ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Oct; 21 (10): 897-904
http://www.chinaphar.com

Dithiolane analogs of lignans inhibit interferon-γ and lipopolysaccharide-induced nitric oxide production in macrophages

Isa M HUSSAINI[1], ZHANG Yi-Hua[2], Jeff J LYSIAK, Tsung Y SHEN[2]
(*Departments of Pathology and [2]Chemistry, University of Virginia, Charlottesville, VA 22908, USA*)

KEY WORDS lignans; interferon type Ⅱ; lipopolysaccharides; platelet activating factor; nitric oxide; nitric-oxide synthase

ABSTRACT

AIM: To investigate the effect of a group of novel synthetic dithiolane analogs of lignans and a well characterized platelet-activating factor (PAF) receptor antagonist, L659,989 on PAF-receptor binding, IFN-γ- and lipopolysaccharide (LPS)-induced NO production, and steady-state inducible nitric-oxide synthase (iNOS) mRNA expression. **METHODS**: PAF-receptor binding study was performed by displacement of ^{3}H-PAF from rabbit platelet membrane; NO production was quantitated by measuring the NO oxidation product, nitrite, in conditioned culture medium; expression of iNOS mRNA was assessed by Northern blot analysis. **RESULTS**: The dithiolane analogs inhibited the production of NO, decreased iNOS mRNA expression and antagonized PAF-receptor binding. L659,989 had no effect on NO production and iNOS mRNA expression. Among the compounds tested, there was no simple correlation between their PAF-receptor antagonistic and iNOS inhibitory activities. **CONCLUSION**: The dithiolane analogs are a new synthetic chemical class of iNOS expression regulators with dual biologic functions: inhibiting iNOS induction and blocking PAF-receptor.

INTRODUCTION

Many potent endogenous mediators are involved in various inflammatory, pulmonary, cardiovascular and renal disorders. These endogenous inflammatory mediators, such as TNF-α[1,2], nitric oxide[3-5], interleukin-1,6 and 8[6,7], eicosanoids[8-10] and platelet activating factor (PAF)[11-13], often act in concert or synnergistically.

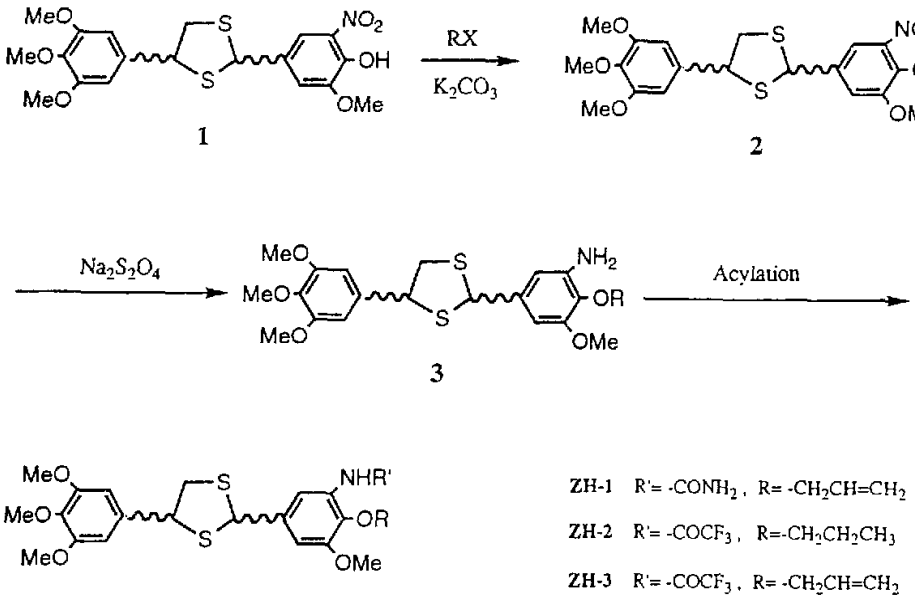
PAF is a potent lipid mediator that is involved in numerous pathophysiologic conditions including platelet aggregation, asthma and endotoxic shock[14,15]. PAF evokes biologic effects by binding to a cell surface receptor that has been characterized and cloned[16]. In the past decade, a number of chemically unrelated but specific PAF-receptor antagonists have been developed[15,17-19]. These compounds block the fatal effects of endotoxin and improve hemodynamic functions in animal experiments[20-22]. In addition, a number of PAF-receptor antagonists have also been reported to partially block LPS-evoked NO generation both *in vivo* and *in vitro*[23,24].

