Oral Administration of Bovine Blood Peptide Generated No Adverse Effect on Healthy Rats

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Abstract: IDA (iron deficiency anemia) is a disease with high incidence in many countries. Inorganic iron supplements, typically ferrous sulfate, are widely utilized in the prevention and treatment of IDA. However, the bioavailability of inorganic iron is only 2% to 20%. In addition, the gastrointestinal side effects occur frequently. Heme iron which is isolated from animal blood is a promising choice for IDA patients. Before extensive utilization of heme iron in clinic, safety evaluation is indispensable. In this study, with untreated rats and ferrous sulfate-treated rats as controls, bovine blood peptide which carries heme iron was applied to normal female rats. After 30 days of gavage feeding, no significant difference in body weight and organ coefficient was observed. Serological examination revealed that oral administration of bovine blood peptide did not disrupt iron metabolism nor caused adverse effects on liver and kidney functions. Pathological HE staining of gastrointestinal tract showed that bovine blood peptide induced much less inflammatory irritation than ferrous sulfate. These results suggest that bovine blood peptide is a kind of safe and reliable organic iron supplement for IDA patients.

1 INTRODUCTION

Iron is one of the trace elements needed by the human body. Due to some congenital or acquired factors, the amount of stored iron in the body is too low to support the synthesis of functional iron (hemoglobin, etc.) and consequently resulted in iron deficiency anemia (IDA) (Lin 2013). The pathogenic factors of IDA include excessive iron loss, iron utilization disorder, iron uptake deficiency, iron malabsorption, iron transport disorder (Chen 2013). As reported, about one billion people worldwide have some form of iron deficiency, and more than half of them have developed IDA (WHO 2001). The WHO report in 2011 mentioned that the prevalence of IDA in children in China was 7.8% (WHO 2011). The prevalence of IDA is more than 20% in women (Zhang 2010). Generally, Infants, growing children and women of childbearing age are vulnerable to IDA (Liu 2012, Dalhøj 1991). Iron deficiency not only leads to decreased red blood cells but also weaken the activity of iron-containing enzyme in cells and triggers clinical symptoms of IDA (Ge 2013).

Supplementation of iron increases the level of hemoglobin (Hb). At present, there are two types of iron supplements for IDA treatment (Ulas 2010). Oral ferrous sulfate is the main drug used in clinical treatment of IDA because of its low price and significant effect. But side effects, such as irritation of the gastrointestinal tract, constipation, or diarrhea cannot be ignored. In addition, it has low bioavailability (He 1995). Studies have shown that

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excessive iron intake can cause changes in intestinal microbiota and oxidative stress injury in the body (Alexeev 2017, Zhang 2017).

The most abundant protein in blood is hemoglobin which can be purified and processed to produce heme iron supplements. Heme iron is better than inorganic iron. Bovine blood peptide also provides human body with useful peptides and amino acids. Therefore, it is a potential substitute of ferrous sulfate. The advantages of blood peptide and heme iron have been extensively reported while the safety and reliability remain to be further clarified.

This study aimed to evaluate the safety of bovine blood peptide in normal female rats. Results of the present study will provide useful information for the clinical application of bovine blood peptide in IDA treatment.

2 MATERIALS AND METHODS

2.1 Animals and Reagents

Forty-eight female SD rats with body weight between 60-80g (purchased from SiPeiFu, Beijing, China) were randomly divided into 6 groups, namely control group, bovine blood peptide low-dose group (2 mg/kg/d), bovine blood peptide medium dose group (20 mg/kg/d), bovine blood peptide high-dose group (100 mg/kg/d), ferrous sulfate low-dose group(100 mg/kg/d) and ferrous sulfate high-dose group(200 mg/kg/d). Bovine blood peptide powder and ferrous sulfate tablets were provided by BIBAU, Hangzhou, China. After adaptive feeding for 5 days, rats were continuously feed with different iron supplements as indicated by gavage for 30 days.

2.2 Measurement of Blood

After gavage administration, rats were sacrificed and blood was collected for the measurement of liver and kidney functions with biochemical or ELISA kits (from Jiancheng, Nanjing, China) on an automatic biochemical analyzer.

2.3 Pathological Examination

Stomach tissues were collected, embedded with paraffin and then sliced for HE staining to examine tissue damage and the infiltration of inflammatory cells.

