Biogerontology **3:** 117–120, 2002.

Hormetic action of mild heat stress decreases the inducibility of protein oxidation and glycoxidation in human fibroblasts *

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Key words: anti-aging, carbonyl, gerontogenes, heat shock, hormesis, protein modification, stress, AGE products

Abstract

Repeated mild heat shock (RMHS) has anti-aging effects on growth and various other cellular and biochemical characteristics of human skin fibroblasts undergoing aging *in vitro*. In this study, we have tested whether RMHS can reduce the accumulation of heavily damaged proteins, such as oxidized and glycoxidized proteins involved in the development of many pathological consequences of aging. Cultured human skin fibroblasts were subjected to RMHS and were subsequently incubated either with glyoxal (0.1–1 mM) generating Nϵ-carboxymethyl-lysine (CML), or with *tert*-butyl-hydroperoxide (t-BHP 10–700 μM) producing oxidized proteins. About 50% more carbonylated-proteins were produced in control cells treated with t-BHP than in cells previously exposed to RMHS. More dramatically, a treatment with 0.1 mM glyoxal for 48 h generated CML only in control cells. Such modulation of the level of damaged proteins is most likely related to the beneficial effects of hormesis resulting from exposure to mild stress.

Introduction

Repeated mild heat stress (RMHS) prevents the onset of several age-related characteristics in human fibroblasts undergoing aging *in vitro* (Rattan 1998; Verbeke et al. 2000). Such modulation of aging is a typical example of hormesis, which is characterized by the beneficial effects resulting from the cellular responses to mild stress (Rattan 2000). We have now investigated whether RMHS can affect protein oxidation and glycoxidation, which are two of the well-known processes behind age-related accumulation of abnormal proteins in cells, tissues and organisms (Verbeke et al. 2000). Post-translational oxidation and glycoxidation generate proteins having heavily modified amino acids, including carbonyls, Nϵ-carboxymethyl-lysine (CML) and pentosidine. These modifications lead to misfolding, loss of activity and either crosslinks or cleavage of proteins (Baynes and Thorpe 2000). Cellular systems can potentially prevent the formation of oxidized and glycoxidized proteins by activating enzymes metabolizing reactive oxygen species (ROS) and α-oxoaldehydes, such as glyoxal, methyl glyoxal and 3-deoxyglucosone (Verbeke et al. 2000). Cellular systems can also protect sensitive proteins against damage and misfolding by activating the heat shock (HS) response involving the HS family of stress proteins (HSP) composed of chaperones. Furthermore, a weakly damaged protein could be either repaired by specific enzymatic systems or routed into appropriate proteolytic pathways (Verbeke et al. 2000). However, inefficiency of cellular processes to repair, refold and/or degrade damaged proteins leads to their aggregation and accumulation during aging.

The ability of a cell to cope with oxidative stress is positively correlated with longevity, suggesting that the status of the antioxidant defenses play a major role in cellular senescence (Finkel and Holbrook 2000). Cells have a complex network of antioxidants and enzymatic systems against oxidation and

* Presented at the 1st ASCMG meeting, Melbourne, Australia, March 19–22, 2001.

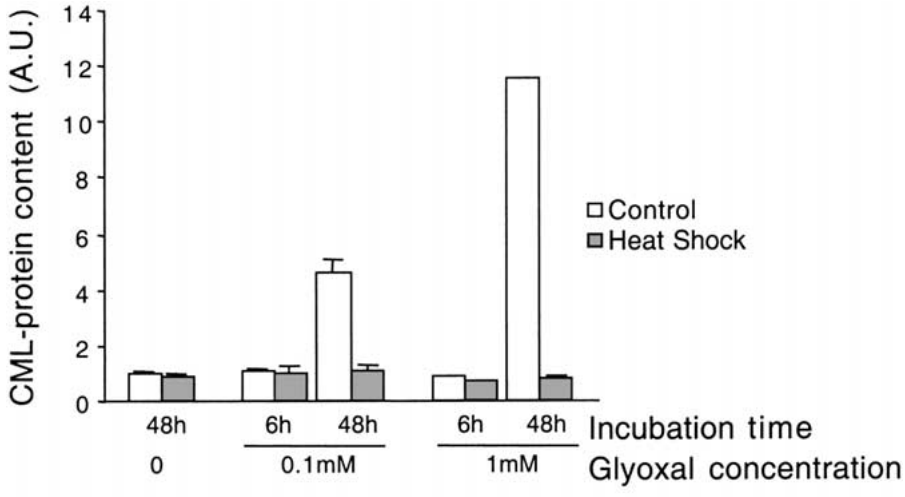

Figure 1. Effect of RMHS treatment on the accumulation of CML-rich proteins in human skin fibroblasts. Early passage (less than 30% lifespan completed) cultures were subjected to RMHS for 4 weeks. At 80% confluency, cultures were incubated with sublethal doses of glyoxal for different times. Proteins were extracted and subjected to a direct ELISA test with a first rabbit polyclonal antibody raised against CML (1/2000), and the HRP-conjugated secondary antibody (1/1000; Dako, DK). HRP activity was revealed with OPD substrate (Dako, DK) according to the instructions of the manufacturer. Data presented are the average ± SD from 3 independent samples.

