Vitamin C Enhances Antioxidant Glutathione and Improves Vascular Function in Cadmium Chloride –Induced Hypertensive Mice

วิตามินซีเพิ่มสารต้านออกซิเดชันกลูทาไทออนและเพิ่มประสิทธิภาพการทำงานของหลอดเลือดในหนูไมซ์ความดันเลือดสูงจากการได้รับสารแคดเมียมคลอไรด์

Wanida Donpunha (วนิดา ดรปัญหา)* Dr.Upa Kukongviriyapan (ดร. ยุพา คู่คงวิริยพันธุ์)**
Dr.Poungrat Pakdeechote (ดร.พวงรัตน์ ภักดีโชติ)***Dr.Veerapol Kukongviriyapan (ดร. วีรพล คู่คงวิริยพันธุ์)****
Dr. Kwanjit Sompamit (ดร. ขวัญจิต สมพมิตร)*****

ABSTRACT

Oxidative stress is a major contributor to the development of vascular dysfunction found in various pathological conditions. Cadmium (Cd) is an important industrial and environmental pollutant that can produce a wide variety of adverse effects in humans and animals. Vitamin C (ascorbic acid) is a powerful water-soluble antioxidant which is found mainly in fruits and vegetables and has a variety of biological activities. The aim of this study was to investigate the protective effect of vitamin C in a mouse model of cadmium chloride ($CdCl_2$)-induced oxidative stress and hypertension. Male ICR mice were received $CdCl_2$ (100 mg/L) in their drinking water for eight weeks whereas normal control mice received deionized water. The other two groups of animals treated with $CdCl_2$ were orally administered with vitamin C at doses of 50 and 100 mg/kg/day. Results showed that mean arterial pressure of $CdCl_2$-treated mice was markedly increased as compared to normal controls. Treatment with vitamin C significantly decreased arterial blood pressure in both doses of 50 and 100 mg/kg/day. The vascular responses to phenylephrine, acetylcholine, and sodium nitroprusside of $CdCl_2$-treated mice were dramatically suppressed and vitamin C dose-dependently restored the vascular responsiveness. Moreover, vitamin C largely protected blood glutathione and suppressed malondialdehyde levels in plasma. These results provide the evidence for the role of antioxidant vitamin C in a reduction of blood pressure and improvement of vascular function in mice with sub-chronic exposure to $CdCl_2$.

บทคัดย่อ

ภาวะเครียดออกซิเดชันมีความสำคัญและเกี่ยวข้องกับภาวะหลอดเลือดทำงานผิดปกติที่พบได้ในพยาธิสภาพของโรคทั้งหลาย แคดเมียมเป็นสารพิษที่พบในโรงงานอุตสาหกรรมและสิ่งแวดล้อมซึ่งส่งผลเสียต่อร่างกายมนุษย์และสัตว์อย่างมาก วิตามินซี หรือ กรดแอสคอร์บิก เป็นสารต้านออกซิเดชันที่สำคัญซึ่งพบได้มากในผักและผลไม้หลายชนิด

* *Ph.D. Student, Department of Physiology, Faculty of Medicine, Khon Kaen University*
** *Associate Professor, Department of Physiology, Faculty of Medicine, Khon Kaen University*
*** *Assistant Professor, Department of Physiology, Faculty of Medicine, Khon Kaen University*
**** *Associate Professor, Department of Pharmacology, Faculty of Medicine, Khon Kaen University*
***** *Lecturer, Faculty of Medicine, Mahasarakham University*

