Inactivation of Kupffer cells by gadolinium administration prevents lipopolysaccharide-induced decrease in liver insulin-like growth factor-I and IGF-binding protein-3 gene expression

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Abstract

Gram-negative bacterial infection or treatment of animals with bacterial lipopolysaccharide (LPS) induces a catabolic state with proteolysis, liver injury and an inhibition of the insulin-like growth factor-I (IGF-I) system. The purpose of this work was to elucidate the role of Kupffer cells in LPS-induced inhibition of the IGF-I/IGF-binding protein-3 (IGFBP-3) system. Adult male Wistar rats were either pretreated with the Kupffer cell inhibitor gadolinium chloride (10 mg/kg, i.v., 24 h prior to LPS exposure) or saline vehicle. Rats received two i.p. injections of 1 mg/kg LPS (at 17:30 and 08:30 h the following day) and were killed 4 h after the second injection. LPS administration induced a significant decrease in body weight and in serum concentrations of IGF-I and IGFBP-3 ($P<0{\cdot}01$), as well as in their gene expression in the liver. LPS-injected rats had increased serum concentrations of ACTH, corticosterone ($P<0{\cdot}05$), tumour necrosis factor-α (TNF-α) and nitrites ($P<0{\cdot}01$). Pretreatment of the animals with gadolinium chloride blocked the inhibitory effect of LPS on body weight, and on serum concentrations of IGF-I, IGFBP-3 and nitrites, as well as growth hormone receptor (GHR), IGF-I and IGFBP-3 gene expression in the liver. In contrast, gadolinium chloride administration did not modify the stimulatory effect of LPS on serum concentrations of ACTH, corticosterone and TNF-α. These results suggest that Kupffer cells are important mediators in the inhibitory effect of LPS on GHR, IGF-I and IGFBP-3 gene expression in the liver, leading to a decrease in serum concentrations of IGF-I and IGFBP-3.

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Introduction

Sepsis and inflammation are associated with negative nitrogen balance and proteolysis (Voerman *et al.* 1993). Inflammation induces several neuroendocrine changes that include an increase in serum concentrations of glucocorticoids along with a decrease in anabolic hormones such as insulin-like growth factor-I (IGF-I; Dahn *et al.* 1988, Katsanos *et al.* 2001). These endocrine modifications contribute to the catabolic state leading to a slower recovery from the illness.

Endotoxin, also known as lipopolysaccharide (LPS), is a component of the wall of Gram-negative bacteria, which triggers the physiological and endocrinological responses to sepsis. Endotoxin administration in rats increases plasma concentrations of corticosterone, whereas it decreases circulating IGF-I and its gene expression in the liver (Soto *et al.* 1998, Defalque *et al.* 1999). The adult liver is the main source of circulating IGF-I and its main serum-binding protein, IGF-binding protein-3 (IGFBP-3), which are synthesized by different cell populations. IGF-I is synthesized mainly by hepatocytes, whereas IGFBP-3 is released by nonparenchymal cells such as Kupffer, endothelial and hepatic stellate cells. The inhibitory effect of LPS on the IGF-I axis is also exerted at the IGFBP-3 level, since there is a decrease in serum concentration of this protein and its synthesis in the liver in LPS-treated rats (Priego *et al.* 2003*a*). Similarly, humans with chronic inflammatory illnesses have decreased serum concentrations of IGF-I and IGFBP-3 (Katsanos *et al.* 2001).

LPS-induced decrease in serum concentrations of IGF-I and IGFBP-3 is due to a direct inhibitory effect of LPS on liver IGF-I and IGFBP-3 gene expression, regardless of pituitary GH secretion (Defalque *et al.* 1999, Priego *et al.* 2003*b*). Induction of inducible nitric oxide synthase (iNOS) during sepsis is involved in the inhibition of IGF-I–IGFBP-3 after LPS administration (Priego *et al.* 2004).

LPS induces liver injury and the death of hepatocytes, which may have a role in liver dysfunction in sepsis (Nolan 1981). The liver plays a central role in the

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LPS response because it clears LPS from circulation and responds to LPS by releasing cytokines and reactive oxygen intermediates. Most of the toxicities of LPS in the liver have been related to those inflammatory mediators (for review see Su 2002). Kupffer cells represent the main cellular mediators of the effects of LPS in the liver. They play an important role in clearing LPS from blood (Mathison & Ulevitch 1979), and in releasing cytokines as well as other inflammatory molecules of the acute phase response. During inflammation, hepatocytes respond to products secreted by Kupffer cells with several metabolic changes, such as a decreased glucose production as a result of inhibition of enzymes involved in gluconeogenesis (Yerkovich *et al.* 2004). Inactivation of Kupffer cells prevents LPS-induced hepatocyte apoptosis and inhibits liver injury (Hamada *et al.* 1999). For that reason, it has been postulated that Kupffer cells or hepatic macrophages mediate most of deleterious effect in liver injury.

