

Effects of Iron Overload on Growth and Intestinal Mucosa in Rats

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Abstract Objective: To detect the mechanism of excess iron intake on growth and intestinal mucosa in rats. **Methods:** Forty-eight male Wistar rats were randomly divided into four groups: Low Iron Group, Normal Iron Group, Medium Iron Group and High Iron Group upon daily iron intake of 7mg/kg, 16.8mg/kg, 35mg/kg, 70mg/kg respectively via fodder for eight weeks. The general condition, body weight and food intake were documented simultaneously. Serum level of Ferritin, IL-6 and IL-10 were detected using ELISA. The observation of morphology of intestine was also included concurrently. **Results:** The weight obtained was significantly lower in the High Iron Group compared with the other three groups while the average weight of the rats in the High Iron Group was lower than the Normal Iron Group. Meanwhile, the average amount food uptake of the High Iron Group had slightly decreased in the eighth week. In addition, the level of IL-6 in small intestine of the High Iron Group is higher than that of the Normal Iron Group ($P<0.05$), while the level of IL-10 in small intestine of the High Iron Group is lower than that of the Normal Iron Group ($P<0.05$). The histopathology results showed that normal morphology was found in Low Iron Group and Normal Iron Group, but the mucosa showed a slight injury in the Medium Iron Group with apical microvilli slightly off. Irregular shape of microvilli and necrosis were found in some epithelial cells of intestinal mucosa in the High Iron Group. **Conclusion:** Excessive dietary iron intake plays a negative effect on the normal growth and development, and resulted in intestinal inflammatory injury in rats.

Keywords: Rat, Iron Overload, Growth and Development, Small Intestinal Mucosa

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Introduction

Iron is an essential trace element of the human body and has important physiological functions. Studies have shown that iron deficiency and excess can cause damage to the body. Iron deficiency can cause iron deficiency anemia, while excessive iron intake can lead to excessive production of oxygen free radicals^[1], which can cause the damage of the tissue and organs^[2,3]. Studies have found that iron deficiency can cause liver fibrosis and hepatocellular carcinoma^[4], while the increasing risk of hemochromatosis patients who suffering from liver cancer is related to abnormal high iron levels in the liver of chronic liver injury^[5]. Epidemiological studies have shown that excessive intake of red meat can increase the risk of colorectal cancer^[6,7], which may play a role in heme iron^[8]. It is confirmed that heme iron does increase the probability of colorectal cancer caused by red meat intake, and it is through the lipid peroxidation pathway to work. Animal

experiments found that excessive intake of iron can cause cecal crypt morphological abnormalities^[9].

In recent years, in order to prevent iron deficiency and iron deficiency anemia, iron fortified food has increased, and with the improvement of people's living standards, rich in heme iron meat intake is growing. In the early stage of the study group, rats were injected with dextran iron by intraperitoneal injection, and it was found that excessive iron supplementation in rats could lead to lymphocyte injury and liver injury in rats^[10,11]. In this study, we will continue to observe the effect of adding excess iron (2 to 4 times the normal amount of iron) to the rats by dietary supplementation to observe the effects on the growth and intestinal mucosa of rats and to explore its possible mechanism.

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1. Materials and methods

1.1 Experimental animals and groups

6 weeks old male Wistar rats (SPF grade) 48, weight 180-220g, purchased in Qingdao Pattersford rat farming professional cooperatives (certificate: 0014108). Forty-eight rats were randomly divided into four groups (n = 12), fed with different doses of iron, low iron group: daily iron intake is 7mg / kg body weight (50mg / kg feed); normal Iron group: daily iron intake is 16.8mg / kg body weight (120mg / kg feed); iron group: daily iron intake is 35mg / kg body weight (250mg / kg feed); high iron group: daily iron intake is 70 mg / kg body weight (500 g / kg feed). Animal feed reference AOAC recommended rodent experimental animal synthetic feed formula, and carries on the appropriate adjustments; Iron use the ferrous sulfate (purchased in Tianjin Beichen Founder Reagent Factory). The rats were allowed to eat and drink deionized water for 8 weeks. The activity, signs and living conditions of all rats were recorded every day.

1.2 Detection of indicators and methods

1.2.1 Serum ferritin

Using the ELISA kit to detect the serum levels of serum ferritin in each group.

1.2.2 IL-6, IL-10 and SIgA in small intestine tissue

The levels of IL-6, IL-10 and SIgA in rat intestinal tissue homogenate were determined by ELISA. The intestinal tissue was rinsed in 0 ~ 4 °C ice saline,

dried, weighed, broken, centrifugated in low temperature 3500r/min 15min, leave the supernatant.

