MINI REVIEW

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

The emerging role of autophagy in the pathophysiology of diabetic neuropathy

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Abstract

Diabetic neuropathy (DN) is a common complication that is associated with diabetic patients. It affects almost half of diabetic patients worldwide. Peripheral DN is associated with neurodegeneration as evidenced by the degenerative loss of fibres in the peripheral nerves with concomitant decrease of nerve conduction Multiple mechanisms were proposed to have role in the pathophysiology of DN including oxidative stress, inflammation and recently autophagy. Autophagy is a cellular process that is present from yeast to mammals for the sake of recycle damaged organelles and protein in autophagosomes. Persistent hyperglycaemia causes an imbalance in the cellular autophagic pathways. Autophagy plays an important role in reserving the cellular homeostasis via its ability to recycle the damaged proteins and organelles. The link between the activation of autophagic flux with the control of the toxic effect of hyperglycaemia on the neuronal cells will be discussed in this review. The present review hypothesizes that upregulation of the autophagic pathways in neuronal cells may aid them to ameliorate the bioenergetic crisis and cellular damage concomitant with DN.

Keywords: Autophagy; Diabetic neuropathy; pathogenesis.

1. Introduction

Long standing hyperglycemia associated with diabetic subjects can result in the development of various microvascular complications including diabetic neuropathy (DN). Symptoms of DN may disturb the quality of life of affected patients as they include tingling sensation in the extremities, burning pain, allodynia and hyperalgesia **(Schreiber et al. 2015)**. Almost half of diabetic subjects of both types of diabetes suffer from DN **(Zimmet 2009)**. Pregabalin and Duloxetine are among FDA approved drugs to control the neuropathic pain which is the main concern in patients suffering from DN. They act mainly by interfering with the neurotransmitters involved in the pain processing pathways, thus they are capable to relieve symptoms associated with DN **(Ziegler 2008)**. Several mechanisms are involved in the pathogenesis of DN including like oxidative/nitrosative stress, inflammation, endoplasmic reticulum stress, metabolic crisis and demyelination **(Zenker et al. 2013)**. However, different drugs targeting any of these pathways fail to control the symptoms on large scale of clinical trials **(Singh et al. 2014)**. This can be attributed to the involvement of more than one signaling pathway in the etiology of DN or to the specific stage of involvement of a certain pathway. Therefore, there is a need to search for drugs that are able to target

many of the involved pathways.

Autophagy is a cellular process that is present from yeast to mammals for the sake of recycling damaged organelles and protein in autophagosomes. Autophagy is additionally involved in clearing cells undergone physiological programmed cell death **(Mizushima 2007)**. Dysregulation of autophagy was reported in the pathogenesis of several diseases including cancer and neurodegenerative disorders **(Ravikumar et al. 2010; Wong and Cuervo 2010)**. Peripheral DN is associated with neurodegeneration as evidenced by the degenerative loss of fibres in the peripheral nerves with concomitant decrease of nerve conduction **(Reichling and Levine 2011)**. In the present review, the theoretical assumption of linking the activation of autophagic flux with the control of the toxic effect of hyperglycemia on the neuronal cells is discussed.

2. Regulation of Basic Machinery of Autophagy

Autophagy is a complex multistep cellular process that occurs with the aid of set of proteins named autophagy related proteins (Atg). The first step is the formation of a phagophore that is derived either from Golgi apparatus or endoplasmic reticulum **(Mizushima et al. 2011)**. The phagophore is made by the combination of Atg 13 and Atg 17 (FIP200) with Atg1or Ulk 1 in mammals. Mammalian target of rapamycin (mTOR) kinase controls this step thus the autophagy is initiated upon the exposure to nutrient starvation and stress signals. In case of nutrient availability, mTOR can phosphorylate Atg13 thus prevents its combination with Atg1 and hence inhibits autophagy **(Jung et al. 2010)**. The elongation and extension of phagophore is further controlled by the activity of class III PI3K enzyme (Vps34) to produce phosphatidyl inositol triphosphate (PI3P) from phosphatidyl inositol (PI). Beclin1 (mammalian homolog of Atg6) also helps to recruit other Atg proteins to the growing phagophore membrane **(Glick et al. 2010)**.

