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Toxicity Study of a Novel Oral Iron Chelator: 1-(N-Acetyl-6-Aminohexyl)-3 Hydroxy-2-Methylpyridin-4-One (CM1) in Transgenic β-Thalassemia Mice

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Abstract

Deferiprone (DFP) (MW=139 Da, Kpart=0.11) is an effective iron chelator used for the treatment of iron overload in thalassemia patients, but the drug is not free from side effects. We have synthesized a novel oral bidentate iron chelator, 1-(N-acetyl-6-aminohexyl)-3-hydroxypyridin-4-one (CM1) (MW=256 Da, Kpart=0.53), which is an analogue of DFP. This compound is more lipophilic than DFP and can bind iron efficiently. Our current results have demonstrated that CM1 reduced iron-induced redox damage and decreased levels of the intracellular iron pool (LIP) in cultured hepatocytes, effectively. However, the toxicity of CM1 remains largely unknown. The aim of this study was to therefore examine the toxicity of CM1 treatment in an animal model under normal and iron overload conditions. To induce iron overload, transgenic ß-thalassemia (BKO) mice were fed with a 0.2% (w/w) ferrocene-supplemented diet (Fe diet) for 240 days. The mice received three doses of CM1 orally (50,100 and 200 mg/kg), every day for 180 days. Blood was collected from the tail vein every 45 days during treatment for the measurement of hemoglobin (Hb) levels, white blood cells (WBC) and platelet numbers. We also determined the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), which are markers of liver damage. Treatment with CM1 at the assigned doses did not markedly alter the numbers of WBC and the platelets, and the Hb level in BKO mice fed with either N diet or Fe diet. Importantly, all the treatments slightly increased the activities of plasma AST, ALT and ALP in BKO mice after 150 days. Nonetheless, hematoxylin and eosin staining results did not show abnormal morphological changes of the spleen, liver and heart tissues. The results imply that CM1 may not be toxic to bone marrow cells and liver cell function in BKO mice under normal and iron overload conditions.

Keywords: 3-Hydroxypyridinone; Iron chelator; Iron overload; Toxicity; ß-thalassemia

Introduction

Iron is absolutely essential to living cells and plays many important roles in energy production, oxygen transport and DNA synthesis. Although iron is crucial for cells, an excess of iron is toxic [1]. In thalassemia patients, multiple blood transfusions and abnormal iron absorption cause iron overload and accumulation in tissues and organs [2]. Consequently, reactive oxygen species (ROS) are produced from the iron-catalyzed Fenton reaction, causing oxidative tissue damage and organ dysfunctions [3,4]. Iron chelation therapy is required, not just to restore iron balance, but also to reduce toxic iron species. Iron chelators, such as desferrioxamine (DFO), deferiprone (DFP) and deferasirox (DFX), are available for clinical use, but these compounds still have many side effects and drawbacks. DFO is not orally active [5], DFX is associated with renal toxicity in some patients [6], and DFP can have side effects, including nausea, vomiting, gastrointestinal tract disturbances, leucopenia, thrombocytopenia and zinc deficiency [7]. We have successfully synthesized a novel oral bidentate iron chelator, 1-(N-acetyl-6-aminohexyl)-3-hydroxypyridin-4-one (CM1). It is DFP analogue and is more lipophilic than DFP. In a previous study, we found that CM1 was able to bind both ferric and ferrous ions, and was also able to chelate plasma non-transferrin bound iron (NTBI), efficiently [8]. Moreover, we found that the CM1 was not toxic to peripheral blood mononuclear cells and liver cells in an *in vitro* study [9]. However, the toxicity of CM1 in the *in vivo* study remains largely unknown. In this study, we examined the toxicity of CM1 on the liver and peripheral blood cells in transgenic ß-thalassemia mice under normal and iron overload conditions.

