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Usage of density analysis based on micro-CT for studying lung injury associated with burn–blast combined injury

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ABSTRACT

Background: Burn–blast combined injury is a kind of injury caused by heat and blast at the same time. The lung injury after burn–blast combined injuries is of primary importance, and investigation of lung injury is needed in the clinical care of patients. Computed tomography (CT) is one of the standard tools used to observe the anatomical basis and pathophysiology of acute lung injury.

Methods: We applied a method of fast 3D (three-dimensional) reconstruction to calculate the density value of the lung injury by CT analysis. Blast-injury group (BL group), burn-injury group (B group), burn–blast combined injury group (BBL group), and sham control group (C group) were established. Each group had 16 rats. The three-dimensional images of the lung tissue were obtained at 6 h, 24 h, and 48 h according to the CT value. The average density of the whole lung, left lung, and right lung were measured. The lung tissues were paraffin-embedded and HE stained. Smith scoring was performed according to the pathological findings.

Results: In the BBL group, the density of the lung tissue was higher than those of the BL group and B group ($P < 0.01$). The lung tissue density values at 24 h after injury were higher than those at 6 h and 48 h after injury ($P < 0.01$). Pathological results confirmed the changes of density analysis of the lung tissue.

Conclusion: The results have indicated that density analysis through a CT scan can be used as a way to evaluate lung injury in a burn–blast injury.

1. Introduction

Burn–blast combined injury is a kind of injury caused by heat and blast at the same time [1]. Burn–blast combined injuries have a high rate of mortality, in both daily life and war places [2–5]. The lung tissue receives a direct mechanical injury from the shock wave, causing vascular permeability and infiltration [6]. After burn–blast combined injury, large amounts of protease and inflammatory mediators play roles [6,7]. Lung injury after the combined injuries is of primary importance [8], and investigation of lung injury is needed in the clinical care of patients.

The combination of the two injuries have been discovered to cause more severe complications than simple burn or blast

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injury. This kind of injury has a more complicated pathological process [9]. Researchers have built an animal model for burn–blast combined injuries by setting up the explosive environment and scald injury in order to analyze the parameters of this kind of injury [6,9]. Pathological examination showed accumulation of interstitial fluid, hemorrhage of the partial alveolar septum and alveolar ruptures in the burn–blast combined injury group [2,10].

Computed tomography (CT) is one of the standard tools used to observe the anatomical basis and pathophysiology of lung injury [11]. Micro-CT is used to scan small animals. With CT or micro-CT, we are able to measure the density of each voxel after three-dimensional reconstruction, which provides us a way to observe the severity of the lung injury [12]. Compared with pathological observation, we are able to obverse the lung injury without having to resort to an autopsy using CT density analysis, which provides us a new way to continuously observe the state of an injury [13]. Furthermore, this analysis could provide us the whole picture of the lung injury instead of relying on incisional biopsy.

In this study, we applied a method of fast 3D (three-dimensional) reconstruction to calculate the density value of the lung injury. Through the analysis of the lung density of the combined injuries in rats, we tried to inquire into the changes in whole lungs, which provided experimental evidence for use in clinical treatment of lung injuries in this type of combined injury.

2. Materials and methods

2.1. Animal models and experimental design

Male Sprague-Dawley rats weighed between 220g and 290g from the Academy of Military Medical Sciences (Beijing, China) were elected. The animals were kept in animal containers with the environment of the temperature 25°C and the relative humidity of 60% for 7 days. All of the 64 rats were separated into different groups based on the randomization method by using a table of random digits: blast-injury group (BL group), burn-injury group (B group), burn–blast combined injury group (BBL group), and sham control group (C group). Each group contained 16 rats. 10 rats of each group were conducted with CT scan. Other 6 rats were autopsied for pathological observation. This experiment was authorized by the Animal Ethics Committee of the First Affiliated Hospital of PLA General Hospital.

2.2. Method of inducing the injury

All the rats were anaesthetized before the injuries. Moderate blast injuries were inflicted on the animals by the explosion source, which was compressed hexogen stick. In the experiments, the left chest was set to face the source of explosion. The pressure received by the left chest was set to be 377kPa (Pressure transducer model 113A21, PCB company). According to the measurement of the Blast Injury Severity National Standard in 2002, we set the degree of blast injury to moderate. Then, 25% of total body surface area burns with full-thickness was conducted on their shaved areas by using 94°C water. The time elapse was 12s. The experiment equipment was provided by the science and technology on transient impact laboratory of China North Industries Group Corporation.

In the burn–blast combined injury (BBL) group, 25% TBSA burns and blast injury caused by explosion were both conducted. In the blast-injury (BL) group, the animals were set at 50cm from the explosion source without 25% TBSA burn injury. In the burn injury (B) group, only 25% TBSA full-thickness burns were conducted. In the sham control (C) group, no injury was conducted.

2.3. Micro-CT scan and 3D reconstruction

The small animal micro-CT (Siemens Medical Solutions, Knoxville, TN) was conducted on all rats at 6h, 24h, and 48h. All of the data was set up at the end of the respiratory cycle and the CT images were transported into the Mimics software (Belgian, version 17.0) to reconstruct the lungs. This software can automatically generate 3D images by setting the CT threshold range and reconstruction area (Fig. 1A). After smoothing the 3D images, all structural data was defined to approach the actual lung tissue. The volume statistics, density analysis, and other follow-up work was conducted after the 3D construction.

2.4. Three-dimensional image density statistics of the lung tissue

The three-dimensional images of the lung tissue were obtained at 6h, 24h, and 48h for each rat according to the CT value. According to the air CT value of −1000Hu and the cortical bone of 1000Hu, the statistics of the lung tissue density was recorded from low to high with an interval of 10Hu (Fig. 1B). The number of pixels in the lung tissue was analyzed to be between −840Hu to −690Hu. The average density of the whole lung, left lung, and right lung of the four groups were measured. The number of pixels of every interval (N_m) was calculated to achieve the average number of CT value. C_{max} or C_{min} (Hu) was defined as the maximum or minimum number of the CT value measured. In this experiment, $C_{max}=-690$Hu, $C_{min}=-840$Hu, $M=(C_{max}-C_{min})/10=15$ and $C_m=C_{min}+10\times(m-1)$. N_m was defined as the number of pixels between C_{m+1} and C_m. The average density (C, Hu) was calculated as the following formula (Fig. 2).

$$C=\frac{\sum_{m=1}^{M}N_m\left(\frac{C_m+C_{m+1}}{2}\right)}{\sum_{m=1}^{M}N_m}$$

2.5. Pathological observation and statistical analysis

Tracheas were clamped immediately when the lung tissue was aerated. Midline sternotomy was performed on each rat. Then the left lung tissue was harvested with tracheas closed. The lung tissue was embedded in paraffin and HE stained obtained after