Hydrogen sulfide plays an important protective role by influencing autophagy in diseases

Jun Wang, Dongdong Wu, Honggang Wang*

School of Basic Medical Science, Henan University, Kaifeng, Henan, 475004, China

*Corresponding author Honggang Wang School of Basic Medical Science, Henan University, Kaifeng, Henan, 475004, China E-mail: whg1975316@sina.com

Short title

HYDROGEN SULFIDE INFLUENCES AUTOPHAGY
Summary

Autophagy can regulate cell growth, proliferation, and stability of cell environment. Its dysfunction can be involved in a variety of diseases. Hydrogen sulfide (H\textsubscript{2}S) is an important signaling molecule that regulates many physiological and pathological processes. Recent studies indicate that H\textsubscript{2}S plays an important protective role in many diseases through influencing autophagy, but its mechanism is not fully understood. This article reviewed the progress about the effect of H\textsubscript{2}S on autophagy in diseases in recent years in order to provide theoretical basis for the further research on the interaction of H\textsubscript{2}S and autophagy and the mechanisms involved.

**KERWORDS:** Hydrogen sulfide; Autophagy; Ischemia reperfusion injury; Metabolic diseases

1. Introduction

Autophagy is a process of self-sustaining internal environment stability in eukaryotic cells, in which pathogens, abnormal proteins and organelles are encapsulated by the bilayer membranes to form autophagosomes and then transferred
to lysosome for degradation (Sir et al. 2016, Qiu et al. 2014, Murrow et al. 2013, Kimura 2014). Autophagy can be classified into macroautophagy, microautophagy, and chaperone-mediated autophagy based on the inducing signals, its timing, types of targets and pathways of delivery of cargo into the lysosome (Gomes et al. 2017, Parzych et al. 2014). Among them, macroautophagy is the most studied autophagy, in which the content is wrapped by bilayer membrane structure to form autophagosome and then fuses with lysosome for degradation. Microautophagy refers to that the lysosomal membrane directly invaginate and then encapsulate the cell contents. Chaperone-mediated autophagy is selective, in which the cytosolic proteins are transported to the lysosomal chamber after binding to molecular chaperones, and then are digested by lysosomal enzymes (Figure1) (Rubinsztein et al. 2012). Under physiological conditions, autophagy is often maintained at the basic level. The internal and external factors such as ischemia, hypoxia, pathogenic infection, hormone therapy, protein misfolding, and nutritional deficiency can induce autophagy (Matsui et al. 2007). When the body is in pathological state, the remarkably enhanced autophagy can remove the abnormal protein in the cell, which is beneficial to the survival of the cell. The effect of autophagy on the cell is in a "double-edged sword" mode, since autophagy can cause autophagic death if the autophagy remains at a high level (Garcia-Huerta et al. 2016, Liu and Levine 2015 ). LC3, Beclin1, and other conserved proteins are involved in the process of autophagy, which are called autophagy related proteins (Penaloza et al. 2008). Recent studies have shown that autophagy plays important roles in maintaining the balance of synthesis, decomposition, and reutilization of cell components (Wei et al. 2016). Abnormal autophagy is involved in the development of the pathological processes such as liver disease, cancer, aging, cardiovascular disease, and kidney disease (Yin and Lu 2017).
Hydrogen sulfide (H\(_2\)S) has long been regarded as a flammable, water-soluble, colorless, and toxic gas with the smell of rotten eggs, but since 90s of last century, mounting researches have convincingly proved that H\(_2\)S is the third gasotransmitter in various biological systems along with nitric oxide and carbon monoxide (Li et al. 2011, Olson 2012, Wang 2012, Kolluru 2013). In mammalian cells, H\(_2\)S is produced by three enzymes: cystathionine \(\beta\)-synthase (CBS), cystathionine \(\gamma\)-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST) (Kimura 2014). The \(\beta\)-replacement reaction of homocysteine with serine is catalyzed by CBS to produce cystathionine(Stipanuk 2004). The \(\alpha\), \(\gamma\)-elimination of cystathionine is catalyzed by CSE to produce cysteine, \(\alpha\)-ketobutyrate and NH\(_3\). H\(_2\)S is generated from cysteine via \(\beta\) elimination reactions catalyzed by CBS and CSE. 3-mercaptopyruvate is produced by the way in which cysteine aminotransferase transfers the amine group from cysteine to \(\alpha\)-ketoglutarate. 3-MST transforms the sulfur of 3-MP into a persulfide to add into the enzyme (3-MST-SSH). The terminal sulfur is released as H\(_2\)S by two endogenous reductants thioredoxin or dihydrolipoic acid (Mikami et al. 2011, Ishigami et al. 2009, Shibuya et al. 2009). The distribution of H\(_2\)S-producing enzymes have tissue specificity. CBS mainly distributes in the liver, kidney, central nerves system, and so on (Distrutti et al. 2006, Kimura 2013, Feliers et al. 2016). CSE mainly distributes in the cardiovascular system (Polhemus and Lefer 2014). In addition, the gut bacteria are important source of H2S and its derivates (Huc et al. 2016, Tomasov et al. 2016). H2S has multiple biological effects depend not on H2S itself but on the formation of new molecules, such as S-nitrosothiols, and its possible mechanisms include reversible protein sulfidation, which alters the function of modified proteins, similar to nitrosation or phosphorylation, direct antioxidant activity and interaction with metalloproteins (Dongó et al. 2017). It has been demonstrated
that H$_2$S plays an important role in many kinds of pathological and physiological processes including development, angiogenesis, carcinogenesis, endoplasmic reticulum stress, and oxidative stress (Huc Paul and Snyder 2012, Kolluru et al. 2013, Szabo 2016, Cao and Bian 2016, Xu et al. 2017, Feliers et al. 2016). Many studies have shown that H$_2$S might have potential therapeutic effects in the diseases by inhibiting or promoting autophagy in a concentration-dependent manner. So it is necessary to clarify the mechanism that H$_2$S acts on autophagy.

