Pretreatment of Curcumin Protects Vascular Dysfunction in Endotoxin-induced Septic Shock in Mice

ผลของเคอร์คูมินในการปกป้องการทำงานของหลอดเลือดที่ผิดปกติในหนูไมซ์ที่ถูกชักนำให้เกิดภาวะช็อคด้วยสารเอ็นโดท็อกซิน

Kwanjit Sompamit (ขวัญจิต สมพมิตร)* Dr. Upa Kukongviriyapan (ดร. ยุพา คู่คงวิริยพันธุ์)**
Dr. Veerapol Kukongviriyapan (ดร. วีรพล คู่คงวิริยพันธุ์)***
Dr. Patchareewan Pannangpetch (ดร. พัชรีวัลย์ ปั้นเหน่งเพ็ชร)****

ABSTRACT

The present study was aimed to investigate whether curcumin (*Curcuma linga*) could protect against vascular dysfunction caused by endotoxin-induced septic shock in mice. Male ICR mice were orally administrated with a single dose of curcumin (100 mg/kg) for three hours before induction of sepsis by intraperitoneal injection with bacterial endotoxin, lipopolysaccharide (LPS) 10 mg/kg. Fifteen hours after injection with LPS, mice were anesthetized for hemodynamic measurement and antioxidant markers analysis. The results showed that LPS caused markedly decreased blood pressure, increased heart rate and increased oxidative stress markers meanwhile pretreatment with curcumin significantly protected vascular dysfunction and oxidative stress. The vascular protective effect of curcumin may be attributable to the free radicals scavenging activity and the maintenance of antioxidant defense system.

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อทดสอบผลของเคอร์คูมินต่อการป้องกันการทำงานล้มเหลวของหลอดเลือดในหนูไมซ์ที่ถูกชักนำให้เกิดภาวะช็อคโดยเอนโดท็อกซินไลโพโพลีแซคคาไรด์โดยหนูไมซ์จะถูกป้อนด้วยเคอร์คูมินในขนาดความเข้มข้น 100 มก./กก. เป็นเวลา 3 ชั่วโมงก่อนฉีดสาร ไลโพโพลีแซคคาไรด์ (LPS), ด้วยขนาดความเข้มข้น 10 มก./กก. เข้าทางช่องท้อง หลังจากฉีด LPS เป็นเวลา 15 ชั่วโมง ทำการสลบหนูไมซ์เพื่อศึกษาพลศาสตร์การไหลเวียนเลือดและ ดัชนีชี้วัดภาวะเครียดออกซิเดชัน จากผลการศึกษาพบว่า LPS มีผลลดความดันเลือด เพิ่มอัตราการเต้นของหัวใจและเพิ่มดัชนีชี้วัดภาวะเครียดออกซิเดชันของหนูไมซ์ ในขณะที่เคอร์คูมินสามารถป้องกันภาวะการทำงานของหลอดเลือดล้มเหลวและลดภาวะเครียดออกซิเดชันได้อย่างมีนัยสำคัญ

**PhD. Student, Department of Physiology, Faculty of Medicine, Khon Kaen University*

*** Associate Professor, Department of Physiology, Faculty of Medicine, Khon Kaen University*

**** Associate Professor, Department of Pharmacology, Faculty of Medicine, Khon Kaen University*

***** Associate Professor, Department of Pharmacology, Faculty of Medicine, Khon Kaen University*

ซึ่งผลการปกป้องหลอดเลือดของเคอร์คูมินนี้อาจเนื่องมาจากฤทธิ์ในการกำจัดอนุมูลอิสระและรักษาระบบต้านออกซิแดนท์ของเคอร์คูมิน

Key Words : Curcumin, Vascular dysfunction, Oxidative stress, Endotoxin, Septic Shock
คำสำคัญ : เคอร์คูมิน การทำงานของหลอดเลือดผิดปกติ ภาวะเครียดออกซิเดชัน ภาวะช็อคจากการติดเชื้อ