glycoxidation contributing to the detoxification of ROS and reactive α-oxoaldehydes. However, the regulation of these systems is altered during aging, due partly to the age-related changes in the inducibility of specific transcription factors, such as NFk-B, AP1, p53 and HSF1 (Finkel and Holbrook 2000). This leads to the imbalance of the cellular redox status, creating an environment favorable to oxidation and glycoxidation processes. The increase of oxidative damages could also be due to a specific decrease in protein repair or protein degradation, especially by the ubiquitin-proteasome- (UPP) and the lysosomal pathways (Cuervo and Dice 2000; Friguet et al. 2000). Lastly, the capacity of cells to cope with environmental and internal stresses by the so-called HS response is gradually decreased during aging (Lee et al. 1996), leading to a decrease in expression and/or a decrease in the activity of most of the HSP and other enzymes involved in defense, maintenance, repair and degradation processes.

Effect of hormesis on experimental induction of damaged proteins

It has been suggested that hormesis, a beneficial effect of repeated mild stress, allows the cells to enhance and maintain their cellular defense processes, and survive in otherwise cytotoxic environments (Rattan 2000). To test this hypothesis, we have developed a model of primary cultures of human skin fibroblasts subjected to RMHS (1 h at 41 °C) twice a week (Rattan 1998). Early passage (less than 30% lifespan completed) fibroblasts were treated for 6 and 48 h with (t-BHP, 10 μM and 700 μM) and glyoxal (0.1 mM and 1 mM), two chemicals capable of generating oxidative and glycoxidative damages, respectively (Krishnamurthy et al. 2000; Niwa et al. 1998). Moreover, t-BHP is known to accelerate the replicative senescence *in vitro* (Toussaint et al. 2000). We have observed that carbonylated-proteins and CML-rich proteins are formed in fibroblasts treated with t-BHP and glyoxal. Figure 1 shows that after 48 h of incubation with low and high glyoxal, the CML concentration was increased respectively 4 and 11 times in control cells, but not in those cells which had undergone 8 RMHS treatments (Figure 1).

RMHS also decreased the appearance of carbonylated-proteins in cells treated with t-BHP in comparison with cells maintained continuously at 37 °C. Figure 2 shows a low concentration of carbonylated proteins in young cells, and a time- and dose-dependent increase of the carbonylated-proteins both in control and RMHS-treated cells. However, after 6 h of treatment with 10 μM of t-BHP and after 48 h of incubation with 10 or 700 μM of t-BHP, cells pretreated with RMHS accumulate 20–70% less carbonylated-proteins than control cells (Figure 2).

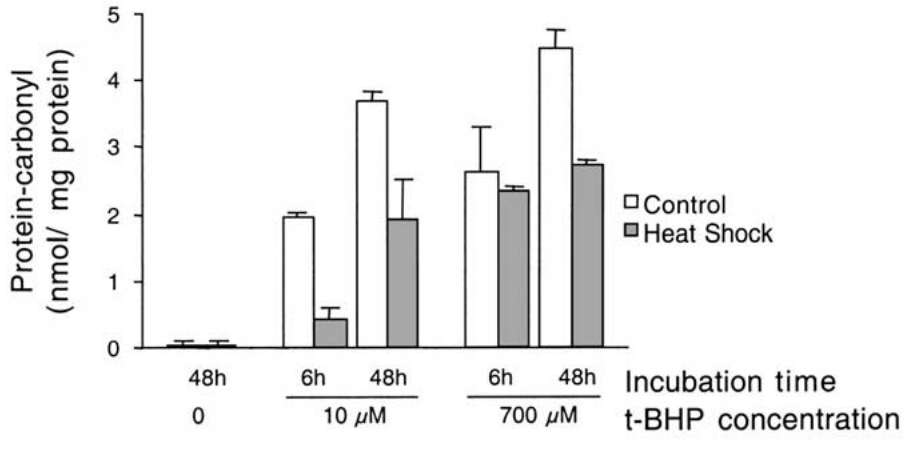

Figure 2. Effect of RMHS treatment on the accumulation of oxidized proteins in fibroblasts. Early passage (less than 30% lifespan completed) cultures were subjected to RMHS during 4 weeks. At 80% of confluence, cultures were incubated with different concentrations of t-BHP for 6 h and 48 h. Proteins were extracted and carbonyl content was measured by ELISA assay as described (Buss et al. 1997). Data presented are the average ± SD from 3 independent samples.