Gadolinium chloride is a Kupffer cell inhibitor. Use of gadolinium chloride is a suitable approach to studying the *in vivo* function of large Kupffer cells, since circulating monocytes and other macrophages are less vulnerable to gadolinium than Kupffer cells (Hardonk *et al.* 1992). Intravenously injected gadolinium chloride blocks Kupffer cell phagocytosis and the subsequent release of its mediators such as cytokines and nitric oxide after LPS administration (Hardonk *et al.* 1992). Furthermore, gadolinium administration reduces mortality (Roland *et al.* 1999) and liver injury (Lee *et al.* 2004) in sepsis.

The aim of this work was to analyse the role of Kupffer cells in LPS-induced inhibition of liver IGF-I and IGFBP-3 and activation of ACTH (corticotropin) and corticosterone secretion. The serum concentrations of tumour necrosis factor-α (TNF-α) and nitrites/nitrates were also analysed, as they are increased during the inflammatory response and may affect the endocrine system. The results suggest that Kupffer cells activated by LPS are involved in LPS-induced inhibition of liver IGF-I and IGFBP-3 gene expression and consequently in the decreased circulating levels of these proteins.

Material and Methods

Animals and experimental protocol

Male Wistar rats weighing 250–300 g (Harlam, Barcelona, Spain) were housed three or four per cage, under controlled conditions of temperature (22 °C) and light (lights on from 07:30 to 19:30 h). Food and water were available *ad libitum*. The procedures followed the guidelines recommended by the European Union for the care and use of laboratory animals.

Rats were randomly assigned to treatment group after 1-week adaptation to environment and diet. The day before start the LPS treatment, 20 rats were i.v. injected with 10 mg/kg gadolinium chloride (Sigma Chemical Co.) and 20 rats were i.p. injected with sterile saline, under halothane anaesthesia. This gadolinium protocol was shown to inhibit phagocytosis by rat liver Kupffer cells, and to reduce their response to LPS stimulation (Hardonk *et al.* 1992). The following day each group was divided in two groups; the first group received an i.p. injection of 1 mg/kg LPS (serotype 055:B5; Sigma Chemical Co.), and the second group received 250 μl sterile saline i.p. Rats received the LPS treatments at 17:30 h and at 08:30 h the following day. All animals were killed by decapitation at 12:30 h, 19:00 h after the first and 4 h after the second LPS, in a separate room, within 30 s of being removed from their cages. Blood was allowed to clot, and the serum was stored at −20 °C for IGF-I, IGFBP-3, corticosterone and nitrite assays, and at −80 °C for ACTH and TNF-α assays. Immediately after decapitation the liver was removed, dissected, frozen in liquid nitrogen and stored at −80 °C until RNA extraction was performed.

Hormone and TNF-α determination

Serum IGF-I concentrations were measured by a double-antibody RIA. The IGF-I antiserum (UB2–495) was a gift from Dr Underwood and Dr Van Wik (University of Maryland School of Medicine, Baltimore, MD, USA), and it is distributed by the Hormone Distribution Program of NIDDK through the National Hormone and Pituitary Program. Levels of IGF-I were expressed in terms of IGF-I from Gropep (Adelaide, Australia). The intra-assay coefficient of variation was 8%. All samples were run in the same assay.

Serum concentrations of corticosterone were determined by a competitive protein-binding assay. ACTH and TNF-α serum levels were measured by RIA and ELISA with commercial kits from Diagnostic System Laboratories (Webster, TX, USA) and Amersham Biosciences.

Nitrite determination

Nitrite and nitrate concentrations in serum were measured by a modified version of the Griess assay, described by Miranda *et al.* (2001). Serum was deproteinized to reduce turbidity by centrifugation through a 30 kDa filter using a Centrifree Micropartition Device with a YM-30 ultrafiltration membrane (Amicon Division, Millipore Corporation, Bedford, TX, USA), at 15 000 r.p.m. for 1 h at 37 °C for 300 μl samples. Filtrated serum (100 μl) or 1/10-diluted culture medium was mixed with 100 μl vanadium chloride, followed quickly by the addition of the Griess reagents. The determination was performed after incubation at 37 °C for 30 min. The absorbance was measured at 540 nm. Nitrite and nitrate concentrations were calculated using a $NaNO_2$ standard curve and expressed in micromolar.

