Curcumin Mitigates Hypertension, Endothelial Dysfunction and Oxidative Stress in Rats Chronically Exposed to Lead

เคอร์คูมินบรรเทาภาวะความดันเลือดสูง เอนโดทีเลียมทำงานผิดปกติ และภาวะเครียดออกซิเดชันในหนูแรทที่ได้รับตะกั่วเป็นเวลานาน

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ABSTRACT

The aim of this study is to evaluate whether curcumin (CUR) could alleviate hypertension, endothelial dysfunction and oxidative stress in rats chronically exposed to lead (Pb). Male Sprague-Dawley rats (n=4/group) received lead acetate (100 mg/L) or deionized water as drinking water for 16 weeks. Rats were intragastrically administered with CUR (100 mg/kg/day) or propylene glycol as vehicle for the final 4 weeks of the experimental period. Results showed that Pb exposure increased arterial blood pressure, elevated peripheral vascular resistance and blunted vascular response to acetylcholine. These alterations were associated with increased superoxide production, elevated plasma malondialdehyde and decreased blood glutathione. CUR significantly improved hemodynamic status and vascular response when compared with untreated Pb controls ($p<0.05$). The improvements of rats exposed to Pb after CUR treatment was associated with a decrease in oxidant formation and an increase of antioxidant glutathione.

บทคัดย่อ

วัตถุประสงค์ของการศึกษานี้เพื่อประเมินว่าเคอร์คูมินสามารถลดภาวะความดันเลือดสูง ภาวะเซลล์เอนโดทีเลียมทำงานผิดปกติ และภาวะเครียดออกซิเดชันได้หรือไม่ในหนูแรทเพศผู้สายพันธุ์ Sprague-Dawley หนูแรทกลุ่มละ 4 ตัว ได้รับสารละลายตะกั่วอะซิเตท (100 มก./ลิตร) หรือน้ำปราศจากไอออน เป็นเวลา 16 สัปดาห์ และใน 4 สัปดาห์สุดท้ายของการทดลองหนูแรทถูกป้อนด้วยเคอร์คูมิน (100 มก./กก./วัน) หรือป้อนด้วยโพรพีลีนกลัยคอลซึ่งเป็นตัวทำละลาย ผลการศึกษาพบว่าตะกั่วสามารถเพิ่มความดันเลือดแดง เพิ่มความต้านทานของหลอดเลือดส่วนปลาย

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และลดการตอบสนองของหลอดเลือดต่อแอซิทิลโคลีน การเปลี่ยนแปลงเหล่านี้สัมพันธ์กับการสร้างซุปเปอร์ออกไซด์ที่เพิ่มขึ้น การเพิ่มระดับมาลอนไดอัลดีไฮด์ในพลาสมา และการลดลงของกลูต้าไธโอนในเลือด เคอร์คูมินสามารถปรับพลศาสตร์ของเลือดและเพิ่มการตอบสนองของหลอดเลือดได้ดีขึ้นเมื่อเปรียบเทียบกับกลุ่มที่ได้รับตะกั่วแต่ไม่ได้รับเคอร์คูมิน ($p<0.05$) ผลดีที่เกิดขึ้นในหนูแรทที่ได้รับตะกั่วภายหลังจากการรักษาด้วยเคอร์คูมินพบว่าสัมพันธ์กับการลดการสร้างออกซิแดนท์และการเพิ่มสารต้านออกซิแดนท์กลูต้าไธโอน

Keywords: Pb-induced hypertension, Curcumin, Vascular dysfunction
คำสำคัญ: ตะกั่วเหนี่ยวนำภาวะความดันเลือดสูง เคอร์คูมิน หลอดเลือดทำงานผิดปกติ

Introduction

Lead (Pb) is widely used in various industries in particularly, battery, paint, electronics manufacturing, vehicle product and welding. Recently, there are several studies reported that Pb contaminates in various wears goods and food stuff in daily life such as cosmetics, plastic, sea and river food, and in tap water [1]. Therefore, most of worldwide populations have unknowingly received low level of Pb in daily lifestyle. When Pb enters into the body it causes toxicity to various systems, especially cardiovascular system [2-4]. Previous studies indicated that Pb promotes hypertension, which is the most common cardiovascular risk [5]. Excessive reactive oxygen species (ROS) formation is one of the major mechanisms of Pb intoxication [6]. In vasculature, over- production of superoxide ($O_2^{\bullet -}$) can quench nitric oxide (NO), via combination to form peroxynitrite ($ONOO^-$) [7]. Moreover, excessive ROS also damage endothelial cell and blunt endothelial function. The imbalance of vasoregulator molecule and endothelial damage can cause the vascular dysfunction leading to increased vascular resistance and finally increased blood pressure [8].