1.2.3 Histomorphological examination

The small intestine was washed with saline for several times and then fixed with 4% neutral paraformaldehyde. After routine dewatering, paraffin was embedded, sliced and stained with HE for small intestinal morphology.

1.3 statistical processing

SPSS16.0 statistical software was used to analyze the data. The results of body mass and serum ferritin were expressed as $\bar{x} \pm s$. Multiple groups were compared with single factor analysis of variance, inspection level for $\alpha=0.05$.

2. Results

2.1 General situation

There was no obvious abnormality and death in the rats after the experiment. The appearance of the rats in each group had no obvious difference.

2.2 weight gain in rats

From the table 1, we found that the body weight of the rats in each group before the experiment has no difference ($p > 0.05$), after the end of the experiment, there were no difference between the low iron group, normal iron group and iron group three groups; Compared with the high-iron group, the average body weight decreased by 20.0%.

Table 1 Changes in body weight and weight gain

group	n	Weight (g)		
		0 weeks	8 weeks	weight gain
Low iron group	12	187.52±13.01	326.90±38.92	139.38±45.54
Normal iron group	12	188.91±14.03	332.18±35.17	143.27±41.64
China Railway Group	12	182.29±16.53	353.17±27.01	170.87±30.97
High iron group	12	191.73±15.39	306.33±12.74	114.60±17.62*

* Compared with the normal iron group, $p < 0.05$

2.3 Comparison of the intake of rats in each group

It can be seen from Table 2 that there was no significant difference in the intake of rats in the rats at the beginning of the experiment, while the average food intake in the high iron group decreased by 11.9% compared with the normal iron group at the 8th week.

Table 2 The intake of rats in each group (g / day)

group	n	0 weeks	8 weeks
Low iron group	12	20.03±1.16	37.15±1.84
Normal iron group	12	19.20±0.91	34.50±2.34
China Railway Group	12	20.80±3.79	35.08±1.55
High iron group	12	22.05±4.10	30.40±1.15*

* Compared with the normal iron group, $p < 0.05$

2.4 serum ferritin levels in each group

Table 3 shows that there was no significant difference in serum ferritin levels between the low iron group and the normal iron group ($p > 0.05$). Compared with the normal iron group, the serum ferritin levels in the high iron group and the iron group were significantly higher.

Table 3 Serum ferritin levels (g/L)

group	n	results ($\bar{x} \pm s$)	<i>P</i>
Low iron group	9	244.3±16.42	
Normal iron group	9	247.3±25.69	
China Railway Group	9	260.6±19.64	0.009
High iron group	10	274.6±24.21	0.002

2.5 Histopathological changes of small intestine in each group

At the end of the experiment, paraffin-embedded small intestine tissue sections were stained with HE under light microscope ($\times 200$). The morphological changes of small intestine were detected in each group. The results showed that the mucosal structure of the small intestine was normal in the low iron group and the normal iron group, the integrity of the

intestinal villi was complete and the edge of the microvilli was clear (Figure 1-A, B). The intestinal mucosa of the middle iron group and the high iron group were slightly damaged, the villus was arranged neatly and the microvilli of the apical group were slightly exfoliated (Figure 1-C). High-iron group showed a small number of irregular hair morphology, top off, part of the intestinal mucosal epithelial cell necrosis (Figure 1-D).

