TREHALOSE AS A POTENTIAL THERAPEUTIC AGENT IN DIFFERENT DISEASES

TREHALOZA KAO POTENCIJALNI TERAPEUTSKI AGENS U RAZLIČITIM BOLESTIMA

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Abstract

Trehalose is a natural, non-reducing disaccharide synthesized in some bacteria, fungi, plants, and insects. Due to its advantageous physical and chemical properties, trehalose can stabilize proteins and membranes, and protect cells from desiccation, heating, and freezing. Vertebrates do not synthesize trehalose, but the beneficial effects of trehalose have been demonstrated in numerous diseases as it eliminates aggregates, misfolded proteins, and damaged organelles, and reduces hyperinflammation and oxidative stress. Trehalose induces autophagy through nuclear translocation and activation of transcription factor EB (TFEB) in an mTOR-independent manner, but increases the expression of SQSTM1/p62 and has antioxidant properties in an autophagy-independent manner. Furthermore, trehalose induces apoptosis in tumor cells by increasing membrane fluidity through the activation of caspase 3, 6 and the JNK (c-Jun N-terminal kinase) pathway. Overall, in this review, previous knowledge on the therapeutic potential of trehalose in various diseases such as dry eye syndrome, neurodegenerative diseases, and tumors, was summarized, focusing on the underlying molecular mechanisms.

Keywords: trehalose, oxidative stress, autophagy, therapy

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Sažetak

Trehalosa je prirodni, neredukujući disaharid koji se sintetiše u pojedinim biljkama, gljivama, bakterijama i insektima. Zbog povoljnih fizičko-hemijskih svojstava, trehalosa stabilizuje proteine i membrane i time štiti celije od izučenja, smrzavanja i toplotne. Iako kičmenjaci ne sintetišu trehalozu, njena primena omogućava eliminaciju proteinškinh agregata, loše savijenih proteina i oštećenih organela i dovodi do smanjenja hiperinflamacije i oksidativnog stresa. Trehalosa indukuje autofagiju nezavisno od mTOR kompleksa usled aktivacije faktora transkripcije EB (TFEB) i deluje antioksidativno tako što pojačava ekspresiju p62 nezavisno od procesa autofagije. Pored toga, trehalosa povećava fluidnost membrane i dovodi do apoptoze tumorskih celija putem aktivacije kaspaze 3 i 6 i JNK (c-Jun N-terminalna kinaza) signalnog puta. U ovom revijalnom radu sumirali smo dosadašnja saznanja o mogućnostima primene trehaloze u terapiji različitih bolesti kao što su sindrom suvog oka, neurodegenerativne bolesti i tumori, sa posebnim osvrtom na glavne molekularne mehanizme koji se nalaze u osnovi njenog dejstva.

Introduction

Trehalose is a natural, non-reducing disaccharide consisting of two glucose molecules. Due to the presence of an α,α-1,1 glycosidic bond, trehalose has numerous advantageous physicochemical properties that allow organisms that synthesize it to survive under extreme conditions such as high and low ambient temperatures, as well as osmotic and mechanical stress (1-5). The advantageous physicochemical properties of trehalose are used in numerous fields, such as agriculture, the cosmetic industry, the food industry, and the pharmaceutical industry (1). Although vertebrates are unable to synthesize trehalose, the use of trehalose is beneficial in many diseases such as neurodegenerative diseases, tumors, dry eye syndrome, infections, and cardiovascular and renal diseases (6-14). Since the exact underlying mechanisms of its protective function are not well understood, certain studies have confirmed that trehalose can remove damaged proteins and aggregates and eliminate reactive oxygen species directly or by stabilizing antioxidant enzymes (6-8, 15, 16). In addition, trehalose induces autophagy and reduces the inflammatory response (9), removes protein aggregates, protects neurons both in vitro and in vivo (6-8, 17), and inhibits tumor cell growth and proliferation (10-14). In this review, the current knowledge on the potential use of trehalose as a therapeutic agent in different diseases was summarized, highlighting its beneficial effects and underlying molecular mechanisms. To search studies related to the topic, the MEDLINE database (PubMed) was used, by using the words trehalose AND cancer, trehalose AND ophthalmology, trehalose AND neurodegenerative disease, trehalose AND Alzheimer’s disease, trehalose AND Parkinson’s disease as tags for search, and further refine search results by choosing filter publication date 10 years.