In this review, we summarize the progress about the effects of H$_2$S on autophagy in diseases in recent years to provide theoretical basis for the further research on the interaction between H$_2$S and autophagy and the mechanism involved.

2. H$_2$S influences autophagy in ischemia-reperfusion (I/R) injury

Tissue ischemia, which often affects autophagy, is an important cause of death and disability in the world. After a period of ischemia, the recovery of blood supply further aggravates the injury of tissue and organ, that is, ischemic reperfusion injury. It has been proved that cell damage induced by free oxygen free radical plays a key role in the IR injury. Ischemia causes hypoxia in the tissue and increases the level of lactic acid, hypoxanthine and lipid peroxide, and the restoring of the oxygen supply produces a large number of free radicals, then free radicals reacts with lipid and mitochondria in cells to produce lipid peroxides, which causes cells death and organ damage (Temiz et al. 2013, Usul et al. 2004). Recent studies have shown that H$_2$S exerted cellular protection through pro-autophagy or anti-autophagy in the process of tissue I/R injury. However, the exact mechanism is not fully understood.

2.1 The pro-autophagy effect of H$_2$S

The upregulation of the autophagy level has been found in a variety of spinal
cord injury models, and might play tissue protective roles (Zhang et al. 2014, Hou et al. 2014). The biological function of miRNA was believed to be widely involved in organic I/R injury (Bijkerk et al. 2014). It has been reported that several miRNA were involved in autophagy regulation by regulating the expression of autophagy-related genes (Wang et al. 2014). In spinal cord I/R injury, the expression of miR-30c increased, exogenous H₂S upregulated the level of autophagy by inhibiting the expression of miR-30c to protect spinal cord injury (Lei et al. 2015). This indicated that miR-30c might be an important target for reducing spinal cord I/R damage. It has been showed that myocardial I/R promoted autophagosome formation, but not lysosome-autophagosome formation, thus inhibited autophagosome clearance to downregulate autophagy and resulted in myocardial ischemia injury (Ma et al. 2012, Zhang et al. 2014). H₂S activated adenosine monophosphate-activated protein kinase (AMPK) in several types of cells (Zhou et al. 2014, Jia et al. 2013). Activated AMPK could induce autophagy through inhibiting mammalian target of rapamycin (mTOR). In myocardial I/R injury, exogenous H₂S upregulated autophagy and activated AMPK to protect cardiomyocytes. In addition, AMPK inhibitors downregulated AMPK activation and abolished the cardioprotective effect of H₂S, suggesting that the activation of AMPK was one pathway for the protection of the heart by H₂S (Xie et al. 2015). The mechanism by which H₂S can increase autophagy by activating AMPK needs further study. It probably provide a new therapeutic strategy for the myocardial I/R injury. Hypoxia ischemia (HI) could lead to neuronal loss and severe neurological deficits in premature infants (Hagberg et al. 2015). Studies have shown that HI inhibited autophagy clearance at the later stage, resulting in cortical neuron death (Cui et al. 2017). LC3 is a marker of autophagy. When autophagy is formed, the cytoplasmic LC3-I will hydrolyze a small fraction of polypeptides and change into (autophagic)
membrane type (LC3-II). The ratio of LC3-II/I can be used to estimate the level of autophagy. Beclin 1, a sign of initiating cell autophagy, is involved in the formation of autophagy by forming a complex with Class III PI3K. P62, which can act as a receptor for vesicles to be degraded by autophagy, is integrated with mature autophagosome, so the level of P62 is negatively correlated with autophagy. In neonatal HI mice, HI could lead to elevated levels of LC3-II and P62, followed by a decrease of Beclin 1, which suggested that the accumulation of LC3-II is due to impaired autophagy fluxes. HI did not affect mTOR phosphorylation, but increased LC3-II, which indicated that HI reduced lysosomal degradation, but did not affect autophagy initiation. L-cysteine, a H₂S donor, significantly increased the expression of LC3-II and Beclin 1, but decreased the expression of p62, promoted autophagy, thereby alleviated hypoxic ischemic injury. Moreover, this protective effect was achieved by lowering the phosphorylation level of mTOR (Xin et al. 2018).

