Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Comparison of the effects of gasoline burn and chromic acid burn on different internal organs and immune functions in rats

Pei Xu, Sida Xu*

Department of Burns, Ningbo No.2 Hospital, Ningbo, Zhejiang 315000, PR China

Article Info

Article history:

Received: November 27, 2023
Accepted: February 25, 2024
Published: April 30, 2024

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Abstract

Burn as physical injury ranks as the fourth most prevalent trauma across the world. In this study, we aimed to compare the impact of gasoline burn and chromic acid burn on the internal organs and immune functions in rats. The results showed that the levels of methemoglobin (MHb) to total hemoglobin (Hb) as well as the Cr6+ content showed significant elevation in the chromic acid burn group relative to the gasoline burn group. HE staining was used to evaluate the histological changes in the injured tissues as well as the tissues excised from internal organs. We found that chromic acid burn-induced more severe damage to rat tissues. Gasoline burn showed no significant impact on the intestinal tissues of rats, while the chromic acid burn-induced increased cell death in rat intestines. Moreover, the results of HE staining also revealed that gasoline burn and chromic acid burn showed no evident impact on rat hearts. Gasoline burn also showed no significant effects on the liver, lungs and kidneys of rats, while the chromic acid burn caused injuries to such internal organs in comparison with the control and gasoline burn groups. In addition, the MPO activity was higher in the liver, intestine, lungs and kidneys of rats with chromic acid burn. Furthermore, the expression of inflammation response cytokines was examined in the serum of rats. The results demonstrated that the levels of IL-6, IL-1β and TNF-α showed a significant increase in both the gasoline burn and chromic acid burn groups of rats relative to the control, and the levels were higher in the chromic acid burn group in comparison with the gasoline burn group. In conclusion, the chromic acid burn-induced more severe organ injury, inflammation and immune response compared with the gasoline burn, which may provide reference data for the clinical treatment of patients with different burn injuries.

Keywords: Burn injury, Chromic acid burn, Gasoline burn, Immune response, Organ function.

1. Introduction

Burn injuries are trauma associated with high morbidity and mortality. It is a global public health problem with over 300 000 injuries or death cases worldwide every year [1]. The injuries can be caused by heat, freezing, friction, electricity, chemicals or radiation, and are often categorized into superficial (first-degree), superficial partial- (second-degree, 2A burns), deep partial- (second-degree, 2B burns) and full-thickness (third-degree) injuries. According to the Lancet, burns sized larger than 60% of the body surface are considered to be related to risk and death [2]. The major/severe burn injuries are in company with an immune and inflammatory response, metabolic changes and distributive shock, leading to multiple organ failure [3]. Advances in skin grafting, fluid resuscitation, infection control and nutrition have contributed to the management of burn injuries [4, 5]. Despite the involvement of tissue destruction due to energy transfer in all burn injuries, different causes are related to diverse physiological and pathophysiological responses. Therefore, understanding the underlying mechanism of different burn injuries is of great significance for the development of effective therapeutic approaches.

Based on the tissue affected, the severity and consequent complications, burn injuries are highly variable. The burn injury severity mainly relies on the size, depth, and location of the injury, and is also influenced by the age and underlying systemic diseases [6]. Different from other types of skin wounds, burns comprise three zones of coagulation, stasis, and hyperemia [7]. The inflammatory reactions are induced in the process and stimulate the immune response, which may lead to multiple systemic effects and cause damage to the organs such as heart, liver, lung and kidney [6, 8, 9].

