ELSEVIER

European Journal of Pharmacology 313 (1996) 129–134

ejp

Tolerance to lipopolysaccharide-induced increase in vascular permeability in mouse skin

Emiko Fujii *, Kaoru Irie, Yoko Uchida, Fujiko Tsukahara, Ken-ichi Ohba, Akira Ogawa, Takamura Muraki

Department of Pharmacology, Tokyo Women's Medical College, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162, Japan

Received 28 May 1996; revised 21 June 1996; accepted 25 June 1996

Abstract

We investigated whether tolerance develops to the lipopolysaccharide-induced increase in vascular permeability of mouse skin on pretreatment with *Salmonella typhimurium* lipopolysaccharide. Lipopolysaccharide-induced plasma extravasation was assessed by determining Pontamine sky blue dye accumulation in the skin where lipopolysaccharide was injected s.c. 2 h previously. When mice were pretreated with lipopolysaccharide (0.15 mg/kg i.p.), the dye leakage induced by s.c. challenge with lipopolysaccharide (400 μg/site) was significantly inhibited for 2–24 h after pretreatment, indicating the development of lipopolysaccharide tolerance. At 4 h after lipopolysaccharide (0.15 mg/kg i.p.), the dose-response curve of dye leakage against the challenge dose of lipopolysaccharide shifted about 2-fold to the higher dose. The dye leakage induced by lipopolysaccharide was inhibited by pretreatment with lipopolysaccharide in a dose-dependent manner (0.05–0.15 mg/kg i.p.). Lipopolysaccharide tolerance was not seen in adrenalectomized mice. When mice were pretreated with lipopolysaccharide and N^G-nitro-L-arginine methyl ester (L-NAME), a nitric oxide (NO) synthase inhibitor, at the same time, the hyporesponsiveness to lipopolysaccharide challenge disappeared. However, L-NAME was ineffective to inhibit the development of lipopolysaccharide tolerance when administered 24 h after lipopolysaccharide pretreatment or just before the lipopolysaccharide challenge. Tumor necrosis factor-α and interleukin-1α but not interleukin-6 induced a similar hyporesponsiveness to lipopolysaccharide. These results suggest that tolerance develops to the lipopolysaccharide-induced increase in vascular permeability in mouse skin after a single lipopolysaccharide administration and that endogenous glucocorticoids and NO are necessary for induction of lipopolysaccharide tolerance. Hyporesponsiveness induced by lipopolysaccharide pretreatment may be mediated by production of some cytokines such as tumor necrosis factor-α or interleukin-1α.

Keywords: Lipopolysaccharide; Endotoxin tolerance; Vascular permeability; Nitric oxide (NO); Cytokine; Adrenalectomy

1. Introduction

Endotoxin, a component of the outer membrane of gram-negative bacteria, is a lipopolysaccharide consisting of a core oligosaccharide and lipid A (Rietschel et al., 1994). Release of lipopolysaccharide from gram-negative bacteria into the systemic circulation is the cause of the clinical disease, sepsis, including septic shock and disseminated intravascular coagulation (Hewett and Roth, 1993). Administration of lipopolysaccharide to animals or human volunteers can reproduce many physiological changes found in gram-negative sepsis (Suffredini et al., 1989; Kuhns et al., 1995).

Lipopolysaccharide binds to CD 14 on monocytes, macrophages and neutrophils with the aid of lipopolysaccharide-binding protein, and thereby induces these cells to produce and release many inflammatory mediators such as tumor necrosis factor (TNF)-α, interleukin-1, nitric oxide (NO) and platelet-activating factor (PAF) (Hewett and Roth, 1993; Labeta et al., 1993). These inflammatory mediators are responsible for many pathophysiological changes observed with sepsis. Lipopolysaccharide and cytokines such as TNF-α activate vascular endothelial cells to produce and release cytokines and other mediators, promote the adherence of neutrophils on the endothelial cells, and increase vascular permeability (Pugin et al., 1995; Yi and Ulich, 1992).

NO is a gaseous mediator which is produced by constitutive or inducible NO synthases in endothelial and many other cells (Moncada et al., 1991; Förstermann et al.,

* Corresponding author. Tel.: +81 3 3353 8111 (extension 22513); fax: +81 3 5269 7417.

