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Regulation of hepatocyte glutathione content by hepatic sinusoidal cells activated with LPS: anatomical restrictions

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Summary. The liver is the main organ for the elimination of bacterial endotoxin involving Kupffer and parenchymal cells. This process is accompanied by the release of free radicals. Parenchymal cells possess especially high levels of glutathione, which make them a key point in the response to free radicals. Sinusoidal cells regulate hepatic function in a very important fashion through the release of cytokines and/or adhesion molecules. These facts suggest the importance of finding new *in vitro* experimental models representing an intermediate step towards *in vivo* models. The treatment with LPS of sinusoidal and parenchymal cell co-cultures on porous membranes provokes an intense reduction of parenchymal cell intracellular glutathione, which does not correspond to *in vivo* results. However, the addition of supernatants of LPS-treated sinusoidal cells to parenchymal cells renders increases in glutathione which agree better with *in vivo* results. We conclude that the regulation of liver hepatocyte glutathione content and NO release in the presence of LPS is strongly modulated by liver non parenchymal cells. The study of this phenomenon requires new *in vitro* models taking into account liver histophysiology and histopathology and anatomical restrictions in cell communication.

Key words: Endotoxin, Glutathione, Hepatocyte, Kupffer cell, Lipopolysaccharide

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Introduction

The liver is the main organ for the elimination of bacterial endotoxin involving Kupffer (KC) and parenchymal cells (HC, hepatocytes). This process is accompanied by the release of free radicals (Curnutte and Babior, 1987) that initiate lipid peroxidation processes, usually inducing irreversible alterations on hepatic cell membranes. The survival of cells in this environment would not be possible without powerful defence mechanisms against oxidant species. The tripeptide glutathione (GSH) stands out among these mechanisms, being the most abundant cellular low molecular weight thiolic compound and the main reducing agent in cells, due to the antioxidant power of the thiolic group in its cysteine (Forman and Fisher, 1981; Meister and Anderson, 1983). Parenchymal cells possess especially high levels of glutathione, which make them a key point in response to free radicals. An inadequate balance between oxidant species and antioxidant mechanisms may constitute the primary mechanisms of a number of pathologies, including endotoxic shock (Wohaieb and Godin, 1987; Halliwell, 1994; Taylor and Piantadosi, 1995; Cesaratto et al., 2004; Tafazoli et al., 2005).

Our previous works have demonstrated that liver GSH is increased during the septic shock acute phase (Portoles et al., 1996). Direct action of LPS on primary cultures of HC also provokes a significant increase of intracellular GSH after short periods (Catalá and Portolés, 2002). However, *in vivo,* other cell types play important roles during endotoxoemia.

Sinusoidal cells (SC) regulate hepatic function in a very important fashion, turning the liver into the major organ of the reticulo-endothelial system (RES)

responsible for the unspecific inflammatory response. RES has an important paracrine action on hepatic function. It has been well established (Kuiper et al., 1988) that hepatic macrophages can modulate protein synthesis and glucose supply by parenchymal cells. Cytokines and/or adhesion molecules can mediate local interaction among these cells. During endotoxaemia LPS is mainly eliminated from circulation by Kupffer cells, inducing the release of inflammation mediators such as TNF-α, IL-1, IL-6, IL-10, IFN-γ, PGs, NO and reactive oxygen species (reviewed in Kmiec, 2001). The parenchymal cells activated by these cytokines produce, amongst other metabolites, NO, which is involved in Kupffer cells' inflammatory response (Harbrecht and Billiar, 1995). One of the major features in endotoxic shock is the induction of iNOS in the liver (Geller et al. 1993). iNOS is expressed by hepatocytes and, under normal basal conditions, iNOS activity is very low but is stimulated by bacterial endotoxins (LPS) and cytokines, such as tumor necrosis factor (TNF) and interleukins (Shiratori et al., 1998).

Recently, using new *in vitro* experimental models, we reported that parenchymal cell glutathione content is strongly regulated by sinusoidal cells in basal conditions (Catala et al., 2007). We used two methods to characterise liver cell cooperation in liver GSH content: a) co-culture of sinusoidal and parenchymal cells using porous membranes, and b) addition of LPS-treated sinusoidal cell supernatants to pure parenchymal cell cultures. Briefly, we demonstrated that the glutathione content of hepatic parenchymal cells in basal conditions is regulated by a sinusoidal-parenchymal cell cross-talk, and suggested the existence of a paracrine circuit in the management of liver oxidative stress. In the present work these models have been used in order to study how LPS affects liver glutathione system regulation in endotoxic conditions.