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Western ligand blot of IGFBP-3

Serum samples (2·5 µl) were subject to 1% SDS/12·5% acrylamide gel non-reducing electrophoresis, and electro-transferred to nitrocellulose membranes (Hybond™-C extra; Amersham Biosciences). The membranes were dried and blocked for 1 h with 5% non-fat dry milk/0·1% Tween (Sigma Chemical Co.), in Tris-buffered saline. Membranes were probed overnight at 4 °C with ^{125}I-labelled IGF-I (5×10^5 c.p.m./ml). The nitrocellulose sheets were then washed, dried and blots were exposed at − 80 °C to X-ray film (Kodak X-Omat AR; Eastman Kodak, Rochester, NY, USA) with two intensifying screens for 1–2 days depending on the signal obtained. Autoradiographs were analysed by densitometric scanning using a PC-Image VGA24 program (Foster Findlay Associates, Newcastle upon Tyne, UK) for Windows. The density of the IGFBP bands in each lane was expressed as the percentage of the mean density of sera from control rats injected with saline.

RNA extraction and Northern blot analysis

Total hypothalamic RNA was extracted by the guanidine thiocyanate method using a commercial kit (Ultraspec™ RNA; Biotecx Laboratories, Houston, TX, USA), according to the protocol supplied by the manufacturer. For Northern blotting, 30 µg denatured RNA from each liver was separated by formaldehyde/agarose gel electrophoresis, transferred to nylon membranes (Hybond-N+; Amersham Biosciences) and fixed by UV crosslinking (Fotodyne, Hartland, WI, USA).

IGF-I and growth hormone receptor (GHR) mRNA hepatic levels were measured by Northern blot hybridization using riboprobes (Roberts *et al.* 1987, Baumbach *et al.* 1989). The rat IGF-I and GHR probes were derived from a HindIII fragment of the pGEM-3 plasmid vector (Promega). ^{32}P-Labelled RNA antisense probes were generated from linearized plasmid with [α-^{32}P]CTP (Nuclear Ibérica, Madrid, Spain) and T7 RNA polymerase (Roche Molecular Biochemicals). Prehybridization was performed for 30 min at 68 °C in ULTRAhyb™ buffer (Ambion, Austin, TX, USA) followed by hybridization for 16 h at the same temperature with 1×10^6 c.p.m./ml IGF-I-labelled riboprobe or 3×10^6 c.p.m./ml GHR-labelled riboprobe, in the same buffer.

The rat IGFBP-3 cDNA probe (Albiston & Herington 1990) was obtained by cutting the PEGEM 4Z plasmid vector using EcoRI and HindIII and labelling it with [^{32}P]dCTP (Nuclear Ibérica, Madrid, Spain) by a random-priming DNA-labelling kit (DECAprimeTM II; Ambion). Prehybridization was performed for 30 min at 42 °C with ULTRAhyb™ buffer followed by hybridization for 16 h at the same temperature with 3×10^6 c.p.m./ml IGFBP-3-labelled probe. To verify that equal amounts of RNA were loaded into each lane within an experimental group, control hybridization was performed with a 28 S DNA probe labelled with [^{32}P]dCTP by random primer.

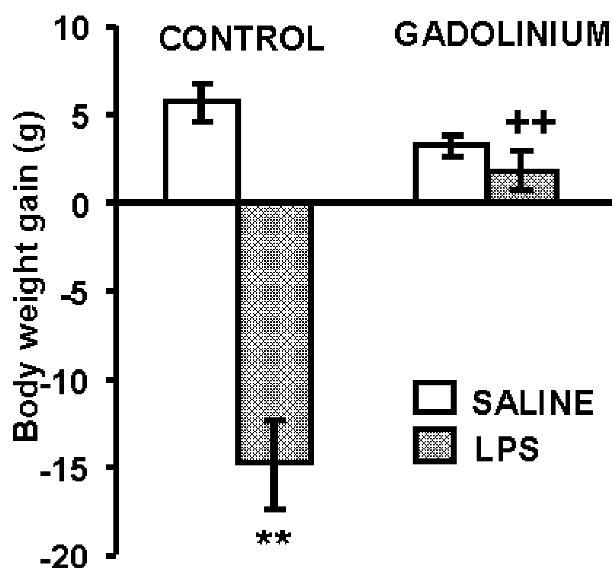

Figure 1 Effect of gadolinium pretreatment on body-weight gain response to LPS administration. There was an interaction between the effect of LPS and gadolinium on body-weight gain ($F_{1,24}$=20, P<0·01), since LPS decreased body weight in control rats, but not in the rats pretreated with gadolinium. Results are expressed as means ± S.E.M. for 5–9 rats per group; **P<0·01 versus control group injected with saline; ^{++}P<0·01 versus control group injected with LPS.