PII S0014-2999(96)00509-2

1995). Induction of NO synthase occurs in many cell types as part of the host response to sepsis and inflammation. NO can have a variety of effects which may be detrimental or beneficial during sepsis or inflammation, depending on amount, duration and anatomic site of synthesis (Morris and Billiar, 1994). It is reported that lipopolysaccharide activates both constitutive NO synthase and inducible NO synthase, and the immediate hypotension in endotoxin shock is mediated by NO produced by constitutive NO synthase; however, the delayed hypotention is due to enhanced NO formation by the lipopolysaccharide-induced enzyme (Szabó et al., 1993).

Tolerance to endotoxin develops after repeated administration of small doses of endotoxin to animals and is characterized by the reduced effect of a subsequent challenge with a high-dose endotoxin. Cardiovascular tolerance to endotoxin is explained by reduced induction of inducible NO synthase due to the elevation of endogenous glucocorticoid levels (Szabó et al., 1994), although there may be glucocorticoid-independent components in other types of endotoxin tolerance (Evans and Zuckerman, 1991). Further, endothelium-dependent arterial relaxation is depressed during sepsis due to depressed NO production from endothelium (Wang et al., 1995). In contrast, Zingarelli et al. (1995) reported that tolerance to lipopolysaccharide is associated with up-regulation of NO synthesis which correlates with acquired resistance to lipopolysaccharide lethality in tolerant rats.

We have studied the role of NO in the microvascular plasma leakage induced by several inflammatory mediators and have reported that NO plays a role in the effect of 5-hydroxytryptamine (5-HT) but not that of PAF in mouse skin (Fujii et al., 1994, 1995). Whereas inhibition of NO production by vascular endothelium was reported to increase the microvascular protein efflux in the feline small intestine (Kubes and Granger, 1992), there are several reports that lipopolysaccharide increases microvascular permeability. Induction of NO synthase by systemic endotoxin is associated with microvascular leakage in the various organs of rats (László et al., 1995). Bradykinin and PAF were reported to mediate the vascular permeability induced by intradermal injection of endotoxin in rats (Ueno et al., 1995). Recently, we reported that subcutaneous (s.c.) injection of lipopolysaccharide induced a delayed plasma leakage in the injection site of mouse skin and that this effect is mediated by production of both NO and prostaglandins (Fujii et al., 1996; Muraki et al., 1996). Here we report that tolerance develops to the increase in vascular permeability induced by lipopolysaccharide in mouse skin.

2. Materials and methods

2.1. Animals

Male ddY strain mice weighing about 35 g (Sankyo Lab Service Inc., Tokyo, Japan) were used. The mice were maintained in an air-conditioned room (temperature 20–24°C, humidity 50–60%) with light on 06.00–20.00 h and with food and water freely available. Some mice were adrenalectomized bilaterally under light ether anesthesia, placed on saline maintenance (0.9% NaCl ad libitum) and stabilized for 7 days before experiments.

2.2. Assessment of vascular permeability

Vascular permeability was determined by the Pontamine sky blue leakage method (Fujii et al., 1994). Briefly, 5 min after intravenous (i.v.) injection of Pontamine sky blue (50 mg/kg), lipopolysaccharide or saline in a volume of 0.1 ml/site was injected s.c. in the back. Mice were killed 2 h later by cervical dislocation and the stained area of the skin was excised. The dye that accumulated in the skin was extracted with acetone-0.5% sodium sulfate mixture (14:6 v/v) and dye concentration was determined colorimetrically at 590 nm.

2.3. Lipopolysaccharide pretreatment

To induce lipopolysaccharide tolerance, lipopolysaccharide at the dose of 0.05–0.15 mg/kg was given to mice intraperitoneally (i.p.) usually 4 h before the s.c. challenge with lipopolysaccharide. The dose of lipopolysaccharide was chosen on the basis of a previous study (Szabó et al., 1994). For the time course study, the dye accumulation for 2 h after the s.c. injection of lipopolysaccharide (400 μg/site) was determined at 2, 4, 24 and 48 h after i.p. administration of lipopolysaccharide (0.15 mg/kg).

2.4. Measurement of blood pressure and heart rate

Blood pressure and heart rate were indirectly determined from the tail with a programmable sphygmomanometer (Riken Kaihatsu PS-200, Tokyo, Japan).

