Biochemistry and Biophysics Reports 7 (2016) 225–229

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Glutathione disulfide liposomes – A research tool for the study of glutathione disulfide associated functions and dysfunctions

Satya S. Sadhu [a], Jiashu Xie [b], Hongwei Zhang [c], Omathanu Perumal [a], Xiangming Guan [a,*]

[a] Department of Pharmaceutical Sciences, College of Pharmacy, South Dakota State University, Brookings, SD 57007, United States
[b] Center for Drug Design, University of Minnesota, Minneapolis, MN 55455, United States
[c] Department of Pharmaceutical Sciences, School of Pharmacy, MCPHS University, Boston, MA 02115, United States

ARTICLE INFO

Article history:
Received 21 February 2016
Received in revised form
18 June 2016
Accepted 20 June 2016
Available online 28 June 2016

Keywords:
Glutathione disulfide
Liposomes
Glutathionylation
Oxidative stress
Thiols

ABSTRACT

Glutathione disulfide (GSSG) is the oxidized form of glutathione (GSH). GSH is a tripeptide present in the biological system in mM concentration and is the major antioxidant in the body. An increase in GSSG reflects an increase in intracellular oxidative stress and is associated with disease sates. The increase has also been demonstrated to lead to an increase in protein *S*-glutathionylation that can affect the structure and function of proteins. Protein *S*-glutathionylation serves as a regulatory mechanism during cellular oxidative stress. Though GSSG is commercially available, its roles in various GSSG-associated normal/abnormal physiological functions have not been fully delineated due to the reason that GSSG is not cell membrane permeable and a lack of method to specifically increase GSSG in cells. We have developed cationic liposomes that can effectively deliver GSSG into cells. Various concentrations of GSSG liposomes can be conveniently prepared. At 1 mg/mL, the GSSG liposomes effectively increased intracellular GSSG by 27.1 $\pm$ 6.9 folds (n=3) in 4 h and led to a significant increase in protein *S*-glutathionylation confirming that the increased GSSG is functionally effective. The Trypan blue assay demonstrated that GSSG liposomes were not cytotoxic; the cell viability was greater than 95% after cells were treated with the GSSG liposomes for 4 h. A stability study showed that the dry form of the GSSG liposomes were stable for at least 70 days when stored at $-80\,^{\circ}C$. Our data demonstrate that the GSSG liposomes can be a valuable tool in studying GSSG-associated physiological/pathological functions.

1. Introduction

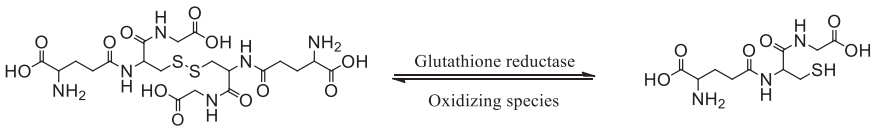
Glutathione disulfide (GSSG) is the oxidized form of glutathione (GSH). GSH is an endogenous three amino acid peptide present in mM concentration in cells and serves as the major antioxidant in the biological system [1]. GSH protects the biological system from oxidizing factors such as reactive oxygen species (ROS) or reactive nitrogen species (RNS) through terminating them while GSH itself is oxidized to GSSG. GSSG is then reduced back to GSH by glutathione reductase (GR) to maintain thiol redox homeostasis (Fig. 1). Under normal conditions, the biological system maintains a high ratio of GSH:GSSG (> 100:1) through effective reduction of GSSG back to GSH. An increase in GSSG is considered as an increase in cellular oxidative stress [2].

An increase in GSSG has also been implicated in various diseases such as neurodegenerative diseases [3] and cystic fibrosis [4]. Further, an increase in GSSG was demonstrated to increase protein *S*-glutathionylation [5]. Protein *S*-glutathionylation is involved in oxidative stress and structural and functional modification of proteins. Protein *S*-glutathionylation also serves as a cellular regulatory mechanism like protein phosphorylation [5]. A study of the effects of GSSG changes on GSSG-associated physiological/pathological states and protein S-glutathionylation remains challenging due to a lack of a research tool to specifically increase intracellular GSSG levels since GSSG is a cell membrane impermeable molecule. Current methods to increase intracellular GSSG levels mainly include a microinjection approach [6] and use of GSSG methyl ester [7]; the latter is expected to be hydrolyzed to yield GSSG intracellularly. The microinjection approach requires an expertize and is not applicable for most *in vitro* and *in vivo* studies. The obvious drawback of GSSG methyl ester is that the rate of hydrolysis by esterases may not be the same in different cells. Also, GSSG methyl ester is not suitable for *in vivo* study since it will be quickly hydrolyzed in plasma before it can reach the targeted site.

