Published in final edited form as:

J Surg Res. 2008 May 1; 146(1): 96–103. doi:10.1016/j.jss.2007.06.013.

Chylomicron-Bound LPS Selectively Inhibits the Hepatocellular Response to Proinflammatory Cytokines

Behzad Kasravi, MD PhD, Diana H. Lee, BS, Jean W. Lee, BS, Stephen Dada, MD, and Hobart W. Harris, MD MPH

UCSF Surgical Research Laboratory at SFGH, San Francisco, California

Abstract

Background/Aims—Pretreatment of rodent hepatocytes with chylomicron-bound LPS (CM-LPS) renders these cells unresponsive to subsequent stimulation by proinflammatory cytokines. We sought to test the selectivity of this response.

Methods—Cellular responses to hypoxia, oxidative stress, apoptosis, and heat-shock response and thermotolerance induced in CM-LPS pretreated hepatocytes were compared with responses in untreated cells.

Results—CM-LPS inhibited the hepatocellular response to proinflammatory cytokines without affecting the response to the other cellular stressors. It did not affect the response to oxidative stress, as measured by mitochondrial activity after hydrogen peroxide was added, or protein induction before or after stimulation with cobalt chloride. Also, induction of heat shock proteins did not differ between the CM-LPS pretreated cells and untreated cells. CM-LPS did not interfere with the adoption of the thermotolerant phenotype, as shown by similar mitochondrial activity between pretreated and untreated cells. Although stimulation with TNF- α and actinomycin D increased activity of the apoptotic enzymes, there were no differences between cells pretreated with CM-LPS and untreated hepatocytes.

Conclusion—When the response to proinflammatory cytokines is inhibited, hepatocellular responses to hypoxia, oxidative stress, heat shock, and apoptosis remain intact after pretreatment with CM-LPS. CM-LPS may have a specific anti-inflammatory effect on hepatocytes.

Keywords

Hepatocyte; McArdle cell; sepsis; hypoxia; oxidative stress; heat shock; thermotolerance; apoptosis

Introduction

Sepsis is the leading cause of death in surgical patients requiring intensive care (1). In response to circulating endotoxins (LPS), the body begins a series of inflammatory reactions that may lead to shock, multiple organ failure, and death. Simultaneously, the body initiates a series of anti-inflammatory processes, including increased production of triglyceride-rich lipoproteins

Corresponding author: Hobart W. Harris MD MPH, UCSF Surgical Research Laboratory at San Francisco General Hospital, 1001 Potrero Avenue, Building 1, Room 210, San Francisco, CA 94110-3518, Telephone: (415) 206-6894, FAX: (415) 206-6997, E-mail: harrish@surgey.ucsf.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

(2). In addition to providing the fuel necessary for the increased energy demands during sepsis, lipoproteins bind to endotoxin and increase their removal from the circulation (3).

Initially, cholesterol was thought to be the primary component of lipoproteins that was responsible for this protective anti-inflammatory phenomenon (4). However, subsequent studies in our laboratory and others have shown that other lipoprotein varieties (LDL, VLDL, chylomicrons), as well as the synthetic lipid emulsion Soyacal®, which does not contain any cholesterol, can bind and neutralize the circulating endotoxins in a rat model of endotoxemia (5,6). We have found that infusion of lipoproteins not only increased the clearance of endotoxin from the circulation, but also increased its delivery into the liver, specifically to the hepatocytes (7). We have also demonstrated that internalization of chylomicron-bound endotoxin (CM-LPS) into the hepatocytes is associated with attenuation of the hepatocellular response to subsequent stimulation by proinflammatory cytokines (8). This phenomenon, which we termed “cytokine tolerance,” is associated with inhibition of NF- κ B activity (9).

We have shown that cytokine tolerance is a transient, time- and dose-dependent process that requires functional LDL receptors (10). Furthermore, we have demonstrated that cytokine tolerance is a selective cellular response and not a reflection of global anergy in response to pretreatment with CM-LPS (9). Despite the fact that the cytokine tolerance attenuates the hepatocellular response to proinflammatory cytokines in cells pretreated with CM-LPS, mitochondrial activity and cyclic AMP response element-binding proteins to forskolin remain unaltered. Nonetheless, although the normal activities of the cells are retained, CM-LPS pretreatment might inhibit the capacity of the hepatocytes to respond to strong cellular stressors.