2.5. Drugs

Lipopolysaccharide from *Salmonella typhimurium* (L6511) and N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) were obtained from Sigma Chemical (MO, USA). Recombinant human TNF-α was provided by Dainippon Pharmaceut. (Osaka, Japan), and recombinant human interleukin-1α by Otsuka Pharmaceut. (Tokushima, Japan), recombinant human interleukin-6 by Ajinomoto Biochemicals (Yokohama, Japan). Other drugs were purchased from commercial sources. Drugs were dissolved in sterile physiological saline solution (0.9% NaCl). All the doses refer to the salt forms of the drugs.

2.6. Statistical analysis

The results, expressed as means ± S.E.M., were usually analyzed by unpaired Student's *t*-test. The dose-response

effect of lipopolysaccharide pretreatment (Fig. 3) was evaluated with Wilcoxon's direct calculation test.

3. Results

3.1. Tolerance in terms of dye leakage

The onset of plasma leakage induced by s.c. injection of lipopolysaccharide was slower than that with another inflammatory mediator, 5-HT (Fujii et al., 1994). The cutaneous dye accumulation was evident by 2 h but not 1 h following the s.c. injection of 400 μg lipopolysaccharide/site in the back of lipopolysaccharide-naive mice. In order to examine the possible development of lipopolysaccharide tolerance, we determined the dye leakage for 2 h after challenge with s.c. administration of lipopolysaccharide at different intervals after a single i.p. pretreatment with lipopolysaccharide (Fig. 1A). The dye leakage induced by s.c. injection of lipopolysaccharide showed a significant decrease by 2 h following lipopolysaccharide pretreatment. The inhibition continued up to 24 h, then disappeared 48 h later. Systemic administration of lipopolysaccharide did not affect the basal cutaneous vascular permeability determined by s.c. injection of saline throughout the 48 h observation period. I.p. injection of this dose of lipopolysaccharide induced neither hypotension nor changes in heart rate until 24 h later, indicating no endotoxin shock was produced (Fig. 1B). We then investigated the effect of a single lipopolysaccharide pretreatment on the dose-response curve of lipopolysaccharide-induced dye accumulation. The amount of dye leakage over 2 h after s.c. challenge with lipopolysaccharide was dependent on the dose between 200–800 μg/site in the lipopolysaccharide-naive mice (Fig. 2). When the mice were pretreated i.p. with 0.15 mg/kg lipopolysaccharide (about 5.3 μg/animal) 4 h previously, the dye accumulation in the skin for 2 h after s.c. lipopolysaccharide challenge was depressed and the dose-response curve shifted to the higher dose about 2-fold, indicating that a single pretreatment

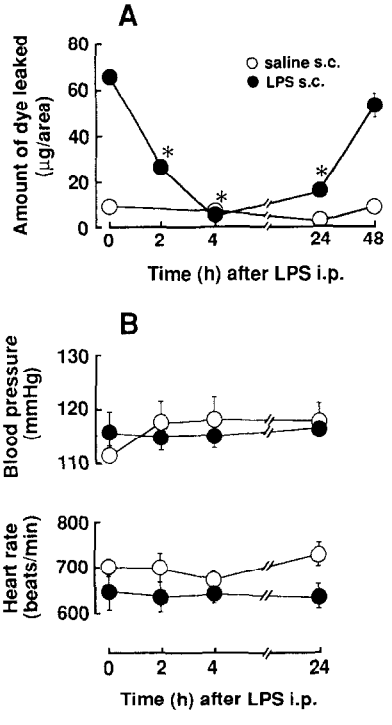

Fig. 1. Effect of systemic administration of lipopolysaccharide on cutaneous dye leakage (A), heart rate and blood pressure (B) in mice. (A) *Salmonella typhimurium* lipopolysaccharide (0.15 mg/kg i.p.) was given to mice at time 0. At the indicated times, mice were given Pontamine sky blue i.v., followed by lipopolysaccharide (400 μg/site s.c.) (●) or saline (0.1 ml/site s.c.) (○) and dye accumulation was determined 2 h later. The point and vertical bar represent mean ± S.E.M. for five mice. * $P < 0.01$ vs. time 0. (B) Immediately after the determination of blood pressure and heart rate, mice received lipopolysaccharide (0.15 mg/kg, ●) or saline (10 ml/kg, ○) i.p. at time 0. Determination of blood pressure and heart rate was then repeated at the times indicated. The point and vertical bar represent mean ± S.E.M. for ten mice.

