

A Single Intravenous Infusion of Human Chorionic Mesenchymal Stem Cell Decreases Early Burn Mortality in a Rodent Model

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Research Article

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Abstract

Background:

Systemic inflammatory responses (SIR) are the main cause of pulmonary dysfunction leading to mortality within hours of extensive burns. Based on previous studies showing that cell entrapment occurs in the lungs following the infusion of human chorionic mesenchymal stem cells (hcMSCs), we hypothesize that the intravenous infusion of hcMSCs, with an immunomodulatory potential, will decrease the risk of SIR induced pulmonary failure leading to mortality in burn patients.

Methods:

Forty adult male Sprague-Dawley rats were randomized into two groups. Group A (sham control, n = 10) received no injury or intervention; the remaining rats (n = 30) were subjected to burns covering 40 % of the total body surface area by immersion of the dorsum in 100 °C water for 15 s under general anesthesia. Injured rats were further randomized into different treatment groups: Group B (saline only control, n = 10), Group C (saline plus culture medium control, n = 10), and Group D (saline plus 2×10^6 hcMSCs, n = 10). Culture medium or hcMSCs were given in a single infusion via the tail vein immediately after burns. Mortality was evaluated on post-burn days 7 and 14.

Results:

The overall mortality among injured rats was 30 % (9/30). In the first week post-injury, four rats in Group C and three in Group B *versus* none in Group D died. In the second week, one rat in both Groups C and D died. Altogether, mortality among Group D rats was 10 %, significantly lower than that in groups B and C combined (40 %; $p < 0.001$).

Conclusions:

We show that a single intravenous infusion of 2×10^6 hcMSCs decreased burn mortality in a severely burned animal model. However, clinical translation requires additional studies to exclude potential adverse effects and to determine the optimal dosage and timing of administration.

Background

Previous studies have mentioned that burns extending over 40 % of total body surface area can trigger a systemic inflammatory response syndrome (SIRS) in the first few hours after injuries, affecting various functions of the systemic organs [1, 2]. Although there has been a great advancement in modern burn care with early burn excision and grafting, aggressive fluid resuscitation, metabolic and nutritional support, and infection control [3–5], mortality remains high even within specialized units, especially in the aged and in cases of truly extensive deep burns [6, 7]. Pulmonary failure is the common cause of mortality. Besides the abovementioned treatments, various therapeutic agents, including high-dose

vitamin C infusions have been used in an attempt to attenuate the inflammation [8–18], but their clinical efficacy remains debatable. Persistent and intractable SIRS has become a challenging clinical problem.

In recent decades, although the immunomodulation and anti-inflammation properties of mesenchymal stem cells (MSCs) has ignited a hope in the treatment of SIRS [19–28], their beneficial effect in reducing the high mortality risk in extensive burns are seldom reported in the literature. Using an animal burn model, the present study investigated the clinical efficacy of human choriodecidual mesenchymal stem cell (hcMSC) in treating extensive burns.

Methods

Rodent burn model

The study protocol was approved by the National Taiwan University College of Medicine and College of Public Health Institutional Animal Care and Use Committee (IACUC 20130331). Animal care and handling was performed in accordance with comprehensive institutional guidelines, including continual animal monitoring and precautions to minimize pain and distress. Adult male Sprague-Dawley rats which were 6 to 8 weeks of age having weight range between 250–300 g were housed in cages at ambient temperature for 7 days to allow acclimatization prior to initiating experimental procedures. In preparation for general anesthesia, rats received nil per os for 4–6 h. Zoletil 50 (Tiletamine combined with Zolezepam) at a dosage of 20 mg/kg was administered via intraperitoneal injection, and satisfactory depth of anesthesia was confirmed using the toe-pinch test. The dorsal aspect of each rat was shaved after securing the animal in a prefabricated mold. Exposed skin was then immersed in 100 °C water for 15 s, followed by fluid resuscitation (intraperitoneal injection of 40 mL/kg of Ringer's lactate solution). Each rat was housed individually with *ad libitum* access to laboratory rodent chow (MFG; BioLASCO Taiwan Co.,Ltd) and water. Cage temperature was maintained at 32 °C and rats were monitored for signs of distress. The toe pinch test was used to monitor post-burn pain, and subcutaneous injection of 0.02 mg/kg buprenorphine was used for analgesia. Early euthanasia was proposed for rats exhibiting fluid shock, respiratory distress, pulselessness, or loss of more than 20 % of body weight. Tissue samples were obtained on post-burn day 0 to confirm burn depth. Mortality was evaluated on post-burn days 7 and 14. The study terminated on post-burn day 14. Early mortality was defined as death occurring within the first post-burn week. Surface area of burned skin was measured using ImageJ, and calculation of total body surface area (TBSA) was based on the method employed by Gouma et al. (2012) [29]. Mortality was the primary outcome measure.

