

Original Article

Canine model of crush syndrome established by a digital crush injury device platform

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Abstract: Objective: To establish a canine model of crush syndrome (CS). Methods: A total of 16 healthy adult female Beagle dogs were randomly divided into the control group (n=8) and the experimental group (n=8). The crush injury was created in the left hind leg of each dog in the experimental group. Results: The biochemical indexes in the experimental group changed significantly compared to the values before extrusion. And they were also significantly different from the values of the control group. The glomerular capillary dilation, renal tubular epithelial cell degeneration, and renal interstitial lymphocytic infiltration were found in the kidneys. Conclusion: The canine CS model established by the digital crush injury device platform was successful according with the diagnosis of CS. It is good for the investigation of the CS mechanism and treatment using this model.

Keywords: Crush syndrome, crush injury device platform, dogs

Introduction

Crush syndrome (CS) also known as traumatic rhabdomyolysis syndrome is defined as traumatic compression of muscle tissue with resulting limb swelling, muscular necrosis, hyperkalemia, myoglobinuria and acute kidney injury (AKI). The main symptom of CS is rhabdomyolysis, leading to the muscle cell contents exuding into the extracellular fluid and blood circulation and causing hypovolemia, electrolyte imbalance and complications like AKI and multiple organ dysfunctions. In 1941, CS was first described in Bywaters's report of patients who were trapped beneath rubble after the air strike in London with symptoms of swollen limbs, circulatory disturbance, dark urine (caused by myohemoglobin in urine) and acute renal failure [1]. Besides the swollen limbs and muscular necrosis, severe secondary systemic pathological changes always appear in CS patients, like serious damages of remote organs including kidney, heart, lung and liver [2]. CS patients without an effective first aid may be multiple organ failure to death. CS may present at patients who were buried or extruded in explo-

sive terrorist attacks, natural disasters (earthquakes and landslides), traffic accidents and other severe disasters (mine accidents and house collapses). Since the incidence rate of CS after an earthquake is about 10% and the mortality rate is up to 40%, CS is the second leading cause of death for patients in earthquakes [3-6].

Some studies indicated that continuous renal replacement therapy (CRRT) is an effective treatment for CS patients [7, 8]. However doctors always use CRRT on the basis of their own experiences for the lack of laboratory experiments for the optimal timing, dose and duration of treatment. Currently animal models of CS are established using small animals like mice, rats or rabbits [9, 10]. But these animals are not suitable for the treatment of CRRT because of their thin cardiac vein and small blood volume. Dogs have rich muscles in their limbs, large blood volume, thick arteries and veins, so it is easy to build access of blood purification. In our study, a canine model of CS was established using the self-made digital crush injury device platform (patent No. 201220049194.0)

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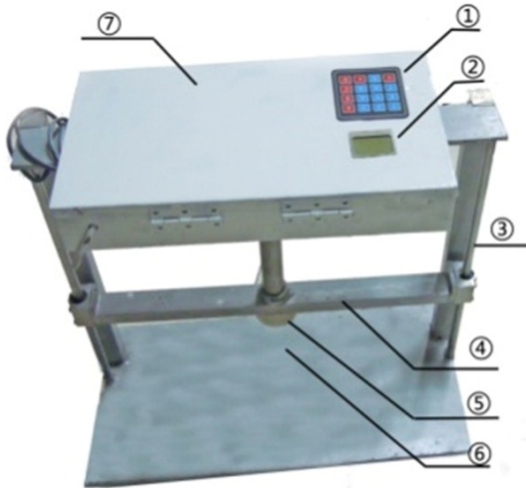


Figure 1. The digital crush injury device platform, picture of the real product. ① controlling keyboard; ② display screen; ③ guiding shaft; ④ lifting lever; ⑤ extrusion sliding block; ⑥ substrate (with pressure sensor); ⑦ control chamber.

