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A novel method to assess the severity and prognosis in crush syndrome by assessment of skin damage in hairless rats

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Abstract

Purpose Crush syndrome (CS), a serious medical condition characterised by damage to the muscle cells due to pressure, is associated with high mortality, even when patients receive fluid therapy during transit to the hospital or admission to the hospital. There is no standard triage approach for earthquake victims with crush injuries due to the scarcity of epidemiologic and quantitative data. We examined whether mortality can be predicted based on the severity of skin damage so that assess the severity and prognosis in crush syndrome by assessment of skin damage in hairless rats because we have previously observed that CS results in oedema and redness of the skin in rats.
Methods Anaesthetised rats were subjected to bilateral hind limb compression [1 kg (mild) and 2 kg (severe) loads] with a rubber tourniquet for 5 h. The rats were then randomly divided into three groups: sham, mild CS, and severe CS.
Results The mild and severe CS groups had mortality rates of 20 and 90%, respectively. The severe CS group demonstrated higher rates of hyperkalaemia, hypovolemic shock, acidosis, and inflammation. Skin damage was significantly worse in the severe CS group compared to the mild CS group. Skin damage showed good correlation with pathological severity.
Conclusions Skin damage is a valid measure of transepidermal water loss and severity of CS. We suggest that these models may be useful to professionals who are not experienced in disaster management to identify earthquake victims at high risk of severe CS.

Keywords Crush syndrome · Skin damage · Severity · Transepidermal water loss · Triage

Introduction

Crush syndrome (CS) is systemic dysfunction resulting from crush injuries and muscle compression. It is characterised by an unpredictable clinical course and high mortality due to cardiac arrest, shock, acute kidney injury (AKI), and inflammatory disorders [1–3].

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In emergency situations, initial management with massive fluid resuscitation is recommended to prevent death due to cardiac arrest and AKI. However, CS is also known to be associated with rescue death with up to 20% of deaths occurring shortly after extrication [4]. Often, patients die during transit to the hospital or admission to the hospital. Therefore, a severity assessment is very important to identify CS and plan the therapeutic strategy at an early stage. Furthermore, the medical approach for disaster management is different from regular clinical approaches due to the lack of medical resources, infrastructure, and information [5, 6]. The medical approach needs to adapt to new developments and changes in the patient's health. Hence, emergency triage must be flawless to provide medical support to victims of CS. Often, triage errors occur in the management of these patients because medical professionals are more focused on assessing more patients in a short period of time than making accurate diagnoses. Given this, establishing a method for rapid and accurate screening for CS is necessary.

Oda et al. [7] reported that CS severity was correlated with muscle damage extent, as reflected by creatine phosphokinase (CPK) levels. However, CPK levels cannot be assessed during triage because the specialised instruments required to draw blood are not available. Crush injury can damage not only the muscles, but also the skin; however, the latter has not been reported as a pathological change. We have previously observed that CS results in oedema and redness of the skin in rats. Pathological changes in the skin are seen in patients with chronic kidney failure, such as decreasing pigmentation, dryness, and decreased tone [8–10], and those with intestinal injury as a result of inflammatory mediators [11]. Namely, we hypothesised that CS severity will be estimated by the severity of skin damage.

In this study, we investigated pathological changes and skin damage in rats with CS with a view to elucidating these changes in detail.

Materials and methods

Experimental design

Experiment 1 (Survival rate and measurements): Rats were randomly divided into the following three groups: (1) sham ($n = 10$), (2) CS with 1.0 kg load (mild CS group) ($n = 10$), and (3) CS with 2.0 kg load (severe CS group) ($n = 12$) and survival period and skin changes were compared between groups.

Experiment 2 (Blood sampling): Vital signs and blood gas and biochemical analyses were carried out for each of the three experimental groups ($n = 6$ each).

Rats were killed for sampling of blood and tissues at the designated time periods by overdose sodium pentobarbital.

Animal model of CS

Male hairless rats weighing 250–300 g were obtained from Japan SLC (Shizuoka, Japan) and housed in a room maintained at a temperature of 23 ± 3 °C and relative humidity of $55 \pm 15\%$. Rats were subjected to a 12:12-h light/dark cycle with free access to food and water. Animal experiments were carried out according to the guidelines for animal use and approved by the Life Science Research Center of Josai University (Approval no. H28025). Anaesthesia was induced by intraperitoneal injection of sodium pentobarbital (50 mg/kg-body weight). Body temperature was maintained throughout the experiment using a heating pad. The CS rat model has been previously described in detail [12]. Briefly, a rubber tourniquet was applied to both hind limbs of each rat. The tourniquet was wrapped five times around either a 1.0 or 2.0 kg (i.e. these differences reflected mild and severe CS, respectively) metal cylinder,

and the end of the band was glued. After a compression of 5 h, the compression was released by cutting the band and removing the tourniquet.

