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Thermographic evaluation of experimental pleurisy induced by carrageenan and modified by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)

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Purpose: The use of a thermal imaging camera may improve the detection of changes during inflammation process propagation in animals and humans that could be caused by numerous factors like 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Methods*: Rats were randomised and divided into two groups, C group, in which experimental pleural inflammatory reaction was evoked and TCDD group, in which a single dose was applied 21 days before administration of 1% carrageenan solution. Infrared thermograms were taken with a microbolometer thermal imaging camera MobIR M8. The surface temperature distribution was measured in three randomly selected animals. *Results*: In the analysis of correlation we found negative results between both groups. In the C group, the pleurisy was developed and allowed to develop freely. It can be observed that both the average maximum temperature and the average minimum temperature were the highest after 48 hours after injection of the 1% carrageenan in solution. In TCDD group, lowered temperature in all days of experiments was noted. However, the increase of temperature after carrageenan injection was similar. The main changes observed in the lungs were oedema, hyperemia with clot formation and changes in lung structure. Several proliferative changes in the lungs were noted. Moreover, increased number of goblet cells as well and increased release of the surfactant was observed. The activation of fibroblasts and synthesis of collagen fibers was noted. *Conclusions*: The TCDD administration results in the reduction of superficial temperature, which is easily detectable by thermal imaging camera that can be effectively used in monitoring the course of inflammation.

Key words: *thermography, pleurisy, TCDD, rats*

1. Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a compound with strong toxic properties, present, among others, in the air coming from the so-called low emissions. Its influence on tissues is widely described but there is no information available in the literature on its effects on the respiratory system. The effect of TCDD and its derivatives, which are accumulated in adipose tissue and in the liver, is the induction of inflammation [7], [11]. It is estimated that about 8% of dioxins get into the body through the respiratory system but there is no information about their irritating effect on the lungs [17], [20].

The toxicity of many dioxin-like compounds, such as polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls, is largely mediated via aryl hydrocarbon receptor (AhR) activation [14]. AhR-mediated gene expression can be tissue-specific, however, the presence of AhR in the lungs remains unclear [1]. Several organs are capable of triggering a regenerative response after acute exposure to harmful agents. Specifically, lung and liver can replenish dead cells and restore tissue architecture and function from the activation of undifferentiated and differentiated precursors. Lung

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exposure to the toxic compound leads to a selective and massive destruction of epithelial cells despite the appearance of a small pool of resistant cells that significantly contribute to the regeneration of the damaged epithelium [18]. Some recent works have suggested that the AhR activation by TCDD interferes with tissue repair at early stages of development in this non-mammalian animal model and, therefore, it may modify the course of the inflammation process [14].

Thermography is a non-invasive, non-contact and quite precise method of measuring thermal radiation emitted by physical bodies in the range of temperatures encountered in natural conditions [9].

Thermography is widely used for example in the industry in order to control technological processes, in power engineering to test high-voltage lines, and in construction to determine the building insulation degree. The wide use of thermography complements its use in scientific research and broadly understood medical diagnostics [16], [21].

Infrared body emission is directly proportional to the intensity of tissue metabolism and associated with blood supply. Reductions in the emission of infrared waves from the body are associated with the thickness of fatty tissue acting as an insulator, the intensity of blood flow through this area and processes occurring in the deeper layers of tissues [2].

The precise measurement of the body temperature, which sometimes has different values in relation to different areas, is an important diagnostic factor and one of the earliest signs of a disease or the disruption of thermoregulatory processes [13].

The obtained thermograms are a functional diagnostic material enabling the detection of lesions in which the variability of blood circulation occurs and which are often impossible to be detected with the use of other methods at the initial stages of the disease [9].

The measurement results obtained are presented in graphical form; each temperature value corresponds to a specific color, as a result of which images are produced as 'temperature maps'.

Recently, infrared cameras have been constantly improved. Currently used models are characterized by much higher resolution, shortened reading time and higher sensitivity, compared to those used in the 20th century. By improving these parameters, a valuable tool has been obtained that can be used in a variety of scientific research. The use of a thermal imaging camera is very helpful in research on the development and course of the inflammatory process [13].

There are numerous methods to induce inflammation, including physical, chemical and biological factors. All of them have some advantages and disadvantages, but for the induction of pleurisy, 1% solution of carrageenan is often used [3], [4]. Therefore, the aim of the study was to assess, using a thermal imaging camera, the temperature of the chest area in rats with pleurisy experimentally induced by carrageenan after the administration of the TCDD solution, as an indicator of the development of the inflammation process.