Abbreviations: GSSG, glutathione disulfide; GSH, glutathione; DDAB, dimethyldioctadecylammonium bromide; PBS, phosphate buffered saline; FBS, fetal bovine serum; BBB, blood-brain barrier

* Correspondence to: South Dakota State University, College of Pharmacy, Box 2202C, Brookings, SD 57007, United States.
E-mail address: Xiangming.Guan@sdstate.edu (X. Guan).

http://dx.doi.org/10.1016/j.bbrep.2016.06.017



GSSG GSH

Fig. 1. Chemical structure of GSSG and its relationship with intracellular antioxidant GSH.

We reported early a method to increase intracellular GSSG through the use of a GR inhibitor [8]. However, the inhibitor was later found also to inhibit other disulfide reductases such as glutaredoxin [9] and thioredoxin reductase [10]. Therefore, the inhibitor is more appropriate as a tool to block all disulfide reduction to increase cellular thiol oxidative stress but not selective enough for the study of the impact caused by GSSG alone.

Here we would like to report a method that effectively delivers GSSG into cells through the use of cationic liposomes. The increase of GSSG led to a significant increase in cellular protein *S*-glutathionylation confirming that the delivered GSSG is functionally effective. This is the first method that delivers specifically GSSG, not in the other forms of GSSG such as GSSG methyl ester, into cells. The method will be valuable in studying the impact of GSSG on GSSG-associated cellular function/dysfunction and provide unambiguous information regarding the roles of GSSG in these function/dysfunction. Similarly, it will be valuable in studying protein *S*-glutathionylation-related function/dysfunction.

2. Material and methods

2.1. Materials

Lecithin (Phospholipon 90 G) was obtained as a gift from Lipoid (Ludwigshafen, Germany). Cholesterol was supplied by MP Biomedicals (Solon, Ohio), dimethyldioctadecylammonium bromide (DDAB) and GSSG were obtained from Applichem (Darmstadt, Germany). GSH was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). Chloroform and LC/MS grade acetonitrile, water and formic acid were obtained from Fisher Scientific (Fair Lawn, NJ). 5-Sulfosalicylic acid was purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ). RPMI 1640 growth medium, penicillin/streptomycin, phosphate buffered saline (PBS), and trypsin were obtained from Mediatech (Herndon, VA). Fetal bovine serum (FBS) was purchased from Atlanta Biologicals (Lawrenceville, GA). NCI-H226 cells (human non-small cell lung carcinoma) were obtained from the National Cancer Institute.

2.2. Preparation of GSSG cationic liposomes

The GSSG liposomes were prepared using a dehydration/rehydration method with minor modification [11]. Briefly, Lecithin (70 mg), cholesterol (10 mg) and DDAB (20 mg) were dissolved in chloroform (10 mL), and evaporated to dryness to form a thin and homogeneous lipid film by rotation in a 250 mL round bottom flask under a reduced pressure at 40 °C. The lipid film was then hydrated with a GSSG PBS loading solution (GSSG dissolved in PBS) (10 mL). Size reduction of the resulting suspension was performed by sonication in a bath sonicator at 40 °C (4 min sonication with 1 min break) for 20 min. After sonication the resulting liposomal suspension was distributed in aliquots of 1 mL each and frozen at −80 °C for 12 h, freeze-dried, and stored at −80 °C for later use.

To use the stored freeze-dried GSSG liposomes, the liposomes in the vial were reconstituted in water (1 mL) (rehydration), vortex mixed for 1 min, and allowed to stand at 40 °C for 10 min, then at room temperature for additional 20 min. The reconstituted liposomes were loaded to a Sephadex column (PD10 Columns, GE Health care, Little Chalfont, UK) and centrifuged to yield the GSSG liposomes. GSSG liposomes were prepared with equal amount of GSSG and lipid in the liposome formulation: GSSG liposomes at 1 mg/mL mean the liposome formulation contained 1 mg GSSG and 1 mg lipid.

