นิพนธ์ต้นฉบับ • Original Article

เททระไฮโดรเคอร์คูมินลดภาวะผิดปรกติของพลศาสตร์การไหลเวียนเลือดและภาวะเครียดออกซิเดชันในหนูแรทที่ได้รับแคดเมียมและตะกั่วขนาดต่ำ

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Tetrahydrocurcumin Attenuates Hemodynamic Disturbance and Oxidative Stress in Rats Exposed to Low Levels of Cadmium and Lead

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หลักการและวัตถุประสงค์: เป็นที่ทราบกันดีว่าการได้รับแคดเมียม และตะกั่วระดับสูงมีผลเพิ่มความเสี่ยงต่อการเกิดโรคหัวใจร่วมหลอดเลือด อย่างไรก็ตาม ผลกระทบที่เป็นอันตรายต่อระบบหัวใจร่วมหลอดเลือดหลังจากได้รับโลหะหนักทั้งสองชนิดในระดับต่ำนั้นยังไม่ทราบแน่ชัด เททระไฮโดรเคอร์คูมินเป็นสารเมแทบอไลต์หลักตัวหนึ่งของเคอร์คูมิน ซึ่งมีคุณสมบัติเด่นในการต้านออกซิเดชัน การศึกษานี้มี วัตถุประสงค์เพื่อตรวจสอบว่าสารเททระไฮโดรเคอร์คูมินสามารถปรับปรุงพลศาสตร์การไหลเวียนเลือด การทำงานของ หลอดเลือด และลดภาวะเครียดออกซิเดชันในหนูแรทที่ได้รับแคดเมียมและตะกั่วในระดับต่ำ

วิธีการศึกษา: แคดเมียมคลอไรด์ (10 มก./ล.) และเลดอะซิเตท (100 มก./ล.) ถูกเตรียมในรูปน้ำดื่มให้แก่หนูแรทเพศผู้สายพันธุ์ Sprague-Dawley เป็นเวลา 16 สัปดาห์ หนูแรทเหล่านี้ถูกป้อนด้วยสารเททระไฮโดรเคอร์คูมิน ขนาด 50 มก./กก. ทุกวัน เริ่มตั้งแต่สัปดาห์ที่ 12 จนถึงสัปดาห์ที่ 16 เมื่อสิ้นสุดการทดลอง หนูทดลองจะถูกนำมาวัดพลศาสตร์ การไหลเวียนเลือด การตอบสนองของหลอดเลือด การแสดงออกของโปรตีน eNOS และ NADPH oxidase ในหลอดเลือดและตัวชี้วัดสภาวะเครียดออกซิเดชัน

Background and objective: It is well known that the exposure to high levels of cadmium (Cd) and lead (Pb) increases risk of cardiovascular diseases. However, the harmful effect on the cardiovascular system after chronic exposure to low levels of these metals has been less defined. Tetrahydrocurcumin (THU), one of the major metabolites of curcumin, possesses strong antioxidant activity. This study aimed to explore whether THU could improve hemodynamics, vascular function and alleviate the oxidative stress in rats chronically exposed to low levels of Cd and Pb.

Methods: Low doses of cadmium chloride (10 mg/L) and lead acetate (100 mg/L) were given via drinking water to male Sprague-Dawley rats for sixteen weeks. THU at 50 mg/kg was intragastrically administered once daily starting from the 12th to 16th week of the experiment. At the end of experiments, hemodynamic status, vascular responsiveness, protein expression of eNOS and NADPH oxidase, and oxidative stress markers were examined.

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ผลการศึกษา: หนูที่ได้รับแคดเมียมและตะกั่วมีความดันเลือดแดงสูงขึ้น ร่วมกับอัตราการไหลเวียนเลือดและการตอบสนองของหลอดเลือดลดลง ภาวะการทำงานของหลอดเลือดที่ผิดปกตินี้พบว่ามีความสัมพันธ์กับการเพิ่มขึ้นของซุปเปอร์ออกไซด์ ($O_2^{\cdot}$) และการลดลงของ eNOS ในเนื้อเยื่อหลอดเลือด นอกจากนี้ยังพบการเพิ่มขึ้นของ $p47^{phox}$ ซึ่งบ่งชี้ว่า NADPH oxidase เป็นแหล่งหลักที่สร้าง $O_2^{\cdot}$ ของหลอดเลือดในภาวะก่อพิษของโลหะ เป็นที่น่าสนใจว่าการให้แคดเมียมร่วมกับตะกั่วนั้น จะมีผลเสริมฤทธิ์กันโดยเพิ่มความรุนแรงต่อค่าตัวชี้วัดต่าง ๆมากขึ้น เททระไฮโดรเคอร์คูมินสามารถเพิ่มประสิทธิภาพการทำงานของหลอดเลือดโดยการลดภาวะเครียดออกซิเดชัน ลดการสร้าง $O_2^{\cdot}$ กดการทำงานของ $p47^{phox}$ ร่วมกับเพิ่มการแสดงออกของ eNOS

