



ORIGINAL ARTICLE

Serial Changes of Heat Shock Protein 70 and Interleukin-8 in Burn Blister Fluid

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Background: It has been reported that heat shock protein 70 (HSP70) and interleukin-8 (IL-8) play an important role in cells during the wound healing process. However, there has been no report on the effect of HSP70 and IL-8 on the blisters of burn patients. **Objective:** This study aimed to evaluate the serial quantitative changes of HSP70 and IL-8 in burn blisters. **Methods:** Twenty-five burn patients were included, for a total of 36 cases: twenty cases on the first day, six cases on the second, five cases on the third, three cases on the fourth, and two cases on the fifth. A correlation analysis was performed to determine the relationship between the concentration of HSP70 and IL-8 and the length of the treatment period. **Results:** The HSP70 concentration was the highest on the first day, after which it decreased down to near zero. Most HSP70 was generated during the first 12 hours after the burn accident. There was no correlation between the concentration of HSP70 on the first day and the length of the treatment period. No measurable concentration of IL-8 was detected before 5 hours, but the concentration started to in-

crease after 11 hours. The peak value was measured on the fourth day. **Conclusion:** While HSP70 increased in the first few hours and decreased afterwards, IL-8 was produced after 11 hours and increased afterward in burn blister fluid. These findings provide new evidence on serial changes of inflammatory mediators in burn blister fluid. (*Ann Dermatol* 29(2) 194~199, 2017)

-Keywords-

Heat-shock protein 70, Interleukin-8, Partial thickness burn, Partial thickness burn blister, Wounds and injuries

INTRODUCTION

Burn wound healing is a complex process that involves various mediators and multiple cell types around the injured zone. A second-degree burn, also called a partial thickness burn, causes the skin to blister. Burn blisters fluid might be important roles in wound healing process¹. Some reports indicate that retention fluid from blister of partial thickness burn, which contains relatively large amounts of cytokines and soluble mediator, may affect the wound healing process²⁻⁴. However, the effect of burn blister fluid on wound healing has not been fully explored. Heat shock protein 70 (HSP70, molecular weight 70 kDa) is the most well understood member of the heat shock family of proteins. HSP is found in all cells and has a chaperone function during the assembly process of protein folding and polypeptide formation as well as a cell protection function. Specifically, extracellular HSP70 is involved in intercellular signaling and is involved in the innate, adaptive immunologic response, interrupts apoptosis, and protects cell structure as a chaperone⁵⁻⁷. HSP70

Received March 11, 2016, Revised July 22, 2016, Accepted for publication July 27, 2016

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can both induce and arrest inflammatory reactions in experimental brain injury and ischemia. It is reported that HSP70 is involved in the healing process, as it has been found in the epidermis and dermis of thermal injury patients⁸⁻¹⁰. If HSP70 is present in burn blisters, it likely affects the damaged cells of thermal injury patients.

Inflammation is an integral phase of wound healing. The local inflammatory response of a skin wound is characterized by increased amounts of several pro-inflammatory cytokines, such as interleukin-8 (IL-8), tumor necrosis factor- α , and prostaglandin E2, and local neutrophil infiltration¹¹. Excessive and ongoing wound inflammation inhibits the healing of burn wounds by fostering an unabated influx of leukocytes to the wound site¹², which directly contributes to the progressive deepening and extension of the wound¹³. This inflammatory response is amplified by an elevated IL-8 level in the wound tissue¹⁴. However, IL-8 has also been shown to act on epithelial cells or endothelial cells to promote migration, invasion, proliferation, and angiogenesis *in vivo* and enhanced wound healing due to induced reepithelialization¹⁵⁻¹⁷.

There are various opinions regarding the treatment method for the blisters of partial thickness burns, including removing the blister roof, extracting the fluid maintaining the blister roof, or leaving the blister intact¹⁸⁻²⁰. A better understanding of the changes in heat shock proteins and pro-inflammatory factors over time is expected to lead to an improved blister management strategy.

This study aimed to determine whether HSP70 and IL-8 are present in burn blisters, the variation in their concentrations over time, and the influence of HSP70 concentration on the length of the treatment period.

