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Trehalose intake induces chaperone molecules along with autophagy in a mouse model of Lewy body disease

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ABSTRACT

The accumulation of mis-folded and/or abnormally modified proteins is a major characteristic of many neurodegenerative diseases. In Lewy body disease (LBD), which includes Parkinson's disease and dementia with Lewy bodies, insoluble α-synuclein is widely deposited in the presynaptic terminals as well as in the neuronal cytoplasm in distinct brain regions. It is well known that the autophagy-lysosome system serves as an efficient degradation pathway for abnormal molecules within cells. To test the possibility that activated autophagy can degrade abnormal molecules, we investigated the effect of trehalose on abnormal aggregation of α-synuclein in a model of LBD. Trehalose is a natural disaccharide composed of two glucose units and functions as an autophagy inducer. Consistent with previous studies, trehalose increased level of the autophagosomal protein LC3, especially a lipidated form LC3-II in cultured cells and mice brain. Also, trehalose increased levels of several chaperon molecules, such as HSP90 and SigmaR1, in the brains of LBD model mice. Further studies revealed that level of detergent-insoluble α-synuclein was suppressed in mice following oral administration of trehalose, despite an apparent alteration was not observed regarding abnormal aggregation of α-synuclein. These results suggest that the oral intake of trehalose modulates propensity of molecules prior to aggregation formation.

1. Introduction

Parkinson's disease (PD) and dementia with Lewy bodies (DLB) share the neuropathological characteristics of the presence of ubiquitin-positive inclusions called Lewy bodies (LBs) in neurons in the central and peripheral nervous systems [1,2]. These disorders are collectively referred to as LB disease (LBD). Large-scale genetic analyses revealed that LBD is strongly associated with genetic mutation of α-synuclein [3,4]. Indeed, α-synuclein has been extensively researched in this field, and its mutation, duplication and triplication results in familial LBD [5,6]. α-Synuclein is originally a presynaptic protein that is solubilized by proteinase K (PK) digestion; however, it becomes resistant to PK digestion in LBD, suggesting that α-synuclein abnormally changes in its conformation. In particular, because PK-resistant α-synuclein is widely deposited in presynaptic terminals in distinct regions [7,8], it is plausible that quantitative and qualitative alterations of α-synuclein are highly involved in the pathogenesis and onset of both familial and sporadic LBD.

Several lines of evidence have reported that increased levels of α-synuclein leads to synaptic dysfunction, memory impairment and neuronal damage in mice [9–11]. Importantly, Lim et al. showed that these abnormalities can be reversed by suppressing α-synuclein transgene expression [12]. Thus, this result implies that the repression of α-synuclein can be a therapeutic strategy for LBD.

Autophagy is a cellular degradation system that is capable of producing nutrients and energy in response to various conditions, such as nutrient starvation [13,14]. Because autophagy also serves as ways of efficiently degrading abnormal proteins [15], activated autophagy could offer a potential therapeutic strategy for diseases

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that involve abnormally aggregated proteins, including LBD. We here examined the effect of activated autophagy induced by trehalose, which is a natural disaccharide composed of two glucose units linked by an α, α-1,1-glycosidic bond, on the aggregation of abnormal proteins. Consistent with previous reports [16,17], trehalose activated autophagic flux and prevented the formation of cytoplasmic inclusions composed of α-synuclein in cultured cells. We therefore applied trehalose to animal model for LBD. Although autophagy was activated by trehalose intake for only 1 week, we failed to find clear differences in aggregated distribution of abnormal α-synuclein between mice with trehalose and maltose. Further analyses, however, revealed that insoluble α-synuclein level was suppressed, and some chaperone molecules were significantly increased in the brains of mice with trehalose compared with maltose. This result suggests that trehalose is not effective to degrade aggregation, rather trehalose modulates propensity of molecules prior to aggregate formation.

2. Materials and methods

2.1. Cell cultures and treatment

HeLa cells (Japanese Collection of Research Bioresources Cell Bank, Osaka, Japan) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics. Cells were treated with trehalose, maltose, sucrose (Wako, Osaka, Japan), rapamycin (Sigma, Saint Louis, MO, USA) or latrepirdine (Tocris Bioscience, Bristol, UK), which is an autophagy inducer.

2.2. Time lapse imaging

Cells were transduced using a Premo™ autophagy tandem sensor mCherry-GFP-LC3B kit (Life technologies, Carlsbad, CA, USA) to monitor autophagic flux. Live-cell imaging was performed at 37 °C using a top incubator (INUG2-TIZ, Tokai Hit, Shizuoka, Japan) and a microscope (Eclipse Ti, Nikon, Tokyo, Japan) equipped with a ×20 objective lens, a spinning disc system (TI-S-EJOY, Nikon) and a CCD camera (CoolSNAP HQ2, Photometrics, Tucson, AZ, USA). For amino acid starvation, cells were cultured in EBSS (Sigma) without amino acids and FBS for 2 h. Images were acquired, analyzed and quantified by NIS-Elements software. Movies were assembled using QuickTime software (Apple, Cupertino, CA, USA).