Curcumin (CUR) is the richest isoform of curcuminoids which is extracted from the turmeric. CUR has been proven for the pharmacological advantages of antioxidant, anti-carcinogenic, anti-inflammatory and anti-hypertensive effects [9-12]. However, the effect of CUR on alleviating Pb-induced vascular dysfunction and oxidative stress in rats has not been explored. Therefore, the objective of this study is to investigate the effect of CUR against oxidative stress and vascular dysfunction in rats chronically exposed to Pb.

Material and Methods

Male Sprague-Dawley rats (150 - 160g) were used in this study. The animals were maintained in a temperature controlled room (25±2 °C) with 12-h dark/light cycle at the Northeast Laboratory Animal Center, Khon Kaen University, Thailand. The animals were given free access to standard chow diet (Chareon Pokapan Co. Ltd., Thailand). The animals were carried out in accordance with recommendation in the Guide for the Care and Use of Laboratory Animal of the National Institutes of Health. This study was passed animal ethics AEKKU 4/2555. All animals were randomly divided into four groups (n=4/group), 1) control group; 2) control+CUR; 3) Pb-treated group; and 4) Pb-treated group+CUR. In group 1 and 2, rats received deionized water for 16 weeks, and the last 4 weeks of experimental period rats in group 1 received propylene glycol (PG) as vehicle while rats

in group 2 were intragastrically administered with CUR 100 mg/kg/day. In group 3 and 4, rats received Pb acetate in concentration of 100 mg/L as a drinking water for 16 weeks, and the last 4 weeks of experimental period rats in group 3 received PG as vehicle while rats in group 4 were intragastrically administered with CUR 100 mg/kg/day.

Hemodynamics and vascular responsive- ness measurements

At the end of experiment, rats were anesthe- tized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg). During the surgical stage of anesthetization, the tracheotomy was performed to facilitate respiration. The body temperature was kept between 37± 2°C throughout the study with the heating pad. The left femoral artery was cannulated with polyethylene tubing connected to a pressure transducer for continuously monitoring of arterial blood pressure.

To test the vascular responsiveness, left femoral vein was cannulated for infusion of acetylcholine (ACh), an endothelium-dependent vasodilator, in concentration of 1, 3 and 10 nM. The changes in mean arterial pressure (MAP) were expressed as percentage of control values obtained immediately before the administration of ACh. The abdominal aorta was approached by minimal opening of intraperitoneal cavity for hindlimb blood flow (HBF) measurement. Hindlimb vascular resistance (HVR) was calculated from MAP and HBF as following equation; HVR = MAP/HBF (mmHg/min/100 g tissue/mL or Peripheral Resistance Unit: PRU).

Biochemical assays

Assay of $O_2^{\bullet-}$ production

Vascular $O_2^{\cdot-}$ production was determined by using luciginin-enhanced chemilu- minescence as previously described [11]. The carotid artery were rapidly excised, cleaned on ice, and incubated in oxygenated Krebs-Ringer bicarbonate solution at 37°C for 30 minutes. The chemiluminescence signals were measured by adding lucigenin and detecting the signals by luminometer (Turner Biosystems, CA, USA).

Assay of malondialdehyde (MDA)

The level of MDA in plasma was assayed following a previous method [11]. The absorbance of the supernatant was measured at 532 nm by spectrophotometer, a standard curve was generated by using 1, 1, 3, 3-tetraethoxy propane.

Assay of glutathione (GSH)

Assay of redox status in whole blood were performed by previously described method [13] with some modifications [14]. Reaction and absorbance were monitored with UV/Visible spectrophotometer (Ultrospec 3600 pro., Biochom Ltd. UK). Optical density reading is set at 412 nm and read every 15 sec for 10 times.

