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Effects of bacterial endotoxin (lipopolysaccharides) on survival and metabolism of cultured precision-cut rat liver slices

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Abstract

The effect of bacterial endotoxin (lipopolysaccharides from *Escherichia coli*, LPS) on cellular metabolism and drug biotransformation was studied in precision-cut rat liver slices (PCLS). Xenobiotic metabolism by PCLS was assessed by measuring phase I (midazolam hydroxylation) and phase II (paracetamol conjugates) enzyme activities. Nitrites formation was used as an indirect way to assess LPS-mediated activation of nitric oxide synthase (iNOS, type 2). PCLS incubation with various LPS doses results in a dose-dependent formation of nitrites. Such a nitrite formation is decreased by dexamethasone. After incubation of PCLS for 24 h LPS addition did not increase the basal nitrite formation, indicating that cells are not responsive any more. Paracetamol conjugation was unaffected by LPS treatment but midazolam hydroxylation was reduced by more than 50%. Such a loss is not due to cell impairment since neither survival (LDH leakage) nor cellular metabolism (protein synthesis or ATP content) were modified by LPS. Indeed, under defined conditions, namely Williams' medium E and O_2/CO_2 (95:5), PCLS maintained both ATP and GSH levels and the capacity of hepatocytes to synthesize proteins. In conclusion, the in vitro model of PCLS reproduces the inhibitory effect of LPS on a CYP3A-dependent activity, allowing a mechanistic approach to study cell–cell interactions. © 2002 Published by Elsevier Science Ltd.

Keywords: Xenobiotic metabolism; Rat liver slices; Kupffer cells; Lipopolysaccharides

1. Introduction

Inflammatory processes may influence both xenobiotic and intermediary metabolism of hepatocytes via reactive intermediates and soluble factors released from non-parenchymal cells (Takemura et al., 1999; Renton and Nicholson, 2000; Shedlofsky et al., 2000; Siewert et al., 2000). Such cellular interactions may have important pharmacological and toxicological consequences that require further investigation. A mechanistic approach by the use of in vitro methods has been, however, a matter of controversy. The co-culture systems of Kupffer cells with hepatocytes have supplied some interesting data (Milosevic et al., 1999), but the lack of normal tissue architecture (the correct proportion in the number of cells, the maintenance of cell polarity, etc.) still remains a critical point. Closely related to in vivo conditions, whole organs appear as the models of choice. However, such an in vitro approach did not represent a substantial development of alternative methods (e.g. no reduction in animal testing). In addition, the requirement of one organ for each experimental condition remains its main inconvenience: for instance, selective elimination of Kupffer cells by gadolinium chloride will make the liver unusable for a subsequent experiment involving such cells (Hardonk et al., 1992). As tissue slices contain all the cell types present in the whole organ in vivo, within their normal spatial relationships, the technological improvements made by Brendel's group (Brendel et al., 1987) make the use of slices very attractive. Indeed, it has been shown that cultured rat precision-cut liver slices (PCLS) are a suitable model to study metabolic pathways and xenobiotic metabolism (Morales et al., 1998; Vanhulle et al., 2001).

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; GSH, reduced glutathione; LDH, lactate dehydrogenase; LPS, lipopolysaccharides; NO, nitric oxide; PCLS, precision-cut liver slices; WME, Williams' medium E

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The aim of this work was to study whether an inflammatory process may affect liver metabolism in cultured rat PCLS. Based on a previous report to define optimized conditions to culture PCLS (Lerche-Langrand and Toutain, 2000), we have selected some biochemical markers to assess the influence of the incubation medium and the oxygen tension during incubation. Thereafter, inflammatory conditions were mimicked by incubating PCLS with various concentrations of bacterial endotoxin (lipopolysaccharides from *Escherichia coli*, LPS). Both survival and metabolic functions were analyzed further. The formation of nitrites was used as an indirect way to assess LPS-mediated activation of nitric oxide (NO) by nitric oxide synthase (iNOS, type 2). Both phase I and phase II metabolizing activities were assessed by monitoring the formation of hydroxylated metabolites of midazolam (mainly a CYP3A-dependent activity), and glucuronide- and sulfate-conjugates of paracetamol, respectively. The results reported here show that LPS did not affect survival, the maintenance of ATP, or the capacity to synthesize proteins and phase II enzyme activities. However, more than 50% of midazolam oxidation was inhibited by LPS.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 250–300 g, were purchased from Iffa-Credo (Les Oncins, France) and housed in individual cages in a temperature- and light-controlled room (12-h dark/light cycle). They received standard diet AO3 (U.A.R., Villemoisson-sur Orge, France) and water ad lib.