Transepidermal water loss (TEWL), erythema, melanin, and blood perfusion (BPF) of rat skin

We aimed to assess the skin of the following five areas: anterior limb (AL), abdomen (AB), crushed part of the hind limb (HL), non-crushed part of the limb that was crushed (nHL), and instep (IS). TEWL was measured with Tewameter TM300 (Courage + Khazaka electronic GmbH, Germany). The IS section could not be assessed because the measurement area was too small, leaving four areas of assessment. Erythema and melanin were measured using Mexameter MX18 (Courage + Khazaka electronic GmbH, Germany). Briefly, the measurement time is until stabilised (approximately 1 min), and three sections from each of the five areas were assessed. Skin BPF was measured using PeriScan PIM3 (PERIMED, USA). BPF measurements were performed under the following conditions: the distance to the skin was 10–15 cm, the measurement area was 10×10 cm, the environmental temperature was 23 ± 5 °C, and the relative humidity was $50 \pm 5\%$. The skin area of AL was excluded.

Vital signs, blood gas levels, and biochemical parameters

The following vital signs were recorded using a Power-Lab data acquisition system (AD Instruments, Nagoya, Japan): systolic blood pressure (SBP), mean blood pressure (MBP), diastolic blood pressure (DBP), heart rate (HR), and body temperature (BT). One carotid artery was cannulated with a polyethylene catheter (PE-50 tubing) connected to a pressure transducer. Arterial blood samples (65 µL) from each group were obtained at 0.083, 0.5, 1, 3, 6, 12, and 24 h after reperfusion using a carotid artery catheter [11]. The arterial levels of sodium (Na^+), potassium (K^+), chlorine (Cl^-), total carbon dioxide (TCO_2), blood urea nitrogen (BUN), glucose (Glu), hematocrit (Hct), pH, partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), bicarbonate (HCO_3^-), base excess (BE), anion gap (AG), and haemoglobin (Hb) were analysed using an i-STAT300F blood gas analyser and EC8+ cartridge (FUSO Pharmaceutical Industries, Osaka, Japan). Haemoconcentration (Hc) ratio was calculated from the Hct levels. Venous blood was collected (100 µL) from the jugular vein and centrifuged to measure plasma CPK (measurements were carried out by SRL Inc., Tokyo, Japan).

Determination of reactive oxygen species (ROS) production and myeloperoxidase (MPO) activity, oedema, and histology

ROS production in the injured gastrocnemius muscle was determined by measuring the concentration of thiobarbituric acid reactive substances (TBARS) [14]. MPO activity in the blood and muscle tissue was measured as previously described [14]. The ratio of wet tissue weight to dry tissue weight (W/D) was determined, and adopted as an index of skin oedema. For histology, the tissues were evaluated using the frozen section method. Sections were cut and stained with hematoxylin and eosin, and were then carefully examined under a microscope. Microscopic photos are representative of three independent experiments (200×).

Statistical analyses

Results are expressed as mean ± standard error of mean (SEM). Differences between the groups were assessed by analysis of variance with Tukey's honest significant difference test or Tukey's test. Survival curves were generated using the Kaplan–Meier method, and survival was compared using the log-rank test. The correction coefficient parameters were calculated using Pearson's test. Correlation was calculated for the experimental period (i.e. 0.083 to 24 h), and considered significant if the possibility of correlation was > 0.7. p values < 0.05 were considered statistically significant.

Results

Survival rate

The survival rate of rats in the mild CS group was 80% at 24 h and 80% at 48 h after reperfusion. In the severe CS group, survival rates decreased over time to 91, 83, 41, 8, and 8% at 1, 3, 6, 24, and 48 h, respectively, after reperfusion. The survival rate in the severe CS group at 48 h after reperfusion was significantly lower than those in the mild CS groups.

Blood analysis

Plasma K^+ and CPK levels in the mild and sever CS group were significantly higher than the sham group, especially at 6 and 24 after reperfusion. Plasma BUN and Hc levels in the severe CS group were significantly higher than those in the sham group. In contrast, pH and MBP levels in the severe CS group were significantly lower than those in the sham and mild CS groups (Table 1). HR, SBP, DBP, BT, HCO_3, and BE levels in the severe CS group were significantly lower than those in the sham and mild CS groups. In contrast, AG levels in the severe CS group were significantly higher than those in the sham and mild CS groups (SDC Table 1).

Skin condition

For HL, TEWL in the sham group was 15.4 ± 1.6–19.0 ± 3.2 g h/m^2 during the experimental period. In contrast, TEWL levels in the mild CS group were lower after 24 h (9.7 ± 1.0 g h/m^2, $p < 0.05$). TEWL was significantly lower in the severe CS group than in the sham group between 0.083 and 3 h after reperfusion (6.0 ± 0.5 to 10.0 ± 2.2 g h/m^2, $p < 0.05$), but was significantly higher than the sham and mild CS groups 24 h after reperfusion (sham: 16.4 ± 1.1, mild CS: 9.7 ± 1.0 vs. severe CS: 27.0 ± 2.6 g h/m^2, $p < 0.05$) (Fig. 1a). In the nHL sections, the changes were similar to those of the crushed part in all groups (Fig. 1b). For AL and AB, there were no significant differences in TEWL levels in the three groups (SDC Fig. 1). For HL, BPF levels in the sham group ranged from 221.5 ± 23.0 PU at 0.083 h to 216.5 ± 9.7 PU at 24 h. The mild and severe CS groups had significantly lower values ($p < 0.05$) (Fig. 2a). For nHL sections, the BPF of the mild CS group was similar to that of the sham group at 0.083–0.5 h after reperfusion, and then gradually decreased. In contract, BPF was significantly lower in the severe CS group than in the sham and mild CS groups at 0.083–6 h after reperfusion. After 24 h of reperfusion, BPF in the mild and severe CS groups was similar to that in the sham group (Fig. 2b).

