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Efficacy of a selective histamine H_2 receptor agonist, dimaprit, in experimental models of endotoxin shock and hepatitis in mice

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Abstract

Dimaprit, a selective histamine H_2 receptor agonist, was examined in experimental models of endotoxin shock and hepatitis in mice. Injection of lipopolysaccharide (8 mg/kg i.v.) into Balb/c mice resulted in an elevation of plasma tumor necrosis factor-α (TNF-α), reaching the maximal level at 1 h post-lipopolysaccharide (1147 U/ml). Oral administration of dimaprit 200 mg/kg, 1 h prior to lipopolysaccharide challenge, inhibited the increase in plasma TNF-α by 71% and also the survival rate was increased to 62.5% from 8.3% in the disease control. In a mouse hepatitis model, simultaneous injection of galactosamine (700 mg/kg i.v.) and lipopolysaccharide (3 μg/kg i.v.) into Balb/c mice caused an increase in plasma TNF-α, peaking at 1 h, followed by an elevation of L-alanine aminotransferase (E.C.2.6.1.2) activity at 4 h onward. Oral administration of dimaprit 200 mg/kg, 1 h prior to galactosamine and lipopolysaccharide, reduced the increase in plasma TNF-α by 99% and L-alanine aminotransferase by 82%. In vitro, dimaprit dose dependently inhibited the production of TNF-α in mouse peritoneal macrophages and human peripheral blood monocytes stimulated with lipopolysaccharide with IC_{50} values of 1 μM. The decrease in TNF-α production by dimaprit was reversed by cimetidine, a histamine H_2 receptor antagonist. Dimaprit dose dependently suppressed TNF-α mRNA in human peripheral blood monocytes. These results suggest that activation of the histamine H_2 receptor downregulates the production of TNF-α, and that histamine may be an important regulator in pathological conditions in which TNF-α plays an important role.

Keywords: Dimaprit; Histamine H_2 receptor; TNF-α (tumor necrosis factor α); Hepatitis; Septic shock; Inflammation

1. Introduction

Tumor necrosis factor α (TNF-α) is a key mediator in immunological and inflammatory responses (Tracey and Cerami, 1994; Tracey, 1995). Accumulating evidence suggests that many of the pathophysiological effects associated with lipopolysaccharide are mediated by TNF-α (DeForge et al., 1990; Dunn, 1991). Injection of lipopolysaccharide induces a rapid and transient appearance of plasma TNF-α in various animal models (Tracey et al., 1987; Sloane et al., 1992). In sepsis, elevated TNF-α in the circulation is considered to contribute to hypotension and organ injury in, for example, the heart, lung and liver (Parrillo, 1989; Tracey and Cerami, 1994). In humans, TNF-α is suggested to be involved in the pathogenesis of liver failure such as alcoholic, fulminant and chronic hepatitis (Muto et al., 1988; McClain and Cohen, 1989; Yoshioka et al., 1989). Experimentally, the importance of TNF-α in galactosamine/lipopolysaccharide-induced toxicity is supported by the fact that neutralization of TNF-α with a specific antibody prevents the lipopolysaccharide-induced lethality (Remick et al., 1990). Additionally, injection of a small amount of TNF-α caused lethal toxicity accompanying fulminant hepatitis in galactosamine-sensitized mice (Lehmann et al., 1987; Hewett et al., 1993).

Histamine is another important mediator in pathophysiological conditions, including gastric acid secretion, vasodilatation, smooth muscle contraction and neurotransmission, and it is a well-known mediator of allergic reactions. Recent studies have shown that interaction of histamine with its specific receptors exerts inflammatory reactions by modulating inflammatory cytokines (Baumann, 1994). The histamine H_1 receptor promotes the production of acute-phase proteins and activates the complement system in

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inflammatory reactions, while the histamine H_2 receptor mediates the immunomodulating effects of histamine by inhibiting the production of effector molecules, such as immunoglobulin, interleukin-6 and interferon-γ (Falus and Meretey, 1992). It has also been reported that histamine inhibits the expression of TNF-α and interleukin-1 in lipopolysaccharide-stimulated human monocytes via activation of the histamine H_2 receptor (Kunkel et al., 1988; Hotermans et al., 1991; Vannier et al., 1991; Vannier and Dinarello, 1993). But these studies are limited to in vitro effects, and the role of histamine in the regulation of TNF-α production in vivo is still poorly understood.