2.2. Chemicals

Williams' medium E (WME) and fetal calf serum (FCS) were purchased from Gibco BRL (Middlesex, UK). Gentamicin sulfate, EDTA, lipopolysaccharides (LPS) from *E. coli*, and Triton X-100 were obtained from Sigma Chemicals (St Louis, MO, USA). Paracetamol (Janssen Pharmaceutica, Beerse, Belgium), 2-acetamidophenol (Aldrich Chemicals Co., Dorset, UK) and insulin (Actrapid HM, Novo Nordisk, Bagsvaerd, Denmark) were available commercially. Midazolam and flunitrazepam were purchased from Roche (Grenzach-Whylen, Germany). [U-^{14}C]leucine was from Amersham (UK). All other chemicals used were of the purest grade available.

2.3. Preparation and incubation of PCLS

Rat surgical procedures were carried out under pentobarbital (60 mg/kg) anesthesia. PCLS (250–300 μm thickness) were prepared by using the Krumdieck tissue slicer according to Goethals et al. (1992). PCLS were placed in 50-ml rubber covered sealing flasks (one slice/2 ml), gassed through a stainless-steel needle, and incubated in a shaking water-bath (80 oscillations/min) at 37 °C. Three incubation media, namely Waymouth, WME and Dulbecco's modified Eagle's medium (DMEM), were tested to optimize culture conditions. In addition, incubations were conducted under a flow of O_2/CO_2 at oxygen concentrations ranging from 21 to 95%. When optimized conditions were defined, standard incubation procedures were as follows: immediately after slicing, PCLS were stored 30 min at 4 °C in WME containing 10% of FCS (v/v), glutamine (2 mM) and insulin (0.1 μM) in order to eliminate cell debris and wash out released inflammatory cytokines. Thereafter, PCLS were incubated at 37 °C for the indicated times under a continuous flow of O_2/CO_2 (95%:5%) in WME supplemented with glutamine (2 mM), insulin (0.1 μM) and 50 μg/ml gentamicin sulfate. After 24 h of incubation, PCLS were washed in saline and reincubated in fresh WME.

2.4. ATP content

PCLS were washed twice in saline and sonicated immediately in 1 ml of 2% perchloric acid. The intracellular ATP content was measured on neutralized PCA extracts using the ATP Bioluminescense Assay Kit CLS II from Boehringer (Mannheim, Germany). The results are expressed as nmol ATP/mg protein.

2.5. GSH content

Reduced glutathione (GSH) was assayed using a modified method of Hissin and Hilf (1976). PCLS were taken, washed twice in saline and sonicated in 1 ml of 6% trichloroacetic acid and centrifuged for 30 min at 3600 ***g*** at 4 °C. Aliquots of the standards and precipitated PCLS were added to tubes containing phosphate/EDTA buffer (pH 8) and 1 mg/ml *o*-phthalaldehyde (200 μl), then stored in the dark (15 min). Fluorescence was measured (excitation 350 nm, emission 420 nm) using a spectrofluorimeter, Perkin-Elmer LC-50B. The results are expressed as nmol GSH/mg protein.

2.6. Nitrites formation

The production of nitric oxide (NO) by slices was quantified by monitoring the formation of nitrites in the incubation medium using Griess reagent (Green et al., 1982). Briefly, after incubation of PCLS with LPS in the absence of dexamethasone, aliquots (200 μl) of incubation medium were mixed with Griess reagent (1% sulfanilamide, 5% H_3PO_4, 0.1% naphthylethylenediamine dihydrochloride) and incubated at 37 °C for 10 min.

The absorbance was measured at 540 nm using $NaNO_2$ as a standard. Results were expressed as nmol NO_2/mg protein.

