Pathophysiological Role of S-Nitrosylation and Transnitrosylation Depending on S-Nitrosoglutathione Levels Regulated by S-Nitrosoglutathione Reductase

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Abstract
Nitric oxide (NO) mediates various physiological and pathological processes, including cell proliferation, differentiation, and inflammation. Protein S-nitrosylation (SNO), a NO-mediated reversible protein modification, leads to changes in the activity and function of target proteins. Recent findings on protein-protein transnitrosylation reactions (transfer of an NO group from one protein to another) have unveiled the mechanism of NO modulation of specific signaling pathways. The intracellular level of S-nitrosoglutathione (GSNO), a major reactive NO species, is controlled by GSNO reductase (GSNOR), a major regulator of NO/SNO signaling. Increasing number of GSNOR-related studies have shown the important role that denitrosylation plays in cellular NO/SNO homeostasis and human pathophysiology. This review introduces recent evidence of GSNO-mediated NO/SNO signaling depending on GSNOR expression or activity. In addition, the applicability of GSNOR as a target for drug therapy will be discussed in this review.

Key Words: Nitric Oxide, S-nitrosylation, Transnitrosylation, GSNO, GSNOR

INTRODUCTION

Nitric oxide (NO) is a free radical that is formed in numerous cell types, including endothelial, muscle, and neuronal cells. NO can play the role of a physiological or pathological effector depending on the target signaling pathway (NamKoong and Kim, 2010; Park et al., 2011; Eo et al., 2013; Ryu et al., 2015). There are three subtypes of NO synthases (NOSs) in mammalian organisms, namely neuronal NOS (nNOS) and endothelial NOS (eNOS), which are constitutive forms, and inductive NOS (iNOS). The biological action of NO is believed to be mediated mainly via guanylate cyclase activation and cyclic guanosine-3',5'-monophosphate (cGMP) production. However, S-nitrosylation, a covalent reaction of an NO group with a reactive cysteine thiol on target proteins, has emerged as another major mechanism for NO bioactivity (Hess et al., 2005). The formation of S-nitrosoproteins (i.e., protein-SNOS) generally regulates protein function by allosteric or direct modification of active site cysteine (Lipton et al., 1993; Hess et al., 2005). Some leading groups discovered and characterized this unique process on N-methyl-D-aspartate-type glutamate receptors (NMDARs) in the central nervous system (CNS) and demonstrated that NO inhibits excessive NMDAR activity via protein S-nitrosylation (Lipton et al., 1993). Presently, over 3,000 proteins have been identified as potential targets for protein S-nitrosylation (Seth and Stamler, 2011). Determination of the specific function of most protein-SNOSs may be helpful for further research, and can support the notion that NO exerts its major biological activity through S-nitrosylation. Notably, S-nitrosylation mediates the protective or toxic effects of NO depending on the action of the target protein. NO produced from NOS efficiently S-nitrosylates neighboring proteins to produce protein-SNO. Along with the proteins located near NOS, NO can react to form low-molecular weight SNOs with cysteine and glutathione (GSH). These low-molecular-weight SNOs, such as S-nitrosocysteine (CysNO) and S-nitrosogluthathione (GSNO), can then function as NO donors under physiological conditions depending on their redox potential (Hess et al., 2005). Until very recently, transnitrosylation, which is the transfer of an NO group from one thiol to another, was believed to take place only between low-molecular-weight SNOs and protein thiols. Some leading groups in this field, however, discovered that transnitrosylation between cell proteins could be a very important enzymatic process of S-nitrosylation (Pawloski et al., 2001; Mitchell and Marletta, 2005; Benhar et al., 2008; Kornberg et al., 2010; Nakamura et al., 2010; Wu et al., 2013).
et al., 2010; Qu et al., 2011). GSNO, a major NO donor in the physiological system, has its own special metabolic process (Fig. 1). GSNO serves as a selective substrate for S-nitroso-glutathione reductase (GSNOR), through which it is fully reduced to glutathione disulfide (GSSG) and ammonia (NH3). At the cellular level, GSNO is in equilibrium with multiple protein-SNOs. GSNOR directly metabolizes GSNO and indirectly controls the amount of protein-SNOs and their related signals (Beigi et al., 2012). Although some other enzymes have been shown to be capable of catalyzing SNO degradation in vitro (Trujillo et al., 1998; Carver et al., 2007), they have not yet been shown to modulate endogenous SNO levels in cells. In contrast, GSNOR has been reported to play an important role in maintaining the balance between GSNO and protein-SNO in physiological conditions (Liu et al., 2004; Que et al., 2005). In this review, we will discuss the pathophysiology of basal SNO changes caused by the decrease or deficiency of GSNOR and examine the potential of GSNOR as a drug target.