In the present study, using a histamine H_2 receptor-specific agonist, dimaprit, we evaluated the physiological role of the histamine H_2 receptor activation in murine models of endotoxin shock and galactosamine/lipopolysaccharide-induced hepatitis, where TNF-α functions as a key mediator.

2. Materials and methods

2.1. Reagents

Dimaprit (*S*-[3-dimethylaminopropyl]-isothiourea dihydrochloride) was prepared in the Department of Medicinal Chemistry at Pfizer (Aichi, Japan). Freund's complete adjuvant and lipopolysaccharide (*E. coli.* 055:B5) used in in vitro experiments were purchased from Difco Laboratories (Detroit, MI, USA). Lipopolysaccharide (*E. coli.* 0111:B4) used in in vivo experiments, galactosamine (D-galactosamine hydrochloride), cimetidine and other reagents were obtained from Sigma (St. Louis, MO, USA) unless specified. RPMI 1640 medium (RPMI 1640), Minimum essential medium (MEM) and Ca^{2+},Mg^{2+}-free phosphate-buffered saline were purchased from Nissui Seiyaku (Tokyo, Japan). L929 cells were obtained from American Type Culture Collection (Rockville, MD, USA).

2.2. Animals

Male Balb/c mice, 6 weeks of age, were purchased from SLC (Shizuoka, Japan). They were housed on a 12:12-h dark-light cycle and were provided with standard food and water ad libitum.

2.3. Production of TNF-α in murine peritoneal macrophages

Murine peritoneal macrophages were prepared from exudates of mice treated i.p. with Freund's complete adjuvant as described by Kunkel et al. (1988). Macrophages were suspended in RPMI 1640 supplemented with 1% fetal bovine serum (Biowhitakker, Walkersville, MD, USA), seeded into 48-well plates at 2×10^5/well and allowed to adhere at 37°C for 1 h. After being washed twice with the medium, the plates were again incubated for 4 h in the presence of lipopolysaccharide at 10 μg/ml with or without a given concentration of dimaprit. After incubation, supernatants were collected and frozen until assayed. TNF-α was quantitated by the L929 bioassay method (Kunkel et al., 1988). In brief, an aliquot of supernatant serially diluted with MEM containing 1% fetal bovine serum was incubated with L929 cells in 96-well flat-bottomed plates (Nunc, Roskilde, Denmark) at 37°C for 24 h. After supernatants were discarded, cell viability was determined by crystal violet staining (0.2% in 20% methanol). Recombinant human TNF-α (specific activity 2×10^7 U/mg, Wako Chemicals, Osaka, Japan) was used as a standard.

2.4. Production of TNF-α in human peripheral blood monocytes

Human peripheral blood mononuclear cells were isolated from fresh heparinized human whole blood by Ficoll-Paque (Pharmacia, Uppsala, Sweden) density centrifugation, washed twice with phosphate-buffered saline, suspended with RPMI 1640 containing 10% fetal bovine serum and plated into 48-well plates (Costar, Cambridge, MA, USA). After incubation at 37°C for 1 h, the supernatant was removed. Adhered monocytes (2×10^5 cells/well) were washed twice with fetal bovine serum-free RPMI 1640, and the supernatant was replaced with RPMI 1640 containing 1% fetal bovine serum. The cells were incubated at 37°C for 4 h in the presence of lipopolysaccharide at 10 μg/ml with or without a given concentration of dimaprit. Supernatants were collected and frozen until assayed for TNF-α. In a separate experiment, to determine the effect of a histamine H_2 receptor antagonist, human peripheral blood monocytes were co-cultured with lipopolysaccharide (10 μg/ml) plus dimaprit in the presence or absence of 300 μM cimetidine at 37°C for 4 h. The plates were centrifuged and the supernatants were assayed for TNF-α by the ELISA.