2.3. Determination of particle size and zeta potential

The above rehydrated liposomes were further subjected to a dilution of 1:100 with deionized water and then examined for particle size and zeta potential by photon correlation spectroscopy and Doppler anemometry using Malvern Zeta One Zeta Potential/Particle Sizer.

2.4. Determination of GSSG loading

The liposome samples prepared above were diluted further using 3% sulfosalicylic acid (1:10 dilution) and sonicated for 1 h. This sonicated sample was further diluted in 0.1% trifluoroacetic acid (1:100 dilution). The resulting solution was centrifuged at 14,000 rpm for 3 min before analysis using LC/MS/MS. LC/MS/MS quantification of GSSG employed an Agilent Eclipse XDB-C18 column (1.0 × 150 mm, 3.5 μm) equipped with an Agilent ZORBAX Eclipse XDB guard column (1.0 × 17 mm, 5 μm). The mobile phase was composed of 0.1% formic acid and acetonitrile at a ratio of 92/8 (v/v). The flow rate was set at 70 μL/min. In MS/MS detection, the electrospray ion source was operated in the positive ionization mode with the following optimized parameters: sheath gas 85 arb, spray voltage 4.5 kV, capillary temperature 350 °C, capillary voltage 3 V and tube lens offset −60 V. Selected reaction monitoring (SRM) was performed to monitor the mass transition of 613→484 (GSSG). Under these conditions, the retention time of GSSG was 3.0 min. The injection volume was 1 μL. GSSG was quantified based on a GSSG standard curve constructed right after the GSSG sample analysis.

2.5. Stability of the stored freeze-dried GSSG liposomes

The GSSG liposomes prepared above were distributed 1 mL each in a 1 mL centrifuge vial, frozen at −80 °C, freeze-dried, and then stored at −80 °C. Periodically, vials containing the freeze-dried GSSG liposomes were reconstituted with water (1 mL), passed through a Sephadex column to remove nonencapsulated GSSG, and analyzed for the encapsulated liposomal GSSG by LC/MS/MS as described above. The stability of the stored freeze-dried GSSG liposomes was determined over a period of 70 days.

2.6. Cellular uptake of GSSG liposomes in NCI-H226 cells

Exponentially growing NCI-H226 cells (4×10^6) were placed in a 75 cm^2 flask in RPMI 1640 growth medium containing 10% FBS and 1% penicillin/streptomycin in a 5% CO_2 incubator at 37 °C for 12 h for attachment before treated with GSSG liposomes[1] (1 mg/mL)[2] in a 5% CO_2 incubator at 37 °C. At the end of the

[1] GSSG liposomes for cell culture use were prepared by reconstituting the stored freeze-dried liposomes with sterile water instead of deionized water.

[2] 1 mg of GSSG and 1 mg of lipid.

treatment, the medium was removed, and the cells were rinsed with PBS thrice and detached by trypsinization. The cell suspension was centrifuged at 1000 g for 5 min. The cell pellet was washed with 1 mL ice-cold PBS containing 1 mM EDTA and resuspended in 0.5 mL of ice-cold 5-sulfosalicylic acid (3%, w/v). Cells were then disrupted by an ultrasonic processor (Qsonica sonicators) with a cup horn probe for 5 min and centrifuged at 20,817 × *g* for 3 min at 4 °C. Protein content was determined using a BCA protein assay method [12]. Quantification of GSSG was achieved by LC/MS/MS as described above using a calibration curve constructed from samples of GSSG prepared by spiking known amounts of GSSG standard solution to pooled cell lysates and diluted with 0.1% formic acid. Three controls (medium alone, aqueous GSSG, and liposomes without GSSG) were conducted in parallel.

2.7. Cell viability determination

Cell viability of NCI-H226 cells treated with GSSG liposomes was determined by the Trypan blue assay according to the manufacture instruction (Sigma).