hcMSC isolation and expansion

Human placentas were donated by women who had undergone cesarean sections with procedures approved by the local ethics committees of Taipei Medical University Hospital (TMU-JIRB 201501063). Written informed consent was obtained from all donors and experiments were performed in accordance

with relevant guidelines and regulations. To isolate hcMSCs, choriodecidual membrane was first physically separated from the placentas and washed with Hank's buffer to remove obvious blood clots. Clear choriodecidual tissues were then shredded with a surgical knife in a digestion buffer (SMEM medium supplemented with 0.5 mg/mL protease, 0.5 mg/mL collagenase B, and 1 mg/mL DNase I) and kept overnight at 4 °C. After pipetting and filtering the digestion buffer containing the tissue fractions through a 100 µm cell strainer, cells were collected by centrifugation and washed with blank medium several times. The cells were subsequently re-suspended in culture medium (MCDB201 supplemented with 1 % insulin transferrin selenium, 10 ng/mL epidermal growth factor, and 1% penicillin/streptomycin) and planted in culture dishes coated with human collagen type IV. After 24 h, the dishes were shaken horizontally and washed with blank medium to remove non-adherent cells. Finally, the adherent cells were kept in the culture medium which was changed every 3–4 days.

Control and experimental groups

Rats (N = 40) were randomized to two groups. Group A (sham control, n = 10) received no injury or intervention. Remaining rats were subjected to burns covering 40 % of TBSA, and further randomized to infusion treatment groups: Group B (saline only control, n = 10), Group C (saline plus culture medium control, n = 10), and Group D (saline plus 2×10^6 hcMSCs, n = 10). Culture medium which consisted of MCDB-201 medium supplemented with L-glutamine and 30 mM HEPES (product number M6770, Sigma-Aldrich) or hcMSCs were given in a single infusion via the tail vein immediately after burns.

Statistical analysis

Analysis of variance (ANOVA) was used for mortality comparisons among independent groups, while the log-rank (Mantel-Cox) test was used to compare survival distributions. Values of $p < 0.05$ were considered statistically significant.

Results

Rat mean body weight (n = 40) was 258 ± 23 g, and mean burn surface area was 49.6 ± 12.9 cm² (constituting 38 ± 4 % of TBSA). Histology confirmed deep dermal to full-thickness burns. The experimental results were shown in Fig. 1 and the survival distributions among groups were compared using log-rank test as in Fig. 2. The survival rate in Group D was significantly higher than that in Groups B or C ($p < 0.01$), and was lowest in Group C. In the first week post-injury, mortality was zero in Group D, while it was 35 % in Groups B (3/10) and C (4/10) combined. In the second week post-injury, one rat each in Group C and Group D died. The overall mortality rate in the present study was 30 % (9/30): 10 % (1/10) of injured rats received an hcMSC infusion, and 40 % (8/20) of injured rats did not receive an hcMSC infusion. The burn healing course was demonstrated by a representative rat in each group as shown in Fig. 3.

Discussion

In the present study, a rat model with a mean full-thickness burn area of 38 ± 4 % of TBSA was generated by immersing the rat dorsum in 100°C water for 15 s. Without therapeutic intervention, the model exhibited a 40 % mortality rate, simulating a clinical picture of severe burn injuries. Given that data regarding the relationship between burn size and mortality in small mammals is lacking [28, 29], it is always challenging to create a regular and uniform reproducible burn model for mortality study. The variations in the burn depth in different model designs and post-burn care protocol resulted in different mortality rates as reported in the literature. While one study demonstrated 62.5 % mortality rate in rats with burns covering 26–30 % of TBSA [30], the others reported 10 % mortality in a 30–40 % of TBSA burn model [31, 32].