2.5. Northern blot analysis for TNF-α mRNA in human peripheral blood monocytes

Human peripheral blood monocytes were prepared as described above. Monocytes (2×10^6 cells in 2 ml RPMI 1640 containing 10% fetal bovine serum) were incubated at 37°C for 2 h in the presence of 10 μg/ml of lipopolysaccharide with or without a given concentration of dimaprit. Total RNA was extracted by acid guanidinium thiocyanate-phenol-chloroform method. RNA was dissolved in 1.2% agarose/formaldehyde gels and transferred to a GeneScreen Plus membrane (Dupont, Boston, MA, USA). cDNA for human TNF-α was isolated by PCR-amplification using Human TNF-α Amplimer Set (Clontech, Palo Alto, CA, USA). This cDNA fragment was labeled with [α-^{32}P]deoxy-CTP (Amersham, Tokyo, Japan)

using the rediprim DNA labeling system (Amersham). Hybridization was performed at 37°C for 2 h with a QuickHyb hybridization solution (Stratagene, La Jolla, CA, USA) containing ^{32}P-labeled probe. The membrane was washed twice with 0.1% sodium dodecylsulfate (SDS) containing 0.1 × standard saline citrate (SSC; 1 × SSC, 150 mM NaCl and 15 mM sodium citrate, pH 7.0) and exposed to an X-ray film (Fuji Photo Film, Tokyo, Japan). Thereafter, the blot was stripped by boiling in 1.0% SDS containing 0.1 × SSC and re-hybridized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) DNA (Oncor, Gaithersburg, MD, USA) as a control. The autoradiogram was scanned by a densitometer (Shimadzu, Kyoto, Japan) for quantitation of transcription levels.

2.6. Lipopolysaccharide-induced shock in the mouse

Dimaprit dissolved in the 0.1% (v/v) methylcellulose in sterile water vehicle was administered to mice 1 h prior to i.v. injection of lipopolysaccharide (8 mg/kg). Mice were bled 1 h post-lipopolysaccharide and plasma TNF-α was determined by the L929 bioassay as described above. Survival of mice was monitored over 72 h after the lipopolysaccharide challenge.

2.7. Galactosamine / lipopolysaccharide-induced hepatitis in the mouse

Galactosamine was dissolved in saline (Ohtsuka Pharmaceuticals, Osaka, Japan) and adjusted to pH 7.0 with NaOH. Male Balb/c mice were orally administered specified doses of dimaprit suspended in 0.1% methylcellulose, 1 h prior to i.v. injection of galactosamine (700 mg/kg) and lipopolysaccharide (3 μg/kg). Mice were bled twice at 1 h and at 8 h for TNF-α and L-alanine aminotransferase determination, respectively. Plasma TNF-α was quantitated by the L929 bioassay, and L-alanine aminotransferase activity was determined by a standard photometric method and expressed in Karmen units (K.U.; Karmen et al., 1955).

2.8. Histological examination

In the hepatitis experiment, mice were killed at 8 h after the challenge with galactosamine and lipopolysaccharide. The liver was removed from individual mice, fixed in 4% formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

2.9. Statistics

Statistical analysis of experimental data was done by using one-way analysis of variance test (ANOVA) and $P < 0.05$ was considered as statistically significant. The IC_{50} value was determined from the plotted curve of concentration (log) versus percentage inhibition (linear) by the method of least squares.

3. Results

3.1. Suppression of TNF-α production by dimaprit in murine peritoneal macrophages and human peripheral blood monocytes

Murine peritoneal macrophages produced 695 U/10^6 cells of TNF-α after lipopolysaccharide-stimulation, while no TNF-α was detected in unstimulated macrophages. Dimaprit suppressed the lipopolysaccharide-induced TNF-α production. At 1 μM, 50% inhibition of TNF-α production was achieved and 90% inhibition was observed at concentrations greater than 10 μM. Similarly, dimaprit decreased the production of TNF-α in human peripheral blood monocytes with an IC_{50} of 1 μM. No TNF-α was detected in unstimulated cells (Fig. 1). Cimetidine (300 μM), a histamine H_2 receptor antagonist, reversed the suppressive effect of dimaprit on TNF-α production in human peripheral blood monocytes (Fig. 2), whereas cimetidine alone did not affect TNF-α production (data not shown).

