Short-term effects of thalidomide analogs on hepatic glycogen and nitric oxide in endotoxin-challenged rats

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Abstract. Hepatic glycogen metabolism is altered by nitric oxide (NO) during endotoxic shock. Thalidomide analogs immunomodulate the endotoxin-induced cytokines which regulate the NO release. We analyzed the short-term effects of some thalidomide analogs on the hepatic glycogen store and on the plasma and hepatic NO in an acute model of endotoxic challenge in rat. An endotoxin dose selection was performed. Rats received vehicle, thalidomide or analogs orally and, two hours after last dose, they were injected with endotoxin (5 mg/kg). Animals were sacrificed 2 h after challenge. Liver glycogen was quantified by the anthrone technique. Plasma and hepatic NO was determined by Griess reagent and HPLC. Hepatic interferon-γ, a NO co-inducer, was measured by ELISA. Endotoxin caused inverse dose-dependent effects on plasma NO and on glycogen. Thalidomide analogs showed short-term regulatory effects on glycogen, some of them increased it. Plasma NO was almost unaffected by analogs but hepatic NO was strikingly modulated. Analogs slightly up-regulated the liver interferon-γ and two of them increased it significantly. Thalidomide analogs may be used as a pharmacological tool due to their short-term regulatory effects on glycogen and NO during endotoxic shock. Drugs that increase glycogen may improve liver injury in early sepsis.

Key words: Endotoxin — Glycogen — Liver — Nitric oxide — Thalidomide

Introduction

Hepatic glycogen perhaps is the main source of energy in the organism. The control on synthesis and degradation of hepatic glycogen is crucial to regulate the plasma glucose level. It is very well known that gram-negative bacterial infections (endotoxic shock) or the treatment of animals with bacterial endotoxin or lipopolysaccharide (LPS) are characterized by biphasic alterations in hepatic carbohydrate homeostasis (Horton et al. 1994). Formerly, there is a hepatic glycogen mobilization by glycogenolysis with consecutive hyperglycemia; afterward, a hypoglycemia is present because of a decreased gluconeogenesis and elevated glucose consumption (Ceppi et al. 1997). Thus, the liver seems to be a major target organ during endotoxic shock.

Nitric oxide (NO) is a short-lived mediator which can be produced in a variety of cell types by inducible NO synthase (iNOS) and generates many physiologic and metabolic changes in target cells (Geller et al. 1993). In liver resident macrophages (Kupffer cells) and hepatocytes, the expression of iNOS is induced by LPS (Billiar et al. 1990; Aono et al. 1997). Cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interferon-γ (IFN-γ), are other important mediators of NO production, because they regulate the expression of iNOS in hepatocytes and Kupffer cells (Geller et al. 1993; Harbrecht et al. 1994). Although IL-1β alone can initiate hepatic iNOS expression, the synthesis of NO in hepatocytes is dependent on the simultaneous synthesis of IFN-γ and synergized with TNF-α (Schroeder et al. 1998). In fact, a large amount of cytokines is quickly

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released from Kupffer cells and hepatocytes after LPS injection as well as in endotoxemia (Luster et al. 1994; Aono et al. 1997; Sang et al. 1999) to later induce the production and release of NO (Titheradge 1999).

Among the many biological effects of NO is the modulation of hepatic glycogen in either processes glycogenolysis or glycogen synthesis (Borgs et al. 1996; Casado et al. 1996; Sprangers et al. 1998). It has been established that NO, as a mediator of endotoxemia, is capable of inhibiting total glucose mobilization, glycogenolysis and gluconeogenesis in hepatocytes, and the expression of iNOS has also correlated with the time of start of the inhibition of these parameters (Horton et al. 1994; Ceppi et al. 1997). Moreover, it has been proposed that iNOS plays a role in LPS-induced hyperglycemia (Sugita et al. 2002). Additionally, the effects on glucose metabolism in hepatocytes elicited by NO are regulated by cytokines and LPS (Ceppi et al. 1996; Ceppi and Titheradge 1998).

