

## Prophylactic effects of tetramethyl pyrazine on mice with endotoxemia and its relationship with platelet-activating factor

FU Yong, HU You-Mei<sup>1</sup>

(Department of Pharmacology, Third Military Medical University, Chongqing 400038, China)

**KEY WORDS** pyrazines; platelet activating factor; endotoxemia; phospholipases A; acetyl-CoA C-acetyltransferase; peritoneal macrophages; lipopolysaccharides

activities of PLA<sub>2</sub> and acetyl-CoA; lyso-PAF acetyltransferase.

### ABSTRACT

**AIM:** To study the prophylactic effects of tetramethyl pyrazine (TMP) on mice with endotoxemia and its relationship with platelet-activating factor (PAF). **METHODS:** LD<sub>50</sub> of lipopolysaccharides (LPS, 15 mg·kg<sup>-1</sup>) was injected into mice (iv) pretreated with TMP (ip). The survival rate and the level of serum PAF were observed. The PAF induced by LPS *in vivo* and *in vitro*, and the activities of PLA<sub>2</sub> and acetyl-CoA; lyso-PAF acetyltransferase were determined. **RESULTS:** TMP (10, 30, 90 mg·kg<sup>-1</sup>) obviously lowered the mortality of mice and also dose-dependently decreased the level of serum PAF [(25.5 ± 1.7), (13.4 ± 3.2), (9.6 ± 2.1) μg·L<sup>-1</sup>, vs control (29.3 ± 2.1) μg·L<sup>-1</sup>, P < 0.01]. TMP (0.05, 0.5, 5, 50 μmol·L<sup>-1</sup>) dose-dependently decreased the release of PAF [(12.7 ± 1.6), (8.9 ± 1.2), (6.9 ± 0.8), (5.5 ± 1.0) μg·L<sup>-1</sup> vs control (11.9 ± 1.8) μg·L<sup>-1</sup>, P < 0.01] from PMØ cultured with LPS (5 mg·L<sup>-1</sup>), reduced the PLA<sub>2</sub> activity [(149.9 ± 2.8), (117.5 ± 2.0), (89.6 ± 2.0), (75.0 ± 2.8) U vs control (170.8 ± 3.9) U, P < 0.01] and acetyl-CoA; lyso-PAF acetyltransferase activity [PAF (9.5 ± 0.7), (5.2 ± 0.7), (2.9 ± 0.3), (2.5 ± 0.3) μg·g<sup>-1</sup> (protein)·min<sup>-1</sup> vs control (11.0 ± 0.7) μg·g<sup>-1</sup> (protein)·min<sup>-1</sup>, P < 0.01] of PMØ lysate. **CONCLUSION:** TMP protected the mice with endotoxemia from the death by decreasing the biosynthesis of PAF through the inhibition of the

### INTRODUCTION

Platelet-activating factor (PAF) is a potent phospholipid inflammatory mediator which involves and plays an important pathophysiologic role in multiple organ failure (MOF) or death caused by endotoxemia<sup>[1,2]</sup>. Some PAF selective antagonists had been used to treat septic shock. However their prophylactic effects on endotoxemia animal are limited, because of PAF molecular heterogeneity<sup>[3]</sup> which still remains a complicated and crucial problem to be solved. So we try to find a drug which can block PAF synthesis and therefore abolish its pathologic role in endotoxemia in order to treat sepsis. Tetramethyl pyrazine (TMP), an effective component of *Ligusticum chuanxiong* Hort, decreased the level of serum PAF in asthma guinea pig<sup>[4]</sup>, which suggested that it might be possible for the prophylactic effects on the endotoxemia. In this paper, the effects of TMP on endotoxemia mice and its relationship with PAF were investigated.

### MATERIALS AND METHODS

**Mice** Kunming mice (♂ 50, ♀ 50, 20 g ± s 2 g) were provided by Experimental Animal Institute of Third Military Medical University (Grade II, Certificate No 24301050).

**Drug and chemicals** TMP (Beijing Institute of Pharmaceutical); LPS (from *E coli* 0111:B4, Sigma); PAF (Sigma); lyso-PAF (Sigma); RPMI-1640 medium (Gibco); acetyl-CoA (BOM, Germany); bovine serum albumin (BSA, Beijing Biochemical Product Factory).

