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Iron distribution and histopathological study of the effects of deferoxamine and deferiprone in the kidneys of iron overloaded β-thalassemic mice

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ABSTRACT

Renal glomerular and tubular dysfunctions have been reported with high prevalence in β-thalassemia. Iron toxicity is implicated in the kidney damage, which may be reversed by iron chelation therapy. To mimic heavy iron overload and evaluate the efficacy of iron chelators in the patients, iron dextran (180 mg iron/mouse) was intraperitoneally (i.p.) injected in heterozygous β-globin knockout mice ($^{mu\beta th-3/+}$, BKO) and wild type mice (C57BL/6J, WT) over a period of 2 weeks, followed by daily i.p. injection of deferoxamine (DFO) or deferiprone (L1) for 1 week. In BKO mice, iron preferentially accumulated in the proximal tubule with a grading score of 0–1 and increased to grade 3 after iron loading. In contrast, iron mainly deposited in the glomerulus and interstitial space in iron overloaded WT mice. Increased levels of kidney lipid peroxidation, glomerular and medullar damage and fibrosis in iron overloaded mice were reversed by treatment with iron chelators. L1 showed higher efficacy than DFO in reduction of glomerular iron, which was supported by a significantly decreased the amount of glomerular damage. Notably, DFO and L1 demonstrated a distinct pattern of iron distribution in the proximal tubule of BKO mice. In conclusion, chelation therapy has beneficial effects in iron-overloaded kidneys. However, the defect of kidney iron metabolism in thalassemia may be a determining factor of the treatment outcome in individual patients.

1. Introduction

Thalassemia is an inhertited hemoglobinopathy that results in mild to severe anemia. As a consequence of frequent blood transfusions and increased gut iron absorption secondary to ineffective erythropoiesis, patients with β-thalassemia develop iron overload that is subsequently implicated in multi-organ dysfunction, including kidney dysfunction.

Renal glomerular and tubular functional abnormalities have been reported in thalassemia major and intermedia. Hyperfiltration, increased creatinine clearance and albuminuria are noted in several studies (see review Bhandari and Galanello, 2012; Ponticelli et al., 2010; Cetin et al., 2003). Increased urinary excretion of N-acetyl-D-glucosaminidase (NAG) and low molecular weight protein as biomarkers of proximal tubular damage as well as malondialdehyde (MDA) as an oxidative marker of membrane lipids by peroxidation have been reported with high prevalence in these patients (Mohkam et al., 2008; Koliakos et al., 2003; Sumboonnanonda et al., 1998). In addition to anemia and hypoxia, abnormalities of renal tubular function were implicated in iron overload because histopathological studies of post-mortem kidney samples of β-thalassemia demonstrated that hemosiderin primarily deposits in tubular systems (Landing et al., 1989; Sonakul et al., 1988).

Iron chelation therapy is necessary to prevent fatal complications due to iron toxicity. Long term use of iron chelators has been proven to reduce iron burden and thus improve quality of life and

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the life span of the patients (Telfer, 2009; Borgna-Pignatti et al., 2005). Currently, there are three clinically available iron chelators: deferoxamine (DFO), deferasirox and deferiprone (L1), which are hexadentate, tridentate and bidentate iron chelators, respectively. The efficacy of iron mobilization from the organs, especially the liver and heart, has been extensively investigated in several models. The renal system is vital for homeostasis of acid-base and electrolyte balance as well as cardio-vascular and hormonal systems. It is also an important route of xenobiotic excretion. Therefore, evaluation of the effect of iron chelators in this organ is important to improve chelation therapy and prevent toxicity from iron chelators.

Recently, heterozygous β-knockout mice ($^{mu\beta th-3/+,}$ BKO) were generated to use as an animal model of β-thalassemia intermedia (Jamsai et al., 2005; Yang et al., 1995). Although the degree of iron accumulation in BKO mice is lower than in patients, various iron loading protocols were successful at mimicking iron overload in thalassemia (Yatmark et al., 2014; Kulprachakarn et al., 2014). The response to iron overload included increased extramedullary erythropoiesis and decreased hepcidin synthesis in BKO mice that was comparable to the response in patients (Nemeth et al., 2004). Recently, the BKO mouse model has been used to study pathology and evaluate novel therapeutic interventions such as gene therapy and antioxidant therapy (Kulprachakarn et al., 2014; Thongchote et al., 2014; Breda et al., 2012; Kumfu et al., 2010; Thephinlap et al., 2009).

Because DFO and L1 are commonly used for iron chelation therapy as either single or combination therapy, this study aimed to investigate the effect of both chelators on iron distribution and oxidative damage in the kidneys of iron-overloaded wild type (WT) and β-thalassemic (BKO) mice. The information on their distinctive effect may help to design chelation therapy for individual patients with different severities of kidney complications.