Thalidomide (α-N-phthalimidoglutarimide) is currently used as an immunomodulatory and anti-inflammatory drug (Marriott et al. 1999). Nowadays, there are novel structural thalidomide analogs which possess improved immunomodulatory effects on cytokines, increased stability, and besides minor adverse effects (Corral et al. 1999; Marriott et al. 1999). Indeed, we have previously showed immunomodulatory effects of thalidomide analogs on LPS-induced plasma and hepatic cytokines in rat (Fernández-Martínez et al. 2004).

All above mentioned data prompted us to assess the short-term effects of some thalidomide analogs on the hepatic glycogen store and on the plasma and hepatic NO in an acute model of endotoxic challenge in rat, as a possible consequence of their immunomodulatory actions on cytokines.

Materials and Methods

Drugs and chemicals

All reagents used for biochemical determinations were acquired of the best quality available commercially. Thalidomide analogs were obtained as we detailed before (Fernández-Martínez et al. 2001, 2004). Synthetic routes to obtain thalidomide analogs were performed as described by Muller and coworkers (Muller et al. 1996, 1998). The reaction conditions were modified and developed in our laboratory based on previously reported methods (Shealy et al. 1968; Kalvin and Woodart 1985). The following thalidomide analogs were synthesized: 3-phthalimido-3-(3,4-dimethoxyphenyl)-propanoic acid (PDA); 3-phthalimido-3-(3,4-dimethoxyphenyl)-propanamide (PDP); methyl 3-phthalimido-3-(3,4-dimethoxyphenyl)-propanoate (PDPme); methyl 3-(4-nitrophthalimido)-3-(3,4-dimethoxyphenyl)-propanoate ($4NO_2$PDPme); methyl 3-(4-aminophthalimido)-3-(3,4-dimethoxyphenyl)-propanoate (4APDPme); methyl 3-tetrafluorophthalimido-3-(3,4-dimethoxyphenyl)-propanoate (TFPDPme); 3-phthalimido-3-(3-ethoxy, 4-methoxyphenyl)-propanitrile (PEMN).

Animals

All animals used in the subsequent experiments were male Wistar rats weighing about 200–250 g. The animals were housed in regular plastic cages at constant temperature, under a 12 h light-dark cycle and had free access to food (standard Purina chow diet; Purina Laboratory, USA) and water *ad libitum*. Rats received human care according to the institutions' guidelines and the Mexican Official Norm (NOM-062-ZOO-1999) regarding technical specifications for production, care and use of laboratory animals as well as according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publ. 86-23, rev. 1985).

Endotoxin (LPS) dose selection

This experiment was carried out in order to choose the LPS dose for the acute septic challenge. The animals had free access to food and water until they were manipulated. Animal groups were as follows ($n = 6$, each group): the control group was injected intraperitoneally (i.p.) with sterile saline solution (NaCl, 0.9% w/v) and the treated groups were i.p. injected with LPS (dissolved in saline) from *Escherichia coli* serotype 026:B6 (Sigma Chemical Co., USA), at the respective doses of 0.5, 1, 5, 10 and 15 mg/kg. Three hours after saline or LPS injection, rats were sacrificed by exsanguination under light ether anesthesia. Blood was collected by cardiac puncture using a syringe containing sodium heparin as anticoagulant. Liver was rapidly removed and rinsed in saline. All samples were frozen at −70°C until analyzed. Plasma samples were used to determine NO and liver sections were obtained to quantify glycogen. Three hours were considered to be a time enough to obtain evident and stable short-term effects on hepatic glycogen and plasma NO, both caused by the different endotoxin doses.

