นิพนธ์ต้นฉบับ • Original Article

เคอร์คูมินเพิ่มการตอบสนองของหลอดเลือดและลดภาวะเครียดออกซิเดชันในหนูแรทที่เหนี่ยวนำให้เกิดภาวะฮีโมลัยติกอะนีเมียด้วยสารฟีนิลไฮดราซีน

ขวัญจิต อภัยจิตต์[1*], วนิดา ดรปัญหา[2], ยุพา คู่คงวิริยพันธุ์[3]
[1]*คณะแพทยศาสตร์ มหาวิทยาลัยมหาสารคาม, มหาสารคาม 44000*
[2]*คณะเทคนิคการแพทย์ มหาวิทยาลัยขอนแก่น, ขอนแก่น 40002*
[3]*ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น, ขอนแก่น 40002*

Curcumin Increases Vascular Responses and Reduces Oxidative Stress in Phenylhydrazine-induced Hemolytic Anemia in Rats

Kwanjit Apaijit[1*], Wanida Donpunha[2], Upa Kukongviriyapan[3]
[1]*Faculty of Medicine, Mahasarakham University, Mahasarakham 44000*
[2]*Facultyof Associated Medical Sciences, Khon Kaen University, Khon Kaen 40002*
[3]*Departmentof Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002*

หลักการและวัตถุประสงค์: เคอร์คูมินเป็นสารสำคัญในขมิ้นชัน (*Curcuma longa*) ซึ่งมีฤทธิ์ต้านออกซิเดชันและฤทธิ์ทางเภสัชวิทยาต่างๆ อย่างไรก็ตามผลของเคอร์คูมินต่อภาวะฮีโมลัยติกอะนีเมียนั้นยังไม่ทราบ ฟีนิลไฮดราซีนเป็นสารที่ทำให้เม็ดเลือดแดงแตกจึงทำให้เกิดภาวะซีดอย่างรุนแรงและรวดเร็ว วัตถุประสงค์ของการศึกษานี้เพื่อตรวจสอบฤทธิ์ของเคอร์คูมินต่อการต้านภาวะฮีโมลัยติกอะนีเมียที่เหนี่ยวนำด้วยสารฟีนิลไฮดราซีนในหนูแรท

วิธีการศึกษา: หนูแรทเพศผู้สายพันธุ์ Sprague-Dawley แบ่งเป็น 6 กลุ่ม กลุ่มละ 8 ตัวถูกฉีดสารฟีนิลไฮดราซีนเข้าทางช่องท้อง (15 มก./กก.น้ำหนักตัว/วัน) และได้รับการป้อนสารเคอร์คูมิน (30 หรือ 100 มก./กก.น้ำหนักตัว/วัน) เป็นเวลา 8 วัน หนูแรทในกลุ่มควบคุมได้รับสารตัวทำละลายด้วยวิธีเดียวกันและระยะเวลาเหมือนกันเมื่อสิ้นสุดการทดลองจะทำการวัดพลศาสตร์การไหลเวียนเลือด การตอบสนองของหลอดเลือดและตัวบ่งชี้ภาวะเครียดออกซิเดชันในหนูทดลองทั้งหมด

ผลการศึกษา: หลังจากฉีดสารฟีนิลไฮดราซีน หนูแรทจะเกิดภาวะซีดและหลอดเลือดทำงานผิดปกติ โดยพบว่าค่าฮีมาโตคริต ความดันเลือดและความต้านทานของหลอดเลือดส่วนปลายลดลงอย่างมาก เมื่อเปรียบเทียบกับหนูแรทในกลุ่มควบคุม ($p<0.05$) นอกจากนี้การตอบสนองของหลอดเลือดต่อสารที่มีผลกระตุ้นหลอดเลือดก็ลดลงอย่างมาก และเคอร์คูมินตามขนาดความเข้มข้นสามารถปรับการตอบสนองของหลอดเลือด

Background and objective: Curcumin, the main chemical compound of turmeric (*Curcuma longa*), possessesstrong antioxidant and various pharmacological activities. However, the effect of curcuminon hemolytic anemic condition is unknown. Phenylhydrazine (PHZ) is ahemolytic agent which causes severe and acute anemia. The aim of this study was to investigate the effect of curcuminagainst PHZ-induced hemolytic anemia in rats.

Methods: Male Sprague-Dawley rats were divided into six groups of 8 rats each which intraperitoneally injected with PHZ (15 mg/kgb.w./day) and intragastrically administered with curcuminat dose of 30 or 100 mg/kgb.w./day for 8 days. Rats in control groups were received vehicles in the same way over the same period. At the end of the experimental period, hemodynamic status, vascular responsiveness and oxidative stress markers were measured in all rats.