In this study, we hypothesized that the hepatocellular response to other cellular stressors is not affected by CM-LPS pretreatment. To test this hypothesis, we compared the hepatocellular responses to oxidative, hypoxic, thermal, and apoptotic stressors after pretreatment with CM-LPS with the responses in unpretreated hepatocytes. Furthermore, by stimulating the hepatocytes by selective cytokines, we tried to understand the mechanism behind the phenomenon of cytokine tolerance.

Methods

Hepatocyte isolation

All procedures involving animals were conducted according to the National Institute of Health guidelines and approved by UCSF Institutional Animal Care and Use Committee. Primary hepatocytes were isolated from Sprague-Dawley rats (225–300 grams) and purified by elutriation, as previously described (10). Hepatocytes with >95% purity and >90% viability were grown as spheroids (multi-cellular aggregates) on culture dishes coated with poly-HEMA (Sigma, St. Louis, MO) for 4–6 days before any experiments.

Cell culture

To produce a reliable cell culture model for the study of cytokine tolerance, rat hepatoma McArdle RH-7777 cells (ATCC, Manassas, VA) were grown in a monolayer on culture dishes coated with poly-L-lysine (Sigma, St. Louis, MO) in DME H-16 50%/F-12 50% medium supplemented with 20% fetal bovine serum and antibiotics (Cell Culture Facilities, UCSF, San Francisco, CA). For experiments, McArdle cells were promoted into spheroid form by plating them on poly-HEMA coated culture dishes for 4–6 days.

Synthetic chylomicron remnant preparation

Synthetic chylomicron remnants were prepared and combined with recombinant human apolipoprotein E₃ (apoE₃) and LPS from *E. coli* 0111:B4 as previously described (11,12).

Pretreatment

Primary rat hepatocyte spheroids or McArdle spheroids were pretreated with CM-LPS (5mg TG/ml) for 2 h, and allowed to recover for 16 h in normal media (10). After the recovery period, the cells were stimulated according to the different stressors examined.

Cytokine tolerance

Sixteen hours after pretreatment, hepatocytes were stimulated with a combination of proinflammatory cytokines (TNF- α 500 U/ml, IL-1 β 100 U/ml, and IFN- γ 100 U/ml, R&D Systems, Minneapolis, MN). As an indication of the hepatocellular proinflammatory response, nitric oxide (NO) was measured 24 h later via the Griess reaction (13) and normalized against the total DNA content.

Oxidative stress

To induce an oxidative stress in the cells, McArdle spheroids were stimulated with 100 mM H₂O₂ (Fisher Scientific, Hampton, NH) for 2 h, 4 h, and 8 h. After an 18-hour recovery period in normal media, mitochondrial activity was measured via alamarBlue™ assay (14) (Fisher Scientific, Hampton, NH) and normalized against the total DNA content.

Hypoxia

A hypoxia stress response was chemically induced in the McArdle spheroids by stimulating them with 200 μ M CoCl₂ for 24 h (15). Nuclear pellets were separated from the cytoplasmic lysate by harvesting the cells in the lysis buffer (10mM HEPES pH 7.9, 10mM KCl, 0.1mM EDTA, 0.4% Nonidet P40). Nuclear proteins were collected by resuspending the pellet in the extraction buffer (20mM HEPES pH 7.9, 0.4mM NaCl, 1mM EDTA) for 15 minutes. One hundred micrograms of nuclear proteins were resolved in 7.5% SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and probed for the HIF-1 α subunit (Novus Biologicals, Littleton, CO) (16).

Heat Stress

Heat shock was induced in pretreated and unpretreated McArdle spheroids by subjecting them to 43°C heat for 45 min (17). After recovering for a period of 18 hours, the cells were lysed and heat-shock protein induction was evaluated via immunoblots, using specific monoclonal antibodies against HSP 25, HSP 32, and HSP 72 (Stressgen, Victoria, British Columbia, Canada). To evaluate the successful induction of a thermotolerant phenotype, another group of pretreated and unpretreated hepatocytes were first subjected to heat shock (45° C, 90 min), allowed to recover, and then challenged at 45°C for 2.5 h (heat kill). Eighteen hours later, mitochondrial activity in each group was measured via alamarBlue™ and normalized against the total DNA content.