Recent studies suggest that hypotension after endotoxemia as well as that induced by TNF-α, a proximal mediator of septic shock, can be partially reversed by nitric-oxide synthase (NOS) inhibitors, suggesting involvement of an NO-dependent pathway[25]. NO is naturally generated from *L*-arginine by NOS, an NADPH-dependent enzyme. There are three NOS isoforms, the constitutive forms (NOS-1 and NOS-3 or cNOS) which are Ca^{2+}/calmodulin-dependent and the Ca^{2+}-independent inducible isoform (NOS-2 or iNOS). By synthesizing NO, the cNOS enzymes optimize microvascular and organ blood flow, with resulting benefits to the host. However, endotoxin- and cytokine-stimulated iNOS seems to play an important role in sepsis-induced hypotension[26]. Expression of the iNOS gene is transcriptionally controlled by a basal promoter (Region Ⅰ) and an enhancer element (Region Ⅱ) that are responsive to LPS in mouse macrophage[27]. The enhancer element is also responsive to IFN-γ[27].

In this study, we demonstrate that a group of dithiolane analogs of lignans block NO production induced by IFN-γ and LPS in macrophages, which appears to occur

[1] Correspondence to Dr Isa M HUSSAINI. Phn 1-804-924-0110. Fax 1-804-924-9177. E-mail imh5c@virginia.edu
Prof ZHANG Yi-Hua, now in *Center of Medicinal Research, China Pharmaceutical University, Nanjing 210009, China*. Phn 86-25-663-5503. E-mail njyihzha@pub.jlonline.com
Received 1999-12-24 Accepted 2000-06-28

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Oct; 21 (10)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629



Synthesis of dithiolane analogs of lignans

at the level of iNOS mRNA. They also inhibit PAF binding to rabbit platelets. Thus, compounds of this type may be therapeutically useful in acute disorders like severe sepsis where the reduction of both NO production and PAF activity might be beneficial.

MATERIALS AND METHODS

Agents Recombinant murine IFN-γ and LPS were purchased from Genzyme Diagnostics (Cambridge, MA) and Sigma Chemical Company (St Louis, MO). The iNOS cDNA in pUC19 was generously provided by Drs Qiao-Wen XIE and Carl NATHAN, Cornell University Medical College, New York, NY. A 645-nucleotide fragment of the cDNA was excised with *Hind* Ⅲ and *Bam*H1 for use as a Northern blot probe. Dithiolane analogs, ZH-1, 2, and 3 are substituted 2,5-diaryl-1,3-dithiolane analogs of the 2,5-diaryltetrahydrofuran lignans which were synthesized by a procedure developed in our laboratories[28]. L-659,989 was a generous gift from the Merck Research Laboratories (Rahway, NJ).

Cell culture Mouse macrophages (RAW 264.7 cells) were obtained from American Type Culture Collection (ATCC) and cultured in RPMI-1640 (Sigma, St Louis, MO) with 10 % fetal calf serum. Cultures were passaged when nearly 100 % confluent, every 4 – 5 days, and distributed into 6-well plates for Northern blot analysis or 96-well plates for nitric-oxide synthesis experiments. When the cells became confluent in the presence of 10 % fetal calf serum, the cultures were washed three times with serum-free RPMI-1640 and then treated with LPS or IFN-γ in the absence or presence of iNOS inhibitors in the same medium.

Nitric-oxide synthesis assay NO synthesis in RAW 264.7 cells was quantitated by measuring the NO oxidation product, nitrite, in conditioned culture medium[29]. Cells were cultured in RPMI-1640 with 10 % serum in 96-well plates until confluent and then cultured in serum-free RPMI, with or without IFN-γ. Dithiolane analogs and L659,989 were added alone or together with the IFN-γ. After 24 h, conditioned medium was recovered. Aliquots of conditioned medium (100 μL) were incubated with 50 μL of 1.0 % (w/v) sulfanilamide in 5.0 % H_3PO_4 and 50 μL of 0.1 % (w/v) naphthyl ethylenediamine dihydrochloride for 5 min. Absorbance was measured at 570 nm.

Northern blot analysis of iNOS mRNA Total RNA was isolated from adherent cells using TRIzol reagent, as described by the manufacturer (Gibco BRL, Gaithersburg, MD). Equal amounts of RNA (20 μg) from each culture were subjected to electrophoresis in 1.0 % (w/v) agarose gels and electroblotted to Zeta

probe nylon membranes (Bio-rad Laboratories, Richmond, CA). Following electroblotting, the RNA was cross-linked by UV irradiation and the nylon membranes were pre-hybridized in a solution containing 50 % formamide, 5 × SSPE (sodium chloride, sodium phosphate and edetic acid), 2 × Denhardt's reagent, 0.5 % SDS and 1 % Salmon testes DNA, at 42 ℃ for 18 – 24 h. The cDNA probe for iNOS was labeled with [α-^{32}P]dCTP (1.85 MBq) by random oligonucleotide-primed synthesis using the kit marketed by Gibco BRL, as described by Hussaini *et al*[30]. The labeled probes were hybridized for 24 h at 42 ℃ in prehybridization solution supplemented with 5 % dextran sulfate. As a control for load, membranes were also hybridized (separately or simultaneously) with [α-^{32}P]dCTP-labeled phosphoglyceraldehyde dehydrogenase (PGAD) cDNA. After hybridization, the membranes were washed twice with 5 × SSPE, 0.5 % SDS at 22 ℃ and twice with 0.1 × SSPE, 1 % SDS at 65 ℃. Specific hybridization was quantitated by PhosphorImager analysis or by exposure to X-ray film (X-omat AR, Kodak, Rochester, NY).