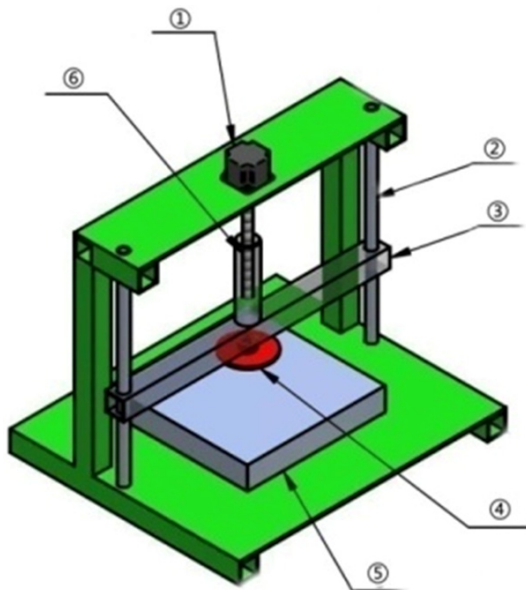


Figure 2. The digital crush injury device platform, diagrammatic drawing of the machinery. ① stepper motor; ② guiding shaft; ③ horizontal axis of the lifting lever; ④ extrusion sliding block; ⑤ pressure sensor; ⑥ vertical axis of the lifting lever.

(Figures 1, 2) for the further investigation of continuous blood purification to treat CS.

Materials and methods

Animals

A total of 16 healthy adult Beagle dogs between 10 and 12 kg body-weight were provided by the

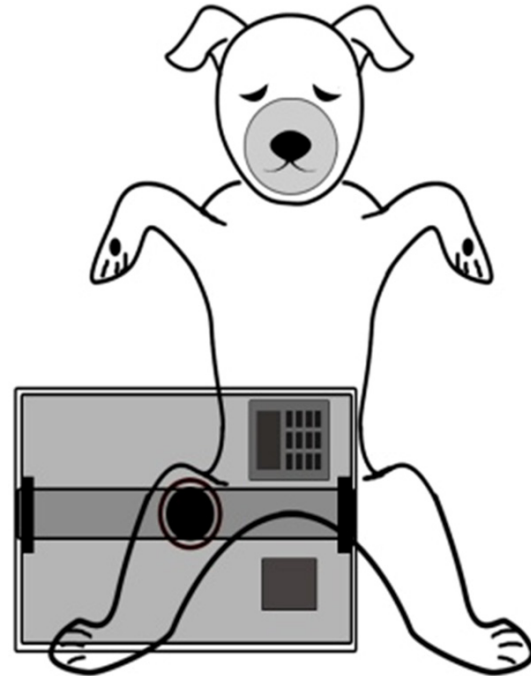


Figure 3. The diagrammatic drawing of the extrusion process.

Animal Center of Academy of Military Medical Sciences (experimental animal production license: SCXK (Army) 2012-0002). Room temperature was maintained at 20-28°C with relative humidity of 40-70%. Experimental procedures were performed in accordance with the Helsinki Convention and the Guide for the Care and Use of Laboratory Animals [11] and were approved by the Animal Ethics Committee of Affiliated Hospital of Logistics University of Chinese People's Armed Police Forces.

Crush injury

Dogs were randomly divided into the control group (n=8) and the experimental group (n=8). Fasting time before anesthesia was 12 hours. Dogs were narcotized using Sumianxin II injection (size: 1.5 ml/L, Veterinary Institute of Changchun Quartermaster University, China). The first intramuscular injection of 1.5 ml was followed by an additional 0.5-1 ml every two hours. Dogs were anesthetized until decompression. Dogs were placed on the crush injury device platform (Figure 3) in the supine position with retention catheterization and restraint straps to fix limbs and mouth. A venous indwelling needle in right femoral vein was used for blood collection and transfusion. Prepare the left hind leg by shaving and continuously extrude the point 1 cm below the left groin by the

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Table 1. Levels of biochemical indexes during the experiment in each group