3.2. Effect of dimaprit on TNF-α mRNA in human peripheral blood monocytes

Human peripheral blood monocytes were treated with a given concentration of dimaprit in the presence of lipopolysaccharide for 2 h. Total RNA was isolated and TNF-α mRNA was determined by Northern blot hybridization using a 450-bp cDNA probe encoding human TNF-α. Dimaprit dose dependently decreased TNF-α mRNA levels (Fig. 3A). The concentration required to produce 50% reduction in mRNA was approximately 1 μM (Fig. 3B), which is in good agreement with the IC_{50} value for inhibition of TNF-α production in human peripheral blood monocytes (Fig. 1).

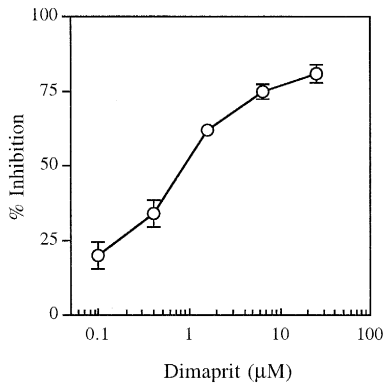

Fig. 1. Inhibition by dimaprit of lipopolysaccharide-induced production of TNF-α in human peripheral blood monocytes. Monocytes were prepared from heparinized fresh human whole blood and stimulated with lipopolysaccharide (10 μg/ml) in the presence of dimaprit. After incubation at 37°C for 4 h, supernatants were collected and assayed for TNF-α by a specific ELISA. Values are means ± S.E.M. of four separate experiments performed in duplicate. The control lipopolysaccharide group produced TNF-α at 0.39 ± 0.07 ng/2 × 10^5 cells ($n = 4$).

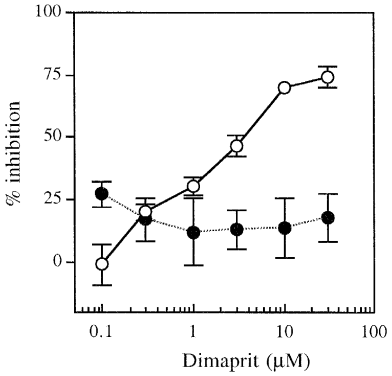

Fig. 2. Effect of a histamine H_2 receptor antagonist, cimetidine, on the dimaprit-induced decrease in TNF-α production. Monocytes were stimulated with lipopolysaccharide (10 μg/ml) and a given concentration of dimaprit in the presence (●) or absence (○) of cimetidine (300 μM). After incubation at 37°C for 4 h, supernatants were collected and assayed for TNF-α by an ELISA. Data are means ± S.E.M. of three separate experiments performed in duplicate. The control lipopolysaccharide group produced TNF-α at 1.09 ± 0.27 ng/2×10^5 cells ($n = 3$).

3.3. Effects of dimaprit on lipopolysaccharide-induced TNF-α production and lethality in the mouse

To determine the inhibitory effect of dimaprit on the production of TNF-α in vivo, dimaprit was orally administered to mice 1 h prior to i.v. injection of lipopolysaccharide (8 mg/kg). Plasma TNF-α levels were measured at 1 h after the lipopolysaccharide challenge. At this time point, the plasma TNF-α reached a plateau with 1147 ± 59 u/ml ($n = 12$) (Table 1). More than 90% of mice died within 72 h after the lipopolysaccharide injection. Oral administration of dimaprit reduced plasma levels of TNF-α at doses greater than 30 mg/kg, and 71% inhibition was achieved at 200 mg/kg. The survival rate was significantly im-