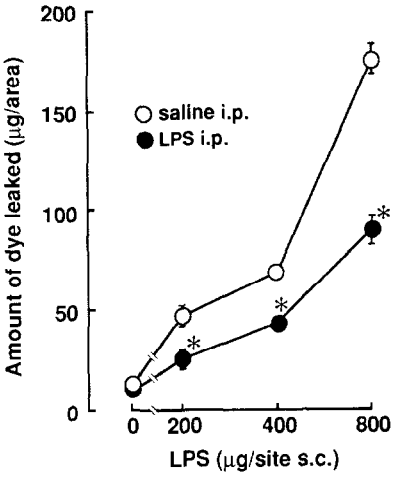

Fig. 2. Suppression by lipopolysaccharide pretreatment of dose-response curve of lipopolysaccharide-induced dye leakage. Lipopolysaccharide (0.15 mg/kg i.p.) (●) or saline (10 ml/kg i.p.) (○) was given to mice 4 h prior to the s.c. injection of increasing dose of lipopolysaccharide and the cutaneous dye accumulation was determined 2 h later. The point and vertical bar represent mean ± S.E.M. for five mice. * $P < 0.01$ vs. mice pretreated with saline.

Table 1
Effect of pretreatment with cytokines on the vascular permeability induced by lipopolysaccharide in mouse skin

Pretreatment	Amount of dye leaked	
	(μg/area)	(%)
Saline	56.1 ± 6.3	100
TNF-α	16.2 ± 3.8 [a]	29
IL-1α	21.1 ± 5.8 [a]	38
IL-6	43.2 ± 6.5	77

TNF-α (0.68 μg/kg), IL-1α (0.32 μg/kg), IL-6 (9.6 μg/kg) or saline (10 ml/kg) was administered i.p. 4 h before the s.c. injection of lipopolysaccharide (400 μg/site). The lipopolysaccharide-induced dye accumulation in the skin was determined 2 h later. Values represent mean ± S.E.M. of five experiments.
[a] $P < 0.01$ vs. saline.

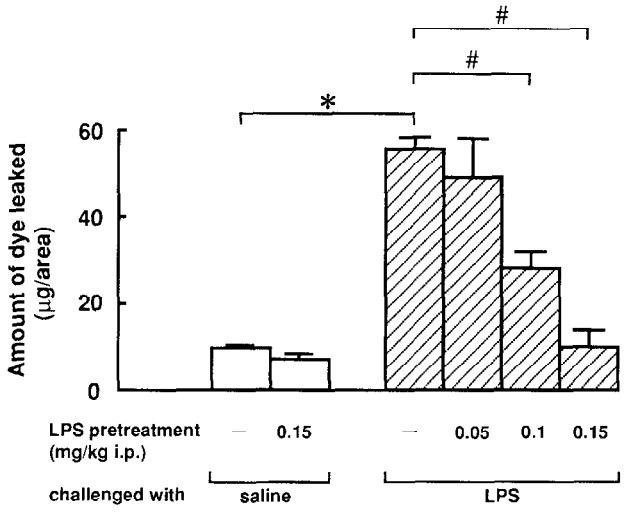

Fig. 3. Effect of dose of lipopolysaccharide pretreatment on lipopolysaccharide-induced dye leakage. 4 h after pretreatment with the indicated doses of lipopolysaccharide i.p., mice were challenged s.c. with lipopolysaccharide (400 μg/site) (hatched columns) or saline (0.1 ml/site) (open columns) followed by determination of cutaneous dye accumulation 2 h later. The column and vertical bar represent mean ± S.E.M. for five mice. * $P < 0.01$ vs. saline alone (Student's t-test), # $P < 0.05$ vs. lipopolysaccharide alone (Wilcoxon's direct calculation test).