	Before extrusion	4 h extrusion	8 h extrusion	1 h after decompression	4 h after decompression	8 h after decompression	12 h after decompression	24 h after decompression
CK (U/L)								
Control group	349.25±21.42	340.01±22.19	324.50±24.68	316.50±15.67	325.25±16.38	331.50±19.36	333.45±19.81	340.00±13.63
Experimental group	345.88±36.39	662.88±197.56 ^{*†}	2470.88±327.03 ^{*†}	17740.88±1393.92 ^{*†}	45456.13±3312.7 ^{*†}	58436.01±3744.87 ^{*†}	50319.75±3195.04 ^{*†}	28319.50±2529.29 ^{*†}
CK-MB (U/L)								
Control group	121.25±25.73	125.12±27.03	122.01±20.90	119.79±22.38	124.67±19.65	121.79±26.12	128.05±27.11	128.91±28.09
Experimental group	124.25±27.73	197.75±42.11 ^{*†}	215.01±39.02 ^{*†}	711.03±106.48 ^{*†}	2162.87±222.61 ^{*†}	3206.38±266.88 ^{*†}	2652.13±238.25 ^{*†}	828.01±137.39 ^{*†}
BUN (mmol/L)								
Control group	4.38±0.62	4.55±0.78	5.52±0.91	5.50±0.81	5.55±1.12	5.60±0.56	5.45±0.69	5.83±1.10
Experimental group	4.26±0.79	5.04±0.99	6.91±0.76	8.54±1.43 ^{*†}	12.01±1.86 ^{*†}	16.45±2.83 ^{*†}	19.05±1.92 ^{*†}	24.50±1.37 ^{*†}
Scr (μmol/L)								
Control group	72.75±12.09	75.75±13.96	72.95±12.18	79.75±14.07	85.25±15.12	82.07±16.27	85.75±16.30	86.50±15.19
Experimental group	78±17.68	77.5±13.52	92.88±19.32	103.38±22.36 ^{*†}	160±52.57 ^{*†}	196.5±61.16 ^{*†}	234.13±50.74 ^{*†}	404.63±53.51 ^{*†}
ALT (U/L)								
Control group	24.50±2.52	28.25±2.63	31.25±3.30	35.75±7.22	34.25±5.38	36.50±7.85	35.75±5.68	35.00±6.68
Experimental group	23.25±2.25	25.50±4.72	31.50±4.04	52.50±19.73 ^{*†}	131.00±27.17 ^{*†}	290.38±92.66 ^{*†}	358.50±51.60 ^{*†}	532.00±32.67 ^{*†}
AST (U/L)								
Control group	32.00±3.16	43.00±3.56	41.75±2.99	44.50±6.02	42.50±3.87	40.50±7.14	47.00±2.44	46.25±1.25
Experimental group	26.88±4.42	53.38±16.00	64.88±24.86	214.50±46.74 ^{*†}	670.25±246.77 ^{*†}	1292.38±238.86 ^{*†}	1629.13±180.11 ^{*†}	2091.50±237.65 ^{*†}
K⁺ (mmol/L)								
Control group	4.17±0.49	4.72±0.36	4.88±0.49	4.68±0.37	4.92±0.43	4.65±0.59	4.22±0.10	4.05±0.44
Experimental group	4.56±0.42	4.88±0.65	5.43±0.59	6.39±0.51 ^{*†}	6.51±0.46 ^{*†}	6.63±0.57 ^{*†}	7.15±0.53 ^{*†}	7.18±0.55 ^{*†}
CO₂CP (mmol/L)								
Control group	24.48±3.30	23.25±3.25	25.38±3.21	24.43±2.77	25.10±3.26	24.01±3.52	24.15±3.22	24.57±3.49
Experimental group	23.59±3.18	22.46±3.33	20.66±3.52 ^{*†}	18.11±3.15 ^{*†}	16.86±3.27 ^{*†}	15.91±3.32 ^{*†}	14.41±3.67 ^{*†}	11.21±3.32 ^{*†}

^{*}, P<0.01 vs. the time before extrusion; [†], P<0.01 vs. control group. CK, creatine kinase; CK-MB, creatine kinase-MB; Scr, serum creatinine; BUN, blood urea nitrogen; ALT, alanine transaminase; AST, aspartate aminotransferase; K⁺, serum potassium; CO₂CP, carbon dioxide combining power.

