Biochemical and Biophysical Research Communications 397 (2010) 420–424

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Inhibitory effects of glutathione on dengue virus production

Yanping Tian [a,1], Wen Jiang [b,1], Na Gao [a], Junlei Zhang [c], Wei Chen [c], Dongying Fan [d], Deshan Zhou [e], Jing An [d,*]

[a] Department of Histology and Embryology, School of Basic Medicine, Third Military Medical University, Chongqing 400038, PR China
[b] Department of Laboratory Animal Sciences, School of Basic Medicine, Third Military Medical University, Chongqing 400038, PR China
[c] Department of Microbiology, School of Basic Medicine, Third Military Medical University, Chongqing 400038, PR China
[d] Department of Microbiology, School of Basic Medical Sciences, Capital University of Medical Sciences, Beijing 100069, PR China
[e] Department of Histology and Embryology, School of Basic Medical Sciences, Capital University of Medical Sciences, Beijing 100069, PR China

ARTICLE INFO

Article history:
Received 5 May 2010
Available online 27 May 2010

Keywords:
Dengue virus
Redox stage
GSH
NF-κB

ABSTRACT

Reduced glutathione (GSH) is the most powerful intracellular antioxidant and also involved in viral infections. The pathogenesis of dengue virus (DV) infection has not been completely clarified. This study investigated the relationship between DV serotype 2 (DV2) infections and host intracellular GSH content. Results showed infection with DV2 resulted in a decrease in intracellular GSH, which caused NF-κB activation and increased DV2 production. Supplemental GSH significantly inhibited activation of NF-κB, resulting in a decreased production of DV2 in HepG2 cells. Furthermore, high activity of NF-κB and increased production of DV2 was observed in HepG2 cells treated with buthionine sulfoximine (BSO), an inhibitor of GSH synthesis. In conclusion, DV2 infection could reduce host intracellular GSH concentration and benefited from this process. Supplemental GSH could inhibit viral production, indicating GSH might be valuable in the prevention and treatment of DV2 infection.

1. Introduction

As obligate intracellular parasites, viral replication occurs exclusively within the host cell and thus depends on numerous factors that control cell machinery and metabolism. Many viral infections are associated with intracellular redox alterations characteristic of oxidative stress responses [1]. Several findings have demonstrated the involvement of the intracellular redox balance in the establishment of viral infection and the progression of virus-induced diseases [2]. Reactive oxygen species (ROS) is increased in virus infected cells and eliminated by antioxidants. Reducing conditions are normally maintained within the cell by antioxidant molecules such as glutathione (GSH), superoxide dismutase (SOD), thioredoxin (Trx), and catalase, which constitute the system developed by cells to counteract oxidation [3]. GSH, a cysteine-containing tripeptide, is the most important and ubiquitous antioxidant molecule of eukaryotic cells.

The resultant decrease in GSH may contribute to pathogenesis of viral infection disease, regulation of viral replication, host defense, and modulation of cellular responses. Previous studies have demonstrated that cultured cells infected with Sendai virus, human immunodeficiency virus (HIV), influenza virus, or hepatitis C virus [4–7] have decreased intracellular GSH levels, increased generation of ROS, and enhanced oxidation of the cellular GSH pool, which activates redox-dependent transcription factors, such as NF-κB, and leads to increased production of various cytokines.

Dengue virus (DV), belonging to the family of Flaviviridae, is one of the most widespread mosquito-borne human pathogens worldwide. There are four serotypes (DV1–4). DV infection may cause a variety of symptoms ranging from mild dengue fever (DF) to the more severe form of dengue hemorrhagic fever or dengue shock syndrome (DHF/DSS). This kind of enveloped, positive-strand RNA virus has spread throughout tropical and subtropical regions worldwide over the past several decades and recently its infection has reemerged as more and more severe threatens in humans health. However, the pathogenesis about DF and DHF/DSS and the interaction between DV and host cell are not clear yet. Recently, it was reported that oxidative damage was observed in dengue fever patients [8]. As an antioxidant molecule, the involvement of host intracellular GSH in DV infection has not been reported.