2.8. Protein S-glutathionylation

Protein *S*-glutathionylation was determined based on a reported procedure with minor modification [13]. Briefly, exponentially growing NCI-H226 cells (6 million) were plated in a medium flask (75 cm^2) and placed in an incubator overnight, and the cells were subjected to GSSG liposomes treatment (1 mg/mL) and incubated for 4 h at 37 °C in an incubator containing 5% CO_2. The cells were processed and subjected for determination of protein *S*-glutathionylation by HPLC as outlined in the literature [13]. Three controls (medium alone, aqueous GSSG, and liposomes without GSSG) were conducted in parallel.

3. Results

3.1. GSSG cationic liposomes

GSSG cationic liposomes were prepared based on a dehydration/rehydration method with minor modification. GSSG encapsulation in liposomes was quantified by an LC/MS/MS method. A study of GSSG encapsulation in liposomes *vs* GSSG concentration in the GSSG loading solution was conducted. Fig. 2 demonstrated that encapsulated GSSG increased with GSSG concentration in the GSSG PBS loading solution. The increase reached to a maximum when GSSG was increased to 100 mg/mL. Further increase in GSSG concentration did not increase GSSG encapsulation in the liposomes. Accordingly, GSSG liposomes prepared with 100 mg/mL GSSG loading solution were used for the rest of the study. The size and zeta potential of the resultant GSSG liposomes were determined to be 190 ± 42 nm and 70 ± 15 mV respectively (mean ± SD, n = 3).

3.2. Stability of freeze-dried GSSG liposomes

The stability of the stored freeze-dried GSSG liposomes was determined over a period of 70 days. As demonstrated in Fig. 3, no significant change was noted in GSSG encapsulation in samples that were stored at −80 °C for 70 days.

3.3. Intracellular delivery of GSSG liposomes

NCI-H226 cells were employed to determine the uptake of GSSG liposomes by cells. After NCI-H226 cells were treated with

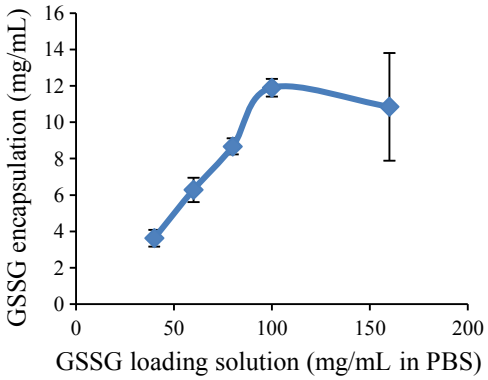


Fig. 2. A plot of encapsulated GSSG in liposomes against the concentration of GSSG in GSSG loading solution used to prepare the GSSG liposomes. GSSG loading solution (GSSG in PBS, 10 mL) was used to wet the lipid layer to prepare crude GSSG liposomes through sonification, dehydration/hydration as described in the experimental procedure part. The crude GSSG liposomes then passed a Sephadex column to remove nonencapsulated GSSG. The quantity of GSSG in the liposomes was determined by an LC/MS/MS method. The results are presented as mean ± SD of independent triplicate experiments.

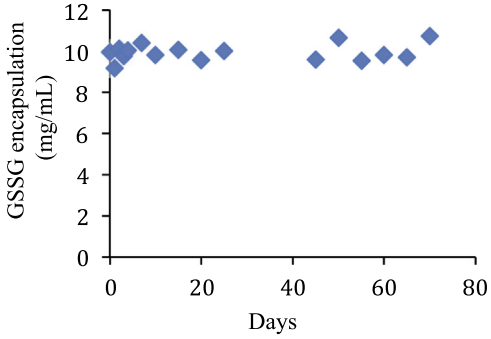


Fig. 3. Stability study of dry GSSG liposomes stored at −80 °C. The freeze-dried GSSG liposomes were reconstituted with water, passed through a Sephadex column to remove nonencapsulated GSSG, and analyzed for the encapsulated GSSG by LC/MS/MS. The results are from one of the duplicate experiments.

GSSG liposomes (1 mg/mL), intracellular GSSG was quantified by an LC/MS/MS method. As presented in Fig. 4, intracellular GSSG increased with time and reached the maximum at 4 h. A maximum of 27.1 ± 6.9 folds (n = 3) increase in intracellular GSSG was observed. No GSSG increase was observed for all three controls (medium alone, aqueous GSSG, and liposomes without GSSG). The cell viability was observed to be > 95% for controls and cells treated with GSSG liposomes at the end of 4 h.