PROTEIN S-NITROSYLATION AND ITS DETECTION

S-nitrosylation is a non-enzymatic reaction that occurs within the range of physiological pH. It can control protein conformations, protein–protein interactions, and other post-translational modifications such as phosphorylation, acetylation, ubiquitination, and disulfide linkage (Hess and Stamler, 2012). Although S-nitrosylation is a generally abundant chemical reaction, initial S-nitrosylation occurs only at preferable cysteines that are proximal to NOS. A major group of S-nitrosylated proteins in this class includes NOS and NOS-interacting proteins. Recent findings on the effects of protein S-nitrosylation reveal a great impact on the related biological and pathophysiological research fields (Stamler et al., 1992; Foster et al., 2009; Broniowska and Hogg, 2012). However, detecting and quantifying protein-SNO and GSNO in experimental systems are not easy. The biotin switch technique (Jaffrey and Snyder, 2001), in which S-nitrosylated cysteine is reduced and biotinylated, offers a clear and powerful method for the qualitative detection of S-nitrosylated proteins. The biotin switch technique and all other protein-SNO measurement techniques have difficulty in identifying each S-nitrosylated thiol clearly (Giustarini et al., 2003). Novel techniques that are precisely quantitative are currently available (Chen et al., 2013; Devarie-Baez et al., 2013), such as tandem mass spectrometry (MS/MS) of S-nitrosylated protein thiols (Murray et al., 2012; Ulrich et al., 2013).

PROTEIN-PROTEIN TRANSNITROSYLATION

As mentioned earlier, the nitrosyl group derived from nitrosylated proteins can then be moved to a remote location via transnitrosylation. Thus, transnitrosylation is also a denitrosylation process of the donor protein, overturning the first SNO-mediated regulation (Fig. 2). To date, fewer than 10 nitrosyl donors have been identified (Hess and Stamler, 2012). The recently discovered protein-protein transnitrosylation reveals that the NO moiety is transferred from protein-SNO to the free thiol of another protein, which regulates the NO regulatory mechanism of specific signaling pathways (Nakamura et al., 2010; Choi et al., 2014). Protein-to-protein transnitrosylation generally occurs when two proteins directly interact and have the appropriate redox potentials to allow electron transfer. In other words, protein binding promotes NO transfer between the two proteins. It is currently believed that physical association of two proteins could promote conformational change, thereby affecting the environment of crucial cysteine residues to assist thiolate anion formation, which is more responsive to S-nitrosylation, followed by further oxidation by ROS. If a protein-SNO interacts with a partner protein having free thiols, the difference in redox potential of the two cysteine residues is a primary determinant of NO transfer. Proteins with higher redox potential tend to be reduced by receiving electrons, which means that they are being denitrosylated. Thus, when a protein with a lower redox potential having a free thiol enclosed by suitable amino acid motifs for S-nitrosylation interacts with a previously nitrosylated protein, transnitrosylation would follow. Considering the above, only a certain subset of proteins is S-nitrosylated to selectively activate or inhibit specific signaling pathways.

Fig. 1. GSNOR controls protein-SNOs by metabolizing GSNO. Metabolizing GSNO is one of the main functions of GSNOR. GSNOR inhibitors increase available GSNO and total protein-SNOs.

Fig. 2. Proposed mechanism of transnitrosylation. Schema illustrates transnitrosylation of an ‘acceptor’ protein (blue) by another S-nitrosylated ‘donor’ protein (orange).
NO often mediates cytoprotection at the physiological level depending on the cellular compartments involved. The most representative example of this protective effect can be found in the relationship between NO/SNO signaling pathway and neurodegenerative disease models. It has also been shown that S-nitrosylation of parkin affects its E3 ligase activity, possibly affecting Lewy body formation in Parkinson’s disease (Chung et al., 2004; Yao et al., 2004).

