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ORIGINAL ARTICLE

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Pulmonary fat embolism induced by exposure to high ambient temperature in rats with a fatty liver

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Abstract The aim of this study was to investigate whether a fatty liver contributes to pulmonary embolism under a high ambient temperature. As an experimental model, we exposed fatty liver rats to a high temperature (45°C) and then looked for fat emboli in the alveolar capillaries using the fat-staining method. Fat emboli were detected in the alveolar capillaries of the fatty liver rats, but not in those of the normal liver rats. Moreover, the degree of pulmonary fat embolism tended to become more severe in proportion to the severity of the fatty liver. In addition, fat emboli did not appear at a core body temperature of 40°C, but were detected at a core body temperature of 44°C. From these results, we conclude that a fatty liver may contribute to the formation of pulmonary fat embolism and that high temperatures act as a trigger for the onset of pulmonary embolism. Moreover, it is possible that fatty liver affects the development of heat stroke induced by exposure to a high ambient temperature and that pulmonary fat embolism is a significant finding which helps to enable a diagnosis of heat stroke in autopsy cases.

Keywords Pulmonary fat embolism · Fatty liver · Core body temperature · Heat stroke · Diagnosis

Introduction

We describe an autopsy case of a homeless male, estimated to be between 40 and 50 years of age, who had been found dead in a sauna room. It is generally said that taking a sauna is safe for most healthy people, yet sudden and unexpected death is not so rare in the sauna room [1–3]. In most of these cases it would seem that the cause of death is circulatory failure following alcohol intake [1, 2]. In the current case, the core body temperature of the deceased was 42.5°C at the start of the autopsy. There was no evidence of blunt force trauma or conspicuous disease such as coronary stenosis, and no alcohol or drugs were detected in the blood. On microscopic examination, however, moderate fat embolism was detected in the lung tissue (Fig. 1a), which was considered to be grade 2 (emboli easily seen) according to the criteria for the histological severity of pulmonary fat embolism [4], and moderate fatty liver was detected in the liver tissue (Fig. 1b).

It is generally accepted not only that pulmonary fat embolism frequently occurs after the fracture of the long bone and trauma to adipose tissue but also that the occurrence of pulmonary fat embolism associates with inflammatory conditions such as acute pancreatitis, osteomyelitis and panniculitis, decomposition sickness and fatty liver [4–9]. It is also said that the degree of pulmonary fat embolism relates to the preexisting diseases [10]. In particular, there have been some reports in which sudden death cases of pulmonary fat embolism with an associated fatty liver seemed to occur due to hepatic necrosis induced by poisoning, acute hepatitis, pregnancy or alcohol intake [11–13]. However, there has never been a report of pulmonary fat embolism that developed by exposure to high temperature, although several mechanisms whereby a fatty liver can lead to pulmonary fat embolism have been reported [4–9].

In this study, to investigate whether fatty liver contributes to pulmonary fat embolism under a high temperature, fatty liver rats were exposed to a high temperature and the existence of fat emboli was then examined in the lung tissue.

Materials and methods

This experiment was reviewed by the Committee of the Ethics on Animal Experiments in the Faculty of Medicine, Kyushu University and carried out under the control of

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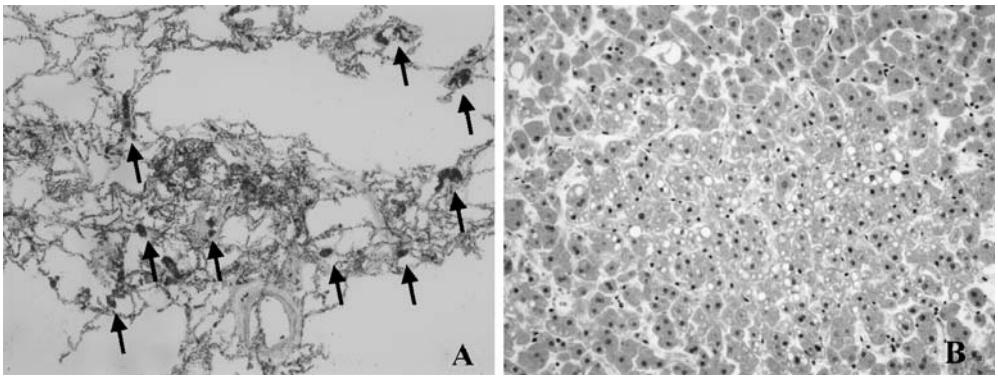

Fig. 1 Lung and liver tissue of an autopsy case of a homeless male found in a sauna room. **a** Fat emboli in alveolar capillaries (*black arrows*) (oil red O stain×40). **b** Moderate fatty liver (H.E. stain×200)