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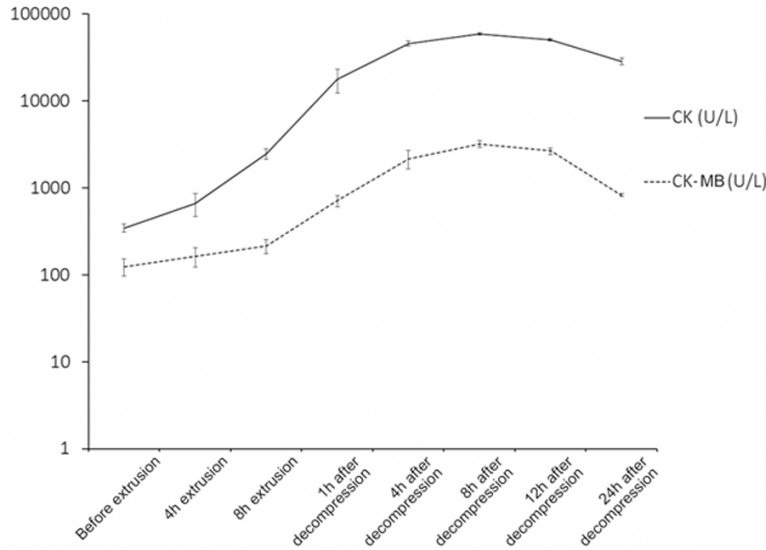


Figure 4. The levels of CK and CK-MB in the experiment.

self-made digital crush injury device platform with the extrusion area of 10 cm×10 cm and the oppressive weight of 50 kg (about 5 times the dog's weight). Decompress dogs following 8 h extrusion. Blood samples were collected before extrusion, at 4 h and 8 h extrusion and at 1 h, 4 h, 8 h, 12 h and 24 h after decompression. Following 24 h decompression, all the dogs were sacrificed by exsanguination under anesthesia. And the kidney, crush-injured muscle and myocardial tissue samples were harvested. Dogs in control group were fixed in the supine position but not extruded.

General measurement

The color and amount of urine, the hemoglobinuria and death were recorded. Physical activities and the perimeter of the crush-injured limbs were observed at 1 h, 4 h, 8 h, 12 h and 24 h after decompression.

Biochemical analysis

Creatine kinase (CK), creatine kinase-MB (CK-MB), serum creatinine (Scr), blood urea nitrogen (BUN), alanine transaminase (ALT) and aspartate aminotransferase (AST) were analyzed using the Beckman-Coulter LX-20 (Beckmann Coulter Inc. Fullerton, CA). Serum potassium (K^+) and carbon dioxide combining power (CO_2 CP) were analyzed using the i-STAT 1 Wireless (Abbott Point of Care Inc., Illinois, USA).

Histological examinations

Tissue samples were fixed in 4% paraformaldehyde, dehydrated in graded ethanol, deparaffinized in xylene, routine paraffin-embedded, sectioned into 5 μ m thick, HE stained and examined for pathological changes.

Diagnostic criteria

According to the human diagnostic criteria of CS [12-14], dogs with muscles rich areas extruded for a long time, continue oliguria or anuria, hemoglobinuria, serum CK 5 times higher than baseline, AKI and one or more complications

like hyperkalemia, metabolic acidosis and etc. were diagnosed with CS.

Statistics analysis

SPSS 16.0 was used for statistical analysis. All data were expressed as mean \pm standard deviation (SD) ($\bar{x} \pm s$). The statistical differences among different groups were assessed by repeated measures ANOVA. Multiple comparisons within groups were performed using LSD test. $P < 0.05$ indicated a significant difference.

Results

General conditions of dogs

Dogs in control group were in good condition with normal breathing and heart rate. Experimental dogs were listless with rapid shallow breathing, increased heart rate and clammy extremities. Dogs in control group had normal physical activities and sensory function with unchanged skin and perimeter of limbs. While experimental dogs were hypoesthesia, and some of them had subcutaneous bleeding, tension blisters or even necrosis. The crush-injured limbs of experimental dogs couldn't act normally and were swelling. The peak of crush-injured limb perimeter appeared at 8 h after decompression and was significantly greater than time before decompression ($P < 0.05$, data are not shown). 3 experimental dogs died in 24