For HL and nHL skin areas, the erythema in the sham group ranged from 120.8 ± 10.4 a.u. (minimum) to 199.4 ± 9.7 a.u. (maximum). These changes in the mild and severe CS groups were significantly higher than those in the sham group at 0.083–6 h after reperfusion. In contrast, the erythema levels of mild and severe CS groups were lower than those in the sham group after 24 h of reperfusion (Fig. 2c, d).

Melanin levels in the sham group ranged from 26.9 ± 4.9 a.u. (minimum) to 76.6 ± 4.0 a.u. (maximum). After 0.083–6 h of reperfusion, melanin was significantly higher in the severe CS group than in the sham and mild CS groups, and after 24 h of reperfusion, melanin was significantly lower in the severe CS group than in the sham group (SDC Fig. 2A, B).

For AB and AL skin areas, BPF, erythema, and melanin levels in the three groups were not significantly different throughout the experimental period (SDC Fig. 3). Skin colour and BPF levels did not differ significantly in the sham group during the experimental period. In the mild CS group, the skin colour changed from red to white in the HL skin areas from 0.083 to 24 h of reperfusion, and in the severe CS group, the skin colour was

Table 1 Changes in CPK, K$^+$, BUN, pH, Hc ratio, and MBP levels after reperfusion from 0.083 to 24 h

	Reperfusion (h)					
	0.083	0.5	1	3	6	24
Plasma CPK ($\times 10^2$ IU/L)						
Sham	1.8 ± 0.3	3.0 ± 1.1	4.2 ± 3.1	1.6 ± 0.5	8.9 ± 8.9	1.4 ± 0.3
Mild CS	13.7 ± 3.9	28.0 ± 5.3*	30.3 ± 3.7*	50.8 ± 12.9*	49.3 ± 12.7*	51.7 ± 12.8*
Severe CS	28.6 ± 1.6*	47.9 ± 8.7*	48.1 ± 6.4*	54.8 ± 11.6*	105.0 ± 15.5*,#	310.6 ± 62.0*,#
Plasma K$^+$ (mEq/L)						
Sham	4.1 ± 0.0	4.4 ± 0.3	4.4 ± 0.3	4.3 ± 0.2	4.6 ± 0.2	3.7 ± 0.1
Mild CS	5.2 ± 0.2*	5.3 ± 0.0*	5.6 ± 0.0*	5.8 ± 0.1*	5.9 ± 0.1*	5.0 ± 0.2*
Severe CS	4.9 ± 0.1*	5.5 ± 0.1*	5.9 ± 0.1*	6.3 ± 0.1*	6.9 ± 0.2*,#	8.3 ± 0.3*,#
BUN (mg/dL)						
Sham	19.0 ± 0.5	21.7 ± 1.5	21.7 ± 1.5	21.0 ± 1.2	21.3 ± 3.4	27.3 ± 5.5
Mild CS	26.0 ± 1.1	30.3 ± 1.4	31.5 ± 1.4*	41.3 ± 1.5*	54.3 ± 5.4*	73.0 ± 17.0*
Severe CS	19.9 ± 2.7	29.1 ± 3.1	31.6 ± 3.1*	42.6 ± 3.1*	59.3 ± 4.2*	125.4 ± 18.8*,#
pH						
Sham	7.47 ± 0.02	7.46 ± 0.02	7.46 ± 0.04	7.48 ± 0.01	7.50 ± 0.01	7.45 ± 0.01
Mild CS	7.41 ± 0.02	7.40 ± 0.02	7.50 ± 0.02	7.47 ± 0.04	7.41 ± 0.04	7.41 ± 0.02
Severe CS	7.44 ± 0.02	7.45 ± 0.02	7.45 ± 0.01	7.41 ± 0.01	7.39 ± 0.04*	7.22 ± 0.08*
Hc						
Sham	1.00 ± 0.01	1.00 ± 0.00	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.03	1.00 ± 0.03
Mild CS	1.06 ± 0.01	1.06 ± 0.02	1.04 ± 0.01	1.13 ± 0.03*	1.13 ± 0.03*	1.45 ± 0.03*
Severe CS	1.10 ± 0.01	1.12 ± 0.01	1.10 ± 0.03	1.22 ± 0.04*	1.23 ± 0.03*	1.68 ± 0.03*
MBP (mmHg)						
Sham	129.8 ± 6.4	136.4 ± 4.4	131.0 ± 6.4	127.8 ± 9.7	112.4 ± 13.9	115.7 ± 11.3
Mild CS	90.9 ± 4.5*	66.3 ± 4.5*	75.2 ± 7.7*	78.8 ± 6.9*	65.2 ± 4.9*	105.6 ± 5.9
Severe CS	105.6 ± 8.1*	61.8 ± 1.9*	54.6 ± 3.9*	49.8 ± 11.5*	43.8 ± 5.6*	48.2 ± 7.6*,#

Values are presented as mean ± SEM ($n = 6$)