Acute animal treatment

Animal groups used for acute septic challenge were as follows ($n = 6$, each group): the group V+S (control) was administered orally twice with an interval of 12 h in between with the vehicle carboxymethylcellulose (CMC, 0.9 % w/v); then, 2 h after last dose of vehicle, rats were i.p. injected with sterile saline (NaCl, 0.9 % w/v). Group V+LPS (damaged control) also received vehicle orally twice within the same interval of time, and 2 h after last dose of vehicle, rats were i.p. injected with LPS (5 mg/kg, dissolved in saline) from *Escherichia coli*

serotype 026:B6 (Sigma Chemical Co., USA). Group T+LPS was administered orally twice with an interval of 12 h in between with thalidomide (100 mg/kg) suspended in CMC 0.9%, and 2 h after last dose of thalidomide, rats were injected i.p. with LPS. The remaining groups underwent the same schedule as thalidomide group; analogs were suspended in vehicle and thus received orally twice administrations as the equimolar dose of 100 mg/kg of thalidomide, again 2 h after last dose of drugs rats were i.p. injected with LPS. Food was immediately withdrawn while rats were injected. Two hours after saline or LPS injection, rats were sacrificed by exsanguination under light ether anesthesia. Blood was collected by cardiac puncture using a syringe containing sodium.

The animal groups treated with thalidomide or its analogs did not show any sign of sickness behavior or side effects elicited by drugs administration during the dosage period previous to LPS challenge; in addition, there was not an evident alteration in animals' health 2 h after LPS injection (5 mg/kg). That time was mainly chosen because the plasma and hepatic levels of the majority of cytokines regulating NO production are significantly increased within the initial 2 h (Fernández-Martínez et al. 2004); also, as commented before, at such dose and time the animals' health is not compromised as it happens later at 3 h after LPS injection of 5 up to 15 mg/kg doses, when the animals show slight hypothermia (data not shown) as well as beginnings of sickness behavior, chiefly for both 10 and 15 mg/kg doses.

Glycogen quantification

Small liver pieces (0.5 g) were separated for glycogen determination using the anthrone-sulfuric acid reagent (Seifter et al. 1950).

Measurement of plasma and hepatic NO

In the case of plasma samples taken from the experimental groups employed to select the LPS dose, NO was indirectly quantified as its oxidized end products NO_2^- + NO_3^- by a method previously described (Muriel 1998), based on the Griess reaction (Green et al. 1982).

NO was also determined as NO_2^- + NO_3^- in the plasma and liver samples obtained from challenged groups and administered with thalidomide analogs. However, a modified high performance liquid chromatography (HPLC) technique was used, based on a previously reported method (Rizzo et al. 1998); that because of analogs showed to cause some interference with the earlier method. Briefly, 400 µl of serum was centrifuged at 12,000 rpm (11,290 × *g*) for 60 min at 4°C, with a micropore filter unit (Ultrafree MC UFC3, Millipore, France) to remove substances larger than 10 kDa. Liver samples underwent an earlier procedure to get liver extracts, such as it is indicated in the following section; subsequently, liver extracts were deproteinized following the same treatment above mentioned for plasma. Once samples were deproteinized, NO_2^- + NO_3^- were determined under optimized chromatographic conditions. The HPLC system consisted of a Waters 600 controller pump (Waters, USA), equipped with a Rheodyne sample injector with a 50 µl sampling loop. Chromatographic separation was performed on a reversed-phase analytical column (3.9 × 300 mm), packed with octadecylsylane-C_{18}, 10 µm particle size (Bondapak, USA). Mobile phase was 20 mmol/l N-octylamine, pH 4.0 (corrected with H_3PO_4) and the flow-rate was 1 ml/min. Detection was achieved by means of a UV detector (Waters, USA). The absorbance was measured at a wavelength of 214 nm. NO_2^- + NO_3^- measurement was carried out using an external standard solution of either NO_2^- or NO_3^-, obtained from solutions of $NaNO_2$ and $NaNO_3$. The retention times obtained were 8.17 min for NO_2^- and 10.34 min for NO_3^-. Liver NO_2^- + NO_3^- levels were expressed as nmol/g of tissue.