Materials and Methods

In the experimental design of this study, the heterozygous ß-thalassemia knockout (BKO, mu?thal-3/+) mice strain C57BL/6, as well as the weight 20 ± 5 g, were recorded. During the entire study period, the mice were housed in hygienic cages in a controlled environment (12 hours light/dark cycle and 25 ± 3°C). The mice were randomized into 2 groups (40 males and 40 females for each group): one group were fed a normal pellet diet (N diet), and the other group were fed a 0.2% (w/w) ferrocene-supplemented pellet diet (Fe diet) to induce iron overload, over 240 days [10]. Both groups of the mice were then segregated as follows: ten mice from each group received the deionized water (DI) placebo, and thirty mice from each group received 1-(N-acetyl-6-aminohexyl)-3 hydroxypyridin-4-one (CM1), and were synthesized by our group [11]. The mice received CM1 at the doses of 50, 100 and 200 mg/kg (10 mice each) orally for 180 days. Clinical observations were performed daily and body weight was recorded weekly. Tail vein blood was collected for prior to beginning the study, at 60 days, and at every 45 days during treatment. The samples were investigated for the hematological and biochemical parameters. Additionally, four organs of the mice, including the heart, liver, kidneys and spleen were collected after termination to perform the pathological examination.

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Hematological parameters

The samples were drawn into a heparin-containing capillary tube. Whole blood was taken to measure hemoglobin concentrations following the cyanmethemoglobin method [12]. White blood cells and platelet numbers were diluted with WBC (1:50) and Platelet (1:200) diluting solution (Biotech Co. Ltd., Bangkok, Thailand), respectively, and were counted with a hematocytometer under microscopy.

Biochemical parameters

Blood samples were centrifuged at 3,000 g for 10 min to separate the plasma. Biochemical parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities, were assessed using enzyme assay kits (Biotech Co. Ltd., Bangkok, Thailand) [13,14].

Pathological examination

At the termination of the study, all groups were weighed and sacrificed for the collection of their organs, including the heart, liver, spleen and kidneys. After that each organ was weighed, parts of the heart, liver and spleen were fixed in 10% neutral buffered formalin. Then, the tissues were dehydrated with a graded series of ethyl alcohol, embedded in paraffin wax and finally sectioned and stained with Hematoxylin & Eosin (H&E) dye. The stained slides were examined under a light microscope by an expert pathologist, and were then photographed with a digital camera.

Statistical analysis

The results were expressed as Means + SEM. Statistical significance was determined using a one-way analysis of variance (ANOVA), in which $p<0.05$ was considered significant.

Results

Clinical observations and body weight

No clinical sign of toxicity was observed in the ß-thalassemia mice that were fed with N and Fe diets at all doses of CM1, over 240 days. Body weight was not different in the placebo and the CM1 treated groups, however, it was slightly increased in all groups of mice, and body weight was similar in both the N and Fe fed groups.

Impact of CM1 on haematological parameters

In the previous *in vitro* study, we found that CM1 is an effective iron chelator that is non-toxic to peripheral blood mononuclear cells (PBMC). However, the toxicity of CM1 in the animal model is unknown. To investigate whether or not CM1 has toxicity to the peripheral cells in the animal study; ß-thalassemia mice were fed with an N diet or Fe diet, and the dietary routine was then intervened with DI water for the placebo group and CM1 at 50, 100 and 200 mg/kg for the test groups. The results showed that haemoglobin levels were not different between the placebo, and/or normally treated mice (Table 1), and the iron-loaded groups (Table 2). That could imply that the type of food and CM1 concentrations were not affected over the 180-day period. We also found that the chelator did not produce a drop in numbers of the WBC and the platelets (Table 1 and 3), there was no difference between the placebo and the treated groups and results were similar among the mice fed with both the N and Fe diets. The experimental stage in this study demonstrated that CM1 was not toxic to peripheral blood cells under normal and iron-overload conditions.

Effect of CM1 on biochemical parameters

To investigate whether CM1 damaged hepatocyte cells in the *in vivo* study, we examined marker enzymes for liver damage, including AST, ALT and ALP using enzymes test kits based on spectrophotometric analysis. The results showed that all of the enzymes in both the placebo and the treated mice were not found to be significantly different, under both dietary conditions. There were also no dose-related effects. In both the normal diet and iron rich consumption groups, AST was slightly increased after 60 days and reached a peak at 150 days, before dropping 45 days later (Table 4). However, the trend was not shown in the ALT and ALP measurements. ALT and ALP slightly increased after 60 days. Nevertheless, these changes were still not dose-dependent (Table 2 and 4).

