyperthermia.

Methods

The research was conducted as a prospective, randomized, controlled, experimental study conducted on an animal model of inducing hyperthermia in rats. The study was conducted at the Faculty of Medicine of the University of Sarajevo and at the Faculty of Veterinary Medicine of the University of Sarajevo, in accordance with valid ethical principles on biomedical research on animals.

In the pilot experiment, 7 adult albino Wistar rats, of both sexes, with a body weight of 250 to 300 g were used. The animals were kept under the same laboratory conditions and 7 days prior to the experiment for acclimatization and adaptation, they stayed in a vivarium with a 12-hour light regime day-night and at room temperature (20°C±2°C). During the duration of the experiment, the animals received commercial feed for laboratory animals and running water ad libitum. The keeping and care of animals, as well as the implementation of all experimental procedures, were carried out in compliance with the international guidelines for biomedical research on animals - CIOMS (The Council for International Organizations of Medical Sciences) and ICLAS (The International Council for Laboratory Animal Science) [17,18), approval Ethical Committee of the Faculty of Medicine of the University of Sarajevo (02-3-4-1253/20) as well as the consent of the Department of Pathological Physiology of the Faculty of Veterinary Medicine of the University of Sarajevo (No. 02-3/720/19).

A sample of seven rats was exposed to a water temperature of 41°C, which is defined in the literature as "heat stroke temperature". The rats were anesthetized with ketamine, by intramuscular injection into the thigh muscle (m. quadriceps) at a dose calculated according to the formula: 1.2 ml/1kg of body weight +/- 10%. Ketaminol 10, 100 mg/ml, manufactured by MSD Animal Health, was used.

Immediately before immersion in water, an esophageal probe for measuring the internal temperature (RET-4 Probe for mice and rats) was placed in the esophagus (5 cm) of an anesthetized rat, and the core temperature was continuously read on a thermometer (Physitemp Thermalert Model TH-8) and recorded before immersion, immediately after immersion, in the 20th minute, and at the moment of death. The thermometer and probe for temperature measurement is manufactured by Physitemp Instruments Clifton, USA.

Induction of hyperthermia in a rat model

Experimental protocol was performed for each anaesthetised rat, sequentially. Each of them was immersed into water with their heads above the surface. Rats of antemortem groups were sacrificed after the expiration of the designated time of 20 min. The survival time was recorded, which included the time from the immersion of the rats in water at a set temperature of 41°C until the moment when death was established. We defined hyperthermia as an increase of 0.5°C in internal temperature, and heat stroke as an increase in internal temperature above 40.5 °C.

Tissue sample and morphological analysis

Tissue samples, obtained by dissection of rats, were fixed in 10% buffered neutral formalin, at room temperature, then incorporated into paraffin blocks, cut at 4-5 microns, mounted and stained with standard hematoxylin-eosin (HE) method. In order to prove/exclude lipid and glycogen accumulation in hepatocytes we did additional histochemical staining, using Sudan black and Periodic Acid Shiff (PAS) method, respectively. We obtained samples from kidney, liver, pancreas, spleen, lung and brain.

Tissue samples were evaluated by two independent pathologist (NC, SK) using light-microscopy (BX40, OLYMPUS, Japan). The most significant morphological changes in the examined tissue samples were photographed and presented in the results section.

Results

Tissue samples of brain showed constant signs of oedema and congestion (**Figure 1.)** in all seven rats, while brain tissue in two rats showed nonspecific reactive change - proliferation of glial cells (gliosis) (**Figure 2**.).

All samples of pancreas showed signs of congestion **(Figure 3.),** while spleen, besides congestion, showed reduction of white pulp **(Figure 4.).**

Morphologic changes of the kidney included congestion, hydropic and vacuolar degeneration (present at least in some minimal degree in all samples) and cystic dilation of proximal tubules filled with homogenic eosinophilic material. In one kidney sample we found phenomenon of "thyroidization" of tubules, with mild chronic interstitial lymphocytic infiltrate, pointing to signs of chronic pyelonephritis **(Figure 5).** Kidney sample of one rat also showed presence of bile pigment in tubular renal epithelium.

The most prominent changes were observed in liver and lung samples.