2.7. Protein synthesis

At different times, PCLS were taken, rinsed with saline and further incubated for 2 h in WME containing radiolabeled leucine. Protein synthesis was estimated by measuring the incorporation of [^{14}C]leucine (sp. act. 94 μCi/mmol, 0.8 mM unlabelled leucine) into the protein pellet obtained by PCA precipitation as described by Seglen (1976). The results were expressed as protein synthesis rates (dpm of leucine incorporated/mg protein/min). The amount of protein was determined by the Lowry method (Lowry et al., 1951), using bovine serum albumin as standard.

2.8. LDH leakage

The viability of slices was assessed by measuring the activity of lactate dehydrogenase (LDH) both in the slice and in the incubation media, as reported elsewhere (Wroblesky and Ladue, 1955). The results were expressed as % of LDH leaked out as compared to activity in the slice.

2.9. Midazolam metabolism

The formation of 1′- and 4-hydroxymidazolam was monitored by capillary HPLC isocratic elution, using aqueous Na_2HPO_4 (10 mM, pH 7.0):acetonitrile (65:35) mixture as eluent, as reported elsewhere (Eeckhoudt et al., 1998). Briefly, at the indicated times, PCLS were incubated for 30 min in the presence of midazolam (25 μg/ml) and aliquots of the incubation mixtures containing the glucuronide conjugates were further incubated at 37 °C for 2 h in acetate buffer (pH 5, 50 mM) containing 10 mU of β-glucuronidase. After alkalization with NaOH (2%), unconjugated metabolites were extracted with freshly distilled ether/cyclohexane (69:31) and analyzed by HPLC. Flunitrazepam (20 μg/ml) was used as internal standard. The retention times were 9–10 min for 4-hydroxymidazolam, 10–11 min for 1′-hydroxymidazolam, 1213 min for flunitrazepam (internal standard) and 15–16 min for midazolam.

2.10. Paracetamol metabolism

Phase II paracetamol conjugates (glucuronide, sulfate and glutathione) were quantified by using reverse-phase HPLC according to the procedure of Lau and Critchley (1994). PCLS were incubated for 2 h in medium supplemented with 5 mM paracetamol. Throughout the 2-h incubation time, the metabolism of paracetamol to its glucuronide, sulfate and glutathione conjugates was linear (data not shown). At the end of incubation, aliquots of incubation medium were stored at −20 °C. After centrifugation, 25 μl of samples together with 50 μl of the internal standard 2-acetamidophenol (400 μg/mg) were injected on a Nova-Pak C18 column. The mobile phase (0.1 M KH_2PO_4/0.1% acetic acid/0.75% propane-2-ol) was delivered to the column at a flow rate of 1.50 ml/min. The results were expressed as μg metabolites/mg protein.

2.11. Statistical analysis

Each experiment was performed in triplicate and the results are mean values ± standard error of the mean (S.E.M.) of at least three rats. Data were analyzed using one-way analysis of variance (ANOVA). For statistical comparison of results at a given time point, data were analyzed using Student's *t*-test. The level of significance was set at $P < 0.05$.

3. Results

3.1. Optimal conditions to culture PCLS

Table 1 shows the capacity of PCLS to synthesize proteins and to maintain ATP and GSH levels when they were incubated either (a) in different media or (b) under different oxygen concentrations.

(a) Similar protein synthesis rates were observed when PCLS were incubated both in WME and Waymouth media. Conversely, protein synthesis was rather depressed when slices were incubated in DMEM. After 3 h of incubation, ATP content of PCLS was quite similar in the three incubation media: 7.6, 9.2 and 8.3 nmol/mg protein in WME, DMEM and Waymouth, respectively (data not shown). After 48 h of incubation in WME, the ATP content was 6.7 nmol/mg protein, but PCLS in DMEM and Waymouth media were unable to maintain its content showing a marked decrease to 3.4 and 1.7 nmol/mg protein, respectively. The intracellular levels of GSH remained fairly constant (around 16 nmol/mg protein), regardless of the incubation medium used. Additional tests were also conducted with RPMI 1640, but WME always showed the best results (data not shown). Therefore, PCLS were further incubated at 37 °C in WME.