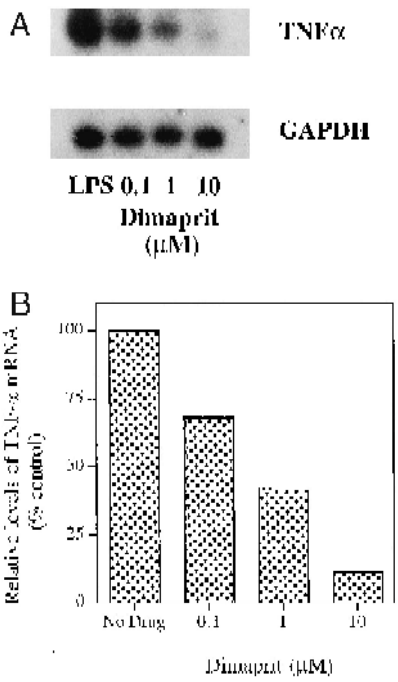

Fig. 3. Effect of dimaprit on TNF-α mRNA in human peripheral blood monocytes. Monocytes were incubated with lipopolysaccharide (10 μg/ml) and a given concentration of dimaprit at 37°C for 2 h. Total RNA was extracted and hybridized with cDNA probe for human TNF-α as described in the text. The blot was stripped and rehybridized with GAPDH as a normal control (A). The film was scanned by a densitometer and normalized to GAPDH (B).

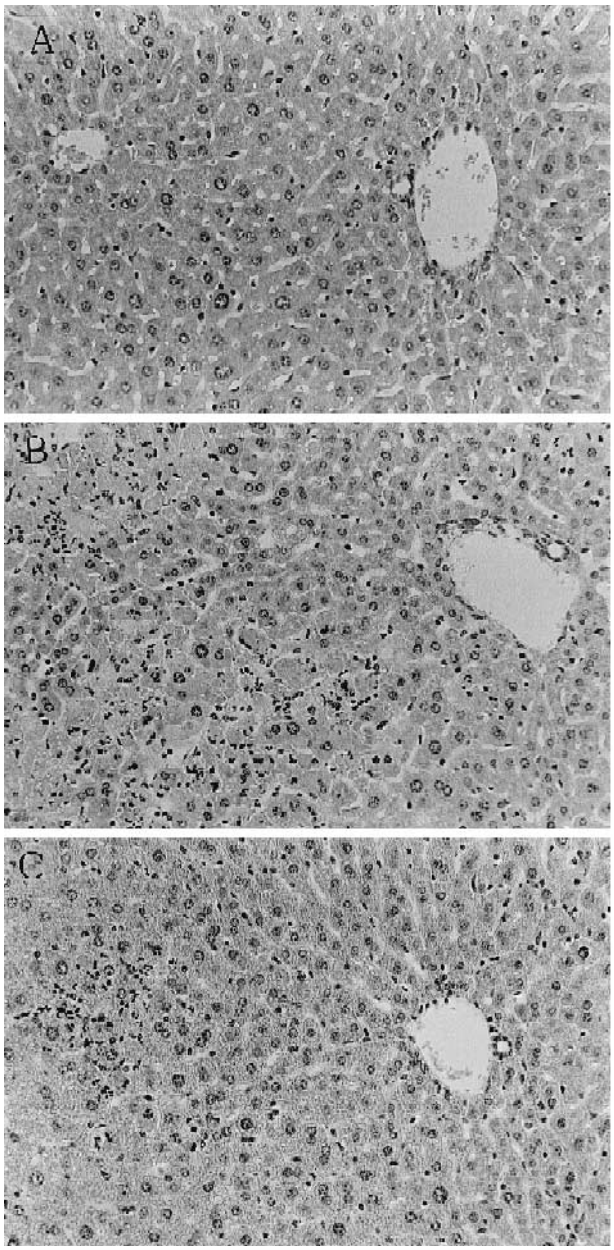

Fig. 4. Histological appearance of the liver from mice challenged with galactosamine and lipopolysaccharide. Balb/c mice were i.v. injected with 700 mg/kg of galactosamine and 3 μg/kg of lipopolysaccharide, and livers were taken 8 h after the challenge. Liver tissues were fixed in formalin, sectioned and stained with hematoxylin and eosin as described in the text. (A) Normal mice injected with saline, (B) galactosamine and lipopolysaccharide-challenged mice and (C) galactosamine and lipopolysaccharide-challenged, dimaprit (100 mg/kg p.o.)-treated mice. Original magnification ×66.