Organ weights, organ/body weight ratio and histopathological examination

After the termination of the study, the mice were collected and

	Hemoglobin concentration (g/dl)				WBC numbers (109/l)				Platelet numbers (109/l)			
	Treatment				Treatment				Treatment			
Days	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)
0	10.25 ± 0.89	11.35 ± 1.28	10.73 ± 0.36	10.05 ± 0.42	7.89 ± 2.30	4.65 ± 1.90	5.83 ± 1.38	3.43 ± 0.68	360.0 ± 39.8	422.0 ± 62.2	385.6 ± 43.4	411.2 ± 40.0
60	10.79 ± 0.50	12.29 ± 0.90	10.82 ± 0.38	10.66 ± 0.26	7.57 ± 1.77	6.67 ± 2.72	6.58 ± 1.67	6.07 ± 1.79	425.6 ± 42.2	424.0 ± 43.5	376.0 ± 18.4	448.8 ± 52.5
105	11.02 ± 0.56	11.53 ± 0.87	10.73 ± 0.35	10.33 ± 0.39	7.60 ± 0.86	4.98 ± 2.03	7.56 ± 1.48	8.57 ± 2.20	249.5 ± 88.5	472.0 ± 145.2	248.0 ± 69.2	286.4 ± 73.6
150	10.14 ± 0.44	9.82 ± 0.65	9.05 ± 1.18	10.92 ± 0.67	8.62 ± 2.14	4.74 ± 2.12	10.54 ± 1.90	11.10 ± 3.60	445.0 ± 199.9	322.4 ± 51.3	265.5 ± 72.4	516.8 ± 265.0
195	9.34 ± 0.87	10.39 ± 0.55	11.10 ± 1.30	10.20 ± 1.11	12.7 ± 2.64	5.63 ± 2.52	11.15 ± 1.44	14.42 ± 2.96	609.0 ± 302.1	534.0 ± 189.8	416.5 ± 126.3	486.5 ± 281.6
240	7.95 ± 0.51	9.10 ± 0.64	8.56 ± 0.61	8.94 ± 0.72	6.25 ± 0.88	2.58 ± 1.15	7.05 ± 1.56	7.33 ± 1.74	375.5 ± 78.2	433.2 ± 84.3	373.5 ± 98.4	490.6 ± 254.7

Table 1: Effect of CM1 treatment on hematological parameters in β-thalassemia mice (BKO) fed with N diet. Data were expressed as mean + SEM.

	Hemoglobin concentration (g/dl)				WBC numbers (109/l)				Platelet numbers (109/l)			
	Treatment				Treatment				Treatment			
Days	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)
0	10.49 ± 0.77	11.28 ± 0.33	10.94 ± 0.40	10.87 ± 0.36	5.05 ± 0.58	3.43 ± 0.39	3.70 ± 0.61	3.31 ± 0.54	425.6 ± 39.8	387.3 ± 52.2	437.7 ± 35.9	387.4 ± 22.0
60	11.44 ± 0.88	11.72 ± 0.63	12.21 ± 0.75	11.76 ± 0.39	10.22 ± 2.82	11.46 ± 3.36	11.73 ± 2.74	12.33 ± 2.19	395.2 ± 35.7	443.3 ± 51.3	363.3 ± 19.5	405.3 ± 36.7
105	9.99 ± 0.70	11.90 ± 0.82	11.20 ± 0.53	11.53 ± 0.58	17.90 ± 2.92	17.67 ± 1.91	13.99 ± 2.54	17.65 ± 3.74	259.2 ± 59.2	290.0 ± 69.1	234.0 ± 79.2	202.0 ± 47.2
150	10.00 ± 0.57	10.87 ± 0.68	10.74 ± 0.57	11.67 ± 0.78	23.08 ± 5.10	26.74 ± 4.72	17.18 ± 3.45	30.24 ± 5.82	218.0 ± 32.5	258.6 ± 59.0	320.6 ± 105.7	170.3 ± 19.84
195	10.77 ± 1.18	9.72 ± 0.95	10.64 ± 0.57	10.38 ± 1.00	16.83 ± 2.31	30.73 ± 2.44	31.55 ± 2.87	37.62 ± 3.11*	332.0 ± 53.3	407.6 ± 129.8	335.0 ± 157.1	364.0 ± 150.36
240	8.61 ± 0.80	9.33 ± 0.38	8.63 ± 0.51	7.94 ± 0.44	13.65 ± 2.96	25.82 ± 6.06	23.25 ± 7.83	22.28 ± 5.30	408.0 ± 28.6	552.8 ± 149.8	398.5 ± 54.2	425.2 ± 66.3