(b) The oxygen tension dramatically influences the maintenance of both ATP and GSH levels as well as the capacity of PCLS to synthesize proteins. At the highest oxygen concentrations (70 and 95%), protein synthesis rates were quite similar (around 12 dpm/mg prot/min), but by decreasing the oxygen concentration to 40 and 21%, such rates dropped to 5.6 and 4.2 dpm/mg prot/min, respectively. PCLS were able to maintain ATP and GSH levels for 48 h only under 95% oxygen. When

Table 1
The effect of medium composition and oxygen concentration on ATP and GSH content and on protein synthesis within cultured PCLS

Conditions tested		Protein synthesis rates (dpm/mg prot/min)	ATP (nmol/mg prot)		GSH (nmol/mg prot)	
			24 h	48 h	24 h	48 h
Medium	WME	15.3±1.6	7.4±0.3	6.7±0.9	13.1±0.8	16.5±2.1
	DMEM	9.3±1.2*	6.3±0.8	3.4±0.7*	12.6±1.1	16.4±1.6
	Waymouth	11.1±1.1	4.9±0.6*	1.7±0.1*	10.1±0.2*	16.7±1.8
Oxygen (%)	95	12.7±1.1	7.1±0.7	5.9±0.3	13.3±2.1	14.7±0.9
	70	12.3±0.9	5.5±0.6**	0.9±0.1**	12.0±1.6	6.9±0.1**
	40	5.6±0.7**	1.2±0.2**	1.0±0.1**	10.7±1.6	6.8±0.5**
	21	4.2±0.5**	1.5±0.5**	0.5±0.1**	8.4±0.8**	5.2±0.8**

PCLS were incubated for 48 h at 37 °C in three different incubation media (under 95% of oxygen) and in the presence of different oxygen concentrations ranging from 21 to 95% (in WME). Afterwards, liver slices were taken and protein synthesis, GSH and ATP content were measured as described in Materials and Methods. The results are expressed as protein synthesis rates (dpm/mg prot/min) and nmol of GSH or ATP/mg protein. The values are means ±S.E.M.

*$P<0.05$ as compared to values of WME; **$P<0.05$ as compared to values of 95% oxygen.

incubated under other oxygen concentrations they were unable to ensure a minimal critical value for both markers. Actually, such a decrease was already observed after 3 h of incubation, since the ATP content was 8.0, 9.7, 5.3 and 3.8 nmol/mg protein for oxygen concentrations of 95, 70, 40 and 21%, respectively (data not shown). Therefore, PCLS were incubated in WME at 37 °C under O_2/CO_2 (95%/5%).

Other studies including the analysis of histological sections (hematoxylin/eosine staining) and the assessment of xenobiotic metabolism (e.g. the hydroxylation of 7-ethoxycoumarin) were also performed. No major differences were observed concerning different incubation media, but at low oxygen concentrations (21 and 40%) some necrotic foci were detected at 24 h of incubation (data not shown).

3.2. Incubation of PCLS in the presence of LPS

Activation of NOS by LPS may be assessed following the formation of NO, which decompose to nitrite and nitrate at different ratios, depending of the experimental conditions. In the absence of hemoglobin, it was assumed that nitrites, while indirect, reflect NO formation and release. Fig. 1 shows dose-dependent formation of nitrites when slices were incubated for 24 h in the presence of various concentrations of LPS ranging from 0.1 to 20 μg/ml. Fig. 2 shows that in PCLS incubated for 24 h in the presence of LPS (10 μg/ml) the nitrites were increased by about 5–7 times as compared to PCLS incubated in the absence of LPS. When such PCLS were rinsed and further incubated in a fresh medium in the absence or in the presence of LPS (10 μg/ml), the formation of nitrites was increased by about 2.5–3 times as compared to their respective control conditions. Such an increase appears likely to correspond to a basal endogenous formation of nitrites, since PCLS incubated for 48 h in the absence of LPS yields about 60 nmol nitrites/mg protein, which is more or less the same amount produced by PCLS incubated with LPS. Finally, Fig. 3 shows that dexamethasone (0.01 μM) was able to decrease by about 50% the formation of nitrites induced by LPS.