CPK creatine phosphokinase, *K$^+$* potassium ion, *BUN* blood urine nitrogen, *Hc* haemoconcentration, *MBP* mean blood pressure, *CS* crush syndrome, *SEM* standard error of the mean

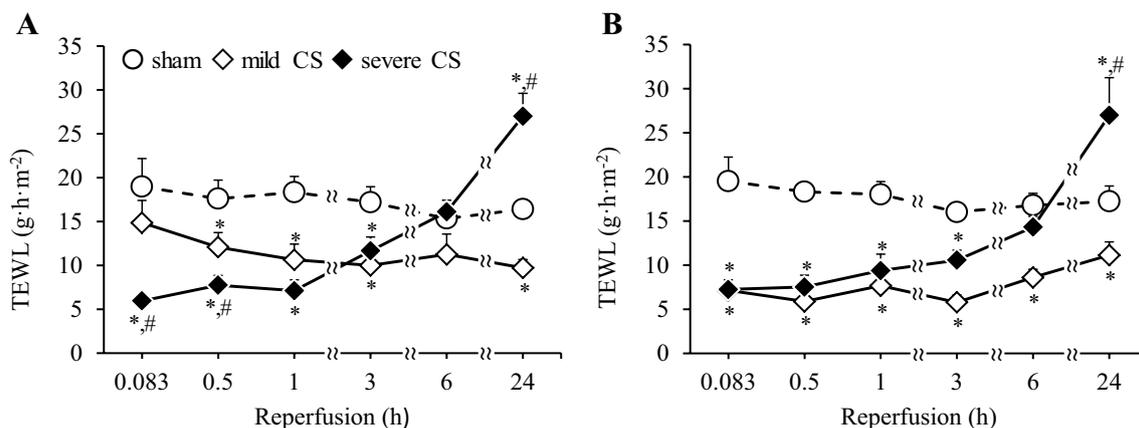*$p < 0.05$ vs. sham group and #$p < 0.05$ vs. mild CS group (Tukey's test)

Fig. 1 Changes in transepidermal water loss (TEWL) after reperfusion from 0.083 to 24 h in the skin of the hind limbs. **a** TEWL level of HL skin area and **b** TEWL level of nHL areas. White circle: sham; white diamond: mild CS; and black diamond: severe CS group. Values represent mean ± SEM ($n = 6$). *$p < 0.05$ vs. sham group and #$p < 0.05$ vs. mild CS group (Tukey's test). *TEWL* transepidermal water loss, *HL* crushed part of the hind limb, *nHL* non-crushed part of the crushed hind limb, *CS* crush syndrome
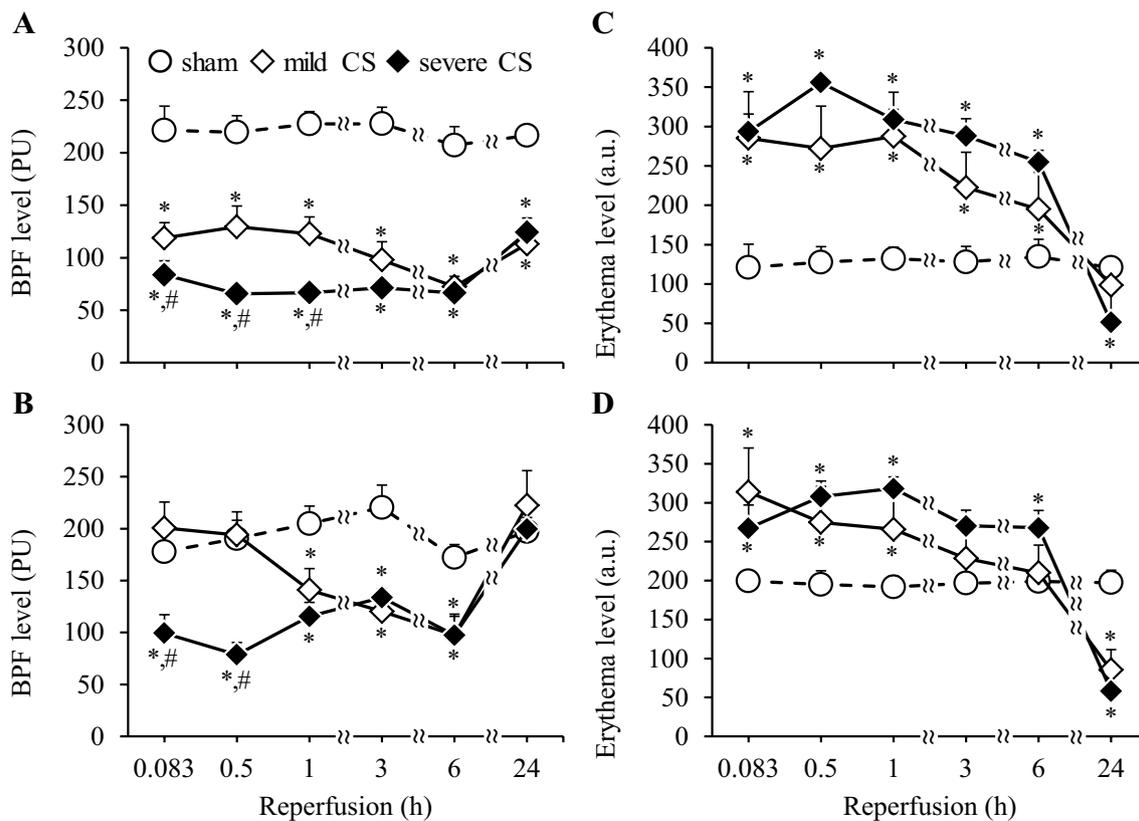
Fig. 2 Changes in BPF and erythema levels after reperfusion from 0.083 to 24 h in the skin of the hind limbs. **a** BPF level in HL, **b** BPF level in nHL, **c** erythema level in HL, and **d** erythema level in nHL. White bar: sham group; grey bar: mild CS group; and black bar: severe CS group. Values represent mean $\pm$ SEM ($n=6$). $*p<0.05$ vs. sham group and $^{\#}p<0.05$ vs. mild CS group (Tukey's test). *BPF* blood perfusion, *HL* crushed part of the hind limb, *nHL* non-crushed part of the crushed hind limb, *CS* crush syndrome