The maturation involves the association of Atg5-Atg12 to Atg16L to form a complex attached to autophagosome. In addition, this Atg5-Atg12-Atg16L complex recruits microtubule associated protein light chain (LC3B) (mammalian homolog of Atg8) into the autophagosome **(Glick et al. 2010)**. The matured autophagosome could be delivered to the lysosomes through endosomal trafficking that involves the activity of Rab7 GTPases and cytoskeletal motor proteins. The hydrolases of lysosomes can digest the engulfed cellular debris and deliver the basic subunits through permeases to cytosol for recycling **(Longatti and Tooze 2009)**.

3. Chaperone-mediated Autophagy in Diabetic Neuropathy

Chaperone-mediated autophagy (CMA) is an intracellular degradation process that involves the activity of lysosomes to breakdown the cytosolic protein **(Kim and Koh 2021)**. Chaperones are mainly implicated in the repair of degraded proteins. Commonly the neuropathic disorders are characterised by the presence of intracellular protein aggregates **(Xilouri et al. 2013).** PMP22 is a key component of myelin sheath that was found to be aggregated in several neuropathies leading to the formation of aggresomes. Stimulation of autophagy contributes in the clearance of aggresomes thus the upregulation of chaperone receptors in the lysosomal membrane could be a useful therapeutic target to clear the protein aggregates **(Ravikumar et al. 2009)**.

Diabetic neuropathy is characterized by the decrease in the nerve conduction and demyelination of either the motor or the sensory nerves or both. Enhanced activity of CMA could attenuate these symptoms **(Mizushima et al. 2004)**. Autophagy can be induced by inhibition of mTORC that acts by phosphorylating the Atg13 and thus prevents its combination with Atg1 and inhibits the formation of autophagosomes **(Jung et al. 2010)**. In addition, autophagy can be stimulated by another mechanism independent to mTOR involving the phosphoinositol and calcium signaling. Therefore, there are several drugs and natural products acting to activate autophagy either as mTOR inhibitors or inositol monophosphatase (IMPase) inhibitors were shown to protect against neuropathies.

Figure 1 shows the postulation for the therapeutic effect of activators of autophagy on diabetic neuropathy. Examples for some drugs and their mode of action in protection against several models of neuropathies are presented in **Table 1**.

Regardless the signalling pathways involved in the activation of autophagy, it is expected that induction of autophagy can ameliorate the deleterious effects associated with DN. Autophagy induction can reduce the oxidative stress, enhance ATP production, and lessen apoptosis through BCL-2

activation **(Choi et al. 2013)**. In addition, it can be postulated that drugs able to activate autophagy either by inhibiting mTOR or inositol monophosphatase can prevent the mitochondrial dysfunction and clear out the protein aggregates.

4. Modulation of autophagy in diabetic neuropathy

There is some evidence of accumulation of autophagosomes upon exposure of sera separated from type 2 diabetic neuropathic patients to neuroblastoma cells. This was confirmed by increased LC3-II immunoreactivity **(Towns et al. 2005)**. In experimental model of DN, it was demonstrated that there was an impairment of autophagy reflux with concomitant mitochondrial dysfunction **(Yang et al. 2014)**.

Melatonin is a potent antioxidant that was shown to reduce the neuronal excitability in a subset of the dorsal root ganglia (DRGs) neurons **(Tan et al. 2015, Oliveira-Abreu et al. 2018)**. Intraperitoneal administration of melatonin alleviates oxaliplatin-induced pain and neuropathic impairments in rats through boosting the autophagy pathway in peripheral neurons and DRG **(Areti et al. 2017)**.

Progranulin is a trophic factor that supports neurons by suppressing microglia activation. It was found in neurons and microglia in the nervous system **(Tanaka et al. 2013)**. Progranulin overexpression in DRG nociceptive neurons improves autophagy and pain behaviour in damaged nerves **(Altmann et al. 2016)**. Another example is Chloroquine (CQ) that can inhibit the lysosomal proteases and autophagosome-lysosomal fusion processes, making it an autophagy blocker **(Geng et al. 2010)**. Therefore, intrathecal injection of CQ in naive mice causes spinal accumulation of microtubule-associated protein 1 light chain 3 (LC3) and p62, as well as significant mechanical hypersensitivity, implying a block in autophagosome clearance and demonstrating the role of the autophagic process in spinal pain processing mechanisms **(Berliocchi et al. 2015)**.

5. Conclusion

Autophagy is an important pathway that involves the activity of lysosomal enzymes for the sake of recycle damaged proteins. In diabetic neuropathy, autophagy is crucial to recycle the damaged protein aggregates and lessen the mitochondrial damage. Thus, autophagy induction can enhance the survival chance in neurons and glia exposed to hyperglycemic conditions. Being a catabolic process, autophagy induction improves the bioenergetic status. Therefore, drugs especially natural ones that are able to induce autophagy could be tested as therapeutic tool to treat neuropathy.