Materials and methods

Materials

Lipopolysaccharide (LPS) from *E.coli* 0111:B4 obtained according to the Westphal method (1952), was supplied by Difco (Detroit, MI, USA). Collagenase and pronase were obtained from Boehringer-Mannheim Diagnostica (Mannheim, Germany). Nycodenz was from Nycomed AS (Oslo, Norway). Monoclonal antibody ED1 conjugated to PE (MCA34LP) was supplied by Serotec (Bicester, UK). Ham's F-12, L-glutamine and antibiotics were from Seromed, Biochrom (Berlin, Germany). Foetal calf serum was from Biowhitaker (France). Dulbecco's Minimal Essential Medium (DMEM) with 25mM HEPES, propidium iodide (PI), William's E medium, 5,5'-Dithio-bis(2-nitrobenzoic acid) (DTNB), glutathione reductase (GR), glutathione reduced form (GSH), vinylpiridine and azide were obtained from Sigma Chemicals (St.Louis, MI, USA). Nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) was from Boehringer Manheim (Germany). Other chemicals were purchased from Merck (Darmstad, Germany). Costar Transwell, permeable polycarbonate membrane (pore diameter 0.4 mm) wells were used for the co-cultures. Pentothal-Na was purchased from Abbot International Ltd (USA).

Animals

Hepatic cells were obtained from male Wistar rats (150-200 g body weight, fasted overnight with water *ad libitum*). The cell isolation procedures were performed in adherence to the EU (86/609) and Ministerio de Agricultura (Spain, BOE 223/1988) guidelines for care and use of laboratory animals. Each animal was initially anesthetised with ether and then injected intraperitoneally with 100 mg Pentothal-Na /100 g weight.

Isolation and culture of hepatocytes

Liver parenchymal cells (HC) were isolated by the perfusion technique, using collagenase in Krebs-Ringer-bicarbonate solution (KRB medium) according to the general method of Berry and Friend (1969) as described previously (Portoles et al., 1987). Cell viability was routinely monitored by the Trypan blue exclusion test obtaining 85-90 % viability. The yield of viable HC was (235±22) x 10^6 per rat liver with a purity above 99%. The isolated HC were cultured in William's E medium in the presence of 10% foetal calf serum, with 500 U/ml penicillin, 100 µg/ml streptomycin, at 37°C under a CO_2 atmosphere for 24 h, up to the formation of a continuous monolayer, and were then used. No collagen or any other coating was used. The cells were cultured on either 25 cm^2 flasks or on 4.5 cm^2 Costar Transwell, membranes ($2.8x10^5$ cells/cm^2). Cell culture was performed under sterile conditions in a Telstar CAM 7001 laminar flow chamber.

Isolation and culture of sinusoidal cells

Liver sinusoidal cells (SC) were isolated by perfusion with collagenase and pronase in Gey's balanced salt solution according to the method of Wisse and Knook (1977), with minor modifications (Catala et al., 1999). After preparation of the sinusoidal cell suspension with a single-step Nycodenz gradient, Kupffer cells were further purified by centrifugal elutriation at 4°C in a JE-6B rotor equipped with a standard separation chamber, using a Beckman model J-6B centrifuge (Beckman Instruments, Fullerton, CA), as previously described (Catala et al., 1999; Gressner and Zerbe, 1987). Cell viability was routinely monitored by the Trypan blue exclusion test or flow cytometry after the addition of PI (0.005% in phosphate buffer saline), obtaining 85-90% viability. The yield of viable sinusoidal cells was (67±20) x 10^6 per rat liver with a purity above 99%. The isolated SC were cultured in

Ham's F-10 medium in the presence of 10% foetal calf serum, at 37°C in either 25 cm^2 flasks or 9.5 cm^2 Costar multiwell (10^5 cells/cm^2) under a CO_2 atmosphere for two weeks. No collagen or any other coating was used. Cell culture was performed under sterile conditions in a Telstar CAM 7001 laminar flow chamber.

Immunolabelling of Kupffer cells and characterization of sinusoidal cell population by flow cytometry and confocal microscopy

In order to characterise SC populations, Kupffer cells (KC) in suspension were labelled with the monoclonal antibody ED1, which specifically recognizes rat macrophages, as previously described in detail (Catala et al., 2007). Briefly, Kupffer cells were sedimented at 208 g for 10 min, suspended in PBS 1% foetal calf serum and incubated for 30 min with ED1 conjugated to PE (10 µl ED1-PE/10^5 cells) and then protected from light. Cells were then diluted 100-fold with buffer, sedimented and resuspended in DMEM with 25 mM HEPES (10^6 cells/ml). After labelling of KC population with ED1-PE, cell suspension was studied exciting the fluorescence of PE linked to ED1 at 488nm and measuring the emitted fluorescence at 575nm in a FACStar Plus Cytometer. Sinusoidal cell suspensions were composed of 30% Kupffer cells and 70% sinusoidal endothelial cells (EC) as evaluated by flow cytometry. The same immunolabelling procedure was adapted to characterize KC in monolayers of cultured liver sinusoidal cells, and the fluorescence of PE linked to ED1 was observed with a Bio-Rad 1024 confocal microscope (Centro de Citometría de Flujo, Universidad Complutense de Madrid). After 2 weeks, the SC cultures presented 50% KC and 50% EC. The procedure is also described in detail in the literature (Catala et al., 2007).