สรุป: ผลการศึกษานี้แสดงให้เห็นว่าเททระไฮโดรเคอร์คูมินสามารถดำรงการทำงานของหลอดเลือดและลดภาวะเครียดออกซิเดชันในหนูแรทที่ได้รับสารแคดเมียมและตะกั่ว

คำสำคัญ: เททระไฮโดรเคอร์คูมิน, แคดเมียม, ตะกั่ว, หลอดเลือดเสียหน้าที่, ภาวะเครียดออกซิเดชัน

Results: Cd and Pb raised arterial blood pressure, reduced blood flow and vascular responsiveness. The vascular dysfunction was associated with enhanced vascular $O_2^{\cdot}$ production and decreased eNOS expression. Up-regulation of vascular $p47^{phox}$ suggested that NADPH oxidase is a major source of $O_2^{\cdot}$ in the metal intoxicated condition. Interestingly, Cd combined with Pb exhibited a synergistic effect on these parameters. THU improved vascular function by alleviating oxidative stress, decreasing $O_2^{\cdot}$ production, suppressing $p47^{phox}$ and increasing eNOS expression.

Conclusions: This study demonstrated that THU has the potential to preserve vascular function and mitigate oxidative stress induced by Cd and Pb in rats.

Key Words: Tetrahydrocurcumin, Cadmium, Lead, Vascular Dysfunction, Oxidative Stress

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Introduction

As a consequence of increasing industrialization especially in developing countries, people are more prone to expose to contaminated toxic metals via occupational and environmental sources. Heavy metals such as cadmium (Cd) and lead (Pb) have been documented to be toxic at even low concentration. Cd and Pb are contaminated in human foodstuffs, drinking water, and polluted air, which have raised public concern[1, 2]. Since the two elements are often released simultaneously in the environment from a number of natural and manmade sources, adverse health effects caused by single or combined exposure to Cd and Pb has provoked a significant public health concern[3], especially risk of cardiovascular disease (CVD)[4, 5]. Cd and Pb are the possible risk factor of hypertension[6]. The pathogenesis of Cd or Pb-induced hypertension involves alterations of vascular function and vascular structural changes[7, 8].

Oxidative stress is one of the major mechanisms behind heavy metal toxicity[9]. Several antioxidants are also used to reduce metal toxicities. Tetrahydrocurcumin (THU) is one of the major colourless metabolites of curcumin. It exhibits a variety of pharmacological activity, including antioxidant, anti-inflammatory, anti-proliferative and cardioprotective properties[10-13]. THU can scavenge reactive oxygen species (ROS) in type 2 diabetes[14]. It has been demonstrated that the β-diketone moiety of THU restored the antioxidant status by increasing activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase[14]. As most studies are dealt with deleterious effects of a single metal exposure such as Cd or Pb, however in reality, people have been continuously exposed to more than one heavy metal at a time. Some metals could cause an additive, synergistic, or antagonistic effects. Therefore, it is of interest to explore the effect of long-term and low level of exposure of Cd and Pb on development of hypertension, vascular dysfunction and oxidative stress.

Materials and Methods

Animal and treatment

Male Sprague-Dawley rats, weighing 160-180 g. were obtained from the National Laboratory Animal Center, Mahidol university, Thailand. The experimental protocol was reviewed and approved by the Animal Ethics Committee of Khon Kaen university. The animals were housed at The Northeast Laboratory Animal Center (Khon Kaen University, Thailand) and maintained on a 12-h dark/ light cycle at room temperature (25 ± 2 °C) with free access to standard rat chow. After seven days of adaptation, animals were randomly assigned into four groups (n=6/group): *(I) control group***;** received deionized water, *(II) THU group***;** received deionized water and orally administered with THU at dose of 50 mg/kg/day, *(III) Pb+Cd group;* received drinking water containing $CdCl_2$ (10 mg/L) plus $PbAc_2$ (100 mg/L), *and (IV) Cd+Pb+THU group;* received drinking water containing $PbAc_2$ plus $CdCl_2$ and orally administered with THU at dose of 50 mg/kg/day. $CdCl_2$ and $PbAc_2$ were dispersed in deionized water as drinking water for sixteen weeks. THU was dissolved in propylene glycol (PG) and orally administered once daily to animals starting from week 12 to 16 of heavy metal exposure.