Chemical burns refer to tissue damage induced by strong acids, gasoline and many other substances [10]. Gasoline as a refined product of petroleum is a mixture of hydrocarbons divided mostly between pentane C_5H_{12} and octane C_8H_{18}, and may also contain olefins, diolefins, cycloparaffins and aromatic hydrocarbons in various concentrations. With a high combustion heat and a low ignition temperature, gasoline is a good fuel and dangerous household substance due to its high volatility [11]. Significant full-thickness burn injuries may be caused by gasoline contact and absorption of hydrocarbons via the skin may cause systemic complications [12–14]. Chromic acid is a

* Corresponding author.
E-mail address: nbeyxusida@126.com (S. Xu).
Doi: http://dx.doi.org/10.14715/cmb/2024.70.4.18

powerful caustic which acts rapidly with the production of considerable heat and the temperature may increase to 125 or 150 degrees [15]. It can cause coagulative necrosis on the skin because of the dehydration of the concentrated acid, and subsequent systemic toxicity such as hepatic or renal failure, gastrointestinal disease and central nervous system disorder [16, 17].

The present study aimed to compare the impact of gasoline burn with chromic acid burn on the internal organs and immune functions in rat models. The findings of this study might deepen the understanding of the underlying mechanisms of different burn injuries.

2. Materials and methods

2.1. Animal experiment

The animal study was under the approval of the Ethics Committee of Ningbo No.2 Hospital. Adult male Sprague–Dawley rats (Sprague–Dawley) were provided by the Vital River (Beijing, China). The animals were fed with standard food and water at a 12-h light/dark cycle at 23 ± 2°C. For the establishment of burn models, rats were randomized into the control (n=10), gasoline burn (n=30) and chromic acid burn (n=30) groups, and the two burn groups were further divided into the 5%, 10% and 20% burn subgroups with ten rats in each group. After anesthesia with pentobarbital sodium (40 mg/kg), rat dorsum was shaved and removed with sodium sulfide to expose 5%, 10% or 30% of the body surface area. before the experiment. Animals in the gasoline burn group were anaesthetized and then the 3% solidified gasoline (1 mL/20 cm^2) was smeared in the exposed area and ignited to burn for 30 seconds with the other area covered using a damp cloth. For rats in the chromic acid burn group, the exposed dorsum was immersed in chromic acid at 90°C for 15 seconds. For rats in the control group, the dorsum was immersed in water at 37°C for 15 seconds. The injured tissue and blood samples were collected from rats in each group. Blood samples were centrifuged at 1000 × g for 10 min. All animals were sacrificed at 48 hours following burn injury. The intestine, heart, liver, lung and kidneys were obtained and subject to histological analysis.

2.2. HE staining

Tissue samples from the intestine, heart, liver, lungs and kidneys were fastened with 10% formalin, embedded with paraffin and then cut into sections (5 μm). The sections were subject to hematoxylin-eosin (H&E) staining using a Hematoxylin and Eosin Staining Kit (Beyotime, Shanghai, China) in accordance with the manufacturer's protocol. Finally, the images were captured using a microscope.

2.3. TUNEL

Apoptosis in rat intestinal tissues was examined using a Terminal deoxynucleotidyl transferase dUTP Nick-End Labeling (TUNEL) kit (Solarbio, China) in accordance with the manufacturer's protocol. Briefly, rat intestinal tissue sections were processed with 1% Triton-X 100 on ice and then cultured with freshly prepared TUNEL detection buffer solution for 60 min at 37°C in the dark. Next, DAPI was applied to stain the nucleus of cells. Finally, the images were photographed using a microscope and the number of TUNEL-positive cells was counted.

2.4. Evaluation of MPO activity

MPO activity in rat intestine, heart, liver, lung and kidney tissues was evaluated using 10-acetyl-3,7-di-hydroxyphenoxazine (AAT Bioquest, Sunnyvale, CA) at an excitation wavelength of 535 nm and an emission wavelength of 590 nm.

2.5. Enzyme-linked immunosorbent assay (ELISA)

The levels of inflammatory cytokines (IL-6, IL-1β, TNF-α) in rat serum were detected using ELISA kits provided by Beyotime (#PI328, #PI303, #PT516) following the manufacturer's instructions.

2.6. RT-qPCR

Total RNA isolation was performed using TRIzol reagent (Thermo Fisher Scientific, USA). Then cDNAs were synthesized using a Reverse Transcription System Bestar qPCR RT Kit. PCR was conducted using an ABI 7500 Real-Time PCR System (Applied Biosystems, USA). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method with GAPDH as an internal reference.

