SHORT REPORT

Microwave hyperthermia treatment increases heat shock proteins in human skeletal muscle

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Objective: To test the hypothesis that microwave hyperthermia treatment (MHT) increases heat shock proteins (HSPs) in the human vastus lateralis muscle.

Methods: Four untrained healthy male volunteers participated in this study. The lateral side of the thigh of one leg (heated leg) was heated with a microwave generator (2.5 GHz, 150 W) for 20 min. At 1 day after the MHT, a muscle sample was taken from the heated leg. A control sample was taken from the unheated leg on another day of the MHT. For both legs, HSP90, HSP72 and HSP27 levels were compared.

Results: The HSP90, HSP72 and HSP27 levels in heated legs were significantly higher than those in control legs ($p < 0.05$).

Conclusions: Application of MHT can increase the levels of several HSPs in human skeletal muscle.

Recent papers have proposed that heat shock proteins (HSPs) can prevent contraction-mediated muscle damage,^{1,2} which reduces muscular strength and engenders muscle soreness.³ Therefore, it is expected that a prior increase in HSPs in the skeletal muscle reduces muscle damage and produces better muscle performance.

Although HSPs can be increased by many types of stresses, heat stress is often used in the case of skeletal muscle. Indeed, various HSPs are increased by elevating muscle temperature to 40–41°C in rat skeletal muscle.^{4,5} However, it has not been investigated whether elevation in muscle temperature increases HSPs in human skeletal muscles. Moreover, the means to increase HSPs have not been established in human skeletal muscle tissue. For these reasons, this study was undertaken to test the hypothesis that microwave hyperthermia treatment (MHT), which is a popular modality of thermotherapy, can increase HSP levels in human skeletal muscle.

METHODS

Subjects

Four untrained healthy men (mean (SD) age 24.8 (1.5) years, height 177.0 (7.6) cm, weight 73.3 (9.3) kg) volunteered for this study, which was approved by the ethics committees of Juntendo University, Inba, Japan and Saitama Medical School, Iruma, Japan. After being informed about this study, the subjects gave written informed consent.

Experimental design

Subjects received the MHT (2.5 GHz, 150 W, ME-7200; OG Giken, Okayama City, Japan) for 20 min on the lateral side of the thigh of one randomly selected leg (heated leg). The applicator of the microwave generator was located 15 cm above the thigh. The other leg served as an unheated control (control leg). One day after the MHT, a muscle sample was taken from the vastus lateralis muscle. Each participant's muscle temperature during MHT was also measured.

Muscle temperature measurement

After anaesthetising the skin surface with 60% lidocaine tape (Penles; Wyeth K.K., Chuo-ku, Tokyo, Japan) for 30 min, a wired thermocouple (IT-23; Physitemp Instruments, Clifton, New Jersey, USA) was inserted in the vastus lateralis muscle (2 cm depth) just under the applicator. Then, the muscle temperature was measured using a calibrated digital thermometer (PTW-301; Unique Medical Co., Komac, Tokyo, Japan), which connects to the wired thermocouple, every 2 or 3 min for 30 min from the onset of MHT. Muscle temperature measurements were carried out on a different day from that of the muscle biopsy to avoid any influence of the thermocouple insertion on HSP levels.

Muscle biopsy

Using a disposable biopsy instrument (14 gauge, Max Core; C. R. Bard, Covington, Georgia, USA), 10 mg of muscle samples were taken from the belly of the vastus lateralis (2 cm depth). The obtained muscle specimens were frozen immediately in liquid nitrogen and stored at –85°C. In the control leg, an identical biopsy procedure was performed on another day of the MHT to avoid disturbing the participant's daily life as much as possible.

Sample preparations and analysis

Frozen muscle samples were thawed, freed from connective tissues and homogenised in ice-cold buffer (10 mM Tris, 10 mM NaCl, 0.1 mM EDTA, pH 7.4). The homogenates were centrifuged at 400g for 15 min, and the supernatants and sediments were used, respectively, for analyses of HSPs and myosin heavy chain (MyHC). The protein concentration of the supernatants was determined using a protein assay reagent (Bio-Rad Laboratories, Foster City, California, USA).

The supernatants were diluted using Laemmli sample buffer (Bio-Rad Laboratories) and boiled at 95°C for 5 min. Then, a

Figure 1 Changes of temperature in the vastus lateralis muscle during and after microwave hyperthermia treatment ($n = 4$). Values are mean (SD).