PAF receptor binding studies The PAF receptor antagonism of L-659,989 and the dithiolane analogs were determined by NOVA Screen (7170 Standard Drive, Hanover, Mariland 21076, USA), using rabbit platelet membrane preparation as described previously[31]. ^{3}H-PAF (1.2 nmol/L) was incubated in absence and presence of different concentrations of L-659,989 and dithiolane analogs and the effect of the compounds on the specific binding was expressed as percent inhibition of the control (no PAF-receptor antagonist or dithiolane analog). The equilibrium dissociation constant (K_i) values of L-659,989 and dithiolane compounds were calculated from the Chen-Prusoff equation:

$$K_i = \frac{IC_{50}}{1 + [PAF]/K_D}$$

Statistics Results show the means ± standard error (s_x) with the number of observations shown in parentheses. Statistical analysis of results was performed with MINITAB for Windows computer software (Addison-Wesley, Reading, MA) using unpaired *t*-test. A probability (P) value of 0.05 or less was taken to indicate statistical significance.

RESULTS

Synthesis of the dithiolane analogs of lignans Compounds ZH-1 to 3 were synthesized from a common intermediate (1), which was prepared by a procedure developed in our laboratory[28]. The nitro phenol (1), obtained as an approximately 1 : 1 mixture of cis and trans isomers, was alkylated with allyl bromide or propyliodide to (2), reduced to the primary amine (3) and acylated to compounds ZH-1 and 3 by established procedures in good yields (Zhang, Hussaini and Shen, manuscript in preparation). The final products were characterized as 1 : 1 mixtures of the corresponding *cis* and *trans* isomers that were not readily separable in several attempts.

Reduction of nitric oxide production by dithiolane analogs IFN-γ (5 – 100 kU/L) and LPS (10 – 100 μg/L), as expected, significantly increased the synthesis of NO in RAW 264.7 cells, as determined by accumulation of the NO oxidation product, nitrite, in the culture medium. IFN-γ (50 kU/L) and LPS (50 μg/L) that induced maximum nitrite accumulation were used to assess the effect of various concentrations of 50 dithiolane analogs. Three analogs (ZH-1, ZH-2, and ZH-3) of dithiolane, out of the 50 compounds tested, were found to be most potent in reducing nitrite formation. Simultaneous addition of dithiolane analogs to the cultures with either IFN-γ (Fig 1) or LPS (Fig 2) produced a concentration-dependent reduction in NO synthesis. The IC_{50} values for ZH-3, ZH-2, and ZH-1 in reducing IFN-γ-induced NO synthesis are (0.3 ± 0.05) μmol/L, (0.4 ± 0.05) μmol/L, and (1.5 ± 0.25) μmol/L ($n = 4$), respectively. Similarly, treatment of macrophages with a single concentration (2.0 μmol/L) of either ZH-1, ZH-2, or ZH-3, reduced LPS (50 μg/L) generated nitrite levels by 11.5 %, 48.0 %, and 55.0 %, respectively (Fig 2). As expected, NOS competitive inhibitor, N^G-monomethyl-*L*-arginine (NMMA), at 5 mmol/L, completely counteracted the effect of both LPS and IFN-γ NO levels (data not shown). PAF (10 nmol/L – 1 μmol/L) had no effect on NO production or iNOS expression. L-659,989 (0.1 – 10 μmol/L), a highly potent PAF-receptor antagonist effective at nanomolar concentrations, did not affect NO synthesis by either LPS or IFN-γ.

iNOS expression in cultures treated with dithiolane analogs In order to elucidate the mechanism involved in the inhibition of NO production by the dithiolane analogs, RAW 264.7 cells were treated with IFN-γ or LPS, in the presence and absence of dithiolane analogs or L-659,989. Northern blot analysis was then performed to assess the basal levels of iNOS mRNA. IFN-γ substantially increased the expression of iNOS mRNA in the RAW 264.7 cells and the dithiolane analogs

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Oct; 21 (10)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