Large burns cause an elevation of serum cytokines levels which have been demonstrated both in humans and animals after thermal burns and associated with mortality. Our results showing that 77.8 % (7/9) of mortalities occurred in the first week after the onset of burn injury was compatible with the statement that the serum IL-6 levels peaked during the first hours after burn injury and were proportionate to the burned size [33]. In animal studies, the serum levels of proinflammatory cytokines were found to increase from days 2 to 7 after infliction of burns of varying degrees [8, 34]. The elevated serum cytokines cause an increase of systemic capillary permeability resulting in protein leakage into the interstitial space, generalized edema, and eventually hypovolemic shock [35, 36].

The present study proved that intravenous infusion of hcMSC attenuated SIRS in large burns. Although some might argue that there was a lack of evidence in serum cytokine level measurement, our study results were supported by cytokine data from other similar studies. Carolina et al. (2016) demonstrated that intradermal subcutaneous injections of MSCs altered plasma cytokine levels in burned rats [37]. Using a 30 % TBSA burned rat model, Liu L. et al transplanted 5×10^6 GFP-labeled human umbilical cord mesenchymal stem cells (HUCMSCs) at day 3 after burn via a tail vein injection [22] and concluded that HUCMSCs remarkably decreased the quantity of infiltrated inflammatory cells and levels of IL-1, IL-6, TNF- α and increased levels of IL-10 and TSG-6 in wound. In another study, a reduction in the plasma levels of proinflammatory cytokines IL-6, IL-1 β and TNF- α was proved to result in a low mortality rate [21].

Although the immune modulation and immune suppression properties of MSCs have been proved in animal studies, their bio-distribution following intravenous injection is a critical issue of discussion. As they are relatively large cells and express various adhesion molecules, our previous study has shown that the majority are trapped within capillaries of various organs, especially in the lungs before the cells reach their target following injections. Although Liu L. et. al mentioned that HUCMSCs migrated to the burn wounds two weeks after injection via the tail vein, Su, LJ et al., by injecting albumin-conjugated fluorescent nanodiamonds (FNDs) pcMSCs via internal jugular vein in a miniature pig for quantitative tracking, mentioned that 80 % of the injected pcMSCs was found in the lung 24 h after intravenous delivery, and decreased to 75 % after a week [38]. Based on a previous study result showing that elevated IL-1 beta was found in the lung tissue after severe burns [39], the entrapment of hcMSCs in lung has

become a therapeutic advantage in treating the deteriorating pulmonary functions caused by cytokine storms [40]. Because of the bioactivities of hcMSCs in the wound [37], we assumed that the entrapped MSCs in the lung decrease neutrophil and macrophage infiltration, as well as proinflammatory cytokine production including levels of IL-1, IL-6 and TNF- α [38, 39, 41, 42], confirming the beneficial effects of the intravenous infusion of hcMSCs on the severely burned rats.

The present study proved that a single dosage of 2×10^6 hcMSCs intravenous infusion was sufficient to have beneficial effects on the burn outcome. Due to the absence of standardized dosage, the dosage used in the present study was based on our previous animal study [40]. As over-dosage may lead to pulmonary embolism and the problem of progressive cell apoptosis may affect efficacy, further investigations are necessary for the study of optimal dosage based on burn severity, half-life of the infused hcMSCs, and body weight of the recipient. Moreover, some may question our choice of using hsMSCs in the study. The reason was because hsMSCs was the only source of stem cells available in our stem cell culture research laboratory. Its advantages include ready availability as a waste product of delivery, low major histocompatibility complex molecule expression, ease of cell isolation and culture, and no requirement of invasive surgical procedures in the donors. However, the associated risk of protumorigenic effects is the main complication that deters its future clinical applications [22, 43], although some argue that the risk is relatively low in hsMSC compared to adipose or bone marrow derived MSCs. Further investigations are necessary to assess the safety and efficacy of the hsMSC treatment and our study results will be useful for the design of future translational researches on infusion stem cell therapy in extensive burns.

The major limitation of the present study was the lack of data showing the impact of hcMSC treatment on serum cytokine levels, although previous studies in the literature have proved that hcMSC decreased the serum cytokine levels. Another drawback was the decrease in sample size resulting from successive mortalities. In addition, in the study, late mortality after 14 days was not investigated and the status of wound healing was not assessed.

Conclusion

We have shown a single intravenous infusion of 2×10^6 hcMSCs decreased burn mortality in a severely burned animal model. However, clinical translation requires additional studies to exclude potential adverse effects, and determine optimal dosage and timing of administration.