Apoptosis

Primary rat hepatocyte spheroids were stimulated by TNF- α (2,000 U/ml) and actinomycin D (400nM; Fisher Scientific, Hampton, NH) (18). After 4 h and 8 h of stimulation, the induction of the enzymatic activities of the apoptotic caspases 3 and 8 were measured via colorimetric assays against their specific substrates (R&D Systems, Minneapolis, MD) (19).

Stimulation of hepatocytes with selective cytokines

Primary rat hepatocytes were grown into spheroid form and were pretreated with CM-LPS for 2 hours at 37 C. Sixteen hours later, cells were stimulated with cytomix or with a combination of IL-1 β /TNF- α , or either IL-1 β , IFN- γ , or TNF- α alone. Twenty-four hours after stimulation, the amount of NO production was measured via the Griess reaction and was compared to the

amount produced by the corresponding unpretreated hepatocytes after normalization with their total DNA content.

Next, a stable cell line of McArdle (RH-7777) cells were produced by transfecting them with a NF- κ B reporter plasmid (Invitrogen, Carlsbad, CA) via a FuGENE technique (QIAGEN, Valencia, CA). Four of the more strongly responding transfected cells were mixed together and used for selective cytokine study. Transfected McArdle cells were grown into spheroid form and pretreated with CM-LPS for 2 hours. After pretreatment, they were allowed to recover for 16 hours and were then stimulated with a combination of IL-1 β /TNF- α , or either IL-1 β or TNF- α alone. After 90 minutes, the luciferase activity was measured, and compared to that of the unpretreated corresponding cells.

Statistical Analysis

Data is presented as mean $\pm$ SD when appropriate. Continuous parametric data were statistically evaluated using unpaired, two-tailed t tests. P values ≤ 0.05 were considered statistically significant. Multiple groups were first evaluated by ANOVA for statistical significance. If any significance was detected, unpaired, two-tailed t tests was used to find the statistically significant groups.

Results

NO production

The response both of primary rat hepatocytes and McArdle cells to stimulation with proinflammatory cytokines was significantly reduced after 2 h of pretreatment with CM-LPS, but not after pretreatment with either CM or LPS alone (Fig. 1).

Oxidative Stress

Addition of 100 mM hydrogen peroxide (H₂O₂) induced a toxic effect on McArdle cells, reducing mitochondrial activity by 50%, as detected by alamarBlue™ assay at 2 h and 4 h (Fig. 2). However, this response was transient; at 8 h, the mitochondrial activity returned to normal levels (0 h time point). Pretreatment with CM-LPS had no effect on the response of the cells to the oxidative stress. Both pretreated and unpretreated cells responded in the same manner at all time points.

Hypoxia

Addition of 200 μ M cobalt chloride (CoCl₂) successfully induced the translocation and upregulation of hypoxia-inducing factor (HIF-1 α) in the McArdle spheroids (Fig. 3). Pretreatment with CM-LPS did not change the protein induction before or after stimulation with CoCl₂.

Heat shock response

Forty-five minutes of 43° C heat induced HSP 25, HSP 32, and HSP 72 proteins in McArdle cells (Fig. 4). There was no detectable difference in the amount of induction of heat shock proteins between the CM-LPS pretreated cells and unpretreated cells. None of the heat shock proteins were detected in the normal non-heat shock cells.

Thermotolerance

Two and a half hours of heat challenge reduced the mitochondrial activity to 60% of control levels, as we had observed before [21]. However, 90 minutes of heat-induced priming prior to a heat challenge increased the mitochondrial activity, establishing the observed thermotolerant

phenotype. CM-LPS did not interfere with the adoption of the thermotolerant phenotype, as shown by similar mitochondrial activity between pretreated and untreated cells (Fig. 5).

Apoptosis

Stimulation with TNF- α and Actinomycin D resulted in the increased expression and activity of the apoptotic enzymes caspase 3 and caspase 8 in primary rat hepatocytes (Fig. 6 A, B and Fig. 7 A, B respectively). Caspase 3 activity was significantly greater at 4 h and 8 h than at baseline in both the pretreated and untreated hepatocytes. Caspase 8 activity was greater at 4 h than at baseline in the pretreated cells, and it was higher than the baseline in both pretreated and untreated cells at the 8 hour time period. However, there were no significant differences in the caspase 8 activity between pretreated and untreated hepatocytes at any time point.