Statistical analysis

Statistics were computed using STATGRAPHICS plus for Windows. Statistical significance was calculated by multifactorial ANOVA with the LPS and gadolinium treatments as factors. When the ANOVA indicated a significant interaction between factors, individual means were compared by LSD multiple comparison test. A *P* value of less than 0·05 was considered significant.

Results

As shown in Fig.1, LPS administration induced a significant decrease (P<0·01) in body-weight gain in control rats, whereas LPS did not modify body-weight gain in rats injected with gadolinium.

The GHR mRNA in the liver was significantly decreased in control rats injected with LPS (P<0·05). Gadolinium treatment did not modify GHR mRNA in the liver of control rats. Gadolinium administration prevented the inhibitory effect of LPS on GHR gene expression in the liver (Fig. 2).

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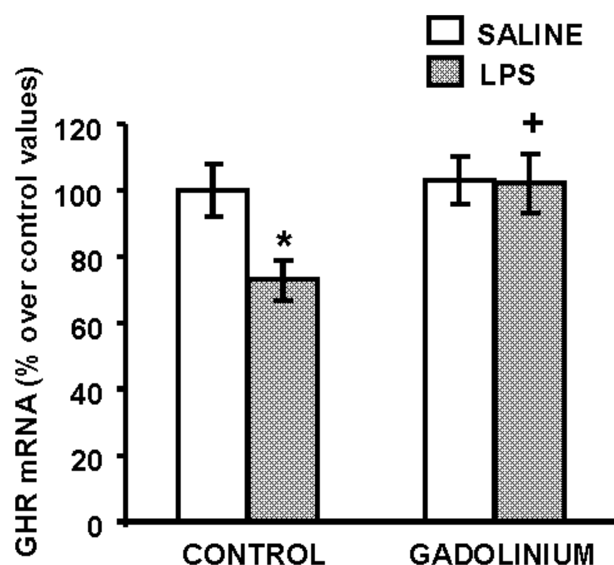

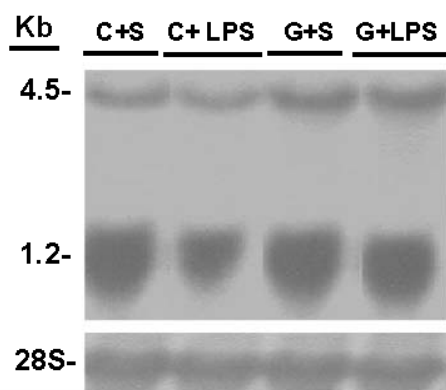

Figure 2 Effect of gadolinium (G) pretreatment on liver GHR mRNA in saline (S)- or LPS-treated rats. Data from 5–8 individual rats were quantified by densitometry and expressed as a percentage of the mean value in control (C) rats treated with saline. A representative Northern blot analysis showing the 4·8 and 1·2 kb GHR transcripts and the 28 S rRNA in each sample is shown below. Results are expressed as means ± S.E.M. for 5–9 rats per group; *$P<0·05$, versus control group injected with saline; $^{+}P<0·05$ versus control group injected with LPS.

The serum concentrations of IGF-I, liver IGF-I and IGF-I mRNA are shown in Fig. 3. In control rats, LPS administration induced a significant decrease in serum concentrations of IGF-I ($P<0·01$), in liver IGF-I ($P<0·01$) and in the IGF-I gene expression in the liver ($P<0·05$). Similar to the effects found in hepatic GHR mRNA, gadolinium pretreatment prevented the inhibitory effect of LPS on liver IGF-I, IGF-I mRNA and serum IGF-I levels (Fig. 3).

LPS administration induced a significant decrease in IGFBP-3 gene expression in the liver ($P<0·01$; Fig. 4), and gadolinium administration prevented the effect of LPS on liver IGFBP-3 mRNA. There was also a significant decrease ($P<0·01$) in serum concentrations of IGFBP-3 in the control rats injected with LPS, whereas gadolinium administration blocked the effect of LPS on serum IGFBP-3 levels (Fig. 4).

As can be seen in Fig. 5, LPS administration induced a significant increase in both ACTH and corticosterone serum levels ($P<0·01$). Gadolinium pretreatment did not modify the serum concentrations of ACTH or corticosterone in control rats or in rats treated with LPS (Fig. 5).

The serum concentrations of nitrites and TNF-α are shown in Fig. 6. Gadolinium pretreatment did not modify the serum concentrations of nitrites/nitrates, whereas LPS induced a significant increase ($P<0·01$) in serum nitrite/nitrate levels in control rats, but not in the rats pretreated with gadolinium chloride. In the rats injected with saline, the serum concentrations of TNF-α were undetectable. Gadolinium pretreatment did not modify TNF-α release after LPS injection, since both control and rats pretreated with gadolinium had similar serum concentrations of TNF-α (Fig. 6).