Co-culture of parenchymal and sinusoidal cells

Primary cultures of hepatocytes (HC) and sinusoidal cells (SC) were used. Before starting the experiments, HC were cultured for 24 h and SC for two weeks, as indicated in "Isolation and culture of hepatocytes" and in "Isolation and culture of sinusoidal cells". SC cultured on 9.5 cm^2 Costar multiwells were washed twice with PBS and then 4.5 ml of medium, with or without LPS, were added. HC cultured on Costar Transwell· membranes were also washed twice with PBS and placed into the wells containing SC (Fig. 1A). Then 1.5 ml of medium, with or without LPS, were added to cover the membrane insert. Co-cultures were incubated at 37°C under a CO_2 atmosphere for various times according to the experiment. After each treatment, the incubation medium was aspirated and HC monolayers were washed twice with PBS to remove any dead cells and kept at -80°C until processed. For each experiment one rat was used for the isolation and growth of SC and another one for the isolation of HC.

The former protocol was modified as follows for the studies of GSH content of HC co-cultured with activated SC (Fig. 1B). After washing the cells and adding medium containing different doses of *E.coli* lipopolysaccharide (0, 10 and 100 µg LPS/ml William's E medium), sinusoidal cells were incubated at 37°C under a CO_2 atmosphere for various times according to the experiment. Then the incubation medium was aspirated and the monolayers were washed twice with Phosphate Buffered Saline (PBS) to remove LPS, and fresh William's E medium was added. Hepatocyte co-culture was performed as described above.

Treatment of cultured parenchymal cells with sinusoidal cell conditioned medium

Cultured sinusoidal cells were incubated for 0, 15 or 120 min in fresh medium, with or without LPS. Supernatants were then gathered and immediately transferred onto HC previously washed twice with PBS to remove cellular debris. Cultured HC remained in SC conditioned media for various times, and then the incubation medium was aspirated and the HC monolayers were washed twice with PBS, and frozen at -80°C. The integrity of the monolayer was monitored by contrast-phase microscopy before and after each experiment. For each experiment one rat was used for the isolation and growth of SC and another one for the isolation of HC. The procedure is illustrated in Figure 1C.

LDH measurement

Lactate dehydrogenase (LDH) activity was measured in the culture medium by an enzymatic method (Monotest LDH from Boerhinger-Mannheim Diagnostica / FRG) using a Beckman DU-8 UV-Visible Spectophotometer.

Glutathione measurement

For HC solubilization, cells were scraped from the plastic surface and homogenized in phosphate buffer 50mM, pH 7.4, with a Pobel glass conical homogenizer on ice for 1 minute at constant speed. After taking an aliquot for protein content determination, each sample was treated with a mixture of trichloroacetic acid (TCA) and HCl. The final concentrations were TCA 5% and HCL 0.01N. Then HC glutathione content was measured by the spectrophotometric recycling method of Tietze (1969) modified by Catala and Portoles (2002), in the presence of 5,5'-ditiobis (2-nitrobenzoic acid) (DTNB), NADPH and glutathione reductase (GR) in a DU70 Beckman spectrophotometer (CA, USA).

Protein content was determined by the method of Lowry et al. (1951).

Nitric oxide measurement

The presence of NO_x species was measured in the

culture medium by a NO kit based on De Griess reaction (from Boerhinger-Mannheim Diagnostica/FRG) using a Beckman DU-8 UV-Visible Spectophotometer.

Statistical analysis

Each experiment was performed three times independently with cells isolated from different animals. During each experiment three or four individual culture flasks or wells were assayed. Thus the mean was finally calculated with the values of at least 9 replicates.

Data given in figures and tables represent the mean values ± SD of the mean, taking into account the degrees of freedom lost with each group of replicates. Comparison between groups has been carried out by the Student's t-test. Results were considered statistically significantly different when $p<0.05$.

Results

Co-culture of parenchymal and sinusoidal cells

The experimental procedure has been detailed in Figure 1A.

Release of lactate dehydrogenase

In order to know the effect of the treatment with LPS of sinusoidal and parenchymal cells co-cultures on the integrity of the cell membrane, the levels of lactate dehydrogenase (LDH) were evaluated in the culture medium. Figure 2 shows that LPS seems not to alter membrane integrity at the doses and times studied, and even seems to protect cells from the stress generated during medium change, although at low doses, 10 µg LPS/ml, this protective effect is not significant. Since, overall, LPS did not greatly alter LDH levels in the co-culture medium, the analysis of the LPS effects on LDH levels from each cell population was not carried out.

Effect of LPS on the glutathione content of parenchymal cells co-cultured with sinusoidal cells

Recently we demonstrated that sinusoidal cells down-regulated parenchymal cell glutathione content when co-cultivated on a porous membrane system. SC respond rapidly to LPS with the release of soluble mediators. It would be very interesting to know whether LPS alters the SC-HC communication that regulates the HC glutathione levels. We used two different doses of LPS, a low dose of 10 mg/ml, closer to endotoxic shock *in vivo,* and a high dose of 100 mg/ml which can help us elucidate the biochemical mechanism of LPS.

The glutathione content of parenchymal cells co-cultured with sinusoidal cells after treatment with different doses of LPS is shown in Figure 3. Control levels ranged from 2.21-7.67 mmol/mg protein. The dose of 10mg LPS/ml provokes a significant momentary increase after 15 min of exposure. At 30 min a sudden decrease to 40% of basal levels is observed, reaching, at 120 min, values as low as 20% of controls. With the dose of 100 mg LPS/ml a significant increase is also observed at 15 min, around 140% above control levels, followed by a decrease to levels of 20%, similar to those reached with 10 mg LPS/ml. However, the decrease of this higher dose is less dramatic, being at 30 min still significantly above control levels.