2.7. Statistical analysis

Data analysis was conducted using GraphPad Prism 8.0 software (San Diego, CA, USA). Results are shown as the mean ± SD. Statistical difference was analyzed using one-way ANOVA. $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Chromic acid burns elevated MHb content and Cr^{6+} level than gasoline burns in rats

Methemoglobin (MHb) is an important indicator of burn injury with significantly higher concentration in severe burns compared with superficial burns [18, 19]. In the gasoline burn rat models, we found that the ratio of MHb to total hemoglobin (Hb) was not significantly changed relative to the control group. However, the chromic acid burn rats showed significantly elevated levels of MHb/Hb in comparison with both the control and gasoline burn groups and the MHb/Hb was increased as the chromic acid

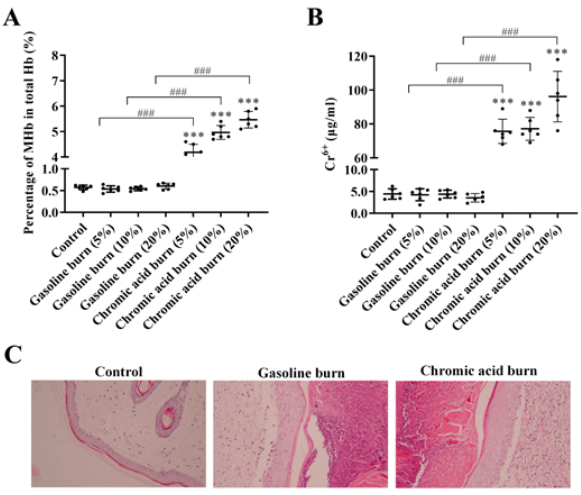

Fig. 1. Effects of gasoline burn and chromic acid burn on MHb content and Cr^{6+} level. (A) The percentage of MHb/Hb in the serum of rats in indicated groups. (B) The serum Cr^{6+} level in each group of rats. (C) The histological changes in rat burn skins were examined using HE staining. ***$P<0.001$ vs control group; ###$P<0.001$ vs gasoline burn groups.

burn area expanded (Figure 1A). Cr^{6+} level was also not significantly altered in the serum of rats with gasoline burn relative to the control and showed significant elevation in the chromic acid groups by over 14-fold in comparison with the control and gasoline burn groups. In addition, rats with the largest chromic acid burn injury (20%) exhibited the highest Cr^{6+} level compared with those with 5% or 10% in the area of burn injury (Figure 1B). Moreover, the results of haematoxylin-eosin (HE) staining of rat burn skin revealed that rats in the chromic acid burn group showed more severe tissue damage with loose muscle layer connection and inflammatory cell infiltration (Figure 1C).

3.2. Effects of gasoline burn and chromic acid burn on intestinal injury

Whether gasoline burn and chromic acid burn-induced internal organ damage was further investigated. As revealed by HE staining, the gasoline burn showed minimal impact on the rat intestinal histology, while the distal ileum of chromic acid exhibited evident blunting and necrosis of the intestinal villi (Figure 2A). Consistently, the results of TUNEL assays demonstrated that the apoptosis of tubular epithelial cells was not significantly changed in the gasoline burn group relative to the control, while the apoptotic rate showed evident increase in chromic acid burn group of rats in comparison with the control and gasoline burn groups (Figure 2B). Furthermore, the expression of apoptosis-associated markers such as Bax and Bcl2 in rat intestinal tissues was examined, and we found that their expression was not significantly altered in the gasoline burn group of rats. By contrast, the Bax mRNA expression was elevated while the Bcl2 mRNA levels were decreased in the intestinal tissues of chromic acid burn rats in comparison with the control and gasoline burn groups (Figure 2C).