3.3. Effects of LPS on survival and metabolism

Table 2 shows the effect of LPS (10 μg/ml) on survival (LDH leakage), ATP content, protein synthesis rates and phase I and II biotransformation reactions during 24 h of incubation. ATP content, protein synthesis ability and survival of PCLS were unaffected by LPS. Such a lack of effect was also observed at 20 μg/ml LPS (data not shown). The formation of metabolites from midazolam (phase I) and paracetamol (phase II) was monitored by HPLC. In the absence of LPS after 24 h of incubation, PCLS are still able to metabolize mid-

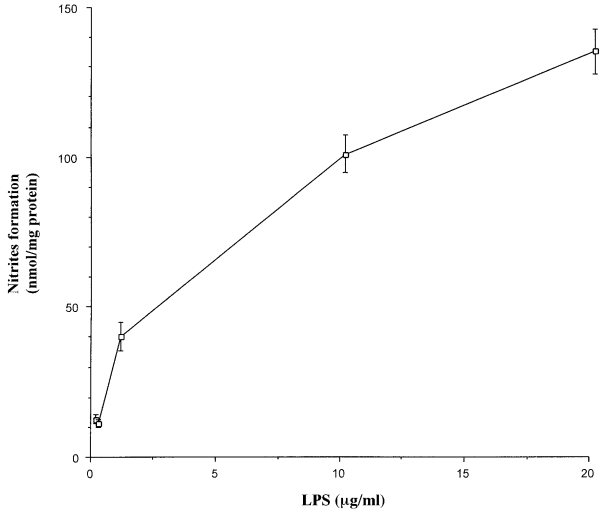

Fig. 1. The effect of LPS on nitrites formation. PCLS were incubated for 24 h at 37 °C with different LPS concentrations ranging from 0.1 to 20 μg/ml. Aliquots of the incubation medium were taken to assess the formation of nitrites as described under Materials and Methods. Values are means±S.E.M.

azolam to its respective hydroxylated derivatives, namely 4-OH- and 1′-OH-midazolam, but their production represented only 31 and 23%, respectively, as compared to metabolites formed at zero time. The addition of LPS strongly enhanced such a loss: actually, the amount of 4-OH and 1′-OH formed after 24 h was further decreased to 8 and 11%, respectively. Indeed, the formation of both metabolites was inhibited by 75 and 50% as compared to control conditions. The metabolism of paracetamol in rat PCLS is characterized by a high sulfation and glucuronidation and low production of glutathione conjugates, but under our experimental conditions, the glutathione conjugates of paracetamol were not detectable. The formation of both paracetamol glucuronide and paracetamol sulfate also decreased after 24 h of incubation of PCLS, falling to 24 and 27%, respectively. However, the addition of LPS did not enhance such a loss in the amount of both glucuronide or sulfate conjugates.

4. Discussion

Both parenchymal and non-parenchymal cells are involved in liver inflammatory processes through a complex and interconnected network of cells and mediators, resulting in a decrease of P450 activities and levels of P450 proteins and mRNAs (Morgan, 1997). The same conditions also induced NO through activation of NOS, which led to the hypothesis that NO was the responsible mediator of this inhibition. However, conflicting results have been reported, making this situation still controversial. As PCLS maintain cell heterogeneity and cell–cell interactions within the original tissue matrix (thus reflecting better the high level of biological organization of the liver), we have explored their application in the study of such parenchymal and non-parenchymal cell interactions.

Actually, several studies have shown that PCLS are a suitable model to study survival and cellular metabolism (Dogterom, 1993; Morales et al., 1998; Vanhulle et al., 2001), drug toxicity (Miller et al., 1993) and xenobiotic metabolism (Gokhale et al., 1997; Oddy et al., 1997; Hashemi et al., 1999b). Moreover, extensive investigations have been conducted to improve slice viability and the maintenance of its metabolic and differentiated functions by modulating the incubation conditions (Olinga et al., 1997; Hashemi et al., 1999a; Lerche-Langrand and Toutain, 2000). On the basis of these reports, some biochemical markers were selected, and the role of incubation media and the oxygen concentration on the maintenance of PCLS metabolic abilities was analyzed. In fact, the best results were observed by incubating PCLS under 95% oxygen in WME, most probably due to the rich amino acid, vitamins and salt composition of this latter milieu. Interestingly, protein synthesis rates were maintained despite the extremely low ATP values measured at 48 h, for instance in Waymouth medium with ATP values falling to 1.7 nmol/mg protein or under 70% oxygen with values of ATP of 0.9 nmol/mg protein. We do not have a clear explanation for the maintenance of this biosynthetic activity under such adverse conditions. In previous studies performed with isolated rat hepatocytes under hypoxic conditions, we