Hepatic IFN-γ determination

With the aim of obtaining liver extracts, pieces of 1 g of liver were homogenized on ice in 5 ml of cold phosphate buffered saline (PBS), pH 7.4, containing a protease inhibitors cocktail tablets (Complete Roche, Germany). The homogenates were centrifuged at 15,000 rpm (17,147 × *g*) for 15 min at 4°C. Supernatants were filtered through a 0.45 µm filter (Millex-HA, Millipore, France) and again centrifuged at 15,000 rpm for 15 min at 4°C. Liver extracts were removed and kept at –70°C until cytokine analysis was performed. Hepatic IFN-γ amounts were determined by enzyme linked immunosorbent assay (ELISA), using a commercial kit selective for this rat cytokine (Biosource International, USA). Manufacturer's directions were followed. Hepatic amounts of cytokines were calculated per 1 g of wet tissue in 5 ml of PBS and expressed as pg/mg.

Statistics

For statistical analysis, ANOVA with the Student-Newman-Keuls test was used to compare experimental groups (Zar 1984). Resulting data are expressed as means ± SEM of five rats in each group at least, and analyzed using SigmaStat software version 3.1 (Systat Software Inc., USA). In all cases a difference was considered significant when $p < 0.05$.

Results

Dose-dependent effects of LPS on glycogen and NO

Three hours after LPS injection, a significant dose-dependent drop of hepatic glycogen from its original content was induced

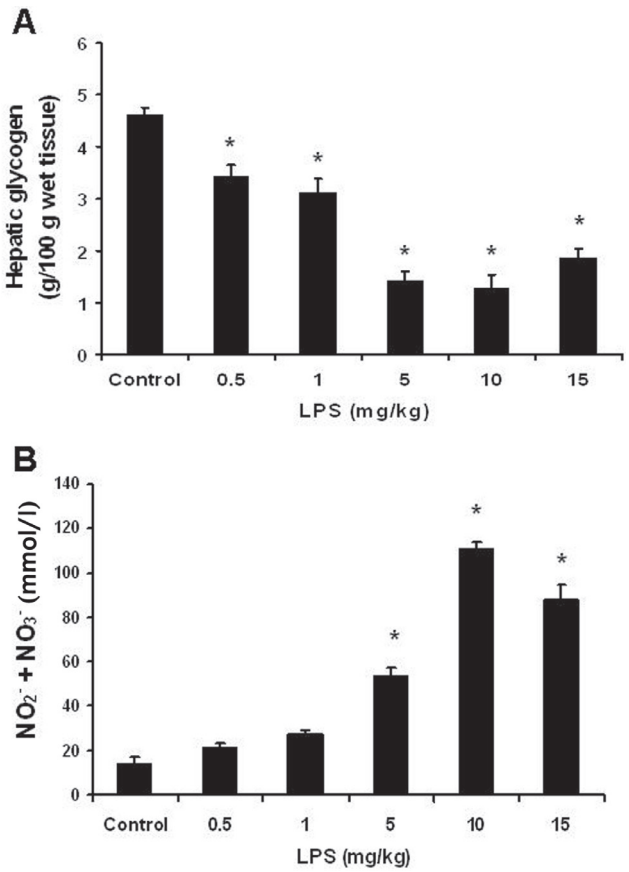

Figure 1. Glycogen content determined in livers (A) and NO end products expressed as NO_2^- + NO_3^- concentration in sera (B), both from rats i.p. injected with saline solution (control group) or different doses of LPS. Each bar represents the mean value of experiments performed in duplicate assays with samples from 5 animals at least ±SEM. * different from control group, ANOVA Student-Newman-Keuls' test, $p < 0.05$.