2.2 The anti-autophagy effect of H₂S

Recent studies have shown that H₂S protected cells against autophagy in the process of tissue I/R injury. Cerebral ischemia is an important cause of death and disability in adults worldwide (Yan et al. 2016). Autophagy was over-activated in rat brains subjected to middle cerebral artery occlusion and PC12 cells subjected to oxygen-glucose deprivation/reoxygenation, resulting in autophagic death of a large number of brain cells. NaHS, a H₂S donor, could inhibit the autophagy to greatly alleviate the damage. The inhibition of autophagy by autophagy inhibitor could further reduce injury, while the upregulation of autophagy by autophagy stimulator could aggravate injury, which suggested that exogenous H₂S could attenuate cerebral I/R injury by suppressing overactivated autophagy (Jiang et al. 2017). Rat cerebral ischemia could increase the expression of LC3-II, reduce the expression of P62 to
promote the aggregation of autophagosome and maintain the autophagy at a high level. Exogenous H$_2$S could reverse the above changes and inhibit autophagic death of the brain cells to protect cerebral against I/R injury by reducing autophagosome. However, the reduction of autophagosome did not mean that the level of autophagy was reduced. The above regulatory role of H$_2$S might be due to reducing the formation of autophagy, or accelerating the degradation of autophagy. In addition, H$_2$S did not affect the expression of Beclin1. Since the level of Beclin 1 did not accurately reflect the activity of Beclin-1-VPS34-AMBRA1, the role of Beclin-1 complex in regulating cell autophagy by H$_2$S needed further study (Shui et al. 2016, Klionsky et al. 2008, Liu et al. 2016). Ischemic heart disease is especially important in the world with high mortality. Reperfusion, which is the main treatment strategy, can cause damage to the heart again. So it is important to find a way to reduce heart reperfusion injury (Sivaraman et al. 2014)[53]. Exogenous H$_2$S could play protective roles through influencing the level of autophagy by affecting different signaling pathways. It has been known that phosphatidylinositol 3-kinase (PI3K) protected cardiomyocytes by influence apoptosis and autophagy in mammalian cells. SGK1, activated by PI3K, is a downstream target protein of PI3K. GSK3β is an important downstream target of SGK1. During myocardial I/R injury in neonatal rats, exogenous H$_2$S activated SGK1 through PI3K and then inhibited GSK3β, regulated the PI3K/SGK1/GSK3β signal pathway, and inhibited autophagy to reduce myocardial injury (Park et al. 1999, Jiang et al. 2016). Previous studies have shown that liver I/R could excessively activate autophagy, which leaded to autophagic death. So blocking the cell death pathways could significantly reduce liver I/R damage (Chen et al. 2013, Shen et al. 2013). In the course of liver I/R injury, the JNK signal pathway was excessively activated and then inhibited the separation of Bcl-2 and Beclin-1 to promote apoptosis. Exogenous H$_2$S
could protect the liver by reducing the JNK signal pathway to inhibit apoptosis and autophagy (Cheng et al. 2014). Spinal cord I/R injury is a serious complication of thoracoabdominal aortic surgery. About 40% of patients suffer from paraplegia (Zhang et al. 2012). Autophagy played an important role in spinal cord injury (Fujita et al. 2015). During the reperfusion injury after 1h in spinal cord ischemia, the oxidative stress was enhanced, the autophagy level was upregulated, the autophagic death was increased and the exogenous H2S could inhibit the oxidative stress level of the cells through influencing the AKT/mTOR signal pathway and reduce the autophagy to mitigate spinal cord I/R injury (Xie et al. 2017). In addition, the time windows for the activation of autophagy exerts influence in I/R injury. Early activation of autophagy could alleviate spinal cord I/R injury by inhibiting apoptosis and inflammatory response. However, later excessively elevated autophagy induced autophagic cell death to aggravate I/R damage (Fang et al. 2016).