2. Materials and methods

2.1. Experimental animals

Male and female wild-type C57BL/6J mice (WT) and heterozygous β-globin knockout mice ($\beta^{th-3/+,}$ BKO) at 7 weeks of age (weight 17–25 g) were obtained from the Institute of Science and Technology for Research and Development, Mahidol University, Thailand. The animals were acclimatized for 1 week before the experiments and were housed under conventional sterile conditions. The rodent diet (082G/15) and water were provided ad libitum. The temperature and humidity were maintained at 25 ± 2 °C and 60 ± 5%, respectively, and the animals were kept on a 12 h light/dark cycle. These experimental protocols were approved by the Animal Ethics Committee at the Faculty of Science, Mahidol University of Thailand (Protocol No. 229).

2.2. Iron overloading and treatment with iron chelators

WT and BKO mice were divided into 4 groups each (5 mice in a group, both male and female): control saline treatment and iron overload with or without treatment of iron chelators. Iron overloading was performed by intraperitoneal injection of iron dextran (Sigma, St. Louis, MO) once daily at a dose of 20 mg iron/mouse for 9 doses over a period of 2 weeks for a total iron administration of 180 mg/mouse (Moon et al., 2011). In the iron chelator-treated groups, 3 days after receiving their last intraperitoneal dose of iron, mice were injected with equimolar concentration of iron binding either 0.2 μmol (125 mg)/g body weight deferoxamine (DFO, deferoxaminemesylate, Merck, San Diego, CA) or 0.6 μmol (80 mg)/g body weight deferiprone (L1, The Government Pharmaceutical Organization, Bangkok, Thailand) for 7 days. The mice were sacrificed 24 h after iron chelation by exsanguination from the heart and were perfused with 10 ml saline before removing the kidney to determine the levels of non-heme iron content using a modification of the method of Foy et al. (1967) described by Yatmark et al. (2014). For histopathological studies, the kidney was removed without perfusion. Control mice received placebo treatment with saline on the same schedule as the iron-overloaded groups.

2.3. Histopathological studies

The kidneys were dissected and preserved for routine histology by fixation in 4% paraformaldehyde, 5% sucrose and 150 mM PBS at pH 7.4. The fixed samples were embedded in paraffin, sectioned to 5 μm, and stained with Perl's Prussian blue for iron deposition, hematoxylin/eosin for morphological analysis and glomerular damage, and Masson's trichrome for evaluation of renal fibrosis (Ikeda et al., 2014). The tissue slides examined at least five non-overlapping areas by a Nikon ECLIPSE E200 light microscope (Tokyo, Japan).

The proximal tubule iron accumulation was graded using the following criteria adapted from Crissman et al. (2004): grade 0 was negative staining; grade 1 was partially diffused in the proximal tubules; grade 2 was diffused in interstitial space, glomerulus, and proximal tubules; and grade 3 was large clumps of all areas of interstitial space, glomerulus, and proximal tubules.

Glomerular damage was identified by glomerular hypertrophy, tubular atrophy, and glomerulosclerosis (Sugimoto et al., 2007). The damaged glomerular was presented as percentage of total glomerular counted from 5 non-overlapping areas of each mice.

Table 1
Kidney weight, iron content, and iron accumulation grading score of wild type and β-knockout mice.

Group	Kidney weight (g)	Kidney iron content (mg/g tissue)	Iron deposition score
Wild type			
Control	0.35 ± 0.10^{a}	$0.02 \pm 0.01^{b,d}$	$0.0 \pm 0.0^{e,g}$
Iron overload	0.27 ± 0.07^{a}	1.16 ± 0.24^{b}	$2.5 \pm 0.3^{e,h,i}$
Iron overload + DFO	0.28 ± 0.06	0.98 ± 0.30	2.0 ± 0.1^{h}
Iron overload + L1	0.26 ± 0.06	1.11 ± 0.21	2.2 ± 0.2^{i}
β-Knockout			
Control	0.30 ± 0.09	$0.07 \pm 0.01^{c,d}$	$0.6 \pm 0.2^{f,g}$
Iron overload	0.28 ± 0.05	1.27 ± 0.70^{c}	$3.0 \pm 0.0^{f,i,k}$
Iron overload + DFO	0.23 ± 0.07	0.86 ± 0.26	2.1 ± 0.2^{j}
Iron overload + L1	0.25 ± 0.08	1.00 ± 0.30	2.3 ± 0.1^{k}

Values are mean ± SD (n = 5).
Significant differences were denoted by the same letter, $p < 0.05$.