deep violet between 0.083 and 1 h after reperfusion and changed to white 24 h after reperfusion (SDC Fig. 4).

Figure 3 shows the TBARS and MPO activity, and oedema levels for all skin areas in the three groups, reflecting the inflammatory response of ischaemia reperfusion injury after 24 h of reperfusion. For AL and AB skin areas, TBARS, MPO activity, and oedema levels in the three groups were not significantly different. The TBARS levels in HL and nHL skin in the severe CS group were significantly higher than those in the sham and mild CS groups (Fig. 3a). MPO activity and oedema levels in the mild and severe CS groups were significantly higher than those in the sham group, and levels in the severe CS group were significantly higher than those in the mild CS group (Fig. 3b, c). With respect to pathological changes, dermal oedema in the mild and severe CS groups were significantly more severe compared to the sham group, and we concluded that the oedema in the severe CS group was not associated with inflammatory factors (i.e. leukocyte, etc.) (Fig. 3d).

Correlation between skin condition and blood parameters in CS

We selected the HL and nHL skin areas because the skin in these areas varies in comparison to skin in the AL and AB areas. Blood parameters selected were CPK, K^+, pH, BE, BUN, Hc ratio, HR, MBP, and BT levels, and HCO_3^-, AG, Hb, Na^+, Cl^-, TCO_2, BUN, Glu, $PaCO_2$, Htc, SBP, and DBP levels were not selected as they did not correlate (data not shown) because CS patients were characterised not only by muscle injury, hyperkalaemia, metabolic acidosis, acute kidney injury, and hypovolemic shock, but also by our rat model.

Table 2 shows the correlation, regression analyses, and r levels based on skin parameters (TEWL, BPF, erythema, and melanin levels) and blood parameters in the HL area. TEWL in the mild CS group was negatively correlated with CPK level. In the severe CS group, CPK, K^+, BUN, and Hc ratio showed a positive correlation, and pH, HR, and BT levels showed a negative correlation with TEWL. The BPF
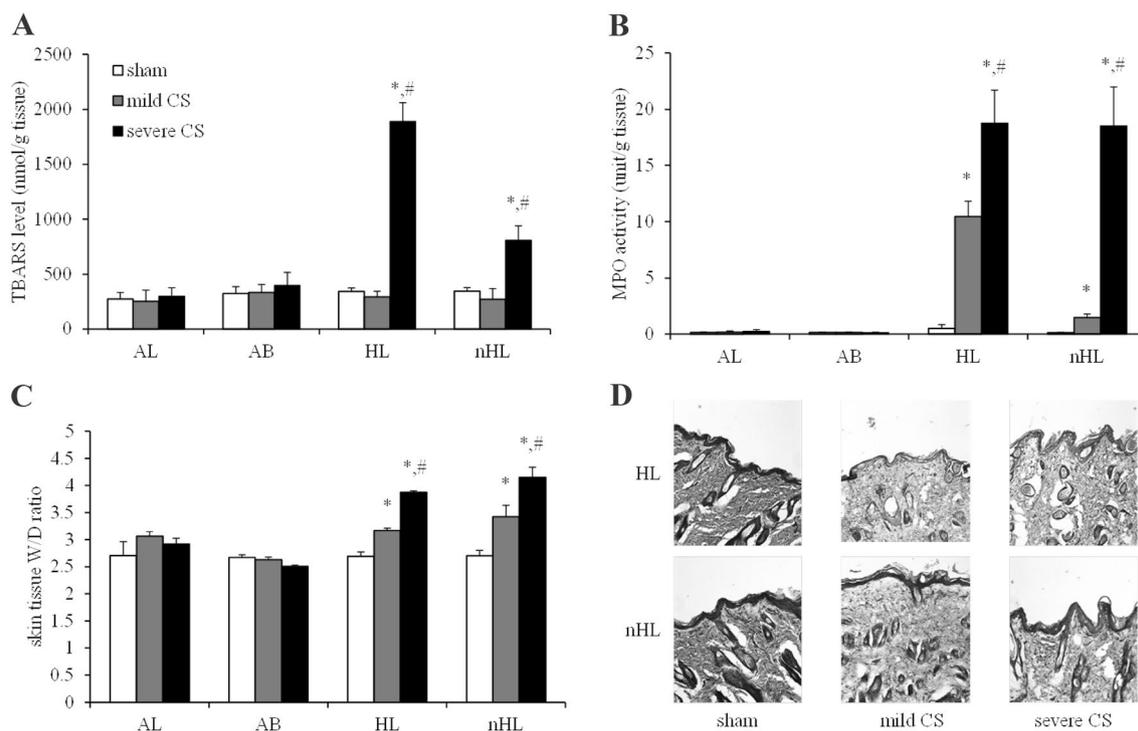
Fig. 3 Changes in TBARS level, MPO activity, oedema, and histopathology after reperfusion from 0.083 to 24 h in the skin of hairless rats. **a** TBARS level, **b** MPO activity, **c** skin tissue *W/D* ratio, and **d** histopathological changes in the skin of HL and nHL areas. Values represent mean ± SEM (*n* = 6). *$p < 0.05$ vs. sham group and #$p < 0.05$ vs. mild CS group (Tukey's test). *TBARS* thiobarbituric acid reactive substances, *MPO* myeloperoxidase, *W/D* wet/dry, *AL* anterior limb, *AB* abdomen, *HL* crushed part of the hind limb, *nHL* non-crushed part of the crushed hind limb, *CS* crush syndrome