Discussion

Our data show that inactivation of Kupffer cells blocked LPS-induced inhibition of the IGF-I/IGFBP-3 system by preventing the LPS-induced decrease in its gene expression in the liver. However, gadolinium administration did not block the stimulatory effect of endotoxin administration on serum concentrations of ACTH and corticosterone. These data suggest that Kupffer cells play an important role in the IGF-I, but not in the glucocorticoid, response to endotoxin.

During sepsis, liver function is impaired and hepatocytes mainly synthesize acute-phase proteins, whereas constitutive protein synthesis is decreased in the liver. The inhibitory effect of LPS on liver IGF-I is well known. LPS is able to block the growth hormone-responsive genes in the liver of hypophysectomized rats (Bergard *et al.* 2000). In addition, cytokines and LPS are able to inhibit GHR and IGF-I gene expression both *in vitro* and *in vivo* (Wof *et al.* 1996, Defalque *et al.* 1999), suggesting that LPS induces GH resistance. Furthermore, LPS, at low doses, decreases liver IGF-I and IGFBP-3 gene expression in rats, whereas it stimulates GH secretion (Priego *et al.* 2003*b*). All these data indicate that one of the mechanisms by which LPS injection inhibits hepatic GHR, IGF-I and IGFBP-3 gene expression is by acting directly on the liver.

The greater decrease in serum concentrations of IGF-I and IGFBP-3 than in their mRNA in the liver can be due to modification in their half-life. IGFBP-3 is the major IGFBP in serum; the decrease in serum concentrations of IGFBP-3 may contribute to increasing IGF-I turnover and then to reducing IGF-I half-life in serum. The binary

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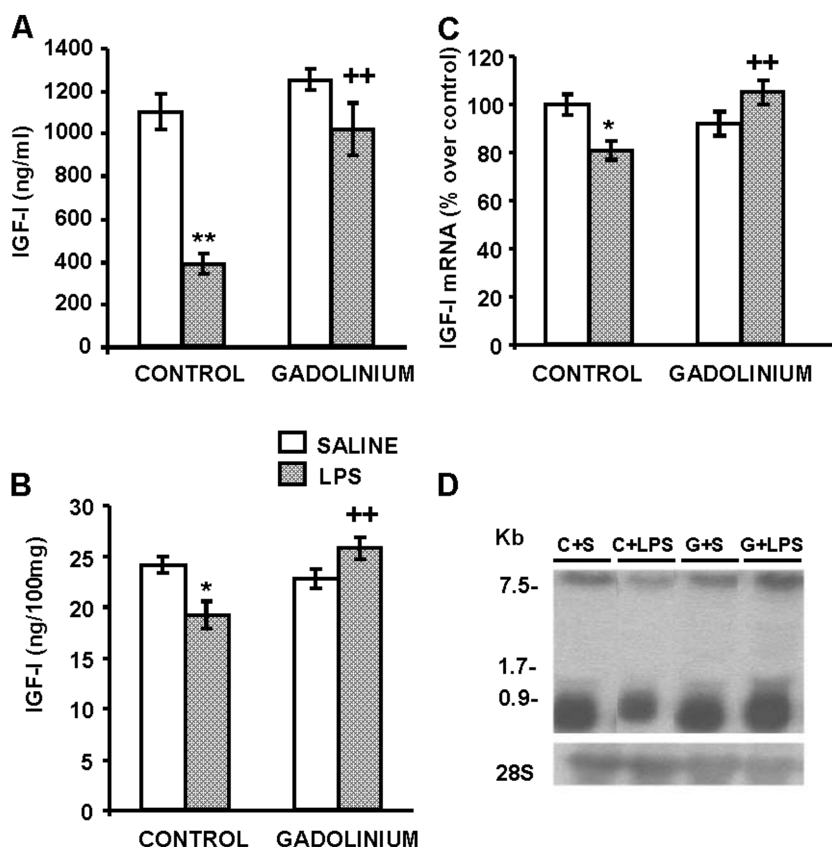