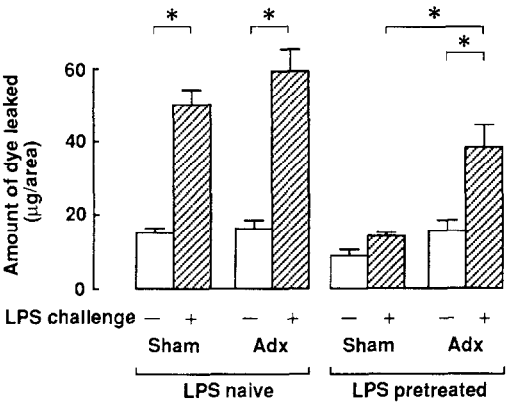

Fig. 4. Reversal by adrenalectomy of tolerance to lipopolysaccharide-induced dye leakage. Adrenalectomized (Adx) or sham-operated (Sham) mice were pretreated with lipopolysaccharide (0.15 mg/kg i.p.) (LPS-pretreated) or saline (10 ml/kg i.p.) (LPS naive) 4 h previously. The mice were then challenged s.c. with lipopolysaccharide (400 μg/site) (hatched columns) or saline (0.1 ml/site) (open columns) followed by determination of the cutaneous dye accumulation 2 h later. The column and vertical bar represent mean ± S.E.M. for five mice. * $P < 0.01$.

with a low dose of lipopolysaccharide was sufficient to make mice tolerant or hyporesponsive to the effects of lipopolysaccharide to increase vascular permeability. When mice were pretreated i.p. with a single administration of increasing doses (0.05–0.15 mg/kg) of lipopolysaccharide 4 h prior to s.c. lipopolysaccharide challenge, the dye accumulation was decreased in a manner dependent on the pretreatment dose of lipopolysaccharide (Fig. 3).

Many effects of lipopolysaccharide are mediated by production of cytokines. Therefore, we examined whether pretreatment with cytokines could induce tolerance to the lipopolysaccharide-induced vascular extravasation. Mice pretreated with TNF-α, interleukin-1α but not interleukin-6 showed a significant hyporesponsiveness to lipopolysaccharide induction of dye leakage (Table 1).

3.2. Role of glucocorticoid and NO

To address the possibility of a role of endogenous glucocorticoids in the establishment of lipopolysaccharide tolerance, we investigated whether adrenalectomized animals become hyporesponsive to lipopolysaccharide after pretreatment with lipopolysaccharide (Fig. 4). In lipopolysaccharide-naive mice adrenalectomized 7 days previously, the basal and lipopolysaccharide-induced dye leakage did not differ significantly from those seen in the sham-operated animals, indicating that lipopolysaccharide-induced dye leakage is not dependent on endogenous glucocorticoids. The hyporesponsiveness after the lipopolysaccharide pretreatment was inhibited in the adrenalectomized mice, indicating that endogenous glucocorticoids play a role in the development of such tolerance.

It was suggested that endotoxin-induced cardiovascular tolerance is associated with attenuation of the induction of inducible NO synthase by endogenous glucocorticoids (Szabó et al., 1994). Therefore, we examined whether L-NAME, a non-selective NO synthase inhibitor, can affect the lipopolysaccharide-induced tolerance of dye leakage (Fig. 5). To minimize the possible direct inhibition by L-NAME of lipopolysaccharide-induced dye leakage, we pretreated mice with L-NAME 24 h prior to the test of plasma extravasation; however, this dose of NO synthase inhibitor was still effective to inhibit the lipopolysaccharide-induced dye leakage in the lipopolysaccharide-naive mice 24 h later. When mice were treated with a concurrent injection of lipopolysaccharide and L-NAME 24 h previously, the decrease in dye leakage induced by lipopoly-