Stimulation of hepatocytes with selective cytokines

Primary rat hepatocytes pretreated with CM-LPS showed an inhibited response to cytomix, or a combination of IL1- β /TNF- α stimulation (Fig. 8). There was no difference in the amount of NO production following IL-1 β or IFN- γ stimulation between the pretreated and nonpretreated groups. TNF- α alone was not able to induce any detectable response in pretreated or nonpretreated hepatocytes. Transfected McArdle cells stimulated with cytomix or TNF- α alone showed a significant reduction of NF- κ B plasmid activity following pretreatment as compared to non-pretreated cells (Fig 9). However, cells stimulated with IL-1 β alone did not show any significant change in the amount of plasmid activity after pretreatment with CM-LPS.

Discussion

In this study, we showed that when the response to proinflammatory cytokines is inhibited, hepatocellular responses to hypoxia, oxidative stress, heat shock, and apoptosis remain intact after pretreatment with CM-LPS. The similar responses to stressors in pretreated and untreated hepatocytes were seen both in primary rat hepatocytes and McArdle cells.

McArdle cells (RH-7777) produced a uniform and reliable cell culture model for primary rat hepatocytes. McArdle (RH-7777) is a transformed rat hepatoma cell line that retains intact membrane receptors and lipoprotein metabolism, which makes these cells ideal for studying hepatic lipoprotein metabolism (20,21). Studies in our laboratory have shown that McArdle cells can be grown into spheroid aggregates in the same manner as primary rat hepatocytes, and that after pretreatment with CM-LPS complexes, these cells exhibit cytokine tolerance in exactly the same time- and dose-dependency pattern as primary hepatocytes (unpublished data).

Oxidative stress is one of the most challenging conditions to cell survival. Although many other compounds are capable of inducing oxidative stress, oxygen is the most ubiquitous oxidant that cells are exposed to (22). Many of the cellular enzymes, structural and membrane proteins, simple and complex sugars, intracellular signaling pathways, and DNA or RNA complexes are susceptible to oxidative damage (23). Antioxidant compounds such as vitamins C and E, ubiquinone, and uric acid, are the first line of defense against oxidative stress. However, when the stress exceeds certain levels, cells adapt in a way to reduce the extent of the damage. Hydrogen peroxide at a concentration of 120 to 150 μ M induces a transient adaptation to oxidative stress in many cells (24). Similarly in this study, we found that H₂O₂ induces a transient but significant reduction in the mitochondrial activity of McArdle hepatocytes at 2 and 4 h after the exposure, and that mitochondrial activity returns to normal levels at 8 h. Cells pretreated with CM-LPS showed a pattern and magnitude of reduced mitochondrial activity that was similar to that of untreated cells, indicating that pretreatment with CM-LPS had no effect on the cellular response to oxidative stress induced by H₂O₂.

In this study, we used mitochondrial activity as a good indicator of hepatocellular response to oxidative stress, instead of measuring the ratio of the reduced to the oxidized form of glutathione (GSH/GSSG). Measurement of GSH and GSSG levels as indicators of oxidative injury in hepatocytes is complicated by the fact that there is shuttling of glutathione, particularly GSSG, out of the cells, resulting in fluctuations in this ratio (25). We have similarly found that oxidative injury incurred by H₂O₂ stimulation did not elevate GSSG levels or change the GSH/GSSG ratio in hepatocytes (unpublished data).

Oxygen homeostasis is critically important for all mammalian cells, which should maintain the oxygen concentration within the tight constraints essential for life. Induction of HIF-1 has been shown to be a central mechanism of oxygen-mediated gene expression in most cells, including hepatocytes (26). As a member of the PAS superfamily of transcription factors, HIF-1 is a heterodimer of α and β subunits (27). The β subunits, also known as hydrocarbon receptor nuclear translocators, are constitutively expressed in the nucleus. When oxygen tension is lowered, HIF-1 α subunits stabilize and translocate to the nucleus, where they dimerize with the β subunit. The HIF-1 complex then binds to the hypoxia response elements and activates transcription of a range of genes. In this study, we have clearly shown that 200 μ M of CoCl₂ induced the nuclear translocation of the HIF-1 α subunits (Fig. 3). Although we have previously shown that NF- κ B activation is inhibited following pretreatment with CM-LPS (9), nuclear translocation of HIF-1 α subunits remains similar in pretreated and untreated cells, showing that pretreatment with CM-LPS has no effect on this hepatocellular response to hypoxia.