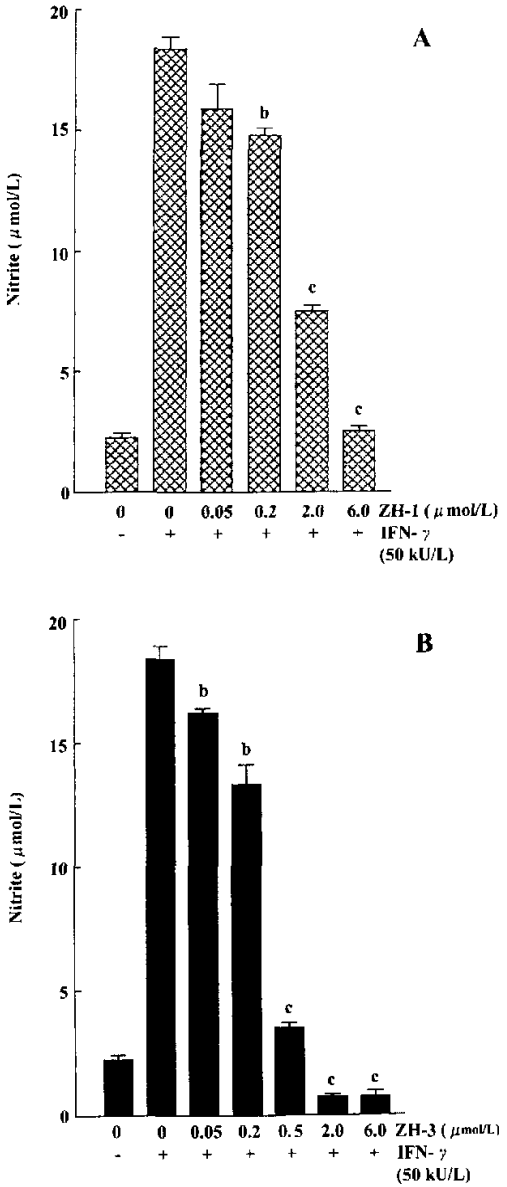


Fig 1. ***Nitrite generation in macrophages treated with*** **IFN-γ in the presence and absence of (A) ZH-1, (B) ZH-3. RAW 264.7 cells were treated with saline (control), 50 kU/L IFN-γ alone or with ZH-1/ZH-3. $n = 4$. $\bar{x} \pm s_{\bar{x}}$. $^bP < 0.05$, $^cP < 0.01$.**

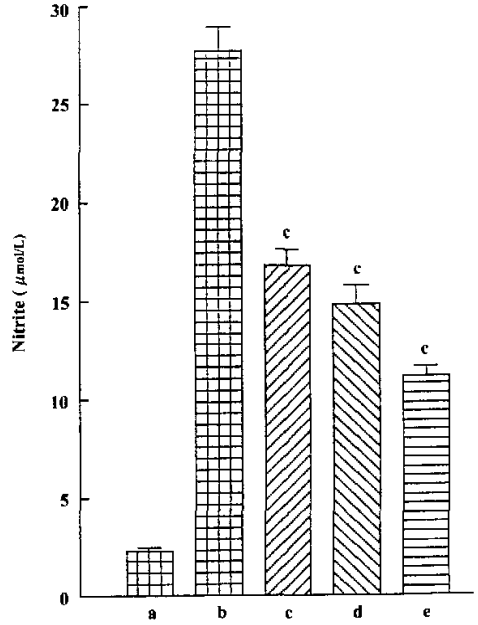


Fig 2. Nitrite generation in macrophages treated with LPS in the presence and absence of dithiolane analogs. RAW 264.7 cells were treated with saline (control), 50 μg/L LPS alone or with 2.0 μmol/L ZH-1 (c), ZH-2 (d), or ZH-3 (e). $n = 4$. $\bar{x} \pm s_{\bar{x}}$. $^cP < 0.01$.

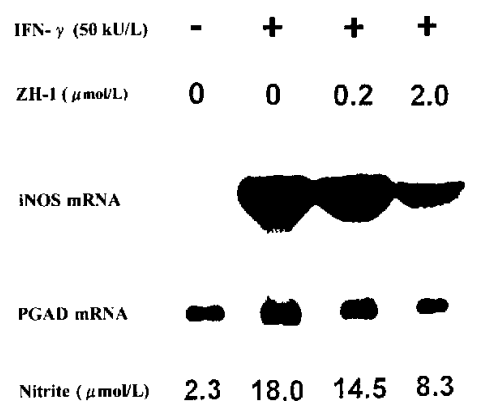


Fig 3. Northern blot analysis of iNOS mRNA expression in RAW 264.7 cells treated with IFN-γ. RAW 264.7 cells were treated with saline (control), kU/L IFN-γ alone or with ZH-1. Total RNA (20 mg/L) was subjected to electrophoresis on a 1.0 % agarose gel and hydridized with cDNA probes for iNOS and PGAD.