2. Materials and methods

2.1. Animals used in the experiment

16 female rats from the Buffalo inbreeding strain (body mass 150–160 g, equally obese, age 10 weeks) were used in the study. The rats come from the rat breeding farm. All animals received human care in compliance with the Guide for the Care and Use of Laboratory Animals as published by the National Institutes of Health (NRC Publication in 2011). All experiments were performed in compliance with the guidelines for the experimentation on animals.

During the experiment, the animals were kept in polystyrene cages with access to the Murigran feed and water in standard physiological conditions. Rats were fasted 2 hours before the thermovision measurements.

The approval of the local commission for conducting the experiment was obtained (permit no. 83/2012).

2.2. Division of animals

The rats were randomized and divided into two groups, 8 animals in each:

- C group, in which the experimental pleural inflammatory reaction was evoked by intrapleural administration of 0.15 mL of 1% carrageenan solution (Sigma-Aldrich, USA) between 5 and 6 intercostal space – region of interest measurement (ROI).
- TCDD group, in which the TCDD solution, in a single dose of 5μg/kg of body mass *i.m.* 21 day, was applied before the administration of 0.15 mL of 1% carrageenan solution (Sigma-Aldrich, USA) between 5 and 6 intercostal space – ROI for lung thermograms.

In this study, infrared thermograms were taken with the use of a microbolometer thermal imaging camera MobIR M8 (Wuhan Guide Infrared, China). The technical specifications of the infrared camera were as

follows: focal plane array microbolometer detector (160 × 120 pixels and 25 μm); spectral range of 8–14 μm; thermal sensitivity of ≤100 mK at 30 °C; field of view and focus of 20.6 × 15.5° and 11 μm; temperature range of 220–250 °C; infrared sensor with high accuracy of ±2 °C or ±2% of reading and 0.01 °C thermal sensitivity; emissivity correction range of 0.01–1.00. The emissivity was set at 0.97 for all the analyzed ROIs. A compatible computer software Guide IR Analyzer (Wuhan Guide Infrared, China) was used for data analysis.

All measurements were taken by the same researcher, in the same experimental room, under the same conditions (controlled temperature range of 22–24 °C and relative humidity level <45%). Any potential interferences disturbing the measurement (heat emitters, ventilators, and air-conditioning system) were excluded. During the infrared detection, the thermal imaging camera was positioned perpendicularly up to 60 cm of the anterior area of the lungs (the assumed ROI) (Fig. 1). The mean, maximum and minimum values of the irradiated temperature on the external surface were determined [°C].

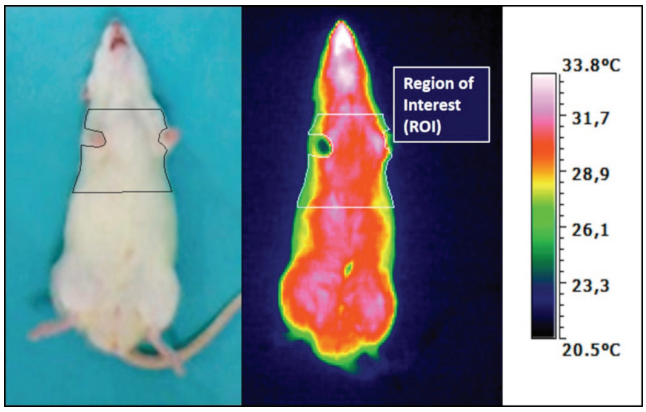

Fig. 1. Area where the temperature was measured in rats involved in the experiment – the so-called region of interest (ROI) for thermographic detection. Image from the thermal imaging camera showing the areas of different temperature values – thermograms with different color palettes (from warmer areas: white, red, yellow; to cooler areas: green, blue, black)

The surface temperature distribution was measured in three randomly selected animals just before the administration of carrageenan solution and after 2, 24, 48, 72 and 96 hours after the administration of carrageenan solution.

The area of the right intercostal space at the level of the third to sixth rib in the line of the elbow joint was chemically depilated with a depilatory cream (Veet, Reckitt Benckiser, Poland) 2 h before the temperature measurement. The animals were immobilized in accordance with the IACUC (Institutional Animal Care and Use Committee) Guidelines modified to visualize the measurement area to meet the requirements of the experiment. The injections were made using 0.5 mm needles. The injection depth was 2–3 mm.

For the histopathological evaluation, fragment of the lung with pleura from the area of injection inducing inflammation was taken.

The material was randomly collected after: 24 h – from 1 animal, 48 h – from 2 animals, 72 h – from 2 animals, 96 h – from 3 animals.