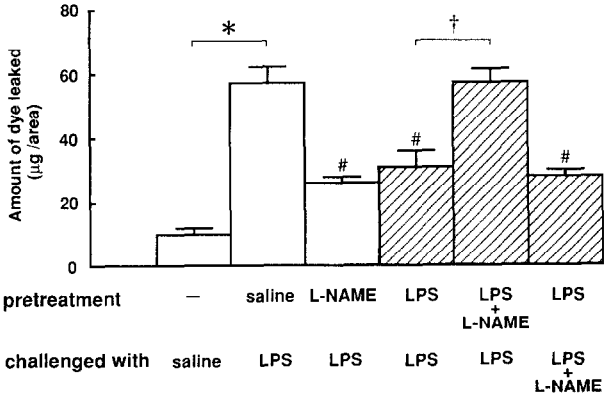

Fig. 5. Effect of a NO synthase inhibitor, L-NAME, on the lipopolysaccharide tolerance. Mice were pretreated i.p. with lipopolysaccharide (0.15 mg/kg), L-NAME (10 mg/kg) or both 24 h previously, then challenged with lipopolysaccharide (400 μg/site) alone, lipopolysaccharide and L-NAME (10 mg/kg i.v.), or saline (0.1 ml/site), then the dye accumulation was determined 2 h later. The column and vertical bar represent mean ± S.E.M. for five mice. * $P < 0.01$, # $P < 0.01$ vs. saline-pretreated, lipopolysaccharide challenged mice; † $P < 0.01$.

saccharide pretreatment was reversed to the level of that in lipopolysaccharide-naive mice, indicating that tolerance had disappeared. However, when L-NAME was administered 24 h after lipopolysaccharide pretreatment or just prior to the s.c. lipopolysaccharide challenge, it inhibited the lipopolysaccharide-induced dye leakage, indicating no reversal of lipopolysaccharide tolerance.

4. Discussion

In this study, we confirmed our previous finding that s.c. injection of lipopolysaccharide increases cutaneous vascular permeability at the injection site in mice. Further, we demonstrated that pretreatment with a single injection of lipopolysaccharide significantly reduced the effect of lipopolysaccharide to elicit plasma leakage, indicating that tolerance develops to the effect of lipopolysaccharide on vascular permeability. The hyporesponsiveness to lipopolysaccharide developed rapidly, by 2 h after lipopolysaccharide pretreatment, and continued up to 24 h, then had disappeared 48 h later. The dose of lipopolysaccharide necessary for making mice tolerant was 0.1 mg/kg or about 3.5 μg/animal, which was more than 10 times less than the dose needed to increase vascular permeability. This dose is less than the dose of lipopolysaccharide that was used for induction of septic shock (Cunha et al., 1994). Circulatory failure due to lipopolysaccharide may not be the cause of tolerance, because no hypotension was observed in the mice treated with this dose of lipopolysaccharide in our study. The extent of tolerance produced was about 2-fold when estimated from the dose-response curve.

Although the plasma extravasation induced by lipopolysaccharide was inhibited by dexamethasone, probably through its direct effect on endothelial cells (Fujii et al., 1996), it was endogenous glucocorticoid-independent because dye leakage occurred in the adrenalectomized mice. On the other hand, lipopolysaccharide tolerance was abolished in adrenalectomized mice, indicating an endogenous glucocorticoid-dependent mechanism. A similar glucocorticoid-dependent mechanism of lipopolysaccharide tolerance was suggested for the lipopolysaccharide-induced increase in serum TNF levels (Evans and Zuckerman, 1991).

Previously, we suggested that NO produced by inducible NO synthase may play a role in mediating the lipopolysaccharide-induced dye leakage, because L-NAME and aminoguanidine inhibit the dye accumulation elicited by s.c. injection of lipopolysaccharide (Fujii et al., 1996). We confirmed our finding for the apparent inhibition of the dye leakage induced by lipopolysaccharide challenge by administering L-NAME 24 h prior to the lipopolysaccharide challenge in the lipopolysaccharide-naive mice or immediately prior to challenge in the lipopolysaccharide-pretreated mice. It is interesting, therefore, that the NO synthase inhibitor cancelled the lipopolysaccharide tolerance when it was administered at the same time as the lipopolysaccharide pretreatment, indicating that NO is also involved in the development of lipopolysaccharide tolerance. Similarly, the lipopolysaccharide tolerance regarding TNF production was prevented by inhibition of NO synthase (Rojas et al., 1993). In addition, it was reported that NO formation is increased during endotoxin tolerance (Chamulitrat et al., 1995) and that endotoxin-induced desensitization of mouse macrophages is mediated by NO production (Fahmi et al., 1995). Inhibition of cytokine-induced corticotropin-releasing factor response by NO synthase inhibitors has been demonstrated previously (Sandi and Guaza, 1995) and it is also known that L-arginine can enhance steroidogenesis in the adrenal gland (Cameron and Hinson, 1993). Szabó et al. (1994) suggested that endogenous NO may be involved in maintaining the glucocorticoid response during the development of tolerance. Although we did not determine the plasma glucocorticoid levels in this study, this all may suggest that L-NAME inhibited the endogenous glucocorticoid response, thereby diminishing the lipopolysaccharide tolerance regarding vascular permeability in our study.