3.3. Chromic acid burn-induced severe injury to the rat liver, lung, and kidney than gasoline burn

We then delved into the impact of chromic acid burn and gasoline burn on other organs of rats. As shown in Figure 3A, both chromic acid burn and gasoline burn showed no significant influence on the cardiac function of rats relative to the control. The results of HE staining of liver tissues indicated that the rat livers showed no evident

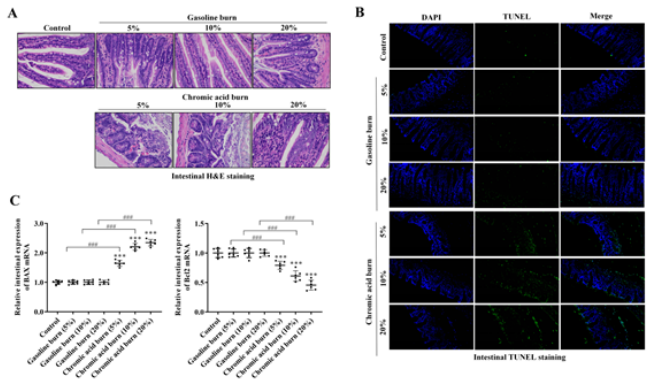

Fig. 2. Effects of gasoline burn and chromic acid burn on intestinal injury. (A) The histological changes in rat intestinal tissues were examined by HE staining. (B) Representative images of intestinal TUNEL staining in each group. (C) The mRNA expression of Bax and Bcl2 in rat intestinal tissues was subject to RT-qPCR analysis. ***P<0.001 vs control group; ###P<0.001 vs gasoline burn groups.

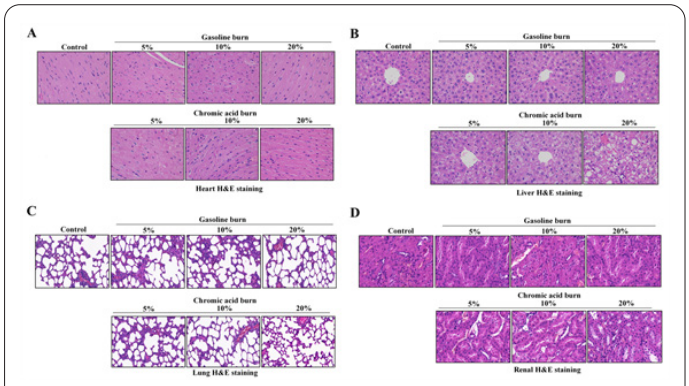

Fig. 3. Effects of gasoline burn and chromic acid burn on rat heart, liver, lung, and kidney tissues. HE staining images of (A) rat cardiac tissues, (B) liver samples, (C) lung samples and (D) renal samples.

histological changes in the gasoline burn and 5% chromic acid burn groups, while the hepatocytes and sinusoidal cells were disarranged, sinusoidal dilatation, pericentral atrophy and inflammatory infiltration was observed in rats with 10% and 20% chromic acid burn (Figure 3B). In addition, we found that gasoline burn and 5% and 10% chromic acid burn did not evidently induce histological changes in the rat lungs, while 20% chromic acid burn was demonstrated to cause rat lung injury, with infiltration of inflammatory cells and edematous alveolar walls (Figure 3C). HE staining of rat renal tissues indicated that gasoline burn showed no significant influence on rat renal histology while 5%, 10% and 20% chromic acid burn-induced cast formation in the tubular and significantly increased the necrotic glomeruli percentage as well as the vacuolation and desquamation of epithelial cells in rat renal tubules (Figure 3D).

3.4. MPO activity was elevated in the heart, liver, lung, kidney and intestine of chromic acid burn rats

Myeloperoxidase (MPO) is highly expressed in neutrophils and serves as a granulocyte-specific marker [20]. We found that the MPO activity was not significantly changed in the heart, liver, lung, and intestine of gasoline-burn rats relative to the control, while the MPO activity in the rat kidney tissues was significantly increased in the 20% gasoline burn group (Figures 4A-E). The chromic acid burn rats also showed no significant impact on the MPO activity in cardiac tissues (Figure 4A), but significantly elevated MPO activity in the rat liver tissues in the 10% and 20% chromic acid burn groups. In addition, chromic acid burn at 5%, 10% and 20% all increased MPO activity in kidney and intestinal tissues relative to the control and gasoline burn groups (Figures 4C, E). MPO activity in the lung tissues of chromic acid burn rats also showed a significant increase relative to the control and gasoline burn groups (Figure 4D).

