Journal of Dermatological Science Supplement (2006) 2, S39—S49

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Journal of Dermatological SCIENCE

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Efficacy of thermal stimulation on wrinkle removal via the enhancement of collagen synthesis

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KEYWORDS
Heat;
Wrinkle;
Sag;
HSP47;
Photoaging;
Collagen

Summary

Background: Heat shock protein 47 (HSP47) is a specific chaperone of procollagen. It is important to elucidate the effects of heat on collagen synthesis both *in vitro* and *in vivo*.
Objectives: We examined the effects of heat on collagen synthesis and the role of HSP47 using an *in vitro* system, and we also characterized the efficacy of wrinkle removal by heat treatment of human skin.
Methods: Normal human fibroblasts were used to evaluate the relationship between heat-induced collagen synthesis and HSP47 using an enzyme-linked immunosorbent assay (ELISA), reverse transcription-polymerase chain reaction (RT-PCR) and antisense. Heat stimulation of 6-week-old hairless mice (HOS:HR-1) was performed at varying temperatures (38, 40 and 42 °C) 3 days a week for 4 weeks, then the amount of collagen was determined by hydroxyproline content. For clinical evaluation, the left side of the face of 31 women (aged 36—55 years), was treated with heat 10 min a day for 2 months using hot steam which kept the skin surface temperature at 40—42 °C. Evaluations were performed using a visual analog scale, by replica taking, and with a Cutometer, prior to and 4 and 8 weeks after the heat treatment.
Results: The *in vitro* study showed that heat treatment enhanced collagen biosynthesis by up-regulating HSP47 mRNA and protein expression but not procollagenα1(I). Antisense inhibition of HSP47 prevented the increase of collagen synthesis induced by heat. Heat treatment at 40—42 °C enhanced hydroxyproline content and improved wrinkles/sags of the facial skin.

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doi:10.1016/j.descs.2006.08.006

Conclusions: These findings indicate that heat treatment at 40—42 °C has a beneficial therapeutic potential to repair wrinkles and sags in the skin through the up-regulation of collagen synthesis.

1. Introduction

Collagen fibers comprise approximately 75% of the dry weight of the dermis [1]. Types I and III collagen account for 80 and 10% of the total collagen in adult human dermis, respectively [2]. Collagen destruction that occurs over decades might underlie characteristic alterations in the appearance of aged skin which results from chronic sun exposure [3]. The amount of elastotic material and associated fibrohexis or fiber breakdown can be quite large and is probably responsible for the wrinkle formation seen in photoaged skin. The molecular mechanisms underlying the collagen deficiency in photoaged skin result from the increased expression of matrix metalloproteinases (MMP), such as MMP-1, 3 and 9, which degrade collagen and other proteins that comprise the dermal extracellular matrix [4—6]. In addition to degrading mature dermal collagen, ultraviolet (UV) irradiation impairs ongoing collagen synthesis, mainly through the down-regulation of types I and III procollagen gene expression, which leads to substantial collagen loss in the skin [7]. Thus, stimulation which induces collagen synthesis in photoaged skin might be effective in repairing wrinkles.

Heat shock protein 47 (HSP47) is a procollagen/collagen-specific molecular chaperone derived from the serine protease inhibitor (serpin) family of proteins which is essential for the early stages of collagen biosynthesis [8]. HSP47 is a constitutive stress response molecule found in the endoplasmic reticulum of cells expressing collagen type I [9,10], and the expression level of HSP47 is known to be proportional to the rate of collagen synthesis [11,12]. HSP47 is also elevated in the presence of toxins that affect collagen metabolism, such as hyperthermia and oxidative stress mediated by light-activation of riboflavin [13,14].

It is of particular interest to determine whether heat could induce collagen formation via HSP47 induction and, if so, whether this induction would remodel photoaged skin by removing the wrinkles. In this study, we first examined the effect of heat on collagen formation in cultured human fibroblasts and in mouse skin, and then we examined the efficacy of heat treatment on wrinkle removal in normal human subjects.

2. Materials and methods

2.1. *In vitro* studies

2.1.1. Reagents and cell culture

Dulbecco's modified Eagle medium (DMEM, from Nikken BioMedical Laboratory, Kyoto, Japan), fetal calf serum (FCS, from Gibco BRL, Grand Island, NY, USA), trypsin (from Difco Laboratories, Detroit, MI, USA), human collagen type I and anti-human collagen type I rabbit polyclonal antibody (from Chemicon International Inc., Temecula, CA, USA) and anti-human Hsp47 mouse monoclonal antibody (from Stressgen Biotechnologies Co., San Diego, CA, USA) were used for this study. All other reagents were purchased from Nakalai Tesque (Kyoto, Japan). Normal human fibroblasts were purchased from Kurabo Industries Ltd. (Osaka, Japan) and were maintained in DMEM with 5% FCS in a humidified incubator at 37 °C in a 5% CO_2 atmosphere.

2.1.2. Heat treatment of human fibroblasts

Human fibroblasts were inoculated at a density of 2×10^4 cells per well in 96-well microplates in DMEM supplemented with 0.5% FCS. After 1 day in culture, cells were exposed to various temperatures for 1 h in Hank's balanced salt solution containing $CaCl_2$ (1.26 mM) and $MgCl_2$ (0.81 mM) (HBS), and were then further cultured with DMEM supplemented with 0.5% FCS for 6 h or 24 h.

2.1.3. Collagen synthesis

The collagen content in the culture medium was determined by an enzyme-linked immunosorbent assay (ELISA) using an anti-type I collagen antibody. The concentration of collagen in the medium 24 h after stimulation was determined using a standard curve.

2.1.4. Heat shock protein47 analysis

The yield of HSP47 was determined by an *in situ* ELISA technique. Briefly, after fixation with 10% formaldehyde, HSP47 protein content was determined using an anti-HSP47 antibody with measurement in a microplate reader at 430 nm.