Our finding that TNF-α and interleukin-1α mimicked lipopolysaccharide in the induction of tolerance to lipopolysaccharide challenge suggests a possible role of these cytokines as mediators for lipopolysaccharide tolerance. Injection of lipopolysaccharide in mice was reported to induce a transient increase in serum TNF, and pretreatment with lipopolysaccharide results in glucocorticoid-dependent tolerance to the rise in serum TNF following the second lipopolysaccharide stimulus (Evans and Zuckerman, 1991). Also, pre-exposure of macrophages to lipopolysaccharide inhibits the production of TNF-α, but not of interleukin-1, by inhibiting the expression of mRNA (Takasuka et al., 1991). Anti-TNF-α antibody inhibited the lipopolysaccharide-induced dye leakage in a preliminary study, suggesting the involvement of TNF-α in the lipopolysaccharide-induced dye leakage; therefore, tolerance to the lipopolysaccharide-induced TNF production may play a role in the tolerance for dye leakage.

In conclusion, we showed that a bolus systemic administration of *Salmonella typhimurium* lipopolysaccharide induced tolerance to lipopolysaccharide for the induction of plasma extravasation. The development of tolerance is dependent on endogenous glucocoticoids and NO. Further work is needed, however, to clarify the role of cytokines in the lipopolysaccharide tolerance.

Acknowledgements

This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan (No. 07670120).

References

Cameron, L.A. and J.P. Hinson, 1993, The role of nitric oxide derived from L-arginine in the control of steroidogenesis, and perfusion medium flow rate in the isolated perfused rat adrenal gland, J. Endocrinol. 139, 415.

Chamulitrat, W., J.-F. Wang and J.J. Spitzer, 1995, Electron paramagnetic resonance investigations of nitrosyl complex formation during endotoxin tolerance, Life Sci. 57, 387.

Cunha, F.Q., J. Assreuy, D.W. Moss, D. Rees, M.C. Leal, S. Moncada and M. Carrier, 1994, Differential induction of nitric oxide synthase in various organs of the mouse during endotoxemia: role of TNF-α and IL-1-β, Immunology 81, 211.

Evans, G.F. and S.H. Zuckerman, 1991, Glucocorticoid-dependent and -independent mechanisms involved in lipopolysaccharide tolerance, Eur. J. Immunol. 21, 1973.

Fahmi, H., D. Charon, M. Mondange and R. Chaby, 1995, Endotoxin-induced desensitization of mouse macrophages is mediated in part by nitric oxide production, Infect. Immun. 63, 1863.

Förstermann, U., I. Gath, P. Schwarz, E.I. Closs and H. Kleinert, 1995, Isoforms of nitric oxide synthase, Biochem. Pharmacol. 50, 1321.

Fujii, E., K. Irie, Y. Uchida, F. Tsukahara and T. Muraki, 1994, Possible role of nitric oxide in 5-hydroxytryptamine-induced increase in vascular permeability in mouse skin, Naunyn-Schmied. Arch. Pharmacol. 350, 361.

Fujii, E., K. Irie, Y. Uchida, K. Ohba and T. Muraki, 1995, Role of eicosanoids but not nitric oxide in the platelet-activating factor-induced increase in vascular permeability in mouse skin, Eur. J. Pharmacol. 273, 267.

Fujii, E., K. Irie, A. Ogawa, K. Ohba and T. Muraki, 1996, Role of nitric oxide and prostaglandins in lipopolysaccharide-induced increase in vascular permeability in mouse skin, Eur. J. Pharmacol. 297, 257.

Hewett, J.A. and R.A. Roth, 1993, Hepatic and extrahepatic pathobiology of bacterial lipopolysaccharides, Pharmacol. Rev. 45, 381.

Kubes, P. and D.N. Granger, 1992, Nitric oxide modulates microvascular permeability, Am. J. Physiol. 262, H611.

Kuhns, D.B., W.G. Alvord and J.I. Gallin, 1995, Increased circulating cytokines, cytokine antagonists, and E-selectin after intravenous administration of endotoxin in humans, J. Infect. Dis. 171, 145.