Table 2: Effect of CM1 treatment on hematological parameters in β-thalassemia mice (BKO) fed with Fe diet. Data were expressed as mean + SEM.

their organs, including the heart, liver, kidneys and spleen were excised and weighed. Organ weights and organ/body weight ratios are shown in Tables 5-8. No significant differences were observed between the placebo and the CM1 treatment groups, in both normal and iron loading conditions. Results of histopathological examination are shown in Figure 2. For the mice treated with DI water and CM1 in the N and Fe diet fed groups, the heart tissue did not show any significant pathological change. The liver tissue showed a moderate degree of spotty necrosis, particularly in the portal area and the neutrophils, and lymphocytes were predominantly associated with numerous hemosiderin-laden macrophages in hepatic sinusoids. The spleen tissue presented numerous hemosiderin-laden macrophages in both the red pulp and white pulp, particularly in the Fe diet fed group. The results suggested that CM1 was not toxic to tissue organs, including the heart, liver and spleen.

	AST (U/L)				**ALT (U/L)**				**ALP (U/L)**			
	Treatment				Treatment				Treatment			
Days	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)
0	16 ± 5	26 ± 7	14 ± 4	14 ± 4	15 ± 2	17 ± 3	9 ± 4	12 ± 3	26 ± 4	25 ± 4	18 ± 6	20 ± 7
60	38 ± 4	39 ± 4	20 ± 7	27 ± 9	35 ± 9	49 ± 17	39 ± 24	54 ± 28	24 ± 2	25 ± 2	16 ± 6	16 ± 5
105	38 ± 7	39 ± 8	25 ± 9	17 ± 5	31 ± 3	36 ± 6	20 ± 6	17 ± 5	36 ± 6	40 ± 3	24 ± 9	22 ± 5
150	111 ± 37*	85 ± 30	77 ± 32	71 ± 26*	19 ± 4	28 ± 10	19 ± 5	32 ± 14	34 ± 4	28 ± 3	21 ± 9	31 ± 16
195	64 ± 9	53 ± 4	36 ± 15	40 ± 12	41 ± 20	62 ± 14	36 ± 19	53 ± 17	41 ± 8	34 ± 5	27 ± 9	23 ± 8
240	56 ± 30	100 ± 36	67 ± 41	65 ± 31	13 ± 12	18 ± 10	14 ± 11	18 ± 10	39 ± 5	40 ± 4	27 ± 10	25 ± 9

Table 3: Effect of CM1 treatment on biochemical parameters in β-thalassemia mice (BKO) fed with N diet. Data were expressed as mean + SEM.