Effect of LPS on NOx

Nitric oxide has been shown to play very important roles in the regulation of oxidative stress and cell communication during inflammation. Since the action of both LPS and cytokines produced by SC has been previously described, in the present study the NO release was only measured in the co-culture model to investigate the simultaneous effects of the different agents which are present in endotoxic liver. We have estimated the production of NO by its endproducts, nitrite and nitrate. Again a low and a high dose of LPS were used.

Figure 4 shows that the activation of co-cultures with 10 µg LPS/ml elicits a decrease in the release of nitric oxide derivatives. The higher dose 100 µg LPS/ml shows a biphasic behaviour provoking a significant 2 fold increase over control values at 15 min. From then on there is a decrease below controls, which becomes significant after 60 min of incubation. Control values ranged from 3.20-9.68 µmol/mg protein.

Cultured parenchymal cells treated with conditioned media from LPS-activated sinusoidal cells

In order to know the joint effect of LPS, together with LPS-activated sinusoidal cell released mediators, on parenchymal cell glutathione content, we performed experiments in which the supernatants of cultured sinusoidal cells treated with LPS are later added to cultured parenchymal cells (Fig. 1C). This system does not allow feedback from HC to KC. We chose two time periods for the incubation of sinusoidal cells with LPS: 15 and 120 min. We estimate that among the mediators released at 15 min we can find mainly reactive oxygen species and arachidonic acid related substances. Longer times such as 120 min allow the synthesis of protein mediators. The supernatants containing LPS and released substances are collected and added onto parenchymal cell cultures for 30 or 60 min. Afterwards the medium is retired and the cells frozen to measure glutathione over the following days.

Figure 5A shows the effect of conditioned media of sinusoidal cells, pre-incubated with LPS for 15 min, on cultured parenchymal cell glutathione content. Control series corresponds to the use of control medium for the pre-incubation, absolute values range from 12.50-20.78 mmol/mg protein. LPS 10 and LPS 100 series correspond to the addition to the pre-incubation medium of 10 µg/ml and 100 µg/ml of endotoxin respectively. Parenchymal cells are therefore treated in these cases