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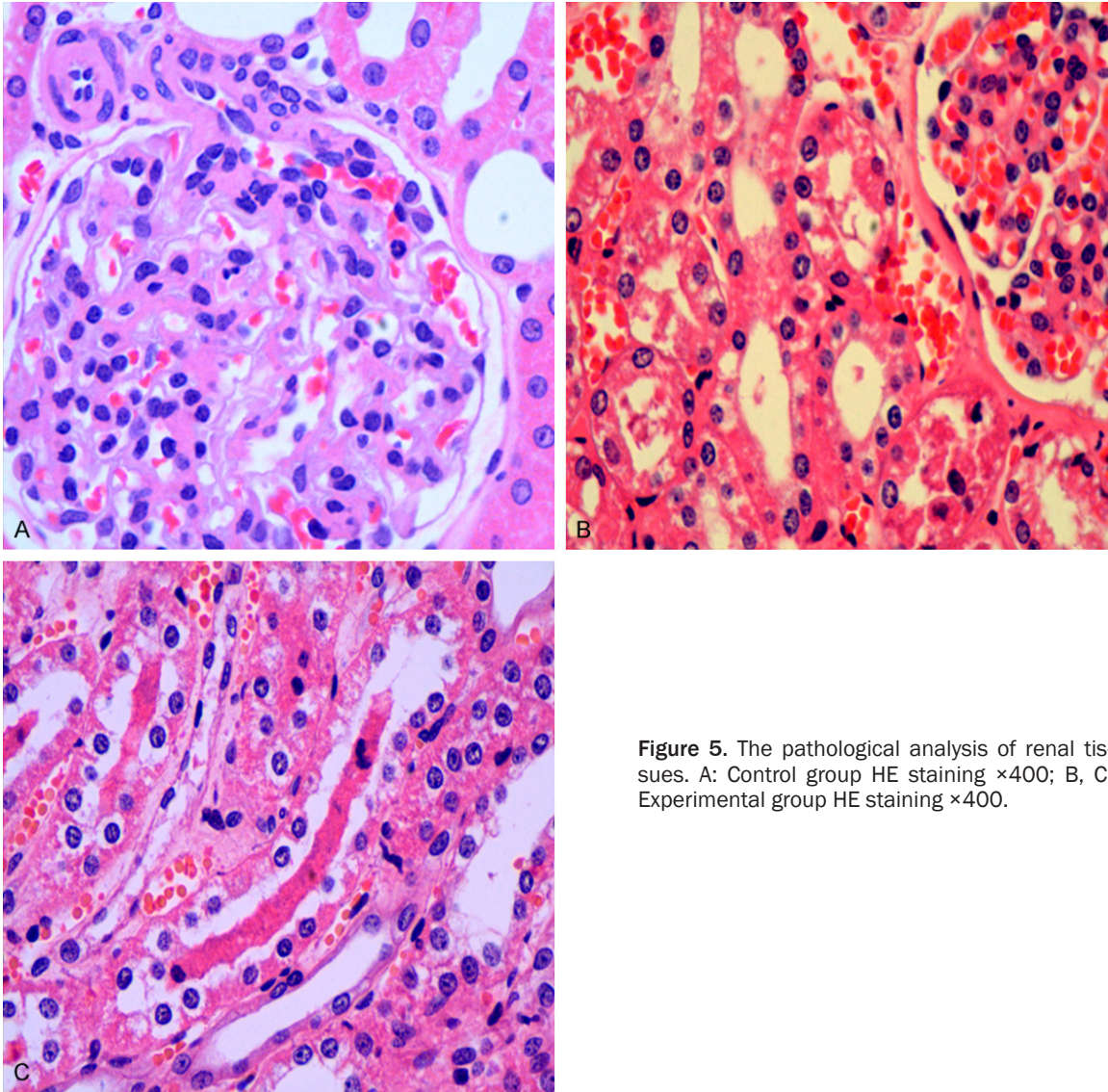


Figure 5. The pathological analysis of renal tissues. A: Control group HE staining $\times 400$; B, C: Experimental group HE staining $\times 400$.

hours after decompression however there was no death in the control group.

Myoglobinuria and hypourcemia occurred in experimental dogs

There was no myoglobinuria in the control group. In the experimental group, there was no myoglobinuria before or at 4 h extrusion, in 1 dog (12.5%) at 8 h extrusion and in the other 7 dogs (87.5%) at 1 h after decompression. The urinary output in the experimental group was significantly poorer than the control group after decompression ($P < 0.05$, data are not shown) and got poorer with the time passing. The urinary output of experimental dogs was < 0.5 ml/Kg/h at 8-24 h after decompression which met the diagnostic criteria of AKI [15].

