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Antioxidant Properties of *S*-Nitrosoglutathione and Nanotechnologies †

Marianne Parent, Yi Zhou, Justine Bonetti, Caroline Perrin-Sarrado, Isabelle Lartaud, Anne Sapin-Minet and Caroline Gaucher *

Université de Lorraine, CITHEFOR, F-54000 Nancy, France; marianne.parent@univ-lorraine.fr (M.P.); yi.zhou@univ-lorraine.fr (Y.Z.); justine.bonetti@univ-lorraine.fr (J.B.); caroline.perrin-sarrado@univ-lorraine.fr (C.P.-S.); isabelle.lartaud@univ-lorraine.fr (I.L.); anne.sapin@univ-lorraine.fr (A.S.-M.)

* Correspondence: caroline.gaucher@univ-lorraine.fr

† Presented at Natural Products and the Hallmarks of Chronic Diseases—COST Action 16112, Luxemburg, 25–27 March 2019.

Published: 16 April 2019

Abstract: Cardiovascular diseases are associated with oxidative stress and a reduced bioavailability of nitric oxide (NO). To counteract both processes, the administration of *S*-nitrosoglutathione (GSNO) can be envisaged. GSNO is able to induce protein *S*-nitrosation (Pr-SNO), which is a post-translational modification of proteins, participating in the storage of NO in tissues, and protect thiol functions from oxidation. However, GSNO antioxidant power is poorly studied, which is probably linked to its low stability. This low stability can be addressed by nanotechnologies that will increase GSNO protection and provide a sustained release of the drug.

Keywords: *S*-nitrosoglutathione; oxidative stress; protein *S*-nitrosation; nanotechnologies

1. Introduction

Cardiovascular diseas is the main cause of mortality in the world with around 17.5 million of deaths in 2012 according to the World Health Organization. In France, it is the second one for men, after cancer, and the first one for women. Risk factors such as high blood pressure, diabetes, smoking, obesity or the lack of physical activities increase the risk of developing a cardiovascular pathology including coronary artery diseases, venous thrombosis, angina pectoris and atherosclerosis. Most of these diseases are associated with oxidative stress and a reduced bioavailability of nitric oxide (NO). Indeed, NO, a physiological gaseous messenger with a short half-live, plays a central role in vascular homeostasis and its biosynthesis decreases during physiopathological processes such as ageing, endothelial dysfunction or oxidative stress [1]. The endogenous *S*-nitrosothiols (RSNOs), one of the main transport and storage forms of NO in the bloodstream and tissues, are good candidates as therapeutics to restore the physiological concentration of NO. Among RSNOs, the *S*-nitrosoglutathione (GSNO), made by *S*-nitrosation of glutathione, was the main NO-donor investigated for its therapeutic potential as antiplatelet agent with arterioselective vasodilator effects and also with well-documented antithrombotic effects. The *S*-nitrosation process apart from its ability to modulate signaling pathways by post-translational modification of proteins (Pr-SNO) is also a protection of thiol functions from oxidation [2–4]. Indeed, GSNO was shown to be able to reduce disulfide bounds forming *S*-nitroso functions, which are a lower oxidation state of thiol functions than disulfide or sulfenic/sulfonic acid functions [2,5]. However, the ability of GSNO to regulate NO bioavailability under oxidative stress is poorly studied. Enzymatic and non-enzymatic degradation of GSNO occurs, reducing its clinical potential to provide a long-lasting effect and to deliver appropriate NO concentrations to target tissues. Therefore, encapsulation of GSNO and other RSNOs

is an interesting approach to overcome these drawbacks. The nanotechnologies may offer a wide range of solutions for RSNO protection and sustained release.

2. Antioxidant Power of NO and GSNO

The protein *S*-nitrosation is a redox reversible process with high spatial and temporal specificity. One determinant that governs the specificity of protein *S*-nitrosation resides in the colocalization of NO sources: NO is provided mostly from NO synthases (NOS) and also from denitrosation of target proteins. Conversely, the *S*-nitrosation process is also a temporal signaling event, which depends on NO synthesis by NOS and its consumption by the soluble guanylate cyclase through the nitrosylation process for example. Cells environment also influences *S*-nitrosation of proteins. Indeed, in endothelial cells, physiological shear stress promotes *S*-nitrosation of protein independently from cGMP-dependent signaling [6,7], whereas TNF-α and oxidized LDL treatments reduce it [8]. Many proteins and transcriptional factors contain cysteine residues, which are potential targets for ROS-dependent or RNS-dependent modifications. NO shows antioxidant effects via NADPH oxidase inhibition, [9] or promotion of thioredoxin-1 activity [10,11] by direct S-nitrosation of their cysteine residues. Transcriptional factors all contain reactive thiols in their DNA binding regions, whose modification alters their binding to DNA. AP-1 activity is altered by *S*-nitrosation [12] and by oxidation of Cys residues [13]. Indeed, H_2O_2 treatment inhibits AP-1 activity and decreases eNOS promoter activity [14].