	AST (U/L)				**ALT (U/L)**				**ALP (U/L)**			
	Treatment				Treatment				Treatment			
Days	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)
0	43 ± 10	22 ± 5	29 ± 6	24 ± 4	17 ± 1	17 ± 2	16 ± 3	15 ± 3	32 ± 5	28 ± 3	26 ± 4	35 ± 5
60	65 ± 2	78 ± 4	74 ± 8	83 ± 6*	65 ± 7	74 ± 7*	79 ± 12	79 ± 14	33 ± 2	28 ± 2	28 ± 3	25 ± 2
105	64 ± 15	72 ± 13	66 ± 12	101 ± 15*	64 ± 14	85 ± 13*	80 ± 8	114 ± 11	42 ± 5	50 ± 8	50 ± 7	44 ± 6
150	157 ± 39*	163 ± 44*	159 ± 40*	174 ± 27*	39 ± 11	53 ± 15	49 ± 17	62 ± 14	36 ± 4	32 ± 2	32 ± 5	31 ± 5
195	100 ± 6	111 ± 12*	109 ± 5	108 ± 12*	39 ± 12	60 ± 17	60 ± 19	54 ± 14	72 ± 13*	58 ± 10	74 ± 15*	54 ± 9
240	94 ± 27	80 ± 22	88 ± 34	57 ± 12	113 ± 57	69 ± 22	81 ± 48	114 ± 62	57 ± 7	49 ± 6	62 ± 10*	51 ± 9

Table 4: Effect of CM1 treatment on biochemical parameters in β-thalassemia mice (BKO) fed with Fe diet. Data were expressed as means + SEM.

	Treatment			
Parameter	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)
Organ weight (g)				
Heart	0.19 ± 0.005	0.17 ± 0.01	0.22 ± 0.02	0.17 ± 0.01
Liver	1.89 ± 0.04	1.69 ± 0.11	1.85 ± 0.08	1.51 ± 0.11
Kidneys	0.46 ± 0.02	0.44 ± 0.03	0.45 ± 0.01	0.42 ± 0.02
Spleen	0.26 ± 0.03	0.29 ± 0.06	0.50 ± 0.16	0.28 ± 0.01
Organ weight index				
Heart	0.0060 ± 0.0002	0.0059 ± 0.0005	0.0074 ± 0.0007	0.0063 ± 0.0005
Liver	0.0603 ± 0.0009	0.0560 ± 0.0030	0.0620 ± 0.0030	0.0540 ± 0.0020
Kidneys	0.0145 ± 0.0006	0.0147 ± 0.0008	0.0150 ± 0.0002	0.0150 ± 0.0010
Spleen	0.0082 ± 0.0009	0.0100 ± 0.0020	0.0170 ± 0.0050	0.0102 ± 0.0009

Table 5: Organ weight and organ weight index in male β-thalassemic mice (BKO) fed with an N diet and treated with CM1 at the assigned doses for 180 days. Data were expressed as means + SEM.

	Treatment			
Parameter	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)
Mean weight (g)				
Heart	0.139 ± 0.007	0.136 ± 0.005	0.133 ± 0.009	0.14 ± 0.01
Liver	1.34 ± 0.06	1.35 ± 0.10	1.31 ± 0.04	1.33 ± 0.04
Kidneys	0.32 ± 0.02	0.33 ± 0.02	0.32 ± 0.02	0.35 ± 0.02
Spleen	0.35 ± 0.02	0.38 ± 0.03	0.37 ± 0.06	0.36 ± 0.02
Mean organ to terminal body weight ratios				
Heart	0.0061 ± 0.0003	0.0057 ± 0.0002	0.0056 ± 0.0005	0.0060 ± 0.0006
Liver	0.058 ± 0.001	0.056 ± 0.003	0.055 ± 0.002	0.0569 ± 0.0003
Kidneys	0.014 ± 0.001	0.0139 ± 0.0007	0.014 ± 0.001	0.0151 ± 0.0007
Spleen	0.015 ± 0.002	0.016 ± 0.001	0.016 ± 0.002	0.015 ± 0.001

Table 6: Organ weight and organ weight index in female mice treated with CM1 and fed with normal diets for 180 days. Data were expressed as means + SEM.