### Table 1. Effects of GSNOR-deficient experimental systems. Positive or negative effects caused by GSNOR deficiency were listed by organs

| Organs | Phenotypes | Effects | References |
|--------|------------|---------|------------|
| Brain  | Neuronal differentiation | Increased | Wu et al., 2014 |
|        | Neuroprotection against PD toxins | Increased | Clements et al., 2006 |
|        | Visual pattern memory | Decreased | Hou et al., 2011 |
| Thymus | Mortality upon endotoxic shock or bacterial challenge | Increased | Liu et al., 2004 |
|        | B and T lymphocyte development | Decreased | Yang et al., 2010 |
| Lungs  | Bronchodiilation | Increased | Que et al., 2005 |
|        | Protection against experimental asthma | Increased | Que et al., 2005 |
| Heart  | Retention of cardiac function after ischemia | Increased | Lima et al., 2009 |
|        | Cardiomyocyte proliferation | Increased | Hatzistergos et al., 2015 |
| Liver  | Hepatic progenitor cells proliferation during development | Increased | Cox et al., 2014 |
|        | Hepatoprotection against acetaminophen intoxication | Increased | Cox et al., 2014 |
|        | Incidence of spontaneous hepatocellular carcinoma (HCC) | Increased | Wei et al., 2010 |
| Skeletal muscle | Strength and fatigue resistance | Increased | Moon et al., 2017 |
|        | Myofiber size and muscle efficiency | Decreased | Montagna et al., 2014 |
| Blood vessels | Vasculogenesis | Decreased | Gomes et al., 2013 |
|        | Peripheral vascular tone and β-adrenergic response | Decreased | Beigi et al., 2012 |
et al., 2010; Guerra et al., 2016). Taken together, dysregulation of GSNOR is associated with several human diseases. By using ADHS\textsuperscript{+} animal models, critical data regarding GSNOR function were obtained. NO-mediated pathway as well as protein-SNO levels are severely affected when GSNOR activity is changed.

**GSNOR AS A THERAPEUTIC TARGET**

When GSNOR activity was regulated, not only the protein-SNO level, but also the NO-mediated pathway varied markedly. GSNOR inhibition increased intracellular GSNOR availability and promoted NO-mediated signal transduction pathways. Drugs that can inhibit the function of GSNOR have been studied (Sanghani et al., 2009; Green et al., 2012; Sun et al., 2012; Jiang et al., 2016). Among them, N6022 and N91115 have been tested in both mild asthma and cystic fibrosis, and have been proved to be potentially safe and effective GSNOR inhibitors. In patients with cystic fibrosis, endogenous GSNO levels were low (Grasemann et al., 1999) and GSNOR inhibition was relatively more effective than direct administration of GSNO (Zaman et al., 2001; Snyder et al., 2002). Since there is no FDA-approved GSNOR inhibitor currently available, attention has been focused on the clinical use of existing drugs that show effects on modulating S-nitrosothiols (RSNOs). The \( \beta \)-adrenergic receptor blocker, nebivolol, used in the treatment of hypertension has been shown to increase total RSNO levels in animal and cell models (Jiang et al., 2016). However, when GSNOR inhibitors are considered as therapeutic agents, it is necessary to consider the level of intracellular NO that can be controlled by enzymes other than GSNOR. Since NO is crucial to the normal functioning of most cell types, there are several complementary mechanisms that regulate NO and RSNO, such as thioredoxin (Trx) (Sengupta and Holmgren, 2013) and carbonyl reductase systems (Bateman et al., 2008). Nevertheless, the direct administration of endogenous NO donors and some exogenous NO donors is not clinically valuable because of rapid degradation and serious side effects such as systemic nitrate accumulation (Al-Sa’doni and Ferro, 2005). Therefore, the therapeutic inhibition of GSNOR for the treatment of patients should be carefully considered in view of potential side effects.

**CONCLUSION AND FUTURE PERSPECTIVES**

Recently, S-nitrosylation has been considered an essential post-translational modification of reactive cysteines. Many proteins have been discovered to be S-nitrosylated, which results in a change in their activity and function. As denitrosylation has been shown to be catalyzed by specific enzymes, Trx and GSNOR have been discovered as enzymes that remove NO from nitrosylated proteins. The discovery of these novel denitrosylation systems has opened new arenas in redox biology and have promoted application studies on related biological and pharmacological signaling pathways. To date, several studies have shown the results of GSNOR-mediated cellular processes or the phenotypes caused by GSNOR gene deletion (Hess and Stamler, 2012). Despite the large amount of information on the involvement of GSNOR in physiological processes, the mechanism by which GSNOR mediates selective denitrosylation is still unclear. By elucidating the mechanism by which GSNOR can act as a selective enzyme, we can expect that the NO/SNO pathway would become a more important target of disease treatment. Given these aspects, it is important to determine the value to be investigated to identify potential post-translational modifications that can modulate GSNOR localization or interaction with other proteins, and to provide a better understanding of future S-nitrosylation dynamics and signaling pathways. Obtaining a better understanding of the precisely regulated denitrosylation pathways and their clinical significance will help not only to discover new targets for drug action, but also to develop new therapeutic agents.