Labeta, M.O., J.-J. Durieux, G. Spagnoli, N. Fernandez, J. Wijdenes and R. Herrmann, 1993, CD14 and tolerance to lipopolysaccharide: biochemical and functional analysis, Immunology 80, 415.

László, F., B.J.R. Whittle, S.M. Evans and S. Moncada, 1995, Association of microvascular leakage with induction of nitric oxide synthase: effects of nitric oxide synthase inhibitors in various organs, Eur. J. Pharmacol. 283, 47.

Moncada, S., R.M.J. Palmer and E.A. Higgs, 1991, Nitric oxide: physiology, pathophysiology, and pharmacology, Pharmacol. Rev. 43, 109.

Morris, S.M. Jr. and T.R. Billiar, 1994, New insights into the regulation of inducible nitric oxide synthesis, Am. J. Physiol. 266, E829.

Muraki, T., E. Fujii, M. Okada, H. Horikawa, K. Irie and K. Ohba, 1996, Effect of *S*-ethylisothiourea, a putative inhibitor of inducible nitric oxide synthase, on mouse skin vascular permeability, Jpn. J. Pharmacol. 70, 269.

Pugin, J., R.J. Ulevitch and P.S. Tobias, 1995, Tumor necrosis factor-α and interleukin-1β mediate human endothelial cell activation in blood at low endotoxin concentrations, J. Inflammation 45, 49.

Rietschel, E.T., T. Kirikae, F.U. Schade, U. Mamat, G. Schmidt, H. Loppnow, A.J. Ulmer, U. Zähringer, U. Seydel, F. DiPadova, M. Schreier and H. Brade, 1994, Bacterial endotoxin: molecular relationships of structure to activity and function, FASEB J. 8, 217.

Rojas, A., J. Padrón, L. Caveda, M. Palacios and S. Moncada, 1993, Role of nitric oxide pathway in the protection against lethal endotoxemia afforded by low doses of lipopolysaccharide, Biochem. Biophys. Res. Commun. 191, 441.

Sandi, C. and C. Guaza, 1995, Evidence for a role of nitric oxide in the corticotropin-releasing factor release induced by interleukin-1β, Eur. J. Pharmacol. 274, 17.

Suffredini, A.F., R.E. Fromm, M.M. Parker, M. Brenner, J.A. Kovacs, R.A. Wesley and J.E. Parrillo, 1989, The cardiovascular response of normal humans to the administration of endotoxin, New Engl. J. Med. 321, 280.

Szabó, C., J.A. Mitchell, C. Thiemermann and J.R. Vane, 1993, Nitric oxide-mediated hyporeactivity to noradrenaline precedes the induction of nitric oxide synthase in endotoxin shock, Br. J. Pharmacol. 108, 786.

Szabó, C., C. Thiemermann, C.C. Wu, M. Perretti and J.R. Vane, 1994, Attenuation of the induction of nitric oxide synthase by endogenous glucocorticoids accounts for endotoxin tolerance in vivo, Proc. Natl. Acad. Sci. USA, 91, 271.

Takasuka, N., T. Tokunaga and K.S. Akagawa, 1991, Preexposure of macrophages to low doses of lipopolysaccharide inhibits the expression of tumor necrosis factor-α mRNA but not of IL-1β mRNA, J. Immunol. 146, 3824.

Ueno, A., T. Tokumasu, H. Naraba and S. Oh-ishi, 1995, Involvement of bradykinin in endotoxin-induced vascular permeability increase in the skin of rats, Eur. J. Pharmacol. 284, 211.

Wang, P., Z.F. Ba and I.H. Chaudry, 1995, Endothelium-dependent relaxation is depressed at the macro- and microcirculatory levels during sepsis, Am. J. Physiol. 269, R988.

Yi, E.S. and T.R. Ulich, 1992, Endotoxin, interleukin-1, and tumor necrosis factor cause neutrophil-dependent microvascular leakage in postcapillary venules, Am. J. Pathol. 140, 659.

Zingarelli, B., P.V. Halushka, A.P. Caputi and J.A. Cook, 1995, Increased nitric oxide synthesis during the development of endotoxin tolerance, Shock 3, 102.