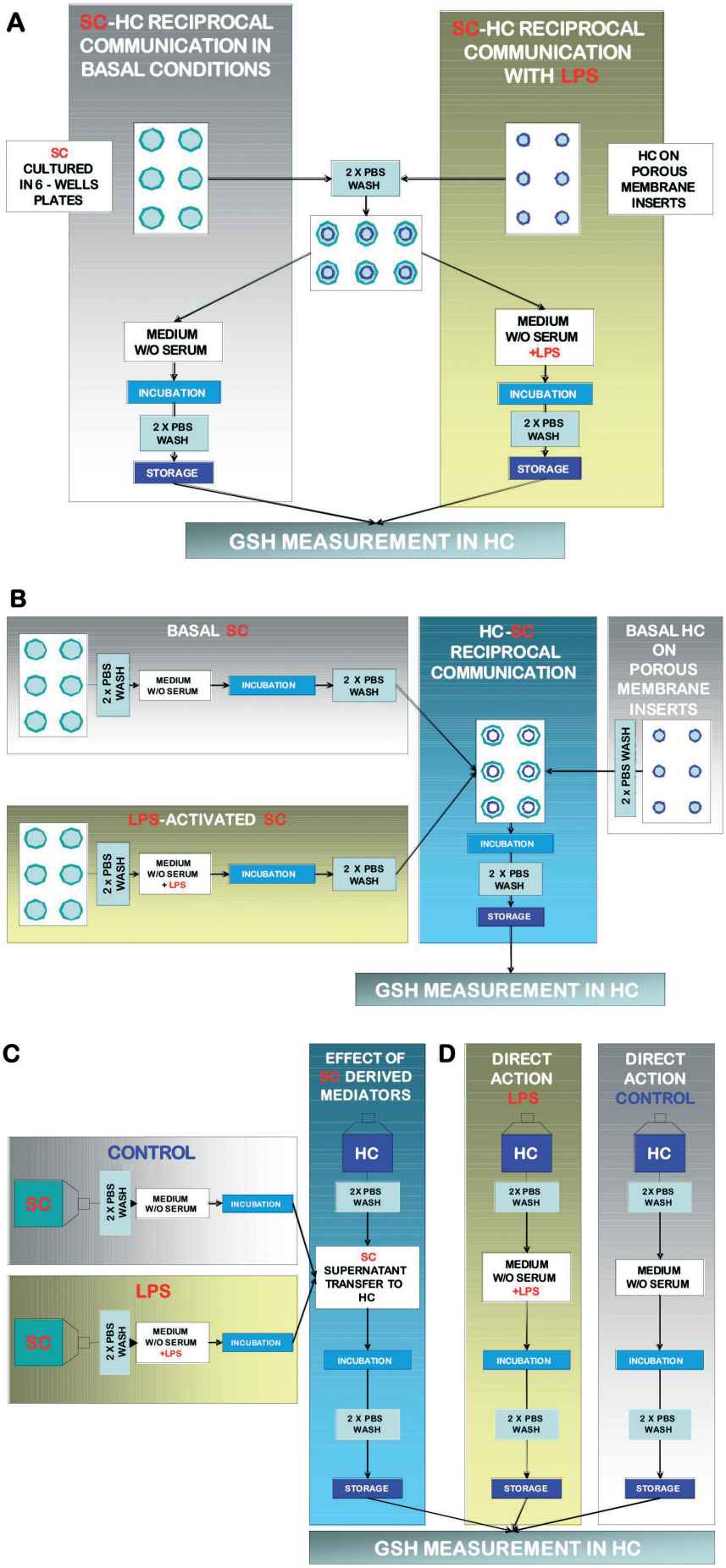

Fig. 1. Schematic representation of the experimental procedure for the evaluation of cell communication on HC glutathione content under LPS challenge. **A.** LPS plus mediators derived from both SC and HC. **B.** Mediators released by LPS-activated SC, no LPS present. **C.** LPS plus SC derived mediators. **D.** LPS direct action on primary pure HC cultures.

with a medium containing LPS and the mediators released by sinusoidal cells during the 15 min of pre-incubation. Pre-incubation of sinusoidal cells for 15 min with 10 µg LPS/ml provokes a dramatic decrease (37%) in hepatocyte glutathione after 30 min, which reverts at 60 min (Fig. 5A). Augmenting the dose up to 100 µg LPS/ml causes a lighter (61%) but longer effect.

Figure 6A shows a control series, with non-LPS supernatants, and two series, LPS 10 y LPS 100, with supernatants containing endotoxin. In this case, pre-incubation lasted 120 min. Control values ranged from 12.50-25.22 mmol/mg protein.

When increasing pre-incubation time up to 120 min the 10 µg LPS/ml dose has a much more gradual effect on GSH content.

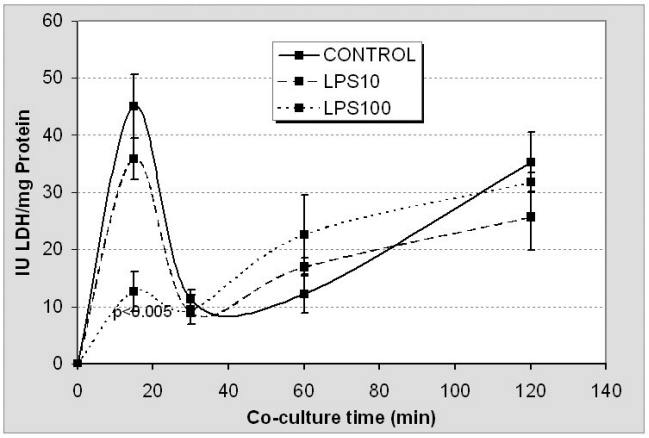

Fig. 2. Determination of LDH in sinusoidal and parenchymal cell co-culture media. Each point is the mean of at least 9 replicates. Bars represent the standard error of the mean.

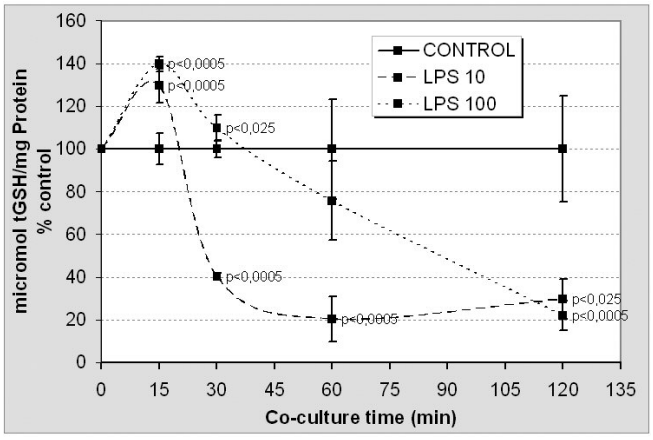

Fig. 3. Effect of LPS on glutathione content of parenchymal cells co-cultured with sinusoidal cells. Each point is the mean of at least 9 replicates. Bars represent the standard error of the mean.

Effect of the co-culture of parenchymal cells with LPS-activated sinusoidal cells

The co-culture system used allows the activation of sinusoidal cells with LPS in a first step. Then, in a second step, LPS can be eliminated and the effect of the released mediators on non-activated HC's glutathione content, can be studied (Fig. 1B). Therefore the role of mediators can be identified independently from LPS.

Figure 5B shows the total glutathione content of parenchymal cells co-cultured with 15 min-LPS-activated SC (controls ranged from 7.07-18.28 mmol/mg protein). In such a short time it is evident that both LPS doses, 10 µg LPS/ml and 100 µg LPS/ml, are able to activate sinusoidal cells to strongly downregulate parenchymal cell glutathione content.