Background and Significance

The term vascular dysfunction describes several pathological conditions, including altered anticoagulant and anti-inflammatory properties of the endothelium, impaired modulation of vascular growth, and dysregulation of vascular remodeling. In much of the literature, however, the term endothelial dysfunction specifically refers to an impairment of endothelium dependent vasodilation caused by decreased nitric oxide (NO) biovailability in the vessel wall (Ferroni *et al.*, 2006). The decline in NO bioavailability may be caused by decrease expression of the endothelial cell NO synthase (eNOS) (Wilcox *et al.*, 1997), a lack of substrate or cofactors for eNOS, alterations of cellular signaling such that eNOS is not appropriately activated and finally accelerated NO degradation by reactive oxygen species (ROS) (Cai and Harrison, 2000). Endothelial dysfunction has been demonstrated in subjects with different risk factors for atherosclerosis, such as hypercholesterolemia, diabetes, hypertension and smoking (Chan *et al.*, 1995; Drexler and Hornig, 1999).

It is now clearly established that bacterial endotoxin, a lipopolysaccharide (LPS) component of the outer membrane of Gram-negative bacteria, is the major mediator of the high morbidity and mortality rates characteristic of gram-negative septic shock. The development of septic shock results in a progressive failure of the circulation to provide blood and oxygen to vital organs of the body leading to impair tissue perfusion and oxygen extraction (Thiemermann, 1997). Moreover, most of the toxicities of LPS, both in the liver and in the systemic circulation, have been related to the release of proinflammatory cytokines such as interleukins (IL-1, IL-6), tumour necrosis factor alpha (TNF-α), and ROS (Arthur *et al.*, 1988; Hartung and Wendel, 1991; Luster *et al.*, 1994). The excess of ROS, especially superoxide anion ($O^{\cdot-}_2$), can oxidize NO and transform it into peroxynitrite (ONOO), an inactive molecule that can lead to more oxidation (Esper *et al.*, 2006) LPS-induced increase in lipid peroxidation, which is an index of oxidative stress, has been described in several studies. Previous study has been also shown that LPS can cause depletion of endogenous liver antioxidants such as reduced glutathione (GSH) in a dose dependent manner (Jaeschke, 1993).

Curcumin, the active component in turmeric, has been shown to possess anti-inflammatory, antioxidant, and antitumor activities (Jin *et al.*, 2007). It also inhibited LPS-induced production of TNFα, IL-1β, and the activation of nuclear factor NF-kB in a human monocytic-derived cell (Jin *et al.*, 2007).

Given a strong evidence of involvement of oxidative stress in endotoxin-induced-septic shock, the present study was aimed to investigating the effect of curcumin on modulation of vascular

dysfunction and oxidative stress caused by bacterial endotoxin, LPS.

Methods

Animals and treatments

The experiments were separated into two main parts, vascular function assessment and biochemical evaluation. Male ICR mice (25-30 g) were obtained from the Animal Care Unit of Faculty of Medicine, Khon Kaen University (Khon Kaen, Thailand) and maintained in a room at 25°C under a 12 h dark/light cycle. The animals were given a standard chow diet (Chareon Pokapan Co. Ltd., Thailand) and tab water ad libitum. Mice were randomly divided to three groups; control, LPS and LPS+curcumin (n=6-10/group):

Control group: mice were treated with vehicle at the same volume as other groups.

LPS group: Mice were injected with LPS (10 mg/kg; i.p.) and rest in metabolic cage for 15 hours for urine sample collection

LPS+curcumin group: Mice were treated with curcumin (100 mg/kg; p.o.) for 3 hours and followed with LPS injection (10 mg/kg; i.p.) and rest in metabolic cage for 15 hours for urine sample collection.