In past several years, it was confirmed that the liver might be one of the important target organs for DV and HepG2 cells, a human hepatocarcinoma cell line, could support DV replication. Therefore, in the present study, the relationship between GSH levels and DV serotype 2 (DV2) productions was investigated to explore the role of cellular redox in DV2 infection in HepG2 cells. Our results showed that infection with DV2 influenced the host's intracellular GSH concentration. Decreased intracellular GSH increased DV2 production. Supplemental GSH significantly inhibited production of DV2 in the cells. Our results thus suggest that GSH have inhibitory effects on DV2 production and may be useful in the prevention and treatment of DV2 infection.

* Corresponding author. Fax: +86 10 8391 1496.
E-mail address: anjing60@yahoo.com.cn (J. An).
[1] These author contributed equally to this work.

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.05.108

2. Materials and methods

2.1. Cells, virus, and reagents

A human hepatocarcinoma cell line, HepG2 (ATCC), was cultured in Dulbecco's modified Eagle's minimum essential medium (DMEM, Gibco) with 10% fetal bovine serum (FBS). Vero cells were cultured in minimal essential medium (MEM, Gibco) with 5% FBS and used for plaque assays. DV2 (strain Tr1751) was isolated from a patient with dengue fever and kindly provided by Dr. Oya A (National Institute of Infectious Diseases, Japan). Buthionine sulfoximine (BSO) and GSH were purchased from Sigma. NF-κB luciferase reporter plasmids and luciferase assay kit were purchased from Beyotime (China). Lipofectamine reagents were from Invitrogen (USA). pReceiver-M01A vector was from Stratagene.

2.2. DV2 infection

HepG2 cells were plated in six-well plates (IWAKI, Japan) about 10^5 per well and grown for 24 h. Cells were washed twice with phosphate-buffered saline (PBS, pH 7.4) and DV2 infection was performed at a multiplicity of infection (MOI) of 10. After incubation for 1 h at 37 °C (adsorption period), un-adsorbed virus was removed, and the monolayer was washed with PBS and then incubated with DMEM supplemented with 2% FBS. At different time points post-infection, as indicated, samples of infected cells and supernatant were collected for determination of GSH concentration. Mock infection was carried out using heat-inactivated DV2 (56 °C, 30 min). Four independent experiments were performed for each time point with triplicate samples for each experiment.

2.3. Sample collection and determination of GSH concentration

For determination of total cellular GSH content, samples of cells and supernatants were collected. First, 0.5 ml of medium was collected at different times after infection. After lyophilization, the sample was resuspended in 50 μl of PBS containing metaphosphoric acid (5% final concentration). Meanwhile, monolayers of infected HepG2 cells in six-well plates were carefully detached and centrifuged at 1000*g* for 5 min at 4 °C. The cell pellet was resuspended in 50 μl PBS containing metaphosphoric acid (5% final concentration). Cell lysates were obtained by repeated cycles of freezing and thawing in liquid nitrogen. After centrifugation at 18,000*g* for 10 min at 4 °C, proteins were precipitated and the low molecular thiols in the supernatant were derivatized. Ten microliters of medium sample or the cell extracts was used for determination of extracellular or intracellular GSH concentrations.

Total cellular GSH content was determined by the method reported by Allen [9], as the colorimetric reaction of 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) with GSH to form 5-thio-2-nitrobenzoic acid (TNB). Briefly, 10 μl of medium sample, cell extracts, or GSH standard solutions were added to the reaction solution, i.e., 0.1 M PBS (pH 7.6) containing 0.24 mM NADPH, 2.5 mM EDTA, and 0.2 U of glutathione reductase per ml. GSH standard solutions containing 0–100 μM GSH were prepared in 5% metaphosphoric acid in PBS and used to generate a standard curve. The reactions were initiated by addition of DTNB. The rate of TNB formation was followed at 412 nm using a TECAN spectrophotometer (Tecan Austria GmbH). The total GSH concentration of each sample was extrapolated from a standard curve of GSH concentrations as a function of the change in absorbance over time. Protein concentrations in cell extracts were determined by the Bradford assay and used to normalize GSH levels.