**CONFLICT OF INTEREST**

There is no conflict of interest.

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**REFERENCES**

Al-Sa’doni, H. H. and Ferro, A. (2005) Current status and future possibilities of nitric oxide-donor drugs: focus on S-nitrosothiols. *Mini Rev. Med. Chem.*, 5, 247-254.

Bateman, R. L., Rauh, D., Tavshanjian, B. and Shokat, K. M. (2008) Human carbonyl reductase 1 is an S-nitrosoglutathione reductase. *J. Biol. Chem.*, 283, 35756-35762.

Beigi, F., Gonzalez, D. R., Minhas, K. M., Sun, Q. A., Foster, M. W., Khan, S. A., Treuer, A. V., Dulce, R. A., Harrison, R. W., Saravia, R. M., Premer, C., Schulman, I. H., Stamler, J. S. and Hare, J. M. (2012) Dynamic denitrosylation via S-nitrosoglutathione reductase regulates cardiovascular function. *Proc. Natl. Acad. Sci. U.S.A.*, 109, 4314-4319.

Benhar, M., Forrester, M. T., Hess, D. T. and Stamler, J. S. (2008) Regulated Protein Denitrosylation by Cytosolic and Mitochondrial Thioredoxins. *Science*, 320, 1050-1054.

Bonaventura, C., Godette, G., Ferruzzi, G., Tesh, S., Stevens, R. D. and Henkens, R. (2002) Responses of normal and sickle cell hemoglobin to S-nitrosocysteine: implications for therapeutic applications of NO in treatment of sickle cell disease. *Biophys. Chem.*, 98, 165-181.

Brennan, A. M., Won Suh, S., Joon Won, S., Narasimhan, P., Kauppinen, T. M., Lee, H., Edling, Y., Chan, P. H. and Swanson, R. A. (2009) NADPH oxidase is the primary source of superoxide induced by NMDA receptor activation. *Nat. Neurosci.*, 12, 857-863.

Broniowska, K. A. and Hogg, N. (2012) The chemical biology of S-nitrosothiols. *Antioxid. Redox Signal.*, 17, 969-980.

Brown-Steinke, K., deRonde, K., Yemen, S. and Palmer, L. A. (2010) Gender differences in S-nitrosoglutathione reductase activity in the lung. *PLoS ONE*, 5, e14007.

Carvalho-Filho, M. A., Ueno, M., Hirabara, S. M., Seabara, A. B., Carvalheira, J. B. C., de Oliveira, M. G., Velloso, L. A., Curi, R. and Saad, M. J. A. (2005) S-nitrosation of the insulin receptor, insulin receptor substrate 1, and protein kinase B/Akt: a novel mechanism of insulin resistance. *Diabetes*, 54, 959-967.

Carver, D. J., Gaston, B., deRonde, K. and Palmer, L. A. (2007) Akt-mediated activation of hif-1 in pulmonary vascular endothelial cells by S-nitrosoglutathione. *Am. J. Respir. Cell Mol. Biol.*, 37, 255-263.

Chen, Y. J., Ching, W. C., Lin, Y. P. and Chen, Y. J. (2013) Methods for detection and characterization of protein S-nitrosylation. *Methods*, 62, 138-150.
Grasemann, H., Gaston, B., Fang, K., Paul, K. and Ratjen, F. (1999) Protein S-nitrosylation: purification and parameters. Nat. Rev. Mol. Cell Biol. 6, 150-166.

Hess, D. T. and Stamler, J. S. (2012) Regulation by S-nitrosylation of protein post-translational modification. J. Biol. Chem. 287, 4411-4418.

Hou, G., Jiang, H., Zhang, X., Guo, C., Huang, B., Wang, P., Wang, T., Wu, K., Li, J., Gong, Z., Du, L., Liu, Y., Liu, L. and Chen, C. (2011) Nitric oxide metabolism controlled by formaldehyde dehydrogenase (fdh, homolog of mammalian GSNOR) plays a crucial role in visual pattern memory in Drosophila. Nitric Oxide 24, 17-24.

Höög, J. O. and Östberg, L. J. (2011) Mammalian alcohol dehydrogenases - a comparative investigation at gene and protein levels. Chem. Biol. Interact. 191, 2-7.