Figure 6B shows the results of a 120 min pre-activation of sinusoidal cells before co-culture (controls ranged 2.76-6.17 mmol/mg protein). Here, the mediators released by sinusoidal cells have a different effect. Pre-treatment with 10 µg LPS/ml activates SC to downregulate HC glutathione during the first 30 min, but not at 60 min. The dose of 100 µg LPS/ml activates SC to regulate HC's tGSH content at 60 min, provoking an induction of tGSH synthesis.

Discussion

Our results show that high doses of LPS (100 µg / ml) significantly reduce LDH release at a short period of incubation (15 min). Previous results of our group demonstrate that the microviscosity of either isolated cells or plasma membranes increased after treatment with LPS (Aracil et al., 1985; Portoles et al., 1987), suggesting that LPS can interact *in vitro* with the lipid bilayer of the cell membrane. This LPS-induced increase of microviscosity could provide a mechanism of membrane protection and avoid LDH leaking.

We demonstrated some years ago that LPS

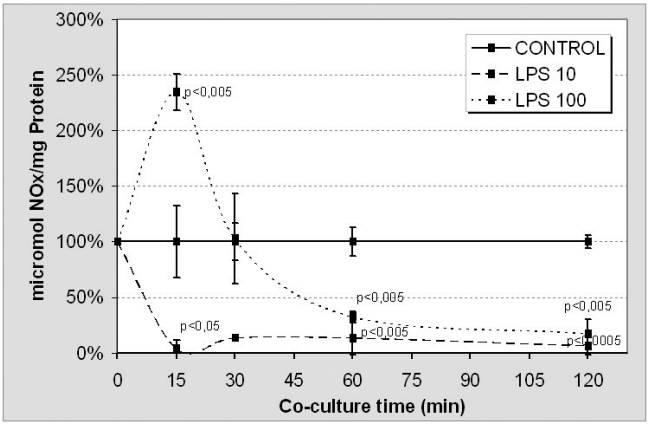

Fig. 4. Determination of NO derived species in supernatants of sinusoidal and parenchymal cell co-cultures. Each point is the mean of at least 9 replicates. Bars represent the standard error of the mean.

upregulates GSH when it is directly added to HC primary cultures (experimental model in Figure 1D) (Catala and Portoles, 2002). However, here we find that HC's intracellular glutathione is dramatically reduced by LPS when co-cultured with SC. We see the same effect when HCs are treated with supernantants of SC treated for a short period (15 min) with LPS. This suggests that LPS, at short incubation times, induces the release of SC-derived factors downregulating HC glutathione content, masking the direct action of LPS.

Several authors have reported similar effects. Spitzer and Zhang (1995) also reported similar decreases of intracellular GSH in parenchymal cells isolated from endotoxemic rats, and in co-cultures of parenchymal cells with PMN or Kupffer cells isolated from endotoxemic livers. Harbrecht et al. (1997) reported the decrease of intracellular content of glutathione in parenchymal cells treated with LPS-activated Kupffer cell's media.

It is important to remark that the simple co-culture of parenchymal and sinusoidal cells provokes a strong decrease in the glutathione content (Catala et al., 2007). Therefore, GSH levels after LPS treatment are manyfold lower than those found in primary parenchymal cell cultures. Such a pronounced depletion in glutathione levels could seriously compromise organic metabolic functions, as well as antioxidant capacity (Ma et al., 1997), and could explain why Hartung and Wendel (1991) reported that HCs were only injured by LPS if KC were also present. However, Clemens et al. (1994)

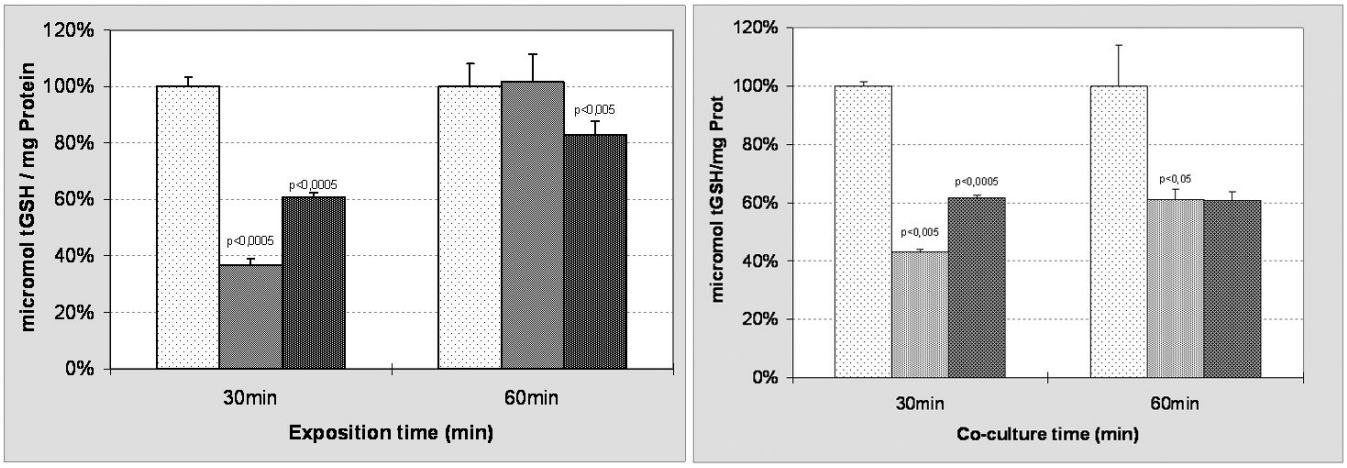

Fig. 5. Modulation of intracellular glutathione of hepatocytes cultured for different times. **A.** With supernatants of sinusoidal cells treated for 15 min with 0, 10 and 100 µg/ml LPS respectively. **B.** In co-culture with activated sinusoidal cells pretreated for 15 min with 0, 10 and 100 µg/ml LPS respectively. Each column stands for the mean of at least 9 replicate HC cultures. The bars represent the standard error of each mean.