List Of Abbreviations

systemic inflammatory response syndrome (SIRS)

mesenchymal stem cells (MSCs)

human chorionic mesenchymal stem cell (hcMSC)

College of Medicine and College of Public Health Institutional Animal Care and Use Committee (IACUC)

total body surface area (TBSA)

Spinner Minimum Essential Medium (SMEM)

Deoxyribonuclease I (Dnase I)

human umbilical cord mesenchymal stem cells (HUCMSCs)

fluorescent nanodiamonds (FNDs)

Declarations

Ethics approval and consent to participate

The study protocol was approved by the National Taiwan University College of Medicine and College of Public Health Institutional Animal Care and Use Committee (IACUC 20130331). Animal care and handling was performed in accordance with comprehensive institutional guidelines, including continual animal monitoring and precautions to minimize pain and distress.

Human placentas were donated by women who had undergone cesarean sections with procedures approved by the local ethics committees of Taipei Medical University Hospital (TMU-JIRB 201501063). Written informed consent was obtained from all donors and experiments were performed in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable

Data availability statement

Raw data were generated at National Taiwan University Hospital. Derived data supporting the findings of this study are available from the corresponding author Ek Yeong on request

Competing interests

The authors declare that they have no competing interests

Funding

Author contributions:

Eng-Kean Yeong: Conception and design, provision of study material or patients, collection and assembly of data, data analysis and interpretation, manuscript writing.

Thai-Yen Ling: Conception and design, provision of study material, data analysis and interpretation.