The antioxidant power of NO and GSNO is also mediated via their ability to reduce disulfide bound forming *S*-nitroso functions. Oxidative stress causes oxidation of the thiol groups of proteins. For example, we showed, using a smooth muscle cell model of oxidative stress, that GSNO prevents oxidation of thiol functions by increasing the level of *S*-nitrosated proteins [4]. Mass spectrometry analysis revealed that these proteins were mainly implicated in cell contraction, morphogenesis and movement, meaning that the redox protection of GSNO will help to maintain smooth muscle cells contractile phenotype.

3. Nanotechnologies Supply

Unfortunately, the low stability of NO and its derivatives such as *S*-nitrosothiols, constitutes a limitation for their therapeutic administration especially for chronic treatment [3]. There are several strategies to protect RSNOs from physicochemical and enzymatic degradations such as assembling of macromolecular RSNO in nanostructures (thiomers), or *S*-nitrosation of encapsulated free thiols (S-nitrosothiol-loaded carrier) [15,16]. The most frequently used approach is the direct encapsulation of RSNOs in liposomes, inorganic or polymeric nanoparticles or films [17,18]. A sustained release of NO is required to maintain stable and physiological NO concentrations. High concentrations should be avoided as they may lead to oxidative/nitrosative stress, especially destructive in a context of cardiovascular diseases. Moreover, in a context of chronic pathologies, oral delivery will be the most acceptable for patients. Here, RSNO will encounter unfavorable environment all along the gastrointestinal tract, ranging from a low pH in the stomach to the difficulty for crossing of the intestinal barrier. We showed that RSNOs have a low intestinal permeability using a passive mode and following a paracellular pathway [19]. In order to design such an oral delivery formulation for RSNO, which can provide protection and sustained release, GSNO loaded nanoparticles made of Eudragit® with a water in oil in water double emulsion method have been developed [20]. These nanoparticles were then embedded into an alginate and chitosan matrix forming a nanocomposite particle (GSNO-acNCP). The nanocomposite protected NO donor from degradation, and the matrix enhanced intestinal absorption of GSNO [21,22]. Indeed, alginate and chitosan were chosen as mucoadhesive polymers increasing the residence time of the formulation on the intestine mucus layer. As alginate has the capacity to penetrate the mucus layer, this will bring the drug closer to the intestinal cells, while chitosan exerts the property to open cells tight junction, helping the drug to cross the intestinal epithelium. Therefore, their combination leads to increased drug permeability through the intestinal barrier.

Acknowledgments: The CITHEFOR EA3452 lab is supported by the "Impact Biomolecules" project of the "Lorraine Université d'Excellence" (Investissements d'avenir—ANR). The PhD thesis of Yi Zhou is financially supported by the Chinese Scholarship Council.