Results: Signs of anemia and vascular dysfunction were present in rats after PHZ injection. A marked decrease in hematocrit, blood pressure and peripheral vascular resistance was found in PHZ-treated rats compared to control rats ($p<0.05$). Moreover, vascular responses to vasoactive agents were dramatically suppressed, and curcumin significantly restored these responses in a dose-dependent manner ($p<0.05$). The restorations of

ให้ดีขึ้นได้อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) การบูรณะพลศาสตร์การไหลเวียนเลือดที่แปรปรวนการทำงานของหลอดเลือดที่ผิดปกติ และสภาวะซีดของหนูทดลองที่ได้รับสารฟีนิลไฮดราซีนนี้มีความสัมพันธ์กับการลดภาวะเครียดออกซิเดชันโดยพบว่าการสร้างซุปเปอร์ออกไซด์และปฏิกิริยาออกซิเดชันของลิพิดและโปรตีนมีค่าลดลง

สรุป: ข้อมูลจากการศึกษานี้ชี้แนะประโยชน์ของเคอร์คูมินในการต้านภาวะหลอดเลือดผิดปกติและภาวะเครียดออกซิเดชันที่พบในสภาวะฮีโมลัยติกอะนีเมีย

คำสำคัญ: เคอร์คูมิน ฟีนิลไฮดราซีน ฮีโมลัยติกอะนีเมีย

hemodynamic disturbance, vascular dysfunction and anemic state during PHZ exposure were related to a reduction of oxidative stress by decreasing superoxide production, lipid peroxidation and protein oxidation.

Conclusion: Data of this study suggestthe beneficial effect of curcuminagainst vascular dysfunction and oxidative stress in a hemolytic anemic condition.

Keywords: Curcumin, Phenylhydrazine, Hemolytic anemia

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Introduction

Phenylhydrazine (PHZ) is a hemolytic and pro-oxidant agent which is widely used in industry and laboratory. This compound is very useful in experimental models studying mechanisms of hemolytic anemia[1]. Theauto-oxidation of PHZ induces free iron release and generates free radicals such as hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$), which initiates the peroxidation of unsaturated fatty acids in endogenous phospholipids of plasma membrane of red blood cells (RBCs) to cause lipid peroxidation and protein oxidation resulting in the destruction of RBCs and hemolytic anemia[2, 3]. Another effect of PHZ is that it induces hypoxia, inflammation, vascular dysfunction and oxidative stress[4, 5]. Previous study reported that rats treated with PHZ (125 mg/kg i. p.) showeda sign of severe hemolytic anemia with a marked reduction of blood hemoglobin (Hb) levels within 48 hours [6]. PHZ also induced hypotension and diminished vascular responsiveness that was associated with increased production of reactive oxygen species (ROS)[5, 7]. Therefore, PHZ can be used as a model of oxidant stress-induced vascular dysfunction and hemolytic anemia.

Curcumin is the major active component of turmeric (*Curcuma longa*), a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. It is widely used in tropical regions of Asia as a spice to give flavor and yellow color to food. Curcuminhas been recommended as food supplements for prevention and treatment of various chronic conditions, including autoimmune, cancer, diabetes, hypertension, cardiovascular, pulmonary, inflammatory, neurological, psychological and metabolic diseases[8-12]. Curcumin and its major metabolites, tetahydrocurcumin also chelate many metal ions such as iron, cadmium, lead and copper [13-16]. Regarding the antioxidant property, curcumin directly scavenges free radicals by using diketone group toreact with $OH^{\bullet}$ and H_2O_2 while the two phenyl methoxy groups of curcumin suppress NF-$_k$B activation[17-19] and inhibits xanthine oxidase/xanthine dehydrogenase conversion, leading to a decrease in superoxide ($O_2^{\bullet-}$) generation[20]. However, data supporting the antioxidant activity of curcumin against oxidative stress and vascular dysfunction in hemolytic anemia is still lacking. Thus, this study aimed to investigate the antioxidant and vascular protective effects of curcumin in an animal model of hemolytic anemia.

Materials and Methods

Chemicals

Curcumin extracted from turmeric (*Curcuma longa*)was generously supplied by the Government Pharmaceutical Organization (Bangkok, Thailand). All chemicals used were of analytical grade quality.

Animals and Treatments

Adult male Sprague-Dawley rats weighing about 200-220 g were obtained from the National Laboratory Animal Center, Mahidol University, NakhonPathom, Thailand. Rats were housed at the Northeast Laboratory Animal Center (KhonKaen University, Thailand)and maintained on a 12-h/light/dark cycle in a controlled temperature environment (25 ± 2ºC) with free access to water and standard rat chow (Chareon Pokapan Co.,

Ltd., Thailand). The study protocol was reviewed and approved by the Institutional Animal Ethics Committee of KhonKaen University (AEKKU 41/2555).

After an adaptation periods for 7 days, rats were randomly assigned to 6 groups(8 animals each) as follows: Group I - Normal control + vehicle (propylene glycol, PG), Group II - Normal control +curcumin (30 mg/kg), Group III - Normal control +curcumin (100 mg/kg), Group IV- PHZ control + PG, Group V - PHZ + curcumin (30 mg/kg), and Group VI - PHZ + curcumin (100 mg/kg). Hemolytic anemia was induced in PHZ-treated groups by intraperitoneal injection of PHZ at dose of 15 mg/kg for 8 days. Curcumin was intragastrically administered throughout the period of PHZ treatment.The doses of curcumin were based on our previous study in animals-induced endotoxic shock [12].

