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Tetrahydrocurcumin in combination with deferiprone attenuates hypertension, vascular dysfunction, baroreflex dysfunction, and oxidative stress in iron-overloaded mice

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ABSTRACT

Excessive iron can generate reactive oxygen species (ROS), leading to oxidative stress that is closely associated with cardiovascular dysfunction. Iron overload was induced in male ICR mice by injection of iron sucrose (10 mg/kg/day) for eight weeks. Iron overload was evidenced by increased serum iron indices. The mice developed increased blood pressure, impaired vascular function and blunted response of the autonomic nervous system. These effects were accompanied by increased malondialdehyde levels in various tissues, increased nitric oxide metabolites in plasma and urine, and decreased blood glutathione. Tetrahydrocurcumin (THU, 50 mg/kg/day), deferiprone (or L1, 50 mg/kg/day) or both was orally administered throughout the period of iron sucrose injection. The treatments significantly alleviated the deleterious cardiovascular effects of iron overload, and were associated with modulation of nitric oxide levels. An imbalance between endothelial nitric oxide synthase (eNOS) and inducible NOS (iNOS) expression in response to iron overload was normalized by THU, L1 or the combination treatment. Moreover, the treatment decreased the upregulated expression levels of $gp91^{phox}$, $p47^{phox}$ and HO-1. The combination of THU and L1 exerted a greater effect than THU or L1 monotherapy. These results suggest beneficial effects of THU and L1 on iron-induced oxidative stress, hypertension, and vascular dysfunction.

1. Introduction

Iron, the most abundant transition metal in the human body, plays an essential role in many physiological processes, such as electron transfer and oxygen utilization reactions. Iron is a necessary component of hemoglobin, myoglobin, and enzymes. However, excessive iron accumulation in the body causes iron overload. This condition is often a secondary side effect of repeated blood transfusions [1] and hemochromatosis [2]. Iron overload induces several health problems including heart disease, liver cirrhosis, diabetes, neurodegenerative disorders, and cancer [3–5].

Iron in the circulation usually forms a complex with heme and non-heme proteins (ferritin and transferrin). In addition to these, another iron species, termed non-transferrin bound iron (NTBI), is found in the circulation and is thought to play a major role in various pathologic conditions that are characterized by iron overload [6]. Excess free iron has the capacity to generate reactive oxygen species (ROS) through the Fenton and Haber Weiss reactions, which are known to cause damage to various organs, including the liver and pancreas, as well as the cardiovascular system [4,7].

In vasculature, endothelial nitric oxide synthase (eNOS) generates endothelial nitric oxide (NO•), which plays a key role in regulation of blood pressure and maintenance of vascular homeostasis [8]. Pathologically, overproduction of superoxide anion ($O_2^{\bullet -}$) derived from increasing vascular NADPH oxidase activity can quench NO• directly via formation of peroxynitrite ($ONOO^-$) [9]. It has been suggested that upregulation of vascular NADPH oxidase not only enhances vascular oxidative stress locally but also depresses arterial baroreflex sensitivity [10].

Arterial baroreflex sensitivity is an important mechanism for blood pressure regulation [11]. Sympathetic overactivity and baroreflex

Abbreviations: ACh, acetylcholine; DBP, diastolic blood pressure; eNOS, endothelial nitric oxide synthase; GSH, glutathione; GSSG, Glutathione disulfide; HO-1, heme oxygenase-1; HR, heart rate; iNOS, inducible nitric oxide synthase; MDA, malondialdehyde; NADPH oxidase, nicotinamide adenine dinucleotide phosphate; NO•, nitric oxide; NTBI, non-transferrin bound iron; $ONOO^-$, peroxynitrite; Phe, phenylephrine; PP, pulse pressure; ROS, reactive oxygen species; SBP, systolic blood pressure; SNP, sodium nitroprusside.

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dysfunction play an important role in the development of hypertension and related cardiovascular disorders [12]. Upregulation of vascular NADPH oxidase not only enhances vascular oxidative stress locally but also suppresses arterial baroreflex sensitivity [10]. Therefore, disruption of the baroreflex may be associated with oxidant stress-induced hypertension. However, there is a lack of information about oxidative stress and baroreflex response in an in vivo model of iron overload.

It has been demonstrated that iron overload exerts deleterious effects mainly through generation of oxidative stress [1], suggesting that antioxidants may be used to treat iron overload either alone or in combination with an iron chelator. Iron chelators, including deferiprone and desferoxamine, have been tested successfully in removal of iron and are effective against iron overload related diseases [13]. Deferiprone or L1, a low molecular weight oral iron chelator, is widely used in patients with β-thalassemia major to prevent the deleterious effects of iron accumulation by decreasing body iron and NTBI [14]. However, L1 may exert adverse effects, especially agranulocytosis and thrombocytopenia [15]. Therefore, combined therapy of antioxidants and iron chelators might reduce the adverse effects of iron chelator and mitigate iron toxicity.

Tetrahydrocurcumin (THU) is derived from curcumin (*Curcuma longa* Linn) by hydrogenation. THU contains the same phenolic and β-diketo moieties as curcumin, but possesses stronger antioxidant activity than curcumin [16]. THU exhibits a variety of biological activities similar to curcumin, including antioxidant, anti-inflammatory, antidiabetic, antihypertensive, and metal chelating properties [17–20]. Interestingly, it has been reported that curcumin improves cardiac function in iron-loaded thalassemic mice [21]. However, the effect of THU against cardiovascular dysfunction in iron overload condition has not been investigated.