Biochemical indexes changed significantly in experimental dogs

As shown in **Table 1**, the levels of CK and CK-MB in experimental dogs at 4 h and 8 h after extrusion and 1 h, 4 h, 8 h, 12 h and 24 h after decompression were significantly higher than the time before extrusion ($P < 0.01$) and higher than the values in the control group ($P < 0.01$) (**Figure 4**). The levels of CK and CK-MB in the experiment group increased to the highest values at 8 h after decompression and then declined but were still significantly higher than the control group. However the levels of CK and CK-MB in the control group didn't change significantly with the time.

The levels of BUN, Scr, ALT, AST and K^+ in experimental dogs at 1 h, 4 h, 8 h, 12 h and 24 h

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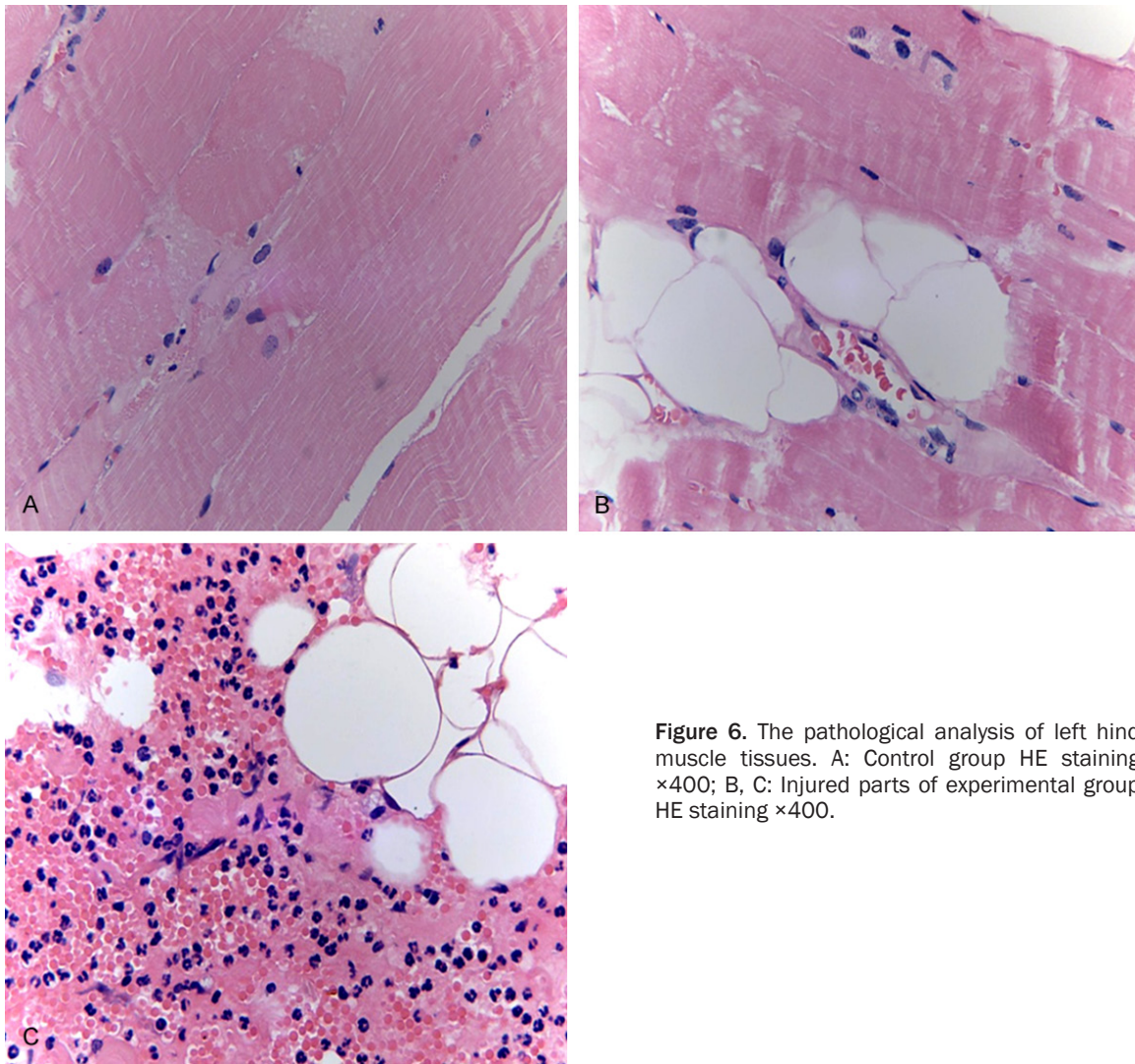


