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Continuous fever-range heat stress induces thermotolerance in odontoblast-leneage cells

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ABSTRACT

Objective: Heat shock during restorative procedures can trigger damage to the pulpodentin complex. While severe heat shock has toxic effects, fever-range heat stress exerts beneficial effects on several cells and tissues. In this study, we examined whether continuous fever-range heat stress (CFHS) has beneficial effects on thermotolerance in the rat clonal dental pulp cell line with odontoblastic properties, KN-3.

Methods: KN-3 cells were cultured at 41 °C for various periods, and the expression level of several proteins was assessed by Western blot analysis. After pre-heat-treatment at 41 °C for various periods, KN-3 cells were exposed to lethal severe heat shock (LSHS) at 49 °C for 10 min, and cell viability was examined using the MTS assay. Additionally, the expression level of odontoblast differentiation makers in surviving cells was examined by Western blot analysis.

Results: CFHS increased the expression levels of several heat shock proteins (HSPs) in KN-3 cells, and induced transient cell cycle arrest. KN-3 cells, not pre-heated or exposed to CFHS for 1 or 3 h, died after exposure to LSHS. In contrast, KN-3 cells exposed to CFHS for 12 h were transiently lower on day 1, but increased on day 3 after LSHS. The surviving cells expressed odontoblast differentiation markers, dentine sialoprotein and dentine matrix protein-1. These results suggest that CFHS for 12 h improves tolerance to LSHS by inducing HSPs expression and cell cycle arrest in KN-3 cells.

Conclusions: The appropriate pretreatment with continuous fever-range heat stress can provide protection against lethal heat shock in KN-3 cells.

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1. Introduction

Heat stress is known to be one of the most severe stresses on dental pulp tissue during restorative procedures.[1] Dental pulp is a delicate specialised connective tissue and has several critical functions, including initiative, formative, protective, nutritive and reparative activities.[2] Therefore, thermotolerance induction of dental pulp may enable the maintenance of healthy teeth.

While severe heat shock exerts toxic effects on cells and tissues, it is known that fever-range heat stress has a beneficial role in various mammalian cells.[3] Body or tissue temperature increases during febrile diseases and fever-range heat stress can be beneficial to organisms, but the mechanisms responsible for this effect are not fully understood. Current hyperthermia studies employ lower temperatures in the range of 40–42 °C, as the majority of clinical hyperthermia treatments do not exceed these temperatures.[4] Fever-range elevation of temperature is presumed to positively regulate cell growth and development[5] and to provoke effects on immune response.[6] Exogenous fever-range heat stress as hyperthermia has been widely used as a physical therapy for a number of diseases, including inflammatory osteoarticular disorders, tendinitis, muscle injury, and malignant tumours.[7]

Odontoblasts form a layer lining the periphery of the dental pulp and are the first pulp cells stimulated by heat stress during restorative procedures. KN-3, a cell line established from rat incisor dental papilla cells, have high levels of alkaline phosphatase activity and expression of dentine sialophosphoprotein mRNA.[8] In addition, KN-3 cells have the ability to form mineralised nodules in medium containing ascorbic acid and β-glycerophosphate.[8,9] These features showed that KN-3 cells exhibited typical odontoblastic properties and the cells are regarded as odontoblast precursor cells,[10] and this cell line can be useful in elucidating the properties of odontoblast.

In the previous studies, we reported that the dental pulp cell line RPC-C2A and the KN-3 have the resistance to heat stress,[9,11] and the thermoresistance were influenced by nutritional status.[9] It was demonstrated that mRNA expression of HSP25 increased in the surviving KN-3 cells after moderate heat stress, at 43 °C for 45 min, and surviving cells underwent cell cycle arrest.[9] Although the KN-3 cells have the resistance to moderate heat stress at 43 °C for 45 min, cell death is entirely induced by the severe heat shock at 49 °C for 10 min.

HSPs, highly conserved polypeptides among species, are induced or enhanced in various cells in response to environmental stresses such as high temperature, as well as ischaemia, infection, radiation and chemical/mechanical stresses. Mammalian HSPs have been classified into five families and the most studied HSPs are HSP90, HSP70 (also called HSP72) and HSP27, a human homolog of rodent HSP25.[12,13] Expression of HSPs by heat stress is mediated by activation of HSF-1.[14] It is known that HSP70 contributes significantly to cellular thermotolerance.[15] The chaperone function of HSP70 assists the folding of newly synthesised polypeptides or misfolded proteins, the assembly of multiprotein complexes and the transport of proteins across cellular membranes.[16,17] Furthermore, HSP70 blocks heat-induced apoptosis by inhibiting signalling events upstream of c-Jun N-terminal kinase (JNK) and p38.[18] In addition, we previously reported that HSP70 has an inhibitory role in the apoptosis of dental pulp cells during wound healing after cavity preparation.[19] Small heat shock proteins HSP25 and HSP27 mediate thermotolerance and inhibit apoptosis,[20,21] and HSP90 also plays a role in thermotolerance.[22]

It has been reported that several mammalian cell strains undergo G1 block or prolongation of G1 phase and delayed entry into S phase when exposed to mild or medium heat stress.[23] p38MAPK, which is induced by several types of stress including heat stress, has also been implicated in down-regulation of cyclin D1 by regulating its transcription and degradation.[24]

Therefore, we hypothesised that the fever-range heat stress for several hours can increase various HSPs and induce cell cycle arrest in KN-3 cells sufficient for the cells surviving against subsequent lethal heat shock. In the present study, we examined whether continuous fever-range heat stress (CFHS) is able to induce thermotolerance to lethal severe heat exposure on the KN-3 cells, and elucidated the mechanisms of thermotolerance induction. The aim of this study is development of a non-cytotoxic method to protect dental pulp cells against lethal heat shock produced by restorative procedures.

2. Materials and methods

2.1. Cell culture

KN-3 cells were seeded at a density of 9.0×10^3 cm^{-2} and cultured in minimum essential medium Eagle alpha modification (Sigma–Aldrich, St Louis, MO, USA) supplemented with 10% foetal bovine serum (JRH Bioscience, Lenexa, KS, USA), 100 U/mL penicillin (Sigma–Aldrich) and 100 μg/mL streptomycin (Sigma–Aldrich) in a humidified atmosphere of 5% CO_2 at 37 °C. Thereafter, KN-3 cells were exposed to heat stress at 24 h after subculture.

2.2. Heat treatment

KN-3 cells were heat-treated at 41 °C for 3, 6, 12 and 24 h (CFHS) in a humidified atmosphere of 5% CO_2. The numbers of cells were counted with a counting chamber at 12 and 24 h. For lethal severe heat stress (LSHS), culture medium was replaced with medium preheated at 49 °C, and culture dishes or plates were placed on aluminium blocks preheated to 49 °C, followed by incubation for 10 min at 49 °C. Thereafter, medium preheated 37 °C was used, followed by incubation at 37 °C.

2.3. Cell growth assay

KN-3 cells were incubated in 96-well plates (AGC Techno Glass, Funabashi, Japan) and exposed to heat stress. At each time point after LSHS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay was carried out using a CellTiter96® Aqueous One Solution Cell Proliferation Assay Kit (Promega, Madison, WI, USA) in accordance with the manufacturer's instructions.