Vascular function assessment and samples collection

After 8 days, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg). The femoral artery was cannulated with polyethylene tubing connected to a pressure transducer for continuously monitoring of arterial blood pressure and heart rate (HR). Hindlimb blood flow (HBF) was measured by placing electromagnetic flow probes around the abdominal aorta connected to an electromagnetic flow-meter. Hindlimb vascular resistance (HVR) was calculated from the mean arterial pressure (MAP) and mean HBF. After HBF measurements, vascular reactivity was assessed by infusing vasoactive agents through an additional catheter in the femoral vein in a stepwise fashion at 5-min intervals. The vasoactive agents tested were acetylcholine (ACh; 10 nmol/kg),sodium nitroprusside (SNP; 3 nmol/kg), and phenylephrine (Phe; 0.1 nmol/kg). Changes in blood pressure were expressed as percentage of control values obtained immediately before the administration of the test substance. At the end of study, rats were sacrificed with an overdose of the anesthetic drug. Blood samples were collected from the abdominal aorta for determinations of hematocrit, plasma malonaldehyde (MDA), plasma protein carbonyl and plasma nitric oxide metabolites (NOx). The carotid arterieswere rapidly excised from the animals and used for analysis of $O_2^{\bullet-}$ production.

Assessment of oxidative stress markers

Vascular $O_2^{\bullet-}$ production was performed in carotid arteries by using the lucigenin-enhanced chemiluminescence technique as previously reported [12]. MDA, a marker of lipid peroxidation, was assessed in plasma, liver, kidney and heart by measuring thiobarbituric acid reactive substances following a previously described method [5]. Protein oxidation in plasma and tissues (liver, kidney and heart) was assessed by the determination of carbonyl groups based on thereaction with dinitrophenyl hydrazine (DNPH) aspreviously described [12]. The plasma NOx level, the end product of NO metabolism, werequantified by an enzymatic conversion method with the Griess reaction as previously described[21].

Statistical Analysis

Data are expressed as mean ± S.E., and *n* refers to the number of animals used. Data comparisons were carried out using one-way analysis of variance (ANOVA), followed by post-hoc Duncan's multiple range test. Difference with a p-value of less than 0.05 was considered as statistical significance.

Results

Effect of PHZ on hematocrit and hemodynamics

After PHZ injection, the hematocrit levels were significantly declined in PHZ-treated rats compared to normal controls ($p<0.05$, Table 1), suggesting that hemolytic anemia wassuccessfully induced. Amarked decrease in arterial blood pressure and HVR andan increase in HBF and HR were observed in the PHZ-treated rats ($p<0.05$, Table 1). Curcumin (30 or 100 mg/kg) in dose-dependent manner improved hemodynamic status and anemia of PHZ-treated rats by increasing blood pressure, HVR and hematocrit levels, but decreasing HR and HBF ($p<0.05$, Table 1). Administration of curcumin did not alter hemodynamics and hematocrit levels in normal control rats (Table 1).

Apart from the induction of hemolytic anemia, PHZ also caused impairment of vascular responsiveness. PHZ significantly blunted the vasodilation to ACh, and SNP and vasoconstriction to Phe ($p<0.05$, Fig. 1). A decreasein vascular responsiveness to Phe, ACh, and

SNP wasabout 30-40 %compared to those found in normal controls ($p<0.05$, Fig. 1). Interestingly, curcumin, especially at dose of 100 mg/kg significantly improved vascular reactivity of PHZ-treated rats ($p<0.05$, Fig.1).

Table 1 Effect of curcumin on hematocrit leveland hemodynamic status in all experimental groups.

Parameters	Normal control	Normal control + Curcumin		PHZ control	PHZ + Curcumin	
	vehicle	30 mg/kg	100 mg/kg		30 mg/kg	100 mg/kg
Hematocrit (%)	41.4 ± 1.1	43.6 ± 1.3	44.0 ± 1.8	27.6 ± 2.1*	35.2 ± 0.7$^{*\#}$	34.8 ± 0.7$^{*\#}$
Systolic blood pressure (mmHg)	126.7 ± 2.4	124.2 ± 2.9	125.1 ± 2.0	84.9 ± 7.4*	106.6 ± 4.3$^{*\#}$	119.3 ± 4.6$^{*\#}$
Diastolic blood pressure (mmHg)	82.9 ± 1.9	75.8 ± 1.9	79.2 ± 1.0	51.1 ± 4.0*	65.0 ± 3.9$^{*\#}$	76.6 ± 3.7$^{\#\dagger}$
Mean arterial pressure (mmHg)	101.0 ± 1.9	95.8 ± 1.6	100.1 ± 1.4	68.3 ± 3.0*	81.9 ± 4.6$^{*\#}$	91.2 ± 3.8$^{*\#\dagger}$
Heart rate (beats/min)	396.5 ± 1.1	382.3 ± 9.9	391.1 ± 4.8	436.7 ± 4.4*	412.8 ± 2.8$^{*\#}$	400.0 ± 6.6$^{\#\dagger}$
Hindlimb blood flow (ml/min/100 g tissue)	10.6 ± 0.7	9.2 ± 0.5	10.4 ± 0.4	17.1 ± 0.8*	13.9 ± 0.7$^{*\#}$	10.4 ± 0.8$^{\#}$
Hindlimb vascular resistance (mmHg/ml/min/100 g tissue)	9.9 ± 0.7	10.4 ± 0.5	9.6 ± 0.3	3.8 ± 0.2*	5.5 ± 0.2$^{*\#}$	9.2 ± 1.0$^{\#\dagger}$