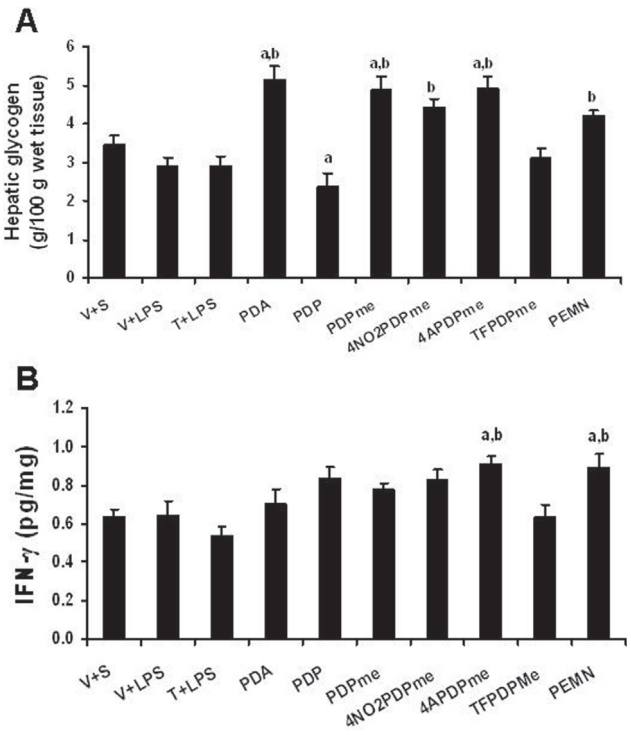

Figure 2. Glycogen content (A) and hepatic amount of IFN-γ (B), both determined in livers from rats treated twice a day with vehicle (V), thalidomide (T, 100 mg/kg) or its analogs in equimolar dose. 2 h after last dose of drugs, rats were injected with saline (S) or LPS (5 mg/kg). Groups administered with thalidomide analogs plus LPS were only named as analog abbreviations for simplification. 2 h after injections rats were sacrificed and liver samples were taken. Each bar represents the mean value of experiments performed in duplicate assays with samples from 5 animals at least ±SEM. [a] different from control group (V+S); [b] different from damaged control group (V+LPS). ANOVA Student-Newman-Keuls' test, $p < 0.05$.

(Fig. 1A). The high doses (5, 10 and 15 mg/kg) elicited similar low values of glycogen in liver, diminishing it almost 70%.

Fig. 1B depicts the serum concentration of NO end products NO_2^- + NO_3^- induced by LPS. Serum NO was increased clearly in a dose-dependent manner and reached statistical significance from 5 up to 15 mg/kg of LPS when compared to control group concentration. It is important pointing out that NO elevation correlates with the hepatic glycogen decrease.

Effects of thalidomide analogs on glycogen and IFN-γ in livers from LPS-challenged rats

As can be seen in Fig. 2A, hepatic glycogen was diminished slightly 2 h after LPS injection, however, such value was not different ($p < 0.05$) from control group and resembled the glycogen content of experimental groups administered with thalidomide and its analog TFPDPme; in addition, the analog PDP lowered the glycogen content scarcely although significantly with respect to V+LPS group. The five remaining thalidomide analogs not only prevented the glycogen diminution but increased its liver content strikingly, even compared to control group.

Two hours after LPS injection, hepatic IFN-γ was almost unaltered. However, thalidomide analogs 4APDPme and PEMN augmented the liver amount of this cytokine in some extent, reaching statistical significance (Fig. 2B).

Effects of thalidomide analogs on plasma and liver NO from LPS-challenged rats

LPS did not increase NO_2^- + NO_3^- serum concentration within the time of determination (Fig. 3A). Although the

majority of thalidomide analogs almost unaffected the levels of $NO_2^- + NO_3^-$ in serum, the thalidomide analogs PDA and PEMN elevated them two-fold ($p < 0.05$).

The injection of LPS induced a soon and remarkable increase in hepatic $NO_2^- + NO_3^-$ amount, three times higher than control group value; whereas thalidomide and most of its analogs regulated the liver $NO_2^- + NO_3^-$ amount elicited by LPS, increasing or diminishing the NO in a significant way (Fig. 3B).