Parameter	Treatment			
	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)
Organ weight (g)				
Heart	0.140 ± 0.003	0.14 ± 0.01	0.13 ± 0.005	0.17 ± 0.02
Liver	3.47 ± 0.12	3.22 ± 0.38	3.34 ± 0.11	3.84 ± 0.32
Kidneys	0.31 ± 0.04	0.290 ± 0.006	0.29 ± 0.02	0.33 ± 0.02
Spleen	0.37 ± 0.04	0.299 ± 0.005	0.40 ± 0.01	0.42 ± 0.04
Organ weight index				
Heart	0.0058 ± 0.0003	0.006 ± 0.003	0.0060 ± 0.0002	0.0066 ± 0.0008
Liver	0.15 ± 0.01	0.14 ± 0.13	0.144 ± 0.0.007	0.151 ± 0.008
Kidneys	0.013 ± 0.001	0.013 ± 0.005	0.0128 ± 0.0005	0.0131 ± 0.0006
Spleen	0.015 ± 0.001	0.013 ± 0.003	0.0176 ± 0.0009	0.017 ± 0.001

Table 7: Organ weight and organ weight index in male mice treated with CM1 and fed with Fe diet for 180 days. Data were expressed as means + SEM.

Parameter	**Treatment**			
	DI	CM1 (50 mg/kg)	CM1 (100 mg/kg)	CM1 (200 mg/kg)
Organ weight (g)				
Heart	0.127 ± 0.007	0.133 ± 0.008	0.12 ± 0.02	0.14 ± 0.01
Liver	2.78 ± 0.32	2.49 ± 0.08	2.91 ± 0.23	2.62 ± 0.14
Kidneys	0.28 ± 0.02	0.263 ± 0.009	0.27 ± 0.02	0.30 ± 0.02
Spleen	0.42 ± 0.03	0.37 ± 0.01	0.38 ± 0.06	0.39 ± 0.01
Organ weight index				
Heart	0.0064 ± 0.0005	0.0066 ± 0.0004	0.0061 ± 0.0007	0.0066 ± 0.0006
Liver	0.14 ± 0.01	0.124 ± 0.008	0.144 ± 0.007	0.129 ± 0.005
Kidneys	0.0139 ± 0.0009	0.0131 ± 0.0004	0.0139 ± 0.0008	0.0146 ± 0.0002
Spleen	0.021 ± 0.001	0.0186 ± 0.0007	0.019 ± 0.002	0.0194 ± 0.0009

Table 8: Organ weight and organ weight index in female mice treated with CM1 and fed with Fe diet for 180 days. Data were expressed as means + SEM.