Exposure of cells to a sublethal increase in temperature activates a cellular stress response that renders the cells more resistant to subsequent lethal insults, a process called thermotolerance (28). Thermotolerance is associated with the synthesis and accumulation of a family of highly conserved proteins referred to as heat-shock proteins (HSP), which are grouped according to their molecular weight. Within the family of heat-shock proteins, we chose three different ones: HSP 25, HSP 32, and HSP 72, to study the hepatocellular response to heat shock following pretreatment with CM-LPS. One of the major functions of heat-shock proteins is acting as a molecular chaperone for protein maturation and integrity (29,30). However, they can also play a more specific role, such as maintaining the redox state of the cell (31). We studied the induction of these heat-shock proteins to get an overall illustration of the heat stress and shock response in hepatocytes. Subjecting both untreated and CM-LPS pretreated hepatocytes to sublethal heat shock resulted in an identical level of expression of all three heat-shock proteins examined. Similarly, cells from both treatment groups were equally capable of adopting a thermotolerant phenotype. These findings indicate that pretreating hepatocytes with CM-LPS does not affect their capacity to initiate a heat shock response or to adopt a thermotolerant phenotype.

Apoptosis, or programmed cell death, is a genetically regulated mechanism. It has been shown to be a highly controlled mechanism involving death factors and death receptors, and is similar to mechanisms that determine cell growth and proliferation (32). TNF- α is a potent inducer of apoptosis, signaling through the TNFR1 receptor (33). TNF- α mediated apoptotic signaling leads to cleavage and activation of the apoptotic enzyme caspase 8, which, through a cascade of events, in turn cleaves and activates caspase 3. Active caspase 3 subunits break down the intracellular proteins that are mainly responsible for cellular proliferation and regulation of the cell cycle, leading to the fragmentation of DNA that is characteristic of apoptosis. In our study, pretreating hepatocytes with CM-LPS complexes did not result in any changes in the activity of either caspase 3 or caspase 8 when compared to untreated cells. These findings indicate that although CM-LPS pretreated cells are less responsive to the stimulatory effects of proinflammatory cytokines, the cellular response to other TNF-mediated processes remains unaltered.

Hepatocytes are hard to stimulate. Multiple proinflammatory cytokines are required to induce an inflammatory response in hepatocytes through a synergistic activity (34). Although TNF- α and IL-1 β have different cell membrane receptors and intracellular pathways, they eventually merge on phosphorylation and activation of the IKK complex (35). Activation of the IKK complex, and specifically the IKK- β subunit of this complex, will lead to activation and intranuclear localization of the NF- κ B heterodimeric complex. In the nucleus, this transcriptional protein promotes the gene activity of many proinflammatory responses, including NO production. In our study, a combination of proinflammatory cytokines (cytomix, TNF- α , IL-1 β , and IFN- γ) could induce a detectable inflammatory response in hepatocytes in terms of NO production. Pretreating these cells with CM-LPS resulted in significant reduction in the amount of NO production. Although the amount of overall NO production was lower in hepatocytes stimulated with a combination of TNF- α and IL-1 β , the inhibitory effect of CM-LPS was still evident in pretreated cells. Stimulating the hepatocytes with IL-1 β or IFN- γ individually also resulted in a much lower amount of NO production, and no tolerance was observed in the pretreated cells. These two findings suggest that pretreatment has no effect on the proinflammatory activity of these two cytokines. However, TNF- α alone could not induce any detectable NO production in the primary rat hepatocytes.

To have a more direct study on the effect of TNF- α on hepatocytes and a better understanding of the possible mechanism of cytokine tolerance, we created a stable cell line of McArdle cells containing a NF- κ B reporter plasmid. Even a small stimulation of the NF- κ B pathway by proinflammatory cytokines could be amplified by the plasmid and generate an appropriate light luciferase response. The transformed McArdle cells were pretreated with CM-LPS and were stimulated with TNF- α and IL-1 β individually or in combination. We found that when TNF- α was used alone or in combination, inhibition of NF- κ B activity was observed in pretreated cells. This finding suggest that pretreatment with CM-LPS inhibits the intracellular signaling pathway of TNF- α and blocks the synergistic activity of this cytokine. Interestingly, while the proinflammatory response of the TNF- α is blocked there is no change in the proapoptotic activity in pretreated cells. This observation further indicates that the effect of CM-LPS on cytokine mediated intracellular signaling is independent of TNF-induced apoptotic signaling.