ZH-1 (Fig 3) and ZH-3 (Fig 4) apparently reduced the induction of iNOS in a concentration-dependent manner. Dithiolane analog ZH-2 also reduced the induction of iNOS mRNA expression by IFN-γ similarly (data not shown). Regarding the specificity of such inhibition, unlike non-specific mRNA synthesis inhibitors (for example, dactinomycin), the dithiolane analogs had no effect on the basal expression of PGAD (house keeping gene)

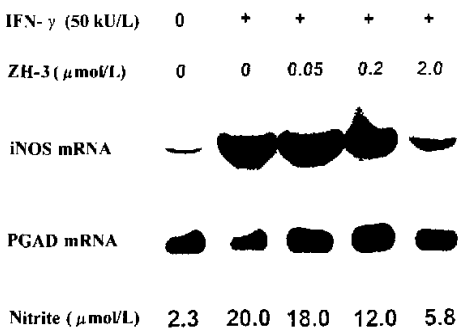


Fig 4. Northern blot analysis of iNOS mRNA expression in RAW 264.7 cells treated with IFN-γ. RAW 264.7 cells were treated with either saline, 50 kU/L IFN-γ alone or with ZH-3. Total RNA (20 mg/L) was subjected to electrophoresis on a 1.0 % agarose gel and hydridized with cDNA probes for iNOS and PGAD.

mRNA. Fig 5 shows the effect of ZH-1, ZH-2, and ZH-3 on LPS induction of iNOS mRNA expression in macrophages. The order of relative potency of these compounds in reducing iNOS mRNA induction by both LPS and IFN-γ is ZH-3 ≥ ZH-2 > ZH-1. Thus, counteraction of IFN-γ and LPS-induced nitrite generation by dithiolane analogs may be mediated at the level of iNOS mRNA. NMMA and L-659,989 did not affect the induction of iNOS (NMMA functions post-translationally by inhibiting the enzyme).

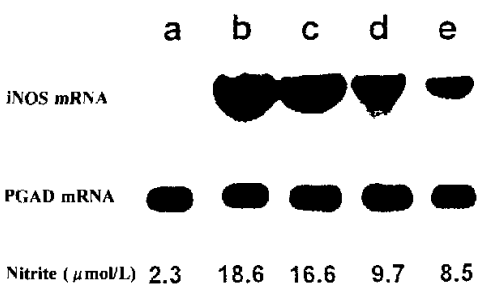


Fig 5. Northern blot analysis of iNOS mRNA expression in RAW 264.7 cells treated with LPS. RAW 264.7 cells were treated with 50 μg/L LPS (b) alone or with 2.0 μmol/L ZH-1 (c), 2.0 μmol/L ZH-2 (d), 2.0 μmol/L ZH-3 (e). Control cells that were not treated with LPS or inhibitor are shown in lane a. Total RNA (20 mg/L) was subjected to electrophoresis on a 1.0 % agarose gel and hybridized with cDNA probes for iNOS and PGAD.

Effect of synthetic compounds on PAF receptor binding ^{3}H-PAF binding studies were performed by NOVA Screen with rabbit platelet membrane preparation at 4 ℃, as an index of functional cell surface expression of PAF-receptor. The affinity (K_D) was 1.7 nmol/L. Displacement experiments were carried out with ^{3}H-PAF (1.2 nmol/L) in the presence of various concentrations of unlabeled PAF, L-659,989 and dithiolane analogs. Equilibrium dissociation constant (K_i) values were determined as previously described[17]. The K_i (mol/L) values for unlabled PAFc-16, ZH-1, ZH-2, ZH-3, and a well-characterized PAF-receptor antagonist, L-659,989 ($n = 3$) are 8.70×10^{-9}, 3.30×10^{-7}, 8.20×10^{-8}, $> 1.00 \times 10^{-6}$, and 0.85×10^{-9}, respectively. The order of relative potency is L659,989 > PAF > ZH-2 > ZH-1 > ZH-3 which is different from the potency for the same compounds in reducing NO formation.

Dithiolane analogs had no effect on brain NOS as determined by ^{3}H-citrulline formation in rat brain cytosol (data not shown). The compounds also did not antagonize the binding of ^{3}H-NMCI and ^{3}H-QNB to the nicotinic and muscarinic receptors, respectively (data not shown).

DISCUSSION

Overproduction of PAF and TNF-α has been implicated separately in the pathogenesis of septic shock[32,33]. LPS, the toxic moiety of the outer cell membrane of gram-negative bacteria, is the active component of endotoxin that mediates septic shock. LPS evokes a complex cascade of events including activation of leukocytes and the concomitant release of PAF and other cytokines such as TNF-α[34,35]. While PAF mediates hypotension in endotoxic shock by receptor activation of arachidonate cascade[36], TNF-α acts indirectly via NO-dependent pathway[37]. Thus, the control of PAF and NO activity will be therapeutically desirable in septic shock.

