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Effects of iron overload condition on liver toxicity and hepcidin/ferroportin expression in thalassemic mice

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ABSTRACT

Aims: Although iron-overload conditions can be found in β-thalassemic patients, resulting in cellular damage, particularly in the liver, the mechanism for this iron-mediated hepatic injury specifically in β-thalassemic (HT) mice is unclear. This study aimed to investigate the roles of L-type calcium channels (LTCC), T-type calcium channels (TTCC) and divalent metal transporter1 (DMT1) in iron-mediated hepatic injury in HT mice.
Main methods: Iron chelator deferoxamine (DFO), LTCC blocker, TTCC blocker and DMT1 blocker were used to determine the roles of these channels regarding liver iron accumulation, apoptosis and iron regulatory protein expression in HT mice.
Key findings: TTCC and DMT1 blockers and DFO decreased liver iron and malondialdehyde (MDA) in HT mice indicating their antioxidant effects, whereas LTCC blocker produced no decrease in liver iron or MDA. However, only DFO decreased liver apoptosis through the reduced Bax/Bcl-2 ratio in wild type (WT) mice. The levels of iron regulatory hormone hepcidin were markedly higher in HT mice even before iron loading while ferroportin levels did not alter. Each of the pharmacological interventions increased ferroportin protein back to normal levels only in WT while HT mice showed no difference.
Significance: Thalassemic mice have different hepcidin/ferroportin and apoptotic protein expression as a defense mechanism to iron-overload compared with those in WT mice. DFO was the most effective intervention in preventing liver apoptosis under iron-overload conditions in WT but did not have the same effect in HT mice.

1. Introduction

One of the most common complications found in thalassemia patients is iron-overload conditions that results in the accumulation of iron in many organs, particularly in the liver which is the main storage site for iron in body, leading to abnormal function in those organs [1–5]. Iron-overload conditions can lead to increased reactive oxygen species (ROS) production, which can damage cellular lipids, proteins, DNA and mitochondria [4–8]. Evidence has demonstrated that iron-overload is associated with an increased risk of liver complications including fibrosis, cirrhosis and hepatocellular carcinoma [4,7,8]. Previous studies demonstrated that hepatocyte apoptosis promotes liver fibrosis [9,10]. Several studies have demonstrated that caspase-3 plays a central role in cellular apoptosis [11,12]. Caspase-3 is activated by a group of signaling cascades, among which the interaction of anti-apoptotic Bcl-2 and pro-apoptotic Bax proteins play a critical role [12–14]. Previous studies have demonstrated that iron-overload conditions can lead to increased hepatocyte apoptosis due to an increased Bax/Bcl-2 ratio and caspase-3 activity [15,16]. However, the mechanism underlying hepatocyte apoptosis in thalassemic mice has never been investigated.

Previous studies demonstrated that the L-type calcium channels (LTCC), T-type calcium channels (TTCC) and divalent metal transporter1 (DMT1) were expressed in liver tissues [17–20]. Although DMT1 is known to be a common pathway for iron uptake in hepatocytes [18,21], under conditions of iron-overload the mRNA and subsequent protein expression of DMT1 in liver were suppressed [18,22]. Recently, previous study demonstrated that DMT1, LTCC, and TTCC played important roles regarding iron entry in thalassemic heart under iron-overload conditions [23]. However, the roles of TTCC, LTCC and DMT1 on iron uptake and apoptosis in hepatocytes under iron-overload conditions in thalassemic mice have never been investigated. Moreover, recent studies have demonstrated that Zinc transporter 14 (Zip14) could be a novel portal for cellular iron uptake in liver [24,25]. However, the expression of liver Zip14 protein in thalassemic mice is still unknown.

Hepcidin is a 25-amino acid peptide hormone which mainly synthesized in liver [26,27]. Under conditions of iron overload and