Vascular function assessment and preparation of Blood sample

Mice were anesthetized with ketamine/xylazine (100:2.5 mg/kg; i.p.). Body temperature, monitoring by the rectal temperature probe, was kept constant at 37°C by using a heat pad. A tracheostomy was performed for spontaneously breathing. The right carotid artery was cannulated with PE tubing which was connected to a pressure transducer for continuously monitoring of arterial blood pressure using the acquisition and analysis software (Biopac system, California, U.S.A.). The left jugular vein was cannulated with another PE tubing for infusion of vasoactive agents. After obtaining stable baseline measurements, an increasing dose of vasodilators; acetylcholine (ACh: 10 nmol/kg) and sodium nitroprusside (SNP: 20 nmol/kg) or the vasoconstrictor; phenylephrine (Phe: 0.03 μmol/kg) were stepwise infused, while blood pressure was continuously monitored. After finish hemodynamic assessment, blood samples were collected from abdominal aorta and thoracic aorta was dissected for measurement of $O_2^{\cdot-}$ production.

Blood samples were kept in a microcentrifuge tube for determination of whole blood GSH ratio and plasma malondialdehyde (MDA).

Biochemical evaluation

Blood and urine samples were kept in a microcentrifuge tube for biochemical assays including malondialdehyde (MDA) and lipid hydroperoxide as lipid peroxidation markers (Luangaram *et al.*, 2007; Long et al., 1999). Assay of ratio of glutathione (GSH/GSSG) as indicator for redox state was modified from Tietze (1969) and Liang et al. (2002) (Liang et al., 2002; Tietze, 1969) and NO metabolite (NOx) as NO production (Kukongviriyapan et al., 2007)

Statistical Analysis

Results were expressed as mean ± S.E.M. The differences among treatment groups were analyzed by one-way analysis of variance (ANOVA) followed by post hoc test. A *p*-value of less than 0.05 was considered significant.

Results

Effect of curcumin on vascular function

LPS induced vascular dysfunction by a marked decline in blood pressure and an increase in heart rate of mice treated with LPS. Meanwhile, LPS+curcumin group showed an improvement of hemodynamics by increasing blood pressure and decreasing heart rate to nearly control values (Table 1). Moreover, injection with LPS attenuated vascular reactivities to Phe, ACh and SNP. These results suggest that LPS impaired endothelium-vasodilation and vasoconstriction, whereas, pretreatment with curcumin significantly improved vascular reactivities to those vasoactive agents when compared with LPS group (Figure 1).

Effects of curcumin on oxidant and antioxidant status

We measured oxidant and antioxidant markers including $O_2^{\cdot-}$ production, NOx, lipid hydroperoxide, MDA, and redox state.

For $O_2^{\cdot-}$ production, mice received LPS injection showed significantly increased in $O_2^{\cdot-}$ production in thoracic aorta when compared with control group whereas pretreatment with curcumin can decrease these $O_2^{\cdot-}$ contents (Figure 2). LPS significantly increased urinary NOx, however curcumin markedly suppressed the urinary NOx to a similar control value (Figure 3A).

Moreover, curcumin significantly decreased urinary lipidhydroperoxide when compared with LPS-treated group (Figure 3B). It was found that the plasma levels of MDA were remarkably increased in LPS-treated group and curcumin administration also reduced MDA level nearly to control value (Figure 4A).

For antioxidant assessment, we found that GSH: GSSG ratio in LPS-treated group was lower than control group but curcumin can significantly improve GSH: GSSG ratio (figure 4B).

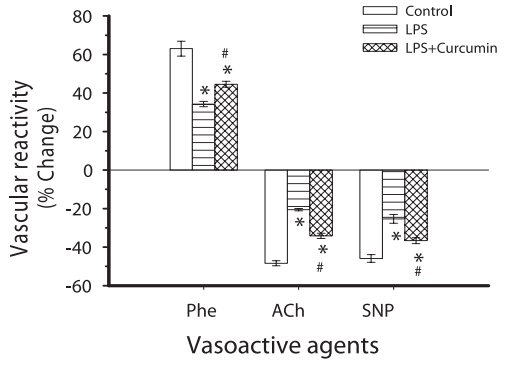

Figure 1 Effect of curcumin on vascular reactivities to vasoactive agents in control, LPS and LPS+curcumin groups. * P < 0.05 vs. control; # *P* < 0.05 vs. LPS