โดยมีฤทธิ์ทางชีววิทยาต่างๆหลายประการ วัตถุประสงค์ของการศึกษาวิจัยนี้เพื่อตรวจสอบฤทธิ์ของวิตามินซีในการปกป้องภาวะเครียดออกซิเดชันและภาวะความดันเลือดสูงในหนูไมซ์ที่ได้รับสารแคดเมียมคลอไรด์ ($CdCl_2$) หนูไมซ์เพศผู้สายพันธุ์ ICR ได้รับ สารแคดเมียมคลอไรด์ขนาดความเข้มข้น 100 มก./ลิตร ผสมในน้ำดื่มเป็นเวลา 8 สัปดาห์ ในขณะที่หนูไมซ์ปกติซึ่งเป็นกลุ่มควบคุมได้รับน้ำดื่มชนิดปราศจากไอออน ส่วนหนูไมซ์ทดลองอีก 2 กลุ่มจะได้รับสารแคดเมียมคลอไรด์ในน้ำดื่มและถูกป้อนด้วยวิตามินซีขนาด 50 และ 100 มก./กก/วัน เป็นเวลา 8 สัปดาห์ ผลการศึกษาพบว่าความดันเลือดแดงของหนูไมซ์ที่ได้รับแคดเมียมคลอไรด์มีค่าสูงขึ้นอย่างมากเมื่อเปรียบเทียบกับหนูทดลองปกติ และวิตามินซีสามารถลดความดันเลือดของหนูทดลองได้อย่างมีนัยสำคัญทางสถิติทั้งวิตามินซีขนาด 50 และ 100 มก./กก/วัน สำหรับการตอบสนองของหลอดเลือดต่อสารกระตุ้นหลอดเลือดได้แก่ phenylephrine, acetylcholine, and sodium nitroprusside ในหนูไมซ์ที่ได้รับแคดเมียมคลอไรด์พบว่าลดลง โดยวิตามินซีสามารถเพิ่มการตอบสนองของหลอดเลือดต่อสารเหล่านี้ได้ นอกจากนี้วิตามินซียังรักษาระดับกลูทาไทออนในเลือด และลดระดับมาลอนไดอัลดีไฮด์ในพลาสมา จากผลการศึกษาวิจัยนี้แสดงให้เห็นถึงบทบาทของวิตามินซีในการลดความดันเลือดและเพิ่มการทำงานของหลอดเลือดในหนูไมซ์ที่ได้รับสารแคดเมียมคลอไรด์เป็นระยะเวลานานกึ่งเรื้องรัง

Key Words: Hypertension, Vitamin C, Vascular dysfunction
คำสำคัญ : ความดันเลือดสูง วิตามินซี หลอดเลือดทำงานผิดปกติ

1. Introduction

Cadmium (Cd) is a toxic heavy metal which causes toxicity to all plants, animals and humans. It is not a redox active metal itself, it can generate free radicals indirectly (Liu *et al.*, 2009). Cd has a high affinity for sulfhydryl groups, inactivating numerous enzymatic reactions, amino acids, and sulfur-containing antioxidants, with subsequently decreased oxidant defense and increased oxidative stress (Eybl *et al.*, 2004; Stohs and Bagchi, 1995). Although there are several antioxidants play an important role in alleviation of oxidative stress, there are limited evidences demonstrating the beneficial effects of the antioxidants against Cd-induced oxidative damage

Vitamin C (ascorbic acid) is known as a natural powerful antioxidant that contains in many fruits and vegetables and it has variety of biological activities, including prevention of ischemia-reperfusion(IR)-induced vascular injury, improvement of endothelial functions and protection against cell damage and/or cell

death from increased oxidative stress (Lavi *et al.*, 2008; Padayatty *et al.*, 2003; Pleiner *et al.*, 2008). Since the effect of vitamin C on endogenous antioxidant glutathione (GSH) and vascular function in an animal model with sub-chronic exposure to Cd has not been evaluated, therefore, the present study was conducted to investigate protective effect of vitamin C in a mouse model of cadmium chloride ($CdCl_2$)-induced oxidative stress and hypertension.

2. Materials and methods

Animals and experimental protocol

Male ICR mice, weighing 25-30 g were obtained from the Animal Care Unit of Faculty of

Medicine, Khon Khaen University, Khon Kaen, Thailand. All animal procedures were reviewed and approved by the Institutional Animal Ethics Committee of Khon Kaen University (AEKKU 20/2550). All of the animals were maintained in a temperature controlled room with a 12 h light: 12 h dark cycle. The animals were given free access to standard chow diet (Chareon Pokapan Co.Ltd., Thailand) and tap water. After an adaptation period of 7 days, the animals were randomly divided into four groups with 5-7 animals per group. Group I —vehicle control, mice were given the antioxidant vehicle alone (deionized water). Group II — mice were received $CdCl_2$ alone. Group III and IV — mice were received $CdCl_2$ and vitamin C at doses of 50 and 100 mg/kg. The animals were exposed to $CdCl_2$ (100 mg/L) via their drinking water and orally administered with vitamin C at dose of 50 or 100 mg/kg for eight weeks.