Funding: This article is based upon work from COST Action NutRedOx-CA16112 supported by COST (European Cooperation in Science and Technology).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Maron, B.A.; Tang, S.S.; Loscalzo, J. S-nitrosothiols and the S-nitrosoproteome of the cardiovascular system. *Antiox. Redox. Signal* **2012**, *18*, 270–287, doi: 10.1089/ars.2012.4744.
2. Sun, J.; Steenbergen, C.; Murphy, E. S-nitrosylation: NO-related redox signaling to protect against oxidative stress. *Antioxid. Redox. Signal* **2006**, *8*, 1693–1705, doi: 10.1089/ars.2006.8.1693.
3. Gaucher, C.; Boudier, A.; Dahboul, F.; Parent, M.; Leroy, P. S-nitrosation/Denitrosation in cardiovascular pathologies: facts and concepts for the rational design of S-nitrosothiols. *Curr. Pharm. Des.* **2013**, *19*, 458–472.
4. Belcastro, E.; Wu, W.; Fries-Raeth, I.; Corti, A.; Pompella, A.; Leroy, P.; Lartaud, I.; Gaucher, C. Oxidative stress enhances and modulates protein S-nitrosation in smooth muscle cells exposed to S-nitrosoglutathione. *Nitric. Oxide* **2017**, *69*, 10–21, doi: 10.1016/j.niox.2017.07.004.
5. Belcastro, E.; Gaucher, C.; Corti, A.; Leroy, P.; Lartaud, I.; Pompella, A. Regulation of protein function by S-nitrosation and S-glutathionylation: processes and targets in cardiovascular pathophysiology. *Biol. Chem.* **2017**, *398*, 1267–1293, doi: 10.1515/hsz-2017-0150.
6. Huang, B.; Chen, S.C.; Wang, D.L. Shear flow increases S-nitrosylation of proteins in endothelial cells. *Cardiovasc. Res.* **2009**, *83*, 536–546, doi: 10.1093/cvr/cvp154.
7. Hoffmann, J.; Dimmeler, S.; Haendeler, J. Shear stress increases the amount of S-nitrosylated molecules in endothelial cells: Important role for signal transduction. *FEBS Lett.* **2003**, *551*, 153–158.
8. Hoffmann, J.; Haendeler, J.; Zeiher, A.M.; Dimmeler, S. TNF-α and oxLDL reduce protein S-nitrosylation in endothelial cells. *J. Biol. Chem.* **2001**, *276*, 41383–41387, doi: 10.1074/jbc.M107566200.
9. Selemidis, S.; Dusting, G.J.; Peshavariya, H.; Kemp-Harper, B.K.; Drummond, G.R. Nitric oxide suppresses NADPH oxidase-dependent superoxide production by S-nitrosylation in human endothelial cells. *Cardiovasc. Res.* **2007**, *75*, 349–358, doi: 10.1016/j.cardiores.2007.03.030.
10. Liu, W.R.; Nakamura, H.; Shioji, K.; Tanito, M.; Oka, S.; Ahsan, M.K.; Son, A.; Ishii, Y.; Kishimoto, C.; Yodoi, Y. Thioredoxin-1 ameliorates myosin-induced autoimmune myocarditis by suppressing chemokine expressions and leukocyte chemotaxis in mice. *Circulation* **2004**, *110*, 1276–1283; doi: 10.1161/01.CIR.0000141803.41217.B6.
11. Haendeler, J.; Hoffmann, J.; Tischler, V.; Berk, B.C.; Zeiher, A.M.; Dimmeler, S. Redox regulatory and antiapoptotic functions of thioredoxin depend on S-nitrosylation at cysteine 69. *Nat. Cell Biol.* **2002**, *4*, 743–749, doi: 10.1038/ncb851.
12. Marshall, H.E.; Merchant, K.; Stamler, J.S. Nitrosation and oxidation in the regulation of gene expression. *FASEB J.* **2000**, *14*, 1889–1900, doi: 10.1096/fj.00.011rev.
13. Xanthoudakis, S.; Miao, G.; Wang, F.; Pan, Y.C.E.; Curran, T. Redox activation of Fos-Jun DNA-binding activity is mediated by a DNA-repair enzyme. *EMBO J.* **1992**, *11*, 3323–3335.
14. Stuehr, D.J.; Griffith, O.W. Mammalian nitric oxide synthases. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1992**, *65*, 287–346.
15. Fleser, P.S.; Nuthakki, V.K.; Malinzak, L.E.; Callahan, R.E.; Seymour, M.L.; Reynolds, M.M.; Merz, S.I.; Meyerhoff, M.E.; Bendick, P.J.; Zelenock, G.B.; et al. Nitric oxide–releasing biopolymers inhibit thrombus formation in a sheep model of arteriovenous bridge grafts. *J. Vasc. Surg.* **2004**, *40*, 803–811; doi: 10.1016/j.jvs.2004.07.007.
16. Tan, L.; Wan, A.; Li, H. Ag_2S quantum dots conjugated chitosan nanospheres toward light-triggered nitric oxide release and near-infrared fluorescence imaging. *Langmuir* **2013**, *29*, 15032–15042, doi: 10.1021/la403028j.
17. Jeh, H.S.; Lu, S.; George, S.C. Encapsulation of PROLI/NO in biodegradable microparticles. *J. Microencapsul.* **2004**, *21*, 3–13, doi: 10.1080/02652040310001619767.

18. Frost, M.C.; Meyerhoff, M.E. Synthesis, characterization, and controlled nitric oxide release from S-nitrosothiol-derivatized fumed silica polymer filler particles. *J. Biomed. Mater. Res. A* **2005**, *72*, 409–419; doi: 10.1002/jbm.a.30275
19. Bonetti, J.; Zhou, Y.; Parent, M.; Clarot, I.; Yu, H.; Fries-Raeth, I.; Leroy, P.; Lartaud, I.; Gaucher, C. Intestinal absorption of S-nitrosothiols: Permeability and transport mechanisms. *Biochem. Pharmacol.* **2018**, *155*, 21–31, doi: 10.1002/jbm.a.30275.
20. Wu, W.; Gaucher, C.; Diab, R.; Fries, I.; Xiao, Y.-L.; Hu, X.-M.; Maincent, P.; Sapin-Minet, A. Time lasting S-nitrosoglutathione polymeric nanoparticles delay cellular protein S-nitrosation. *Eur. J. Pharm. Biopharm.* **2015**, *89*, 1–8, doi:10.1016/j.ejpb.2014.11.005
21. Wu, W.; Gaucher, C.; Fries, I.; Hu, X.-M.; Maincent, P.; Sapin-Minet, A. Polymer nanocomposite particles of S-nitrosoglutathione: a suitable formulation for protection and sustained oral delivery. *Int. J. Pharm.* **2015**, *495*, 354–361, doi: 10.1016/j.ijpharm.2015.08.074.
22. Wu, W.; Perrin-Sarrado, C.; Ming, H.; Lartaud, I.; Maincent, P.; Hu, X.-M.; Sapin-Minet, A.; Gaucher, C. Polymer nanocomposites enhance S-nitrosoglutathione intestinal absorption and promote the formation of releasable nitric oxide stores in rat aorta. *Nanomed. Nanotechnol.* **2016**, *12*, 1795–1803, doi: 10.1016/j.nano.2016.05.006.