Available online at www.sciencedirect.com
ScienceDirect
Free Radical Biology & Medicine 41 (2006) 1817–1828
www.elsevier.com/locate/freeradbiomed

Original Contribution

Nitric oxide modulates glutathione synthesis during endotoxemia

Seyedmehdi Payabvash [a], Mohammad Hossein Ghahremani [b,c], Ardeshir Goliaei [b], Ali Mandegary [b,d], Hamed Shafaroodi [e], Massoud Amanlou [f], Ahmad Reza Dehpour [a,*]

[a] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran
[b] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[c] Medical Nanotechnology Research Center, Tehran University of Medical Sciences, Tehran, Iran
[d] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran
[e] Department of Pharmacology, Tehran Medical Unit, Islamic Azad University, Tehran, Iran
[f] Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Received 4 October 2005; revised 16 August 2006; accepted 9 September 2006
Available online 16 September 2006

Abstract

Nitric oxide is known to modulate intracellular glutathione levels, but the relationship between nitric oxide synthesis and glutathione metabolism during endotoxemia is unknown. The present study was designed to examine the effects of increased nitric oxide formation on hepatic glutathione synthesis and antioxidant defense in endotoxemic mice. Our results demonstrate that hepatic glutathione synthesis is decreased for 24 h following injection of lipopolysaccharide (LPS). Administration of the cysteine precursor, L-2-oxothiazolidine-4-carboxylic acid (OTZ), failed to normalize hepatic glutathione concentration, and suggests that decreased γ-glutamylcysteine ligase activity is primarily responsible for the decrease in hepatic glutathione levels during endotoxemia. Inhibition of nitric oxide synthesis prevented the endotoxin-induced changes in hepatic and plasma glutathione status and up-regulated liver glutathione and cysteine synthesis pathways at the level of gene expression. Furthermore, whereas the activity of glutathione peroxidase and glutathione *S*-transferase decreased during endotoxemia, both of these changes were prevented by inhibition of nitric oxide synthesis. In conclusion, increased nitric oxide synthesis during endotoxemia causes marked changes in glutathione flux and defenses against oxidative stress in the liver.

Keywords: Nitric oxide; Glutathione biosynthesis pathway; γ-Glutamylcysteine ligase; Cysteine; Cystathionase; Antioxidant enzymes

Introduction

Glutathione (GSH) is a major intracellular antioxidant. Glutathione metabolism involves pathways among the liver, kidneys, and small intestine [1]. The liver plays the major part in the interorgan homeostasis of GSH, since hepatic sinusoidal efflux is the main source for plasma GSH and cysteine [2].

Considerable evidences implicate the role of oxidative injury and decreased antioxidant defense in the pathogenesis of endotoxemia and sepsis leading to organ dysfunction. Endotoxemia is known to decrease hepatic GSH levels and inhibit its synthesis and conjugation pathways as well as down-regulating the activity of antioxidant enzymes [3–5]; however, the underlying mechanism is unknown.

Increased inducible nitric oxide synthase (iNOS) expression is observed in hepatocytes, Kupffer, and stellate cells following exposure to LPS [6]. Nitric oxide can be present in several redox forms. As the nitrosonium ion (NO+) it can react with protein thiols leading to *S*-nitrosation of cysteine residues or it may produce nitrogen-centered free radical species, such as peroxynitrite (ONOO−), which can cause oxidative injury to lipids, proteins, or DNA [7].

Glutathione (γ-glutamylcysteinylglycine) synthesis is regulated by the activity of γ-glutamylcysteine ligase, the rate-limiting enzyme of GSH synthesis, and the availability of its constituent amino acid, cysteine [2]. There are controversial

Abbreviations: LPS, lipopolysaccharide; OTZ, L-2-oxothiazolidine-4-carboxylic acid; GSH, glutathione; NOS, nitric oxide synthase; L-NAME, N^{ω}-nitro-L-arginine methyl ester; AMG, aminoguanidine; GSSX, acid-soluble oxidized glutathione; GCLh, γ-glutamylcysteine synthetase heavy subunit; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; RT-PCR, reverse transcription polymerase chain reaction.

* Corresponding author. Fax: +98 21 6640 2569.
E-mail address: Dehpour@medscape.com (A.R. Dehpour).

0891-5849/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.freeradbiomed.2006.09.010

data on the effects of nitric oxide on GSH synthesis and metabolism. While nitric oxide donors are reported to enhance the expression of rate-limiting enzyme of GSH synthesis in some cell culture studies [8,9], in vivo studies suggest that increased nitric oxide synthesis leads to a decrease of hepatic GSH levels during endotoxemia [10]. However, the effects of nitric oxide on glutathione synthesis are unknown.

