



Local Hyperthermia Affects Murine Contact Hypersensitivity around Elicitation Phase

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Dear Editor:

Murine contact hypersensitivity (CHS) is an animal model of human allergic contact dermatitis triggered by twice exposures to contact allergens. It is a prototype of delayed-type hypersensitivity reaction, and is classically divided into two distinct phases, sensitization and elicitation. In the sensitization phase, cutaneous dendritic cells capture antigens, migrate to draining lymph nodes, and present antigens to T cells to sensitize them. In the elicitation phase, sensitized T cells migrate to the skin and produce inflammatory mediators¹. Many factors can influence this progress, such as ultraviolet radiation, stress and mild thermal stimuli²⁻⁴.

Danno and Sugie⁵ demonstrated that single exposure of mouse skin to near-infrared radiation reversibly suppressed the proliferation of epidermis, the density of Langerhans cells (LCs), and the induction of CHS. Ostberg et al.⁶ found that mild fever-range whole body hyperthermia (FR-WBH) before sensitization could reduce CHS, while mild FR-WBH after elicitation could enhance the CHS. In our previous study, local hyperthermia was pre-, concurrent- or post-applied in the sensitization phase, respectively. After the elicitation phase, the severity of ear swelling was inhibited in pre-heated group, and the suppression showed a temperature-dependent manner. In contrast, the intensity of CHS was exacerbated by concurrent-heated treatment or post-heated treatment⁷.

There is a lack of local hyperthermia data on elicitation phase in CHS. In this study, sixty-five 6~8 weeks old fe-

male BALB/c mice, weighting at 20 ± 2 g were randomly allocated into pre-heated, concurrent-heated, post-heated and control groups. Twenty mice were allocated to each of the first three groups and 5 mice were allocated to control group. Each of the first three groups were divided into 4 subgroups; each subgroup consists of 5 mice was subjected to local hyperthermia at temperatures of 37°C, 39°C, 41°C, and 43°C respectively by a patented device (patent name: a hyperthermia device; patent no.: ZL 200720185403.3; patent holder: China Medical University). The mice were sensitized with 100 μ l of 0.5% FITC (Fanbo Biochemicals, Beijing, China) on the shaved abdominal side at size of 1×1 cm². The mice were challenged on the shaved dorsal side with same size on Day 6 with the above solution. Local hyperthermia was applied to the challenged site on Day 3 (pre-heated), Day 6 (concurrent-heated) and Day 8 (post-heated) at defined temperature (37°C, 39°C, 41°C, or 43°C) for each subgroup for 20 minutes, respectively. The dorsal skin thickness was measured on Day 8 with Quick Mini thickness-gauge calipers (Mitutoyo Corporation, Kanagawa, Japan). Each site was measured 3 times and an average value was calculated. The difference value of the dorsal skin thickness was calculated by the thickness of each temperature subgroup minus the thickness of control group. Biopsies of dorsal skin were taken on Day 8 and the HE stain slices were observed under microscope (Olympus BX51; Olympus Corporation, Tokyo, Japan). All experiments were performed in accordance with the in-

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stitutional and national guidelines for the care and use of laboratory animals.

All the data were analyzed with IBM SPSS Statistics ver. 21.0 (IBM Co., Armonk, NY, USA). Quantitative results were expressed as $\bar{X} \pm SD$. The data was conducted by two-way analysis of variance (ANOVA) with Turkey test. If the interaction between treatment timing and temperature is significant, ANOVA model was included. The level of significance for multiple comparison tests was set at 0.05.

The dorsal skin thickness between treatment timing and temperature is significant ($p < 0.05$). The thickness of control was 0.776 ± 0.108 mm. In no matter pre-heated group, concurrent-heated group, or post-heated group local hyperthermia at 37°C did not change the thickness ($p > 0.05$). In contrast, local hyperthermia at 39°C, 41°C, or 43°C has significantly increased the dorsal skin thickness in all the three groups ($p < 0.05$).

In all groups, difference values of 39°C/41°C/43°C were higher than that of 37°C, respectively ($p < 0.05$). In pre-heated group, difference value of 43°C was higher than those of 39°C and 41°C, respectively ($p < 0.05$). No significant difference was seen between 39°C and 41°C ($p > 0.05$). There were no statistic significances among different temperatures in other groups ($p > 0.05$) (Fig. 1A).

At 37°C, no significant difference was seen among the three groups ($p > 0.05$). The difference value of post-heated group were significantly higher than that of pre-heated group at 39°C or 41°C ($p < 0.05$). Other comparisons showed no statistic significances ($p > 0.05$). At 43°C, no significant difference was observed among the three

groups ($p > 0.05$) (Fig. 1B).

The histological manifestations of the three groups were similar. The manifestations of control and 37°C were almost the same. Slight to moderate intracellular and/or intercellular edema was seen in epidermis and slight increment of lymphocytes were seen in both epidermis and dermis at 39°C, 41°C, and 43°C (Fig. 2).