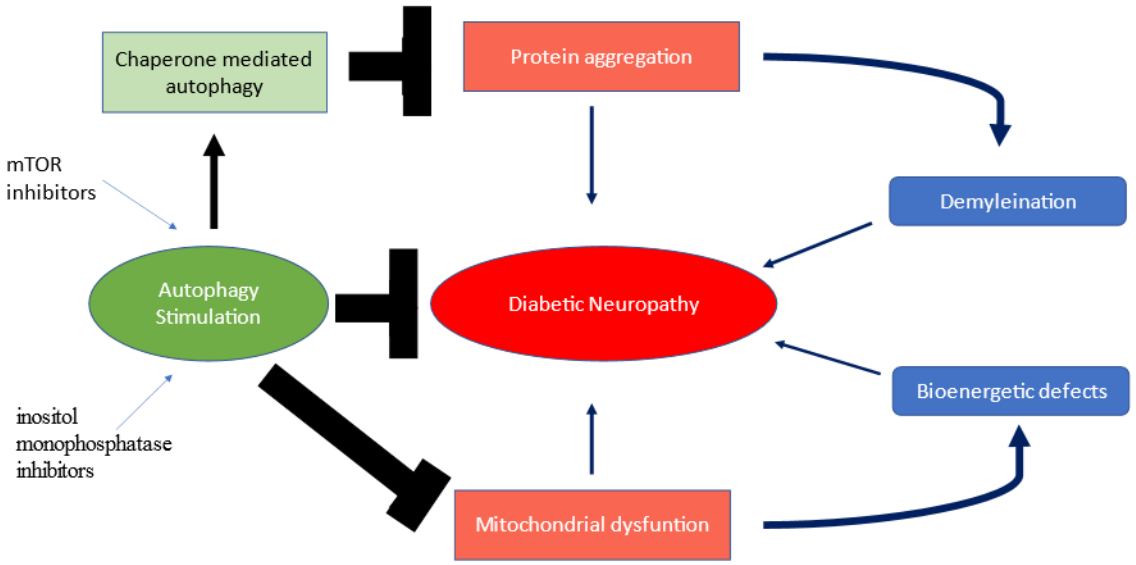

Figure 1: Proposed theory for potential effect of activators of autophagy on diabetic neuropathy **(Yerra et al. 2016)**

Table 1: Examples of some mTOR inhibitors and inositol monophosphatase inhibitors experiencing neuroprotection and the proposed mode of action

Drug	Model	Mode of action	Major finding	Reference
Rapamycin	Spinal cord injury (SCI) in mice	Inhibition of mTOC1	Reduced neuronal damage and locomotor impairment through autophagy activation	**(Sekiguchi et al. 2012)**
Cystatin C	murine primary cortical neuronal cell line and N2a cells	mTOR inhibition	Increased cell survival and had anti-Aβ amyloidogenetic property	**(Tizon et al. 2010)**
Lamotrigine	Animal model of Alzheimer's disease (AD) in AbPP/PS1 mice	mTOR inhibition and CREB activation	Reduced Ab production by reducing the mRNA levels of β-site AβPP-cleaving enzyme 1 (BACE)	**(Wu et al. 2015)**
Curcumin	Alzheimer's disease in APP/ PS1 double transgenic mice	Down regulating PI3 K/Akt/ mTOR signaling pathway	Inhibit the generation and deposition of Aβ	**(Wang et al. 2014)**
Sodium valproate	Drosophila and Zebra fish models of Huntington's disease	Reduced ionositol and IP3 levels	Reduced accumulation of huntingtin and other mutant proteins	**(Williams et al. 2008)**
Resveratrol	Rotenone-induced neurotoxicity in dopaminergic SH-SY5Y cells	Heamoxygenase 1 (HO-1) dependent autophagic flux	Reduced apoptosis and oxidative damage to dopaminergic neurons	**(Lin et al. 2014)**
β-Asarone	6-Hydroxy DopamineInduced Parkinsonism in rats	through modulation of JNK/ Bcl-2/Beclin-1 pathway	β-Asarone improved the behavioral symptoms of rats in the open field, rotarod test, initiation time, and stepping time	**(Zhang et al. 2016)**

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