Tissue samples of the lung of all seven rats showed signs of oedema and inflammation - acute bronchitis and bron-

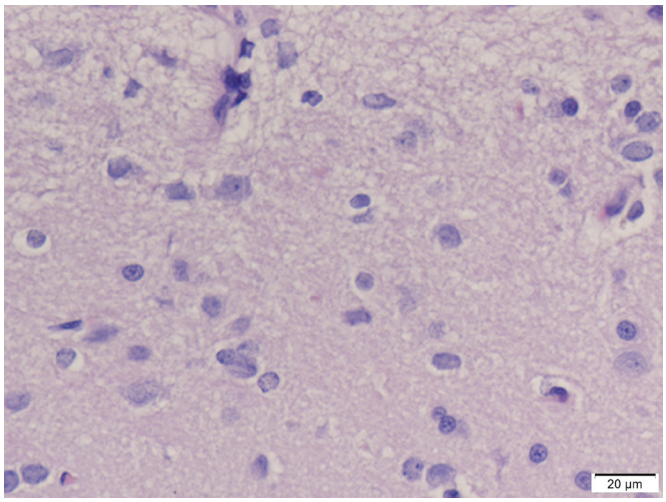

Fig. 1. Signs of severe brain oedema with expanded pericellular and virtual Robin- Virchow perivascular spaces (HE, x400).

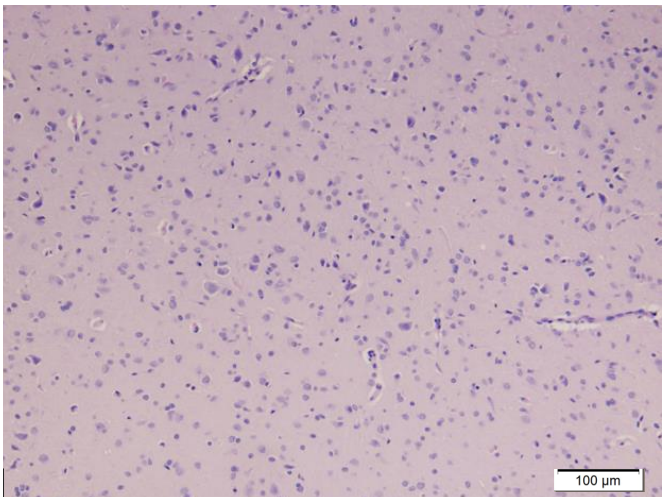

Fig. 2. Gliosis, HE x100

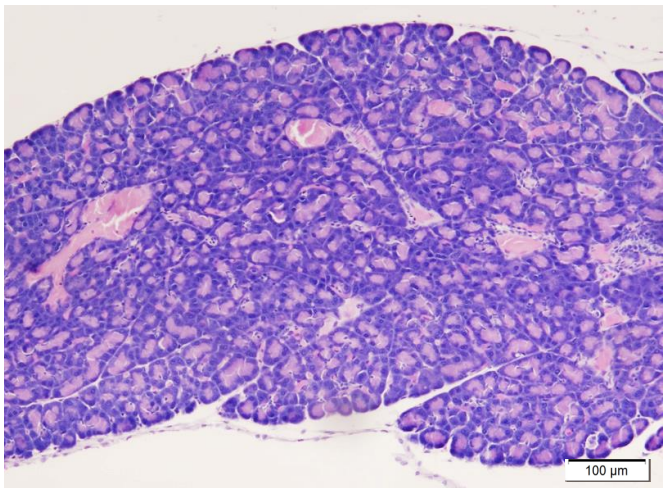

Fig. 3. Signs of vascular congestion in pancreas (HE, x100)

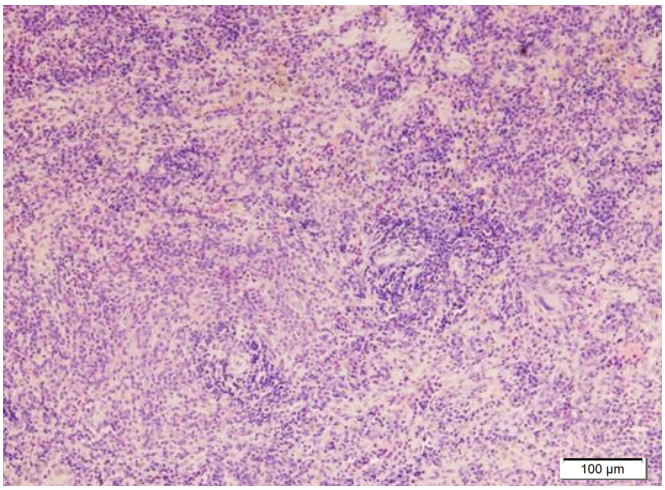

Fig. 4. Reduction of white pulp in spleen (HE, x100)

chiolitis, together with signs of initial bronchopneumonia with consolidation of lung tissue **(Figure 6.),** together with the focal accumulation of foamy macrophages and signs of focal acute emphysema (**Figure 7.).**