**Endotoxemia** LPS 15 mg·kg<sup>-1</sup> was injected

<sup>1</sup> Correspondence to Prof HU You-Mei. Phn 86-23-6875-3008. Fax 86-23-6532-0699. E-mail chentmin@public.cta.cq.cn  
Received 1998-07-08 Accepted 1998-12-14

by tail vein. The survival rate of mice was observed for 25 h.

**Bioassay of serum PAF** Mice were pretreated (ip) with TMP, and 30 min later LPS was injected by tail vein. Samples of blood were collected at 5 min. PAF was measured<sup>5)</sup>.

**Mice PMØ isolation** The mice were decapitated. RPMI-1640 medium (8 mL) with HEPES (25 mmol · L<sup>-1</sup>) were injected into the peritoneal cavity, the abdomen was massaged several times and the fluid was aspirated. The cells were collected by centrifugation (500 × g, 4 °C, 5 min), washed twice with the RPMI-1640, and then the cells were suspended in RPMI-1640 medium. The peritoneal macrophages (PMØ) in the collected cells (Wright's stain) were >90 %.

**Bioassay of PAF<sup>6)</sup>**

**Assay for PMØ lysate PLA<sub>2</sub>** The cultivated PMØ cells were scraped from the surface of the well and immediately homogenized using a sonicator (needle probe for 3 pulses of 25 W for 15 s). Activity of PLA<sub>2</sub> was measured<sup>7)</sup>.

**Assay for acetyl-CoA: lyso-PAF acetyltransferase activity** The PMØ were cultured with TMP. The cells were homogenized. PMØ homogenate 100 µL (50 µg protein) was added to 400 µL of reaction mixture (containing HEPES 4.2 mmol · L<sup>-1</sup>, pH 7.4; NaCl 137 mmol · L<sup>-1</sup>; KCl 2.6 mmol · L<sup>-1</sup>; CaCl<sub>2</sub> 1.3 mmol · L<sup>-1</sup>; MgCl<sub>2</sub> 1 mmol · L<sup>-1</sup>; BSA 0.25 g; acetyl-CoA 40 mmol · L<sup>-1</sup>; lyso-PAF 150 µmol · L<sup>-1</sup>) and incubated at 37 °C for 15 min. The reaction was stopped by the addition of iced acetic acid 50 mmol · L<sup>-1</sup> (0.5 mL in methanol). The PAF was extracted and bioassayed for the activity<sup>8)</sup>.

## RESULTS

**Effect of TMP on survival rate of endotoxemia mice** The survival rate of mice pretreated with TMP was much higher than that of mice untreated with TMP (Tab 1).

**Effect of TMP on serum PAF of mice with endotoxemia** TMP 10, 30, 90 mg · kg<sup>-1</sup> dose-dependently decreased the level of serum PAF (Tab 2).

**Effect of TMP on release of PAF from PMØ, and activities of PLA<sub>2</sub> and acetyl-CoA: lyso-PAF acetyltransferase of PMØ** TMP 0.05 -

**Tab 1. Effect of tetramethyl pyrazine (TMP) on survival rate of mice after iv LPS 15 mg · kg<sup>-1</sup>. n = 8 mice. <sup>b</sup>P < 0.05 vs control.**

TMP/ mg · kg <sup>-1</sup>	Number of survived mice				
	5 h	10 h	15 h	20 h	25 h
0	7	4	3	1	1
10	8	6	4	4	3
30	8	7	6	6 <sup>b</sup>	5 <sup>b</sup>
90	8	8 <sup>b</sup>	8 <sup>b</sup>	7 <sup>b</sup>	7 <sup>b</sup>

**Tab 2. Effect of TMP on serum platelet-activating factor (PAF) of mice after iv LPS 15 mg · kg<sup>-1</sup>. n = 8 mice.  $\bar{x} \pm s$ . <sup>c</sup>P < 0.01 vs LPS group.**

Drugs/mg · kg <sup>-1</sup>	PAF/µg · L <sup>-1</sup>
LPS	29.3 ± 2.1
LPS + TMP 10	25.5 ± 1.7 <sup>c</sup>
LPS + TMP 30	13.4 ± 3.2 <sup>c</sup>
LPS + TMP 90	9.6 ± 2.1 <sup>c</sup>

50 µmol · L<sup>-1</sup> dose-dependently inhibited the PAF release from PMØ induced by LPS and also decreased the activities of PLA<sub>2</sub> and acetyl-CoA: lyso-PAF acetyltransferase in PMØ lysate (Tab 3).