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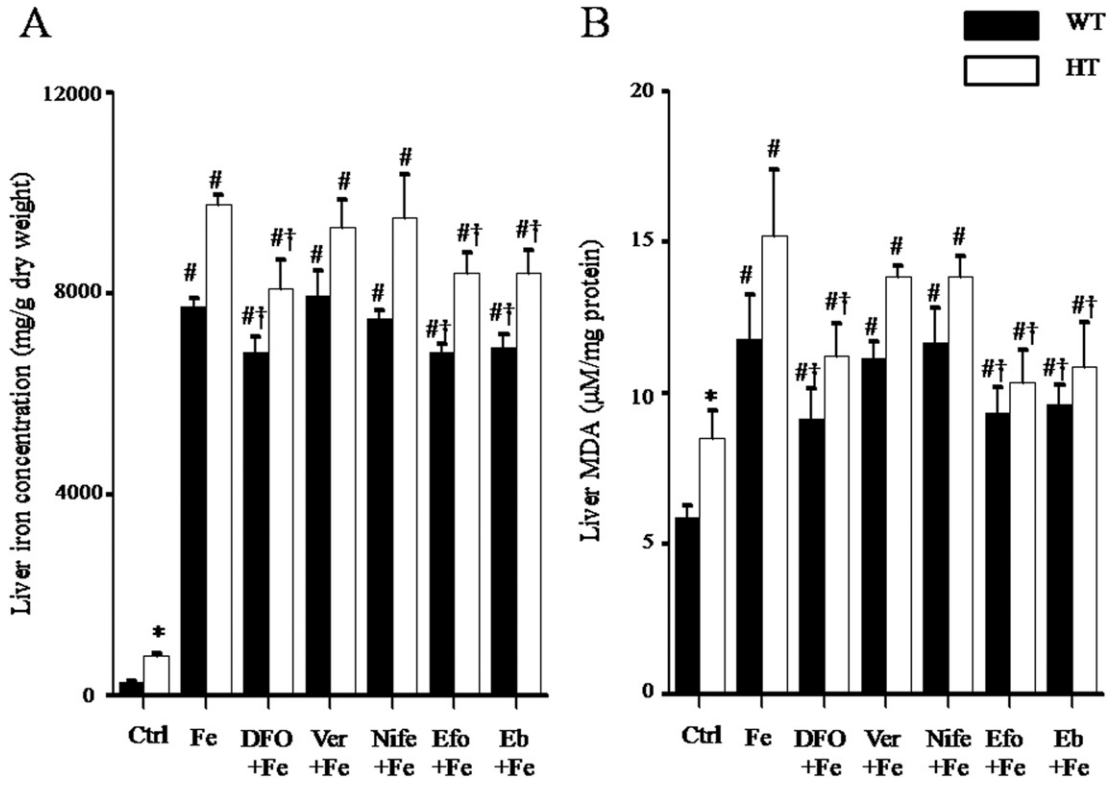

Fig. 1. Effects of pharmacological intervention on iron concentration in the liver (A) and liver malondialdehyde (B) in wild-type (WT) and thalassemic mice (HT) ($n = 6$/group). $^{*}P < 0.05$ vs. WT, $^{\#}P < 0.05$ vs. control, $^{\dagger}P < 0.05$ vs. FE.

inflammation, hepcidin level is high resulting in the reduction of serum iron due to iron being trapped within macrophages and liver cells, and also decreased gut iron absorption [26,27]. Hepcidin controls iron levels by interacting directly with iron exporter ferroportin (FPN), resulting in internalization and degradation of FPN when iron levels are high [26, 27]. However, the expression of liver hepcidin and ferroportin protein in thalassemic mice, as well as the alteration of these proteins under conditions of iron overload, are still unknown. Investigation into and clarification regarding these novel findings could lead to better treatment and prevention strategies for iron overload induced liver toxicity in thalassemia patients in very near future.

Therefore, in this study, the hypotheses tested are that: (1) thalassemic mice display more severe liver iron-overload, and altered expressions of Zip14, hepcidin and ferroportin, and (2) these alterations can be attenuated by administration of iron chelator and blockers of LTCC, TTCC and DMT1 in thalassemic mice.

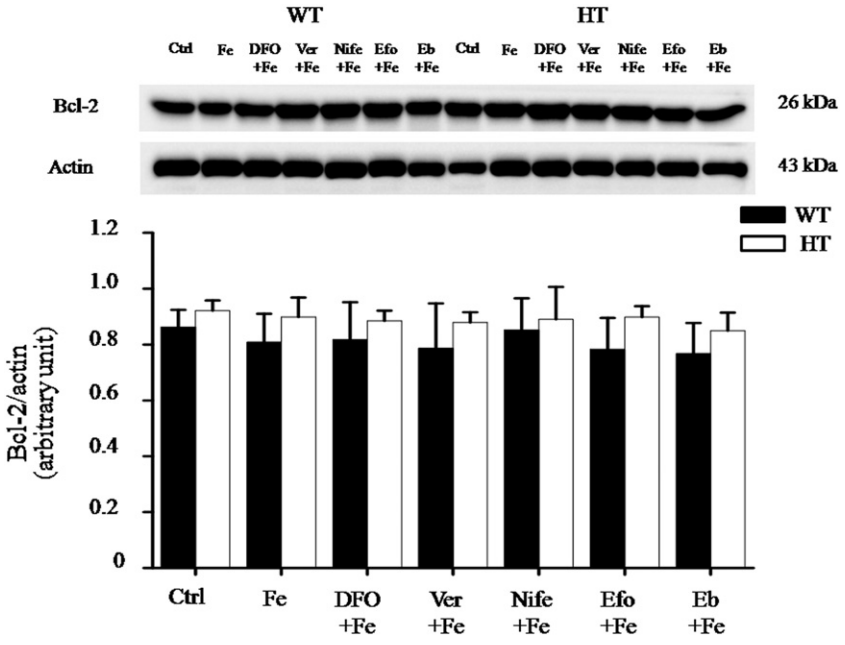

Fig. 2. Effects of pharmacological intervention on *anti*-apoptotic Bcl-2 protein expression in liver tissue in wild-type (WT) and thalassemic mice (HT) ($n = 6$/group). Loading control; actin.