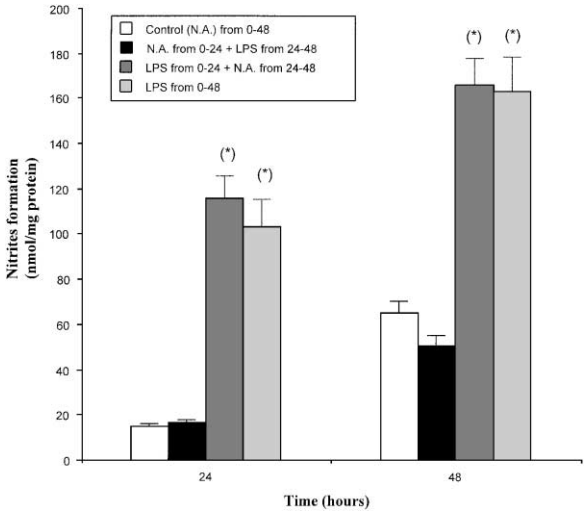

Fig. 2. The effect of incubation time on nitrites formation by LPS. PCLS were incubated for 48 h at 37 °C in the absence or in the presence of LPS (10 μg/ml) for 24 h and further incubation for 24 h with or without 10 μg/ml LPS. At the indicated times, aliquots of the incubation medium were taken to assess the formation of nitrites as described under Materials and Methods. Values are means±S.E.M. *$P<0.05$ as compared to control conditions.

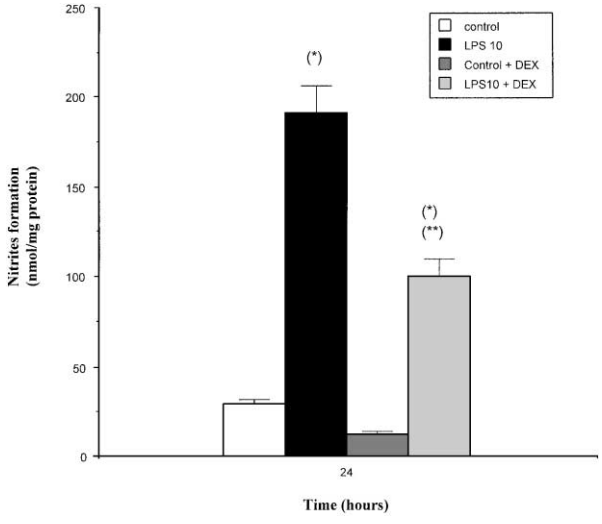

Fig. 3. The effect of dexamethasone on nitrites formation by LPS. PCLS were incubated for 24 h at 37 °C with LPS (10 μg/ml) in the absence or in the presence of 10 nM dexamethasone. Aliquots of the incubation medium were taken to assess the formation of nitrites as described under Materials and Methods. Values are means±S.E.M. *$P<0.05$ as compared to control (no addition) conditions; **$P<0.05$ as compared to LPS conditions.

have found that the capacity of hepatocytes to synthesize proteins is dependent on pO_2 rather than on ATP content (Lefebvre et al., 1993). In this sense, since PCLS are reincubated for 120 min in fresh medium under well-oxygenated conditions, it could be possible that cells "sense" the new pO_2 and the energetic stores might be replenished enough to allow protein biosynthesis to be operating again.

PCLS were incubated with bacterial endotoxin (LPS), under such optimized conditions, and its putative influence on survival and metabolic functions (including phase I and II enzymatic activities) was studied. Different pathways may explain how LPS affects cellular metabolism: they include its interaction with CD-14 receptor (Liu et al., 1998), the formation of nitric oxide (NO) produced by nitric oxide synthase (NOS) (Ignarro, 1997), and the release of cytokines such as IL-1β (Ferrari et al., 1993) or TNF-α (Milosevic et al., 1999). It has been reported that NO production by hepatocytes is not significantly enhanced by LPS, but was markedly enhanced by cytokines and even LPS-activated Kupffer cells conditioned medium (Kurose et al., 1996; Taylor et al., 1998). Moreover, Kupffer cells have been reported as the main source of NO during endotoxemic shock (Alexander, 1998). On the basis of such previous reports, and since cell activation by LPS did not occur after a preincubation of PCLS for 24 h (while hepatocytes remained in a functional state for at least 48 h), we suggest that LPS-targeted cells are mainly Kupffer cells rather than hepatocytes.