Huang, Y., Man, H. Y., Sekine-Aizawa, Y., Han, Y., Julius, K., Luo, H., Cheah, J., Lowenstein, C., Huganir, R. L. and Snyder, S. H. (2005) S-nitrosylation of N-ethylmaleimide sensitive factor mediates surface expression of AMPA receptors. Neuron 46, 533-540.

Hur, M. W. and Edenberg, H. J. (1992) Cloning and characterization of the ADH5 gene encoding human alcohol dehydrogenase 5, formaldehyde dehydrogenase. Gene 121, 305-311.

Iyer, A. K. V., Rojanasakul, Y. and Azad, N. (2014) Nitrosothiol signaling and protein nitrosation in cell death. Nitric Oxide 42, 9-18.

Jaffrey, S. R. and Snyder, S. H. (2001) The biotin switch method for the detection of S-nitrosylated proteins. Sci. STKE 2001, p17.

Jelski, W., Orywal, K., Panek, B., Gacko, M., Mroczko, B. and Szmitkowski, M. (2009) The activity of class I, II, III and IV of alcohol dehydrogenase (ADH) isoenzymes and aldehyde dehydrogenase (ALDH) in the wall of abdominal aortic aneurysms. Exp. Mol. Pathol. 87, 59-62.

Jelski, W. and Szmitkowski, M. (2008) Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in the cancer diseases. Clin. Chim. Acta 385, 1-5.

Jiang, H., Polhemus, D. J., Islam, K. N., Tornegrosa, A. C., Li, Z., Potts, A., Lefer, D. J. and Bryan, N. S. (2016) Nebivolol acts as a S-nitrosogluthione reductase inhibitor. J. Cardiovasc. Pharmacol. Ther. 21, 478-485.

Kornberg, M. D., Sen, N., Hara, M. R., Julius, K. R., Nguyen, J. V. K., Snowman, A. M., Law, L., Hester, L. D. and Snyder, S. H. (2010) GAPDH mediates nitrosylation of nuclear proteins. Nat. Cell Biol. 12, 1094-1100.

Laniewska-Dunaj, M., Jelski, W., Orywal, K., Kochanowicz, J., Rutkowski, R. and Szmitkowski, M. (2013) The activity of class I, II, III and IV of alcohol dehydrogenase (ADH) isoenzymes and aldehyde dehydrogenase (ALDH) in brain cancer. Neurochem. Res. 38, 1517-1521.

Lima, B., Lam, G. K., Xie, L., Diesen, D. L., Villamizar, N., Nienaber, J., Messina, E., Bowles, D., Kontos, C. D., Hare, J. M., Stamler, J. S. and Rockman, H. A. (2009) Endogenous S-nitrosothiols protect against myocardial injury. Proc. Natl. Acad. Sci. U.S.A. 106, 6297-6302.

Lipton, S. A., Cui, J., Strongin, A., Smith, J. S. (2004) Protein S-nitrosylation: purview and parameters. Nat. Chem. Biol. 10, 474-480.

Methner, C., Chouchani, E. T., Buononcontri, G., Pell, V. R., Sawai, S. J., Murphy, M. P. and Krieg, T. (2014) Mitochondria selective S-nitrosation by mitochondria-targeted S-nitrosothiols protects against post-infarct heart failure in mouse hearts. Eur. J. Heart Fail. 16, 712-717.

Mitchell, D. A. and Marletta, M. A. (2005) Thioredoxin catalyzes the S-nitrosation of the caspase-3 active site cysteine. Nat. Chem. Biol. 116, 617-628.

Ozep, K., Kaul, M., Yan, B., Kridel, S. J., Cui, J., Strongin, A., Smith, J. S., Green, L. S., Chun, L. E., Patton, A. K., Sun, X., Rosenthal, G. J. and Gomes, S. A., Rangel, E. B., Premer, C., Dulce, R. A., Cao, Y., Florea, Giustarini, D., Milzani, A., Colombo, R., Dalle-Donne, I. and Rossi, R. (2015) S-nitrosoglutathione reductase deficiency enhances the proliferative expansion of adult heart progenitors and myocytes post myocardial infarction. J. Am. Heart Assoc. 4, e001974.

Reade, H., Cho, D., Marshall, H. E. and Stamler, J. S. (2005) Protein S-nitrosylation: purview and parameters. Nat. Rev. Mol. Cell Biol. 6, 150-166.