Biochemical properties and biological function of trehalose

Trehalose is a natural disaccharide synthesized in certain plants, fungi, bacteria, roundworms, and insects (18). There are at least five identified trehalose biosynthetic pathways, the most common being that involving two enzymatic steps catalyzed by trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TP6PP). Trehalose-6-phosphate synthase catalyzes the transfer of glucose from UDP (uridine diphosphate) or GDP (guanosine diphosphate) to glucose-6-phosphate forming trehalose-6-phosphate and UDP or GDP. Trehalose-6-phosphate is a major intermediate of trehalose biosynthesis, which is dephosphorylated by trehalose-6-phosphate phosphatase to form trehalose and inorganic phosphate (figure 1) (18). According to its chemical properties, trehalose is a non-reducing sugar consisting of two glucose molecules linked by an α,α-1,1-glycosidic bond. Due to the presence of this glycosidic bond, the molecule of trehalose exhibits exceptional thermodynamic stability and does not react with amines, amino acids, and proteins (18). Trehalose is an osmoprotectant with high glass transition temperature and numerous hydroxyl groups forming hydrogen bonds with polar groups of phospholipids (1,18). Therefore, it can replace the water in plants to provide drought stress tolerance and stabilize proteins and lipid bilayers preventing its damage, membrane leakage, and fusion (19). Thus, organisms that synthesize this disaccharide can survive in extremely adverse conditions such as dehydration, high and low ambient temperatures, and osmotic and mechanical stress (1-5).

The useful physicochemical properties of trehalose are used in numerous fields, such as agriculture to improve the resistance of plants to drought and frost, or in the cosmetic industry to increase skin hydration (1). In the food industry, trehalose is used as a sweetener, to mitigate unpleasant food odors and tastes, and to dry fruits and vegetables to extend shelf life and preserve nutritional value (1). In the pharmaceutical industry, trehalose can be used to store and stabilize vaccines, monoclonal antibodies, and long-distance diagnostic kits. It is also used in the cryopreservation of human cells and organelles such as hepatocytes, oocytes, sperm, erythrocytes, hematopoietic and embryonic stem cells, mitochondria, adipocytes, as well as an eyedrop to protect corneal epithelia from desiccation (1). Vertebrates do not have enzymes for the biosynthesis of trehalose but can degrade it to glucose in the small
intestine by the enzyme trehalase found in the intestinal villi (18). Numerous studies indicate the protective effects of trehalose in neurodegenerative diseases, tumors, dry eye syndrome, infections, and cardiovascular and renal diseases (6-14, 20-22). The beneficial effects of trehalose result from its ability to reduce hyperinflammation, prevent aggregates and misfolded proteins accumulation, and eliminate reactive oxygen species directly, or by stabilizing antioxidant enzymes (6-9, 16, 17).

Process of autophagy

Autophagy is an evolutionarily conserved process of degradation of protein aggregates, damaged organelles, and macromolecules in lysosomes, which plays an essential role in cell growth, proliferation, and differentiation (23). There are three types of autophagy: microautophagy, chaperone-mediated autophagy, and macroautophagy. In microautophagy, lysosomes directly through the invagination of the lysosomal membrane, engulf the cytoplasmic material. In chaperone-mediated autophagy, the LAMP2A (lysosome-associated membrane protein 2A) receptor on the surface of the lysosomal membrane recognizes a complex of the target protein and chaperone (Hsc 70), which is then translocated across the lysosomal membrane and broken down within the lysosome by the hydrolytic enzymes. In the process of macroautophagy (hereinafter autophagy), different cellular components are encompassed by a double membranous structure - autophagosome, which merges with the lysosome to form an autophagolysosome, where the cell content is degraded (23).

In addition to Ulk1/Ulk2 and Atg13, other molecules involved in the initial phase of autophagy are FIP200 and Atg101 (27). After the initiation step, an assembly of the complex of Atg molecules with phosphatidylinositol 3-kinase 3-kinase activity enables the formation of nascent autophagosomes or phagophores and it recruits other Atg proteins to the phagophore assembly site (PAS), usually located on the endoplasmic reticulum-mitochondria contact site (28). The most important phosphatidylinositol 3-kinase is Vps34 (vesicular protein sorting 34), which participates in the formation of three complexes such as Atg14L-Beclin1-hVsp34-p150 and UVRAG-Beclin1-hVsp34-p150 which positively regulate autophagy, as well as Rubicon-UVRAG-Beclin1-hVsp34-p150 which negatively regulates autophagy (27, 29, 30). By the action of two ubiquitin-like conjugation systems, Atg12-Atg5 and LC3, newly formed phagophores will elongate to expand their size (31). First, Atg4 leaves proLC3 to form LC3-I which is further conjugated to phosphatidyl-ethanolamine (PE) to form LC3-II, a major feature of the autophagosome widely used as a marker of autophagy (27).