Changes in the liver ranged from mild congestion and vacuolar cell change, over zonal necrosis which affected in some samples zone 3 (centrilobular) of the liver lobule, to extensive confluent necrosis affecting centrilobular zones of multiple lobules, or almost whole lobule (**Figure 8).** These areas of confluent necrosis were accompanied by massive haemorrhage. Sudan black and PAS staining were negative (no fatty change or glycogen accumulation in the hepatocytes). In liver samples of two rats, we found signs of subacute nonspecific hepatitis.

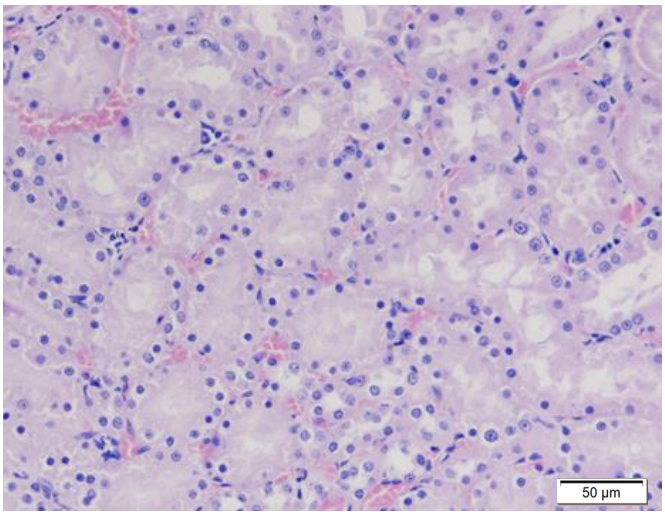

Fig. 5. Hydropic and vacoular degeneration and congestion in the kidney (HE, x200)

Discussion

Multiple organ dysfunction syndrome (MODS) is very often a complication of heat stroke, but also the leading cause of death. Hyperthermia triggers a coordinated stress response which provides protection against tissue injury and promotes cell repair. Hubbard et al. [7] induced rat hyperthermia by heating the cage at high temperature, measuring rectal temperature [19]. Weshler et al. [20] investigated the development of thermotolerance in the development of hyperthermia in rats in the aquatic environment.

Analysing tissue samples of different organs obtained from seven Wistar rats, we gained insight into morphologi-