Guidelines for Animal Experiments in the Faculty of Medicine, Kyushu University and the Law (No. 105) and Notification (No. 6) of the Government of Japan. Moreover, this study followed the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

Preparation of fatty liver rats

In order to produce rats with a fatty liver, 3-week-old male Sprague–Dawley (SD) rats (Kyudo Co. Ltd, Kumamoto, Japan) were reared for 6 weeks, during which time they ate only special laboratory feed which contained a high level of lipid, a low level of choline and a low level of protein (Japan SLC, Inc., Shizuoka, Japan). However, water was available ad libitum until the rats were needed for the experiment. All the rats were housed in a temperature-controlled room on a 12:12 light–dark cycle (light cycles started at 9:00 A.M.).

Procedure

The experiment was performed to compare three groups: A, rats with a fatty liver as the experimental group (n=9); B, 9-week-old male SD rats as an age-matched control group (n=9); and C, 140–150 g male SD rats as a body weight (body weight)-matched control group (n=5). We made a wooden box (40×40×50 cm), and the temperature and humidity of the interior of the box were regulated by a water bath to a maximum of 45°C and above 90% humidity. To prevent hypoxia, the inside of the box was fully ventilated. The rats were anaesthetized with an intraperitoneal injection of 5% sodium pentobarbital solution at 50 mg/kg body weight, and after being attached to an electrocardiograph the rats were placed in the box that contained a thermometer. The electrocardiogram (ECG) and rectal temperature as the core body temperature continued to be measured by the operating monitor (BP-508; COLIN Medical technology Co. Ltd, Aichi, Japan) throughout the experiment. The duration from the start of the experiment until cardiac arrest was defined as the survival time. Immediately after cardiac arrest, the lungs and liver were removed, fixed in 4% formaldehyde, embedded in paraffin, and then 3.0-μm thick sections were cut and stained with hematoxylin-eosin (H&E) for light microscopy analysis. To investigate fat emboli in the alveolar capillaries, each lobe of the bilateral lungs fixed in 4% formaldehyde solution were stained using the Sudan IV method for fat staining. On the basis of the histological severity for human [4], the degree of pulmonary fat embolism was estimated as below: slight, emboli found sparsely; severe, emboli present in large numbers; and moderate, between slight and severe. In addition, the degree of fatty infiltration was classified as follows: slight, fatty infiltration was only seen in zone 3; moderate, fatty infiltration invaded zone 2; and severe, fatty infiltration reached into zone 1 [14].

Relationship between core body temperature and fat emboli

To investigate the relationship between core body temperature and fat emboli in lung tissue, fatty liver rats which had been anaesthetized with an intraperitoneal injection of 5% sodium pentobarbital solution at 50 mg/kg, were placed in the wooden box and exposed under the conditions described above until their core body temperature reached 40°C, after which they were immediately killed in a vacuum chamber to prevent to be injured (n=3). Moreover, to confirm that fat emboli were not present in the alveolar capillaries of the fatty liver rats before the experiment, other fatty liver rats were killed without any exposure to a high temperature in a vacuum chamber (n=3). They were then treated using the same methods.

Statistical analysis

The measurements are expressed as mean±SD. All analyses were performed using JMP® version 5, Japanese Edition (SAS Institute Inc., Cary, N.C., USA). The difference between multiple groups was determined with a one-way analysis of variance (ANOVA). The significance of individual differences was evaluated using Tukey–Kramer's procedure as a post hoc test. A P-value <0.05 was considered statistically significant.

Results

Relationship between survival time and core body temperature

The survival time of rats in the experimental, age-matched control and body weight-matched control groups was 46.2±1.5, 53.3±1.1 and 52.0±1.9 min, respectively. The survival time in the experimental group was significantly different from the two control groups; however, there was no difference between the age-matched control group and the body weight-matched control group.

Before the experiment, the core temperature of rats in the experimental, age-matched control and body weight-matched control groups was 36.06±0.32, 36.1±0.5 and 35.7±0.8°C, respectively, with no significant difference between the three groups. At cardiac arrest, the core temperature in the experimental, age-matched control and body weight-matched control groups was 44.07±0.23, 44.46±0.2 and 44.2±0.43°C, respectively, with no significant difference between the three groups.

Microscopic examination of the lung and liver

The livers in the experimental group showed a centrilobular fatty infiltration. Seven of the nine livers showed moderate or severe fatty infiltration in the experimental group (Table 1 and Fig. 2d and e), whereas there were no cases of fatty liver in either the age-matched or the body weight-matched control groups. The reduction in staining of nuclei of hepatocytes, eosinophilic change of cytoplasm and/or hemorrhage were diffusely seen in some liver tissues of the experimental and two control groups, which considered to be hepatocellular necrosis (Fig. 2f).