2.4. Effects of GSH or BSO treatment on DV2 infection

HepG2 cell lines were treated with GSH or BSO in order to assess their effects on DV2 infection. First, the cytotoxicity of these drugs to HepG2 cells was determined by monitoring their morphology and their ability to exclude the Trypan blue. From these experiments, 10 and 20 mM GSH and 0.2 and 1 mM BSO were chosen as working concentrations.

For treatment with GSH, HepG2 monolayers in six-well plates were washed twice with PBS to remove residual FBS and then infected with DV2 at MOI of 1 in serum-free DMEM for 1 h. After removing DV2, the infected cells were cultured in 1 ml DMEM with 2% FBS, either with 10 or 20 mM GSH for 24 h after infection or without additive. According to the purpose of experiments, GSH was added either before or during viral inoculation. For treatment with BSO, 0.2 or 1 mM BSO was added to cells 18 h before infection and maintained in the media for 24 h after infection. At the end of the treatments, i.e., at 24 h after infection, supernatants were harvested for virus titration, and cells were collected and prepared for GSH determination. Mock-infected cells treated with GSH or BSO or untreated cells were run as parallel controls. Virus production was determined by plaque assay using monolayers of Vero cells under 1% methylcellulose overlay medium. Mock infection was performed by using heat-inactivated DV2 (56 °C, 30 min). Three independent experiments were performed for GSH or BSO treatments.

2.5. Luciferase reporter assays

One day prior to transfection, HepG2 cells were seeded in 24-well plates (IWAKI, Japan). The pNF-κB-luc containing four NF-κB binding motifs (GGGAATTTCC) was transfected into HepG2 cells using Lipofectamine 2000 reagents (Invitrogen, USA). At 24 h post-transfection, DV2 was inoculated at MOI of 10 in serum-free DMEM for 1 h, the cells were washed twice with PBS and then cultured in 1 ml DMEM with 2% FBS for 24 h. The luciferase activity of cell extracts from each sample was measured using a luciferase assay kit according to the manufacturer's protocol (Beyotime, China).

To further investigate the relationship between intracellular levels of GSH and NF-κB activity, HepG2 cells transfected with pNF-κB-luc were infected with DV2 and treated with 20 mM GSH. The cells were collected 24 h post-infection and prepared for measurement of luciferase activity. Meanwhile, the pNF-κB-luc-transfected cells were treated with 0.2 mM BSO at 18 h before infection and then infected with DV2. Luciferase activity of these cells was also measured at 24 h after infection as described above. Three independent experiments were performed to assess luciferase activity after infection.

2.6. Data analysis

Data from separate experiments are expressed as means ± standard deviation. The statistical significance of observed differences between means was determined using Student's *t*-test (for comparing the means of two samples) or ANOVA (for comparing means of three or more samples, to avoid the error inherent in performing multiple *t*-tests) and was defined as $p < 0.05$.

3. Results

3.1. Alterations of intracellular GSH levels during DV2 infection

In order to investigate the effect of DV2 infection on the intracellular level of GSH, HepG2 cells were infected with DV2 and levels of intracellular GSH were assayed at different time points post-infection. As shown in Fig. 1, DV2 infection caused

a time-dependent alteration in the intracellular GSH content. At very early stages of the infection, the decrease of GSH levels occurred at the beginning of DV2 adsorption and the lowest GSH value, about 73% as compared with mock infection ($p < 0.05$), was seen at 30 min after adsorption. At the end of adsorption (1 h), the GSH levels tended to recover, but the values were always significantly lower than those observed in mock-infected cells (Fig. 1A). At late stages of infection, a significant decrease in intracellular GSH levels was detected in DV2-infected cells at different time points, and the values at 2, 6, 12, and 24 h after infection were as low as 83%, 91%, 89%, and 67%, respectively, compared with mock-infected cells (Fig. 1B, $p < 0.05$). GSH levels tended to recover and reached normal levels at 48 h after infection.