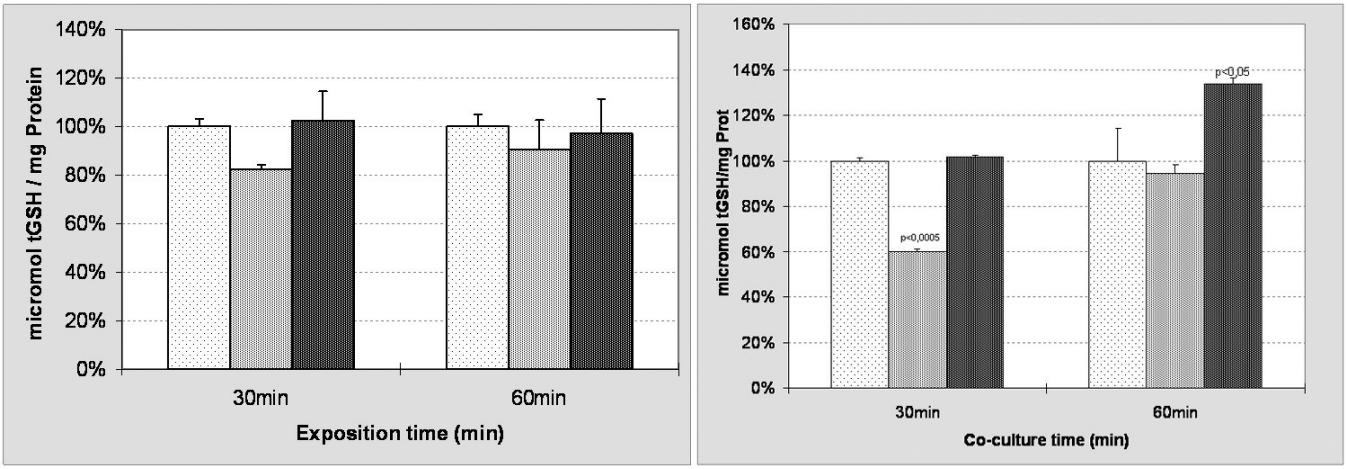

Fig. 6. Modulation of intracellular glutathione of hepatocytes cultured for different times. **A.** With supernatants of sinusoidal cells treated for 120 min with 0, 10 and 100 µg/ml LPS. **B.** In co-culture of activated sinusoidal cells pretreated for 120 min with 0, 10 and 100 µg/ml LPS. In each series, the columns respectively stand for the mean of at least 9 replicate HC cultures. The bars represent the standard error of each mean.

remark that, *in vivo,* the strict hepatic cell organisation imposes certain restrictions in the types of communication (see Fig. 7). In particular, they argue that the vascular, as well as the perivascular compartment, are subject to a convective flow in the periportal-to-perivenous direction and therefore the communication via soluble mediators only affects cells downstream. Under this premise, soluble mediators released by downstream HC cannot reach upstream KC directly. This restriction may be modulated by conductance due to intercellular canalicular juctions of HC, which is diffusive instead of convective, but during inflammatory states these intercellular cannalicular communications are strongly depressed via negative regulation of the polypeptidic subunits of the channels (conexins).

Then, taking into account these restrictions in cell communication imposed by anatomy, the "supernatant" model is more realistic than the "co-culture" model for the study of liver cell communication during endotoxemia. In fact, our results show that LPS-activated SC supernatants, at longer treatments, can induce the recovery of glutathione levels, closer to what can be observed *in vivo* during the acute phase of endotoxic shock (Portoles et al., 1996). In this sense, a

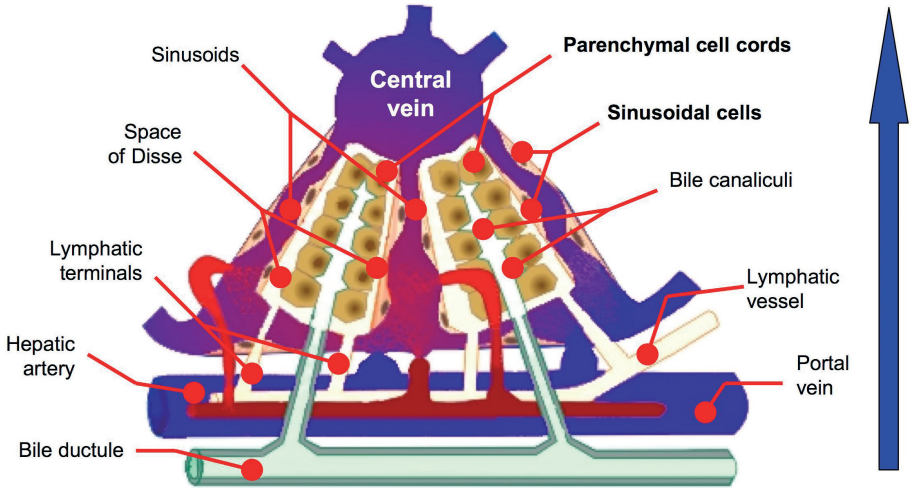

Fig. 7. The anatomical organization of hepatic lobule imposes a flow in the periportal to perivenous direction.