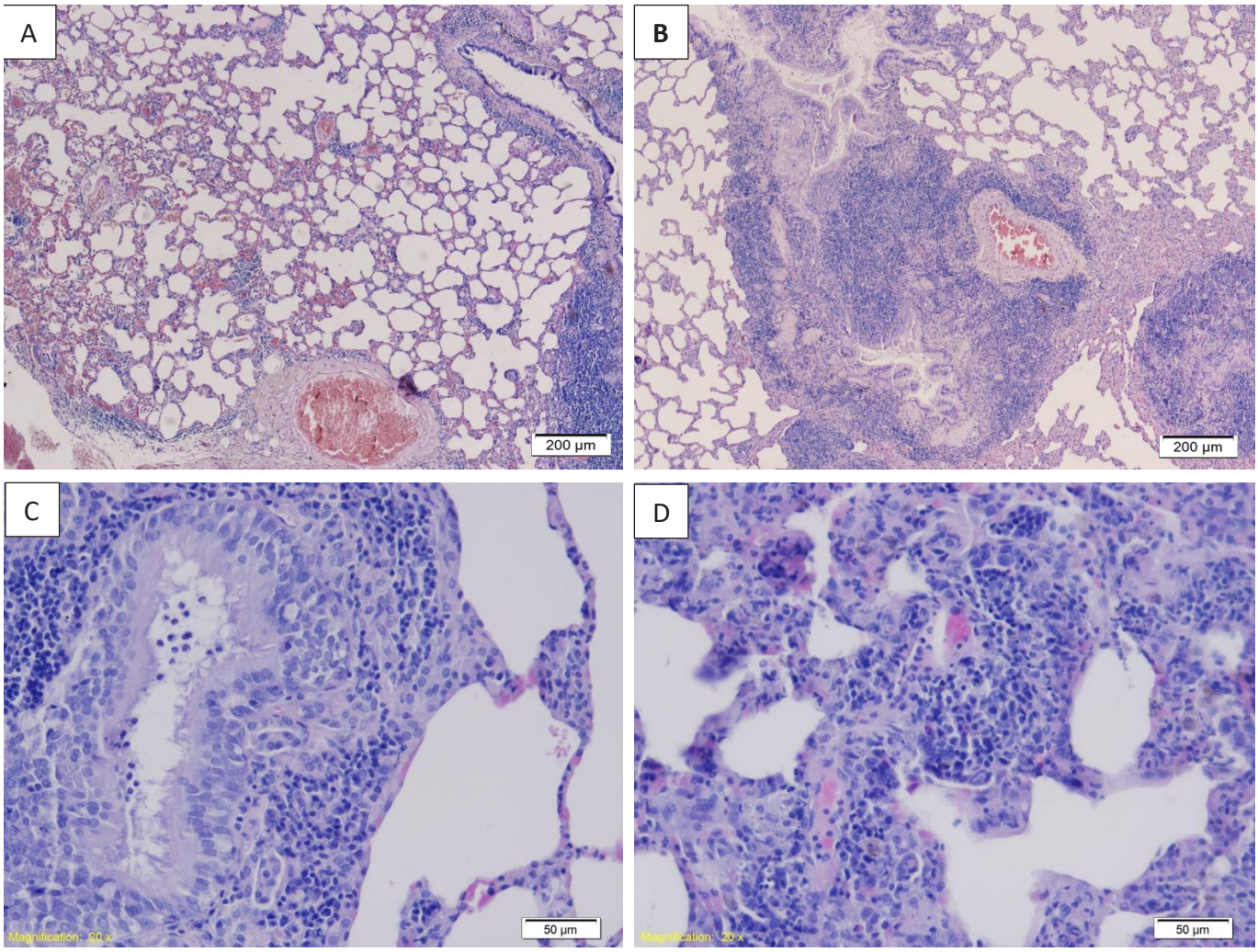

Fig. 6. SIgns of congestion, inflammation and consolidation of lung parenchyma (A-D) with inflammatory cells in the wall and in the lumen of the bronchiole (C) (HE, A and B x40, C and D x200).

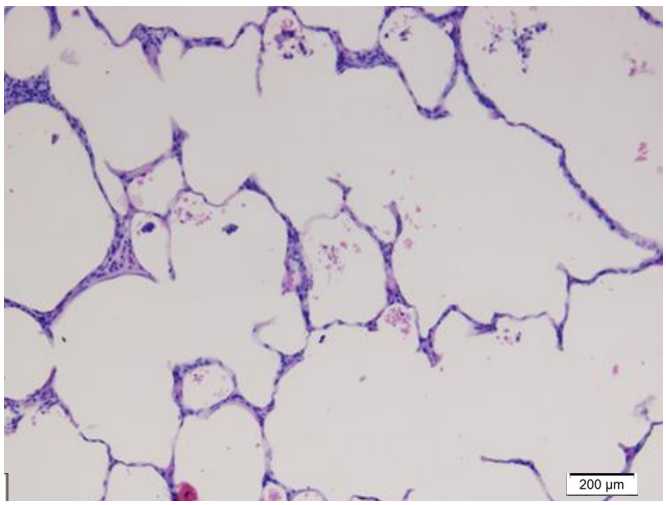

Fig. 7. Signs of lung emphysema (HE, x40)

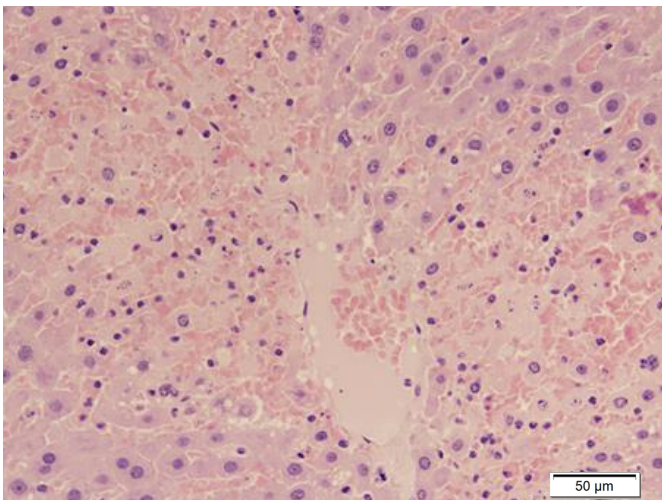

Fig. 8. Centrilobular necrosis (HE, x200)

cal changes caused by induced hyperthermia. All sampled organs showed congestion and at least some degree of oedema. Among seven examined rats, morphologic changes on same organ were of varying degree of severity with the most prominent changes observed on liver and lung.