Meanwhile, extracellular GSH level was measured at different time points post-infection and large amounts of GSH were detected in supernatants of infected cells at 30 min ($p < 0.05$), but not at 24 h and other time points ($p > 0.05$), as compared with that of controls and mock-infected cells (Fig. 1C). It is presumed that glutathione is extruded from the cell during virus infection at 30 min. These results indicate that DV2 infection could affect the host cells' intracellular levels of GSH.

3.2. Effect of exogenous GSH on intracellular GSH and DV2 infection in HepG2 cells

To further investigate the effects of GSH on DV2 infection in vitro, HepG2 cells were infected with DV2 and treated with GSH at the concentration of 10 mM and 20 mM, respectively. Subsequently, the cells were cultured for 24 h in the presence of GSH and then intracellular viral titers were assessed. As shown in Fig. 2, the addition of 20 mM GSH did not affect intracellular GSH levels in mock-infected cells, but significantly inhibited viral production in a dose-dependent manner in DV2-infected HepG2 cells. The decrease of intracellular GSH seen at 24 h after DV2 infection was blocked by adding exogenous GSH. Meanwhile, DV2 titers in supernatants decreased significantly, to 62% and

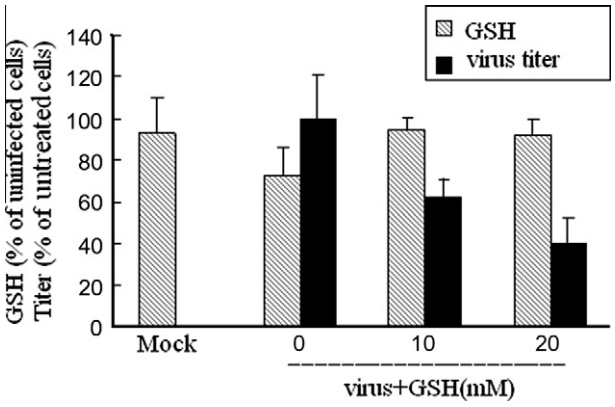

Fig. 2. Effect of exogenous GSH on intracellular GSH and DV2 titers in HepG2 cells. Cells were infected with DV2 at 1 MOI for 1 h. After removal of viral inoculation and washing, cells were maintained in DMEM containing 2% FCS and GSH at the indicated concentrations for 24 h. Viral production in the medium and intracellular GSH were determined as described in Section 2 ($p < 0.05$, $n = 3$).

