proceedings

MDPI

Proceedings

Nano or Micro: 3 Different Particles to Deliver and Protect *S*-Nitrosoglutathione for Oral Route Administration †

Yi Zhou, Caroline Gaucher *, Isabelle Fries and Marianne Parent

Université de Lorraine, CITHEFOR, F-54000 Nancy, France; yi.zhou@univ-lorraine.fr (Y.Z.); isabelle.raeth@univ-lorraine.fr (I.F.); marianne.parent@univ-lorraine.fr (M.P.)

* 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: As a physiological nitric oxide donor, *S*-nitrosoglutathione (GSNO) is a promising candidate for several diseases (e.g., stroke and atherosclerosis). However, its clinical application has been limited by its low stability. In order to protect GSNO suitable for oral route administration and to achieve sustained release, 3 different particles from nano-size to micro-size were obtained by a water-in-oil-in-water (W/O/W) or solid-in-oil-in-water (S/O/W) double emulsion/solvent evaporation method. The 3 different particles tuned out to have similar encapsulation efficiency while the microparticles showed longer release time. Finally, the 3 formulations have been successfully lyophilized for long term stability.

Keywords: *S*-nitrosoglutathione; nitric oxide donor; oral delivery; sustained release; storage stability

1. Introduction

Nitric oxide (NO) is a gaseous radical, which plays important roles in different physiological activity of human beings [1,2]. The imbalance of NO may result in several disfunction and diseases (e.g., stroke and atherosclerosis). S-nitrosoglutathione (GSNO) is a promising candidate as a physiologic NO donor [3,4]. However, with a short half-time GSNO needs to be protected especially for oral administration. In a previous study, an oral delivery system made of nanoparticles (NP) has been found to protect and provide a sustained release of GSNO [5]. NP are then embedded into an alginate/chitosan matrix to form GSNO-nanocomposite particles (GSNO-acNCP). The GSNO-acNCP showed high encapsulation efficiency, a sustained release effect and led to the formation of a NO store in the aorta wall after a single oral administration to rat [6]. However, this formulation has several limitations (1) insufficient batch-to-batch reproducibility (due to the complex structure); (2) multi-step and time-consuming preparation; (3) immediate use only (no stability as suspensions). Our previous work also showed that *S*-nitrosothiols intestinal permeability (in vitro model) is passive and follows paracellular pathway [7]. So, by increasing the amount of GSNO brought to the intestine and by opening intestinal cells tight junctions, the GSNO-acNCP is perfectly adapted. The formulation protocol needs modification to get a higher reproducibility, longer stability during storage and a higher encapsulation efficiency (EE) with sustained release.

2. Materials and Methods

Two different GSNO loaded microparticles (GSNO-MPs) types were prepared by a water-in-oil-in-water (MP-W) or a solid-in-oil-in-water (MP-S) double emulsion solvent evaporation method and

compared to the previously developed GSNO-NP. These particles were characterized as regards to their size (dynamic light scattering/laser diffraction and scanning electron microscopy), zeta potential, GSNO EE, in vitro release kinetic, cytocompatibility and intestinal permeability using Caco-2 cells monolayers [7]. Moreover, the three formulations (GSNO-NP, GSNO-MP-W and GSNO-MP-S) were obtained as dried powders by optimized lyophilization using sucrose as cryoprotectant. These lyophilized particles were also characterized.

3. Results and Discussion

Through the modification of nanoparticles (NP) preparation, two types of GSNO-MPs were obtained in micro-size (GSNO-MP-W: 80.2 ± 10.2 μm, $n = 9$; GSNO-MP-S: 118.3 ± 16.4 μm, $n = 7$) with similar GSNO EE. However, these two GSNO-MPs exhibited a longer release time and a slower release profile during the 2 first hours compared with GSNO-NP or free GSNO. The freeze-dried powders had similar EE, size and zeta potential to fresh preparations. Freeze-dried particles maintain all their physico-chemical characteristics for more than 1 month (3 months for NP, 4–6 weeks for the two MP) of storage at 4 °C under inert atmosphere.

All formulations were cytocompatible with Caco-2 cells for up to 8.5 mg/mL of freeze-dried powder (corresponding to 2.8 mg/mL of polymer and 0.017 mg/mL of GSNO, i.e., 50 μM of GSNO) after 24 h. The apparent permeability coefficient (in vitro intestinal barrier model) of NOx species released from the particles was higher than for free GSNO meaning that these GSNO delivery systems are enhancing GSNO intestinal permeability.

In summary, two different GSNO-MP were obtained through the modification of nanoparticles preparation process. All three GSNO loaded particles were successfully converted into dried and stable powders through the freeze-drying method, which facilitated their storage and mode of application. Among these particles, the S/O/W microparticles is an attractive avenue as it's EE and release kinetics may be improved by reducing the size of GSNO powder (currently 40 μm). This challenging procedure is in progress using supercritical fluid technology.

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

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

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

References

1. Bogdan, C. Nitric oxide and the immune response. *Nat. Immunol.* **2001**, *2*, 907–916.
2. Naghavi, N.; de Mel, A.; Alavijeh, O.S.; Cousins, B. G.; Seifalian, A.M. Nitric oxide donors for cardiovascular implant applications. *Small* **2013**, *9*, 22–35.
3. Broniowska, K.A.; Diers, A.R.; Hogg, N. *S*-Nitrosoglutathione. *Biochim. Biophys. Acta (BBA)—Gen. Subj.* **2013**, *1830*, 3173–3181.
4. Maron, B.A., Tang, S.-S.; Loscalzo, J. S-Nitrosothiols and the S-Nitrosoproteome of the Cardiovascular System. *Antioxid. Redox Signal.* **2013**, *18*, 270–287.
5. 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.

6. 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. Biol. Med.* **2016**, *12*, 1795–1803.
7. 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.



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).