Original Research Article

Effect of hot fomentation on plasma HSP70 levels and body temperature

ABSTRACT

Objective: Our previous study reported that thermal stimulation on thigh muscle in vivo and skeletal muscle-derived cell (SMDC) in vitro favorably changed plasma proteins and several gene expression, respectively, to prevent atherosclerotic cardiovascular disease (ASCVD). In our previous in vitro experiment using transcriptome, hot stimulation caused a slight decrease in SMDC density, associated with higher gene expression of apoptosis-related factors as well as HSP70. In the present study, plasma HSP70 was compared before and after hot fomentation methods as a marker of heat stress. Furthermore, skin temperature was measured to assess whether the hot effect was local or systemic.

Method: Subjects were volunteers of 3 men and 7 women aged 21.8 ± 1.31 (mean ± SD). Hot fomentation consisted of four types: hot towels, hot packs, red bean bags, and thermal sheets. Plasma HSP70 concentration was determined by ELISA. Skin temperature was measured by an infrared thermometer.

Results: Plasma HSP70 concentration was significantly reduced by thermal loading using hot towels (pre: 18.46 ± 6.55 vs. post: 14.7 ± 6.66 ng / ml, $p = 0.011$), but not by those using the other 3 types. Thermal stimulated thigh skin temperature in the opposite side of the hot fomentation was significantly increased by hot towels, hot packs and red bean bags. There was no significant change in axillary temperature.

Conclusion: These results suggest that thermal sheets might not cause heat stress at least in this condition. Hot towels, hot packs and red bean bags provide systemic thermal effect. Further evaluation is required for safe and effective application of hot fomentation to ASCVD prevention.

Keywords: Hot fomentation, heat stimulation, skeletal muscle, atherosclerotic cardiovascular disease

1. INTRODUCTION

We have verified the effect of thermal stimulation on skeletal muscle as a preventive method of atherosclerotic cardiovascular disease (ASCVD). We reported that thermal loading of the femoral skeletal muscles of healthy men and women increased plasma adiponectin concentration [1], suggesting that the thermal stimulatory effect targeting skeletal muscle can be utilized for the prevention of ASCVD. In order to precisely know the mechanism, we tested the effect of thermal loading on gene expression of human skeletal muscle-derived cells (SMDC) by in vitro experiments using microarray. Thermal loading on SMDC could change gene expression to prevent ASCVD, particularly on glucose metabolism related genes such as many insulin dependent/independent glucose uptake-related factors and insulin resistance-related factors [2].

On the other hand, we found a slight decrease of SMDC density by thermal loading at 42 °C for 21 hours, where thermal loading caused several apoptosis-related gene expressions and markedly increased HSP70 gene expression [3]. Thus, it was considered that the thermal loading in this condition caused somewhat heat stress by apoptosis-related mechanism and introduced repair mechanism by HSP70. Soluble HSP70 has no intrinsic circadian rhythm at rest [4]. Therefore, changes in plasma HSP70 levels due to thermal stimulation may reflect in vivo reactions, although the origin of HSP in blood is unknown [4]. There is a time lag until mRNA expression is reflected in blood protein levels. The time difference varies depending on individual factors. It has also been reported that after bathing, there was a change in blood HSP70 concentration from 15 minutes to 2 days [5] and that there was a change in blood HSP70 concentration immediately after 60 minutes of exercise [6]. The plasma high-sensitivity HSP70 measured in this study is affected by several physiological processes, including recovery from exercise, the process of repairing damaged tissue, and the reduction of oxidative stress due to inflammation [7]. Therefore, measuring plasma HSP70 before and after applying the hot fomentation will verify the degree of heat stress in vivo by the hot fomentations. This could be an indicator of the safety of the hot fomentation.

Hot fomentation is one type of hyperthermia treatment that increases local blood flow throughout the body [8]. It is well known that hot fomentation affects skin blood flow, skin temperature, and the autonomic nervous system. Measurement of skin temperature was considered useful to know how the effect of hot fomentations extend. In the present study, plasma HSP70 and skin temperature were compared before and after hot fomentation methods to verify which equipment is safe and systemically effective.

2. METHODS

Subjects

Three men and 7 women of age 21.8 ± 1.31 (mean ± SD) were recruited by poster and volunteered to participate. They have no medical history, including metabolic disorders. Their BMI was 21.19 (± 3.12).

Intervention

The temperature 22 °C (± 2) and humidity (40-60%) were controlled in the room.

Changes in plasma HSP70 concentration were determined using four types of hot fomentation (hot towels, hot packs, red bean bags, and thermal sheets). In this study, all hot fomentations were applied to the participants' right thighs.