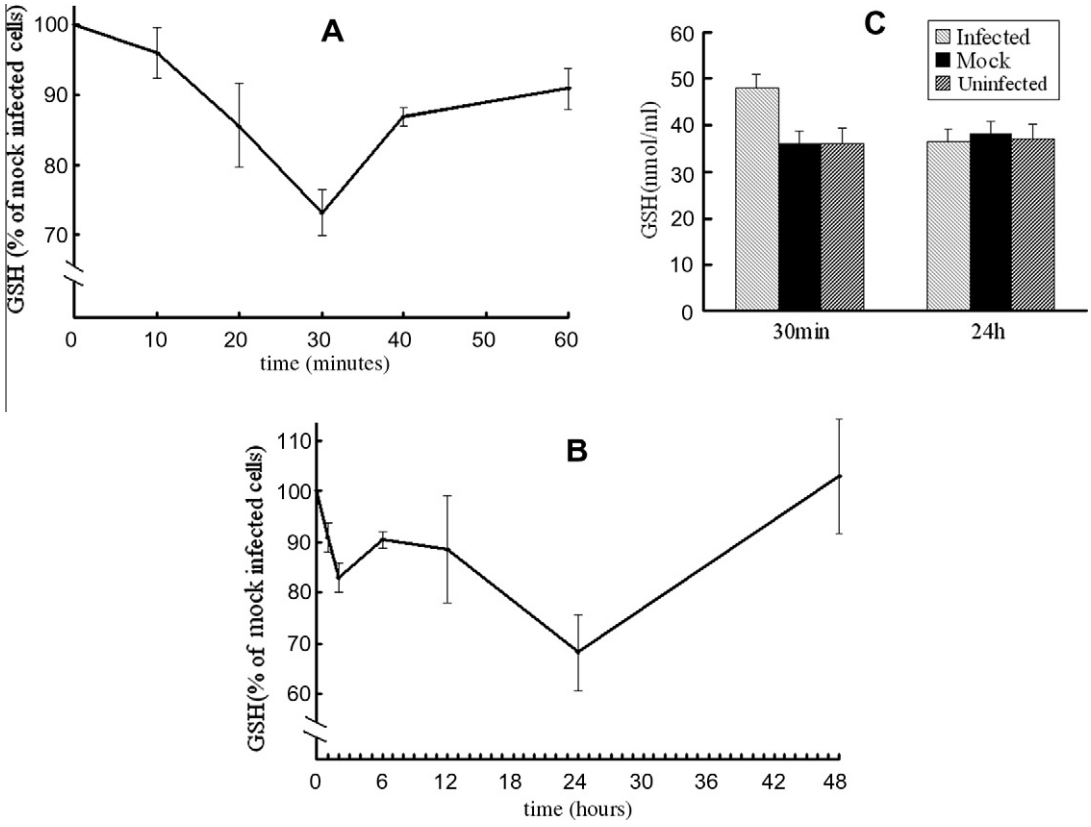

Fig. 1. Changes of intracellular GSH content in HepG2 cells during DV2 infection. (A,B) Confluent monolayers of HepG2 cells were infected with DV2. The cells were collected at various times either during or after the infection period and intracellular GSH content was measured. Values are four independent experiments with triplicates for each experiment. The difference in GSH content between DV2-infected and mock-infected cells were statistically significant ($p < 0.05$). (C) Effect of DV2 infection on extracellular GSH content in HepG2 cells at 30 min and 24 h after infection. Confluent monolayer was infected with DV2, and cells and media were collected at time points as indicated. GSH was assayed as described in Section 2 ($n = 4$).

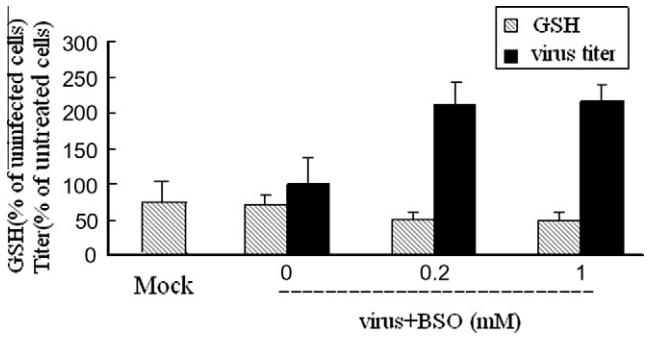

Fig. 3. Effect of BSO on intracellular GSH and DV2 infection in HepG2 cells. HepG2 monolayers were treated with 0.2 or 1 mM BSO for 18 h before infection and for 24 h thereafter. Cells and medium were collected for measurement of intracellular GSH levels and viral production, respectively. Values are three independent experiments with triplicates for each experiment ($p < 0.05$, $n = 3$).

40%, respectively, as a percentage of infection alone ($p < 0.05$) when 10 or 20 mM GSH was added. This indicates that treatment of cells with exogenous GSH inhibits virus production, but not alters intracellular GSH levels ($p > 0.05$, Fig. 2). Therefore, we suggest that GSH confer substantial protection against DV2 infection in HepG2 cells.