Krishna described different patterns of liver necrosis pointing that patterns of necrosis together with inflammation, may reveal different underlaying causes of the disease, although these patterns are not entirely specific [21]. In a study of Nadesan and colleagues on human patients affected by heatstroke, liver changes were related to multiple organ dysfunction and coagulation disorders, with liver, kidneys and lungs being most commonly congested [22].

The most common pathologic change on hepatocytes injured by heat stroke is massive degeneration and necrosis, but the pathogenesis of hepatocyte injury in hyperthermia hasn't been fully elucidated [23]. We also confirmed the presence of vacuolar change and necrosis as part of liver injury caused by hyperthermia. Necrosis ranged from focal and zonal necrosis, affecting in some samples zone centrilobular or peripheral areas of the liver lobule, to massive confluent necrosis affecting centrilobular zones of multiple lobules. These areas of confluent necrosis were accompanied by massive haemorrhage.

The heatstroke-induced reduction in intestinal blood flow causes gastrointestinal ischemia, adversely affecting cell viability and cell-wall permeability. The resulting oxidative and nitrosative stress damages cell membranes and opens tight cell-to-cell junctions, allowing endotoxins and possibly pathogens to leak into the systemic circulation, overwhelming the detoxifying capacity of the liver and resulting in endotoxemia [24,25].

From a forensic point of view, postmortem morphological changes in the pancreas are more often caused by trauma and various diseases, such as diabetes mellitus, acute and chronic pancreatitis, as well as by malignant tumours [26]. In previous studies, the effects of hyperthermia on experimentally induced acute necrotizing pancreatitis were studied, in which it was mainly found that hyperthermia has a protective effect through increased expression of heat shock proteins, which results in a reduction of pancreatic injuries and leads to a better outcome [27]. In our study, all samples of pancreas showed signs of congestion.

In studies examining the effects of hyperthermia on numerous organs of rats, morphologic changes of the spleen included hyperemia with impaired and reduced white pulp [28]. Our results are in concordance with these results, since spleen, besides congestion, showed reduction of white pulp.

On gross examination, lungs of rats died by hyperthermia were heavy, congested and showed focal subpleural haemorrhages. On histologic examination, tissue samples of the lung of all seven rats showed signs of inflammation - acute bronchitis and bronchiolitis, together with signs of initial bronchopneumonia. With the increase in temperature, the congestion became more pronounced, with the presence of bleeding in the alveolar spaces, desquamation of epithelial cells and damage to the structure of the lung alveoli. We also noticed signs of focal acute emphysema as well as focal accumulations of foamy macrophages. Pulmonary oedema with pulmonary congestion is a frequent complication that develops in patients after heat stroke, especially if not adequately treated in terms of hydration. Involvement of the lungs is actually part of the systemic response [29].

Hyperthermia leads to damage to neurons, both on a structural and functional level, where the mitochondria and plasma membrane are most sensitive to the effects of high temperatures, with irreversible cellular changes at temperatures above 40°C [30]. Tissue samples of brain showed constant signs of oedema and congestion in all seven rats, while brain tissue in two rats showed nonspecific reactive change - proliferation of glial cells (gliosis).

In studies examining morphological changes in various organs of neonate rats exposed to hyperthermia, histological examination of kidney revealed signs of hyperplastic glomeruli, hydropic and vacuolar changes of the tubular epithelium with obliteration of the lumen, and periglomerular deposition of proteins with oedema and infiltration of inflammatory cells [28]. The most prominent changes in our study were also hydropic and vacuolar change, together with congestion.

In conclusion, our results confirm findings from previous studies that hyperthermia induces multiple organ dysfunction leading to death, causing changes in many organs that vary from oedema and congestion, to inflammation and necrosis.

Authors contributions

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; ED (Conceptualization, Data curation, Methodology, Project administration, Resources), NČ (Software, Supervision, Validation, Visualization, Writing – original draft), SK-V(Software, Supervision, Validation, Visualization, Writing – review & editing), MD (Validation, Visualization, Writing – original draft, Writing – review & editing)

Conflict of interest

None to declare.

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