Statistical analyses

Results are expressed as mean ± SEM. Statistical evaluation was performed by one-way analysis of variance (ANOVA) followed by Newman-Keuls post-hoc test to show specific group differences. Statistical significance was determined at a level of $p<0.05$.

Results

Effect of CUR on hemodynamic status and vascular responsiveness

Administration of CUR at dose of 100 mg/ kg did not alter hemodynamics or vascular function in normal controls. Daily intake of Pb acetate at 100 mg/L concentration caused a significant increase in systolic, diastolic, MAP, vascular resistance and a decrease in

blood flow when compared with the normal control group (Fig. 1).

CUR at tested dose significantly decreased MAP of rats exposed to Pb ($p < 0.05$, Fig. 1). Importantly, administration with Pb impaired the vascular responses to ACh (Fig. 2). These results indicate that Pb caused an impairment of vasorelaxation. CUR significantly restored the response of ACh in high tested dose about 10.1% compared with Pb-treated controls ($p < 0.05$, Fig 2). Altogether, CUR protected against hypertension and prevented impairment of vascular responsiveness to ACh induced by Pb.

Effects of CUR on oxidant and anti-oxidant status

To evaluate whether increase of blood pressure and blunt of vascular responsiveness in Pb-exposed rats was associated with oxidant formation and antioxidant effect of CUR, we measured the following parameters related with oxidative stress and antioxidant redox status, including vascular $O_2^{\cdot-}$ production, plasma MDA, and the blood level of GSH.

Importantly, chronic exposure to Pb caused approximately 2.5-fold increase of $O_2^{\cdot-}$ production in the carotid artery and plasma MDA level when compared with normal controls rats ($p < 0.05$, Fig. 3). GSH plays an important role in regulating various redox-sensitive molecules. Therefore, alteration of cellular functions may be due to changes in redox status. Rats-treated with Pb showed a reduction in blood GSH. These data provide evidence of oxidative stress in rats exposed to Pb.

CUR at tested dose significantly lowered the rate of $O_2^{\cdot-}$ production in carotid artery and decreased plasma MDA level in comparison to normal control values ($p < 0.05$, Fig. 3B). These results indicate that CUR reduces oxidative stress and lipid peroxidation caused by Pb exposure. In addition Pb-treated rats that received CUR also show significant prevention of loss of GSH in the blood cells (Fig. 3C).

Discussion

Considerable evidences suggest that the hypertensive effect of Pb exposure might be resulted from a complex action on the vascular endothelial cells and vascular smooth muscle cells [15]. The insight mechanism of endothelial dysfunction which resulting in blunting response of ACh-stimulated vascular relaxation and increasing blood pressure in Pb exposed rats can be described by the suppression of eNOS expression in the vascular tissues [16], thereby reduced NO bioavailability and causing hypertension [17]. In this study, CUR attenuated high blood pressure and increased vascular responses to ACh, suggesting its effect on improvement of endothelial function and increase in NO bioactivity [18].

Pb increased oxidative stress by inducing ROS generation through activation of $O_2^{\cdot-}$ production and increasing oxidative damage by altering the membrane integrity and fatty acid compositions via increase of MDA level, whereas the antioxidant defense system was reduced as shown by a depletion of blood GSH level [19-20]. CUR decreased $O_2^{\cdot-}$ production, reduced plasma MDA and increased the blood GSH. Results indicated that CUR could protect the oxidative damage-induced by Pb via a suppression of oxidative stress and improvement of antioxidant status. Moreover, previous study revealed that CUR also possesses the metal chelating property [11], therefore, CUR might reduce Pb accumulation in the target organs. Overall findings of this study suggest that

CUR is a potent antioxidant that can be used as a dietary supplement to reduce risk of Pb-induced hypertension and vascular dysfunction. The precise mechanisms remain to be elucidated by further investigation.

Conclusion

CUR prevented development of hypertension-induced by Pb in rats via its antioxidant property.