3.3. Effect of BSO on intracellular GSH and DV2 infection in HepG2 cells

To further confirm the relationship between intracellular GSH levels and DV2 infection, HepG2 cells were treated with BSO, which is a well-known inhibitor of GSH synthesis, and then infected with DV2. As shown in Fig. 3, treatment of cells with 0.2 or 1 mM BSO caused a decrease in intracellular GSH levels at about 20% compared with that in HepG2 cells infected alone ($p < 0.05$). And DV2 titers were twofold higher than those of untreated infected cells, reaching 211% and 215% as a percentage of untreated infected cells ($p < 0.05$, Fig. 3), indicating that DV2 infection is closely associated with intracellular GSH levels.

3.4. DV2 induces NF-κB activation in DV2-infected cells

To investigate the effect of alteration of intracellular GSH levels induced by DV2 infection on the redox-responsive transcription factor, NF-κB, HepG2 cells transfected with vectors pNF-κB-luc were infected with DV2 and transcription activity was assayed. NF-κB activity was recorded as a percentage of mock infection.

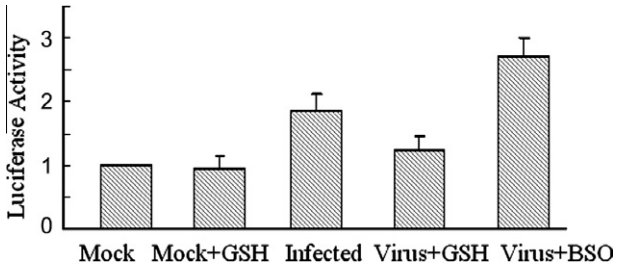

Fig. 4. Effect of DV2 on NF-κB-controlled luciferase expression. HepG2 cells were transfected with NF-κB luciferase plasmids pNF-κB-luc. Luciferase activity was tested at 24 h after DV2 infection. This experiment includes five groups, e.g., HepG2 cells mock-infected (Mock), mock-infected and treated with 20 mM GSH (Mock + GSH), DV2-infected (Infected), DV2-infected and treated with 20 mM GSH (Virus + GSH, added immediately after virus adsorption), or DV2-infected and treated with 0.2 mM BSO (Virus + BSO, added 18 h before infection and maintained for 24 h thereafter). The percent of luciferase activity in experimental samples was compared to that measured in mock-infected cells ($p < 0.05$, $n = 3$).

The results are shown in Fig. 4. NF-κB activity in DV2-infected HepG2 cells and GSH-treated infected cells was 184% and 124%, respectively, as compared to mock-infected HepG2 cells. Interestingly, NF-κB activity was as high as 271% in BSO-treated infected HepG2 cells ($p < 0.05$). NF-κB activity of GSH-treated mock-infected cells was no difference compared with that of mock-infected cells ($p > 0.05$). These results indicate that NF-κB activation could be induced by DV2 infection and is closely associated with GSH levels in host cells.

4. Discussion

Oxidative stress, either systemic or localized within infected tissues and cells, might be a common consequence of RNA virus infection. Therefore, antioxidants are potentially useful strategies against either viral infection or infection-associated symptoms [10]. GSH is a major water-soluble antioxidant that is normally present in epithelial cells at millimolar concentrations and regulates a variety of cellular functions by redox-dependent mechanisms [11]. In the present study, we found that decrease of intracellular GSH levels occurred in DV2-infected HepG2 cells at several time points after infection. Consistently, GSH decrease was also observed during the infection of Vero cells with clinically isolated herpes simplex type 1 virus [12]. Those indicate a more general relationship between GSH and viral replication.

