Marine Environmental Research 107 (2015) 35–44

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Anti-oxidative cellular protection effect of fasting-induced autophagy as a mechanism for hormesis

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ARTICLE INFO

Article history:
Received 22 February 2015
Received in revised form
1 April 2015
Accepted 7 April 2015
Available online 8 April 2015

Keywords:
Anti-ageing
Age-pigment
Autophagy
Caloric-restriction
Cell network model
Cytoprotection
Hormesis
Lipid peroxidation
Lipofuscin
Lysosome
Lysosomal membrane stability
Mollusc
mTOR
Protein aggregates
Reactive oxygen species
Stress tolerance

ABSTRACT

The aim of this investigation was to test the hypothesis that fasting-induced augmented lysosomal autophagic turnover of cellular proteins and organelles will reduce potentially harmful lipofuscin (age-pigment) formation in cells by more effectively removing oxidatively damaged proteins. An animal model (marine snail - common periwinkle, *Littorina littorea*) was used to experimentally test this hypothesis. Snails were deprived of algal food for 7 days to induce an augmented autophagic response in their hepatopancreatic digestive cells (hepatocyte analogues). This treatment resulted in a 25% reduction in the cellular content of lipofuscin in the digestive cells of the fasting animals in comparison with snails fed *ad libitum* on green alga (*Ulva lactuca*). Similar findings have previously been observed in the digestive cells of marine mussels subjected to copper-induced oxidative stress. Additional measurements showed that fasting significantly increased cellular health based on lysosomal membrane stability, and reduced lipid peroxidation and lysosomal/cellular triglyceride. These findings support the hypothesis that fasting-induced augmented autophagic turnover of cellular proteins has an anti-oxidative cytoprotective effect by more effectively removing damaged proteins, resulting in a reduction in the formation of potentially harmful proteinaceous aggregates such as lipofuscin. The inference from this study is that autophagy is important in mediating hormesis. An increase was demonstrated in physiological complexity with fasting, using graph theory in a directed cell physiology network (digraph) model to integrate the various biomarkers. This was commensurate with increased health status, and supportive of the hormesis hypothesis. The potential role of enhanced autophagic lysosomal removal of damaged proteins in the evolutionary acquisition of stress tolerance in intertidal molluscs is discussed and parallels are drawn with the growing evidence for the involvement of autophagy in hormesis and anti-ageing processes.

1. Introduction

Normal metabolic generation of reactive oxygen species (ROS), including oxy-radicals, can cause oxidative attack on the protein machinery and organelles of the cell (Livingstone, 2001; Regoli, 2000). Increased removal of damaged cellular constituents by autophagy will conserve cell function; and also reduce the amount of age-pigment (lipofuscin) produced (Cuervo, 2004; Moore et al., 2006a, b, c; 2007). Consequently, an effective capability to up-regulate the autophagic process will be advantageous to organisms exposed to environmental influences such as many environmental toxins and pollutants which can contribute to increased generation of ROS (Moore, 2008; Moore et al., 2006c). Lipofuscin accumulates in lysosomes as a result of peroxidation of autophagocytosed proteins associated with protein aggregates and oxidatively damaged organelles; and was previously considered to be just cellular junk (Fig. 1; Brunk and Terman, 2002). However, recent evidence indicates that lipofuscin binds iron, which generates ROS, probably resulting in exacerbation of oxidative damage and sequestration of proteases, thereby, inhibiting lysosomal degradation (Brunk and Terman, 2002; Grune et al., 2004). This in turn may

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http://dx.doi.org/10.1016/j.marenvres.2015.04.001
0141-1136/

lead to "incomplete or failed autophagy" with autophagic accumulation of essentially undegradable damaged organelles, proteins, phospholipids and lipids that will produce more lipofuscin (Brunk and Terman, 2002; Cuervo, 2004; Grune et al., 2004; Lüllmann-Rauch, 1979; Moore et al., 2006a, b, c; 2007).

Molluscan species such as bivalve mussels and marine snails provide useful models for studying autophagic function, as autophagy can be readily induced by starvation, salinity change, hyperthermia and hypoxia in the cells of the hepatopancreas or digestive gland, which is the liver analogue in molluscs (Bayne et al., 1978; Lowe et al., 2006; Moore, 2008; Moore and Halton, 1973, 1977; Moore et al., 1986, 2007; Owen, 1970). These species have been extensively investigated, particularly with respect to the harmful effects of pollutant chemicals such as toxic metals and polycyclic aromatic hydrocarbons (Moore et al., 1985). Previous studies using bivalve molluscs have indicated that fasting-induced autophagy has a cytoprotective effect against oxidative stress (Moore, 2004; Moore et al., 2006b, 2007); and Moore and Stebbing (1976) demonstrated that autophagy was involved in hormesis induced by very low concentrations of copper, cadmium and mercury in a colonial hydroid. Hormesis is a biphasic dose response to an environmental agent characterized by low dose stimulation or beneficial effect and a high dose inhibitory or toxic effect (Mattson, 2008).