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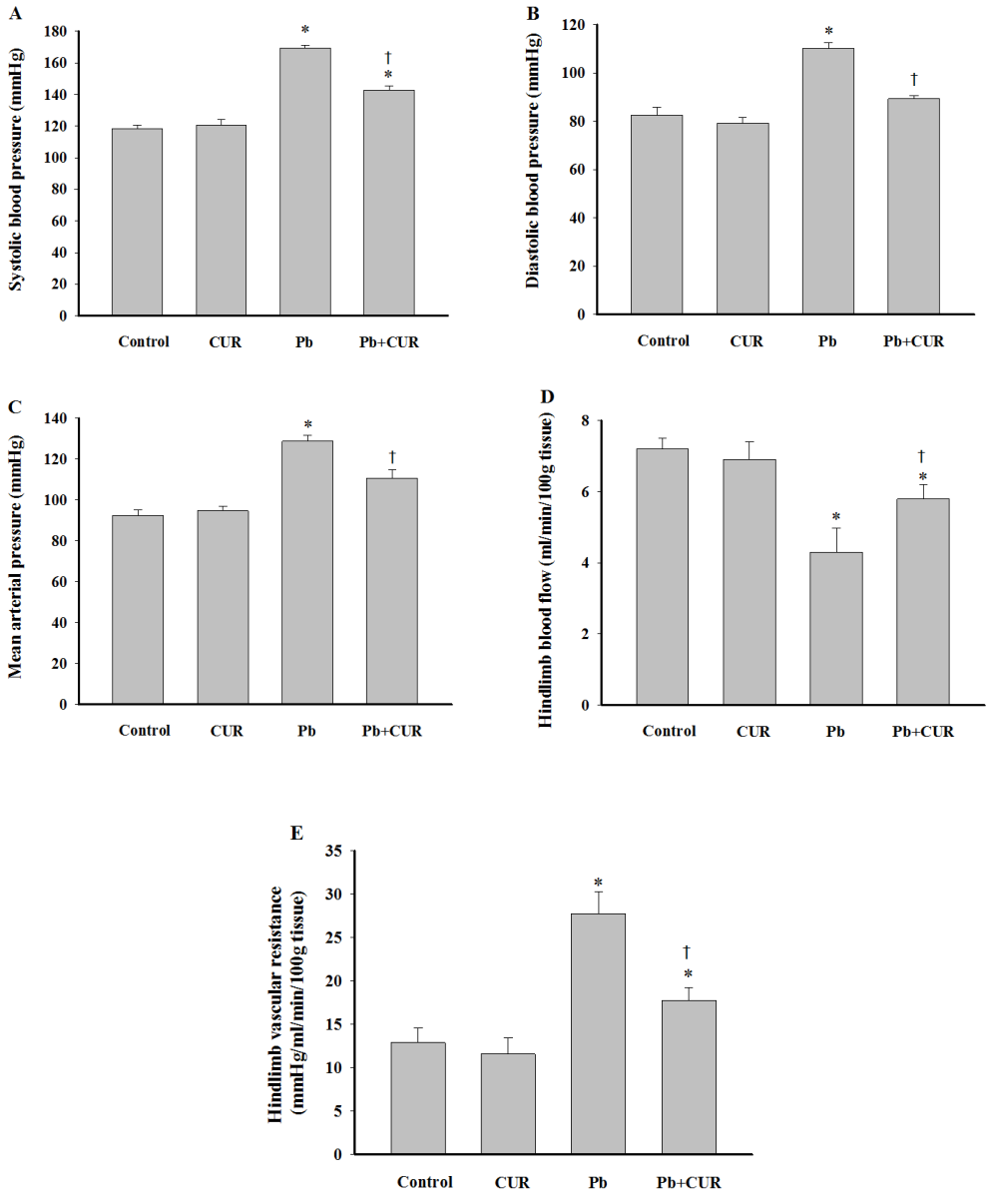

Figure 1 Effect of CUR on hemodymamic status. Systolic blood pressure and (A), diastolic blood pressure (B), mean arterial pressure (C), hindlimb blood flow (D), hindlimb vascular resistant (E). Data are expressed as mean ± S.E., each column represents the mean of 4 experiments. $^{*}p < 0.05$ compared with normal control group, $^{\dagger}p < 0.05$ compared with Pb treated group.