Previous studies indicated that pre-treatment (whether systematic or local treatment) with hyperthermia at suitable temperature at sensitization phase could inhibit the CHS intensity⁶⁻⁸. In current study, local hyperthermia at 39°C, 41°C or 43°C has significantly increased the dorsal skin thickness in the three groups. The changes of histological manifestations were in line with those of skin thickness. During the elicitation phase, antigen-reactive/memory T cells can be stimulated by keratinocytes or mast cells, and while less dependent on LCs expressing major histocompatibility complex class II (MHC II)⁹. Haptens stimulate keratinocytes or mast cells to produce pro-inflammatory cytokines or neutrophil-recruiting chemokines. The pro-inflammatory cytokines activate vascular endothelial cells to express adhesion molecules. The latter can guide T cells transigrate from blood to tissues. The neutrophil-recruiting chemokines are crucial for neutrophil recruitment and subsequent T-cell infiltration¹⁰. We proposed that one or more of these processes in elicitation phase were enhanced by local hyperthermia.

In conclusion, when hyperthermia was locally applied around the elicitation phase, there were temperature-dependent increases of the intensity of CHS. Our study con-

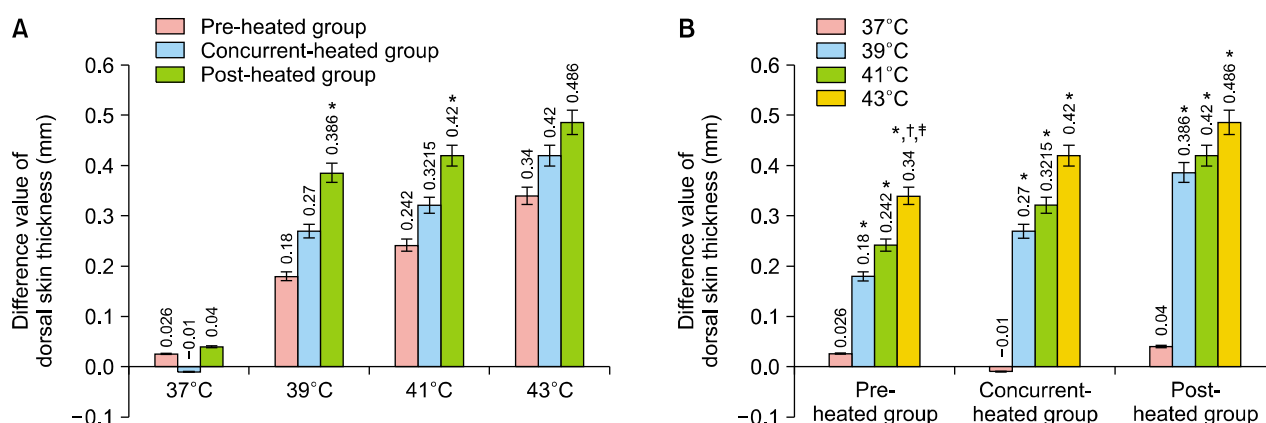


Fig. 1. (A) Comparison of the difference values at different temperatures. The difference values of 39°C, 41°C, or 43°C were higher than that of 37°C, respectively. The difference value of 43°C was higher than those of 39°C or 41°C, respectively. No significant difference was seen in concurrent-heated group and post-heated group among different temperatures. *Represented significant difference comparing to 37°C ($p < 0.05$), †represented significant difference comparing to 39°C ($p < 0.05$), ‡represented significant difference comparing to 41°C ($p < 0.05$). (B) Comparison of the difference values of different groups. No significant difference was seen among the three group at 37°C or 43°C. The difference value of post-heated group were significantly higher than that of pre-heated group at 39°C or 41°C. Represented significant difference comparing to pre-heated group ($p < 0.05$).