MATERIALS AND METHODS

Study subjects

For this study, patients with partial thickness burn with bullae who were aged 18 years and over and agreed to cooperate during the period from October 1, 2013 to March 31, 2014 were selected. Patients with immune-related diseases, with diseases that can induce an immune unbalance, and who were undergoing cancer treatment or other hormonal treatments were excluded. For all patients, information regarding sex, age, treatment period, total burn surface area (TBSA), and the abbreviated burn severity index (ABSI) score was obtained. The protocol for this study was reviewed and approved by the institutional Ethics Committee for human studies of Hangang Sacred Heart Hospital, Burn Center, Seoul, Korea (2013-068). We considered p -values < 0.05 to be statistically significant.

Collection of blister fluid

After informed consent was obtained, blister fluid was aspirated from the intact burn blisters of patients with thermal burns who were admitted to our Emergency Department of Hangang Sacred Heart Hospital. Aspiration of the blister fluid was performed with an 18-gauge needle. The bullous fluid was first collected while leaving the blister roof intact. On the next visit, the fluid from the same bullae was collected if fluid remained. The collected amount for each case was 1 ml. We set the time after injury to sampling as the collected time. The first day was defined as the time between 1 hour and 12 hours after the damage occurred, the second day was between 25 and 36 hours, the third day was between 49 and 60 hours, the fourth day was between 73 and 84 hours, and the fifth day was between 97 and 108 hours. To ensure consistent conditions, any case that did not meet the criterion was excluded from the analysis. The end of the treatment period was determined by a plastic surgeon according to the treatment results.

Heat shock protein 70 and interleukin-8 quantification in blister fluid

The concentrations of HSP70 and IL-8 were measured using enzyme-linked immunosorbent assay (ELISA) kits. ELISA was used to measure IL-8 and HSP70 in the bullae of burn patients. The quantitative measurement of IL-8 and HSP70 was performed in duplicate samples using commercial ELISA kits (Cat. No. KAC1301; BioSource, Nivelles, Belgium) for IL-8 and HSP70 (Cat. No. ab13306; Abcam, Cambridge, UK) according to the manufacturer's instructions. Each sample was diluted 100-fold (HSP70) or 5-fold (IL-8) with dilution solution, and the samples were centrifuged for 5 minutes at 4°C and 12,000 rpm to remove other debris and blood cell components. Then, 100 μ l of the standard and bullae samples were placed in an antigen-coated 96-well plate and left for 2 hours at room temperature, after which they were washed four times with 400 μ l washing solution. Next, anti-HSP70 and anti-IL-8 conjugates were placed in the wells followed by incubation for 2 hours at room temperature. After washing, each well was filled with a freshly prepared chromogenic solution, and the plate was incubated for 30 minutes. Finally, the reaction was stopped with by adding stop solution (1.8 N H₂SO₄), and the plate was read at 450 nm using a DTX880 multimode detector (Beckman Coulter, Brea, CA, USA).

Statistical analysis

All statistical analyses were conducted using PASW Statis-

tics 18.0 (IBM Co., Armonk, NY, USA). The results were expressed as means ± standard deviations or median values and interquartile range (IQR). A correlation analysis was performed to determine the relationship between the concentration of HSP70 and IL-8 and the length of the treatment period. The correlation analysis was performed using the Spearman test. We used the Mann-Whitney U-test to compare non-normally distributed variables.

RESULTS

Among 53 total cases (29 patients), 36 cases (25 patients) that met the inclusion criteria were analyzed. Twenty cases from the first day, 6 cases from the second day, 5 cases from the third day, 3 cases from the fourth day, and 2 cases from the fifth day were evaluated. Table 1 presents the demographic data of the patients. Sixteen patients were male, and 9 patients were female. The average age was 44.20 years old (minimum, 18 years old; maximum, 90

Table 1. Demographic data

| Variable | Value |
|----------------------|---------------|
| Sex (male/female) | 16/9 |
| Age (yr) | 44.20 ± 18.21 |
| TBSA (%) | 3.12 ± 2.15 |
| ABSI | 4.00 ± 1.15 |
| Treatment period (d) | 15.08 ± 7.92 |

Values are presented as number only or mean ± standard deviation. TBSA: total burn surface area, ABSI: abbreviated burn severity index.

years old), the average TBSA was 3.12% (minimum, 1%; maximum, 8%), and the average ABSI score was 4.00 (minimum, 2; maximum, 7). The average treatment period was 15.08 days (minimum, 6 days; maximum, 37 days). Age, TBSA, ABSI score, and treatment period were not normally distributed, and there was no significant correlation between them and HSP70 and IL-8.