**Tab 3. Effects of TMP on release of PAF from PMØ and activities of PMØ PLA<sub>2</sub>, acetyl-CoA: lyso-PAF acetyltransferase induced by LPS 5 mg · L<sup>-1</sup>. n = 8 mice.  $\bar{x} \pm s$ . <sup>c</sup>P < 0.01 vs LPS group.**

Drugs/ µmol · L <sup>-1</sup>	PAF/ µg · L <sup>-1</sup>	PLA <sub>2</sub> / U	Acetyltransferase PAF µg · g <sup>-1</sup> (protein) · min <sup>-1</sup>
LPS	11.9 ± 1.8	170.8 ± 3.9	11.0 ± 0.7
LPS + TMP 0.05	12.7 ± 1.6	149.9 ± 2.8 <sup>c</sup>	9.5 ± 0.7 <sup>c</sup>
LPS + TMP 0.5	8.9 ± 1.2 <sup>c</sup>	117.5 ± 2.0 <sup>c</sup>	5.2 ± 0.7 <sup>c</sup>
LPS + TMP 5.0	6.9 ± 0.8 <sup>c</sup>	89.6 ± 2.0 <sup>c</sup>	2.9 ± 0.3 <sup>c</sup>
LPS + TMP 50.0	5.5 ± 1.0 <sup>c</sup>	75.0 ± 2.8 <sup>c</sup>	2.5 ± 0.3 <sup>c</sup>
LPS + Qui 100.0	7.0 ± 0.7 <sup>c</sup>	68.7 ± 7.7 <sup>c</sup>	-

Qui; quinaquine as a positive control.

## DISCUSSION

PAF is known to have a potentially detrimental impact on endothelial cells leading to increase vascular permeability and to promote the cells to release IL-1,

TNF, LT, oxygen free radicals, protease, thrombin, ATP etc. PAF regulates the adhesion molecules to express in leukocyte and endothelial cells resulting in cell chemotaxis and tissue damaged. PAF decreases the blood pressure in the shock animal by inhibition of the myocardium resulting from inhibition of exchange of  $\text{Na}^+ - \text{Ca}^{2+}$ . PAF also activates the  $\text{PLA}_2$  which is an enzyme of PAF synthesis through its combination with GTP protein. These products can activate the cells to produce more PAF. As a result an adverse feedback action was established. This adverse action played an important part of septic shock.

The structures of PAF molecule are heterogeneity including alkyl-PAF, acyl-PAF, and alkenyl-PAF, and in which the PAF molecules differ in the lengths of the carbon chains and have polar head groups other than choline. These indicate that the structures of PAF are very complicated. In addition PAF receptors exist in multiple subtypes. So, the pathophysiological effects of PAF are variety. It had been demonstrated that a selective PAF antagonist only affected some actions of PAF but not the others. We found a drug TMP which inhibited the release of PAF from  $\text{PM}\emptyset$  and lowered the level of serum PAF of mice. Therefore, the drug can minimize the pathophysiological actions of PAF, and protect the mice from death.

The synthesis of PAF have 2 pathways: remodeling pathway and alternative pathway. It has been commonly accepted that PAF was synthesized on remodeling pathway in sepsis.  $\text{PLA}_2$  and acetyl-CoA; lyso-PAF acetyltransferase were 2 key enzymes in this pathway.  $\text{PLA}_2$  activation occurred after inflammatory cell stimulation and resulted in the hydrolysis of membrane phospholipids to generate a variety of 2-lysophospholipids which may then serve as the immediate precursors of PAF. The acetylation of free hydroxyl at the *sn*-2 position by PAF acetyltransferase and acetyl-CoA produced various molecular species of PAF. In our studies, we found that TMP inhibited the activities of  $\text{PLA}_2$  and acetyl CoA; lyso-PAF acetyltransferase and resulted in decreasing the release of PAF from  $\text{PM}\emptyset$  stimulated with LPS obviously.