3.4. Protein S-glutathionylation

An increase in intracellular GSSG by a GR inhibitor was reported to increase protein *S*-glutathionylation [13]. To check whether GSSG delivered to cells by liposomes is functionally effective, cellular protein *S*-glutathionylation was determined. When NCI-H226 cells were treated with GSSG liposomes (1 mg/mL) for 4 h, intracellular GSSG was increased by 20 folds. The treatment also led to a significant increase in protein *S*-glutathionylation; protein *S*-glutathionylation was found to be 1.72 ± 0.74 nmol/million cells (n = 3) in the treated cells while no protein *S*-glutathionylation was detected for cells treated with medium alone (control), aqueous GSSG (1 mg/mL), or liposomes without GSSG. The results demonstrate that GSSG delivered by liposomes is functionally effective.

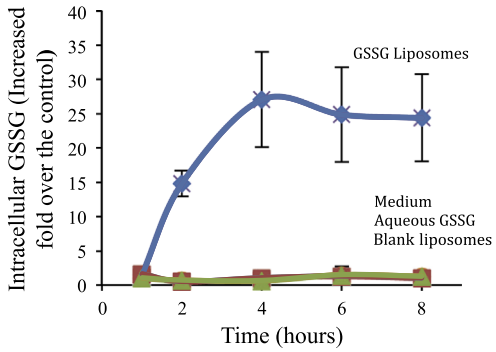


Fig. 4. A time course of intracellular GSSG delivered by GSSG liposomes. Exponentially growing NCI-H226 cells (4×10^6) were treated with GSSG liposomes* (1 mg /mL)**. Intracellular GSSG was determined by an LC/MS/MS method. The results are presented as fold increase over the medium control and as mean ± SD of independent triplicate experiments for GSSG liposomes. The data obtained from medium, aqueous GSSG, and liposomes are presented as one representative of two independent experiments. *GSSG liposomes for cell culture use were prepared by reconstituting the stored freeze-dried liposomes with sterile water instead of deionized water. **1 mg of GSSG and 1 mg of lipid.

4. Discussion

Liposomes consist of an aqueous core surrounded by one or multiple bilayers composed of natural or synthetic lipids [14–17]. The lipids are biologically inert and weakly immunogenic with low toxicity [14–17]. Liposomes are able to encapsulate compounds with different lipophilicities with water-soluble compounds entrapped into aqueous core and lipophilic ones in phospholipid bilayer [14–17]. Liposomes are widely used to aid absorption of cell membrane impermeable compounds. Since its discovery by Alec D Bangham in the 1960s at the Babraham Institute, University of Cambridge, liposomes have been extensively investigated for the delivery of drugs, diagnostics, vaccines, nutrients and other bioactive agents and considered as the most successful drug-carrier system known to date for its efficient delivery, biodegradability, and biocompatibility [15–19]. Depending on the charge on liposomal surface, liposomes can be divided into negatively charged (anionic), positively charged (cationic), and neutral liposomes [14–17]. Cationic liposomes have been extensively used in gene delivery due to the electrostatic interaction with the negatively charged DNA [15–17,20]. Cationic liposomes help increase the uptake of the entrapped materials through electrostatic interaction between positively charged liposomes and negatively charged cell membrane [15–17,21]. In general, the cellular uptake of liposomes occurs through membrane fusion and receptor-mediated endocytosis [15–17,22,23]. The cationic lipid DDAB also helps in escaping the endolysosomal pathway which would degrade proteins or peptides to constituent amino acids [24]. Although positively charged lipids are reported to be cytotoxic, the composition of liposomes employed in this method was reported to be less toxic [25].