Data are expressed as mean ± S.E. (n=8/group).Rats received PHZ (15 mg/kg, i.p.) alone or combined with curcumin (30 or 100 mg/kg, p.o.) for 8 days.$^{*}p< 0.05$ vs. normal control, $^{\#}p< 0.05$ vs. PHZ control, $^{\dagger}p<0.05$ vs.PHZ+curcumin 30 mg/kg.

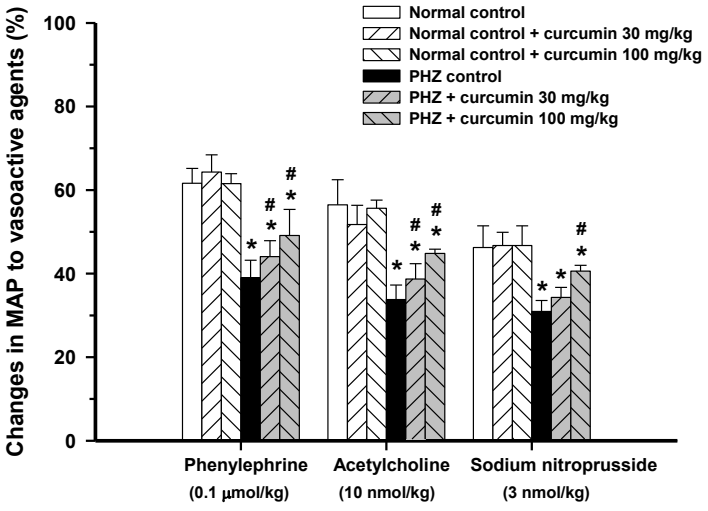

Figure 1 Effect of curcumin on mean arterial pressure responses to various vasoactive agents, including phenylephrine (Phe; 0.1 μmol/kg), acetylcholine (ACh; 10 nmol/kg), and sodium nitroprusside (SNP; 3 nmol/kg) in all experimental groups. Results are expressed as mean ± S.E., n=8, $^{*}p< 0.05$ vs.normal control; $^{\#}p<0.05$ vs. PHZ control.

Effects of PHZ on oxidative stress

A marked increase in$O_2^{\bullet-}$ in vascular tissues and plasma NOxwere found in PHZ-treated rats ($p<0.05$, Fig. 2A and 2B), suggesting that oxidative and nitrosative stresses were present after PHZinjection. PHZ also increased oxidative damage by elevating MDA and protein carbonyl levels in plasma and tissues (liver, kidneys, heart) ($p<0.05$, Fig. 3 and Fig. 4). Curcumin, especially at high dose significantly normalized the rate of $O_2^{\bullet-}$ and NO productions to near basal values in PHZ-treated rats($p<0.05$,Fig. 2A and 2B). Moreover, curcuminalso alleviated oxidative damage by reducing

lipid peroxidation and protein oxidation in a dose dependent manner ($p<0.05$, Fig. 3 and Fig. 4). Administration of curcuminat tested doses did not increase oxidative stress in normal control rats (Fig. 2-4).

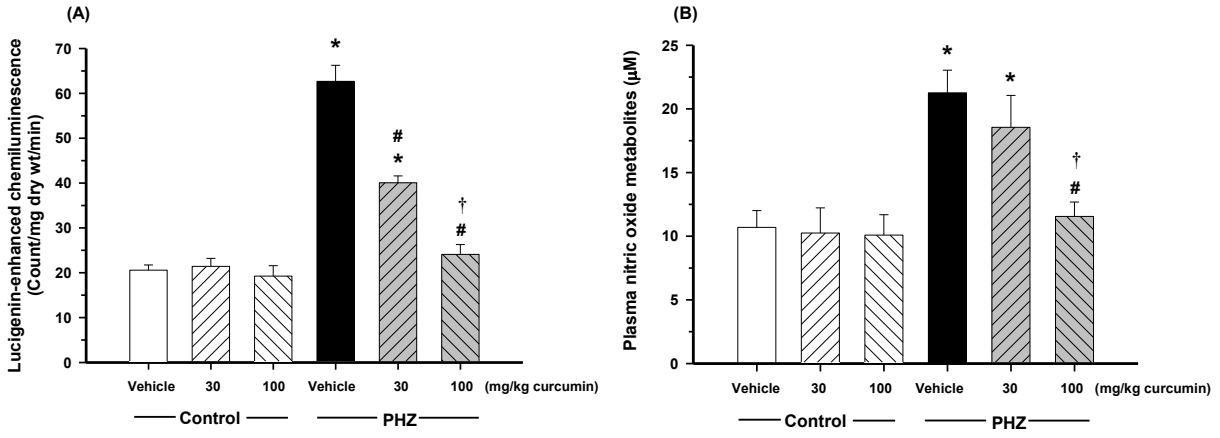

Figure 2 Effect of curcumin on superoxide production in carotid arteries (A) and plasma nitric oxide metabolites (B) in all experimental groups. Results are expressed as mean ± S.E., n=8, $^{*}p< 0.05$ vs. normal control; $^{\#}p<0.05$ vs. PHZ control, $^{\dagger}p<0.05$ vs.PHZ+curcumin 30 mg/kg.