Sample collection and haemodynamic and vascular reactivity assessments

After 8 weeks of the experiment, haemodynamics and vascular reactivity were measured by previuosly described method (Sompamit *et al.*, 2009). Briefly, mice were anaesthetized 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 heating pad. A tracheostomy was performed for spontaneously breathing and then the right carotid artery was cannulated with polyethylene (PE) 10 tubing which was connected to a pressure transducer for continuously monitoring of arterial blood pressure, using the Acknowledge data acquisition and analysis software (Biopac System, California, U.S.A.). Another PE 10 tubing was inserted into the left jugular vein for infusion of vasoactive agents. Arterial blood pressure and heart rate were recorded after obtaining stable baseline measurements, vascular reactivity was assessed by testing responsiveness to the vasoconstrictor; phenylephrine (Phe, 0.03 μmol/kg), vasodilators; acetylcholine (ACh, 10 nmol/kg) and sodium nitroprusside (SNP, 10 nmol/kg). Each vasoactive agent was infused at 5-min interval. After last dose of vasoactive agents, blood samples were collected from abdominal aorta for measurements of oxidative stress markers, plasma malondialdehyde (MDA) and antioxidant GSH.

Biochemical assays

Assay of GSH: Blood GSH was measured as previously described method (Sompamit *et al.*, 2009). Briefly, 100 μl of whole blood was reacted with 10 μl 33 mM 1-methyl-2vinyl-pyridinum trifate (M2VP, the GSH scavenger) or deionized water, and subsequently treated with 5% cold meta phosphoric acid to precipitate protein. The supernatant obtained after centrifugation was used in the enzymatic coupling assay for GSH.

Assay of MDA: Plasma lipid peroxidation measured as MDA was estimated using thiobarbituric acid, as previously described (Luangaram *et al.*, 2007). In brief, 150 μl plasma was reacted with 125 μl 10% trichloroacetic acid, 125 μl 5 mM EDTA, 125 μl 8% sodium dodecylsulfate, and 10 μl 0.5 μg/ml of butylated hydroxytoluene. The mixture was incubated for 10 min at room temperature, 535 μl 0.6% thiobarbituric acid was then added, and the mixture was boiled for 30 min. After cooling to room temperature, the mixture was centrifuged at 2800 g for 20 min. The absorbance of the supernatant was

measured at 532 nm. A standard curve was generated with appropriate concentrations of 1,1,3,3-tetraethoxypropane standards (0.3–10 μmol/l).

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 comparison test. A *p*-value of less than 0.05 was considered significant.

3. Results and discussion

3.1 Effect of vitamin C on haemodynamics and vascular function

Mice treated with $CdCl_2$ (100 mg/L) in drinking for 8 weeks showed significantly increase in mean arterial pressure (MAP), systolic blood pressure (SBP) and diastolic blood pressure (DBP) when compared with the control group. It was found that vitamin C at doses of 50 and 100 mg/kg/day significantly decreased arterial blood pressure (Table 1; $P<0.05$). However, changes in heart rates (HR) among groups were not significantly different. A previous study showed that long-tern exposure with low dose of Cd induced hypertension in rats, and this finding was associated with increased sodium retention (Perry and Erlanger, 1981). However, the specific mechanism for Cd-induced hypertension has not yet been cleared, but there are several supporting evidences, including a decrease in vasodilating activities (Skoczynska and Martynowicz, 2005), an activation of sympathetic nervous system (Fadloun and Leach, 1980), and an interaction of Cd with Ca^{2+} channel (Balaraman *et al.*, 1989).

Effect of vitamin C on vascular function; endothelial-dependent vasodilator acetylcholine (ACh), endothelial-independent vasodilator sodium nitroprusside (SNP) and also vasoconstrictor phenylephrine (Phe) have been impaired in mice treated with $CdCl_2$. Vitamin C (100 mg/kg) significantly improved vascular responses to Phe, ACh and SNP (Figure 1, 2 and 3; $P<0.05$).

ACh, endothelial-dependent vasoactive agent, is common used to test endothelial function that produced vasodilator, nitric oxide (NO) lead to decrease vascular resistance and decrease MAP. The result in this study shown that the vascular response to ACh was decreased after $CdCl_2$ exposure, indicated that $CdCl_2$ induced endothelial dysfunction. Moreover , SNP, endothelial-independent vasodilator , is NO donor which directly induced vascular smooth muscle cell (VSMC) relaxation. However, effect of SNP to VSMC was decreased by $CdCl_2$, this result suggested that $CdCl_2$ also induced VSMC dysfunction.