Hemodynamic status and vascular responsiveness

Systolic blood pressure (SBP) of rats in all experimental groups was measured every two weeks throughout the sixteen weeks by tail-cuff plethysmography (IITC Life Science Inc. Victory Blvd Woodland Hills, CA). At the end of experiments, rats were anesthetized with pentobarbital sodium (60 mg/kg, i.p.). SBP, diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (HR) and hindlimb blood flow (HBF) were measured followed a previous described method [15]. Hindlimb vascular resistance (HVR) was obtained from MAP divi ded by HBF. Thereafter, vascular responsiveness to endothelium-dependent (acetylcholine, 30 μmol/kg) and–independent vasodilators (sodium nitroprusside, 30 μmol/kg) was determined. Arterial blood samples were collected from the abdominal aortas for assays of oxidative stress and antioxidant biomarkers. The aortas and carotid arteries were rapidly excised from the animals and used for analysis of endothelial nitric oxide synthase (eNOS) and $p47^{phox}$, a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunit protein expression, and vascular superoxide anion ($O_2^{\bullet-}$) production.

Assay of oxidative stress markers and antioxidant glutathione

The production of $O_2^{\bullet}$ in rat aortas was detected by lucigenin-enhanced chemiluminescence method as previously described[10]. The malondialdehyde (MDA), a lipid peroxidation marker, was determined in the plasma using the thiobarbituric acid (*TBA*) *assay*[16]. Reduced *Glutathione* (*GSH*) in the whole blood was determined spectrophotometrically following a previously described method[16].

Western blot analysis

The protein expression levels of endothelial nitric oxide synthase (eNOS) and NADPH oxidase ($p47^{phox}$ subunits) were measured in the aortic homogenates by Western blotting as previous described[16]. The expressions of eNOS and $p47^{phox}$ were normalized to β-actin protein expression from the same sample. The data were expressed as a percentage of normal controls

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Statistical analysis

Results are expressed as mean ± SEM, and n refers to the number of animals used. Statistical analysis 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 less than 0.05.

Results

Effect of THU on hemodynamic status

The effect of THU on SBP of rats exposed to Cd and Pb is shown in Figure 1. Long-term exposure to low level of Cd plus Pb caused a progressive increase in SBP throughout the 12-week of administration compared with normal control group ($p<0.05$, Figure 1). Treatment of THU for 4 weeks significantly decreased SBP ($p<0.05$). Results indicated that THU can attenuate

high blood pressure in heavy metal-intoxicated condition. This is not due to hypotensive effect of THU as normal control rats treated with THU alone did not experience a change in SBP (Figure 1). A marked increase in SBP, DBP and MAP was observed in Cd plus Pb-treated group ($p<0.05$, Table 1). The increase in arterial blood pressure of rats exposed to Cd and Pb was accompanied by decreased HBF and increased HVR (Table 1). THU significantly improved hemodynamics by decreasing MAP, increasing HBF and decreasing HVR in rats exposed to Cd and Pb ($p<0.05$, Table 1).

Effect of THU on vascular responsiveness

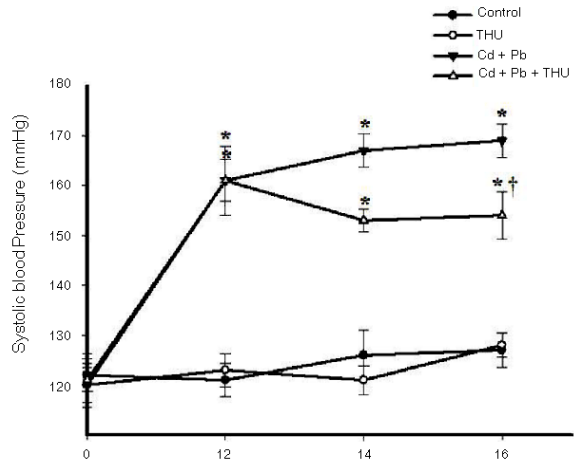

Figure 1 Effect of THU on systolic blood pressure during heavy metals exposure for 16 weeks. Results are expressed as mean ± SEM.*$p < 0.05$ vs. control group; †$p < 0.05$ vs. Cd+Pb- treated group.