Abbreviations: HSP, heat shock protein; MHT, microwave hyperthermia treatment; MyHC, myosin heavy chain

Figure 2 Electrophoretic identification of myosin heavy chain isoforms. Control and heated legs of each subject are denoted, respectively, C and H. P+S is a sample mixture produced from the rat plantaris and soleus muscles.

standard western blot analysis was performed for analysing HSPs using the following primary antibodies: anti-HSP90 (SPA-835; Stressgen Biotechnologies, Victoria, British Columbia, Canada), anti-HSP72 (SPA-810; Stressgen), and anti-HSP27 (SPA-800; Stressgen) as described previously.⁶

The sediments were diluted using MyHC sample buffer (30% glycerol, 5% β -mercaptoethanol, 2.3% sodium dodecyl sulphate, 0.05% bromophenol blue, 62.5 mM Tris, pH 6.8) and boiled at 60°C for 10 min. The MyHC compositions (percentage of type I, IIa and IIx) of each biopsy sample were determined using one-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis (8% separating gel) as described previously.⁷

Figure 3 Heat shock protein (HSP) 90 (top), HSP72 (middle) and HSP27 (bottom) in control (C) and heated (H) legs of each subject ($n=4$). Thin and thick lines, respectively, indicate individual and group mean (SD) values.

Table 1 Myosin heavy chain composition in control and heated legs ($n=4$)

	Myosin heavy chain composition (%)		
	I	IIa	IIx
Control leg	32.0 (7.1)	36.4 (7.1)	31.6 (6.6)
Heated leg	29.3 (6.9)	38.3 (7.6)	32.5 (7.1)

Values are mean (SD).

Statistics

Data of HSPs were expressed as a value relative to the group mean of the control leg. A paired *t* test was performed for statistical analysis ($p<0.05$).

RESULTS

Muscle temperatures were increased rapidly by MHT; they remained 4–5°C above the basal level from the 6th minute to the end of the MHT (fig 1). Significant differences between legs were not found for any type of MyHC isoform (table 1 and fig 2). The HSP90, HSP72 and HSP27 levels in heated legs were significantly higher than those in control legs ($p<0.05$; fig 3). Mean (SD) augmentations (%) of HSP90, HSP72 and HSP27 were, respectively, 68.6% (44.4%), 71.7% (27.7%) and 40.4% (6.0%).

DISCUSSION

The novel finding of this study is that MHT can increase the levels of HSP90, HSP72 and HSP27 in human skeletal muscle. Although our sample size is small, it is noteworthy that HSP levels were increased in all subjects.

Temperature elevation was a major factor in increasing HSP levels in skeletal muscles.^{4,5} For this study, we increased the muscle temperature to 40°C and maintained it for 10–15 min using MHT. Therefore, it seems probable that the increase in HSPs here is induced by the MHT-elevated muscle temperature.

The HSP levels of skeletal muscle are known to depend on their fibrous composition, and oxidative fibres contain higher levels of HSPs than do glycolytic fibres.^{6,8} In this study, however, as the MyHC composition of the examined samples was similar between control and heated legs, we have concluded that high levels of HSPs in the heated legs are the results of MHT.

Although no studies have investigated the effect of passive heating on the induction of HSPs in human skeletal muscles, some studies have investigated effects of exercise on the induction of HSPs.^{9,10} Exercise imparts various stresses that can increase HSPs in skeletal muscles. Interestingly, these studies found that HSPs were not increased after 24 h of a single bout

What is already known on this topic

Heat shock proteins (HSPs) play important physiological roles and might prevent muscle damage. However, the means to increase HSPs have not been established in human skeletal muscles.

What this study adds

This study shows that application of microwave hyperthermia treatment can increase the levels of several heat shock proteins in human skeletal muscles.

of non-damaging exercise (70% of maximal oxygen uptake for 45 min), but were increased after ≥ 48 h of exercise.⁹⁻¹⁰ In our study, although we do not know the levels of HSPs at the latter time frame, 24 h after the MHT was sufficient to show an increase all HSPs studied.

Currently, little is known regarding clinical effects of the induction of HSPs in human skeletal muscles. Future investigations should examine how much of an increase in HSPs is adequate to prevent muscle damage and maintain muscle functions.

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COMMENTARY

This study shows that microwave treatment, which is one of the treatment modalities in rehabilitation medicine, results in the induction of heat shock proteins (HSPs) in human skeletal muscle. Considering the possible role of HSPs in various types of cells, the findings of this study may have great impact on the prevention of muscle injury. Induction of HSPs using microwave treatment 24 h before some stressful event such as surgery or high-intensity exercise may have benefits in terms of facing the outcome with less muscle damage.

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