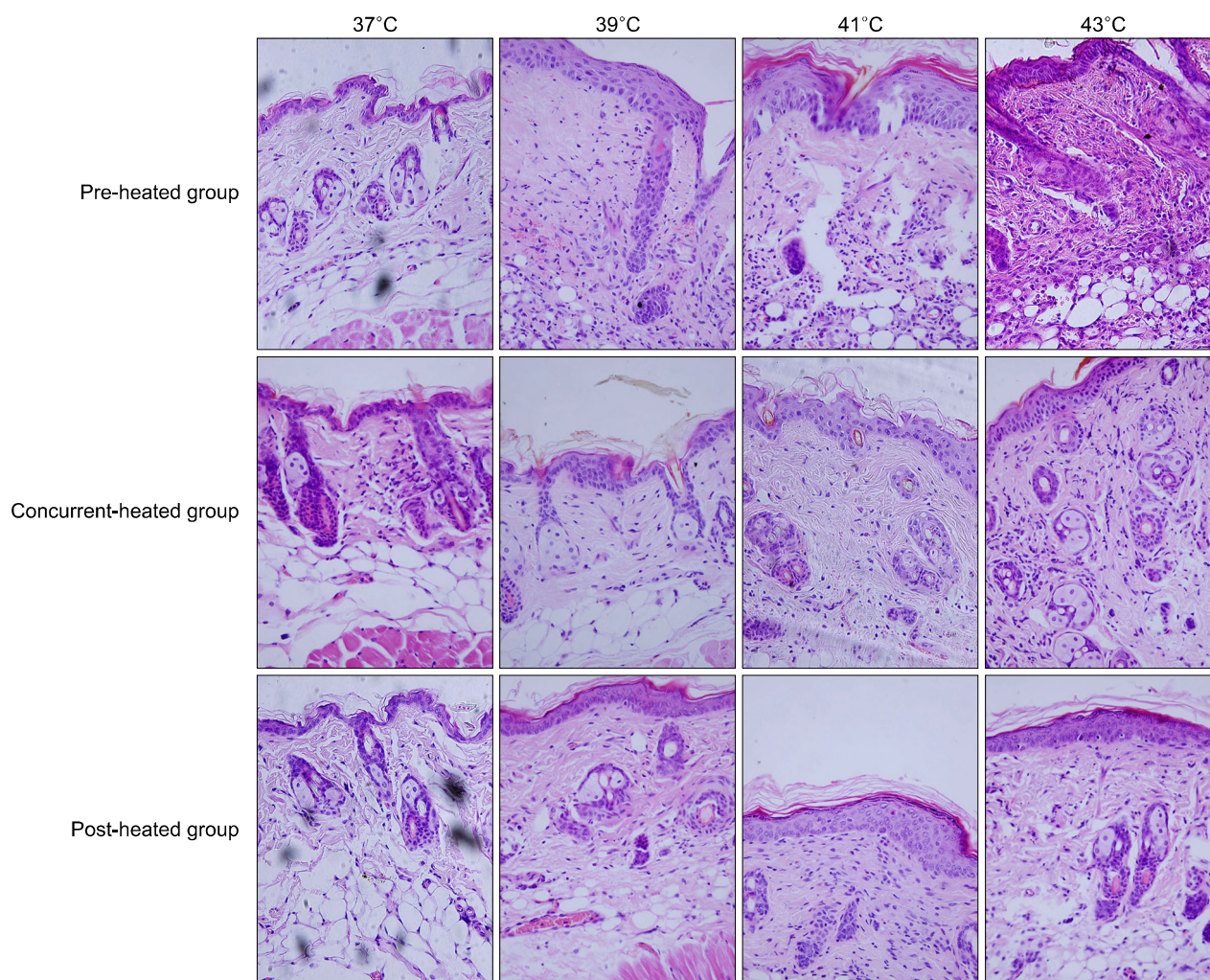


Fig. 2. Histological examination (H&E, $\times 40$). The histological manifestations of pre-heated group, concurrent-heated group and post-heated group were similar. The manifestations of control and 37°C were almost the same. The intracellular and/or intercellular edema of epidermis was slight at 39°C, and moderate at 41°C and 43°C. Increment of lymphocytes were seen in both epidermis and dermis, at 39°C, 41°C, and 43°C.

tributes to telling the complete story about the influence of local hyperthermia on CHS.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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Re: Histologic Evidence of New Collagen Formulation Using Platelet Rich Plasma in Skin Rejuvenation: A Prospective Controlled Clinical Study

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Dear Editor:

I read the article entitled "Histologic Evidence of New Collagen Formulation Using Platelet Rich Plasma in Skin Rejuvenation: A Prospective Controlled Clinical Study" published in the *Annals of Dermatology*¹ with great interest. I would like to draw your attention to two important points. First of all, while platelet rich plasma (PRP) is prepared by differential centrifugation protocol, the force exerted on the blood sample varies depending on the diameter of the centrifuge: G-force or RCF (relative centrifugal force) = $1.12 \times \text{radius of rotation in mm} \times (\text{revolutions per minute [RPM]} / 1,000)^2$. Therefore, when talking about centrifugation, one should either use the term G-force or RCF, or if RPM is used, the radius of the rotor should be provided. Secondly, the total amount of growth factor and the efficiency of the treatment depends on the platelet concentration in PRP. Accordingly, the platelet count should be

>1,000,000 platelets/ μl for an effective treatment^{2,3}. While the platelet concentration in PRP depends on the baseline platelet count of an individual, the device used, and the technique applied; it is not possible to achieve the desired PRP concentration if platelets are diluted with more than 10% of the blood sample. In the current study, however, the authors report that they obtained a PRP concentration of 2 ml (25%) from an 8 ml blood sample. Did the authors perform a platelet count from this sample to confirm that the sample was really platelet-rich?

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The methodology of this manuscript has some misstatement. I would like to draw attention for readers.

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