Lung sections were taken and fixed in 4% buffered formaldehyde at pH 7.2 and after rinsing in tap water – dehydrated in an alcohol series, embedded in paraffin and stained with hematoxylin and eosin. The material was analyzed using the Nikon Eclipse 80i microscope. For better evaluation of the material, the following morphological criteria were used for scoring: 0 – normal lung structure; 1 – minimal edema or infiltration of bronchiolar or alveolar walls; 2 – moderate edema and presence of inflammatory cell; 3 – intensive infiltration without obvious damage to lung architecture; 4 – severe inflammatory cell infiltration with obvious damage to lung architecture. Histological studies were performed in a blinded fashion.

3. Results

3.1. Thermography examination results

The results of the thermography examination are presented in graphs (Figs. 2 and 3). There is a significant difference in the results obtained in both groups. Another significant difference was observed in both the C and TCDD group between 2 and 24 h, and in the TCDD group between 48 and 72 and 96 h.

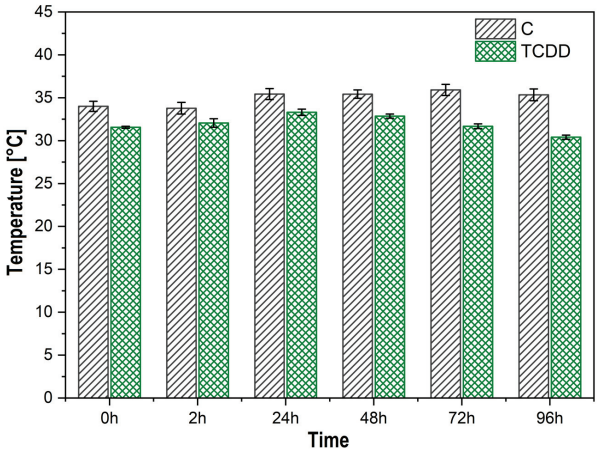

Fig. 2. Average temperature of the rat chest in the C and TCDD groups ($p < 0.05$)

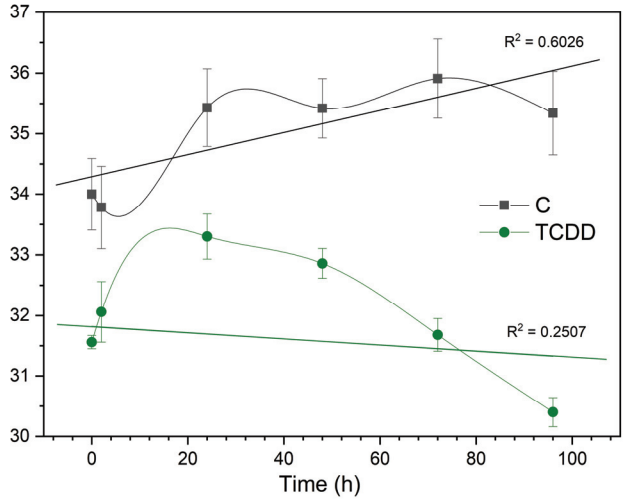

Fig. 3. Trend lines of temperature changes in the C and TCDD groups

In the correlation analysis, we found negative results in both groups. In the C group, the pleurisy was developed and allowed to develop freely. It can be observed that both the average maximum temperature and the average minimum temperature were the highest after 48 hours after the injection of 1% carrageenan solution. This may suggest that a time interval is needed to mobilize the immune system of animals subjected to the experiment. In addition, it can be observed that on the following day the mean temperature value in this group was slightly lower, close to the physiological temperature of the rat.

In case of TCDD intoxication, lower temperature was observed on all the days of the experiments. However, the increase in temperature after the injection of carrageenan was similar. After 48 hours, a subsequent decrease in temperature was observed in this group.

3.2. Histology results

The main changes observed in the lungs were edema, hyperemia with clot formation and changes in lung structure during first 48 hours in both groups. Several proliferative changes were detected in the lungs such as alveolar hyperplasia and alveolar-bronchial hyperplasia. Bronchiolar hyperplasia and alveolar hyperplasia