Hormetic effects of RMHS on protein oxidation and glycoxidation are clearly demonstrated in this study where cells have been exposed to different doses of harmful chemicals. Although the exact mechanism of the hormetic effects of RMHS are yet to be understood, it can be suggested that RMHS increases the expression of HSP and/or activate various HSP, protecting cellular proteins from misfolding and from susceptibility to attacks by ROS and α-oxoaldehydes. Some enzymes such as catalase and glutathione reductase have been reported to be protected from oxidative damages by different HSP (Hook and Harding 1997). The effect of RMHS could also be due to an up-regulation of the synthesis and/or the activity of some crucial proteins or enzymes which belong to anti-oxidants pathways and/or the α-oxoaldehydes catabolism. Indeed, genes of some proteins modulating the cellular redox status, for example, γ-glutamylcysteine synthetase, gluthatione synthetase, catalase, SOD and thioredoxin, contain either a HS element or a stress responsive element and are regulated by different stresses (Leppa et al. 1997; Nakagawa et al. 1999; Sugiyama et al. 2000; Yoo et al. 1999). Finally, it can also be hypothesized that proteins are damaged to the same extent in both heat-shocked- and control cells, but that the proteolytic machineries such as the UPP or the lysosomal pathways are more efficient in degrading the oxidized and the glycoxidized proteins in the HS-treated cells. It has been shown that heat and changes in the intracellular redox homeostasis can modulate the activity of the proteasome (Andersson et al. 1999). Moreover, several stress-regulated proteins, such as ubiquitin, ubiquitin-carrier protein, and HSC73 are involved in either UPP or the lysosomal pathways (Cuervo and Dice 2000). Further studies are in progress investigating how young fibroblasts treated with RMHS can limit the accumulation of cytosolic damaged proteins and also whether old fibroblasts undergoing RMHS during their lifespan can maintain their ability to cope with such stresses.

Acknowledgements

We thank Dr T. Miyata (Tokai University, Japan) for the gift of anti-CML antibodies. This project is a part of the shared cost action programmes GENAGE and PROTAGE under the EU Biomed & Health Programme and Quality of Life Projects. Marianne Deries was an E.R.A.S.M.U.S. exchange student from the Grenoble I University (Grenoble, France).

References

Andersson M, Sjostrand J and Karlsson JO (1999) Differential inhibition of three peptidase activities of the proteasome in human lens epithelium by heat and oxidation. Exp Eye Res 69: 129–138

Baynes JW and Thorpe SR (2000) Glycoxidation and lipoxidation in atherogenesis. Free Radic Biol Med 28: 1708–1716

Buss H, Chan TP, Sluis KB, Domigan NM and Winterbourn CC (1997) Protein carbonyl measurement by a sensitive ELISA method. Free Radic Biol Med 23: 361–366

Cuervo AM and Dice JF (2000) When lysosomes get old. Exp Gerontol 35: 119–131

Finkel T and Holbrook NJ (2000) Oxidants, oxidative stress and the biology of ageing. Nature 408: 239–247

Friguet B, Bulteau AL, Chondrogianni N, Conconi M and Petropoulos I (2000) Protein degradation by the proteasome and its implications in aging. Ann N Y Acad Sci 908: 143–154

Hook DW and Harding JJ (1997) Molecular chaperones protect catalase against thermal stress. Eur J Biochem 247: 380–385

Krishnamurthy PK, Mays JL, Bijur GN and Johnson GV (2000) Transient oxidative stress in SH-SY5Y human neuroblastoma cells results in caspase dependent and independent cell death and tau proteolysis. J Neurosci Res 61: 515–523

Lee YK, Manalo D and Liu AY (1996) Heat shock response, heat shock transcription factor and cell aging. Biol Signals 5: 180–191

Leppa S, Pirkkala L, Chow SC, Eriksson JE and Sistonen L (1997) Thioredoxin is transcriptionally induced upon activation of heat shock factor 2. J Biol Chem 272: 30400–30404

Nakagawa Y, Koide K, Watanabe K, Morita Y, Mizuguchi I and Akashi T (1999) The expression of the pathogenic yeast Candida albicans catalase gene in response to hydrogen peroxide. Microbiol Immunol 43: 645–651

Niwa H, Takeda A, Wakai M, Miyata T, Yasuda Y, Mitsuma T, Kurokawa K and Sobue G (1998) Accelerated formation of N epsilon-(carboxymethyl) lysine, an advanced glycation end product, by glyoxal and 3-deoxyglucosone in cultured rat sensory neurons. Biochem Biophys Res Commun 248: 93–97

Rattan SIS (1998) Repeated mild heat shock delays ageing in cultured human skin fibroblasts. Biochem Mol Biol Int 45: 753–759

Rattan SIS (2000) Biogerontology: the next step. Ann N Y Acad Sci 908: 282–290

Sugiyama K, Izawa S and Inoue Y (2000) The Yap1p-dependent induction of glutathione synthesis in heat shock response of Saccharomyces cerevisiae. J Biol Chem 275: 15535–15540

Toussaint O, Medrano EE and von Zglinicki T (2000) Cellular and molecular mechanisms of stress-induced premature senescence (SIPS) of human diploid fibroblasts and melanocytes. Exp Gerontol 35: 927–945

Verbeke P, Clark BFC and Rattan SIS (2000) Modulating cellular aging in vitro: Hormetic effects of repeated mild heat stress on protein oxidation and glycation. Exp Gerontol 35: 787–794

Yoo HY, Chang MS and Rho HM (1999) The activation of the rat copper/zinc superoxide dismutase gene by hydrogen peroxide through the hydrogen peroxide-responsive element and by paraquat and heat shock through the same heat shock element. J Biol Chem 274: 23887–23892