Each hot fomentation was performed as described previously [9]. The hot towels were prepared by researchers based on previous studies [9]. The hot pack consisted of a medical carboxymethylcellulose (CMC) product. The red bean bags utilized the thermal effect of steam when the red beans were warmed. *(KOBAYASHI PHARMACEUTICAL CO., LTD. Japan)*. Thermal sheets were performed using a heat steam generating (HSG) sheet *(Kao corporation Japan)*. The HSG sheets produces a heat of about 40 °C for 5-8 hours.

The time to apply the hot fomentation was determined by pretesting with reference to previous studies [9]. The hot fomentation application time was 10 minutes for hot towels, 20 minutes for hot packs, 20 minutes for red bean bags, and 5 hours for thermal sheets.

In addition, since the surface of each heating device encounters the skin, the temperature was set to 40 °C. This temperature was determined from a previous study on safety [10].

Measurement of plasma HSP70

Plasma sampling for measurement of plasma HSP70 was performed 30 minutes after removal of the hot fomentation. Plasma HSP70 concentration was measured by enzyme-linked immunosorbent assay using HSP70 ELISA Kit *(StressMarq Biosciences INC)*.

Measurement of body temperature

The skin temperature on the application site and the opposite side was measured by an infrared thermometer *(MonotaRO Co., Ltd.)* before the intervention and every 5 minutes during the application of the hot fomentation. Axillary temperature was measured before and after the intervention.

Statistical analysis

Statistical significance was assessed using 'IBM SPSS' Statistics Desktop version.

3. RESULTS

No significant change in plasma HSP70 concentration was detected by 3 thermal loading methods (hot packs: 15.17 ± 3.71 vs. 13.27 ± 6.82 ng / ml, red bean bags: 14.66 ± 5.1 vs. 14.13 ± 3.7 ng / ml, thermal sheets: 15.16 ± 5.88 vs. 14.4 ± 3.97 ng / ml). Plasma HSP70 concentration was significantly reduced after hot towels (pre: 18.46 ± 6.55 vs. post: 14.7 ± 6.66 ng / ml, $p = 0.011$) (Wilcoxon matched-pairs signed-rank test) (Figure. 1. a-d). Plasma HSP70 levels were not associated with bathing habits, gender, or BMI (Mann-Whitney U test) (Data not shown).