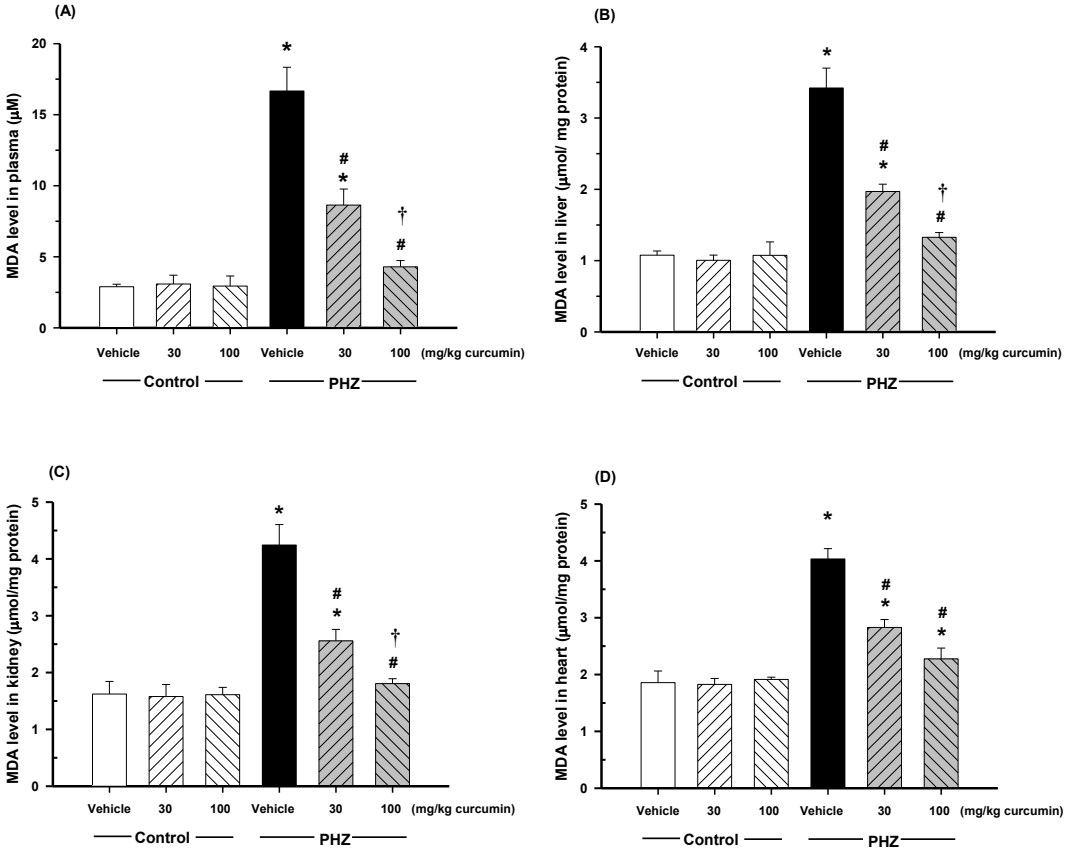

Figure 3 Effect of curcumin on MDA level in plasma (A), liver (B), kidney (C) and heart (D) in all experimental groups. Results are expressed as mean ± S.E., n=8, $^{*}p< 0.05$ vs. normal control; $^{\#}p<0.05$ vs. PHZ control, $^{\dagger}p<0.05$ vs. PHZ+curcumin 30 mg/kg.