3.5. Gasoline burn and chromic acid burn-induced inflammatory response in rats

We then explored the effects of Gasoline burn and chromic acid burn on the expression of inflammatory cytokines in rat serum. The results indicated that the concentration and mRNA expression of IL-6 showed significant elevation in the gasoline burn rats, as well as the chromic acid burn rats relative to the control, and IL-6 levels, were significantly higher in the chromic acid burn rats in

comparison with the gasoline burn group (Figures 5A, D). The IL-1β concentration showed a significant increase in the 10% and 20% gasoline burn group and the chromic acid burn groups, and its mRNA expression was significantly increased in all gasoline burn and chromic acid burn groups, with higher levels in the chromic acid burn groups relative to the gasoline burn groups (Figures 5B, E). TNF-α concentration was revealed to be elevated in the 10%, and 20% gasoline burn groups and the chromic acid burn groups relative to the control, and its mRNA levels were increased in all experiment groups relative to the control, with higher expression in the chromic acid burn groups in comparison with the gasoline group (Figures 5C, F).

4. Discussion

In this study, the effects of gasoline burn and chromic acid burn on the organ and immune function of rats were compared. The results indicated that rats with the chromic acid burn showed elevated MHb content and Cr6+ level, and induced histological changes in the intestine, liver, lung and kidney compared with the control and gasoline burn groups. Moreover, we found that the MPO activity and levels of inflammatory cytokines were higher in the chromic acid burn group compared with the gasoline burn group.

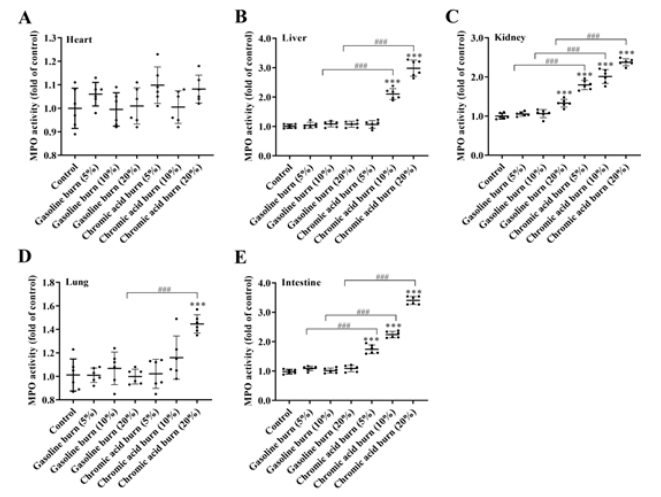

Fig. 4. Effects of gasoline burn and chromic acid burn on MPO activity in heart, liver, lung, kidney, and intestine tissues. MPO activity in the (A) heart, (B) liver, (C) kidney, (D) lung and (E) intestine tissues of rats in indicated groups. ***P<0.001 vs control group; ###P<0.001 vs gasoline burn groups.