Throughout this study, we examined the cellular response to a variety of strong stressors in an attempt to understand how CM-LPS complexes affect hepatocytes. Our choice of stimulants was based on a variety of different stressors that could produce a strong and reliable cellular response through different mechanisms. Although, each of these stressors can be analyzed by different techniques or groups of assays, in this study, by carefully selecting key proteins and assays, we tried to use the most reliable and acceptable indicators of the hepatocellular response to these stressors.

In conclusion, increased circulating triglyceride levels may provide a protective role in sepsis, and cytokine tolerance is likely a means of negatively regulating the cytokine-mediated hepatic response to infection. As this study shows, induction of cytokine tolerance is a selective process, and this phenomenon is due to blocking of the TNF- α signaling pathway and disruption of the synergistic activity of the proinflammatory cytokines on hepatocytes. Although the hepatocellular response to proinflammatory cytokines is inhibited after pretreatment with CM-LPS complexes, hepatocytes are still capable of responding to many other cellular stressors in a normal manner. In addition, this study shows that McArdle cells are a suitable cell culture model for the study of cytokine tolerance in hepatocytes.

References

1. Effect of high-dose glucocorticoid therapy on mortality in patients with clinical signs of systemic sepsis. The Veterans Administration Systemic Sepsis Cooperative Study Group. *N Engl J Med* 1987;317:659–665. [PubMed: 2888017]
2. Feingold KR, Serio MK, Adi S, Moser AH, Grunfeld C. Tumor necrosis factor stimulates hepatic lipid synthesis and secretion. *Endocrinology* 1989;124:2336–2342. [PubMed: 2707158]
3. Harris HW, Grunfeld C, Feingold KR, Rapp JH. Human very low density lipoproteins and chylomicrons can protect against endotoxin-induced death in mice. *J Clin Invest* 1990;86:696–702. [PubMed: 2394827]
4. Ulevitch RJ, Johnston AR, Weinstein DB. New function for high density lipoproteins. Their participation in intravascular reactions of bacterial lipopolysaccharides. *J Clin Invest* 1979;64:1516–1524. [PubMed: 227936]
5. Harris HW, Grunfeld C, Feingold KR, Read TE, Kane JP, Jones AL, Eichbaum EB, Bland GF, Rapp JH. Chylomicrons alter the fate of endotoxin, decreasing tumor necrosis factor release and preventing death. *J Clin Invest* 1993;91:1028–1034. [PubMed: 8450032]
6. Read TE, Grunfeld C, Kumwenda ZL, Calhoun MC, Kane JP, Feingold KR, Rapp JH. Triglyceride-rich lipoproteins prevent septic death in rats. *J Exp Med* 1995;182:267–272. [PubMed: 7790821]
7. Harris HW, Rockey DC, Chau P. Chylomicrons alter the hepatic distribution and cellular response to endotoxin in rats. *Hepatology* 1998;27:1341–1348. [PubMed: 9581689]
8. Kasravi FB, Brecht WJ, Weisgraber KH, Harris HW. Induction of cytokine tolerance requires internalization of Chylomicron-Bound LPS into hepatocytes. *J Surg Res* 2003;115:303–309. [PubMed: 14697298]
9. Kumwenda ZL, Wong CB, Johnson JA, Gosnell JE, Welch WJ, Harris HW. Chylomicron-bound endotoxin selectively inhibits NF-kappaB activation in rat hepatocytes. *Shock* 2002;18:182–188. [PubMed: 12166784]
10. Kasravi FB, Welch WJ, Peters-Lideu CA, Weisgraber KH, Harris HW. Induction of cytokine tolerance in rodent hepatocytes by chylomicron-bound LPS is low-density lipoprotein receptor dependent. *Shock* 2003;19:157–162. [PubMed: 12578125]
11. Martins IJ, Vilcheze C, Mortimer BC, Bittman R, Redgrave TG. Sterol side chain length and structure affect the clearance of chylomicron-like lipid emulsions in rats and mice. *J Lipid Res* 1998;39:302–312. [PubMed: 9507991]
12. Morrow JA, Arnold KS, Weisgraber KH. Functional characterization of apolipoprotein E isoforms overexpressed in *Escherichia coli*. *Protein Expr Purif* 1999;16:224–230. [PubMed: 10419818]
13. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Anal Biochem* 1982;126:131–138. [PubMed: 7181105]
14. Tiballi RN, He X, Zarins LT, Revankar SG, Kauffman CA. Use of a colorimetric system for yeast susceptibility testing. *J Clin Microbiol* 1995;33:915–917. [PubMed: 7790460]
15. Gorlach A, Fandrey J, Holtermann G, Acker H. Effects of cobalt on haem proteins of erythropoietin-producing HepG2 cells in multicellular spheroid culture. *FEBS Lett* 1994;348:216–218. [PubMed: 8034045]
16. Yuan Y, Hilliard G, Ferguson T, Millhorn DE. Cobalt inhibits the interaction between hypoxia-inducible factor-alpha and von Hippel-Lindau protein by direct binding to hypoxia-inducible factor-alpha. *J Biol Chem* 2003;278:15911–15916. [PubMed: 12606543]
17. Schamhart DH, van Walraven HS, Wiegant FA, Linnemans WA, van Rijn J, van den Berg J, van Wijk R. Thermotolerance in cultured hepatoma cells: cell viability, cell morphology, protein synthesis, and heat-shock proteins. *Radiat Res* 1984;98:82–95. [PubMed: 6718697]
18. Li J, Bombeck CA, Yang S, Kim YM, Billiar TR. Nitric oxide suppresses apoptosis via interrupting caspase activation and mitochondrial dysfunction in cultured hepatocytes. *J Biol Chem* 1999;274:17325–17333. [PubMed: 10358093]
19. Cleveland JL, Ihle JN. Contenders in FasL/TNF death signaling. *Cell* 1995;81:479–482. [PubMed: 7758103]