2.4 Statistical Analysis

All data were expressed as mean ± standard deviation. Prism 8.0 was used for statistical analysis. One-way analysis of variance (ANOVA) with $P < 0.05$ was used to consider the difference statistically significant.

3 RESULTS

3.1 Body Weight of Rats Was Not Affected

The body weight of rats after 30 days of iron supplementation was shown in Fig. 1. At the end of the experiment, the body weight of rats in each group was about 200g, and there was no significant difference among different groups ($P > 0.05$). The results showed that bovine blood peptide did not affect the normal growth of rats, which was consistent with the results of traditional iron supplementation agent ferrous sulfate.

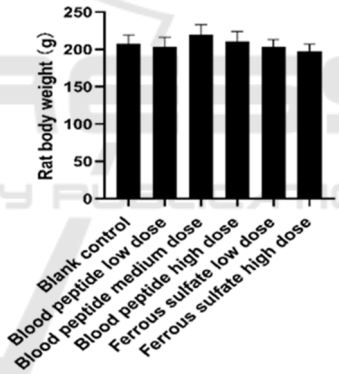


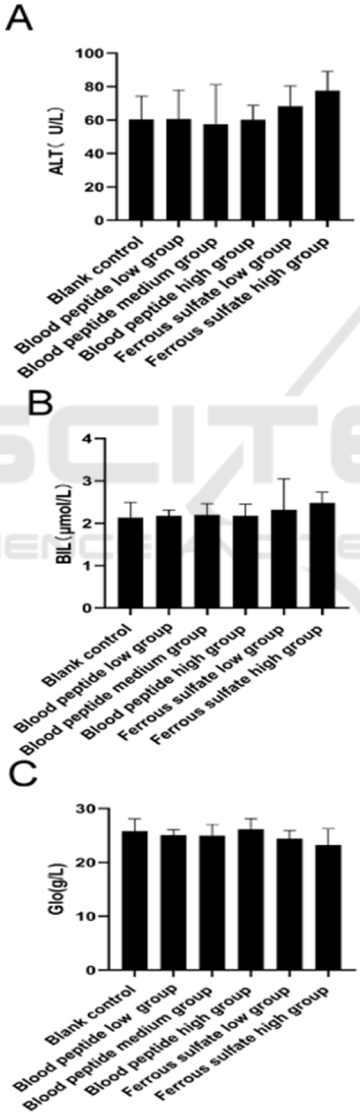
Figure 1: Rat body weight post 30-day feeding.

3.2 Liver and Kidney Organ Coefficients and Functions Were Unaffected

To evaluate the safety of bovine blood peptide, size of liver and kidney was measured. As shown in Table 1, compared with the blank control, liver and kidney organ coefficients of various experimental groups were not significantly different ($P > 0.05$). By detecting ALT, bilirubin and Glo (Figure 2), it was found that there was no significant difference in liver function in each group supplemented with iron ($P > 0.05$). The results showed that the functions of liver and kidney were not affected by bovine blood peptide or ferrous sulfate.

Table 1: Organ coefficient of rats fed with iron supplements.

Group	Organ coefficient(g/100g), mean ± SD	
	liver	kidney
Blank control group	4.67±0.47	0.57±0.06
Blood peptide low dose group	4.92±0.25	0.69±0.03
Blood peptide medium dose group	4.57±0.33	0.71±0.08
Blood peptide high dose group	4.76±0.41	0.58±0.05
Ferrous sulfate low dose group	4.87±0.27	0.51±0.08
Ferrous sulfate high dose group	4.75±0.14	0.46±0.01

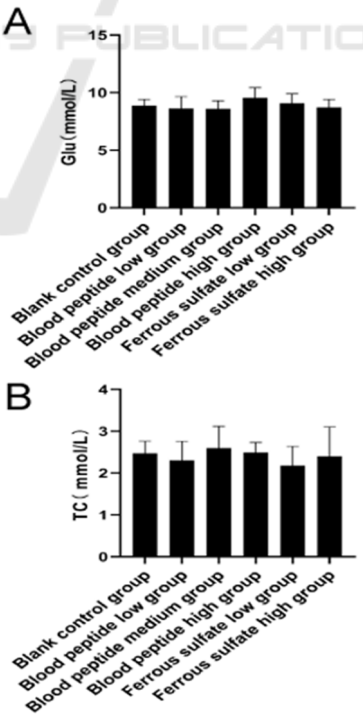


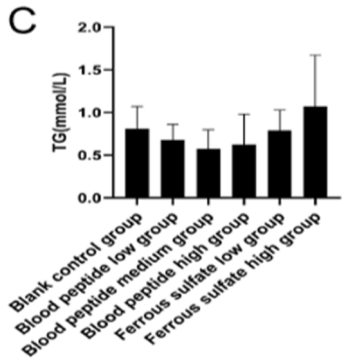
A. Alanine aminotransferase, B. total bilirubin, C. globulins

Figure 2: Rat liver function.