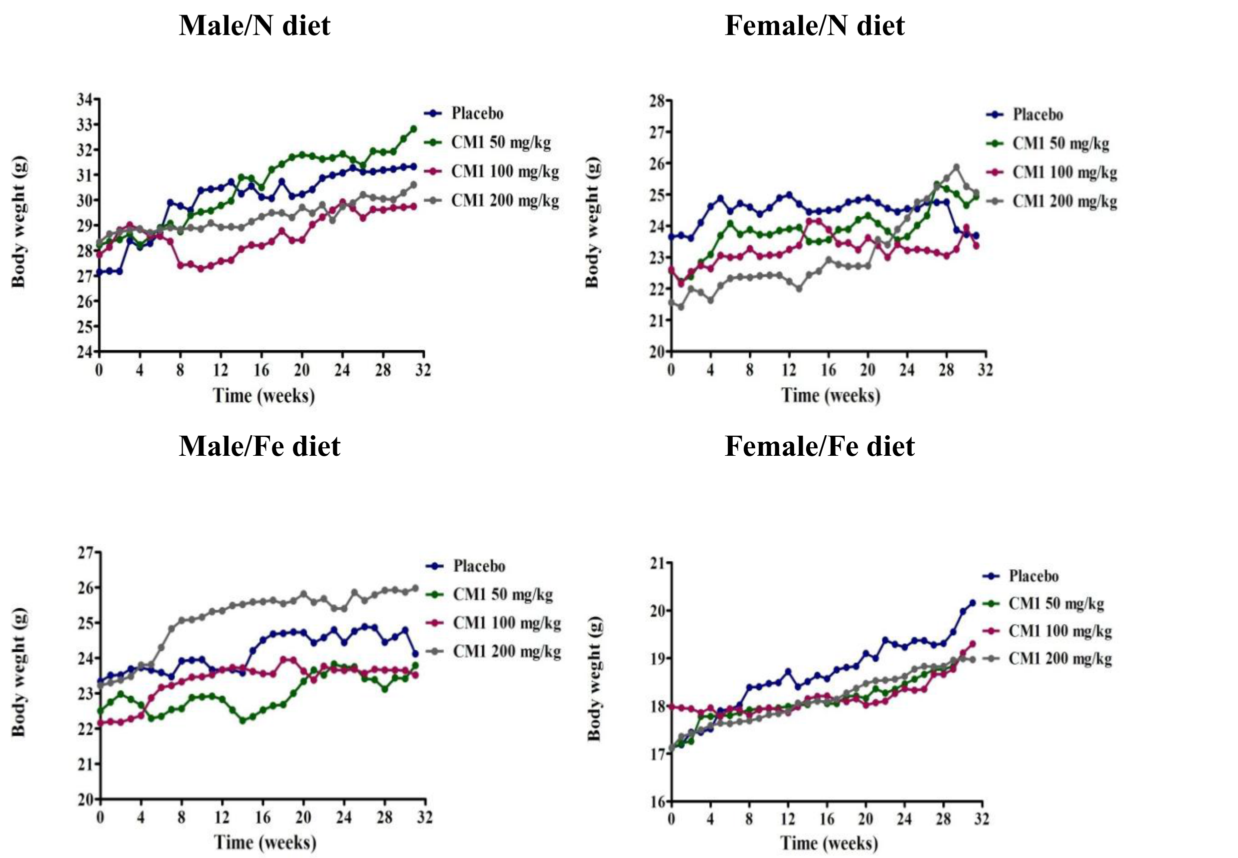

Figure 1: Changes in body weight (average values) of male and female β-thalassemia (BKO) mice fed with N and Fe diets and treated with CM1 (0-200 mg/kg) for 8 months.

Discussion

Iron is vital to cells, but an excess of iron is toxic. The genetic disorder, ß-thalassemia, is a secondary iron overload disease that requires repeated blood transfusions [15], and shows abnormal intestinal iron absorption. Iron is able to accumulate in many tissues and organs leading to tissue damage and organ dysfunction [16]. One way to remove excess iron is through iron chelation therapy. Nowadays, there are several widely used iron chelators that are available for iron chelation therapy. However, these compounds still have many adverse effects [17]. CM1 is a bidentrate synthetic oral iron chelator that is an analogue of DFP. In the previous work, we found that CM1 was an effective iron chelator. It can chelate iron efficiently in both *in vitro* and *in vivo* studies, but its toxicity is unknown. In this work, we investigated the toxicity of CM1 in transgenic ß-thalassemia mice under normal and iron overload conditions. The results indicated that there was no sign of toxicity under the clinical observation and there was no effect of CM1 on body weight under both conditions (Figure 1 and 2). Hemoglobin levels were not significantly different between the placebo and the treated groups, and this was similar in terms of the white blood cells and the platelet numbers (Table 1 and 3). The activity of AST, ALT and ALP slightly increased after 60 days for all groups and peaked on day 150, especially among mice fed with an iron containing diet, but there was still no difference between the treated groups and the placebo groups. Moreover, the enzyme activities were observed to react in a dose-independent manner. In the pathological