An increase in intracellular GSSG has been associated with various normal and abnormal cellular functions [3]. A direct outcome of an increase in intracellular GSSG is protein *S*-glutathionylation resulted from a thiol-disulfide exchange reaction of a protein thiol with GSSG. Physiologically, protein *S*-glutathionylation serves as a mechanism to protect a protein thiol from irreversible oxidation during oxidative stress [5]. Protein *S*-glutathionylation also serve as a cellular regulatory mechanism like protein phosphorylation [5]. An increase in GSSG has been reported in a number of disease states such as neurodegenerative diseases. All neurodegenerative diseases are characterized by progressive loss of neurons [3]. These diseases confront the growing elderly population and have become a major public health issue and economic burden due to the need for extensive long-term health care. A common neuropathological feature of these diseases is associated with increased oxidative damage to biological macromolecules such as proteins, lipids, and nucleic acids in neurodegenerative tissue. Increased levels of GSSG were observed in postmortem brain samples of patients [3]. Recent studies show that dysregulation of redox signaling and sulfhydryl homeostasis likely contributes to onset/progression of neurodegeneration [3]. *S*-Glutathionylation of key enzymes and proteins has been implicated in neurodegenerative diseases. However, the exact roles of sulfhydryl homeostasis and protein *S*-glutathionylation in the pathology of these diseases are not fully understood. This field is evolving and much remains to be explored.

The inability of GSSG to pass through cell membrane and a lack of effective method to deliver it into cells has hampered our ability to investigate the role of GSSG in its normal and abnormal cellular functions. Liposomes as one of the most effective and widely used delivery systems have been employed to aid the delivery of bioactive agents through various biological membrane and biological barriers including blood-brain barrier (BBB) [26]. GSSG liposomes reported here are the first method that delivers specifically GSSG into cells. We demonstrated that different concentrations of GSSG liposomes can be conveniently prepared through the use of a PBS solution containing different concentrations of GSSG; providing us the ability to manipulate intracellular GSSG concentration. The data from protein *S*-glutathionylation confirmed that GSSG delivered to cells by GSSG liposomes were functionally effective. Further, cell viability study showed that GSSG liposomes exhibited no significant cytotoxicity. Therefore, the GSSG liposomes can serve as a valuable tool in studying the role of GSSG in GSSG-associated cellular function and dysfunction. These studies can help identify approaches to prevent GSSG-associated diseases and potentially provide effective treatments. GSSG liposomes can also undoubtedly help the investigation of the role of protein *S*-glutathionylation in various cellular regulatory mechanisms.

5. Conclusion

GSSG liposomes reported here provide the first method that delivers GSSG into cells. Since the method delivers only GSSG into cells, it avoids any complications caused by other indirect way to increase intracellular GSSG such as the use of a GR inhibitor. The method provides an unambiguous tool in investigating GSSG-associated roles in cellular function and dysfunction. The simple procedure for preparation and storage stability of the dry GSSG liposomes provides convenience and also makes the product commercialization possible.

Acknowledgment

This work was supported by a grant from the National Institutes of Health (1R15GM093678–01).

Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2016.06.017.

References

[1] A. Meister, M.E. Anderson, Glutathione, Annu. Rev. Biochem. 52 (1983) 711–760.
[2] Y.M. Go, D.P. Jones, Thiol/disulfide redox states in signaling and sensing, Crit. Rev. Biochem. Mol. Biol. 48 (2013) 173–181.