3.3 Glycolipid Metabolism Were Not Affected by Iron Supplements

Bovine blood peptide used in this study was a mixture of peptide in different length prepared from blood protein digestion. It was previously reported that some peptide might affect blood glucose. To test whether the blood glucose and lipid of normal rats altered after ingestion of bovine blood peptide, rat sera were examined. There was no significant difference in serum glucose, total cholesterol and triglyceride levels among groups supplemented with iron and the control group (Figure. 3) ($P > 0.05$). The results showed that bovine blood peptide did not affect the glycolipid metabolism of normal rats after intragastric administration.





A. glucose, B. total cholesterol, C. triglyceride

Figure 3: Rat Glucose and Lipid Metabolism Index

3.4 Iron Metabolism Was Not Affected by Iron Supplements

To test whether bovine blood peptide would increase the iron concentration in serum in normal rats, serum iron was determined. As show in Figure 4, there was no difference in serum iron concentration among different groups ($P > 0.05$), and there was no significant dose-response relationship. The results indicate that the supplementation of blood peptide iron or ferrous sulfate did not significantly change the status of iron metabolism in healthy rats fed normal diet.

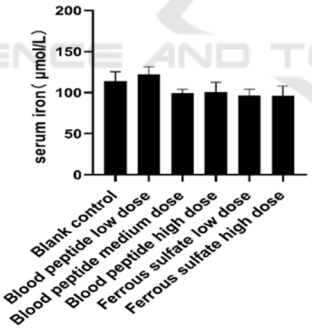


Figure 4: The serum iron content of rats in different groups.

3.5 Bovine Blood Peptide Caused Less Gastrointestinal Side Effects than Ferrous Sulfate

A major disadvantage of ferrous sulfate is severe gastrointestinal side effects after taken orally. To determine the effects of iron supplements on gastrointestinal tract, HE staining was performed.

Compared with the normal control group, the number and distribution of inflammatory cells in the low dose and medium dose blood peptide groups were not obvious (Figure 5). In the high-dose group, the mild inflammatory reaction was mainly in the mucosa layer, and there was no increase of inflammatory cells in the submucosa. Whereas gastric tissues from low and high dose ferrous sulfate groups displayed infiltration of neutrophils and lymphocytes in the mucous and the submucosa layers. In ferrous sulfate high dose group, interstitial edema and vascular dilatation and congestion were observed. These results demonstrated that bovine blood peptide is less irritating to the gastrointestinal tract than ferrous sulfate.

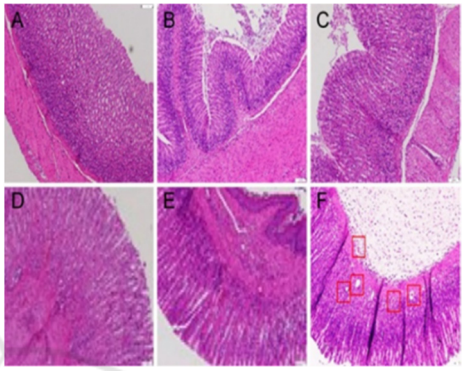


Figure 5: Changes in the stomach tissue in rats (HE staining, 100×): (A) blank control; (B) blood peptide low dose; (C) blood peptide medium dose; (D) blood peptide high dose; (E) ferrous sulfate low dose; (F) ferrous sulfate high.

4 CONCLUSIONS

In this study, the body weight, liver and kidney function, liver and kidney size, glucose and lipid metabolism, and iron metabolism of normal rats were not significantly affected by the supplementation of bovine blood peptide or ferrous sulfate. Different from ferrous sulfate, bovine blood peptide didn't lead to obvious inflammatory change in gastrointestinal mucosa. Histological examination demonstrated that compared with ferrous sulfate, bovine blood peptide is a type of safer iron supplement for the treatment of IDA and can avoid the gastrointestinal irritation caused by inorganic iron supplements.

CONFLICT OF INTEREST

We have no conflicts of interest to disclose.

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