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Figures

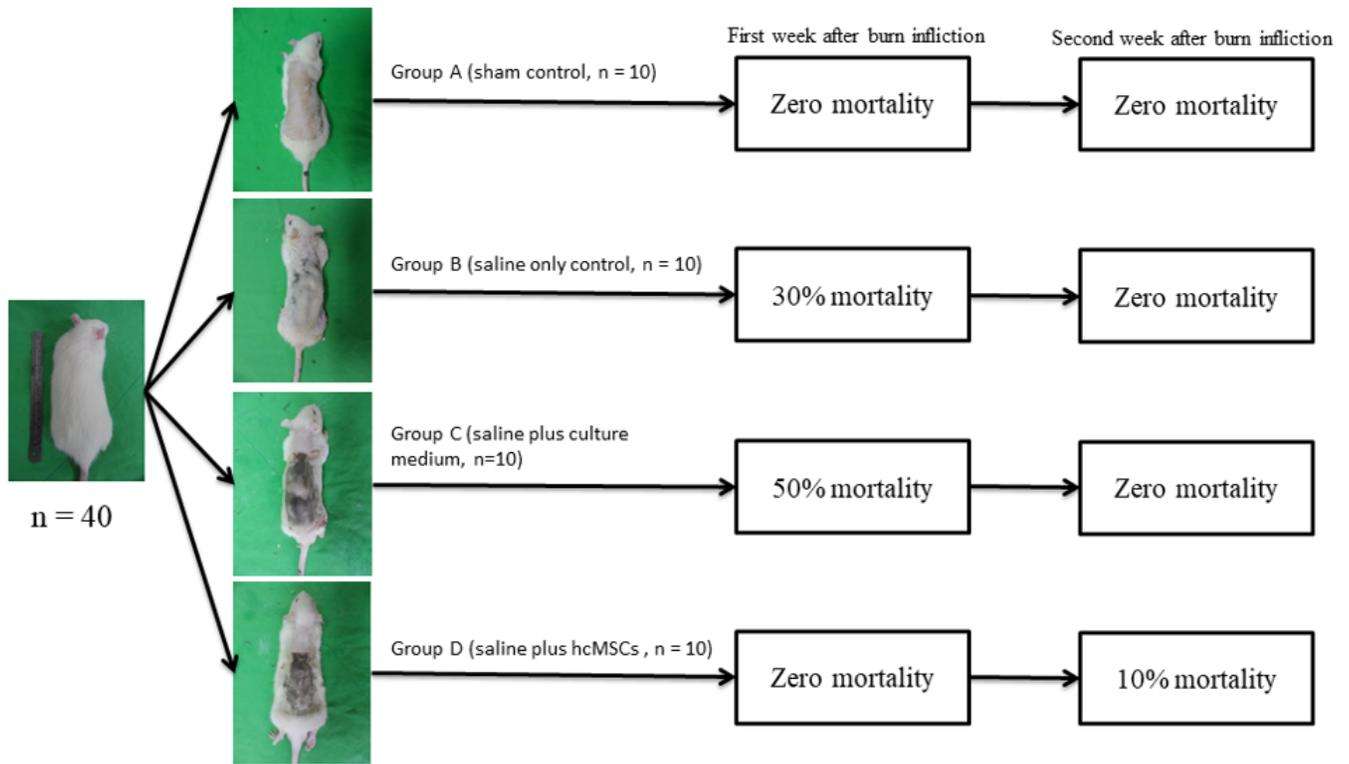


Figure 1

Experimental results of each group.

Log-Rank Test

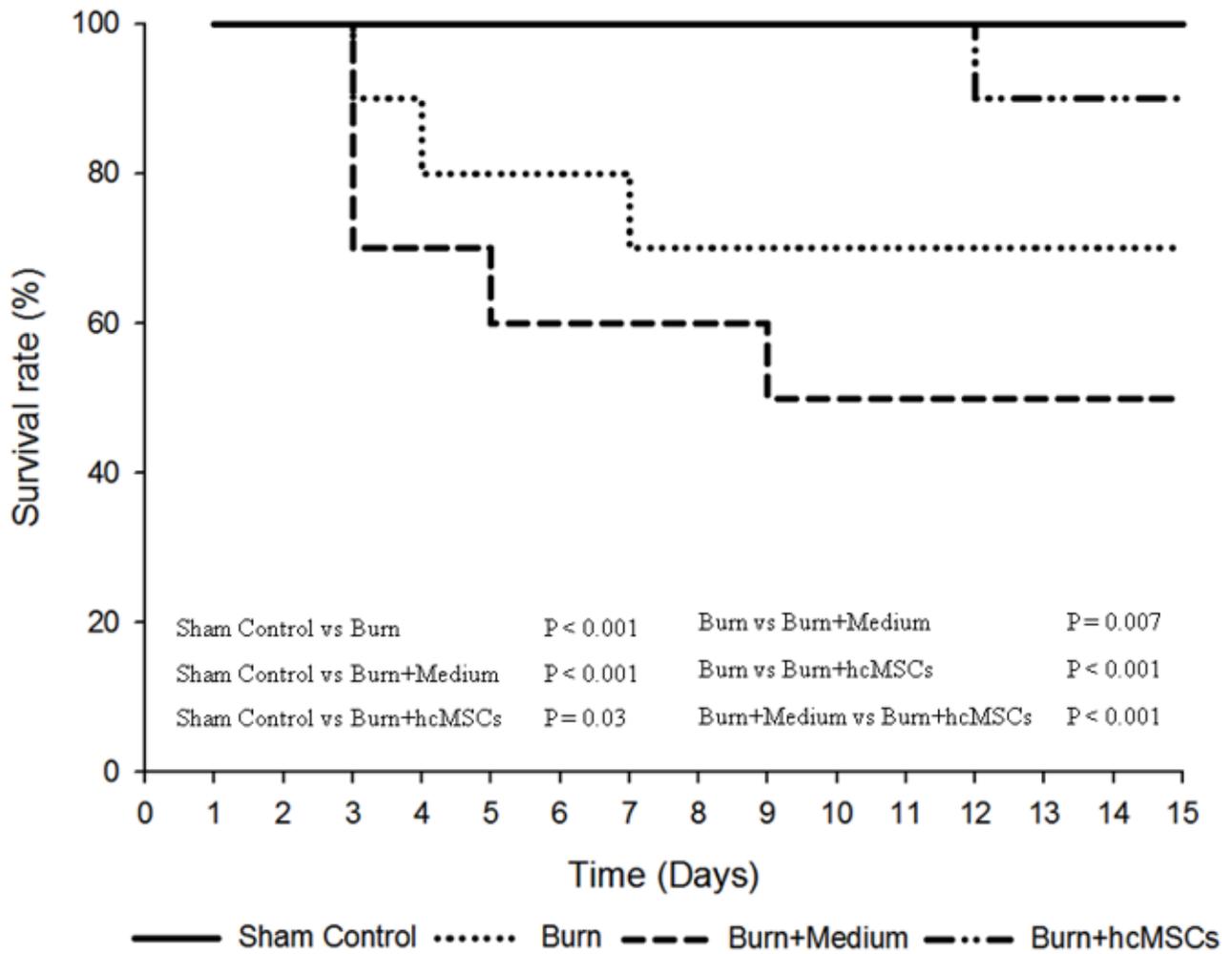


Figure 2

Survival distribution among the groups as per the log-rank test.