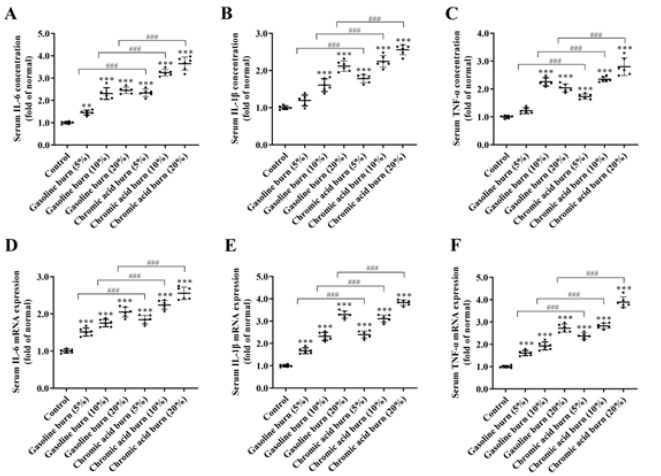

Fig. 5. Effects of gasoline burn and chromic acid burn on levels of serum proinflammatory cytokines. ELISA was conducted to examine the concentrations of (A) IL-6, (B) IL-1β and (C) TNF-α in the serum of rats in each group. RT-qPCR was performed to examine the mRNA expression of (D) IL-6, (E) IL-1β and (F) TNF-α in the rat serum. **P<0.01, ***P<0.001 vs control group; ###P<0.001 vs gasoline burn groups.

It has been reported that burn injury can induce alteration and injury in other organs due to the systemic response to the burn, including brain and gut atrophy, renal failure, live failure, cardiac dysfunction and pulmonary damage and some others [3, 21]. Methemoglobin (MHb) indicates the oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) within the hemoglobin (Hb) molecule, which reduces the ability of Hb to transport oxygen and carbon dioxide and results in tissue hypoxemia and possibly death [22]. Previous studies have revealed that the concentrations of MHb are directly correlated with the severity of burn after the injury [18, 19, 23]. The thermal insult can disrupt the vascular network, with blood trapped in the affected tissue. MHb is the byproduct of trapped Hb breakdown and can be used as a functional parameter of the burn tissue [23]. Solutions with chromium ions are industrially applied in the plating, painting and dyeing, and the hexavalent form of chromium (Cr^{6+}) is pretty hazardous and can cause membrane damage. The content of the chromium in the involved tissues depends on the severity of the burn [24]. In our study, we found that the level of MHb/Hb and the content of Cr^{6+} were not significantly altered in rats with gasoline burn (5%, 10%, 20%), while rats with chromic acid burn showed significant elevated MHb/Hb and Cr^{6+} content compared with gasoline burn. The HE staining of the burn tissues demonstrated that chromic acid burn caused more severe tissue damage than the gasoline burn in rats.

Burn injury can also induce a massive inflammatory response, with an increase in the circulating cytokines such as TNF-α, IL-6 and IL-1β [25–27]. The major burn-induced activation of a proinflammatory cascade can contribute to immune dysfunction, susceptibility to sepsis, and multiple organ dysfunction [21]. Immune cells such as neutrophils, monocytes and macrophages respond to the inflammation after thermal injuries by releasing considerable growth factors and signaling proteins for modulation of proliferation and differentiation in the wound healing process [6]. In our study, the gasoline burn showed no significant impact on the intestine, heart, liver, lung and kidney of rats, while the chromic acid burn-induced intestinal injury and cell death in rats in comparison with the control and gasoline burn groups. Moreover, chromic acid burn caused more severe liver, lung and renal injury in rats relative to the control and gasoline burn groups. In addition, the MPO activity of rats was increased in the liver, lung, kidney and intestinal tissues of chromic acid burn rats relative to the gasoline group. The levels of inflammatory cytokines were elevated in the serum of both the gasoline burn and chromic acid burn groups of rats, and the levels were higher in the chromic acid burn group relative to the gasoline burn group.

In conclusion, chromic acid burn caused more severe internal organ injury and inflammation and immune responses than gasoline burn in rats. The findings of our study might deepen the understanding of the different impacts

and underlying mechanisms of the two types of burn injury, which may provide clues for the specific management of patients with different burn injuries.

Informed consent
The authors report no conflict of interest.

Availability of data and material
We declared that we embedded all data in the manuscript.

Authors' contributions
XP conducted the experiments and wrote the paper; XP and XS analyzed and organized the data; XS conceived, designed the study and revised the manuscript.

Funding
The current study was supported by the Zhejiang Medical and Health Science and Technology Project (No. 2022498346).

Acknowledgements
We thanked Ningbo No.2 Hospital for approving our study.

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