Heat shock protein 70

The median concentration of HSP70 was the highest on the first day (median, 5.555 ng/dl; IQR, 1.602 ~ 12.164 ng/dl). On the second day and third day, the median values were 0.038 ng/dl (IQR, 0.007 ~ 0.058 ng/dl) and 0.170 ng/dl (IQR, 0.058 ~ 0.552 ng/dl), respectively. HSP70 was not detected on the fourth and the fifth days (Fig. 1A). The majority of the HSP70 was found in the first 12 hours. The highest median HSP70 value was measured 1 hour after the damage, at 9.261 ng/dl (IQR, 7.347 ~ 15.345 ng/dl). After 1 hour, the concentration decreased rapidly: 7.285 ng/dl (IQR, 1.732 ~ 14.515 ng/dl) after 2 hours, 2.038 ng/dl after 4 hours, 0 ng/dl after 5 hours, 1.264 ng/dl after 11 hours, and 0.182 ng/dl after 12 hours (Fig. 1B). There was no correlation between the concentration of HSP70 and treatment period during the first 12 hours ($\rho = -0.316, p = 0.175$).

Interleukin-8

The concentration of IL-8 was measured from the first day to the fifth day. The median concentration of IL-8 was 0 pg/dl on the first day, 1,477.245 pg/dl (IQR, 733.622 ~ 2,302.500 pg/dl) on the second day, 511.429 pg/dl (IQR,

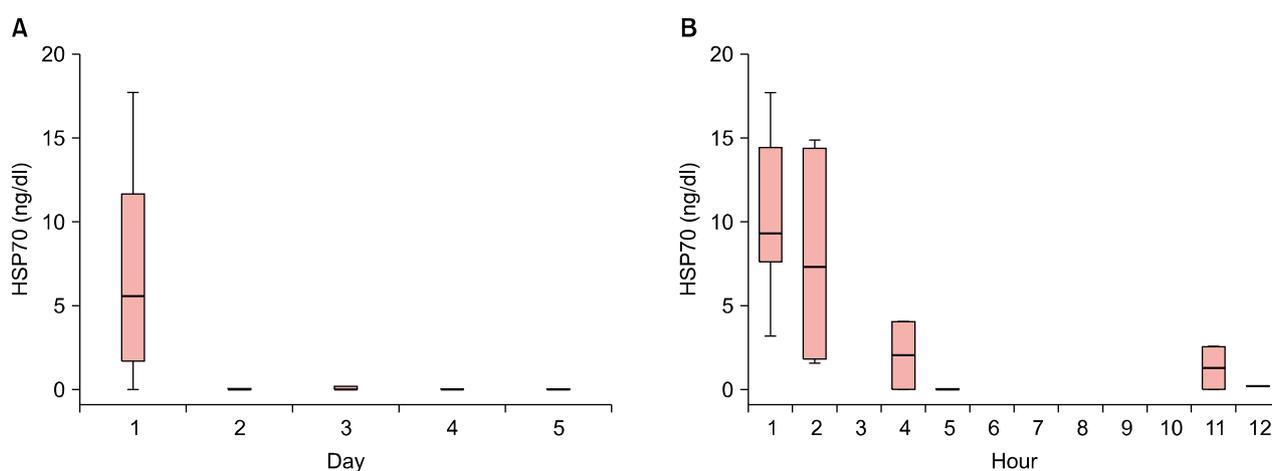


Fig. 1. (A) The heat shock protein (HSP) concentration variation within partial thickness burn blister. The HSP70 concentration was the highest on the first day (up to 12 hours after the damage). Then, the value drastically dropped down to near zero on the second day. There was a small increased on the third day and no significant amount was found on the fourth and fifth day. (B) The concentration of HSP70 within partial thickness burn blister of up to 12 hours after damage. The HSP70 concentration after one hour of the damage was measured to be the highest. After one hour, the concentration drops rapidly.