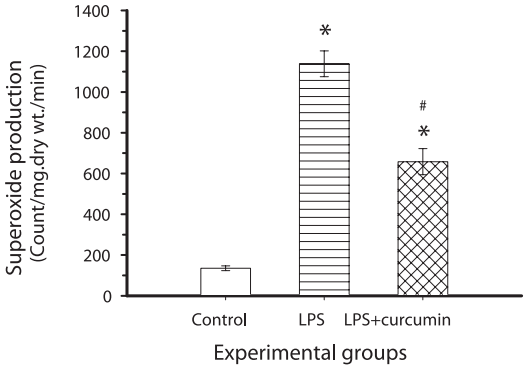

Figure 2 Effect of curcumin on superoxide production in thoracic aorta from 3 experimental groups. * P < 0.05 vs. control; # P< 0.05 vs. LPS.

Table 1 Effects of curcumin on hemodynamic status in LPS-induced septic shock in mice

Parameter measurements	Control	LPS	LPS+curcumin
Systolic blood pressure (mmHg)	119.8 ± 1.8	86.0 ± 1.5*	97.2 ± 1.1
Diastolic blood pressure (mmHg)	89.4 ± 2.2	49.6 ± 0.4*	61.2 ± 0.5* #
Mean arterial blood pressure (mmHg)	103.2 ± 1.7	61.2 ± 0.5*	86.8 ± 2.1* #
Heart rate (beat/minute)	330.6 ± 2.9	397.1 ± 6.1*	354.3 ± 2.8 #

Values are mean± S.E.M. (* $P < 0.05$ vs. control; # $P < 0.05$ vs. LPS)

Discussion

The present data showed that pretreatment with curcumin improves vascular function which is evidented by an improvement of hemodynamics and vascular function. Sepsis is a systemic response to infection, and septic shock is one of the most common causes of death in intensive care unit (Titheradge, 1999). The most common cause of sepsis is an exposure to the structural component of a Gram-negative bacterial membrane LPS. Bacterial LPS in the bloodstream induces overexpression of various inflammatory mediators such as interleukin 1β, TNF-α, NO and prostaglandin E_2 (PGE_2) (Takano *et al.*, 1997). Inaddition, LPS is known to express an inducible isoform of NOS (iNOS), followed by production of large amount of NO in various cells, including smooth muscle cell, endothelial cells and macrophage, which contributes importantly to several key features in septic shock, such as hypotension and vascular hyporeactivity to vasoconstrictor (Parratt, 1998) and vasodilator agents (Farias *et al.*, 2002; Piepot *et al.*, 2003). Our results also showed hypotension and hyporeactivity in LPS-treated which indicated that iNOS mediates impaired constrictor and dilator response in vessels after exposure to LPS. Interestingly, curcumin can prevented by its antioxidant and anti-inflammatory properties partially restore vascular reactivities and alleviate oxidative stress (Gunnett *et al.*, 1998; Karimi *et al.*, 2006).

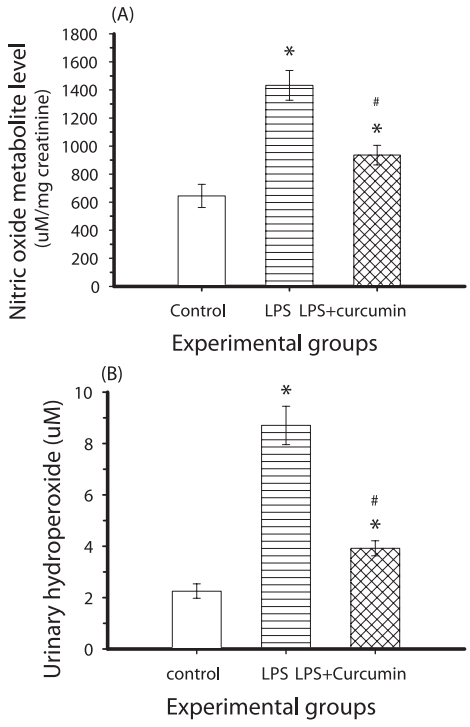

Figure 3 Effects of curcumin on urinary nitric oxide metabolite (A) and urinary hydroperoxide (B). * $P < 0.05$ vs. control; # $P < 0.05$ vs. LPS group