Impairment of vascular responsiveness to pharmacological vasoactive agents including ACh and SNP was found in rats exposed to Cd plus Pb ($p<0.05$, Figure 2 A, B), indicating heavy metals-induced vascular damage [17]. Interestingly, THU significantly restored the vascular responses to both ACh and SNP ($p<0.05$, Figure 2A, B) in rats exposed to Cd and Pb. This suggests that THU protects against metals-induced vascular dysfunction.

Effect of THU on oxidative stress and antioxidant status

Increased oxidative stress was found in rats chronically exposed to low level of Cd and Pb as indicated by increasing $O_2^{\cdot-}$ production in thoracic aorta as compared to normal control levels ($p<0.05$, Figure 3A). Increased lipid peroxidation was also found in rats treated with Cd and Pb as shown by a marked increase in plasma MDA (Figure 3B). THU significantly reduced the rate of aortic $O_2^{\cdot-}$ production and decreased plasma MDA level when compared with rats treated with heavy metals alone ($p<0.05$, Figure 3A, B). Regarding the antioxidant status, the blood GSH level was reduced remarkably after 16 weeks exposure to Cd and Pb ($p<0.05$ vs. normal controls, Figure 4), and THU significantly restored the GSH level (Figure 4). As for the normal control groups, there was no change in the basal levels of $O_2^{\cdot-}$, MDA and GSH after THU treatment (Figure 3 and 4).

Table 1 Effect of THU on hemodynamic status.

Parameters	Group			
	Control	THU	Cd+Pb	Cd+Pb+THU
SBP (mmHg)	113.5 ± 2.1	112.4 ± 1.2	171.3 ± 1.4*	151.3 ± 2.8*†
DBP (mmHg)	79.3 ± 1.7	80.4 ± 3.2	109.2 ± 1.5*	92.2 ± 2.2*†
MAP (mmHg)	92.2 ± 2.0	91.2 ± 1.7	127.8 ± 3.5*	111.9 ± 3.2*†
HR (beats/min)	340.6 ± 3.2	343.8 ± 2.1	431.2 ± 6.9*	393.2 ± 5.1*†
HBF (ml/min/100 g tissue)	7.8 ± 0.9	8.1 ± 1.4	4.1 ± 0.2*	5.7 ± 0.6*†
HVR (mmHg/ml/min/100 g tissue)	12.5 ± 1.5	12.9 ± 1.7	27.7 ± 2.5*	19.7 ± 3.1*†

Values are mean ± SEM. SBP systolic blood pressure, DBP diastolic blood pressure, MAP mean arterial blood pressure, HR, heart rate; HBF, hindlimb blood flow; HRV, hindlimb vascular resistance.*$p < 0.05$ vs. control group; †$p < 0.05$ vs. Cd+Pb- treated group.

Figure 4 Effect of THU on blood glutathione levels. Results are expressed as mean ± SEM. *p < 0.05 vs. control group; †p < 0.05 vs. Cd+Pb- treated group.

hypertension, vascular dysfunction and oxidative stress in rats. Previous study suggested that down-regulation of eNOS expression can lead to a reduction in NO production and subsequently impaired NO-derived vasodilation[19], therefore, one of the mechanisms involved with these metals-induced hypertension is a depletion in NO bioavailability. Other mechanisms of Pb and Cd-induced hypertension might be explained by the increase in plasma angiotensin II and the disturbance of calcium ion homeostasis [20, 21]. In this study, THU restored the eNOS expression and decreased oxidative stress, thereby improved the vascular function. Structurally, THU has β-diketone group which can directly scavenge excessive ROS [22], therefore, it might prevent ROS-mediated eNOS uncoupling.

It has been demonstrated that heavy metals can directly stimulate the vascular NADPH oxidase[23], which is the dominant enzymatic source of vascular $O_2^{\bullet -}$ production under the regulation of NF-κB [24]. We found that NADPH oxidase ($p47^{phox}$) of rats exposed to Cd and Pb was down-regulated after THU treatment and $O_2^{\bullet -}$ levels were reduced as an outcome from suppression of NF-κB activation[25].

In conclusion, this study provides the evidences of the beneficial effects of THU on alleviation of hypertension, improvement of hemodynamics and endothelial function, and attenuation of oxidative stress in rats with chronic exposure to low levels of Cd and Pb. Therefore, THU may be useful as an antioxidant agent against toxic metals-induced hypertension and vascular dysfunction.

Figure 5 Effect of THU on the protein expression of eNOS (A), and p^{47phox} (B) in aortic tissues. β-actin was served as an internal control. Results are expressed as mean ± SEM. *p < 0.05 vs. control group; †p < 0.05 vs. Cd+Pb- treated group.

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