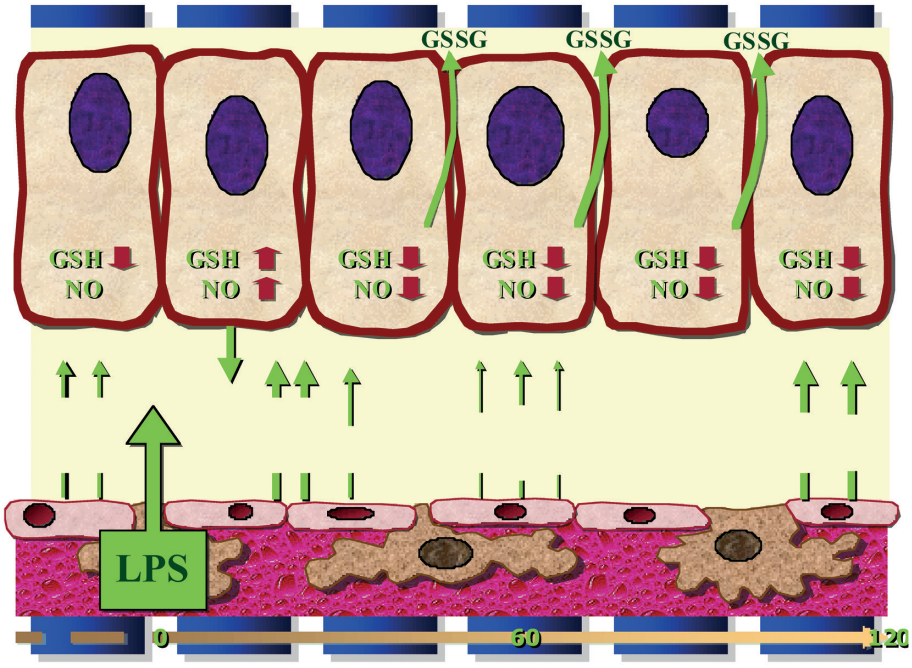

Fig. 8. Hypothetical model of communication between hepatic sinusoidal cells and hepatocytes.

certain cross-talk from HC to KC could also help raise glutathione levels at longer times once LPS has been cleared (Fig. 6B).

Our results reveal a parallel behaviour between glutathione content and NO production in response to endotoxin in SC and HC co-cultures. Diverse studies conclude that iNOS is regulated by glutathione cellular levels, participating not only in parenchymal cell iNOS activity regulation, but also in iNOS mRNA expression (Adamson and Billings, 1993; Duval et al., 1995; Kuo and Abe, 1996; Harbrecht et al., 1997). It has also been demonstrated that NO levels transcriptionally regulate glutathione synthesis and plasma efflux in normal and endotoxic conditions (Kuo and Abe, 1996; Payabvash et al., 2006). All this suggests that NO effects are partly mediated by glutathione redox status (Trauner et al., 1997), and that its protective action may be due to glutathione synthesis (Kuo and Abe, 1996; Kuo and Slivka, 1994; Molina-y-Vedia et al., 1992; Padgett and Whorton, 1997).

Figure 8 presents a tentative hypothetical model based on our own results and those by other authors referred to above, which could help us understand hepatic cell communication for glutathione regulation. In basal conditions, sinusoidal cells (endothelial and Kupffer cells) downregulate HC glutathione content and NO production. Upon LPS detection, sinusoidal cells rapidly release inflammation factors such as NO, derivatives of arachidonic acid and ROS. These mediators, together with LPS, induce GSH, NO and glucose synthesis which are then released by HC. At longer times LPS-activated sinusoidal cells synthesise protein factors, such as interleukins and TNF, which downregulate HC glutathione content and induce GSSG secretion.

From the results presented above we can draw the following conclusions. First, the regulation of liver hepatocyte glutathione content and NO release in the presence of LPS is strongly modulated by liver non parenchymal cells. LPS, at short incubation times, induces the release of sinusoidal cell - derived factors, dramatically downregulating HC glutathione content and masking its own direct action. If the experimental model allows feedback from HC, such as co-culture models, sinusoidal cells induce a prolonged depletion of glutathione levels. However, anatomical configuration of the hepatic sinusoid imposes a restrictive flow in the periportal-to-perivenous compartment which strongly reduces the probability of a direct feedback from HC towards sinusoidal cells. Therefore, the study of such complex organs as the liver needs the simultaneous use of *in vitro* models that take into account liver histophysiology, and histopathology representing an accurate intermediate step towards *in vivo* models.

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