Table 1

Effects of vitamin C on blood pressure in all experimental groups

Parameter measurements	Normal control	$CdCl_2$ control	$CdCl_2$ + vitamin C	
			50 mg/kg	100 mg/kg
Mean arterial blood pressure (mmHg)	96.2 ± 2.3	136.5 ± 2.5*	96.9 ± 5.8†	92.2 ± 6.7†
Systolic blood pressure (mmHg)	117.0 ± 3.0	155.1 ± 1.8*	121.5 ± 5.7†	122.0 ± 1.9†
Diastolic blood pressure (mmHg)	82.30 ± 3.2	113.7 ± 2.4*	81.0 ± 6.5†	84.9 ± 1.5†
Heart rate (bpm)	327.1 ± 3.4	333.3 ± 4.7	329.5 ± 2.5	334.1 ± 4.6

$CdCl_2$-treated mice showed a significant increase in mean arterial pressure (MAP), systolic blood pressure (SBP) and diastolic blood pressure (DBP). Supplementation with vitamin C significantly decreased SBP, DBP and MAP of mice treated with $CdCl_2$.

* $P < 0.05$ vs. control; † $P < 0.05$ vs. $CdCl_2$, n = 5/group

Interestingly, the impairment of endothelial-dependent and -independent vasodilation was improved by vitamin C 100 mg/kg. Moreover, this result also shown that the vasoconstriction effect of Phe, which is an α_1-adrenergic receptor on VSMC was decreased by $CdCl_2$, while treatment with vitamin C trend to improve vascular function. Previous study suggest that the Cd exposure induced both endothelial and VSMC dysfunction resulting in hypertension (Prozialeck *et al.*, 2008). Additionally, Cd decrease the NO bioavailability and lead to increase blood pressure (Skoczynska and Martynowicz, 2005; Wolf and Baynes, 2007). In addition, it was reported that the decreasing in endothelial-dependent vasorelaxation may play a role in cadmium-induced hypertension in rats model (Gokalp *et al.*, 2009). Several studies indicate that a decrease in vascular reactivity is an important marker of vascular dysfunction which is found in various pathological conditions. It has been demonstrated that vitamin C improves vascular responses in many pathological conditions such as atherosclerosis, and this effect is associated with an increase in endothelial NO bioavailability (Ajay and Mustafa, 2006; Matsumoto *et al.*, 2003)

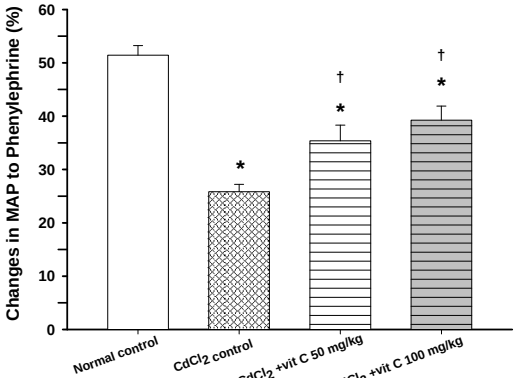

Figure 1 Change in mean arterial pressure (MAP) in response to vasoconstrictor phenylephrine (Phe, 0.03 μmol/kg) in all experimental groups. * $P < 0.05$ vs. control; † $P < 0.05$ vs. $CdCl_2$ control, n = 5/group

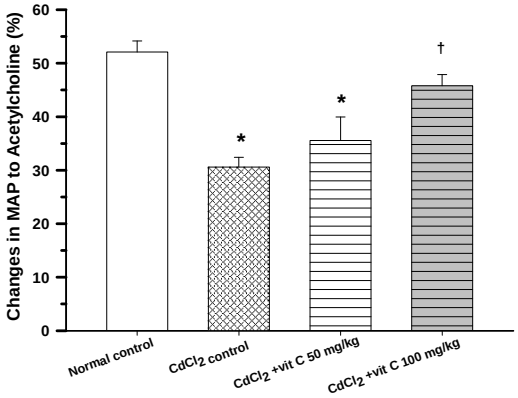

Figure 2 Change in mean arterial pressure (MAP) in response to vasodilator acetylcholine (ACh, 10 nmol/kg) in all experimental groups.