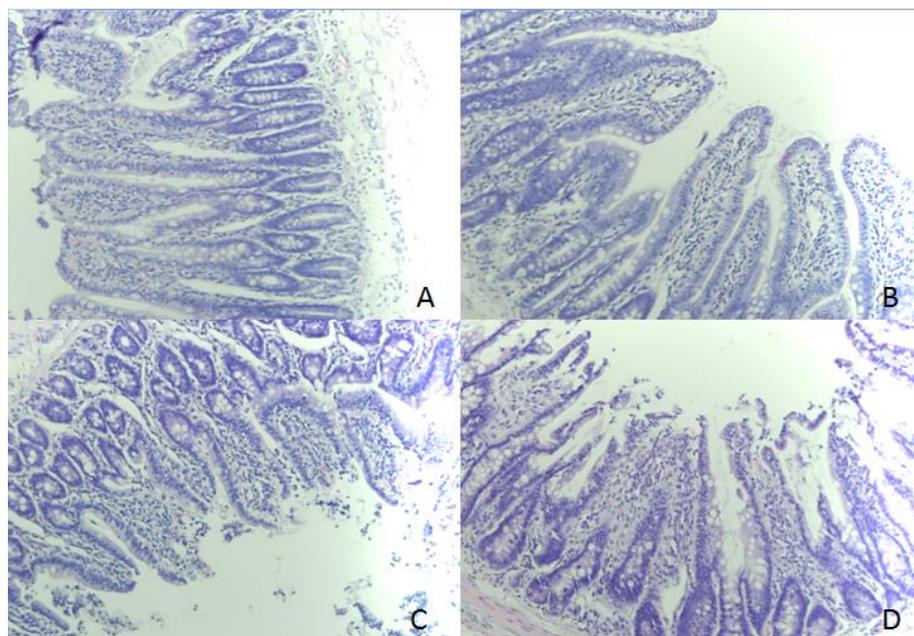


Figure 1 Changes of intestinal histomorphology in each group (HE $\times 200$)
(A: low iron group B: normal iron group C: iron group D: high iron group)

2.6 The comparison between the levels of IL-6 and IL-10 in small intestine of rats

The levels of IL-6 and IL-10 in small intestine were measured by ELISA. The levels of IL-6 in the small intestine of the high iron group were significantly higher than those in the normal iron group ($p < 0.05$), while the level of IL-10 was significantly decreased ($p < 0.01$).

Table 4 IL-6 and IL-10 levels in small intestine

group	IL-6(pg/ml)	IL-10(pg/ml)
Low iron group	84.68±7.68	71.69±15.35
Normal iron group	86.49±11.43	69.89±19.60
China Railway Group	92.37±6.42	67.22±6.59
High iron group	108.60±15.22*	50.47±8.31*

* Compared with the normal iron group, $p < 0.05$

3 Discussion

Ferrous sulfate is commonly used for the treatment of iron deficiency anemia iron, the conventional dose is 0.9g/day during the treatment, equivalent to an additional 180mg daily iron element, and some patients will have varying degrees of gastrointestinal symptoms, mainly nausea. In the course of the study, we found that the average body weight of the rats in the high-iron group decreased by 20.0% compared with the normal iron group at the end of the experiment, and we found that the rats in the high-iron group had a significant decrease in the food intake, this might be because excessive iron sulfate supplement to reduce the appetite of rats, thus affecting the high iron group rats growth and development. In addition, some studies have pointed out that excessive intake of iron will hinder the body of zinc and other nutrients absorption and use, thus affecting the normal development of embryos^[12]. And the corresponding changes in iron and other necessary divalent cations, such as zinc, copper and ion balance, may be involved in iron-induced growth retardation^[13]. Foreign studies have also shown that the addition of excess iron in baby food may affect the growth and development of children^[14]. While the specific mechanism of action is not clear.

In this study, the morphological results of small intestine mucosa showed that the intestinal mucosa of rats in the middle and high iron groups had different degrees of damage. In the high iron group, the villous morphology was irregular and some intestinal mucosal epithelial cells were necrotic. Suggesting that excessive iron supplement (up to 4 times the normal requirement) can cause local mucosal tissue damage. It has been suggested that chronic iron intake can affect intestinal homeostasis by altering intestinal microbial composition, oxidative stress, inflammation, etc.^[13,15,16,17-19].

In this study, we found that the levels of IL-6 in

intestinal tissue of high-iron group were significantly higher than those of normal iron group, and the level of IL-10 was lower than that of normal iron group by detecting the level of inflammatory factors in small intestine tissue. IL-6 is an important mediator of inflammation and pathophysiology in vivo, which can aggravate the inflammatory response. IL-10 is an anti-inflammatory, anti-immune cytokine that reduces damage caused by inflammatory responses. Therefore, this study also confirmed that excessive iron intake can cause intestinal mucosal inflammatory response. Li et al also found that high-speed iron can cause porcine duodenal mucosal cytokines expression increased^[21], its possible mechanism is associated with increased oxidative stress-induced intestinal mucosal permeability^[22,23], increased permeability of intestinal mucosa can lead to antigen and pathogen epithelial cell translocation^[24], causing intestinal mucosal inflammation reaction.

In summary, the excessive intake of dietary iron can affect the normal growth and development of rats, and cause intestinal mucosal injury in rats, and excessive iron caused by intestinal inflammatory response may be one of the mechanisms, the result is In the future to study the impact of iron on the intestinal tract provides a certain basis. The results of this study suggest a reasonable diet, a reasonable supplement iron fortified food, to avoid blind iron on the health of the body have a negative impact.

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