20. Ji ZS, Lauer SJ, Fazio S, Bensadoun A, Taylor JM, Mahley RW. Enhanced binding and uptake of remnant lipoproteins by hepatic lipase-secreting hepatoma cells in culture. *J Biol Chem* 1994;269:13429–13436. [PubMed: 8175774]
21. Tanabe S, Sherman H, Smith L, Yang LA, Fleming R, Hay R. Biogenesis of plasma lipoproteins in rat hepatoma McA-RH7777: importance of diffusion-mediated events during cell growth. *In Vitro Cell Dev Biol* 1989;25:1129–1140. [PubMed: 2481672]
22. Davies KJ. The broad spectrum of responses to oxidants in proliferating cells: a new paradigm for oxidative stress. *IUBMB Life* 1999;48:41–47. [PubMed: 10791914]
23. Cadenas E. Biochemistry of oxygen toxicity. *Annu Rev Biochem* 1989;58:79–110. [PubMed: 2673022]
24. Laval J. Role of DNA repair enzymes in the cellular resistance to oxidative stress. *Pathol Biol (Paris)* 1996;44:14–24. [PubMed: 8734295]
25. Catala M, Portoles MT. Action of *E. coli* endotoxin, IL-1 β and TNF- α on antioxidant status of cultured hepatocytes. *Mol Cell Biochem* 2002;231:75–82. [PubMed: 11952168]
26. Maxwell PH, Pugh CW, Ratcliffe PJ. Inducible operation of the erythropoietin 3' enhancer in multiple cell lines: evidence for a widespread oxygen-sensing mechanism. *Proc Natl Acad Sci U S A* 1993;90:2423–2427. [PubMed: 8460154]
27. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proc Natl Acad Sci U S A* 1995;92:5510–5514. [PubMed: 7539918]
28. Hahn GM, Li GC. Thermotolerance and heat shock proteins in mammalian cells. *Radiat Res* 1982;92:452–457. [PubMed: 7178414]
29. Jakob U, Gaestel M, Engel K, Buchner J. Small heat shock proteins are molecular chaperones. *J Biol Chem* 1993;268:1517–1520. [PubMed: 8093612]
30. Welch WJ. Heat shock proteins functioning as molecular chaperones: their roles in normal and stressed cells. *Philos Trans R Soc Lond B Biol Sci* 1993;339:327–333. [PubMed: 8098537]
31. Minowada G, Welch WJ. Clinical implications of the stress response. *J Clin Invest* 1995;95:3–12. [PubMed: 7814629]
32. Grutter MG. Caspases: key players in programmed cell death. *Curr Opin Struct Biol* 2000;10:649–655. [PubMed: 11114501]
33. Osawa Y, Banno Y, Nagaki M, Nozawa Y, Moriwaki H, Nakashima S. Caspase activation during hepatocyte apoptosis induced by tumor necrosis factor- α in galactosamine-sensitized mice. *Liver* 2001;21:309–319. [PubMed: 11589767]
34. Nussler AK, Di Silvio M, Liu ZZ, Geller DA, Freeswick P, Dorko K, Bartoli F, Billiar TR. Further characterization and comparison of inducible nitric oxide synthase in mouse, rat, and human hepatocytes. *Hepatology* 1995;21:1552–1560. [PubMed: 7539395]
35. Scheidereit C. IkappaB kinase complexes: gateways to NF-kappaB activation and transcription. *Oncogene* 2006;25:6685–6705. [PubMed: 17072322]