Table 1
Suppression by dimaprit of plasma TNF-α and lethality in lipopolysaccharide-challenged mice

Treatment	Dose (mg/kg)	*n*	TNF-α (U/ml)	Percentage inhibition	Percentage survival
Lipopolysaccharide	–	12	1147 ± 59	0	8.3
Dimaprit	30	8	618 ± 39 [a]	46	37.5
	100	8	726 ± 105 [a]	37	62.5
	200	8	328 ± 35 [a]	71	62.5

Balb/c mice were orally administered a given dose of dimaprit 1 h prior to lipopolysaccharide challenge (8 mg/kg i.v.). Mice were bled for the determination of plasma TNF-α by the L929 assay at 1 h post-lipopolysaccharide. Detection limit of TNF-α was 10 u/ml. Survival rate was assessed at 72 h after the lipopolysaccharide challenge. *n* = number of animals per group. Values are means ± S.E.M. [a] $P < 0.01$.

proved at doses greater than 30 mg/kg, and 62.5% survival was seen at 200 mg/kg, while only 8.3% of mice survived in the disease control.

3.4. Effects of dimaprit on galactosamine / lipopolysaccharide-induced liver injury

Simultaneous i.v. injection of galactosamine (700 mg/kg) and lipopolysaccharide (3 μg/kg) caused a transient increase in plasma TNF-α followed by a sustained elevation of transaminase activity (data not shown). The efficacy of dimaprit was examined in the galactosamine/lipopolysaccharide-induced hepatitis model. Galactosamine or lipopolysaccharide alone produced little effect on plasma TNF-α and L-alanine aminotransferase levels. Combination of both exerted a synergistic effect on plasma TNF-α, as well as L-alanine aminotransferase levels (Table 2). The increase of L-alanine aminotransferase was correlated well with histopathological findings, observed as hepatic necrosis and infiltration of inflammatory cells (Fig. 4A,B). Oral administration of dimaprit reduced plasma TNF-α and L-alanine aminotransferase levels in a dose-dependent manner (Table 2). At a dose of 30 mg/kg p.o. dimaprit exhibited 50% inhibition, while at doses above 100 mg/kg p.o. restored plasma TNF-α and L-alanine aminotransferase levels to the normal levels ($P < 0.01$). Histologically, dimaprit (100 mg/kg p.o.) clearly diminished necrotic lesions, while infiltration of inflammatory cells was partially inhibited (Fig. 4C).

4. Discussion

Our results demonstrated that oral administration of dimaprit, a specific histamine H_2 receptor agonist, decreases the plasma TNF-α concentration and protects mice from endotoxic shock. Passive immunization of mice with a specific antiserum against TNF-α prevented lipopolysaccharide-induced lethality (Remick et al., 1990) and subsequent studies demonstrated that reduction of TNF-α in the circulation resulted in the prevention of lethal shock in sepsis models (Zabel et al., 1993; Proctor et al., 1994). Dimaprit also displayed an inhibitory effect against TNF-α production in lipopolysaccharide-stimulated murine peritoneal macrophages and human monocytes (Fig. 1). The suppressive effects of dimaprit on TNF-α production were reversed by a histamine H_2 receptor antagonist, cimetidine (Fig. 2). Thus the efficacy of dimaprit in the lipopolysaccharide-induced shock model suggests that dimaprit suppresses the production of TNF-α via interaction with the histamine H_2 receptor in vivo.

