Effect of Combined Tetrahydrocurcumin and Deferiprone on Oxidative Stress and Vascular Dysfunction in Iron Overloaded Mice

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Background and Objective: Iron overload is an excessive accumulation of iron in the body. This condition can generate reactive oxygen species (ROS), which leads to oxidative stress and subsequently vascular dysfunction via the mechanism of reducing nitric oxide (NO), a key regulator of vascular homeostasis. Deferiprone (or L1) is used to prevent iron toxicity by chelating labile iron. Moreover, several studies indicated that tetrahydrocurcumin (THU) possesses strong antioxidant and vasculoprotective effect in various stress conditions. The present study was aimed to investigate whether supplementation with L1 plus THU can mitigate oxidative stress and vascular dysfunction in iron overloaded mice, a model represented iron overload condition.

Methods: Iron sucrose (10 mg/kg/day, i.p.) was injected to Imprinting Control Regio (ICR) mice for eight weeks. . L1 or THU at dose of 50 mg/kg/day was intragastrically administered through the period of iron overload induction. Blood pressure, vascular responsiveness to various vasoactive agents, lipid peroxidation markers were measured. The thoracic aortas were excised for assessment of superoxide radical ($O_2^{\cdot-}$) production.

Results: Iron overloaded mice exhibited high arterial blood pressure and impaired vascular responses to vasoconstrictor and vasodilators, Increase in $O_2^{\cdot-}$ production in vascular tissues and plasma malondialdehyde (MDA) concentration were found in iron overloaded mice. Treatment with L1 or THU partially alleviated these deleterious effects. Interestingly, combined therapy with L1 and THU exerted a greater effect than L1 or THU monotherapy.

Conclusion: Iron overload-induced by iron sucrose enhanced oxidative stress and vascular dysfunction in mice. L1 plus THU restored the blood pressure, decreased oxidative stress, attenuated vascular dysfunction.

Key words: Iron overload, Oxidative stress, Vascular dysfunction, Deferiprone, Tetrahydrocurcumin

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Introduction

Iron, the most abundant transition metal in human body is primarily present in protein-bound forms, such as heme and non-heme protein which plays a crucial role in electron transfer and oxygen utilization reactions. However, excessive iron accumulation in the body or iron overload, frequently occurs in patients with sickle cell disease and blood transfusions dependent thalassemia patients. Iron overload is associated with increased iron-binding proteins transferrin which leads to formation of highly reactive non-transferrin bound iron (NTBI) and enhances the formation of reactive oxygen species (ROS) through Fenton and Haber Weiss reaction.[1]

A large number of evidences indicated that ROS is implicated in cardiovascular risk factors such as hypertension which have a close linkage with vascular dysfunction. [2] Endothelial nitric oxide synthase (eNOS) generates nitric oxide ($NO^{\bullet}$) radical which plays a key role on vascular function. ROS can quench $NO^{\bullet}$ directly with formation of peroxynitrite which leads to decrease $NO^{\bullet}$ bioavailability.

Deferiprone or L1, a low molecular weight oral iron chelator, is widely used in patients with -thalassemia major and has the capacity to decrease body iron and NTBI. [3] However, there is a little information about the effect of L1 on oxidative stress and vascular function in iron overload condition

In addition, it has been demonstrated that heavy metal-induced oxidative stress is attenuated by antioxidants. Tetrahydrocurcumin (THU), a reduced derivative of curcumin from *Curcuma Longa* (Turmeric), has been shown to possess a variety of biological activities, such as anti-inflammation, anti-proliferative response, strong antioxidant and cardioprotective properties.[4] Nevertheless, the beneficial effect of THU against hypertension, vascular dysfunction and oxidative stress is required further exploration.

Objective

The present study was aimed to investigate whether supplementation with L1 plus THU can mitigate oxidative stress and vascular dysfunction in iron sucrose-induced iron overloaded mice.

Methods

Adult male ICR mice weighing 25-30 g. were obtained from National Laboratory Animal Center, Salaya, Nakornprathum, Thailand. All animal experimental treatment protocols were reviewed and approved by the Animal Ethics Committee of Khon Kaen University (AEKKU 62/2555).

After an adaptation periods, the animals were induced iron overload by intraperitoneal injection of iron sucrose (10 mg/kg/day), three days/week for eight weeks. L1 (50 mg/kg) or THU (50 mg/kg) were intragastrically administered once daily for five days per week throughout the period of iron sucrose administration. After eight weeks of the experiment, mice were anesthetized with ketamine/xylazine (100:2.5 mg/kg, i.p.), arterial blood pressure was recorded through carotid artery by previously described method [5]. Vascular responsiveness was investigated by using vasoactive agents, including phenylephrine (Phe; 0.03 ตmol/kg), aetylcholine (ACh; 10 nmol/kg), and sodium nitroprusside (SNP; 10 nmol/kg). At the end of experiment, the animals were euthanized with overdose of anesthetic drug. Blood samples were rapidly collected from the abdominal aorta for measurements of malondialdehyde (MDA), a lipid peroxidation marker, reduced glutathione (GSH) and liver tissues were collected for evaluating glutamate cysteine ligase (GCL) activity. The thoracic aortas were immediately excised for measurement of superoxide ($O_2^{\cdot-}$) production by using lucigenin-enhanced chemiluminescence technique [5].