Trehalose as an autophagy inducer and antioxidant

The unique feature of trehalose, which significantly
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contributes to its protective function, is its ability to induce autophagy in cells such as neurons, hepatocytes, fibroblasts, endothelial, and epithelial cells (32). Although one study has demonstrated that trehalose inhibits autophagy flux and increases the number of autophagosomes in SH-SY5Y neuroblastoma cells, derived from a bone marrow biopsy taken from a four-year-old female with neuroblastoma (33, 34), yet many more studies have shown its ability to stimulate autophagy degradation (35-38). Mechanisms by which trehalose induces autophagy are not sufficiently explored. One study showed that trehalose through TFE2 activation could increase the expression of the genes for BECN1, ATG10, ATG12, SQSTM1/p62, and MAP1LC3B in mouse motor neuron-like hybrid NSC cells (35). In addition, it is known that overexpression of heat shock protein B8 (HspB8) protects NSC cells during the stress period through autophagic removal of misfolded proteins (35, 36). Trehalose increases the level of HSPB8 which facilitates degradation of lipid droplets in primary murine hepatocytes and HepG2 cells derived from the liver tissue of male with hepatocellular carcinoma, through the inhibition of a glucose transporter SLC2A8/GLUT8 (solute carrier family 2, [facilitated glucose transporter], member 8). The reduction of glucose uptake leads to energy depletion, which stimulates autophagy through AMPK and activation of ULK (37, 39). However, in COS-7 fibroblast-like cell lines derived from male monkey kidney tissue, and T-REx 293, derived from female Human Embryonic Kidney (HEK) cells, trehalose induces autophagy but independently of mTOR, since the levels of mTOR substrates, ribosomal S6 protein kinase (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) were unchanged (38).

Trehalose also has antioxidant properties, although the underlying mechanisms of its antioxidative activity are not well understood. One study emphasized the role of p62 in the antioxidative activity of trehalose through the activation of the Keap1/Nrf2 signaling pathway manner in Hepa1-6 (a mouse hepatoma derived from the BW7756 hepatoma that arose spontaneously in C57L/J mice), in differentiated 3T3-L1 adipocytes derived from mouse, and Atg5-knockdown and wild type mouse embryonic fibroblasts (MEFs) (16). A cargo receptor SQSTM1/p62 functions as a linkage molecule between LC3-labeled autophagosomes and ubiquitinated proteins that are targeted to be degraded in autolysosomes. Since p62 is a substrate for selective autophagic degradation, the decreased p62 level is used as a marker of autophagy degradation, while p62 accumulation indicates the blockage of the autophagic flux. In this study, trehalose activates autophagy degradation but also upregulates p62 expression in an autophagy-independent manner (16). p62 competitively interacts with Nrf2 (nuclear factor erythroid 2-related factor 2) inhibitor Keap, which allows Nrf2 to be released into the nucleus and be activated. Activated Nrf2 enhances the expression of antioxidant genes heme oxygenase-1 (Ho-1) and nicotinamide adenine dinucleotide phosphate quinone dehydrogenase 1 (Nqo1) (16). In that manner, p62 is a linkage molecule between autophagy and oxidative stress and has an important role in the antioxidative action of trehalose. Furthermore, trehalose can reduce endoplasmic reticulum stress by inhibiting AMPK activity, as well as oxidative stress by maintaining the activity of two antioxidant enzymes, superoxide dismutase, and catalase in SH-SY5Y cells, treated with hydrogen peroxide (40).

**Trehalose as an anticancer agent**

Trehalose is a natural compound that is suggested to be an efficient anticancer agent by targeting cell growth and proliferation, angiogenesis, and metastasis at the molecular level (41). Trehalose exhibits antitumor activity in *in vitro* models of human hepatocellular carcinoma, colon cancer isolated from the colon of an adult male with colon cancer, gastric cancer, and in vivo model of acute lymphoblastic leukemia (10, 11). In a xenograft lung cancer model induced by the transplantation of A549 malignant cells (isolated from the lung tissue of a male with lung cancer) into mice, liposomes with trehalose reduced tumor growth and increased the level of apoptotic cells in tumor tissue (12). Recent data indicate that liposomes with trehalose also stimulate apoptosis by increasing membrane fluidity leading to the activation of caspase 3, 6 and JNK (Jun-c-Jun N-terminal kinase) signaling pathway, and inactivation of transcription factor NF-κB (nuclear factor-κB) (13). In two different melanoma cell lines (female A375, and male human melanoma cell line SK-Mel-28) trehalose significantly reduced tumor growth through autophagy.
Trehalose in ophtalmology