The mechanism(s) by which intracellular GSH levels are altered by DV infection is not clear. Nevertheless, this phenomenon occurs during different types of viral infections, both in vivo and in vitro systems, or very early with respect to virus challenge. Suliman et al. found that influenza A virus infection induced oxidative stress in mice [13,14]. HIV-Tat protein could also induce oxidative stress and deplete intracellular GSH [15]. More recently, oxidative stress was observed in DF patients [8]. In the present study, decreased intracellular levels of GSH was observed and large amounts of GSH were detected in the media of infected cells at 30 min, but not 24 h, after infection. It may indicate a possibility that the decrease of intracellular GSH seen at very early stages of infection may result mainly from efflux of intracellular GSH due to disruption of cell membranes induced by DV2 adsorption and penetration. The slight recovery of GSH levels was probably due to the stimulation of the GSH synthesis subsequent to decreased GSH levels (Fig. 1A). However, decreased intracellular levels of GSH with unchanged extracellular GSH levels observed at 24 h after infection may result from GSH depletion induced by DV2 replication. Our results indicate a close association between oxidative stress and viral infection. The rapid biphasic drop in GSH content, leading to changes in the redox state in a very short time, may easily lead to different cell responses, which may associate with pathogenesis of DV infection. Moreover, the different GSH levels in the medium at 30 min and 24 h may indicate that either the rate of GSH extrusion is significantly different or that GSH leaves the cells with different modality at the two infection phases examined.

To further confirm the relationship between GSH and viral infection, GSH or BSO was added exogenously to cell medium. The decrease of intracellular GSH in infected cells was prevented by exogenous GSH. Several GSH-specific transporters were identified on the cellular and mitochondrial membranes of different cellular systems. Because of not cell-permeable, the balance of intracellular GSH is retained by the specific GSH carrier [16]. When added extracellularly, GSH had a dose-dependent inhibitory effect on DV2 infection in cultured cells but intracellular GSH levels maintained unchanged. Moreover, chemical inhibition of the GSH synthesis by BSO led to very slow GSH decrease (20% reduction was reached by at least 18 h of treatment) and drastically increased DV2 production. These further support the close

association of GSH levels with DV2 infection and suggest that the impairment of intracellular redox status is essential for the initiation and maintenance of viral multiplication. This is a primary event produced by viral infection other than the consequence of chronic exposure to inflammatory cytokines.

The resultant decrease in GSH levels may contribute to pathogenesis of viral infection diseases. GSH is an abundant natural antioxidant and thought to inhibit the activation of NF-κB [17,18]. It is now clear that viruses can benefit from activated NF-κB in different ways, which may result in host responses closely linked with pathogenesis of viral infection [19]. Our results also displayed that intracellular GSH decrease induce higher activity of NF-κB and more virus production. Conversely, addition of GSH to the medium of DV2-infected HepG2 cells inhibited the activity of NF-κB and led to decreased virus production. Decreased GSH levels and high NF-κB activity stimulate inflammatory responses leading to increased production of various cytokines that contribute to tissue damage and the symptoms of DHF/DSS, which may be the principal mechanisms of the etiology of DHF/DSS. Our results suggest that GSH provides an alternate strategy for prevention and treatment of DV infection. The present study only investigated relationship between the viral production and intracellular GSH levels in vitro. Studies about GSH-mediated protection against DV infection in vivo will be performed in near future.

In conclusion, the present results showed that DV2 infection altered host intracellular GSH content. Supplemental GSH significantly inhibited DV2 production whereas drastically increased production of DV2 was observed by decreasing intracellular GSH. Our data may provide clues to understanding the complex interrelationship between GSH status and DV replication, as well as a rationale for the oxidative stress mediated by DV. Thus, further research concerning the potential clinical efficacy of GSH treatment for DV infection is warranted.

Acknowledgments

This work was supported by Grants 30872227, 30800983, and 30972596 from the National Natural Science Foundation of China; 2009ZX10004-305 from S&T grand special program; 20090616119, 200907112, and 201007113 from PHR (IHLB).