Figure 3 Effect of gadolinium (G) administration on IGF-I concentrations in serum (A) and liver (B) and on liver IGF-I mRNA (C) in rats injected with LPS or saline (S). There was an interaction between the effect of gadolinium and LPS on serum IGF-I ($F_{1,21}$=4,6, P<0·05), on liver IGF-I ($F_{1,22}$=8, P<0·01), and on liver IGF-I mRNA ($F_{1,22}$=7·8, P<0·05), since gadolinium administration prevented the inhibitory effect of LPS. Each bar represents the mean ± S.E.M. for at least 5–9 rats. *P<0·05, **P<0·01 versus control rats injected with saline; ^{++}P<0·01 versus control group injected with LPS. (D) A representative Northern blot of IGF-I mRNA hybridization. Total RNA (30 µg) was hybridized with an RNA probe for rat IGF-I mRNA. Each lane corresponds to an individual animal from the indicated group; the 28 S rRNAs are shown below. Detectable IGF-I mRNA species are indicated and consist of a group of transcripts ranging from 7·5 to 0·8 kb. Because all these transcripts may potentially be translated to IGF-I, the densitometric results corresponded to the sum of all IGF-I transcripts. Quantitative analyses of Northern blots are expressed as percentages of control rats (C) injected with saline.

complexes form a ternary complexes with the acid-labile subunit, or ALS, and endotoxin administration also induces a decrease in serum concentrations of ALS (Barreca *et al.* 1998, Kong *et al.* 2002). Then the decrease in circulating IGFBP-3 and ALS might contribute to the decline in serum IGF-I observed after LPS administration.

Gadolinium treatment prevents liver injury after LPS by inhibiting superoxide production of large Kupffer cells (Kono *et al.* 2001), and completely prevents the increase in oxygen uptake due to endotoxin. Gadolinium pretreatment prevents iNOS induction by LPS in Kupffer cells (Roland *et al.* 1996, Hamada *et al.* 1999). This inhibitory effect on LPS-induced iNOS expression has also been reported in hepatocytes (Tirmenstein *et al.* 2000) and in other tissues such as the lung (Fujii *et al.* 1998). Thus the ability of gadolinium to prevent LPS-induced increase in serum concentrations of nitrite/nitrate can be the result of blocking iNOS induction in several tissues. We have previously observed that inhibition of iNOS by aminoguanidine treatment prevents LPS-induced decrease in IGF-I and IGFBP-3 gene expression in the liver (Priego *et al.* 2004). Therefore, prevention of iNOS induction and nitric oxide release in the liver may be one of the possible mechanisms by which gadolinium administration prevents the effect of LPS on serum and liver IGF-I and IGFBP-3.

It can be seen in our data that gadolinium administration also prevented an LPS-induced decrease in

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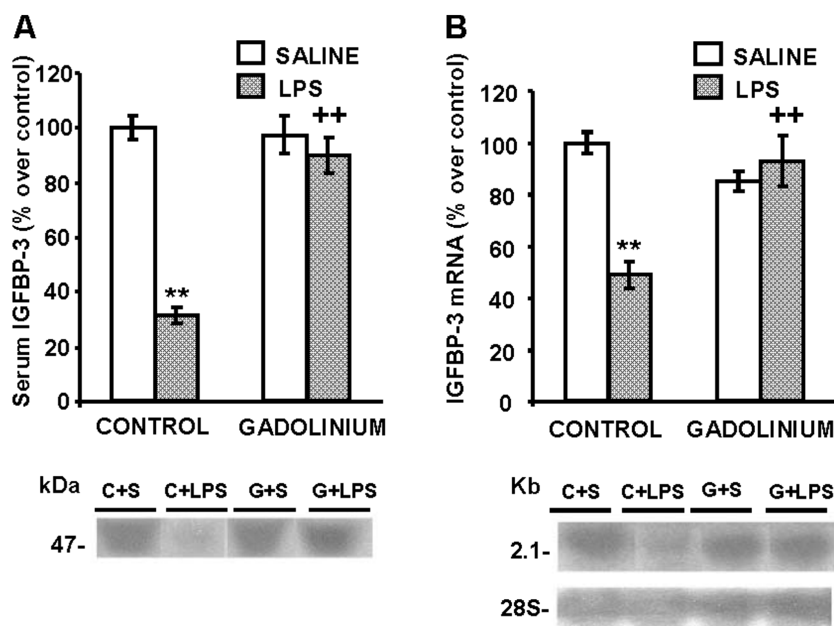

Figure 4 Effect of gadolinium (G) administration on serum concentrations of IGFBP-3 and on liver IGFBP-3 mRNA in rats injected with LPS or saline (S). (A) Western ligand blot analysis and quantification of serum IGFBP-3. Serum IGFBP-3 was separated by SDS/PAGE on a 12·5% acrylamide gel, transferred to nitrocellulose, ligand blotted with ^{125}I-IGF-I, and visualized via autoradiography. Data from 5–9 individual rats were quantified by densitometry and expressed as the percentage of the mean value in control rats (C) injected with saline. (B) A representative Northern blot of IGFBP-3 mRNA hybridization of 30 µg total liver RNA. The size of the hybridization band (in kb) is indicated. 28 S, 28 S rRNA. Quantitative analyses are expressed as percentages of control rats injected with saline, for five rats per group. There was an interaction between the effect of gadolinium and LPS on serum IGFBP-3 ($F_{1,22}=19$, $P<0·01$), and on liver IGFBP-3 mRNA ($F_{1,18}=10$, $P<0·01$), since gadolinium administration prevented the inhibitory effect of LPS. $^{**}P<0·01$ versus control rats injected with saline; $^{++}P<0·01$ versus control group injected with LPS.