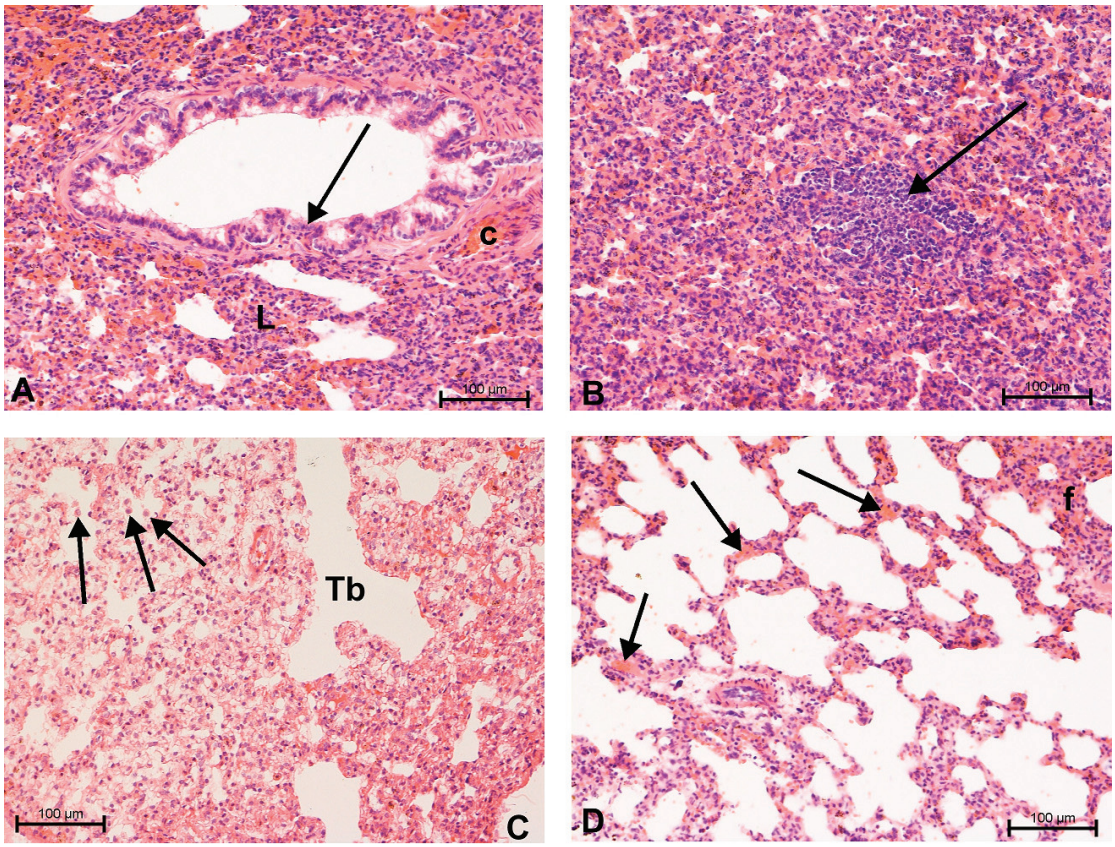

Fig. 4. Examples of pictures presenting animals from the C group (A, B) and from the TCDD group (C, D) 48 h after the administration of carrageenan, H&E mag 200×. A – hyperplasia of bronchiolar epithelium (arrow) and severe changes in the lung structure (l), including blood vessels containing a clot (c); B – lymphocyte infiltration in the lungs (arrow); C – numerous macrophages (arrow) in lung alveoluses in the vicinity of the terminal bronchiole (Tb); D – mild changes in the lung structure. Hyperemia (arrow) and some areas of an increased fibroblast activity (f) can also be observed

were found at the terminal bronchiolar alveolar duct junction (Fig. 4A). Bronchiolar hyperplasia is a focal proliferative lesion of epithelium confined to the bronchiole. Severe changes in lung architecture were observed 48 hours after the administration of carrageenan. Hyperplasia as the proliferation of mucociliary epithelial cells with extension into the alveolar spaces was observed. There was an intense infiltration of leukocytes into the lung (Fig. 4B). The increased activity of fibroblasts was observed 24 h after the administration of carrageenan in both groups. In case of the C group, their activity increased in the following days, whereas the amount of collagen fibers in the TCDD groups decreased due to their degradation by neutrophils. The main immune cells found in the lungs were macrophages and numerous lymphocytes (Fig. 4C). In the TCDD group, neutrophils were found instead of lymphocytes. Moreover, an increased number of goblet cells as well an increased release of the surfactant were observed. Severe changes in the lung structure were observed 72 hours after the administration of carrageenan. The activation of fibroblasts and the synthesis of collagen fibers were also observed. The hyperemia with clots in the vessels were found in all the groups up to 96 hours of the observation (Figs. 4A, 4D). Scoring has revealed more profound changes in the lungs in the C group than in the TCDD group (Fig. 5).