Discussion

Sepsis and septic shock are substantial for morbidity and mortality. When sepsis results in hypotension and multiorgan dysfunction, it is referred to as septic shock (Das 2003); in fact, since liver is a target organ, the hepatic failure is mediated by LPS-induced activation of cytokines and NO release. Furthermore, cirrhotic individuals and rats may be predisposed to endotoxic shock due to their impaired defense mechanisms; therefore they may show an even more compromised carbohydrate metabolism, increased hepatic damage (Rackow and Astiz 1991; Harry et al. 1999; Titheradge 1999) and augmented plasma level of nitrite and nitrate. Such increase in NO level is directly related to endotoxemia (Guarner et al. 1993; Harry et al. 1999).

Three hours after LPS injection, the hepatic glycogen showed a dose-dependent drop which correlates with the dose-dependent increase in plasma $NO_2^- + NO_3^-$, given that liver glycogen is very sensitive to the stress induced by endotoxic challenge and to the consequent NO rise. This prompt response is well documented; administration of LPS to rats causes hypoglycemia, which is associated with a depletion of hepatic glycogen content and an impairment of the gluconeogenic capacity of the liver (Lang et al. 1987). Sprangers et al. (1998) found that the glycogen content of isolated rat hepatocytes falls down within the first hour after a NO donor exposure and they also confirmed that NO inhibits the glycogen synthesis. In addition, other authors pointed out a drop in liver glycogen content in rats treated 1–6 h earlier with 1 mg/kg of LPS (Casado et al. 1996). Furthermore, NO as $NO_2^- + NO_3^-$ is increased significantly in plasma 3–6 h after the i.p. administration of 0.5 mg/kg of LPS (Harry et al. 1999). Also, the hepatic iNOS expression and its activity are augmented within 2–5 h following similar endotoxic challenges (Casado et al. 1996; Heller at al. 2000). The dose of 5 mg/kg was chosen to be used in the endotoxic challenge due to its capability of diminishing the hepatic glycogen in a similar extent than higher doses, as well as increasing the NO content significantly ($p < 0.05$), but without compromising the animal's health.

After the 2 h of the challenge with LPS, interesting short-term effects of the thalidomide analogs were observed in liver