body weight. A decrease in serum concentrations of IGF-I is associated with negative nitrogen balance and hypermetabolism (Jeschke *et al.* 2005). In addition, IGF-I and IGFBP-3 administration increases protein synthesis in the skeletal muscle in burned humans (Debroy *et al.* 1999) and in septic rats (Svanberg *et al.* 2000). Taking into account that the IGF-I/IGFBP-3 system plays an important role in anabolic reactions, it is logical to think that, in the LPS-treated rats, the effects of gadolinium on body weight and on the IGF system are related.

In contrast to the IGF-I system, the stimulatory effect of LPS on ACTH and corticosterone secretion was not modified by gadolinium pretreatment. Cytokines and their receptors are expressed both in the hypothalamus and in the pituitary (Faggioni *et al.* 1995, Arzt *et al.* 1999). During inflammation, cytokines stimulate the hypothalamus–pituitary–adrenal axis, and upon increased corticosteroid secretion they antagonize their peripheral proinflammatory action (for review, see Chesnokova & Melmed 2002). In our study, gadolinium administration does not seem to affect cytokine release in tissues outside the liver, since it did not modify serum concentrations of TNF-α after LPS injection. Similar results have previously been observed by other authors (Kohno *et al.* 1997, Rizzardini *et al.* 1998, Kono *et al.* 2001). Furthermore, Kono *et al.* (2002) have reported that although gadolinium administration prevented LPS-induced increase in TNF-α gene expression in the liver, it did not modify the serum concentrations of TNF-α. However, it has recently been reported that gadolinium treatment decreased superoxide generation, but conversely increased Kupffer cell TNF-α production after LPS stimulation (Kinoshita *et al.* 2005). These data indicate that gadolinium treatment decreases phagocytosis and superoxide production in Kupffer cells, whereas its effect on TNF-α and cytokine release is less clear.

It has been reported that depletion of macrophages did not prevent pituitary–adrenal activation after 2·5 mg/kg LPS administration (Derijk *et al.* 1991), suggesting that during LPS-induced sepsis, other mechanisms apart from macrophage-derived products are involved in the activation of the hypothalamus–pituitary–adrenal axis.

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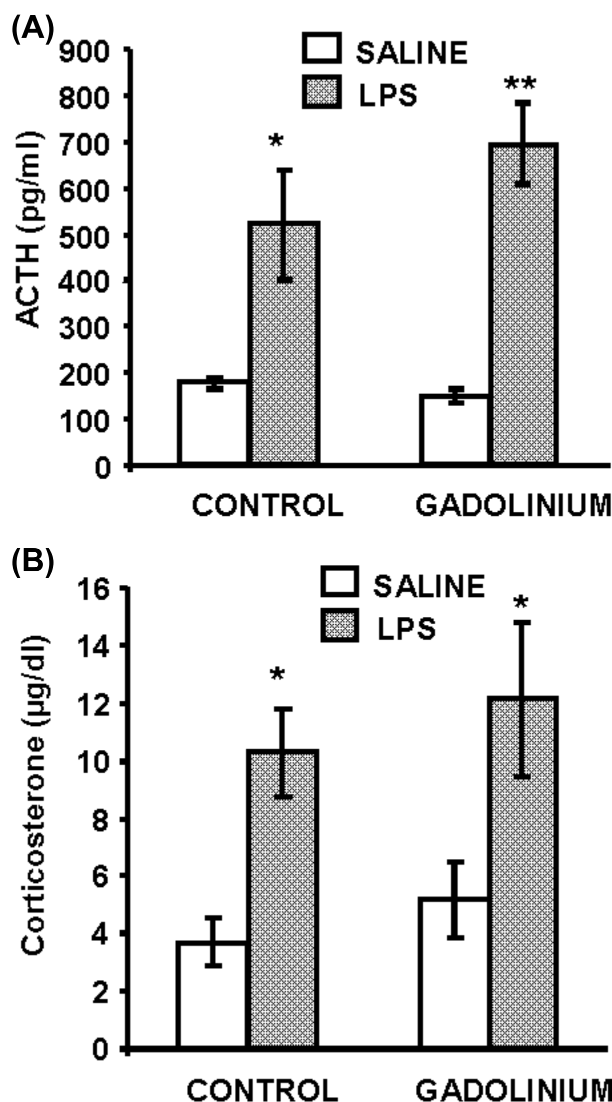

Figure 5 Serum concentrations of ACTH (A) and corticosterone (B) after LPS administration in control or gadolinium-treated rats. LPS administration increased the serum concentrations of ACTH ($F_{1,19}$=12·8, P<0·01) and corticosterone ($F_{1,23}$=10·7, P<0·01), whereas gadolinium had no significant effect. Results are expressed as means ± S.E.M. for 5–9 rats per group; **P<0·01, *P<0·05 versus the respective group injected with saline.