The present studies demonstrate the ability of a small group of novel dithiolane analogs of lignans to act both as PAF-receptor antagonists and inhibitors of iNOS induction. In PAF binding studies, these analogs of dithiolane, unlabeled PAF and L-659,989 antagonized the binding of labeled PAF to cell surface receptors. The relative PAF receptor blocking potency of the compounds tested was L-659,989 > PAF > ZH-2 > ZH-1 > ZH-3. Using IFN-γ and LPS, we have shown that only the dithiolane analogs but not L-659,989 reduced the generation of nitrite and iNOS mRNA expression. The dithi-

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Oct; 21 (10)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

olane analogs may be blocking NO synthesis by interfering with iNOS induction by LPS or IFN-γ. The PAF receptor antagonism by the dithiolane compounds seems unrelated to the mechanism responsible for their ability to inhibit iNOS induction since L-659,989, a well-characterized PAF receptor antagonist, had no effect on nitrite formation. In addition, there was no simple correlation in the relative potencies in terms of PAF receptor antagonism and inhibition of IFN-γ or LPS-induced nitrite generation by these compounds. A variety of drugs, including glucocorticoid[38], high concentrations of salicylate[39], antioxidants[40], and proteasome inhibitors[41], have been reported to block the induction of iNOS by preventing the translocation of NF-κB from the cytosol into the nucleus. Whether the dithiolane analogs of lignans used in the present studies, affect the translocation of NF-κB or other mechanisms remains to be determined.

PAF receptor antagonists have been used in a number of human disease models[15]. It was recognized early on that PAF receptor antagonists had positive effect on LPS-induced responses (hypotension, increased vascular permeability, organ damage and high mortality) in rats and mice. PAF receptor antagonists have been tested in large animals such as pigs, sheep, and monkeys, which, like human, are highly sensitive to LPS[15] and in a phase III septic shock trial[42]. The results were variable, with PAF receptor antagonists being more effective in endotoxin-induced lung injury and not consistently effective in the cardiovascular and hemodynamic responses in septic shock. In contrast, NO synthesis inhibition exerts beneficial hemodynamic and cardiovascular effects and improves survival in endotoxic shock in various species[43]. Thus, a PAF receptor antagonist with iNOS inhibitory activity may be more effective in controlling both lung injury, cardiovascular and hemodynamic responses associated with severe sepsis. The dual acting property is analogous with a group of lignan analogs developed recently, which inhibit both the PAF receptor and 5-lipoxygenase as more effective anti-asthmatic and anti-allergic agents[44].

In summary, the present studies constitute the first report of dithiolane analogs of lignans as a new class of inhibitors of iNOS induction, some having significant dual PAF receptor antagonism and iNOS inhibitory property. We believe that these compounds will be useful pharmacologic tools to control the pathophysiologic activities of both PAF and NO and as promising medicinal chemical lead for developing regulators of iNOS induction. Further chemical and biologic studies of related compounds are in progrees.

REFERENCES

1 Tracey KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, *et al*. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteremia. Nature 1987; 330: 662 – 4.
2 Verhoef J, Hustinx WM, Frasa H, Hoepelman AI. Issues in the adjunct therapy of severe sepsis. J Antimicrob Chemother 1996; 38: 167 – 82.
3 Okusawa S, Gelfand JA, Ikejima T, Connolly RJ, Dinarello CA. Interleukin-1 induces a shock-like state in rabbits: synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. J Clin Invest 1988; 81: 1162 – 72.
4 Wolkow PP. Involvement and dual effects of nitric oxide in septic shck. Inflamm Res 1998; 47: 152 – 66.
5 Nakae H, Endo S, Inada K, Yaegashi Y, Takakuwa T, Yamada Y, *et al*. Nitrite/nitrate (NOX) and type Ⅱ phospholipase A2, leukotriene B4, and platelet-activating factor levels in patients with septic shock. Res Commun Mol Pathol Pharmacol 1996; 92: 131 – 9.
6 Dinarello CA. Biology of interleukin-1. FASEB J 1988; 2: 108 – 13.
7 Mariano F, Guida G, Donati D, Tetta C, Cavalli PL, Verzetti G, *et al*. Production of platelet-activating factor in patients with sepsis-associated acute renal failure. Nephrol Dial Transplant 1999; 14: 1150 – 7.
8 Jacobs ER, Soulsby ME, Bone RE, Wilson FJ Jr, Hiller FC. Ibuprofen in canine endotoxin shock. J Clin Invest 1982; 70: 536 – 41.
9 Heller A, Koch T, Schmeck J, van Ackern K. Lipid mediators in inflammatory disorders. Drugs 1998; 55: 487 – 96.
10 Klabunde RE, Calvello C. Inhibition of endotoxin-induced microvascular leakage by a platelet-activating factor antagonist and 5-lipoxygenase inhibitor. Shock 1995; 4: 368 – 72.
11 Anderson BO, Bensard DD, Alden HH. The role of platelet activating factor and its antagonists in shock, sepsis and multiple organ failure. Surg Gynecol Obstet 1991; 172: 415 – 24.
12 Ayala A, Chaudry IH. Platelet activating factor and its role in trauma, shock and sepsis. New Horiz 1996; 4: 265 – 75.
13 Mustafa SB, Howard KM, Olson MS. Platelet-activating factor augments lipopolysaccharide-induced nitric oxide formation by rat Kupffer cells. Hepatology 1996; 23: 1622 – 30.
14 Braquet P, Touquil L, Shen TY, Vargaftig BB. Perspective in platelet-activating factor research. Pharmacol Rev 1987; 39: 97 – 145.
15 Summers JB, Albert DH. Platelet activating factor antagonists. In: August JT, Anders MW, Murad F, Coyle JT, editors. Advances in Pharmacology. San Diego: Acad Press; 1995. 32: 67 – 168.
16 Honda Z, Nakamura M, Miki I, Minami M, Watanabe T, Seyama Y, *et al*. Cloning by functional expression of platelet-activating factor receptor from guinea-pig lung. Nature 1991; 349: 342 – 6.
17 Shen TY, Hussaini IM. Kadsurenone and other related lignans as antagonists of PAF receptor. In: Murphy RC, Fitzpatrick FA, editors. Methods in Enzymology. San Diego:

Acad Press; 1989. 187: 446 – 54.

18 Hwang SB. Specific receptors of platelet-activating factor, receptor heterogeneity, and signal transduction mechanisms. J Lipid Mediat 1990; 2: 123 – 58.

19 Shen TY. Chemical and biochemical characterization of lignan analogs as novel PAF receptor antagonist. Lipids 1991; 26: 1154 – 6.

20 Sanchez-Crespo M, Fernandez-Gallardo S. Pharmacological modulation of PAF: a therapeutic approach to endotoxin shock. J Lipid Mediat 1991; 4: 127 – 43.

21 Albert DH, Magoc TJ, Tapang P, Luo G, Morgan DW, *Curtin M, et al*. Pharmacology of ABT-491, a highly potent platelet-activating factor receptor antagonist. Eur J Pharmacol 1997; 325: 69 – 80.

22 Balsa D, Merlos M, Giral M, Ferrando R, Garcia-Rafanell J, Forn J. Effects of a new platelet-activating factor antagonist, UR-12670, on several endotoxic shock markers in rats. Drugs Exp Clin Res 1997; 23: 191 – 9.

23 Szabo C, Wu CC, Mitchell JA, Gross SS, Thiemermann C, Vane JR. Platelet-activating factor contributes to the induction of nitric oxide synthase by bacterial lipopolysaccharide. Cir Res 1993; 73: 991 – 9.

24 Arthur JF, Shahin S, Dusting GJ. PAF antagonists block induction of nitric oxide synthase in cultured macrophages and vascular smooth muscle cells. Clin Exptl Pharmacol Physiol 1995; 22: 452 – 4.

25 Thiemermann C, Vane JR. Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharide in the rat *in vivo*. Eur J Pharmacol 1990; 182: 591 – 5.

26 Szabo C. Alterations in nitric oxide production in various forms of circulatory shock. *New Horizons* 1995; 3: 2 – 32.

27 Lowenstein CJ, Alley EW, Raval P, Snowman AM, Snyder SH, Russell SW, *et al*. Macrophage nitric oxide synthase gene: two upstream regions mediate induction by interferon-gamma and lipopolysaccharide. Proc Natl Acad Sci USA 1993; 90: 9730 – 4.

28 Goldstein D, Shen TY. Dual inhibitors of platelet-activating factor and 5-lipoxygenase. Ⅱ. Novel 2,4-diaryl-1,3-dithiolanes with iron chelating functionalities. Med Chem Res 1992; 2: 451 – 6.

29 Lysiak JJ, Hussaini IM, Webb DJ, Glass II WF, Allietta M, Gonias SL. α_2-Macroglobulin functions as a cytokine carrier to induce nitric oxide synthesis and cause nitric oxide-dependent cytotocity in the RAW 264.7 macrophage cell line. J Biol Chem 1995; 270: 21919 – 27.

30 Hussaini IM, LaMarre J, Lysiak JJ, Karns L, VandenBerg SR, Gonias SL. Transcriptional regulation of low density lipoprotein receptor-related protein expression by interferon-γ and the antagonistic activity of transforming growth factor-β1 in *RAW 264.7 macrophage-like cells*. *J Leuk Biol 1996*; 59: 733 – 9.

31 Hwang SB, Lee CSC, Cheah MJ, Shen TY. Specific receptor sites for 1-*O*-alkyl-2-*O*-acetyl-sn-glycero-3-phosphocholine (platelet-activating factor) on rabbit platelet and guinea pig smooth muscle membranes. Biochem 1983; 22: 4756 – 63.

32 Marks JD, Marks CB, Luce JM, Montgomery AB, Turner J, Metz CA, *et al*. Plasma tumor necrosis factor in patients with septic shock. Am Rev Respir Dis 1990; 141: 94 – 7.

33 Bussolino S, Porcellini MG, Varese L, Bosia A. Intravascular release of platelet-activating factor in children with sepsis. Throm Res 1987; 48: 619 – 20.