Curcumin might reduce some of the toxic effect from LPS by quenching ROS and reactive nitrogen species (RNS) acting on vascular and other tissues. Curcumin has phenol rings that act as electron traps to scavenge peroxyradicals, O_2^- and hydroxyl radicals and prevent oxidation of iron which they can also chelate. Moreover, curcumin also has a diketone group that can react with hydroxyl radicals and hydrogen peroxide. Therefore, at least one of the mechanisms of curcumin may be due to an inhibition of peroxynitrite formation through its oxidative reactions (Chan *et al.*, 1995).

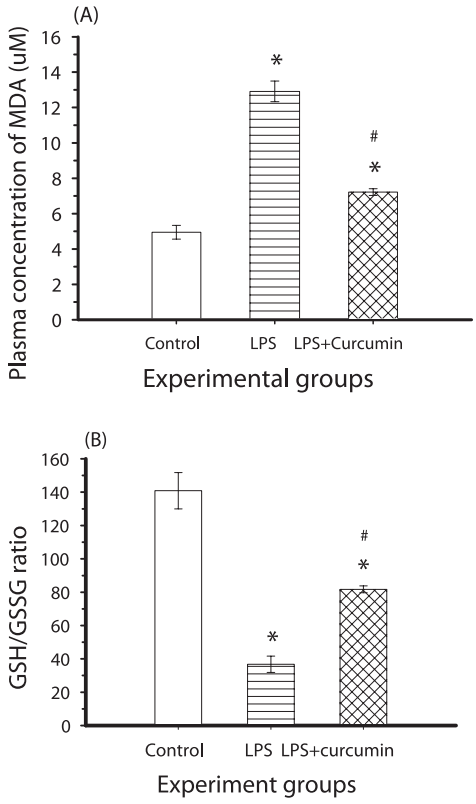

Figure 4 Effect of curcumin on plasma concentration of MDA (A) and GSH/GSSG ratio (B). * P < 0.05 vs. control; # P < 0.05 vs. LPS groups.

In conclusion, the present study provides the evidence that curcumin could reduce oxidative stress in LPS-induced septic shock model and this is associated with prevention of vascular dysfunction by restoration of blood pressure, heart rate and vascular reactivity. Nonetheless, further study is required for investigation the mechanisms involed of action of curcumin in maintenance of vascular function.

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References

Arthur, MJ., Kowalski-Saunders, P., Wright, R. 1988. Effect of endotoxin on release of reactive oxygen intermediates by rat hepaticmacrophages. Gastroenterology. 1588-94.

Cai, H., Harrison, DG. 2000. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res; 87: 840-4.

Chan, MM., Ho, CT., Huang, HI. 1995. Effects of three dietary phytochemicals from tea, rosemary and turmeric on inflammation-induced nitrite production. Cancer Lett ; 96: 23-9.

Drexler, H., Hornig, B. 1999. Endothelial dysfunction in human disease. J Mol Cell Cardiol; 31: 51-60.

Esper. RJ., Nordaby, RA., Vilarino, JO., Paragano, A., Cacharron, JL., Machado, RA.2006. Endothelial dysfunction: a comprehensive appraisal. Cardiovasc Diabetol; 5: 4.

Farias, NC., Borelli-Montigny, GL., Fauaz, G., Feres, T., Borges, AC., Paiva, TB. 2002. Different mechanism of LPS-induced vasodilation in resistance and conductance arteries from SHR and normotensive rats. Br J Pharmacol ; 137: 213-20.