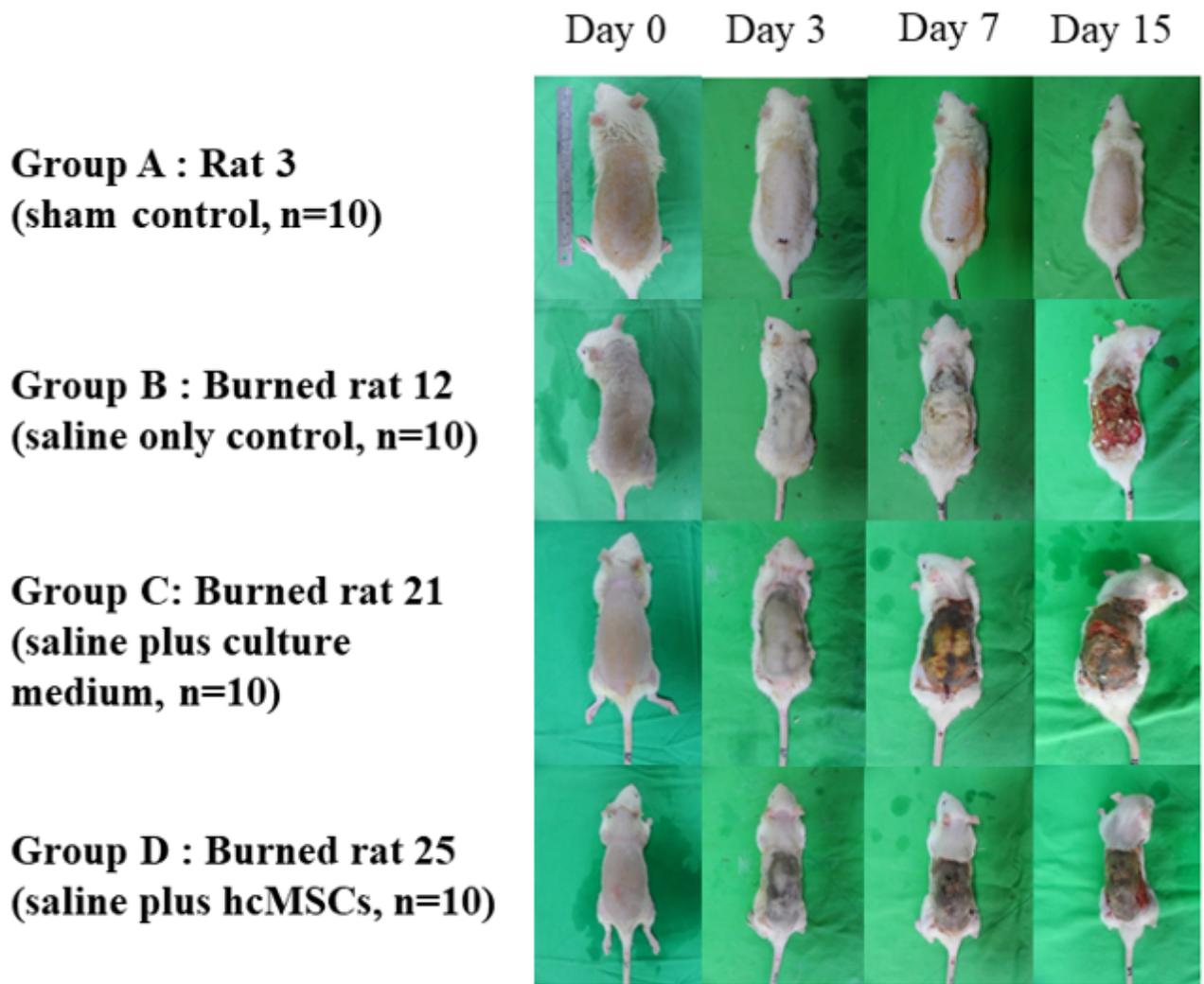


Figure 3

Representative images showing the burn healing course in each group