in the severe CS group was positively correlated with CPK, BUN, and Hc ratio, and negatively correlated with pH and BT levels. Erythema and melanin were negatively correlated with CPK, BUN, and Hc ratio in the mild and severe CS groups, while erythema and melanin were positively correlated with pH, BE, and BT levels in the severe CS group. The r levels for TEWL (compared to BPF, erythema, and melanin in the severe CS group) demonstrated a significant correlation (> 0.7) similar to the mild CS group, which demonstrated low levels of correlation. In the nHL skin area, the correlation between skin condition and blood parameters was similar to that for the HL area (SDC Table 2).

Judgement of severe CS by skin insult score

Table 3 shows the skin insult score which TEWL ratio that were fixed (re-calculation) with the TEWL level of each skin part at experimental periods. In the sham group, scores were stable within each area of the skin. For HL/AL, AB, and nHL areas, the mild CS group had significantly higher scores than the sham group (i.e. ratio level > 1.0). In contrast, during the early reperfusion period, the severe CS group had significantly lower scores compared to those in the sham and mild CS groups at 0.083–0.5 h after reperfusion (i.e. score level < 1.0). Scores were similar to those in the mild CS group at 24 h after reperfusion (i.e. score level > 1.0).

Discussion

The survival rate in CS varies significantly depending on the initial treatment and strategy; therefore, it is important to accurately assess the severity of CS. Oda et al. proposed a method for judging CS severity using shifts in CPK levels [7], and these shifts are important to evaluate treatment outcomes in hospital. However, these measurements are difficult to make during on-the-spot emergency triage. Therefore, judging the severity of CS during triage depends on the experience of the doctor and medical staff. In this study, we have demonstrated an easy method to judge the severity of CS by assessing the severity of skin damage.

In this study, the hairless rat CS model was similar to Wistar model results that we reported previously [12, 15]. The possible reasons for the low survival rate were hyperkalaemia, hypovolemic shock, acidosis, and kidney failure (Table 1). In other words, the high survival rate in the mild CS group suggested that these complications did not occur in the mild CS group. We demonstrated a difference in changes

Table 2 Regression analysis and correlation between crushed skin severity and blood parameters