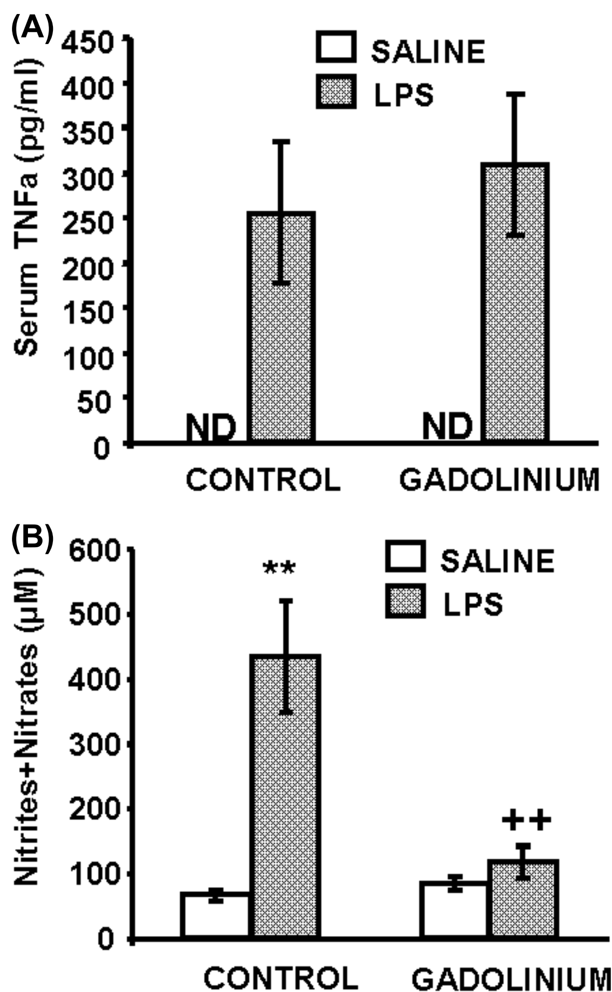

Figure 6 Serum concentrations of TNF-α (A) and nitrites/nitrates (B) in control and gadolinium-treated rats injected with saline or LPS. There was an interaction between the effect of gadolinium and LPS on serum concentrations of nitrites/nitrates ($F_{1,28}$=6·12, P<0·05), since LPS increased the serum concentrations of nitrites/nitrates in control, but not in the rats pretreated with gadolinium. ND, no detectable values. Results are expressed as means ± S.E.M. for 5–9 rats per group; **P<0·01 versus control rats injected with saline; ^{++}P<0·01 versus control group injected with LPS.

These authors postulated that high doses of endotoxin may directly stimulate endothelial cells to produce cytokines and prostaglandin-E_2 and thereby activate the hypothalamic–pituitary–adrenal axis in a macrophage-independent manner (Tilders *et al.* 1994).

Inactivation of Kupffer cells by gadolinium pretreatment is not able to prevent all the physiological reactions induced by LPS injection. It has previously been reported that gadolinium administration is not able to prevent the haemodynamic alterations of acute endotoxaemia (Fujii *et al.* 1998). Similarly, despite improved survival, gadolinium chloride failed to prevent laboratory and clinical signs of disseminated intravascular coagulation in endotoxaemic rats (Ruttinger *et al.* 1998).

On other hand, IGF-I has an important role in the early stages of liver tissue repair (Scharf *et al.* 2004). IGF-I administration results in effective prevention of lethal acute liver failure induced by D-galactosamine plus LPS treatment in rats (Inoue *et al.* 2003). For that reason, the therapeutic potential of IGF-I in the prevention of acute liver failure has been suggested. In addition,

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transgenic mice overexpressing IGF-I have attenuated fibrogenesis and accelerated liver regeneration after liver injury (Sanz *et al.* 2005).

Since the IGF-I system has been involved in tissue regeneration, these findings suggest that one of the possible mechanisms by which gadolinium prevents liver injury and metabolic derangement in septic rats is through normalization of the IGF-I system.

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