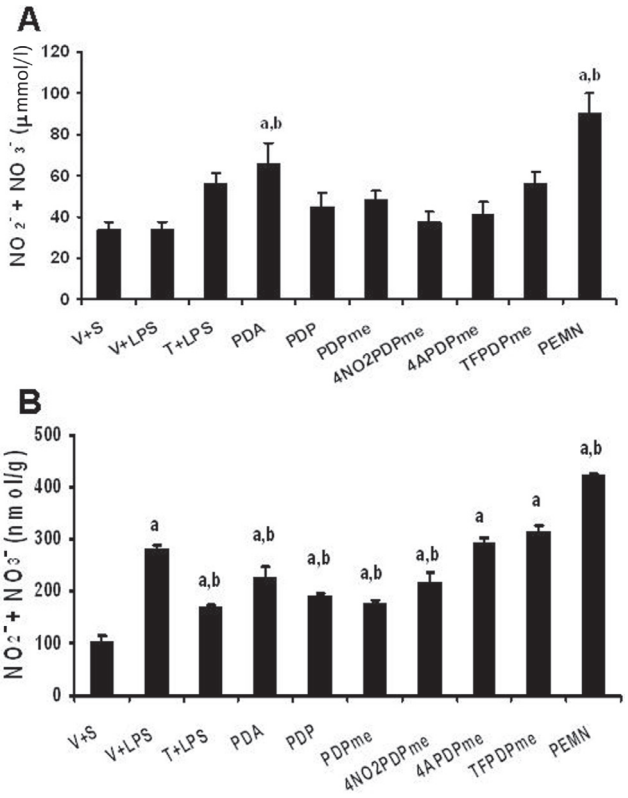

Figure 3. Serum concentration (A) and hepatic amount of NO end products expressed as $NO_2^- + NO_3^-$ (B), both determined in sera and livers from rats treated twice a day with vehicle (V), thalidomide (T, 100 mg/kg) or its analogs in equimolar dose. 2 h after last dose of drugs, rats were injected with saline (S) or LPS (5 mg/kg). Groups administered with thalidomide analogs plus LPS were only named as analog abbreviations for simplification. 2 h after injections rats were sacrificed and liver samples were taken. Each bar represents the mean value of experiments performed in duplicate assays with samples from 5 animals at least ±SEM. [a] different from control group (V+S); [b] different from damaged control group (V+LPS). ANOVA Student-Newman-Keuls' test, $p < 0.05$.

on glycogen, $NO_2^- + NO_3^-$, IFN-γ and in plasma on $NO_2^- + NO_3^-$. Although at 2 h post-endotoxin injection there was a slight trend to lower the glycogen liver content, this was not statistically significant it might be due to a longer time required (3 h) to observe a more severe effect as we discussed in the previous paragraph. Two hours were the selected time since it has been reported to be enough to induce a short-term significant glucose output by glycogenolysis and plasma NO rise after LPS injection (Casado et al. 1996; Sugita et al. 2002), as well as to trigger the release of plasma and liver cytokines such as TNF-α, IL-1β and IL-6 which are involved in glycogen metabolism and NO production (Ceppi et al. 1996; Kanemaki et al. 1998; Fernández-Martínez et al. 2004). Hence, despite the fact that at 2 h after challenge

there was not a significant difference between the glycogen content from V+S and V+LPS groups, the short-term effects on liver glycogen by thalidomide analogs deserve to be analyzed. Hepatic glycogen was increased notably by the administration of five thalidomide analogs, in spite of their very well known inhibitory activity on phosphodiesterase-4, what may explain the reduction on glycogen content by the analog PDP (Muller et al. 1998; Corral et al. 1999). There are several reports on the glycogenolytic effect exerted by diverse phosphodiesterase (PDE) inhibitors or adenylyl cyclase stimulants, which are adenosine 3′,5′-cyclic monophosphate (cAMP)-elevating drugs that showed similar effects when compared with glucagon, a peptide hormone activator of adenylyl cyclase (Iijima et al. 1985; Hermsdorf and Dettmer 1998; Abdollahi et al. 2003). PDE-3 and PDE-4 inhibitors increase serum glucose levels formed by glycogenolysis and/or gluconeogenesis as a consequence of decreasing cAMP degradation and stimulating high cAMP intracellular levels that determine the phosphorylation state of many key enzymes in the metabolism of liver glycogen (Abdollahi et al. 2003). Additionally, increasing of cAMP levels by PDE inhibitors provokes immunomodulatory effects on cytokines that induce liver damage and NO overproduction; therefore PDE inhibitors protect from liver injury in rodent models (Gantner et al. 1997; Fernández-Martínez et al. 2001). On the other hand, the glycogen-augmenting effects observed herein by the administration of the thalidomide analogs may be explained as follows: i) A paradoxical stimulation by the thalidomide analogs on rat liver PDE-3 or PDE-4 enzyme activity, both regulated by guanosine 3′,5′-cyclic monophosphate (cGMP), protein kinase A (PKA) and protein kinase C (PKC) (Vasta and Beavo 2004). Then, thalidomide analogs might be a kind of substrate competitors in a given subcellular compartment and at low local concentration; this effect has been observed with methylxanthines, very well known as PDE-inhibitors (Erneux et al. 1982). ii) The second possible explanation is related to the first one and to the increment of NO during LPS-induction. When NO concentration is elevated, it paradoxically inhibits glycogenolysis; this situation is reminiscent of the anti-glucagon action of NO. It has been proposed that NO would stimulate guanylyl cyclase and consequently cGMP-stimulated cAMP PDE, which should result in hydrolysis of cAMP (Borgs et al. 1996). iii) Indeed, the rise in glycogen by thalidomide analogs may be the result of a complex net of processes, such as the increase in cAMP and the consequent feedback inhibition or immunomodulation on the glycogen synthesis-inhibitory cytokines TNF-α, IL-1β and IL-6. Since TNF-α inhibits the transcriptional rate of glucose-6-phosphatase as well as IL-1β and IL-6 are capable of diminishing the glycogen synthase activity, elevating in turn the glycogen phosphorylase activity at the same time (Metzger et al. 1997; Kanemaki et al. 1998; Fernández-Martínez et al. 2004).