In this study, the time course of changes in hepatic and plasma glutathione was determined. We evaluated the effects of NOS inhibitors on hepatic GSH synthesis and consumption pathways as well as antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) in endotoxemic mice. To determine whether hepatic GSH synthesis was impaired due to decreased availability of cysteine, we cotreated endotoxemic mice with OTZ. In this study we observed that glutathione and cysteine synthesis decreases for at least 24 h during endotoxemia which was due to a marked decrease in the activity and expression of γ-glutamylcysteine ligase. The observed changes were significantly abrogated by the inhibition of nitric oxide synthesis.

Materials and methods

Chemicals

Ninhydrin, *o*-phthalaldehyde, glutathione disodium salt, glycylglycine, 1-chloro-2,4-dinitrobenzene, sulfanilamide, and naphthylethylenediamine dihydrochloride were purchased from Merck (Darmstadt, Germany). The dNTP mixures and *Taq* DNA polymerase were from Roche Diagnostic (Mannheim, Germany). Agarose and ethidium bromide were obtained from Amersham Pharmacia Biotech (Buckinghamshire, UK). All other materials, including OTZ, were purchased from Sigma (St. Louis, MO).

Experimental procedure

Male Swiss mice, each weighing 24 to 27 g, were housed in a controlled temperature room with 12:12 h light-dark cycle. Food and water were provided ad libitum. All animal procedures were in accordance with *Guide for the Care and Use of Laboratory Animals* (NIH US Publication No. 85-23 revised 1985). For all studies, six mice per each group were treated, unless otherwise stated, and the mice that died were replaced with extra mice from the same shipment. For time course study, six groups of mice received intraperitoneal injection of 5 mg/kg LPS, *Escherichia coli* serotype 055:B5 (Sigma). The animals were sacrificed 1, 3, 6, 12, 24, and 48 h after injection. Liver and blood samples were collected then. Normal group received no treatment.

To investigate the role of nitric oxide on hepatic metabolism of GSH and antioxidant defense, during endotoxemia, the animals were treated with either a nonspecific NOS inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME) or a specific inhibitor of iNOS, aminoguanidine (AMG), both dissolved in normal saline. Six groups of animals were studied; the mice were sacrificed 24 h after intraperitoneal injection of LPS or vehicle and the liver and blood samples were obtained. L-NAME or AMG was injected subcutaneously 30 min before LPS or vehicle injection in corresponding groups. Control group received vehicle (normal saline); LPS group received LPS injection at dose of 5 mg/kg; LPS + L-NAME group received 10 mg/kg L-NAME and 5 mg/kg LPS injections; LPS + AMG group received 100 mg/kg AMG and 5 mg/kg LPS injections; L-NAME and AMG groups received 10 and 100 mg/kg injections of L-NAME and AMG, respectively, 30 min before vehicle injection.

To evaluate the role of hepatic cysteine levels on the formation and levels of GSH in the liver, groups of mice were treated with OTZ (which is converted to cysteine by the intracellular enzyme, oxoprolinase) [11]. In LPS+OTZ group, mice were treated with OTZ (1 g/kg as 10% solution subcutaneously) 24 h and again 30 min prior to intraperitoneal injection of LPS (5 mg/kg).

We also tested the possible role of nitric oxide in early changes of hepatic and plasma GSH concentrations after endotoxin injection. For this purpose six groups of mice were treated as follows; 6h-Control group, received vehicle (normal saline); 6h-LPS group, received 5 mg/kg LPS injection; 6h-LPS + L-NAME group, received 10 mg/kg L-NAME and 5 mg/kg LPS injections; 6h-LPS+AMG group, received 100 mg/kg AMG and 5 mg/kg LPS injections; 6h-L-NAME group received 10 mg/kg injection of L-NAME, and 6h-AMG group received 100 mg/kg injection of AMG. Injections of L-NAME or AMG were performed 30 min before injection of either LPS or vehicle. The mice were sacrificed 6 h after injection of LPS or vehicle, and the liver and blood samples obtained.

Since endotoxemia induces anorexia, all control mice were pair-fed the mean intake of the endotoxemic animals. We used fresh liver tissue for the determination of γ-glutamylcysteine ligase and cystathionase enzyme activities, but other measurements were carried out using snap-frozen hepatic specimens. Blood samples were obtained via cardiac puncture.

Determination of γ-glutamlylcysteine ligase activity in hepatic tissue

γ-Glutamylcysteine ligase activity was measured as described by Seelig and Meister [12] with slight modifications. γ-Glutamylcysteine ligase catalyses the formation of α-aminobutyrylglutamate from L-α-aminobutyrate and glutamate which requires ATP. The method coupled ATP consumption with oxidation of nicotinamide adenine dinucleotide phosphate. The assay measures the change in absorbance at 340 nm.

Determination of cystathionase activity in hepatic tissue

The activity of the enzyme in liver samples (μmol/h/mg protein) was determined by monitoring the rate of cysteine synthesis from cystathionine [13]. Dithiothreitol (final concentration of 10 mM) was added to bring all cystine in the mixture to the reduced form. The amount of cysteine was determined by method of Gaitonde [14].