Results were expressed as mean $\pm$ 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.

Results

Iron overloaded mice showed a significant increase in mean arterial blood pressure ($p<0.001$) when compared with normal control animals. Treatment with L1 partially restored the blood pressure. Interestingly, administrations of L1 together with THU attenuated the high blood pressure to near normal control values as shown in Figure 1.

Administration with iron sucrose impaired the vascular responses to different vasoactive agents, Phe, ACh, and SNP shown in Figure 2. These results indicate that iron L1 partly attenuated vascular dysfunction. Interestingly, L1 plus THU administration significantly restored the vascular responses to Phe (45.53 % vs. 27.15 %), ACh (40.23% vs. 24.56%) and SNP (29.01 % vs. 17.29 %) when compared with those found in iron overload-treated

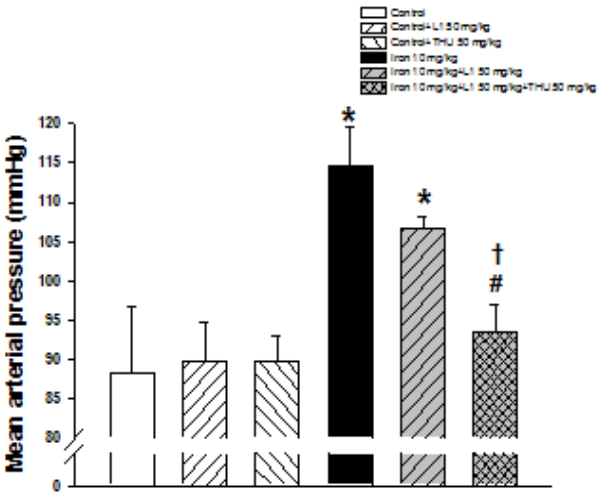

Figure 1 Effect of L1 and THU on arterial blood pressure in iron-treated mice. * p< 0.05 vs. control, # p< 0.05 vs.

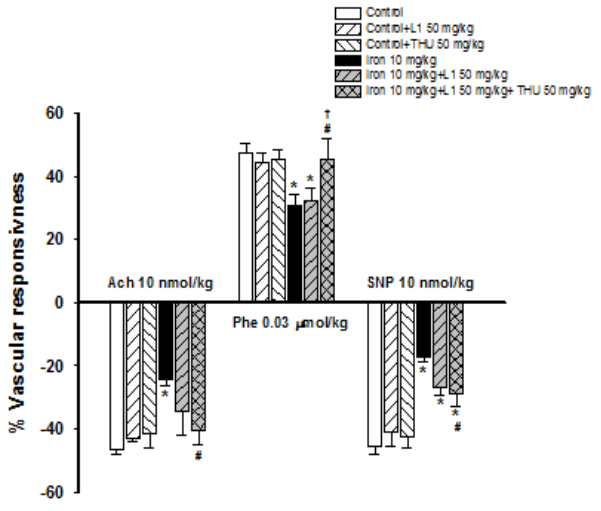

Figure 2 Effect of L1 and THU on vascular responsiveness in iron-treated mice. *P < 0.05 vs. control; # p< 0.05 vs. iron control; □ p< 0.05 vs. Iron with L1 50 mg/kg, n = 6/group

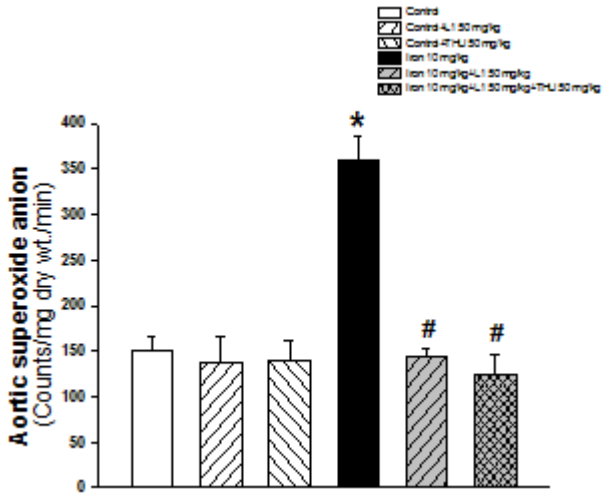

Figure 3 Effect of L1 and THU on $O_2^{\cdot}$ production of thoracic aorta in iron-treated mice. * p< 0.05 vs. control, n =

