Hematological Response of Anemic Rats to hemosiderin and ferrous sulfate

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Abstract

Biovailability of iron in ferrous sulfate and hemosiderin, prepared from pork liver in our laboratory, was evaluated in terms of hematological indices. Twenty-one days old fifty-four male Wistar weanling rats were made anemic by feeding a casein-based, iron-deficient diet for 1 week and withdrawing blood from retro-ocular veins twice a week. Anemic rats were divided into 9 groups and fed, for additional 6 weeks, with the iron-deficient diet or the diet supplemented with ferrous sulfate and hemosiderin at 6, 12, 18, 24 mg iron/kg diet. Significant depression was observed in blood parameters of both anemic rats fed with the iron-deficient diet and those diets supplemented with ferrous sulfate or hemosiderin at 6 mg iron/kg diet than other groups ($p<0.05$). No significant difference was observed in blood parameters between the groups on the ferrous sulfate and the hemosiderin diets; However, the hemosiderin diet gave slightly higher but statistically insignificant values than in animal fed with the ferrous sulfate. Therefore, these data indicate that hemosiderin is a good source of iron for nutrition.

Introduction

Iron deficiency remains a common cause of anemia in infants, children and pregnant women despite increasing availability of iron-fortified in food[1]. However, anemia due to iron deficiency is still a common problem recognized throughout of the world[2]. Anemia due to iron deficiency is caused not only by low iron intake, but more often, from poor availability of iron from diet, due to iron interaction with or inhibition by other dietary constituents[3]. Many dietary constituents affect on iron bioavailability[3]. For example, protein, vitamin C and citric acid have been known to enhance iron availability in diet[4], whereas, fibre, phytate and tannin have been showed to inhibit iron availability in food[5]. Previous studies have shown that the hemosiderin iron was less available in normal subjects than in anemic subjects about 1.9 and 7.5%, respectively but when administered with ascorbic acid, the availability of hemosiderin iron was markedly increased[6]. However, there is limited information on biovailability of hemosiderin

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iron in human and in animals. To our knowledge, there is no report on hemosiderin iron bioavailability as assessed in terms of hematological response in anemic rats. Therefore, the purpose of this study was to evaluate the effect of hemosiderin on iron bioavailability in anemic rats by using hematological indices.

Materials and Methods

Hemosiderin was prepared from pork liver by a modified method of Stephan et al[7] and Gabrio et al[8]. Analyses showed that the hemosiderin preparation used in this study contained 70% protein, and 34 mg Fe/100 g. *Diets and animals* : The basal diet was formulated according to AIN-76[9]. Fifty-four male weanling Wistar albino rats, 21 days old (purchased from Shimizu Laboratory Supplies, Ltd., Kyoto) were individually housed in stainless steel wire-meshed cages. Housing was temperature-controlled at 23°C with a 12 hour light and dark cycle. Anemic rats were produced by feeding a casein based diet for 1 weeks and by withdrawing blood from retro-ocular vein on the day four and sixth. The animals were assigned to 9 groups of six rats each, on the basis of hemoglobin concentrations (50g/l) and body-weight (74.5g). During the repletion phase, the animals were given free access to the diet and water for 6 weeks. The iron-deficient group continued to receive the iron-deficient diet. The reference groups received the diet containing ferrous sulfate at 6, 12, 18, 24 mg Fe/kg diet and the experimental groups received the diet containing hemosiderin at 6, 12, 18, 24 mg Fe/kg diet, respectively. Body-weight, food intake and water were recorded every day in the morning. After 6 weeks of repletion diets, rats were deprived of food for 16-20 h before collection of blood and organ tissues.

Estimation of iron availability in experimental diets by in vitro digestion.

The effect of different level of hemosiderin and the ferrous sulfate on relative iron availability was estimated by using a modification of Miller et al[10]. The method involves a two-stage (pepsin and pancreatin) digestion. The amount of iron that passed into a dialysis tube (dialyzable iron), which is present in the stage 2 is used as an indicator of iron availability.