This investigation was designed to test the hypothesis that augmented autophagic turnover of oxidatively damaged proteins reduces lipofuscin (age-pigment) formation in hepatopancreatic digestive cells of the marine snail or periwinkle *Littorina littorea*. Snails were subjected to fasting (nutritional deprivation) for a period of seven days in order to induce autophagy (Moore and Halton, 1973; Moore et al., 1986), and the relative content of intralysosomal lipofuscin was then determined cytochemically in comparison to fed control snails. Additional parameters measured included lysosomal membrane stability, cytoplasmic and lysosomal neutral lipid (triglyceride) and lipid peroxidation.

Modelling of whole biological systems from cells to organs is gaining momentum in cell biology and disease studies. This pathway is essential for the derivation of explanatory frameworks that will facilitate the development of a predictive capacity for estimating outcomes or risk associated with particular disease processes and therapeutic or stressful treatments (Moore and Noble, 2004). In this context, a parallel modelling exercise used a modified version of the generic cell network model described by Moore (2010) in order to accommodate the available biomarker data. The original generic model was developed from extensive published data in the environmental toxicology and biomedical literature, and the large-scale organisation of metabolic networks (Cuervo, 2004; Di Giulio and Hinton, 2008; Jeong et al., 2000; Klionsky and Emr, 2000; Zhang and Zhang, 2009). This cellular interaction network was constructed around the essential processes of feeding, excretion and energy metabolism (Moore, 2010). Protein synthesis and degradation, including lysosomal autophagy, are also incorporated in the model as are the major protective systems (Cuervo, 2004; Di Giulio and Hinton, 2008; Livingstone

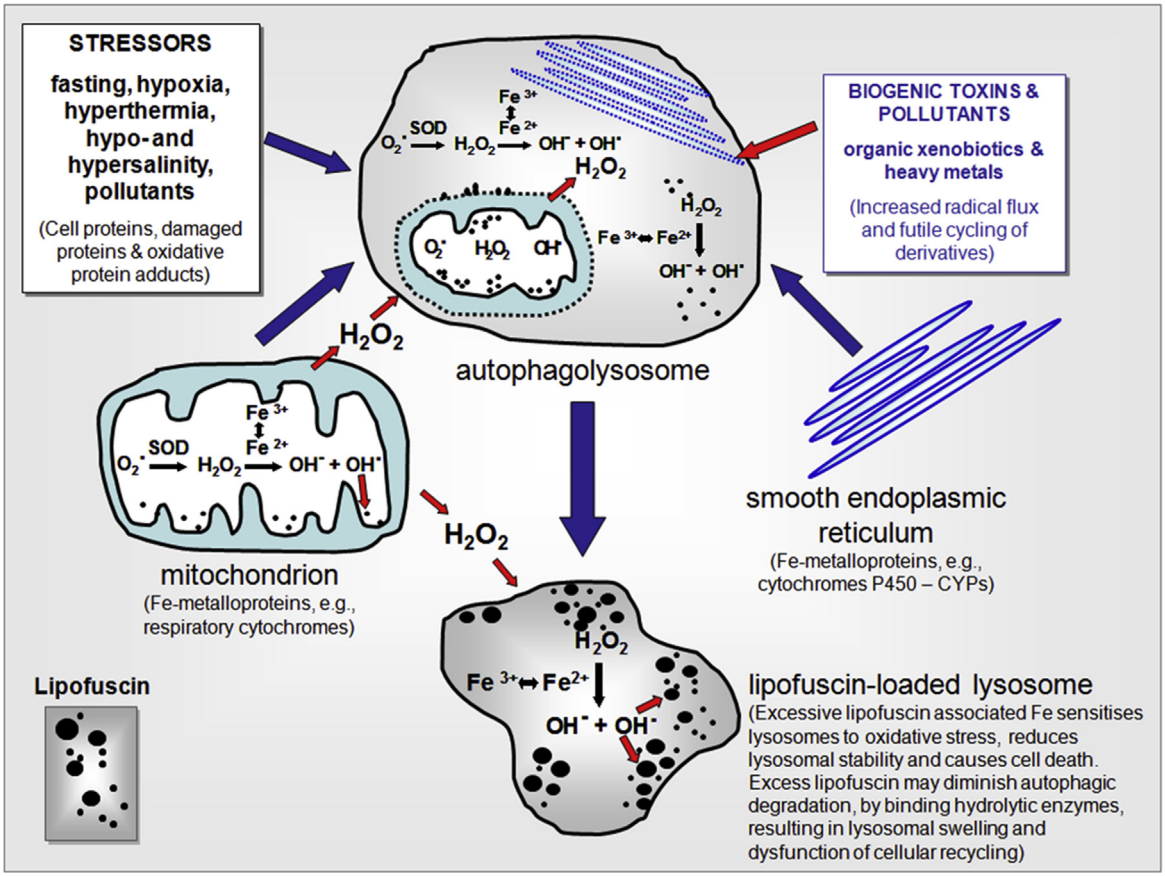

Fig. 1. Conceptual model for the role of reactive oxygen species (ROS) and lipofuscin in lysosomal autophagy and oxyradical-mediated cell injury. This model draws on one proposed by Brunk and Terman (2002) and adapted by Moore et al. (2006a). Fe^{2+} – ferrous cation; Fe^{3+} – ferric cation; $O_2^{\cdot}$ – superoxide; OH^- – hydroxyl anion; $OH^{\cdot}$ – hydroxyl radical; SOD – superoxide dismutase.