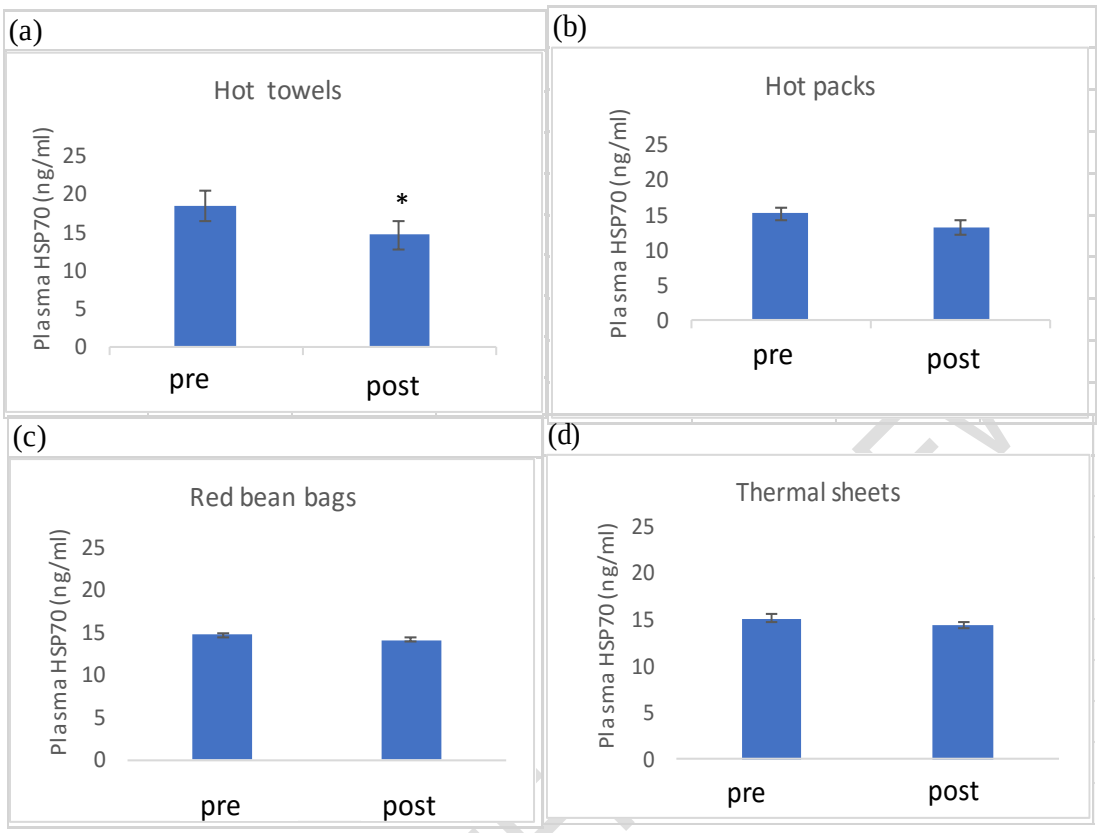

Figure.1. Effect of 4 type hot fomentation on plasma HSP70 levels. *(a) Hot towel for 10 min, (b) Hot pack for 20 min, (c) Red bean bag for 20 min, (d) Thermal sheet for 5h. *p<0.05 vs. pre*

Local skin temperature in the opposite side of the hot fomentation was significantly increased by hot towels, hot packs and red bean bags (Wilcoxon matched-pairs signed-rank test) as shown in Table.1. It has been confirmed that the skin temperature before the thermal load did not differ among the thermal stimulation methods (Data not shown). There was no significant change in axillary temperature. (Wilcoxon matched-pairs signed-rank test) (Data not shown).

Table.1. Effects of hot fomentation on skin temperature

	Right thigh skin temperature (heat load site)			Left thigh skin temperature (no thermal load)		
	pre (°C)	post	p-value	pre(°C)	post	p-value
Hot towels	29.29 (2.2)	31.3 (1.15)	0.021*	29.58 (1.77)	31.37 (0.98)	0.008**
Hot packs	30.27 (1.41)	30.48 (0.92)	0.959	29.97 (1.75)	31.85 (1.15)	0.005**
Red bean bags	30.63 (1.48)	32.09 (0.97)	0.019*	30.54 (1.47)	32.36 (1.32)	0.007**
Thermal sheets	30.58 (1.07)	30.58 (0.97)	0.813	30.34 (1.09)	30.49 (1.16)	0.635

Each value represents the mean (±SD). p<0.05, p<0.01***

4. DISCUSSION

In this study, no change or rather decrease in plasma HSP70 was found with thermal loading with hot fomentation. Thermal sheets were loaded for 5 hours. The duration was not long but possible to induce muscle HSP70 gene expression followed by increase in muscle production of HSP70 protein as well as plasma HP70 levels. Therefore, it is possible to speculate that thermal sheets method might not cause heat stress. However, hot packs, and red bean bags were loaded for 20 min and hot towels for 10 min. The time to measure, 50 or 40 minutes after start of fomentation, is too short to increase muscle HSP70 gene expression followed by increase in muscle production of HSP70 as well as plasma HP70 levels. Therefore, it cannot be concluded that methods of hot towels, hot packs and red bean bags do not cause heat stress, although changes in mRNA expression of HSPs by thermal stimulation to the skeletal muscle in vivo were also confirmed at 39 °C for 30 minutes [11] and at 41 °C for 60 minutes [12] in the previous reports by others. Thermal loading by hot towels decreased plasma HSP70 concentration. Since the time to measure was 40 minutes after the start, we cannot exclude the possibility that the clearance of HSP70 from plasma is increased by an increase in local blood flow.

Hot fomentation is often used in nursing with the expectation of relaxation and parasympathetic activity [9]. In this study, hot towels, hot packs and red bean bags significantly increased the skin temperature on the opposite side of thermal stimulation. This result may be caused by an increase in surface skin temperature due to cutaneous vasodilation through suppression of α1-adrenergic receptor stimulation [13]. In addition, the axillary temperature did not increase significantly, suggesting that the heat stress is not severe enough to change core temperature.

These results indicate that the hot fomentations used in this study have low invasion *in vivo* and that the effects expected by nursing can be obtained. The four hot fomentations used in this study are the most frequently used hot fomentation used by nursing [9]. It is beneficial in nursing to be able to confirm its safety and its effect *in vivo*. In the future, it will be necessary to verify the effect of continuous use of the hot compress in order to utilize the thermal effect in preventing ASCVD.

The limitation of this study is that the intervention time is short due to the characteristics of the heating device. Therefore, changes in plasma HSP70 concentration need to be verified in the future. Participants in this study were healthy young people. In the future, verification will be required even for those suffering from underlying diseases related to ASCVD and the elderly.

5. CONCLUSION

These results suggest that hot fomentation by thermal sheets is unlikely to cause heat stress at least in this condition and that hot towels, hot packs and red bean bags provide a systemic thermal effect. The best selection of safe methods of hot fomentation effective for ASCVD prevention requires further studies. This should briefly state the major findings of the study.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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