Whether the autophagy plays a beneficial or harmful role after I/R is elusive. Autophagy induced by ischemia can promote survival by degrading lipid and protein in the cells into reusable free fatty acids and amino acids. On the other hand, autophagy also promotes cell death and aggravates I/R damage. Generally, mild injury at the early stage of I/R can activate autophagy to play protective role, and excessive activation of autophagy caused by severe injury at the late stage of I/R can play a destructive role. H2S not only activates autophagy but also inhibits autophagy, and what role it plays depends on the type of tissue and the basal level of autophagy in the tissue.

3. H2S influences autophagy in glycometabolic diseases

Hyperglycemia often causes organic lesions by affecting autophagy. It was
reported that the decrease of H\textsubscript{2}S caused the deposition and hypertrophy of renal matrix protein in diabetic nephropathy. Exogenous H\textsubscript{2}S could improve the above hyperglycemic effect (Lee et al. 2012). AMPK is activated through phosphorylation by its upstream liver kinase B1 (LKB1) complex with two other subunits, STE20 related adapters (STAD) and mouse protein 25 (MO25) (Hardie. 2008, Alessi et al. 2006). In the lomerular endothelial cells of the high blood glucose mice model, hyperglycemia reduced the expression of CBS, CSE, and H\textsubscript{2}S level, thereby inhibiting the activation of LKB1 and AMPK. Inactive AMPK further reduced autophagy and promotes matrix protein synthesis. Exogenous H\textsubscript{2}S could reverse the above changes to protect the renal, which suggested that it played protective role in matrix remodeling (Kundu et al. 2014). The activation of AMPK inhibits mTOR, a negative regulator of autophagy, and subsequently stimulates autophagy. In diabetic cardiomyopathy, H\textsubscript{2}S-induced activation of AMPK inhibited hyperglycemia-induced cardiomyocyte apoptosis and prevented cardiac dysfunction by promoting autophagy via the AMPK/mTOR pathway, which indicated that autophagy was an important target for the treatment of diabetic cardiomyopathy (Yang et al. 2017). Further studies are needed to study the relationship between H\textsubscript{2}S-induced autophagy and AMPK/mTOR pathway in type I or type II diabetes. In the vascular endothelial cells of type 2 diabetic model rats, exogenous H\textsubscript{2}S could promote the transfer of Nrf2 into the nucleus, inhibit high glucose-induced oxidative stress, reduce the phosphorylation level of AMPK, inhibit autophagy and protect the vascular endothelial cells (Liu et al. 2016). These above results suggested that the hyperglycemia could affect autophagy and cause tissue damage by changing the phosphorylation level of AMPK, and the use of H\textsubscript{2}S could reverse the role of hyperglycemia, which suggested that the activation of AMPK by H\textsubscript{2}S could be a new strategy for the treatment of diabetic vascular complications.
Persistent hyperglycemia could induce metabolism, to cause cardiac structural remodeling and myocardial fibrosis (Wang et al. 2014). Studies have shown that high glucose could reduce the generation of endogenous H₂S, inhibit PI3K/AKT1 pathway, activate autophagy and induce myocardial fibrosis. Exogenous H₂S could reverse the above changes and protect the myocardium of diabetic myocardium (Xiao et al. 2016).

4. H₂S influences autophagy in lipid metabolism

Recent studies have shown that autophagy was involved in the regulation of hepatic lipid metabolism (Czaja et al. 2010, Settembre and Ballabio 2014, Singh et al. 2009). H₂S has been proved to have a protective effect on hypertriglyceridemia (HTG) and nonalcoholic fatty liver disease (NAFLD) (Luo et al. 2014, Polyzos et al. 2012). It has been shown that the level of H₂S and the level of autophagy in the hepatocyte of hyper-triglyceride patients were significantly lower than normal. In the hyper-triglyceride hepatocytes, NaHS could reduce the triglyceride in plasma by promoting autophagy, which indicated that the activation of liver autophagy may be a therapeutic target for HTG. In addition, the phosphorylation of AMPK was promoted and the phosphorylation of mTOR was inhibited by H₂S, which suggested that H₂S had exerted the protective role through influencing the AMPK/mTOR pathway (Sun et al. 2015). A previous study in our laboratory has shown that in steatosis hepatocytes induced by oleic acid, H₂S could promote the formation and degradation of lipid autophagosomes, improve the expression of autophagy-related protein LC3 II and further promote the degradation of triglyceride by autophagic pathway (Wang et al. 2017). Further study is needed to illustrate the mechanism about the effects of H₂S on lowering serum TG and improving NAFLD.