*Ligusticum chuanxiong*, a natural drug used in our country for many years has been used as "removing blood stasis to promote circulation". In recent years, the effective constituent of *Ligusticum chuanxiong*, TMP, was investigated in experimental animals and in

clinic trial for this purpose. It had been proved that TMP had notable protective effects on postischemia reperfusion myocardium of rabbit heart and against pulmonary edema induced by adrenalin in rat, cerebral vascular disease, and circulating shock induced by LPS etc. The mechanisms were found that the TMP had anti-lipid peroxidative reaction and scavenged oxygen free radicals<sup>[9]</sup>; promoted the  $\text{PGI}_2$  synthesis and minimized the release of  $\text{TXA}_2$ <sup>[10]</sup>; inhibited the release of ET-1 from tissue, and then attenuated the constriction of coronary arteries<sup>[11]</sup>. TMP not only blocked the entry of extracellular calcium through calcium channels but also inhibited the release of intracellular stored calcium in the vascular smooth muscle cells<sup>[12]</sup>. TMP at low concentration stimulated the pulmonary arteries to produce  $\text{NO}$ <sup>[13]</sup>, resulting in dilatation of the blood vessels and pulmonary microvasculature. TMP inhibited the endothelial cells of cerebral vascular to express the ICAM-1 induced by  $\text{TNF}_\alpha$  and LPS *in vivo*<sup>[14]</sup> that would minimize the adhesion of leukocytes. TMP lowered the  $\text{TNF}_\alpha$  and  $\text{NO}$  level in LPS-induced circulating shock rats<sup>[15]</sup>. In addition, TMP has been known to have anti-aggregation of blood platelet due to inhibition of releasing intracellular stored calcium and enhancement of intracellular cAMP by inhibiting cAMP phosphodiesterase, attenuating the synthesis and activity of  $\text{TXA}_2$ , inhibiting the release of serotonin, and reducing glycoprotein II b/III a on the surface of activated platelet. In our studies, TMP also inhibited the activities of  $\text{PLA}_2$  and acetyl-CoA lyso-PAF acetyltransferase, resulting in lowering the synthesis of PAF. These effects play an important part of the mechanism of protecting effect against endotoxemia shock. In conclusion, TMP protected the mice with endotoxemia induced by iv LPS from the death. TMP decreased the biosynthesis of PAF through inhibition of the activities of  $\text{PLA}_2$  and acetyl-CoA acetyltransferase on the remodeling pathway of PAF.

## REFERENCES

- 1 Prescott SM, Zimmerman GA, McIntyre TM. Platelet-activating factor. *J Biol Chem* 1990; 265: 17381-4.
- 2 Koltai M, Hosford D, Braquet PG. Platelet-activating factor in septic shock. *New Horiz* 1993; 1: 87-95.
- 3 McManus LM, Woodard DS, Deavers SI, Pinckard RN.