Galactosamine, an amino sugar, is known to induce

Table 2
Effects of dimaprit on plasma TNF-α and L-alanine aminotransferase levels in galactosamine and lipopolysaccharide-induced hepatitis

Treatment	Dose (mg/kg)	*n*	TNF-α (U/ml)	Percentage inhibition	L-Alanine aminotransferase (K.U.)	Percentage inhibition
Disease [a]	–	24	321 ± 96	0	336 ± 16	0
Dimaprit	30	12	160 ± 55 [c]	53	190 ± 24 [b]	49
	100	12	35 ± 6 [c]	94	113 ± 14 [c]	75
	200	12	20 ± 3 [c]	99	92 ± 11 [c]	82
Saline		6	16 ± 10		40 ± 4	
Galactosamine		6	14 ± 8		58 ± 9	
Lipopolysaccharide		6	56 ± 9		50 ± 4	

Balb/c mice were dosed orally with dimaprit 1 h prior to i.v. injection of galactosamine (700 mg/kg) and lipopolysaccharide (3 μg/kg). Mice were bled at 1 h and 8 h for the determination of plasma TNF-α and L-alanine aminotransferase, respectively. Percentage inhibition was calculated by comparison to the disease group, after the values for the saline group were subtracted. *n* = number of animals per group. Data are means ± S.E.M. [a] Galactosamine and lipopolysaccharide treatment, [b] $P < 0.05$; [c] $P < 0.01$.

hepatitis similar to human viral hepatitis in the presence of lipopolysaccharide (Keppler et al., 1968). In humans, TNF-α is a key mediator responsible for the pathogenesis of chronic viral hepatitis (Gonzalez-Amaro et al., 1994). Galactosamine alone did not induce the production of significant amounts of TNF-α in the plasma nor symptoms of hepatitis. But the combination of galactosamine and lipopolysaccharide resulted in an increase of plasma TNF-α that peaked at 1 h. This was subsequently followed by an increase of transaminase activity that lasted from 4 to 24 h post-challenge, resulting in death of animals (data not shown). In this study we showed that oral pretreatment with dimaprit, 1 h prior to galactosamine and lipopolysaccharide challenge, significantly decreased plasma TNF-α levels and L-alanine aminotransferase activity in doses from 100 mg/kg p.o. (Table 2).

Histologically, lesions of hepatocytes were significantly reduced at doses greater than 100 mg/kg (Fig. 4), indicating that inhibition of TNF-α production is effective for prevention of hepatic injuries. Our results are in agreement with a previous study in which passive immunization against TNF-α improved the survival rate of mice with galactosamine/lipopolysaccharide-induced septic shock (Barton and Jackson, 1993).

Histamine is a vasoactive amine and an important effector molecule in allergic and inflammatory processes. Histamine reportedly attenuates the production of TNF-α in lipopolysaccharide-stimulated human monocytes, and this effect is reversed by a histamine H_2 receptor antagonist cimetidine, indicating the involvement of the histamine H_2 receptor in the regulation of TNF-α synthesis (Hotermans et al., 1991; Vannier et al., 1991). In the central nervous system, histamine is also present and may regulate the production of TNF-α (Alvarez et al., 1994). Histamine receptors are expressed on a variety of immune cells, including lymphocytes, monocytes and neutrophils (Falus and Meretey, 1992). Dimaprit is a highly specific functional histamine H_2 receptor agonist (Parsons et al., 1977; Hill, 1990), which increases cAMP at a micromolar concentration in a variety of cells (Sawutz et al., 1984; Borchard et al., 1986; Foreman et al., 1986). Evidence presented in this study that dimaprit exerts a protective effect in galactosamine/lipopolysaccharide hepatitis strongly suggests that the histamine H_2 receptor is involved. Generally, macrophages secrete a number of cytokines upon the exposure to lipopolysaccharide (Johnston, 1988). In endotoxic shock, monocytes, Kupffer cells and hepatic sinusoidal cells are considered to be a main source of inflammatory cytokines (Chensue et al., 1991).

It is concluded that the inhibition of TNF-α is a major mechanism in the hepatoprotective effect of dimaprit in vivo and that the histamine H_2 receptor may play an important role in the regulation of the pathogenesis of galactosamine/lipopolysaccharide-induced hepatitis. This is the first report demonstrating hepatoprotective activity of a histamine H_2 receptor agonist in vivo and suggests a potential therapeutic use of a histamine H_2 receptor agonist in TNF-α-mediated diseases.

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