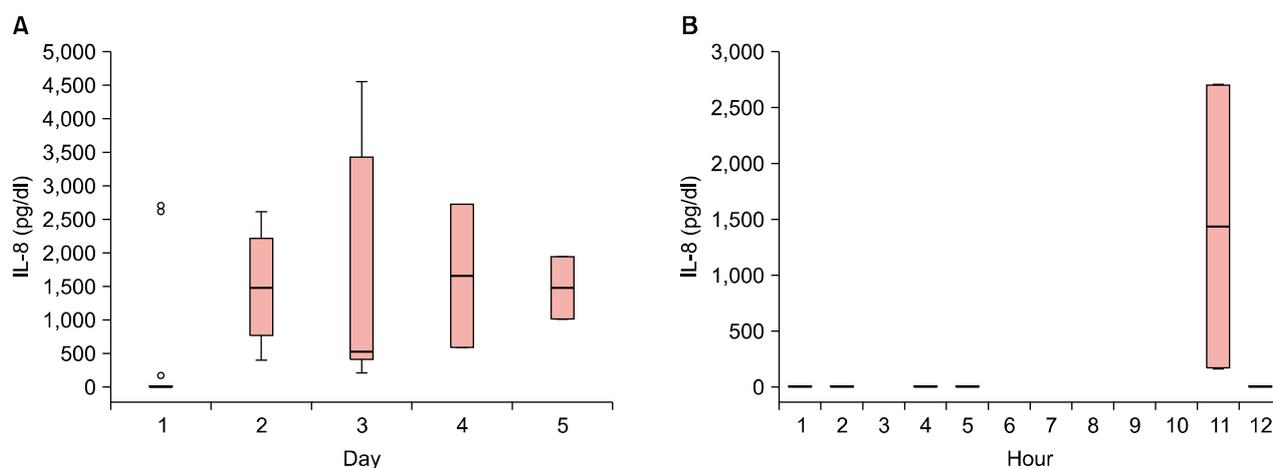


Fig. 2. (A) Variation of the interleukin-8 (IL-8) concentration within partial thickness burn blister over the time. The IL-8 concentration was measured to be the highest on the fourth day. (B) The IL-8 concentration within partial thickness burn blister up to 12 hours after damage. IL-8 was not observed up to 5 hours of the occurrence.

322.483~4,021.122 pg/dl) on the third day, 1,653.010 pg/dl (IQR, 584.388~2,187.322 pg/dl) on the fourth day, and 1,473.6735 pg/dl (IQR, 1,006.071~1,707.477 pg/dl) on the fifth day (Fig. 2A). When the concentration of IL-8 within the first 12 hours was analyzed, IL-8 was not detected up to 5 hours after the damage (Fig. 2B). There was no correlation between the concentration of IL-8 and treatment period ($\rho = -0.326$, $p = 0.053$).

DISCUSSION

HSP70 is released in a passive manner in necrotic cells and is also secreted through an active mechanism involving lysosomal vesicles and lipid rafts²¹. The functions of HSP70 include acting as a molecular chaperone to facilitate the assembly of proteins, targeting misfolded and damaged proteins for degradation, disassembling protein aggregates, acting as a regulatory molecule in protecting cells from thermal or oxidative proteins, and inhibiting apoptosis⁸. In addition, HSP70 is involved in inflammatory reactions and the healing process of the epidermis and dermis following thermal injury⁸⁻¹⁰. HSP70 has an extracellular function that appears to potentiate the immune response, whereas the intracellular mechanisms appear to be anti-inflammatory. HSP70 can also interrupt the cleavage of pro-matrix metalloproteinases to an active form, which in turn may reduce immune signaling²².