In this study, we used a model of iron sucrose-induced iron overload in experimental animals. The physiological and biochemical parameters associated with iron sucrose-induced iron overload, including hemodynamics, vascular reactivity, baroreflex sensitivity, and levels of oxidant and antioxidant status were examined with or without supplementation of THU, L1, or THU plus L1. Results of this study provide the first information about the antioxidant and chelating effects of THU under iron overload conditions.

2. Material and methods

2.1. Chemicals

THU and L1 were generously provided by the Government Pharmaceutical Organization, Bangkok, Thailand. Iron sucrose (Venofer®) was purchased from Vifor (International) Ltd., St. Gallen, Switzerland. Phenylephrine (Phe), acetylcholine chloride (ACh), and sodium nitropusside (SNP) were obtained from Fluka Chemika Co., Ltd., Buchs, Switzerland. β-actin was purchased from Sigma-Aldrich Co., Ltd., St. Louis, MO, USA. A mouse monoclonal anti-eNOS antibody was acquired from BD Biosciences, Qume Drive, San Jose, CA, USA. A rabbit-monoclonal anti-$p47^{phox}$, a mouse-monoclonal anti-$gp91^{phox}$, a goat anti-rabbit antibody, a mouse monoclonal anti-actin, and a goat anti-mouse antibody were obtained from Santa Cruz Biotechnology, UK. A rabbit polyclonal anti-iNOS antibody was purchased from Abcam, Cambridge, UK. A rabbit-monoclonal anti-HO-1 was obtained from Enzo life Science, Lausen, Schweiz, Switzerland. All chemicals used were of analytical grade quality.

2.2. Animal and treatment

Adult male ICR mice weighing 25–30 g were obtained from the National Laboratory Animal Center, Mahidol University, Nakornpathom, Thailand. Mice were housed at the Northeast Laboratory Animal Center (Khon Kaen University, Thailand) and maintained on a 12-h/light/dark cycle in a controlled temperature environment (23 ± 2 °C) with free access to water and standard rat chow (Chareon Pokapan Co., Ltd., Thailand). The study protocol was evaluated and approved by the Institutional Animal Ethics Committee of Khon Kaen University (AEKKU 62/2555).

Mice were randomly assigned to eight groups (10 animals per group) as follows: *group I* - Normal control + vehicle, *group II* - Normal control + L1 (50 mg/kg), *group III* - Normal control + THU (50 mg/kg), *group IV* - Normal control + L1 (50 mg/kg) + THU (50 mg/kg), *group V* - Iron control, *group VI* - Iron + L1 (50 mg/kg), *group VII* - Iron + THU (50 mg/kg), and *group VIII* - Iron + L1 (50 mg/kg) + THU (50 mg/kg). Chronic iron overload was induced in the iron-treated groups by intraperitoneal injection of iron sucrose at a dose of 10 mg/kg/day every other day for eight weeks. To induce iron-overload, we used a concentration of iron sucrose-induced corresponding to the minimum dose that induced oxidative stress and vascular dysfunction in a previous study [20]. L1 was administered at dose of 50 mg/kg. This lower dose of L1 is used in transfusion-dependent patients [22] and is effective based on our preliminary observation [20]. The dose of THU (50 mg/kg) was based on previous studies in animals exposed to metal-induced toxicity [23]. Mice from the normal control group were injected with saline solution. L1, THU, L1 + THU or vehicle (deionized water) was intragastrically administered throughout the period of iron sucrose treatment.

2.3. Assessment of hemodynamics

At the end of the treatment, mice were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (2.5 mg/kg). A tracheotomy was performed to facilitate respiration. Using a dissecting microscope, the right carotid artery was approached and subsequently cannulated with a polyethylene catheter connected with a pressure transducer for continuously monitoring arterial systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure (PP). Another polyethylene tube was inserted in the left jugular vein for drug injection. An electrocardiogram lead II was connected for determination of the heart rate (HR). The mean arterial pressure (MAP) and HR were analyzed by using the Acknowledge Data Acquisition System for the Windows Operating System (Biopac System Inc., CA, USA).

2.4. Assessment of vascular function and arterial baroreflex sensitivity

After obtaining baseline blood pressure recordings, the vascular responses were evaluated following a previously described method [24]. Briefly, mice were injected intravenously with a bolus of an endothelium-dependent vasodilator, acetylcholine (ACh, 10 nmol/kg); an endothelium-independent vasodilator, sodium nitroprusside (SNP, 10 nmol/kg), or an α_1-adrenergic receptor agonist, phenylephrine (Phe, 0.03 μmol/kg). Each drug was administrated after MAP and HR had returned to baseline values, resulting in approximately 15 min intervals between injection of drugs. Change in vascular responsiveness in response to vasoactive drugs was measured as the change in MAP from the baseline. The peak values of MAP and HR in response to Phe and SNP injections were used to evaluate the baroreflex response as described previously [25]. The maximal changes in MAP and HR were assessed and the baroreflex gain-coefficient was calculated by the following equation: Gain $= \Delta_{max}HR / \Delta_{max}MAP$, where $\Delta_{max}MAP$ is the maximal (peak response) change in MAP after injection of Phe or SNP; and $\Delta_{max}HR$ is the maximal change in HR due to the changes in MAP caused by the pharmacological treatment. The bradycardic or tachycardic peak after testing with Phe or SNP were used as an indication of parasympathetic or sympathetic component of the baroreflex gain.

2.5. Measurement of vascular $O_2^{\bullet -}$ production

The animals were euthanized with an overdose of an anesthetic drug. The level of vascular $O_2^{\bullet -}$ production was determined using the