Trehalose can reduce hyperinflammation and oxidative stress, and protects cells and organelles from dehydration and damage. Therefore, 3% trehalose solution has been used as an effective eye drop for the treatment of moderate to severe dry eye syndrome (48–50). It is shown that in HeLa cells (derived from cervical carcinoma), trehalose-mediated osmoprotection depends on its ability to induce autophagy to maintain epithelial cell homeostasis in dried-out conditions (51). Moreover, combination of sodium hyaluronate with trehalose showed a synergistic effect in the induction of autophagy (51). In human corneal endothelial cells (HCECs) obtained from donors 18 to 65 years of age, trehalose induces autophagy by activating TFEB (transcription factor EB) through Akt inhibition thus suppressing increased levels of TNF-α, IL-1β, IL-6, and IL-8 induced by hyperosmolarity (9).

Trehalose in neurodegenerative diseases

Trehalose is a promising neuroprotective agent, and its neuroprotective effects have been investigated in numerous studies, highlighting the various mechanisms by which trehalose exerts its neuroprotective function. Some results suggest that trehalose can increase the expression of progranulin, a neuronal growth factor, in H4 cells derived from male neurogliomas, and in 3-month-old mice with haploinsufficiency independent of transcription factor EB (TFEB) (52). In the immortalized motoneurons NSC34, trehalose increases the expression of autophagy-related genes for SQSTM1/p62, BECLIN1, LC3B, ATG10, and ATG12, and induces lysosome synthesis through activation of transcription factor EB (TFEB) to prevent autophagosome accumulation and neuronal damage (35). Accordingly, trehalose enables the elimination of misfolded proteins and aggregates in a number of models of proteinopathies, both in vitro and in vivo, underscoring that autophagy is an important mechanism to prevent neurodegeneration (53, 54).

Increasing evidence suggests that defects in autophagic flux, or specific autophagy-regulatory processes, as well as reduced autophagic degradation, contribute to motor neuron degeneration and death in amyotrophic lateral sclerosis (ALS) (55–57). Mutant SOD1 protein, which is accumulated in the familial form of the disease could damage motor neurons through abnormal axonal transport, mitochondrial dysfunction, and activation of apoptosis (55, 56). In addition, mutant SOD1 through inhibition of dynein/dynactin function disrupts the fusion of autophagosomes with lysosomes and inhibits autophagic flux (55). In vitro study demonstrated that trehalose induces autophagy and decreases the levels of mutant SOD1-G93A in NSC34 cells (58, 59). In a SOD1-G93A transgenic mouse model of ALS, trehalose reduced the levels of mutant SOD1 (Gly93 to Ala) in the spinal cord via autophagy stimulation, and improve motor function during the early stages of the disease (58, 59). Another study showed that
in peripheral blood mononuclear cells (PBMCs) of ALS patients, there is inhibition of fusion between autophagosome and lysosomes which impaired mitophagy (selective autophagic degradation of mitochondria) leading to the accumulation and clusterization of mitochondria. In this study, trehalose could remove accumulated lysosomes which could restore mitophagy in cells (60).

In immortalized motoneuronal models of spinal and bulbar muscular atrophy, trehalose through autophagy activation promotes the clearance of mutant androgen receptor (AR) with an abnormally long polyglutamine tract (ARpolyQ) in cells overexpressing HspB8, thus maintaining motoneuron proteostasis and viability (36). While silencing of heat shock protein family B (HspB8) in the presence of trehalose resulted in a significant accumulation of ARpolyQ (AR.Q46) - insoluble species compared with trehalose alone, it doesn’t stop completely degradation process which suggests that HspB8 is not indispensable for trehalose induced autophagy in NSC34 cells, but it is an important pro-autophagic mediator which augments and facilitates the autophagic degradation (36).