The concentration of HSP70 was found to be very high only on the first day. It was weakly detected on the second day. A small amount was found on the third day. Finally, no detectable amount was found on the fourth and the fifth days. Because the concentration of HSP70 was the highest in the first 12 hours, the results from 1

hour to 12 hours after the burn injury were analyzed in detail. The concentration was the highest 1 hour after the injury (Fig. 1B). Then, the concentration decreased rapidly afterwards. It was weakly detected after 5 hours. Even though there were not many cases included in this study, the results indicated a pattern that HSP70 is present in blisters for the first few hours, after which the amount decreases. In this study, no correlation was found between the concentration of HSP70 during the first 12 hours and the length of the treatment period ($\rho = -0.316$, $p = 0.175$). A major burn injury induces an inflammatory response, which is accompanied by the release of various cytokines²³. The chemokine IL-8 induces chemotaxis in neutrophil granulocytes (polymorphonuclear leukocytes, PMNs), causing them to adhere to endothelial cells and then move into tissue cells to induce PMN-mediated damage²⁴. Therefore, many inflammatory mediators are involved in multiple steps of the systemic inflammatory response syndrome to a trauma or severe burn, and they may be used as an index to estimate the extent of the inflammatory response or prognosis. IL-8 has been shown to play a pivotal role in the activation of the pro-inflammatory cascade in necrotizing enterocolitis (NEC)^{25,26}, and it has been recently demonstrated that IL-8 may serve as a potential predictive marker in diagnosing NEC. Hans-Oliver reported that IL-8 increases the amount of the intracellular integrin $\alpha 6$ subunit, keratinocytes proliferation, and the number of mitotic keratinocytes and enhances reepithelialization¹⁷. Berney et al.²⁷ reported that the serum IL-8 concentration increased after several days or the first day in acute pancreatitis, reaching a plateau between days 2 and 5, and also reported that the concentration of serum IL-8 in a severe case increased on the day the patient visited the

emergency department. Lee et al.²⁸ reported that immunomodulatory effect of interleukine expressed.

In this study, the changes in IL-8 levels in burn blister fluid were investigated. The IL-8 concentration in partial thickness burn cases was found to be the highest on the fourth day. IL-8 was not detected up to five hours, but it was detected after 11 hours. Cases for 6 to 10 hours are not included in this study. However, no relationship between the IL-8 concentration in burn blisters and the length of the treatment period was found.

Our results suggest that IL-8 is an important mediator in inflammatory changes following a burn injury but that various factors may influence the concentrations of this cytokine. Luo et al.²⁹ demonstrated a novel role for exogenous human HSP70 in suppressing the production of IL-6, IL-8, and monocyte chemoattractant protein-1 in fibroblast-like synoviocytes that involves inhibiting the activation of the mitogen-activated protein kinases and nuclear factor- κ B signaling pathways.

There is still a debate regarding how to best treat the blisters of burn patients¹⁹⁻²¹. This study showed that HSP70 increased in the first few hours and decreased afterwards and that IL-8 was produced after a few hours and increased afterwards. It is speculated that HSP70 present in a blister has a pro-inflammatory effect outside the cell and a protective effect in the intracellular space of the ischemic cell zone³⁰. If this is the case, then the proper treatment would be to remove the blister fluid during the period when HSP70 is present. In addition, a better understanding of the changes in IL-8 concentration in partial thickness burn blisters would help to improve blister management.

Because this study was based on patient schedules, it was not possible to obtain all of the data on the cases for the targeted times. In addition, the number of cases was limited because we counted the first 12 hours as one day; thus, we likely lost some cases, and there were many cases for which we could not re-sample the fluid from the same blister.

This study had limitations and could not clearly define the healing mechanism in burn blister fluid, but the results demonstrate the changes in the concentrations of HSP70 and IL-8 and may help determine the guidelines for when blister fluid should be removed.

Various inflammatory mediators function during the healing process. To better understand the immunological processes during wound healing and how to best treat wounds, additional studies on the changes in HSP70 and IL-8 levels over time as well as those of other mediators are necessary.

ACKNOWLEDGMENT

This work was supported by National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2014R1A1A3A04049491), a grant from the Hallym University Medical Center Research Fund (01-2007-13), Hallym University Hangang Research Fund (2013-068), and Hallym University Research Fund 2014 (HURF-2014-58).

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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