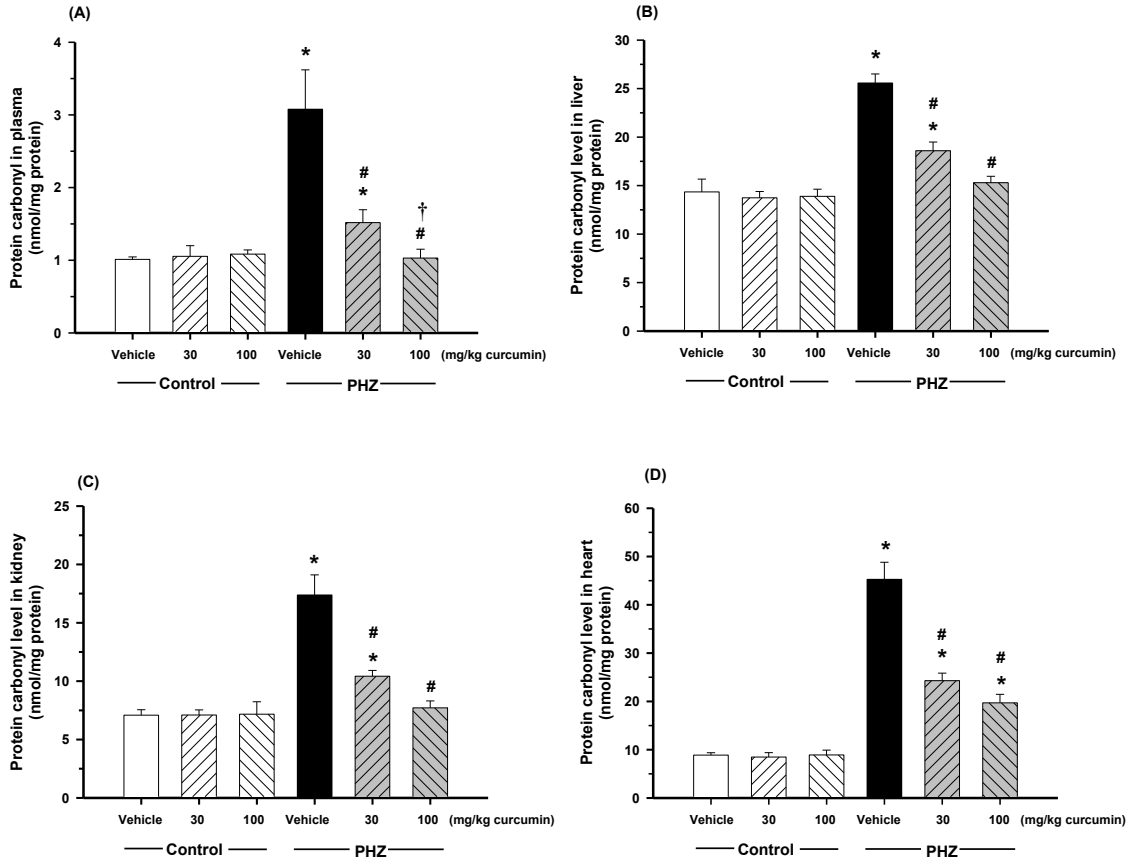

Figure 4 Effect of curcumin on protein carbonyl levels in plasma (A), liver (B), kidney (C) and heart (D) in all experimental groups. Results are expressed as mean ± S.E., n=8, $^{*}p< 0.05$ vs. normal control; $^{\#}p<0.05$ vs. PHZ control, $^{\dagger}p<0.05$ vs.PHZ+curcumin 30 mg/kg.

Discussion

The present study demonstrated that intraperitoneal injection of PHZ at dose of 15 mg/kg for eight days induced severe hemolytic anemia and vascular dysfunction, manifested by a reduction of hematocrit, blood pressure, vascular responsiveness to vasoactive agents and HVR while acceleration of HBF and HR. Interestingly, these alterations were associated with increased oxidative stress. Concurrent treatment with curcumincould alleviate hemolytic anemia and vascular dysfunction, as indicated by a restoration of hemodynamic and oxidative stress status and partially increase hematocrit. Overallfindings support the idea that antioxidants prevent free radicals-mediated vascular dysfunction in cardiovascular diseases[22].

Previous studyreported that PHZ induced vascular smooth muscle relaxation by increasing cGMP production through the activation of guanylatecyclase which is mediated via the formation of iron-phenyl heme complexes[23]. Therefore, the hypotensive state after PHZ treatment might be due to increased formation of iron-phenyl heme complex. Many studiesalso suggested that inflammatory response caused by free heme and hemoglobin released from lyzed erythrocytes, subsequently induced oxidative stress andinflammation[24, 25]. Thus, oxidative stress and inflammatory mediators released from inflammatory cells are also involved in hemolytic anemia induced by PHZ[6]. In this study, we found that $O_2^{\bullet-}$ and NO were increased in PHZ-treated rats.Normally, $O_2^{\bullet-}$ and NO rapidly interact and form peroxynitriteanion ($ONOO^-$), a highly reactive free

radical[26], thereby, $ONOO^-$ and inflammatory cytokines released by inflammatory cells might induce vascular dysfunction[27]. It has been suggested that increased $O_2^{\bullet-}$ production may lead to decreased NO bioactivity for the vascular endothelial cells[28, 29], resulting in impairment of vascular reactivity to endothelial-dependent vasodilator, ACh. Regarding the vascular response to SNP, there is a slightly decrease in vasorelaxation, this isin consistent with other study in sickle cell mice[30]. Interestingly, not only vasodilation was impaired, butalso vascular response to a vasoconstrictor, Phewas also blunted. These findingsaresupported by previous studies, which found an impairment of vasoconstrictionin hypovolemic and septic shock conditions[31, 32].

It is suggested that curcumincan increase hemoglobin formation, thereby; it may preventerythroid precursor apoptosis and red blood cellshemolysis[33, 34]. Therefore, the hemolytic anemia condition of PHZ-treated rats is recovered after curcumin administration.