[3] E.A. Sabens Liedhegner, X.H. Gao, J.J. Mieyal, Mechanisms of altered redox regulation in neurodegenerative diseases – focus on S-glutathionylation, Antioxid. Redox Signal. 16 (2012) 543–566.
[4] A.J. Cooper, J.T. Pinto, P.S. Callery, Reversible and irreversible protein glutathionylation: biological and clinical aspects, Expert Opin. Drug Metab. Toxicol. 7 (2011) 891–910.
[5] P. Ghezzi, Protein glutathionylation in health and disease, Biochim. Biophys. Acta 2013 (1830) 3165–3172.
[6] H.A. Park, S. Khanna, C. Rink, S. Gnyawali, S. Roy, C.K. Sen, Glutathione disulfide induces neural cell death via a 12-lipoxygenase pathway, Cell Death Differ. 16 (2009) 1167–1179.
[7] M. Tajima, Y. Kurashima, K. Sugiyama, T. Ogura, H. Sakagami, The redox state of glutathione regulates the hypoxic induction of HIF-1, Eur. J. Pharmacol. 606 (2009) 45–49.
[8] T. Seefeldt, Y. Zhao, W. Chen, A.S. Raza, L. Carlson, J. Herman, A. Stoebner, S. Hanson, R. Foll, X. Guan, Characterization of a novel dithiocarbamate glutathione reductase inhibitor and its use as a tool to modulate intracellular glutathione, J. Biol. Chem. 284 (2009) 2729–2737.
[9] S.S. Sadhu, E. Callegari, Y. Zhao, X. Guan, T. Seefeldt, Evaluation of a dithiocarbamate derivative as an inhibitor of human glutaredoxin-1, J. Enzym. Inhib. Med. Chem. 28 (2013) 456–462.
[10] W. Chen, Z. Jiang, N. Lin, Z. Zheng, Z. Chen, X. Zhang, X. Guan, Evaluation of N-acetyl-S-(p-chlorophenylcarbamoyl)cysteine as an irreversible inhibitor of mammalian thioredoxin reductase1, J. Enzym. Inhib. Med. Chem. (2015) 1–7.
[11] N. El Kateb, L. Cynober, J.C. Chaumeil, G. Dumortier, L-cysteine encapsulation in liposomes: effect of phospholipids nature on entrapment efficiency and stability, J. Microencapsul. 25 (2008) 399–413.
[12] K.J. Wiechelman, R.D. Braun, J.D. Fitzpatrick, Investigation of the bicinchoninic acid protein assay: identification of the groups responsible for color formation, Anal. Biochem. 175 (1988) 231–237.
[13] Y. Zhao, T. Seefeldt, W. Chen, X. Wang, D. Matthees, Y. Hu, X. Guan, Effects of glutathione reductase inhibition on cellular thiol redox state and related systems, Arch. Biochem. Biophys. 485 (2009) 56–62.
[14] P. Milla, F. Dosio, L. Cattel, PEGylation of proteins and liposomes: a powerful and flexible strategy to improve the drug delivery, Curr. Drug Metab. 13 (2012) 105–119.
[15] K.A. Ghaffar, A.K. Giddam, M. Zaman, M. Skwarczynski, I. Toth, Liposomes as nanovaccine delivery systems, Curr. Top. Med. Chem. 14 (2014) 1194–1208.
[16] D. Tila, S. Ghasemi, S.N. Yazdani-Arazi, S. Ghanbarzadeh, Functional liposomes in the cancer-targeted drug delivery, J. Biomater. Appl. 30 (2015) 3–16.
[17] G. Bozzuto, A. Molinari, Liposomes as nanomedical devices, Int. J. Nanomed. 10 (2015) 975–999.
[18] R.A. Schwendener, Liposomes in biology and medicine, Adv. Exp. Med. Biol. 620 (2007) 117–128.
[19] L.K. Muller, K. Landfester, Natural liposomes and synthetic polymeric structures for biomedical applications, Biochem. Biophys. Res. Commun. 468 (2015) 411–418.
[20] N.J. Zuidam, Y. Barenholz, Electrostatic parameters of cationic liposomes commonly used for gene delivery as determined by 4-heptadecyl-7-hydroxycoumarin, Biochim. Biophys. Acta 1329 (1997) 211–222.
[21] R. Bajoria, S.R. Sooranna, S.F. Contractor, Endocytotic uptake of small unilamellar liposomes by human trophoblast cells in culture, Hum. Reprod. 12 (1997) 1343–1348.
[22] J. Rejman, A. Bragonzi, M. Conese, Role of clathrin- and caveolae-mediated endocytosis in gene transfer mediated by lipo- and polyplexes, Mol. Ther. 12 (2005) 468–474.
[23] C.R. Miller, B. Bondurant, S.D. McLean, K.A. McGovern, D.F. O'Brien, Liposome-cell interactions in vitro: effect of liposome surface charge on the binding and endocytosis of conventional and sterically stabilized liposomes, Biochemistry 37 (1998) 12875–12883.
[24] Y. Xu, F.C. Szoka Jr., Mechanism of DNA release from cationic liposome/DNA complexes used in cell transfection, Biochemistry 35 (1996) 5616–5623.
[25] A. Manosroi, N. Khositsuntiwong, C. Komno, W. Manosroi, R.G. Werner, J. Manosoi, Chemical stability and cytotoxicity of human insulin loaded in cationic DPPC/CTA/DDAB liposomes, J. Biomed. Nanotechnol. 7 (2011) 308–316.
[26] K.B. Johnsen, T. Moos, Revisiting nanoparticle technology for blood-brain barrier transport: unfolding at the endothelial gate improves the fate of transferrin receptor-targeted liposomes, J. Control. Release 222 (2016) 32–46.