Geroprotection by Glycerol

Insights to Its Mechanisms and Clinical Potentials

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ABSTRACT: Chaperones, particularly the heat-shock proteins, are considered as key players in the maintenance of protein homeostasis and are associated with longevity and cellular immortalization. In this study, we investigated the geroprotective activity of the chemical chaperone glycerol. Glycerol showed significant chaperoning activity in refolding heat-denatured luciferase *in vivo* and in protecting cells from heat stress-induced cytotoxicity. This was accompanied by decrease in p53, an up-regulation of a stress chaperone mortalin/mtHsp70, and an increase in proteasome activity in the presence of oxidative stress.

KEYWORDS: glycerol; chemical chaperone; protection; antiaging; life-span extension

INTRODUCTION

Accumulation of protein aggregates and misfolded moieties is a nearly universal phenomenon during aging. Such aggregates have been specifically referred to as amyloids, lysosomal lipofuscin, ceroid bodies, advanced glycation end-products (AGEs), and cytoplasmic inclusions observed in senescent cells, as well as in affected tissues of age-associated diseases such as diabetes and Alzheimer's disease.[1] Although it is not exactly clear how these protein aggregates are formed, the dysregulation of innate chaperoning forces, involving

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mainly the heat-shock proteins (HSPs), have emerged to be the major critical factors. These HSPs are evolutionarily conserved life-essential housekeeping molecules that play a protective role against proteotoxic stress. Several studies further suggest that an increase in the levels of the molecular chaperones is a common denominator for the extension of cellular life span, immortalization and species' longevity.[2]

Recently, chemical chaperones that are of commercial and medical relevance have been introduced to aid protein refolding of various aggregation-prone proteins. A group of these small molecules comprise the osmolytes, such as glycerol, which are known to stabilize native proteins by altering solvent properties of water, protein polarity, and diffusion, and favor formation of native protein oligomers.[3] The chaperone activity of glycerol, specifically, has been used to repair various mutant or misfolded proteins of p53,[4] prions from familial Creutzfeldt-Jakob disease,[5] and the cystic fibrosis transmembrane conductance regulator protein (CFTR),[6] among others. In this study, we provide evidence in support of the action of glycerol as a potential stress modulator.

CHAPERONE ACTIVITY OF GLYCEROL

To establish the minimal levels of glycerol to be used for this study, we first performed an *in vivo* chaperone assay using human osteosarcoma (U2OS) cells transfected with the firefly luciferase construct pGL3.[7] Our observations indicated that 0.4 M glycerol ($P < 0.05$) is sufficient to refold luciferase after heat denaturation at 45°C (FIG. 1A). Meng *et al.* have indicated higher concentration requirements (>1.25 M) to effectively inhibit heat-induced aggregation of creatine kinase. Such levels, albeit, are suitable for maintaining integrity of proteins during purification and long-term storage and are otherwise toxic for cells.[8] Glycerol concentrations higher than 0.5 M were progressively toxic to cells (FIG. 1B). Alternatively, we found that the lower doses of glycerol are effective refolding aids *in vivo* as opposed to *in vitro*. Biologically, this was also evident from the survival curves of heat-stressed (45°C) cells treated with 0.4 M glycerol (FIG. 1C). The increased tolerance of cells to thermal as well as oxidative killing (data not shown) in the presence of 0.4 M glycerol could likely be due to cellular mechanisms other than direct chaperoning effects of glycerol with a target protein.

DOES GLYCEROL MODULATE PROTEASOME AND MORTALIN?

Diamant *et al.* demonstrated that the protection by osmolytes also occurs in the same low physiologic concentrations that also activate the molecular