Hepatic IFN-γ amount was similar in almost all groups after 2 h of endotoxic challenge, but the analogs 4APDPme and PEMN increased it significantly, although this family of derivatives does not possess stimulatory properties on Th1 cytokines as IFN-γ (Corral et al. 1999). Sang et al. (1999) reported an increase in IFN-γ mRNA in rat liver within 30 min after an i.p. injection of LPS (4 mg/kg) and this was still observed at 3 h post-challenge, but we did not observe a marked expression of that cytokine. Nevertheless, IFN-γ was assessed in our study because it is the sole dominant iNOS-inducing agent in macrophages (Aono et al. 1997). The IL-1β-mediated hepatocyte synthesis of NO is dependent on the simultaneous endogenous synthesis of IFN-γ (Schroeder et al. 1998) and this cytokine mainly enhances the inhibition of gluconeogenesis and glycogen breakdown in hepatocytes elicited with TNF-α, IL-1β plus LPS, this emulates what happens in endotoxic shock (Ceppi and Titheradge 1998).

Plasma and liver NO_2^- + NO_3^- levels showed different patterns after LPS-induction. While plasma NO_2^- + NO_3^- concentration was almost unaffected within 2 h, reflecting the systemic production, the hepatic amount was remarkably three-fold augmented. That suggests that liver is one of the most sensitive target organs during septic shock (Titheradge1999), and that hepatic NO is perhaps one of the main and fastest regulators of short-term hepatic responses induced by LPS. Thalidomide and most of its analogs trended to increase the plasma concentration of NO_2^- + NO_3^- but only PDA and PEMN reached statistical significance, perhaps by synergy with LPS challenge. On the other hand, thalidomide and four of its analogs partially but significantly prevented the elevation of hepatic NO_2^- + NO_3^- amount, while 4APDPme and TFPDPme were unable to achieve it. There are two likely explanations: i) hepatic NO_2^- + NO_3^- levels are the outcome from their immunomodulatory effects as PDE-4 inhibitors (Fernández-Martínez et al. 2004) or ii) authors as Shimazawa et al. (2004) have recently reported that thalidomide and some homophthalimide derivatives possess NOS-inhibitory activity. They propose that one or more of the thalidomide metabolites or decomposition products might be more potent as NOS inhibitors; the family of compounds evaluated herein was designed resembling thalidomide metabolites (Muller et al. 1996). Liver NO inhibition by some of the thalidomide analogs may be correlated to increased hepatic glycogen. Particularly, PEMN was the analog that enhanced the LPS-induced plasma and liver NO_2^- + NO_3^- levels; this may be in part due to the high hepatic IFN-γ amount (which we observed) and low hepatic quantity of the NOS inhibitory cytokine IL-10 (Fernández-Martínez et al. 2004).

Finally, a reason for dissimilarities in the effects on glycogen, NO and IFN-γ of these thalidomide analogs may be found in different half-lives, stability of each compound, solubility and potency (Fernández-Martínez et al. 2004). In

conclusion, thalidomide analogs may be used as a pharmacological tool due to their short-term regulatory effects on hepatic glycogen and NO during endotoxic shock. Drugs that increase hepatic glycogen may improve liver injury in early sepsis.

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