In Huntington’s disease, long polyglutamine tails in the mutant huntingtin protein caused by the expansion of CAG repeats in the huntingtin gene, are cleaved to N-terminal fragments forming protein aggregates. Trehalose as a chemical chaperone and inducer of autophagy could promote the elimination of aberrant proteins, and since the inclusion is much larger than autophagosomes, autophagy could degrade only smaller aggregate precursors such as soluble and oligomeric species (35). In doxycycline-inducible PC-12 cells derived from a pheochromocytoma of the rat adrenal medulla, expressing EGFP-HDQ74, trehalose promotes degradation of soluble EGFP-HDQ74 and insoluble mutant huntingtin (38). The inhibition of autophagy by 3-methyl adenine didn’t further reduce EGFP-HDQ74 aggregates in trehalose treated COS-7 cells, while inhibition of proteasome with lactacystin significantly decreased the accumulation of misfolded proteins which suggests that trehalose-induced clearance of protein aggregates was mediated only by autophagy degradation, but not by proteasome activity (38). On the other hand, another study has shown that trehalose inhibits aggregates of two different polyglutamine-bearing proteins, Mb-Gln35, and truncated huntingtin, without inducing cell stress pathways in Neuro 2A cells derived from mouse neural crest (7). Additionally, this study showed that in the R6/2 transgenic mouse model of Huntington’s disease treated with 2 % (w/v) orally administered trehalose, significantly decreases polyglutamine aggregates consequently reducing the brain damage which improves motor dysfunction and extending lifespan (7).

In addition to the ubiquitin-proteasome system and the endosome-lysosome system, autophagy is one of the main mechanisms involved in the elimination of tau aggregates and beta-amyloid (Aβ) deposits in various Alzheimer’s disease (AD) models, especially in the late phase of the disease (61-64). Trehalose as a chemical chaperone stabilizes molecules to prevent protein aggregation, while as an autophagy activator trehalose enables the degradation of misfolded proteins and aggregates (62). Trehalose reduces neurotoxicity by activating autophagy that allows the degradation of tau aggregates in tau-overexpressing rat cortical neurons isolated from Sprague-Dawley rat embryos at day 18 (6). In three-month-old mice with overexpressed tau protein and parkin deletion (PK−/-/TauWt), trehalose through autophagy reduces the amount of Aβ plaques and phosphorylated tau protein and consequently improves behavior and motor-neuron function (63). Moreover, trehalose inhibits oligomeric aggregation of Aβ16-22, and Aβ40 and the formation of fibrillar structures of Aβ40 and Aβ42 by preventing β-sheet conformational state (62, 66, 67). In the AD mouse model APP/PS1, trehalose reduced secreted Aβ level in an autophagy-independent manner, by reducing colocalization of Aβ precursor protein (APP) and beta-secretase (BACE) in the cell, without changing lysosome acidity (68).

Numerous studies demonstrate a neuroprotective effect of trehalose in various models of Parkinson’s disease (PD) as well (69). Trehalose selectively reduces the accumulation of A53T α-synuclein aggregates by increasing the number of autophagosomes, lysosomes, and autolysosomes and enhances LC3-II level in PC-12 cells (70). In the mouse model of Lewy body disease, where mice were housed under standard conditions (12 h light, 12 h dark; food and water available ad libitum), 2% (w/v) orally administrated trehalose decreases the level of insoluble α-synuclein while increasing the level of LC3-II and chaperon molecules, HSP90 and SigmaR1 (71). Trehalose also suppresses inflammation in a mouse model of PD, generated from 8-week-old male mice (C57BL6), by inhibiting activation of microglia and astrocytes in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (71). Furthermore, in this study, trehalose prevents the downregulation of two tight junctional proteins, ZO-1, and occludin in endothelial cells, as well as the glucose transporter-1 protecting the blood-brain barrier in MPTP-treated mice (72). A recent study showed that trehalose decreased α-synuclein and p62 accumulation in the brain and improve prodromal non-motor signs such as olfactory dysfunction and depressive-like behaviors of 8-week-old male C57BL/6 mice exposed to rotenone (17). Furthermore, the combination of trehalose with sodium butyrate, a pan histone deacetylase inhibitor, decreased the level of pro-inflammatory cytokines reducing the motor dysfunction in PD induced by preformed fibrillar form (PFF) of α-syn in Wister rats (73).

**Conclusion**

Overall, this review suggests that trehalose, as a nonreducing sugar that stabilizes and protects molecules and organelles from damage, may also show beneficial effects in many diseases, especially tumors, dry eye syndrome, and neurodegenerative diseases due to its...
autophagy-promoting, anti-inflammatory ability and p62-mediated antioxidant properties. Since the exact mechanisms of its antitumor and neuroprotective effect have not yet been elucidated, further research is needed to uncover the molecules and signaling pathways involved in these processes. In addition, further knowledge in this area could provide a basis for using this natural disaccharide to develop new effective therapies.

Literature


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