Figure 6. The pathological analysis of left hind muscle tissues. A: Control group HE staining $\times 400$; B, C: Injured parts of experimental group HE staining $\times 400$.

after decompression were significantly higher than the time before extrusion ($P < 0.01$) and higher than the values in the control group ($P < 0.01$). However those levels in the control group didn't change much.

Blood CO_2CP in the experimental group was weaker at 8 h extrusion and at 1 h, 4 h, 8 h, 12 h and 24 h after decompression than the time before extrusion ($P < 0.01$) and weaker than that in control group ($P < 0.01$) which didn't change significantly.

Pathological changes in kidney, crush-injured muscle and myocardial tissues of experimental dogs

As show in renal pathological sections, in the control group, the structure of glomerulus was

normal, the renal tubular epithelial cells were integral and there were no casts in lumen (**Figure 5A**). In the experimental group, part of the renal capsule thickened, part of the glomerular capillary dilated or congested, part of the renal tubular dilated, epithelial cells swelled and some were vacuolar degeneration or necrosis, necrotic cell debris and exudates were found in part of the renal tubular lumen, myoglobin casts were seen and there was an infiltration of renal interstitial lymphocytes (**Figure 5B, 5C**).

As shown in muscle pathological sections, in the control group, the structure of muscle fibers was normal, horizontal grains were clear and cells were completed (**Figure 6A**). In the experimental group, bleeding was in muscle tissues, degeneration, swelling and necrosis appeared

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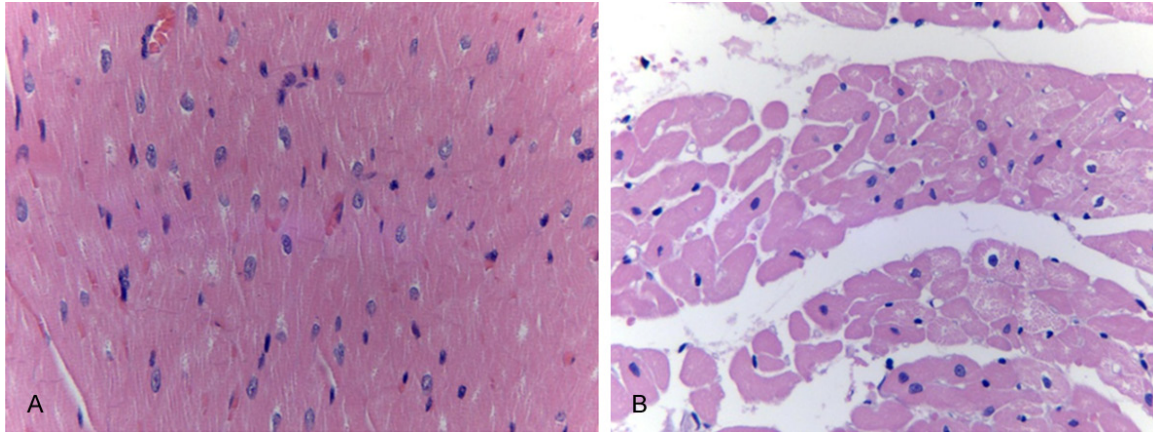


Figure 7. The pathological analysis of myocardium tissues. A: Control group HE staining $\times 400$; B: Experimental group HE staining $\times 400$.

in part of the muscle fibers and lymphocytic infiltration and fat cell infiltration were seen (Figure 6B, 6C).

As shown in Figure 7, part of the heart capsule thickened, fat cell infiltration and inflammatory cell infiltration were found and some myocardial cells were necrosis.