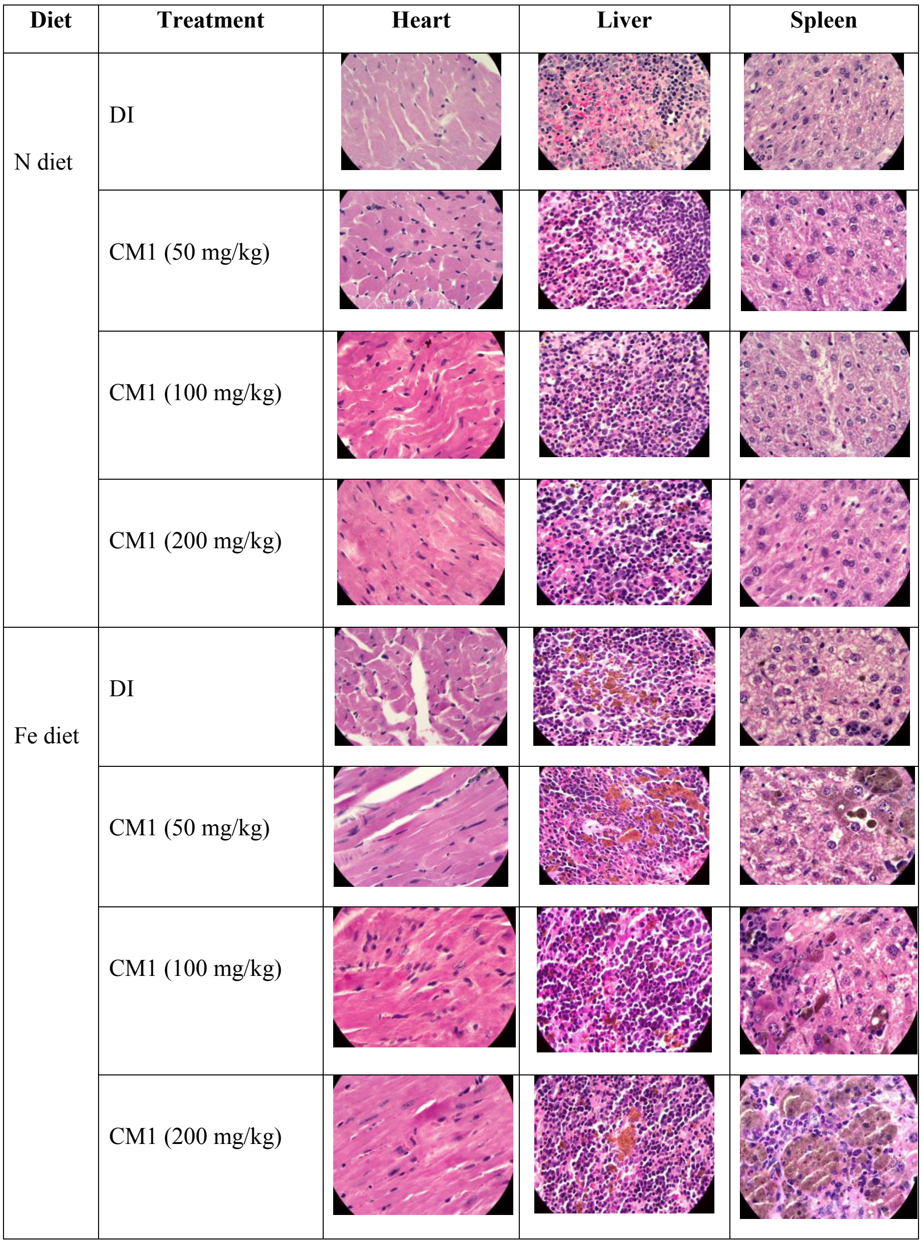

Figure 2: H&E stained heart, liver and spleen tissues of male and female β-thalassemia (BKO) mice fed with N and Fe diets and treated with CM1 (0-200 mg/kg) for 8 months.

examination, no significant pathological changes were observed in the hearts of the mice that received all doses of both feeding conditions, but their livers were still predominantly composed of neutrophil and lymphocytes. The spleen presented numerous scattered hemosiderin-laden macrophage, especially among then iron loaded ß-thalassemia mice. This is probably due to the liver being the major organ for iron storage. High iron accumulation induces liver damage, and thus, liver enzymes are released [18,19]. More parameters could not be investigated due to the limited blood volumes available in the mice.

Conclusions

CM1 is an effective iron chelator, and is not toxic to the peripheral blood and liver cells of ß-thalassemia mice under normal and iron overload conditions. This may indicate that CM1 treatment is free from the severe side effects, such as leukopenia and thrombocytopenia.

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