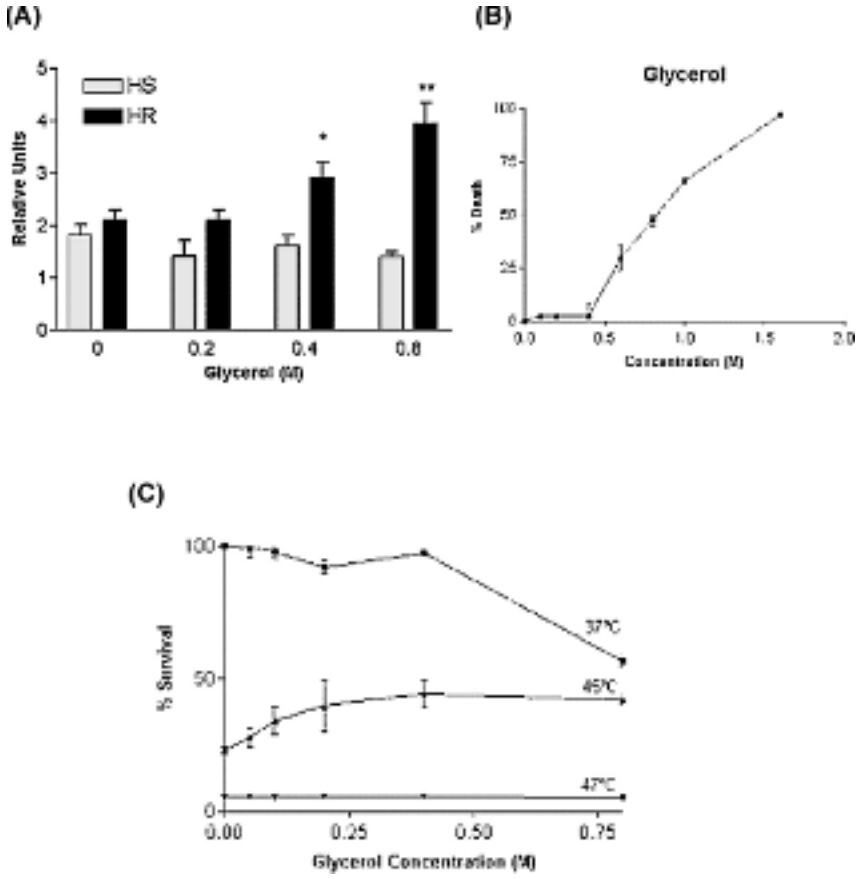

FIGURE 1. Chaperone activity of glycerol protects cells from heat stress. (**A**) *In vivo* chaperone assay in U2OS cells in the presence of different concentrations of glycerol in the medium. After heating cells at 45°C for 30 min (HS), plates were placed back in the CO_2-incubator for 3 h to allow recovery/refolding of heat-denatured luciferase at 37°C (HR). Refolding values were obtained by dividing luciferase activity of heat-shocked and heat-recovered cells by activity of nonheated (control) cells. (**B**) Effect of increasing concentrations of glycerol on survival of U2OS cells. (**C**) Effect of glycerol on survival of heat-shocked cells.

chaperones GroEL, DnaK, and ClpB in *E. coli*.[3] We observed that glycerol causes a modest upregulation of the molecular chaperone mortalin (also known as mthsp70 or grp75) and proteasome activity in the presence of doxorubicin (data not shown). Interestingly, mortalin overexpression has also been associated with longevity and immortalization in human fibroblasts.[9] Both chaperone upregulation and enhancement of proteasome indicate that glycerol, independent of its chemical chaperone activity, may stimulate innate homeostatic maintenance systems that may be useful for healthy aging.

GLYCEROL THERAPY FOR HUMAN LONGEVITY?

The osmotic effect of glycerol has been exploited therapeutically for the acute treatment of brain cortical infarct and edema. Improvement in survival time of patients under glycerol therapy was achieved without serious adverse effects, except for mild, subclinical hemolysis. Stroke patients treated with intravenous 10% glycerol solution experienced only a brief period of plasma hyperosmolarity that returned to baseline levels after a few hours. Its beneficial effects, however, have been facilitated by the rapid glycerol accumulation in the brain.[10,11]

As clinical findings support that glycerol therapy is effective in managing brain pathologies and is well tolerated in humans, a study by Bai *et al.* showed, however, that the concentration of supplemental oral dosing of 10% glycerol in mice would unlikely result in the targeted serum concentration of 0.4 M.[12] The obstacle of increasing systemic availability is mainly due to the rapid metabolism of glycerol resulting to an estimated circulating half-life ($T_{1/2}$) of only 3.8 h both in mice and humans.[12] Because several tissues in the brain, trachea, kidney, eye, nasopharnx, digestive tract, skeletal (but not smooth or cardiac) muscles, and the skin express high levels of the aquaporins, a family of transmembrane transporters that rapidly transports exogenous glycerol into a cell,[13] a rapid intracellular accumulation of glycerol may likely offset the difficulty in maintaining high glycerol serum levels. In light of our preliminary data, glycerol can be tested as a potential antiaging drug.

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