In agreement with previous reports (Takemura et al., 1999; Renton and Nicholson, 2000; Shedlofsky et al., 2000; Siewert et al., 2000), showing a decreased activity of P450 drug-metabolizing enzymes by endotoxin, we found that CYP3A-dependent activity (midazolam hydroxylation) in PCLS was strongly inhibited by LPS. While it is not clear to what extent each individual cytokine contributes to the overall reduced expression of CYP isoforms, it seems that in our conditions NO plays a major inhibitory role. Such an inhibition may be explained by the ability of NO to react with iron complexes which renders the cytochrome P450 microsomal enzymes natural targets for inhibition by NO (Ignarro, 1997). Supporting that view, it has been reported that NO reacts readily with heme proteins such as cytochrome P450, decreasing the intensity of the low spin signal of ESR responsible for the ferric form of P450 with a concomitant increase in heme-iron nitrosyl complexes in the liver (Takemura et al., 1999). Although Hoebe et al. (2001) have reported that LPS decreased UDPG transferase activity in direct contact co-cultures between Kupffer cells and hepatocytes, the capacity of PCLS to conjugate drugs (phase II) was not impaired by LPS. The significant differences between the experimental models (co-cultures and slices) may explain why phase II were unaffected by LPS.

It can be argued that NO is not the only mediator since TNF-α, for instance, is also released by activated Kupffer cells. Actually, the release of soluble factors such as cytokines, eicosanoids, and reactive oxygen species may be deleterious to the neighboring cells when non-parenchymal cells are activated. Nevertheless, since LPS did not affect either survival and the capacity of PCLS to synthesize proteins or glucuronidation and sulfation reactions, it is highly probable that inflammatory cytokines are not involved in such LPS-mediated responses. LPS may affect in a NO-independent way some P450 enzymes via its interaction with CD-14 receptor (Liu et al., 1998). However, such interaction is unlikely to occur in the presence of non-parenchymal cells and at such a high LPS concentration (Ferrari et al., 2001). In addition, it has been suggested that hepatic iNOS expression functions as an adaptive response in order to minimize the inflammatory injury (Taylor et al., 1998), indicating that activation of Kupffer cells may have protective effects.

In summary, by using the in vitro model of PCLS we confirmed the inhibitory effect of LPS on P450 enzymes.

Table 2
The effect of LPS on survival, protein synthesis and xenobiotic metabolism

Measurements		Values obtained	
		Control	LPS (10 μg/ml)
LDH leakage	(% of slice activity)	36.0±3.0	42.3±3.8
ATP content	(nmol/mg protein)	14.9±1.5	13.8±1.9
Protein synthesis	(dpm/mg prot/min)	16.8±1.12	16.3±1.94
Phase I:	4-hydroxymidazolam	31%±3.6[a]	8%±0.9[a]
	1′-hydroxymidazolam	23%±2.2[a]	11%±0.8[a]
Phase II:	Paracetamol-glucuronide	24%±3.6[a]	21%±1.5[a]
	Paracetamol-sulfate	27%±2.8[a]	29%±3.1[a]

PCLS were incubated under standard conditions for 24 h at 37 °C in the absence and in the presence of LPS at 10 μg/ml. Afterwards, aliquots of the incubation medium were taken to assess LDH leakage as described under Materials and Methods. The slices were rinsed and further incubated in a fresh medium containing [^{14}C]leucine for 120 min, midazolam for 30 min, or paracetamol for 120 min. The analysis of metabolite formation was performed as described under Materials and Methods. Values are means±S.E.M.

[a] Amount of metabolites (expressed in%) as compared to their respective formation at zero time.

The activation of enzymes such as iNOS (and the subsequent release of NO and other cytokines) might dramatically affect phase I metabolizing enzymes, a fact of important pharmacological and toxicological consequences. Therefore, a more mechanistic approach to study conditions (such as inflammation, ischemia reperfusion, etc.) leading to that activation is of major importance. Because of its unique characteristics, PCLS therefore appear as a suitable model to assess cellular interactions and its influence on hepatic metabolism and drug biotransformation.

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