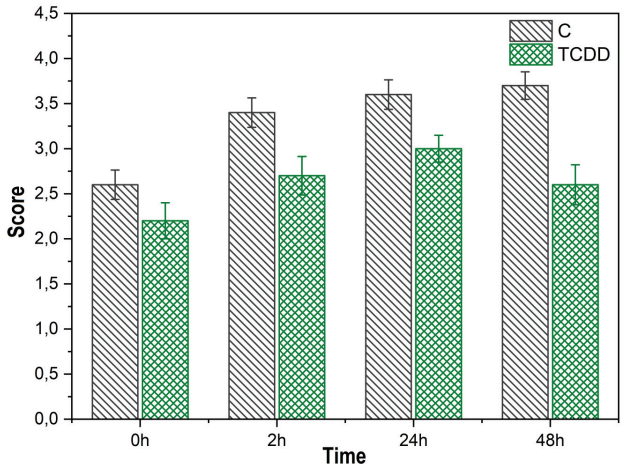

Fig. 5. Average scoring of histological picture of the lungs in the C and TCDD groups

4. Discussion

The pleura covers the lungs and chest wall and is a thin well-vascularized membrane, with numerous lymph vessels. The pleura is also subject to inflammatory processes that spread with blood, lymph or contact. The resulting exudation following the introduction into the pleura of one of the irritants can be inhibited by agents exhibiting anti-inflammatory properties. The dynamics of inflammation can be determined at various stages of blocking inflammatory mediators, evaluation of leukocyte migration and assessment of acute-phase protein concentrations in blood [4]. Therefore it is a very good model for course of inflammatory process examination.

The results obtained from histopathological examinations revealed the growing nature of pleurisy and focal lungs with associated exudate. In addition, the number of macrophages and lymphocytes accumulated in the inflammatory focus was proportional and their contribution depends on the duration of the inflammatory process. Further consequences of the inflammatory process included the accumulation of fibrin deposits and the occurrence of erythrocytes debris. The process observed may explain the decrease in the number of erythrocytes trapped in the fibrinogen network in the inflammatory focus found by the other authors. The decrease in plasma fibrinogen concentration and the decrease in the number of platelets were associated with the diagnosed occurrence of DIC (disseminated intravascular clot) as observed in the examined lungs [6]. The activation of fibroblasts resulted in the increased synthesis of collagen fibers.

The transport of TCDD from the muscles, when deposited by injection to the lungs, bypasses the liver, where in most animal species, dioxins are accumulated. In the liver, 25–75% of the administered dose of these compounds is deposited during one day. These compounds are excreted during the animal life. The elimination of TCDD may range from 75 days to several years in rats [8], [11], [15]. Moreover, TCDD may accumulate in adipose tissue, especially located in the subcutis and around the organs (visceral adipose tissue) [22]. The presence of the increased TCDD content in adipocytes may modify the metabolism of the local cells, such as endothelial cells and myocytes of vessels or skin fibroblasts. As mentioned above, the decrease in the skin temperature may be observed due to the modified blood circulation [21]. This could explain the occurrence of decreased temperature in the TCDD group, compared to the carrageenan group. It is worth stating that TCDD does not accumulate in the lungs directly and that it does not act on the lung structure in the direct manner. This is supported by the study carried out by Santostefano et al. who found that in the kidneys and lungs there is no CYP1A2 protein which is responsible for the sequestration of TCDD. This gene is one of the products of AhR-activation the presence of which in the lungs is under debate [1], [18], [19].

TCDD used in the experiment significantly affects liver metabolism, including the production of acute phase proteins [5], [10], as a result of which the body's response to an inflammatory factor may change significantly. By activating other inflammation signals, TCDD alters the immune system response process and the morphological picture of the lung [1]. Moreover, TCDD affects the surface temperature in the thermographic evaluation. This is probably because of decreased thyroxine level which results in hypothermia in adult rats [12]. Interestingly, after the administration of carrageenan in the TCDD group, the increase in the local skin temperature was the same as in the C group within the first 24 h. It shows that TCDD does not interfere with inductive signals but that it has an indirect influence on the inflammatory process propagation. The use of a thermal imaging camera indicates that the surface temperature measurement may be used to monitor tissue inflammation.

5. Conclusions

1. TCDD modulates the inflammation process and therefore it affects the final lung morphology.
2. The overall results of TCDD intoxication lead to the reduction in the surface temperature.
3. The decrease in temperature may be recorded using a thermal imaging camera.
4. Thermography as a non-invasive technique may be effectively used to monitor the course of inflammation.

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