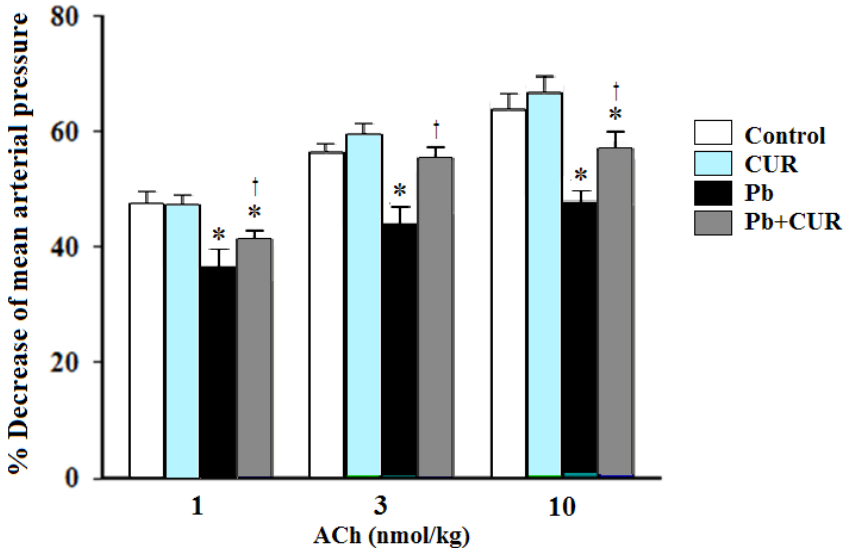

Figure 2 Effect of CUR on vascular responses to acetylcholine (ACh). Results are expressed as mean ± S.E., each column represents the mean of 4 experiments. *p < 0.05 compared with normal control group, †p < 0.05 compared with Pb treated group.

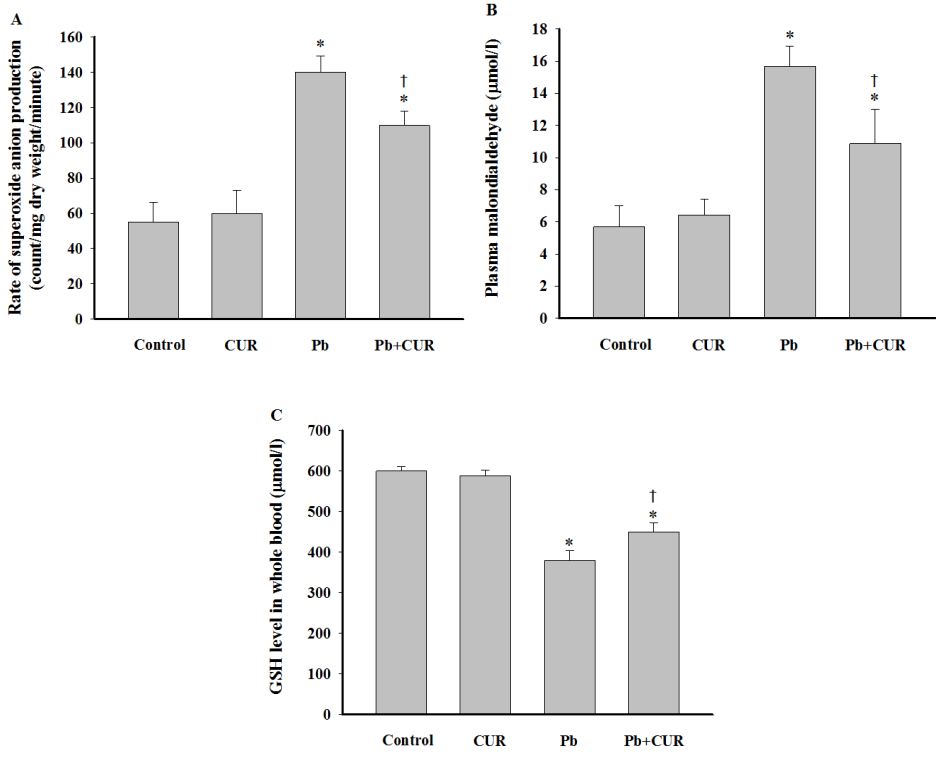

Figure 3 Effect of CUR on oxidant and antioxidant status. Rate of superoxide production (A), plasma malondialdehyde (B) and blood glutathione level (C). Results are expressed as mean ± S.E., each column represents the mean of 4 experiments. *p < 0.05 compared with normal control group, †p < 0.05 compared with Pb treated group.