2.3. Animals

All studies and procedures were carried out with the approval of the Committee of Medical Ethics of Hirosaki University Graduate School of Medicine, Hirosaki, Japan. Animals were housed under standard conditions (12 h light, 12 h dark; food and water available *ad libitum*). Normal or transgenic mice ($n = 55, 12–32$ weeks of age) were orally subjected to 2% (w/v) trehalose, maltose, sucrose or water. Disaccharides were dissolved in the drinking water, and these solutions were changed every two days.

2.4. Biochemical and pathological analyses

Organs were fixed with 4% paraformaldehyde for 48 h. Blocks were cut, embedded in paraffin, sectioned, and then stained with hematoxylin and eosin. Immunohistochemical study were performed as described in supplementary method. The Western blot and filter trap analyses were performed as described in detail (supplementary method). The following antibodies were used in this study. Rabbit antibodies against p62 (MBL, Nagoya, Japan), GABRAPAP/L1 (MBL), LC3 (Sigma), phosphorylated α-synuclein (Abcam, Cambridge, UK), which recognizes α-synuclein with phosphorylated at Ser 129, β-actin (Sigma), HSC70 (Cell Signalling Technology Inc., Danvers, MA, USA), Beclin1 (Novus Biologicals, Littleton, CO, USA), and phosphorylated Beclin1 (Abbiotec, San Diego, CA, USA), which recognizes Beclin1 with phosphorylated at Ser 15 (Ser 14 in mice) [18], mouse monoclonal antibodies against HSP90 (Santa Cruz Buitechnology), pan-synuclein (4D6, GeneTex, Irvine, CA, USA; LB509, Zymed, South San Francisco, CA, USA) and phosphorylated α-synuclein (pSyn#64; Wako), which recognizes α-synuclein with phosphorylated at Ser 129, GFP (Life Technologies), and goat polyclonal antibodies against anti-SIGMAR1 (Santa Cruz Biotechnology, Dallas, TX, USA), and anti-Bip (GRP78; Santa Cruz Biotechnology). Quantitative and statistical analyses were described in detail (supplementary method).

3. Results

3.1. Trehalose efficiently degrades aggregate formation through activated autophagy in cells

Cultured cells were infected with baculovirus containing mCherry-GFP-LC3B to visualize autophagic flux (Fig. 1a). The GFP is sensitive to acidic conditions; therefore, the signal disappears in lysosomes, whereas the mCherry signal remains stable even in lysosomes [19]. Based on this property, colocalization of the mCherry and GFP signal was detected as yellow in vesicles that had not fused with lysosomes: phagophores and autophagosomes, whereas mCherry signal without GFP was localized to amphisomes or autolysosomes (Fig. 1b). Actually, LC3 puncta with both mCherry and GFP signal were clearly detected, and their number increased with time under conditions of rapamycin treatment (1 μM at the indicated time, Fig. 1b, middle columns) and starvation (data not shown). Thus, this system is useful to monitor autophagic flux.

Previous studies showed that trehalose activates autophagic flux and inhibits aggregate formation in cultured cells [16,20]. To examine the effect of trehalose on autophagic flux, cells were treated with trehalose (Fig. 1b, lower columns). Signal number and intensity were increased at the later stage, indicating that LC3-positive structures were accumulating in the cells (Fig. 1c). In support, immunoblotting revealed that levels of lipidated forms of LC3 (LC3-II) and GABARAP/L1, which are also responsive to autophagosomal formation, were increased in cells with trehalose. Latrepirdine was also increased in a concentration-dependent manner. In contrast, less of an effect on LC3 and GABARAP/L1 induction was observed in cells with maltose (Fig. 1d, e, f). To examine the effect of activated autophagy on protein aggregation, we next used a cell-based system bearing LB-like inclusions [21] and compared the number of inclusions in the presence of trehalose (Fig. 1g). The number of inclusions was significantly decreased by 65% in cells treated with trehalose (80 mM) for 24 h compared with the number of inclusions in cells with maltose (data not shown). This result was also confirmed by the filter trap assay, which showed fewer aggregates with the higher concentration of trehalose (Fig. 1h). Collectively, these results suggest that trehalose can prevent protein aggregation through activated autophagy.

3.2. Trehalose intake for a short period activates autophagy in the brain

Autophagy is well known to be activated under conditions of starvation. Consistent with a previous report [22], autophagy was induced in the hearts and livers of mice starved for 48 h, whereas it was not clearly detected in the brain (Fig. 2a). We examined whether trehalose could activate autophagy in the brain. Like the results of the cell-based assay, trehalose (1-week intake) increased

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