	TEWL (g h/m^2)		BPF (PU)		Erythema (a.u.)		Melanin (a.u.)	
	Regression equation $y=$	r	Regression equation $y=$	r	Regression equation $y=$	r	Regression equation $y=$	r
CPK (IU/L)								
Sham	$-1.26x+25.3$	-0.58	$-0.25x+58.7$	-0.67	$0.42x+-49.5$	0.82	$0.12x+-0.5$	0.19
Mild CS	$-7.15x+119.0$	-0.86	$-0.48x+89.6$	-0.64	$-0.17x+75.7$	-0.79	$-0.88x+56.3$	-0.61
Severe CS	$12.90x+-63.4$	0.96	$4.15x+-231.2$	0.89	$-0.97x+349.4$	-0.97	$-2.12x+282.3$	-0.90
K$^+$ (mEq/L)								
Sham	$-0.05x+5.1$	-0.20	$-0.01x+5.7$	-0.16	$0.05x+-2.3$	0.91	$-0.02x+5.0$	-0.33
Mild CS	$-0.05x+6.0$	-0.25	$-0.01x+6.7$	-0.68	$0.00x+5.2$	0.25	$0.02x+5.1$	0.54
Severe CS	$0.15x+4.4$	0.97	$0.04x+3.4$	0.68	$-0.01x+8.8$	-0.89	$-0.02x+8.0$	-0.74
pH								
Sham	$0.00x+7.6$	-0.36	$0.00x+7.6$	-0.34	$0.00x+7.2$	0.64	$0.00x+7.5$	-0.16
Mild CS	$-0.01x+7.5$	-0.39	$0.00x+7.4$	0.06	$0.00x+7.4$	0.34	$0.00x+7.4$	0.36
Severe CS	$-0.01x+7.5$	-0.97	$0.00x+7.7$	-0.91	$0.00x+7.2$	0.98	$0.00x+7.2$	0.93
BE (mmol/L)								
Sham	$0.48x+-1.8$	0.47	$0.01x+4.6$	0.05	$-0.03x+10.1$	-0.12	$-0.22x+13.6$	-0.74
Mild CS	$-0.01x+1.9$	-0.01	$0.07x+-5.8$	0.57	$-0.01x+5.1$	-0.42	$-0.15x+5.0$	-0.65
Severe CS	$-0.54x+4.6$	-0.89	$-0.09x+5.0$	-0.42	$0.03x+-10.5$	0.72	$0.06x+-6.9$	0.52
BUN (mg/dL)								
Sham	$-1.02x+39.7$	-0.48	$-0.08x+40.6$	-0.23	$-0.14x+40.2$	-0.29	$0.55x+4.6$	0.87
Mild CS	$-6.07x+112.1$	-0.64	$-0.39x+85.4$	-0.46	$-0.24x+97.8$	-0.99	$-1.38x+72.3$	-0.83
Severe CS	$4.80x+-9.2$	0.99	$1.42x+-62.0$	0.84	$-0.35x+141.5$	-0.96	$-0.74x+115.4$	-0.87
Hc								
Sham	$0.00x+1.0$	-0.04	$0.00x+1.0$	0.45	$0.00x+1.0$	0.05	$0.00x+1.0$	-0.11
Mild CS	$-0.04x+1.6$	-0.53	$0.00x+1.3$	-0.14	$0.00x+1.6$	-0.96	$-0.01x+1.4$	-0.95
Severe CS	$0.03x+0.9$	0.97	$0.01x+0.5$	0.90	$0.00x+1.8$	-0.97	$0.00x+1.6$	-0.91
HR (bpm)								
Sham	$11.47x+187.4$	0.52	$2.40x+-142.1$	0.64	$-1.82x+617.6$	-0.36	$-1.57x+436.0$	-0.24
Mild CS	$6.53x+282.7$	0.51	$-0.26x+385.2$	-0.22	$-0.05x+368.2$	-0.15	$0.06x+356.2$	0.03
Severe CS	$-4.70x+331.9$	-0.75	$-0.95x+348.2$	-0.43	$0.29x+198.0$	0.62	$0.50x+229.7$	0.45
MBP (mmHg)								
Sham	$5.84x+24.3$	0.82	$0.90x+-72.3$	0.73	$-0.19x+149.6$	-0.11	$-1.75x+181.3$	-0.83
Mild CS	$-0.40x+84.8$	-0.05	$0.19x+59.4$	0.26	$-0.12x+106.5$	-0.54	$-0.92x+100.1$	-0.64
Severe CS	$-1.57x+80.4$	-0.55	$0.00x+60.7$	0.00	$0.06x+43.8$	0.30	$0.04x+57.5$	0.07
BT (°C)								
Sham	$-0.07x+37.5$	-0.45	$0.00x+36.6$	-0.04	$0.02x+34.3$	0.43	$0.01x+36.0$	0.22
Mild CS	$0.18x+32.5$	0.55	$0.01x+33.5$	0.33	$0.00x+34.7$	-0.09	$-0.01x+34.8$	-0.19
Severe CS	$-0.33x+37.6$	-0.97	$-0.10x+41.1$	-0.81	$0.02x+27.4$	0.94	$0.05x+29.2$	0.83

CPK creatine phosphokinase, *K$^+$* potassium ion, *BE* base excess, *BUN* blood urine nitrogen, *Hc* haemoconcentration ratio, *HR* hart rate, *MBP* mean blood pressure, *BT* body temperature, *CS* crush syndrome, *TEWL* transepidermal water loss, *BPF* blood perfusion

in the HL and nHL areas. For example, despite TEWL levels being lower in the mild CS group than in the sham group, in the severe CS group it decreased and then significantly increased. Immediately after reperfusion, the suggested cause of low TEWL levels in the mild and severe CS groups was decreasing blood flow due to the crush injury (Fig. 1), and the matched BPF levels in the severe CS group were lower than that in the mild CS group (Fig. 2). Furthermore, the skin barrier function may have broken down due to inflammation because high TEWL levels reflected not only direct injury to skin secondary to the crush injury, but also induction of TBARS and MPO activities due to ischaemia reperfusion injury (Fig. 3). Interestingly, in the severe CS group, despite the low BPF levels at 6 h after reperfusion, erythema and melanin levels had increased. In other words, skin colour changed to dark violet in the severe CS group