Ferroni, P., Basili, S., Paoletti, V., Davi, G. 2006. Endothelial dysfunction and oxidative stress in arterial hypertension. Nutr Metab Cardiovasc Dis; 16: 222-33.

Gunnett, CA., Chu, Y., Heistad, DD., Loihl, A., Faraci, FM. 1998. Vascular effects of LPS in mice deficient in expression of the gene for inducible nitric oxide synthase. Am J Physiol; 275: H416-21.

Hartung, T., Wendel, A. 1991. Endotoxin-inducible cytotoxicity in liver cell cultures'I. Biochem Pharmacol; 42: 1129-35.

Jaeschke, H. 1993. The therapeutic potential of glutathione in hepatic ischemia-reperfusion injury. Transplantation; 56: 256-7.

Jin, CY., Lee, JD., Park, C,, Choi, YH., Kim ,GY. 2007. Curcumin attenuates the release of pro - inflammatory cytokines in lipopolysaccharide-stimulated BV2 microglia. Acta Pharmacol Sin; 28:1645-51.

Karimi, G., Fatehi, Z., Gholamnejad, Z. 2006. The role of nitric oxide and protein kinase C in lipopolysaccharidemediated vascular hyporeactivity. J Pharm Pharm Sci; 9: 119-23.

Kukongviriyapan, U., Luangaram, S., Leekhaosoong, K., Kukongviriyapan, V., Preeprame, S. 2007. Antioxidant and vascular protective activities of Cratoxylum formosum, Syzygium gratum and Limnophila aromatica. Biol Pharm Bull; 30: 661-6.

Liang, Q., Carlson, EC., Donthi, RV., Kralik, PM., Shen, X., Epstein, PN. 2002. Overexpression of metallothionein reduces diabetic cardiomyopathy. Diabetes; 51: 174-81.

Long, LH., Evans, PJ., Halliwell, B. 1999. Hydrogen peroxide in human urine: implications for antioxidant defense and redox regulation. Biochem Biophys Res Commun; 262: 605-9.

Luangaram, S., Kukongviriyapan, U., Pakdeechote, P., Kukongviriyapan, V., Pannangpetch, P. 2007. Protective effects of quercetin against phenylhydrazine-induced vascular dysfunction and oxidative stress in rats. Food Chem Toxicol; 45: 448-55.

Luster, MI., Germolec, DR., Yoshida, T., Kayama, F., Thompson, M. 1994. Endotoxin-induced cytokine gene expression and excretion in the liver. Hepatology; 19: 480-8.

Parratt, JR. 1998. Nitric oxide in sepsis and endotoxaemia. J Antimicrob Chemother; 41 Suppl A: 31-9.

Piepot, HA., Groeneveld, AB., van Lambalgen, AA., Sipkema, P. 2003. Endotoxin impairs endothelium-dependent vasodilation more in the coronary and renal arteries than in other arteries of the rat. J Surg Res; 110: 413-8.

Takano, H., Yoshikawa, T., Yamaguchi, T., Hongo, H., Fujii, T., Boku, Y., et al. 1997. Effects of aminoguanidine on systemic inflammatory response syndrome induced by platelet activating factor and by lipopolysaccharide in rats. Eur J Pharmacol; 323: 107-10.

Thiemermann, C. 1997. Nitric oxide and septic shock. Gen Pharmacol; 29: 159-66.

Tietze, F. 1969. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem; 27: 502-22.

Titheradge, MA. 1999. Nitric oxide in septic shock. Biochim Biophys Acta; 1411: 437-55.

Wilcox, DT., Glick, PL., Karamanoukian, HL., Morin, FC., 3rd., Fuhrman, BP., Leach, C. 1997. Partial liquid ventilation and nitric oxide in congenital diaphragmatic hernia. J Pediatr Surg; 32: 1211-5.