Discussion

Establishing animal model of CS is the basic method to investigate the mechanism and treatments of CS. Nowadays there are two main methods to build an animal model of CS: the first is tying up the certain part of animals by ropes or springs to block the blood flows [16, 17]; the second is extruding the certain part of animals by heavy things to cause necrosis of muscle tissue. The second method is similar to the way causing CS in earthquakes, landslides and other disasters or accidents. In 2005, Akimau [18] designed a rat model of CS by putting heavy things on some parts of the rat. But the area of thrust surface in the model couldn't be controlled accurately. In 2013, a new crush injury device was made by Chen, et al. [19] so that rats could drink and eat in the extrusion. However the device could not show the area of thrust surface and the real time pressure. In our study, we used a single chip microcomputer Intel 89C52 (Intel Corporation, USA) as the control unit of our crush injury device platform, used a pressure sensor to measure the pressure and used different extrusion modules to control the area of thrust surface. The pressure and time for extrusion could be set in our self-

made digital crush injury device and the temperature, pressure and the remaining time for extrusion could be shown.

The lower limbs have rich muscles and they are easily injured in earthquakes, landslides and traffic accidents resulting in a CS. In our research, the left hind legs of Beagle dogs were extruded by a 50 kg weight for 8 h using the self-made digital crush injury device platform. And then they were found to be congestion, swelling and perimeter increasing. Myoglobinuria was found in 1 h after decompression in the experimental group. In some studies, myohemoglobin in blood and urine were used as the important diagnostic criteria [20]. CK and CK-MB as sarcoplasmic proteins in myocytes will enter into the blood when myocytes are damaged. They are closely related to the injured range and degree of the skeletal muscle necrotic. In our study, the levels of CK and CK-MB increased and reached to the peak level beginning with the extrusion until 8 h after decompression. The peak value of CK in the experimental group was >50000 U/L significantly higher than the control group at the same time reaching the diagnostic criteria of CS. So CK and CK-MB were the sensitive indicators of CS. The levels of CK and CK-MB declined after the peak in the experimental group but still higher than the control group. That was the same as the previous reports, and that might be related to the amount and the injury degree of striated muscle cells damaged by the extrusion [21].

The necrotic cell lysates of injured muscle tissues were absorbed into blood leading to the

hyperkalemia, metabolic acidosis and AKI [22]. In our study, dogs in experimental group suffered from hyperkalemia after the decompression and the level of serum potassium was significantly higher than the control group ($P < 0.01$). The reason seems to be that a large number of intracellular potassium ions outflow into the blood when muscle cells injured by the extrusion. Blood gas analysis for the experimental dogs showed that the value of CO_2CP at 8 h extrusion and after decompression was significantly lower than the time before extrusion ($P < 0.01$). It indicated that metabolic acidosis aggravated with the process of the extrusion. AKI is one of the outstanding features of CS and is an important prognostic factor of CS [23]. The mechanism of AKI induced by CS is still not fully clear. There were reports suggesting that myoglobin entering into kidney with the blood flow after the rhabdomyolysis was filtered in glomerulus and then resolved in proximal tubule. It became into jelly in an acid environment and then formed casts with shedding tubular epithelial cells to block the renal distal convoluted tubule and the collecting duct leading to AKI. In an internal environment of metabolic acidosis, the iron ligand valence of myoglobin changed and ferrous myoglobin was generated to induce a dose-dependent apoptosis of renal tubular epithelial cells [24, 25]. In our study, the urine color of experimental dogs changed from light red to dark brown after decompression. Experimental dogs got urinary output significantly reduced at 5 to 8 h after decompression and even anuria at 8 to 24 h after decompression. All the experimental dogs met the TIFLE diagnosis of AKI after decompression. And the pathological analysis further confirmed the diagnosis of AKI.

As was reported that liver dysfunction could be found in CS patients [7]. The levels of ALT and AST of experimental dogs in this study increased significantly suggesting a liver dysfunction. Myocardial damage was rarely reported so far. In our study, edema, necrosis and inflammatory cell infiltration were observed in part of the myocardial cells of experimental dogs. It indicated that a myocardial damage was induced by CS. That should be confirmed by further studies with more tests of myocardial injury markers.

In conclusion, dogs in the experimental group suffering from 8 h extrusion got obvious swell-

ing and muscle trauma in injured limbs, significant changes of blood biochemical indexes and pathological changes in kidney, crush-injured muscle and myocardial tissues, meeting the diagnostic criteria of CS. The further studies about the mechanism and treatment of CS could be performed easily using this canine CS model established by the digital crush injury device platform.

Disclosure of conflict of interest

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