References

[1] Christoph J. Burckhardt, Urs F. Greber, Redox rescues virus from ER trap, Nat. Cell Biol. 10 (2008) 9–11.
[2] M.A. Beck, J. Handy, O.A. Levander, The role of oxidative stress in viral infections, Ann. NY Acad. Sci. 917 (2000) 906–912.
[3] E. Cadenas, Biochemistry of oxygen toxicity, Annu. Rev. Biochem. 58 (1989) 79–110.
[4] M.R. Ciriolo, A.T. Palamara, S. Incerpi, E. Lafavia, M.C. Bue, P. de Vito, E. Garaci, G. Rotilio, Loss of GSH, oxidative stress, and decrease of intracellular pH as sequential steps in viral infection, J. Biol. Chem. 272 (1997) 2700–2708.
[5] Miriam Garland, Wafaie W. Fawzi, Antioxidants and progression of human immunodeficiency virus (HIV) disease, Nutr. Res. 19 (1999) 1259–1276.
[6] J. Cai, Y. Chen, S. Seth, S. Furukawa, R.W. Compans, D.P. Jones, Inhibition of influenza infection by glutathione, Free Radic. Biol. Med. 34 (2003) 928–936.
[7] Maher Y. Abdalla, Iman M. Ahmad, Douglas R. Spitz, Warren N. Schmidt, Bradley E. Britigan, Hepatitis C virus-core and non structural proteins lead to different effects on cellular antioxidant defenses, J. Med. Virol. 76 (2005) 489–497.
[8] R.C. Seet, C.Y. Lee, E.C. Lim, A.M. Quek, L.L. Yeo, S.H. Huang, B. Halliwell, Oxidative damage in dengue fever, Free Radic. Biol. Med. 47 (2009) 375–380.
[9] S. Allen, J.M. Shea, T. Felmet, J. Gadra, P.F. Dehn, A kinetic microassay for glutathione in cells plated on 96-well microtiter plates, Methods Cell. Sci. 22 (2001) 305–312.
[10] Ernst Peterhans, Oxidants and antioxidants in viral diseases: disease mechanisms and metabolic regulation, J. Nutr. 127 (1997) 962S–965S.
[11] D.P. Jones, Redox potential of GSH/GSSG couple: assay and biological significance, Methods Enzymol. 348 (2002) 93–112.
[12] A.T. Palamara, C.F. Perno, M.R. Ciriolo, D. Luciana, E. Balestra, C. D'Agostini, P. Di Francesso, C. Favalli, G. Rotilio, E. Garaci, Evidence for antiviral activity of glutathione: in vitro inhibition of herpes simplex virus type 1 replication, Antiviral Res. 27 (1995) 237–253.
[13] A.T. Palamara, L. Nencioni, K. Aquilano, G. De Chiara, L. Hernandez, F. Cozzolino, M.R. Ciriolo, E. Garaci, Inhibition of influenza A virus replication by resveratrol, J. Infect. Dis. 191 (2005) 1719–1729.
[14] H.B. Suliman, L.K. Ryan, L. Bishop, R.J. Folz, Prevention of influenza-induced lung injury in mice overexpressing extracellular superoxide dismutase, Am. J. Physiol. Lung Cell. Mol. Physiol. 280 (2001) L69–L78.
[15] M. Toborek, Y.W. Lee, H. Pu, A. Malecki, G. Flora, R. Garrido, B. Hennig, H.C. Bauer, A. Nath, HIV-Tat protein induces oxidative and inflammatory pathways in brain endothelium, J. Neurochem. 84 (2003) 169–179.
[16] S. Vilella, L. Guerra, C. Helmle-Kolb, H. Murer, Characterization of basolateral Na/H exchange (Na/H-1) in MDCK cells, Pflugers Arch. 420 (1992) 275–280.
[17] A. Meister, M.E. Anderson, Glutathione, Annu. Rev. Biochem. 52 (1983) 711–760.
[18] L.W. Sharon, M.W. Louise, L.H. Maureen, et al., Oxidative stress and thiol depletion in plasma and peripheral blood lymphocytes from HIV-infected patients: toxicological and pathological implications, AIDS 11 (1997) 1689–1697.
[19] J. Hiscott, T.L. Nguyen, M. Arguello, P. Nakhaei, S. Paz, Manipulation of the nuclear factor-kappaB pathway and the innate immune response by viruses, Oncogene 25 (2006) 6844–6867.