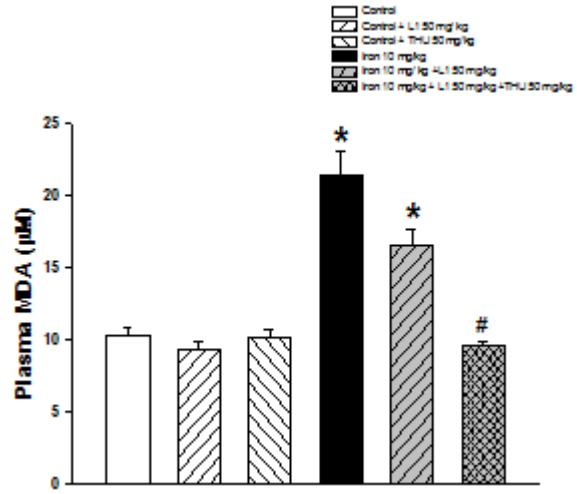

Figure 4 Effect of L1 and THU on plasma MDA in iron-treated mice. * p< 0.05 vs. control; # p< 0.05 vs. iron control, n = 6/group

L1 or L1 plus THU-treated mice reduced the rate of $O_2^{\cdot}$ production (p<0.05; Fig.3). A marked increase of

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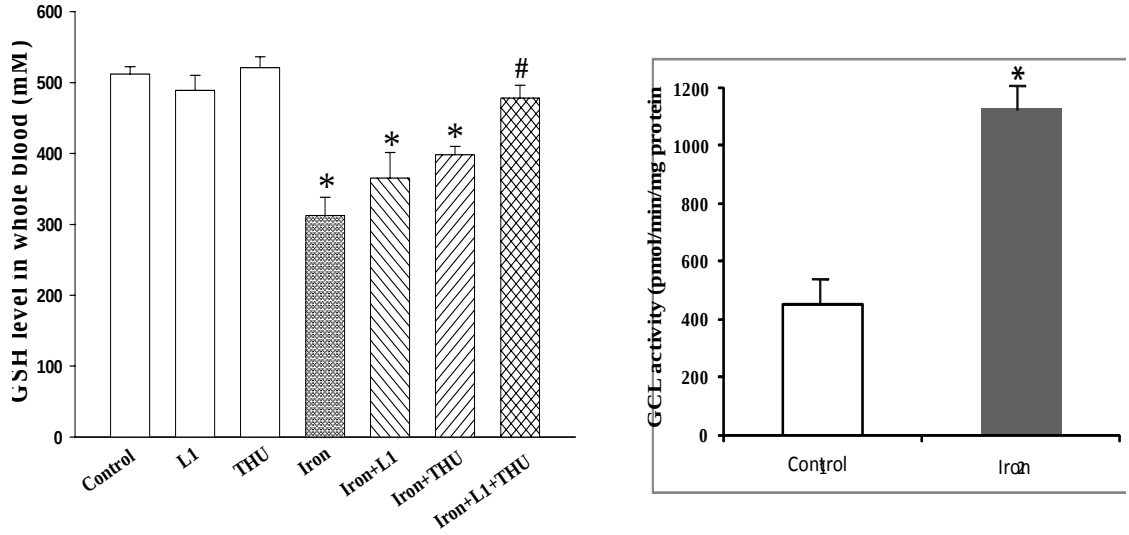

Figure 5 (A) Effect of L1 and THU on blood GSH in iron-treated mice. * p< 0.05 vs. control; # p< 0.05 vs. iron control, n= 6/group; (B) Iron overload induced GCL activity,* p< 0.05 vs. control

plasma MDA was also found in mice treated with iron sucrose. Interestingly, it was apparent that administration of L1 concurrent with THU significantly decreased plasma MDA to near normal controls (p<0.05; Fig. 4).

Apart from oxidative stress markers, we also investigated blood GSH, an antioxidant defense status, in mice treated with iron sucrose. It was found that blood GSH was dramatically reduced when compared with normal controls (Fig. 5). L1 plus THU partially prevented losses of blood GSH. These results suggested that combination of L1 and THU reduced the oxidant generated during development of iron overload. Moreover, GCL, the rate limiting enzyme of GSH was found that the activity of GSH was increased in iron overloaded mice (*p< 0.05 vs. control) indicating that decreasing GSH level may stimulate GCL activity.

Conclusion

Iron overload enhanced oxidative stress and vascular dysfunction. This stress condition was correlated with the elevation of arterial blood pressure, vascular superoxide production, plasma MDA and lowering the GSH levels. The mechanism of vascular dysfunction and subsequently hypertension might be resulted from ROS-suppressed NO activity. L1 or L1 plus THU alleviated these effects by restoring the blood pressure, decreasing vascular superoxide production, and reducing MDA levels. The overall findings of this study suggest the beneficial effects of L1 and THU on reducing vascular dysfunction and oxidative stress in the iron overload condition.

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References

1. Esposito BP, Breuer W, Sirankapracha P, Pootrakul P, Hershko C, Cabantchik ZI. Labile plasma iron in iron overload: redox activity and susceptibility to chelation. Blood 2003; 102:2670-7.

Proceeding

2. Li L, Smith A, Hagen TM, Frei B. Vascular oxidative stress and inflammation increase with age: ameliorating effects of alpha-lipoic acid supplementation. Ann N Y Acad Sci 2010; 1203:151-9.

3. Sajid R, Ghani F, Adil S, Khurshid M. Oral iron chelation therapy with deferiprone in patients with Thalassemia Major. J Pak Med Assoc 2009;59:388-90.

4. 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.

5. 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.