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Iron-chelating and anti-lipid peroxidation properties of 1-(N-acetyl-6-aminohexyl)-3-hydroxy-2-methylpyridin-4-one (CM1) in long-term iron loading β-thalassemic mice

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PEER REVIEW

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Comments

This manuscript has evaluated that CM1 on the iron removal capacity and lipid peroxidation in long-term iron loaded β-thalassaemia mice before translating the compound to the β-thalassaemia patients, which is very common in southeast Asia. The results are interesting.
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ABSTRACT

Objective: To evaluate the iron-chelating properties and free-radical scavenging activities of 1-(N-acetyl-6-aminohexyl)-3-hydroxy-2-methylpyridin-4-one (CM1) treatment in chronic iron-loaded β-thalassemic (BKO) mice.

Methods: The BKO mice were fed with a ferrocene-rich diet and were orally administered with CM1 [50 mg/(kg·day)] for 6 months. Blood levels of non-transferrin bound iron, labile plasma iron, ferritin (Ft) and malondialdehyde were determined.

Results: The BKO mice were fed with an iron diet for 8 months which resulted in iron overload. Interestingly, the mice showed a decrease in the non-transferrin bound iron, labile plasma iron and malondialdehyde levels, but not the Ft levels after continuous CM1 treatment.

Conclusions: CM1 could be an effective oral iron chelator that can reduce iron overload and lipid peroxidation in chronic iron overload β-thalassemic mice.

KEYWORDS

Iron-chelating, Iron overload, β-thalassemia, Iron chelator, Non-transferrin bound iron, Lipid peroxidation

1. Introduction

Iron is a component of many metalloproteins and plays a crucial role in a range of vital biochemical activities, such as oxygen sensing and transport, electron transfer, and catalysis[1]. When present in excess, cellular iron overload leads to toxicity and cell death via free radical formation and lipid peroxidation[2]. Non-transferrin bound iron (NTBI),

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and labile plasma iron (LPI) are toxic forms of the iron that appear in plasma when the transferrin saturation increases. Changes in the labile iron pool (LIP) can be considered a cytosolic equivalent of plasma NTBI influence on intracellular ferritin (Ft) levels[3]. Thus, elevated levels of the LIP lead to an increased accumulation of Ft iron and in extreme cases to the formation of hemosiderin[4]. Iron chelation therapy is required to prevent iron-mediated injury to cells and to reduce the levels of NTBI, LPI, LIP and plasma Ft[5,6].

At present, the treatment of iron overload diseases especially in β-thalassemia patients commonly involves the administration of deferiprone (DFP), desferioxamine (DFO) and deferasirox (DFX)[7-9]. Effectiveness, cost, compliance, quality of life and side effects of the chelators are all relevant considerations. Many adverse effects of these chelators include: nausea, vomiting, gastrointestinal tract disturbance, leukocytopenia, thrombocytopenia, arthopathy, zinc deficiency and agranulocytosis from DFP, skin redness, local irritation, mild pain at the applied sites from DFO, renal toxicity, Fanconi syndrome, formation of rashes and gastrointestinal tract disturbance from DFX[10].

We have been studying the properties of a specific novel orally active iron chelator, 1-(N-acetyl-6-aminohexyl)-3-hydroxy-2-methylpyridin-4-one (CM1). Our previous studies have illustrated that the CM1 (MW=256, K_{part}=0.53) is an effective bidentate chelator and is slightly more lipophilic than the DFP (MW=139, K_{part}=0.11)[11]. Preliminary results have established that CM1 is relative non-toxic in acute studies and can reduce the levels of malondialdehyde (MDA), LIP and reactive oxygen species in both mouse primary hepatocytes and human hepatocellular carcinoma (HepG2) cells[12,13]. Furthermore, CM1 was found not to be toxic to the peripheral blood mononuclear cells and liver cells of β-thalassemia mice under normal and iron overload conditions after 240 d exposure[14]. Srichairatanakool *et al*.[15] reported that CM1 removed excess iron in the blood compartment and tissues of iron loaded wild type C57BL/6 mice. These preliminary studies have now been extended to include β-thalassemic mice.

2. Materials and methods

2.1. Animals

The heterozygous β-thalassemia knockout (BKO, $^{mu}\beta^{th-3/+}$) mice strain C57BL/6 aged between 6-10 weeks and having a body weight (20±5) g were kindly supplied by the Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Thailand[16]. The animals were housed in polyethylene cages and maintained in a clean air-conditioned room under the controlled conditions of 12-h day/12-h night cycle at (25±3) °C and at 40%-70% humidity. The study protocol that was used has been approved by the Animal Ethical Committee of the Medical Faculty, Chiang Mai University, Thailand (Reference Number -3/2554).

2.2. Iron overload in mice and chelation treatment

The mice were fed a normal pellet diet (N diet) and an N diet supplemented with 0.2% (w/w) ferrocene (Fe diet) to induce iron overload, over 240 d[17]. The iron-loaded mice were randomly subdivided into 5 groups. The study group was fed with the Fe diet along with treatments of deionized water placebo, DFP [50 mg/(kg·day)] and CM1 [50 and 100 mg/(kg·day)] orally for 180 d (5 mice in each group)[18]. The control group was fed with N diet throughout the study. Blood samples were collected from the tail vein and collected into Na-heparin tubes. Plasma was separated immediately and kept frozen -20 °C for further analysis.

2.3. Quantification of plasma NTBI

Plasma NTBI was quantified based on nitrilotriacetic acid (NTA) chelation/HPLC technique with slight modifications[19]. Briefly, plasma was incubated with a weak chelator; NTA solution (80 mmol/L, at final concentration, pH 7.0) for 30 min at room temperature to produce the Fe^{3+}-$(NTA)_2$ complex from NTBI. Subsequently, the complex was filtered through a membrane (Nano-Sep®, 10-kDa cutoff, polysulfone type; Pall Life Sciences, Ann Arbor, MI, USA) at 12 000 r/min for 60 min and analyzed using a non-metallic HPLC system. NTBI was fractionated on a glass analytical column (ChromSep-ODS1, 100 mm×3.0 mm, 5 μm), eluted with a mobile phase solvent (3 mmol/L CP22 in 20% acetonitrile/MOPS pH 7.0) at a flow rate of 1.0 mL/min and the optical density (OD) was monitored at 450 nm using a flow cell detector (SpecMonitor 2300; LDC Milton-Roy Inc., Riviera Beach, FL, USA). Data analysis was conducted with BDS software (BarSpec Ltd., Rehovot, Israel). NTBI concentration represented by Fe^{3+}-$(CP22)_3$ peak area was calculated with a calibration curve constructed from Fe^{3+}-$(NTA)_2$ in 80 mmol/L NTA (0-32 μmol/L).

2.4. Quantification of LPI

In principle, redox-active LPI can convert non-fluorescent dihydrorhodamine (DHR) to oxidized form rhodamine (R), resulting in an increase of fluorescence intensity (FI)[20]. In the assay, plasma was incubated with/without 5 mmol/L DFP at 37 °C for 30 min and then the DHR solution containing ascorbic acid was added. Kinetics of increasing FI was followed immediately for 40 min, with readings every 2 min at 37 °C using a 96-well plate spectrofluorometer ($\lambda_{excitation}$ 485 nm, $\lambda_{emission}$ 538 nm). The slope of the FI was plotted against a reaction time of between 15-40 min. A calibration curve was constructed from the standard ferrous ammonium sulfate solution (0-20 μmol/L). Difference in rate of DHR oxidation represents a component of redox active LPI. The