The present study demonstrated that curcumin decreased $O_2^{\bullet-}$ and NO formation which might be associated with down regulation of NAD(P)H oxidase and iNOS expressions via inhibition of NF-$_\kappa$B activation and many inflammatory cytokines[35, 36]. Therefore, curcumin improves vascular function and protects vascular injuries from ROS, reactive nitrogen species (RNS) and other radicals such as PHZ. Moreover, our study revealed that curcuminin a dose-dependent manner alleviated lipid peroxidation and protein oxidation. A marked increase in oxidative stressis well documented to subsequently induce oxidative damage through a destruction of lipid, protein and DNA in the organ systems of animals and humans[37]. Previous studies demonstrated that curcumincan increase glutathione and restore redox status in oxidative damage condition. Moreover, curcuminalso increases γ-glutamyl cysteine ligase, a rate limiting enzyme for GSH synthesis and reduces gene of Nrf2-ARE signaling pathway where curcumin is served as a strong inducer of transcription factor Nrf2 activator [38].

Conclusion

In conclusion, data of this studyprovide the beneficial effects of curcumin in a rat model of PHZ-induced hemolytic anemia and vascular dysfunction. The plausible mechanisms involved with these effects might be due to the capability of curcumin to scavenge peroxyl radicals, prevent hemolysis of RBCs and inhibit the generation of free radicals, thereby improved vascular function and vascular reactivity and oxidative stress. Collectively, curcumin supplementation may be useful in hemolytic anemic condition. However, further investigation regarding the mechanisms of curcumin in maintenance of vascular function is still required.

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References

1. Berger J. Screening of toxic-haemolyticanaemia in laboratory rats: a model of phenylhydrazine-induced haemolysis. Haematologia (Budap) 1985; 18: 193-200.
2. Chakrabarti S, Sonaye B, Naik AA, Nadkarni PP. Erythrocyte membrane protein damage by oxidation products of phenylhydrazine. Biochem Mol Biol Int 1995; 35: 255-63.
3. Biswas S, Bhattacharyya J, Dutta AG. Oxidant induced injury of erythrocyte-role of green tea leaf and ascorbic acid. Mol Cell Biochem 2005; 276: 205-10.
4. Nicolas G, Chauvet C, Viatte L, Danan JL, Bigard X, Devaux I, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. J Clin Invest 2002; 110: 1037-44.
5. Luangaram S, Kukongviriyapan U, Pakdeechote P, Kukongviriyapan V, Pannangpetch P. Protective effects of quercetin against phenylhydrazine-induced vascular dysfunction and oxidative stress in rats. Food Chem Toxicol 2007; 45: 448-55.
6. Swann JW, Contrera JF. Depletion of cardiac norepinephrine during two forms of hemolytic anemia in the rat. Circ Res 1976; 38: 179-84.