Figure 1. Effect of different pretreatments on NO production in primary rat hepatocytes and McArdle cells. Open bars represent primary rat hepatocytes and shaded bars represent McArdle cells; Control, untreated and stimulated; LPS, endotoxin alone; CM, chylomicron alone; CM-LPS, chylomicron-endotoxin complexes. Data represent the mean $\pm$ SEM of 6 samples from 2 separate experiments (* $p \leq 0.05$ compared to their respective controls, t test).

Figure 2.

Effect of CM-LPS pretreatment on the hepatocellular response to oxidative stress. Open bars represent cells with no pretreatment, shaded bars represent cells pretreated with CM-LPS. Mitochondrial activity is shown as a percent of unstimulated Control. There was no significant difference between the CM-LPS pretreated and unpretreated groups. Data represent the mean $\pm$ SEM of 3 samples per condition from 2 separate experiments (* $p \leq 0.05$ compared to 0 time point, t test).

Figure 3.
Effect of CM-LPS pretreatment on the hepatocellular response to hypoxia induced with 200μM CoCl₂. This immunoblot of the nuclear fraction is a representative sample from two experiments.

Figure 4. Effect of CM-LPS pretreatment on the hepatocellular response to heat stress. This immunoblot of cytoplasmic and nuclear HSP 25, 32, and 72 in pretreated and non-pretreated hepatocytes after heat shock ($43^{\circ}\text{C} \times 45 \text{ min}$) is a representative sample of three separate experiments.

Figure 5.

Effect of CM-LPS on induction of thermotolerant phenotype in primary hepatocytes. Data represent the mean $\pm$ SEM of mitochondrial activity measured by alamarBlue™ assay of 9 samples from 3 separate experiments. The table below the figure reflects different interventions in each experimental group.

Figure 6.

Effect of CM-LPS pretreatment on the enzymatic activity of (A). caspase 3 and (B). caspase 8 stimulation with TNF- α (2,000 U/ml) and actinomycin D (400 nM) in pretreated and non-pretreated cells. Caspase 3 (20 kD and 17 kD bands) and Caspase 8 (43 kD and 41 kD bands).

Figure 7. Effect of CM-LPS pretreatment on the enzymatic activity of the apoptotic enzymes caspase 3 (A) and caspase 8 (B). Open bars represent cells with no pretreatment and shaded bars represent cells pretreated with CM-LPS. Data represent the mean $\pm$ SEM of 6 samples from 2 separate experiments (* $p \leq 0.05$ compared to their respective 0 h controls, t test).

Figure 8. Effect of different cytokines on primary rat hepatocytes pretreated with CM-LPS. Data represent mean $\pm$ SEM of 6 samples from 2 different experiments (* $p < 0.05$ compared to corresponding nonpretreated groups, t test).

Figure 9. Effect of different cytokines on transformed McArdle cells. Data represent mean $\pm$ SEM of 6 samples from 2 different experiments (* $p < 0.05$ compared to corresponding nonpretreated group, t test).