Table 3 Judging severe CS from the skin insult score

	Duration of reperfusion (h)					
	0.083	0.5	1	3	6	24
HL/AL						
Sham	0.92 ± 0.10	0.86 ± 0.06	1.07 ± 0.11	0.94 ± 0.06	0.90 ± 0.04	0.98 ± 0.11
Mild CS	1.99 ± 0.20*	1.37 ± 0.17*	1.52 ± 0.19	1.95 ± 0.36*	1.62 ± 0.36	1.75 ± 0.32*
Severe CS	0.40 ± 0.05*,#	0.77 ± 0.10#	0.51 ± 0.11*,#	0.82 ± 0.14*,#	1.04 ± 0.13	2.01 ± 0.26*
HL/AB						
Sham	1.06 ± 0.09	0.92 ± 0.07	1.09 ± 0.06	1.11 ± 0.10	1.12 ± 0.09	1.14 ± 0.09
Mild CS	1.47 ± 0.25*	1.22 ± 0.28	1.43 ± 0.20	1.83 ± 0.32*	1.73 ± 0.46	1.91 ± 0.41*
Severe CS	0.45 ± 0.05*,#	0.64 ± 0.09*,#	0.99 ± 0.58	0.95 ± 0.14#	1.26 ± 0.17	2.21 ± 0.40*
HL/nHL						
Sham	0.96 ± 0.05	0.95 ± 0.06	1.02 ± 0.08	1.07 ± 0.06	0.91 ± 0.05	0.96 ± 0.07
Mild CS	2.86 ± 0.24*	2.35 ± 0.38*	1.98 ± 0.35*	3.20 ± 0.87*	1.88 ± 0.24	1.59 ± 0.19
Severe CS	0.86 ± 0.11#	1.22 ± 0.39#	0.80 ± 0.11#	1.21 ± 0.26#	1.17 ± 0.17	1.09 ± 0.16

Values represent mean $\pm$ standard error of the mean (SEM) ($n = 6$ each)

HL crushed part of the hind limb, *AL* anterior limb, *AB* abdomen, *nHL* the non-crushed part of the crushed hind limb

*$p < 0.05$ vs. sham group and #$p < 0.05$ vs. mild CS group (Tukey's test)

and red in the mild CS group (Fig. 2 and SDC Fig. 4). After 24 h of reperfusion, decreasing erythema and melanin levels suggested the return of colour to white due to the development of oedema, as shown by the recovered levels of BPF and pathological changes.

Of note in this study was the correlation between skin damage and symptom development. For HL areas, TEWL correlated positively with CPK, K^+, HCO_3^-, and the Hc ratio for the severe CS model and negatively in the mild CS group (Table 2). Of note, CPK levels showed a significantly higher correlation ($r > 0.8$) in both groups. Our results are consistent with Oda et al.'s suggestion that severity is correlated with CPK levels [7]. However, BPF, erythema, and melanin were not correlated with several blood markers, because the sham group also tended to correlate (Tables 2 and S2). CS is a medical condition characterised by muscle cell damage as a result of pressure; hence, high CPK levels reflect leakage of potassium, myoglobin, and cytokines from the insulted muscle cells. In fact, we demonstrated that high levels of these agents could be judged using TEWL, which is important for estimation of cardiac failure, AKI, shock, and inflammation. We can assess TEWL severity based on skin properties because the absolute value differs between various types of skin. Between the skin of AL and AB, the insult score was approximately 1 in the experimental period (data not shown). Hence, affected parts of the skin that are distant (i.e. AL and AB) are not affected by the whole body. In nHL skin, the AL and AB skin insult scores were significantly higher in the mild and severe CS groups than in the sham group (ratio level 1) (data not shown). Interestingly, the HL skin scores in the severe CS group were completely different to those in the mild CS group. With respect to the HL skin

ratio calculated from AL and AB skin parameters, the severe CS group had a ratio > 1 and the mild CS group had a ratio < 1 (Table 3). We can use these cut-off figures to make a simple judgement about CS severity. Furthermore, these tendencies remained until 6 h after reperfusion. In other words, the severity of CS can be judged by TEWL levels.

According to a report by Aoki et al. [16], in a disaster situations, a triage model which includes pulse rate (> 120/min), delayed rescue (> 3 h), and abnormal urine colour, are valid assessments for patients with CS. Similarly, we suggest that the severity may be judged using a combination of skin colour and vital signs (HR and BT). Immediately after rescue, skin colour assessment is limited in humans. However, broadly speaking, a red colour indicated a milder type of CS, while deep violet indicated a more severe type.

Since these colour changes are due to decreasing BPF levels, it may be important to assess the pulse in the affected limb (Fig. 2). We demonstrated that changes in BPF levels could not be observed in the HL area in mild and severe CS groups immediately after reperfusion. Therefore, immediately after rescue, a mild type of CS may be denoted by red skin colour and decreasing pulse, whilst a severe type of CS may be denoted by deep violet colour and decreasing pulse. It is suggested that changes in skin colour result from cyanosis secondary to low perfusion. Furthermore, as shown in Table 2, decreases in vital signs such as HR and BT were able to reflect a severe type of CS because CS rats demonstrated that cardiac failure was due to hyperkalaemia. Hence, we suggest that decreasing HR and BT were caused by cardiogenic shock.

In summary, the skin insults in hairless rats with CS were induced by crush injury and ischemia reperfusion injury,

and the skin conditions and severity of CS were predictable. These models may be especially useful for distinguishing earthquake victims at high risk of severe CS from those at lower risk (SDC Fig. 5). We believe that better triage assessment techniques can be developed from these results.

Conclusion

We have demonstrated that the severity of CS and the prognosis of CS can be predicted by assessing skin damage in rats. We suggest that these findings may be useful to assist medical professionals who are not experts in disaster situations to identify earthquake victims who are at high risk of severe CS by skin colour of red, deep violet or white either.

Compliance with ethical standards

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