5. H₂S influencing autophagy in tissue fibrosis diseases
It has been reported that the high level of autophagy leaded to massive loss of myocardial cells, which played an important role in the development of cardiac remodeling and myocardial fibrosis (Ma et al. 2015, Jie et al. 2015, Zhang et al. 2016). In the process of myocardial fibrosis induced by alcohol, H₂S inhibited autophagy to alleviate the myocardial fibrosis caused by alcohol via regulating the expression of miR21, miR211 and the PI3K/AKT1/TGF-β1 signal pathway (Liang et al. 2017). Hyperthyroid heart disease is characterized by obvious clinical symptoms and pathological changes, such as myocardial hypertrophy, myocardial fibrosis, arrhythmia, and cardiac dysfunction (Freitas et al. 2013, Kaminski et al. 2012). Myocardial fibrosis is an important sign of myocardial remodeling in hyperthyroid heart disease, and it is also the main cause of left ventricular dysfunction (Kim et al. 2013, Dillmann 2010). Current studies showed that autophagy was involved in the pathogenesis of the myocardial fibrosis and myocardial remodeling. In the model of rat myocardial fibrosis induced by high concentration thyroxine, the protein expression of autophagy related genes was reduced, which suggested that the decreased autophagy was involved in the development and the progression of myocardial fibrosis. H₂S-intervention could significantly reduce collagen deposition, increase autophagy and inhibit myocardial fibrosis. PI3K/AKT is a signal pathway of negative regulation of autophagy. H₂S could reduce the protein expression of PI3K and AKT, which suggested that H₂S promoted autophagy by inhibiting PI3K/AKT signaling pathway (Liu et al. 2018). The PI3K/AKT signaling pathway may become a target for the intervention of myocardial fibrosis.

6. H₂S influences autophagy in cancer

H₂S has an important biphasic effect in cancer(Hellmich and Szabo 2015, Wu et al. 2015). Autophagy is a double-edged sword, which can both promote and inhibit tumor
growth and development in different experimental environments. In the hepatocellular carcinoma, the treatment of NaHS at a final concentration of $10^{-3}$ M for 24 h inhibited cell migration, proliferation and cell cycle progression and promoted apoptosis and autophagy. The PI3K/Akt/mTOR signaling pathway played an important role in autophagy. Further studies showed that the use of NaHS could inhibit PI3K/Akt/mTOR signaling pathway. Given that rapamycin induced autophagy by inhibiting the expression of mTOR, rapamycin plus H$_2$S treatment could enhance autophagy more obviously, which indicated H$_2$S promote autophagy through the PI3K/Akt/mTOR signaling pathway (Wang et al. 2017). These above results suggested a new strategy for the treatment of liver cancer.

7. Conclusion

Autophagy maintains a balance between protein degradation and synthesis, which can protect or destroy cell. The effect of autophagy is determined by specific pathological processes. Nowadays, more and more studies suggest that H$_2$S related drugs can have potential application in many kinds of diseases by influencing autophagy. However, the mechanism that H$_2$S affects autophagy has not been thoroughly studied, such as the role of Becline-1 receptor in the regulation of autophagy by H$_2$S, and whether H$_2$S can regulate the fusion of autophagosomes and lysosomes. The role of H$_2$S in influencing autophagy in more diseases needs further study. In addition, the donor of H$_2$S used in researches of recent years are usually NaHS, considering that NaHS can not release H$_2$S for a long time, the new and efficient H$_2$S-donors need to be further developed and applied to research.

In conclusion, autophagy may be a potential target for H$_2$S therapy with the in-depth study of the effect of H$_2$S on autophagy.
Conflict of Interest statement

The authors declare that there are no conflicts of interest.

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### Table 1. H$_2$S influences autophagy in ischemia-reperfusion injury

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<td>Decreases high</td>
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**Table 2. H₂S influences autophagy in metabolic diseases**
The general process of macroautophagy, microautophagy, and chaperone-mediated autophagy.

In the process of macroautophagy, the content is wrapped by bilayer membrane structure to form autophagosome and then fuses with lysosome for degradation. Microautophagy refers to that the lysosomal membrane directly invaginate and then encapsulate the cell contents. In the process of chaperone-mediated autophagy, the cytosolic proteins are transported to the lysosomal chamber after binding to molecular chaperones, and then are digested by lysosomal enzymes.