* $P < 0.05$ vs. control; † $P < 0.05$ vs. $CdCl_2$ control, n = 5/group

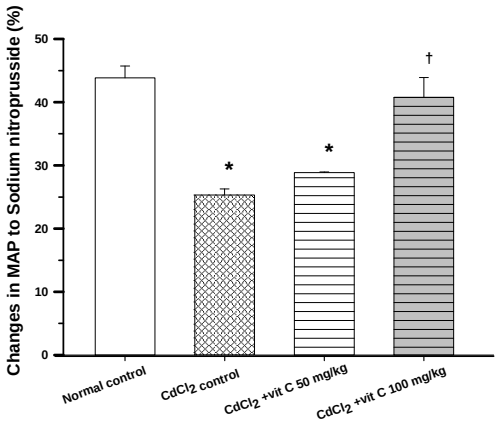

Figure 3 Change in mean arterial pressure (MAP) in response to vasodilator sodium nitroprusside (SNP, 10 nmol/kg) in all experimental groups. * $P < 0.05$ vs. control; † $P < 0.05$ vs. $CdCl_2$ control, n = 5/group

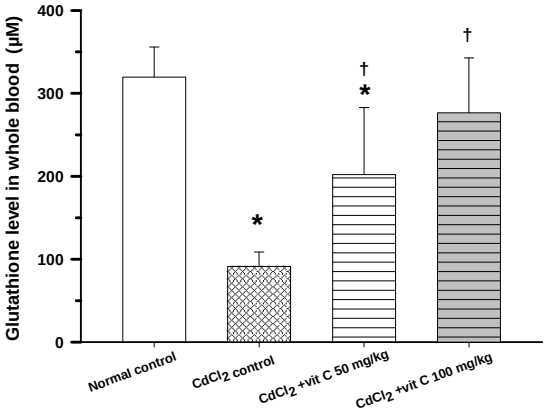

Figure 5 Effect of vitamin C on the improvement of blood glutathione in mice exposed to $CdCl_2$. * $P < 0.05$ vs. control; † $P < 0.05$ vs. $CdCl_2$ control, n = 5/group

3.2. Antioxidant activities of vitamin c

Oxidative stress was found in mice treated with $CdCl_2$ as shown by an increase in plasma MDA level and a reduction in blood GSH when compared with the controls (Figure 4 and 5; $P<0.05$). Vitamin C reduced lipid peroxidation and increased antioxidant GSH in $CdCl_2$ -treated mice as depicted in Figure 4 and 5 ($P<0.05$).

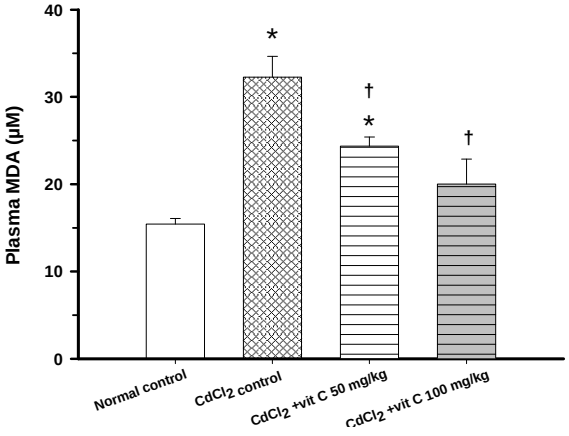

Figure 4 Effect of vitamin C on a reduction of plasma malondialdehyde (MDA) in mice exposed to $CdCl_2$. * $P < 0.05$ vs. control; † $P < 0.05$ vs. $CdCl_2$ control, n = 5/group

A depletion of intracellular antioxidant GSH is one of the important markers of oxidative stress (Schulz *et al.*, 2000). It is reported that a reduction in GSH might be associated with the development of hypertension (Vaziri *et al.*, 2000). Our data support this evidence since a depletion of GSH in $CdCl_2$-treated mice appeared with the increase in blood pressure.

Vitamin C is an electron donor and a powerful water-soluble antioxidant. It is recognized as the free radical scavenger because it can reduced reactive free radicals through a formation of the less reactive compound (Padayatty *et al.*, 2003). Moreover, it is demonstrated that vitamin C is able to improve vascular responsiveness and endothelium function (Taddei *et al.*, 1998). These effects may be mediated via modulation of the antioxidant enzyme systems (Chen *et al.*, 2001) and preservation of endothelium-derived NO (Ajay and Mustafa, 2006).

In this study, we have found the strong antioxidant effect of vitamin C against $CdCl_2$-induced oxidative stress, and this effect was related to a decrease in blood pressure and an improvement of vascular function in mice treated with $CdCl_2$ and vitamin C.

4. Conclusion

Data from this study showed that Cd induced oxidative stress and caused a significant elevation of blood pressure and impairment of vascular function. Vitamin C supplementation protected the increase in blood pressure and the blunted vascular responses in mice with sub-chronic exposure to $CdCl_2$. The beneficial effect of vitamin C might be due to its metal chelating and strong anti-oxidative effects.

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