Fat emboli were detected in the alveolar capillaries of lung tissues in the experimental group, the degree of which tended to become more severe in proportion to the severity of the fatty liver (Table 1 and Fig. 2a–c). The size of pulmonary fat embolus was almost similar to that of fat deposits in hepatocytes. However, the degree of pulmonary fat embolism in the three rats with fatty liver that were killed at a core body temperature of 40°C was slight in all cases in spite of the severity of the fatty liver in all three rats. In addition, the livers of the three rats did not show any reduction in staining of nuclei of hepatocytes, eosinophilic change of cytoplasm and hemorrhage. On the other hand, pulmonary fat embolism was not detected in either of the control groups. In addition, pulmonary fat embolism was not seen in the rats sacrificed in the vacuum chamber without exposure to a high temperature.

Table 1 Degree of fatty liver and pulmonary fat embolism in the experimental group

Rat no.	Fatty liver	Pulmonary fat embolism
F1	+++	+++
F2	++	++
F3	+	−
F4	+	−
F5	+++	+
F6	++	+
F7	+++	++
F8	+++	++
F9	+++	+++

(+++), (++), (+) or (−) indicates the degree of fatty liver and pulmonary fat embolism
+++ Severe, ++ moderate, + slight, − not detected

Discussion

The results of the present study highlight the following two points: (1) pulmonary fat embolism was detected in rats with a fatty liver who were exposed to a high ambient temperature, and (2) the degree of pulmonary fat embolism tended to be exacerbated in proportion to the severity of the fatty deposits. These results suggest that a fatty liver may contribute to pulmonary fat embolism under a high temperature and that a high temperature may be one of the triggers for the onset of pulmonary fat embolism in individuals with a fatty liver. It has been said that elevation of the core body temperature leads to hepatocellular necrosis [15–17]. Consistent with those reports, hepatocellular necrosis did not appear in the liver of the three rats killed at 40°C, but was detected in the liver of some of the rats killed at over 44°C in this study. It has also been reported that elevation of the core body temperature induces injury to the vascular endothelium [15], which would enable the invasion of fat particles from hepatocytes into the vascular systems through the damaged endothelium. Therefore, one of the mechanisms behind the development of pulmonary fat embolism can be considered to be that hepatic cells with fatty droplets are induced to necrosis by elevation of the core body temperature, and that many fat particles leak into the hepatic vein and, thereby, embolize within the pulmonary capillaries.

The size of pulmonary fat embolus was almost the same as that of fat deposits in hepatocytes in this study, but some fat emboli in alveolar capillaries were obviously greater than fat deposits in hepatocytes in our autopsy case described in the Introduction. These findings may suggest that the mechanism of pulmonary fat embolism is not simple [7–9]. A high core body temperature induces central nervous system abnormalities such as delirium, convulsions or coma, often resulting in death, which is commonly referred to as heat stroke, and there is fairly wide agreement that heat stroke evokes systemic inflammatory responses [15]. According to Hulman [9], a C-reactive protein that gradually increases during acute inflammation agglutinates chylomicrons in the presence of calcium. Thus, it can be considered that the inflammatory response induced by heat stroke contributes to the formation of fat emboli from plasma in addition to the inflow of fatty droplets. Namely, it is possible that the severe hyperlipidemia which is present with a fatty liver gives rise to the danger of pulmonary fat embolism at a time of heat stroke. Moreover, there has been a report that elevation of the core body temperature injured the vascular endothelium. Thus, it is possible that systemic adipose tissue, including subcutaneous fat tissue, flows into the vein and then pulmonary fat embolism occurs