- PAF molecular heterogeneity: pathobiological implications. *Lab Invest* 1993; 69: 639-50.
- 4 Yu HP, Li PS, Yong FJ. The variation of PAF in allergic asthma of guinea pig and the effect of tetramethyl pyrazine. *Chin J Tuber Resp Dis* 1993; 16: 152.
- 5 Grandel KE, Farr RS, Wanderer AA, Eisenstadt TC, Wasserman SI. Association of platelet-activating factor with primary acquired cold urticaria. *N Engl J Med* 1985; 313: 405-9.
- 6 Song SQ, Hu YM. Effects of tetrandrine, Fura 2-AM, and Bay K 8644 on platelet-activating factor release from rat peritoneal macrophages stimulated by lipopolysaccharides. *Acta Pharmacol Sin* 1996; 17: 230-7.
- 7 Chen SF, Wu ZL. A convenient and rapid assay method for PLA<sub>2</sub> in body fluid and tissues. *Acad J Second Military Med Univ* 1989; 10: 254-6.
- 8 Holland MR, Venable ME, Whatley RE, Zimmerman GA, McIntyre TM, Prescott SM. Activation of the acetyl-coenzyme A: lysoplatelet-activating factor acetyltransferase regulates platelet-activating factor synthesis in human endothelial cells. *J Biol Chem* 1992; 267: 22883-90.
- 9 Xu ZJ, Wang WT, Li D. Protective effects of *Ligustrazine* on myocardial ischemia-reperfusion injury in rabbits and its mechanism. *Basic Med Sci Clin* 1997; 17: 308-11.
- 10 Feng J, Liu R, Wu G, Tang S. Effects of tetramethylpyrazine on the release of PGI<sub>2</sub> and TXA<sub>2</sub> in the hypoxic isolated rat heart. *Mol Cell Biochem* 1997; 167: 153-8.
- 11 Zeng Z, Zhu W, Zhou X, Jin Z, Liu H, Chen X *et al*. Tetramethylpyrazine, a Chinese drug, blocks coronary vasoconstriction by endothelin-1 and decreases plasma endothelin-1 levels in experimental animals. *J Cardiovasc Pharmacol* 1998; 31 Suppl 1: S313-6.
- 12 Pang PK, Shan JJ, Chiu KW. Tetramethylpyrazine, a calcium antagonist. *Planta Med* 1996; 62: 431-5.
- 13 Peng W, Hucks D, Priest RM, Kan YM, Ward JP. Ligustrazine-induced endothelium-dependent relaxation in pulmonary arteries via an NO-mediated and exogenous L-arginine-dependent mechanism. *Br J Pharmacol* 1996; 119: 1063-71.
- 14 Liu Y, Xu YG. Effect of ligustrazine on the expression of ICAM-1 of cerebral vascular endothelial cells. *Basic Med Sci Clin* 1997; 17: 313.
- 15 Liao MH, Wu CC, Yen MH. Beneficial effects of tetramethylpyrazine, an active constituent of Chinese herbs, on rats with endotoxemia. *Proc Natl Sci Counc Repub China B* 1998; 22: 46-51.

四甲基吡嗪对内毒素血症小鼠的保护作用  
及其与血小板活化因子的关系

傅勇, 胡友梅

(第三军医大学药理教研室, 重庆 400038, 中国)

关键词 吡嗪类; 血小板活化因子; 内毒素血症;  
磷脂酶 A 类; 乙酰辅酶 A C-乙酰基转移酶; 腹腔  
巨噬细胞; 脂多糖

目的: 研究四甲基吡嗪(TMP)对内毒素血症小鼠的保护作用及其与血小板活化因子(PAF)的关系。  
方法: 给 TMP 处理的小鼠 iv LPS, 观察其存活率及血清 PAF 水平。体外用 LPS 刺激小鼠 PMØ, 测定 PAF 及 PLA<sub>2</sub> 和乙酰辅酶 A 乙酰基转移酶的活性。结果: TMP 明显提高小鼠存活率和降低血清 PAF 水平。体外, TMP (0.05-50 μmol·L<sup>-1</sup>) 剂量依赖性减少 PMØ 释放 PAF [(12.7 ± 1.6), (8.9 ± 1.2), (6.9 ± 0.8), (5.5 ± 1.0) μg·L<sup>-1</sup>, P < 0.01], 降低 PLA<sub>2</sub> 活性 [(149.9 ± 2.8), (117.5 ± 2.0), (89.6 ± 2.0), (75.0 ± 2.8) U, P < 0.01] 和乙酰辅酶 A 乙酰基转移酶活性 [PAF (9.5 ± 0.7), (5.2 ± 0.7), (2.9 ± 0.3), (2.5 ± 0.3) μg·g<sup>-1</sup> (protein)·min<sup>-1</sup>, P < 0.01]。结论: TMP 对内毒素血症小鼠有保护作用, 其机制是通过抑制 PLA<sub>2</sub> 和乙酰辅酶 A 乙酰基转移酶的活性而抑制 PAF 的合成。

(责任编辑 杨雪芳)