34 Chang SW, Feddersen CO, Henson PM, Voekel NF. Platelet-activating factor mediates hemodynamic changes and lung injury in endotoxin-treated rat. J Clin Invest 1987; 79: 1498 – 509.

35 Mathison JC, Wolfson E, Ulevitch RC. Participation of tumor necrosis factor in the mediation of gram negative bacterial lipopolysaccharide-induced injury in rabbits. J Clin Invest 1988; 81: 1925 – 37.

36 Yamanaka S, Miur K, Yurimura T, Okumura M, Yamamoto T K. Putative mechanism of hypotensive action of platelet-activating factor in dogs. Cir Res 1992; 70: 893 – 901.

37 Kilbourn G, Gross SS, Jubran A, Adams J, Griffith OW, Levi R, *et al*. N^G-methyl-*L*-arginine inhibits tumor necrosis factor-induced hypotension: implications for involvement of nitric oxide. Proc Natl Acad Sci USA 1990; 87: 3629 – 32.

38 Kleiner H, Euchenhofer C, Ihrig-Biedert I, Forstermann U. Glucocorticoids inhibit the induction of nitric oxide synthase Ⅱ by down-regulating cytokine-induced activity of transcription factor nuclear factor-κB. Mol Pharmacol 1996; 49: 15 – 21.

39 Pierce JW, Read MA, Ding H, Luscinskas FW, Collins T. Salicylates inhibit I-kappa-B-alpha phoshporylation, endothelial-leukocyte adhesion molecule expression, and neutrophil *transmigration*. *J Immunol 1996*; 156: 3961 – 69.

40 Meyer M, Shreck R, Baeuerle PA. H_2O_2 and antioxidants have opposite effects on activation of NF-κB and AP-1 in intact cells: AP-1 as secondary antioxidant responsive factor. EMBO J 1993; 12: 2005 – 15.

41 Finco TS, Beg AA, Baldwin Jr AS. Inducible phosphorylation of IkBα is not sufficient for its dissociation from NF-κB and is inhibited by proteasome inhibitors. Proc Natl Acad Sci USA 1994; 91: 11884 – 8.

42 Tenaillon A, Dhainaut JF, Letulzo UY, Schlemmer B, Solet JP, Wolff M, *et al*. Efficacy of PAF antagonist BN52021 in reducing mortality of patients with severe gram negative sepsis. Am Rev Respir Dis 1993; 147: A97.

43 Thiemermann C. The role of the *L*-arginine: nitric oxide pathway in circulation shock. In: August JT, Anders MW, Murad F, Coyle JT, editors. Advances in Pharmacology. San Diego: Acad Press; 1994. 28: 45 – 79.

44 Cai X, Scannell RT, Yaeger D, Hussoin MS, Killian DB, Qian C, *et al*. *trans*-2-[3-Methoxy-4-chlorophenylthioethoxy)-5-(*N*-methyl-*N*-hydroxyureidyl) methylphenyl]-5-(3,4,5-trimethoxyphenyl) tetrahydrofuran (CMI-392), a potent dual 5-lipoxygenase inhibitor and platelet-activating factor receptor antagonist. J Med Chem 1998; 41: 1970 – 9.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Oct; 21 (10)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

木酚素二硫戊环类似物抑制 γ-干扰素和脂多糖在巨噬细胞中诱生一氧化氮

Isa M HUSSAINI[1], 张奕华[2], Jeff J LYSIAK, Tsung Y SHEN[2]

(*Departments of Pathology and* [2]*Chemistry*, *University of Virginia*, *Charlottesville*, *VA 22908*, *USA*)

关键词 木酚素类; 干扰素Ⅱ型; 脂多糖类; 血小板活化因子; 一氧化氮; 一氧化氮合酶

目的: 研究木酚素二硫戊环类似物及血小板活化因子(PAF)受体拮抗剂 L-659,989 对 PAF-受体结合, 对 γ-干扰素和脂多糖诱导的一氧化氮(NO)生成, 以及对 iNOS mRNA 表达的影响. **方法**: 通过对兔血小板膜中^{3}H 标记的 PAF-受体竞争结合试验来研究对 PAF-受体的拮抗作用; 测定 NO 的氧化产物亚硝酸盐来定量 NO 的生成. 用 RNA 印迹分析来研究对 iNOS mRNA 表达的影响. **结果**: 二硫戊环类似物抑制 NO 的生成, 减少 iNOS mRNA 的表达, 拮抗^{3}H-PAF 受体. L-659,989 对 NO 生成和 iNOS mRNA 表达没有影响. 受试物的 PAF 受体拮抗活性和 iNOS 抑制活性之间无简单关联. **结论**: 木酚素二硫戊环类似物是 iNOS 表达的新型调控剂, 具有抑制 iNOS 诱导和拮抗 PAF 受体的双重活性.

(责任编辑 刘俊娥)