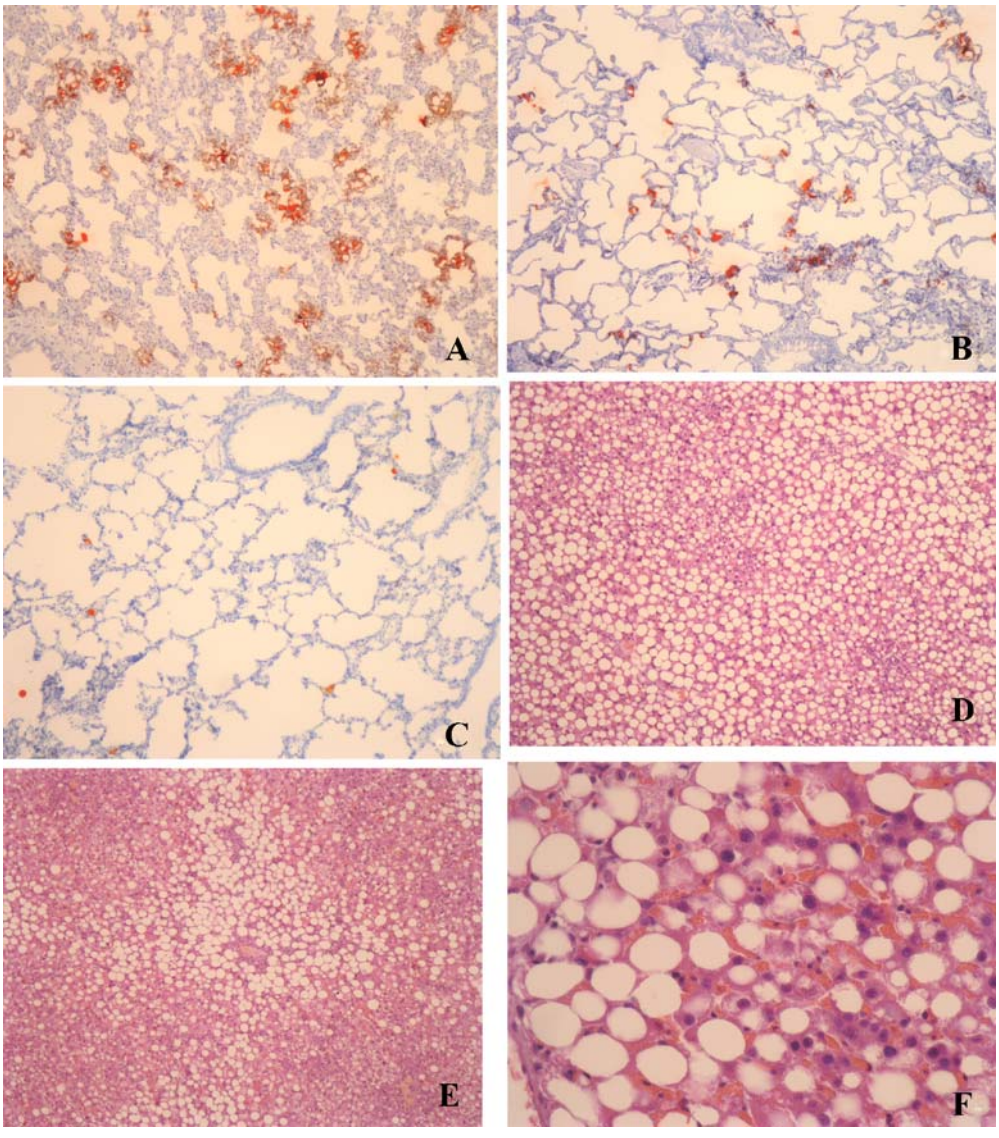

Fig. 2 Lung tissues using Sudan IV staining in the experimental group (**a**–**c**). Liver tissues in the experimental group (**d**–**f**). **a** Severe pulmonary fat embolism (F1, Sudan IV stain×100). **b** Moderate pulmonary fat embolism (F2, Sudan IV stain×100). **c** Slight pulmonary fat embolism (F5, Sudan IV stain×100). **d** Severe fatty liver (F1, H.E. stain×100). **e** Moderate fatty liver (F2, H.E. stain×100). **f** Reduction in staining of nuclei of hepatocytes, eosinophilic change of cytoplasm and hemorrhage are seen in the liver tissue (F1, H.E. stain×400)

under the condition of high core body temperature. To make a diagnosis of heat stroke is difficult in forensic medicine, due to the absence of any significant findings other than an elevation of core body temperature [17, 18]. However, the detection of pulmonary fat embolism may be a significant finding that helps to enable a diagnosis of heat stroke in autopsy cases.

In this study, the survival time of fatty liver rats was significantly shorter than that of the rats in the two control groups. These results would seem to suggest that the survival time of heat stroke is affected by the existence and the degree of fatty liver, and/or the malnutrition due to producing fatty liver. Therefore, the cause of death of all rats in this study was not pulmonary fat embolism alone, but should be heat stroke in itself. Accordingly, it is considered that the autopsy case described in the Introduction demonstrates the same phenomenon as the results of our experiment and that the cause of death in that case was heat stroke.

We conclude that an excessive elevation of core body temperature leads to pulmonary fat embolism in rats with a severe fatty liver. Accordingly, we consider that the results of the present study serve as a very strong warning that pulmonary fat embolism is likely to occur under high ambient temperatures in people with fatty liver. Moreover, the detection of pulmonary fat embolism would seem to be a significant finding for a diagnosis of heat stroke in autopsy cases.

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