7. Ferrali M, Signorini C, Caciotti B, Sugherini L, Ciccoli L, Giachetti D, et al. Protection against oxidative damage of erythrocyte membrane by the flavonoid quercetin and its relation to iron chelating activity.FEBS Lett 1997; 416: 123-9.
8. Sharma RA, Gescher AJ, Steward WP. Curcumin: the story so far. Eur J Cancer 2005; 41: 1955-68.
9. Aggarwal BB, Sung B. Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. Trends Pharmacol Sci 2009; 30: 85-94.
10. Nakmareong S, Kukongviriyapan U, Pakdeechote P, Donpunha W, Kukongviriyapan V, Kongyingyoes B, et al. Antioxidant and vascular protective effects of curcumin and tetrahydrocurcumin in rats with L-NAME-induced hypertension. Naunyn Schmiedebergs Arch Pharmacol 2011; 383: 519-29.
11. Garcia-Nino WR, Pedraza-Chaverri J. Protective effect of curcumin against heavy metals-induced liver damage. Food Chem Toxicol 2014; 69: 182-201.
12. Sompamit K, Kukongviriyapan U, Nakmareong S, Pannangpetch P, Kukongviriyapan V. Curcumin improves vascular function and alleviates oxidative stress in non-lethal lipopolysaccharide-induced endotoxaemia in mice. Eur J Pharmacol 2009; 616: 192-9.
13. Kukongviriyapan U, Apaijit K, Kukongviriyapan V. Oxidative Stress and Cardiovascular Dysfunction Associated with Cadmium Exposure: Beneficial Effects of Curcumin and Tetrahydrocurcumin. Tohoku J Exp Med 2016; 239: 25-38.
14. Kukongviriyapan U, Pannangpetch P, Kukongviriyapan V, Donpunha W, Sompamit K, Surawattanawan P. Curcumin protects against cadmium-induced vascular dysfunction, hypertension and tissue cadmium accumulation in mice. Nutrients 2014; 6: 1194-208.
15. Sangartit W, Kukongviriyapan U, Donpunha W, Pakdeechote P, Kukongviriyapan V, Surawattanawan P, et al. Tetrahydrocurcumin protects against cadmium-induced hypertension, raised arterial stiffness and vascular remodeling in mice. PLoS One 2014; 9: e114908.
16. Sangartit W, Pakdeechote P, Kukongviriyapan V, Donpunha W, Shibahara S, Kukongviriyapan U. Tetrahydrocurcumin in combination with deferiprone attenuates hypertension, vascular dysfunction, baroreflex dysfunction, and oxidative stress in iron-overloaded mice. Vascul Pharmacol 2016; 87: 199-208.
17. Sandur SK, Pandey MK, Sung B, Ahn KS, Murakami A, Sethi G, et al. Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism. Carcinogenesis 2007; 28: 1765-73.
18. Singh S, Aggarwal BB. Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. J Biol Chem 1995; 270: 24995-5000.
19. Somparn P, Phisalaphong C, Nakornchai S, Unchern S, Morales NP. Comparative antioxidant activities of curcumin and its demethoxy and hydrogenated derivatives. Biol Pharm Bull 2007; 30: 74-8.
20. Manikandan P, Sumitra M, Aishwarya S, Manohar BM, Lokanadam B, Puvanakrishnan R. Curcumin modulates free radical quenching in myocardial ischaemia in rats. Int J Biochem Cell Biol 2004; 36: 1967-80.
21. Kukongviriyapan V, Somparn N, Senggunprai L, Prawan A, Kukongviriyapan U, Jetsrisuparb A. Endothelial dysfunction and oxidant status in pediatric patients with hemoglobin E-beta thalassemia. Pediatr Cardiol 2008; 29: 130-5.
22. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 2000; 87: 840-4.
23. Vidrio H, Fernandez G, Medina M, Alvarez E, Orallo F. Effects of hydrazine derivatives on vascular smooth muscle contractility, blood pressure and cGMP production in rats: comparison with hydralazine. VasculPharmacol 2003; 40: 13-21.
24. Jeney V, Balla J, Yachie A, Varga Z, Vercellotti GM, Eaton JW, et al. . Pro-oxidant and cytotoxic effects of circulating heme. Blood 2002; 100: 879-87.
25. Setty BN, Betal SG, Zhang J, Stuart MJ. Heme induces endothelial tissue factor expression: potential role in hemostatic activation in patients with hemolytic anemia. J Thromb Haemost 2008; 6: 2202-9.
26. Miles AM, Bohle DS, Glassbrenner PA, Hansert B, Wink DA, Grisham MB. Modulation of superoxide-dependent oxidation and hydroxylation reactions by nitric oxide. J Biol Chem 1996; 271: 40-7.
27. Cuzzocrea S, Mazzon E, Di Paola R, Esposito E, Macarthur H, Matuschak GM, et al. . A role for nitric oxide-mediated peroxynitrite formation in a model of endotoxin-induced shock. J Pharmacol Exp Ther 2006; 319: 73-81.
28. Cai H. NAD(P) H oxidase-dependent self-propagation of hydrogen peroxide and vascular disease. Circ Res 2005; 96: 818-22.
29. Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part II: animal and human studies. Circulation 2003; 108: 2034-40.
30. Hsu LL, Champion HC, Campbell-Lee SA, Bivalacqua TJ, Manci EA, Diwan BA, et al. Hemolysis in sickle cell mice causes pulmonary hypertension due to global impairment in nitric oxide bioavailability. Blood 2007; 109: 3088-98.

31. Altavilla D, Saitta A, Guarini S, Galeano M, Squadrito G, Santamaria LB, et al. . Nuclear factor-kappaB as a target of cyclosporin in acute hypovolemic hemorrhagic shock. Cardiovasc Res 2001; 52: 143-52.
32. Piepot HA, Groeneveld AB, van Lambalgen AA, Sipkema P. The role of inducible nitric oxide synthase in lipopoly-saccharide-mediated hyporeactivity to vasoconstrictors differs among isolated rat arteries. ClinSci (Lond) 2002; 102: 297-305.
33. Banerjee A, Kunwar A, Mishra B, Priyadarsini KI. Concentration dependent antioxidant/pro-oxidant activity of curcumin studies from AAPH induced hemolysis of RBCs. Chem Biol Interact 2008; 174: 134-9.
34. Thephinlap C, Phisalaphong C, Lailerd N, Chattipakorn N, Winichagoon P, Vadolas J, et al. . Reversal of cardiac iron loading and dysfunction in thalassemic mice by curcuminoids. Med Chem 2011; 7: 62-9.
35. Nirmala C, Anand S, Puvanakrishnan R. Curcumin treatment modulates collagen metabolism in isoproterenol induced myocardial necrosis in rats. Mol Cell Biochem 1999; 197: 31-7.
36. RalayRanaivo H, Carusio N, Wangensteen R, Ohlmann P, Loichot C, Tesse A, et al. Protection against endotoxic shock as a consequence of reduced nitrosative stress in MLCK210-null mice. Am J Pathol 2007; 170: 439-46.
37. Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 2003; 192: 95-117.
38. Thimmulappa RK, Lee H, Rangasamy T, Reddy SP, Yamamoto M, Kensler TW, et al. Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis. J Clin Invest 2006; 116: 984-95.