Stage 1 : pepsin digestion. The experimental diets were homogenized in a mixer blender to a creamy consistency. After blending, the homogenized diets were adjusted to pH 2 with 6N HCL, mixed with pepsin, and incubated for 2 h in a 37°C shaking water bath.

Stage 2 : pancreatin digestion. After 2 hours pepsin of digestion, aliquots of the pepsin digest were divided into four proportion of 20-g aliquots each and transferred to beakers. Two of these aliquots were frozen. Another two proportion of the pepsin digest were mixed with 5ml of the pancreatin-bile suspension and titrated to pH 7.5 with 0.5N KOH. Dialysis tube containing 25ml of redistilled water and amount of 0.1N $NaHCO_3$ equal to the volume of 0.5N KOH required to titrate the aliquot-pancreatin-bile mixture to pH 7.5 were prepared for each diets. The frozen aliquots were thawed in a 37°C water bath and a dialysis tube containing redistilled water and $NaHCO_3$ were placed in each aliquots beaker. The beakers were sealed with parafilm and incubated in a 37°C shaking water bath until the pH reached ab-

out 5.

Pancreatin-bile extract mixture was added to each beaker and the incubation was continued for an additional 2 hours. After the pancreatin digestion, aliquots from the dialysates were analyzed by using bathophenanthroline reaction iron. The percentage of dialyzable iron in experimental diets were calculated as follows :

$$\frac{\mu g \text{ iron/ml dialysate} \times \text{ml dialysate}}{\mu g \text{ non-heme iron/g of sample} \times \text{weight of sample}} \times 100$$

Chemical analysis : Serum and TIBC was quantified by methods proposed by the International Committee for Standardization in Hematology[11]. Non-heme iron in tissues were determining by the method of Schricker et al.,[12]. Hemoglobin concentration was measured by the cyanomethemoglobin method using a commercial kit (hemoglobin-test-Wako, Wako Pure Chemical Ind., Osaka, Japan). Red blood cells were counted under microscope. Mean corpuscular volume was calculated as described by Wintrobe[13].

Statistical analysis : The data are reported as the mean ± SEM. The data was analyzed by one-way ANOVA to detect significant difference. Difference was considered significant at $p < 0.05$. The significance of differences means was evaluated by Scheffe's test. All analysis were performed using Stat View version. 4 (Abacus concepts, Inc., Berkeley, CA).

Results and discussion

After 6 week of repletion period, a significant difference in body-weight and food consumption was observed among the iron-deficient diet group and other group (data not shown). However, the body-weight in the groups were supplemented with either ferrous sulfate or hemosiderin as iron source in diet above the level of 12 mg iron/kg diet were not significantly different, although body-weight in the group supplemented with hemosiderin was slightly higher than the group with ferrous sulfate.

The hemoglobin concentration and hematocrit, red blood cell numbers and mean corpuscular volume in the rats fed with 6 mg iron/kg diet were lower than those of rats administered with iron above 12 mg iron/kg diet (Fig. 1). Non-heme iron in liver and spleen were depressed both in the iron deficient diet as well as in supplemented groups which contained less than 6 mg iron/kg diet. Serum iron and percentage of transferrin saturation also decreased, transferrin saturation dropped to below 15% (Figs. 2 and 3).

No significant difference was observed in blood parameters and non-heme iron values in spleen and liver in rats on ferrous sulfate and hemosiderin diet; hemosiderin diet gave slightly higher but statistically insignificant values than anemic rats administered with ferrous sulfate.

Dialyzable iron. In vitro results for percent dialyzable iron in experimental diets are showed in Fig. 4. The percent dialyzable iron showed no significant difference between the experimental diets which contained iron from ferrous sulfate and hemosiderin. However, overall, percent of dialyzable iron from hemosiderin diets were slightly better than ferrous sulfate. The data obtained in this study shows that hemo-

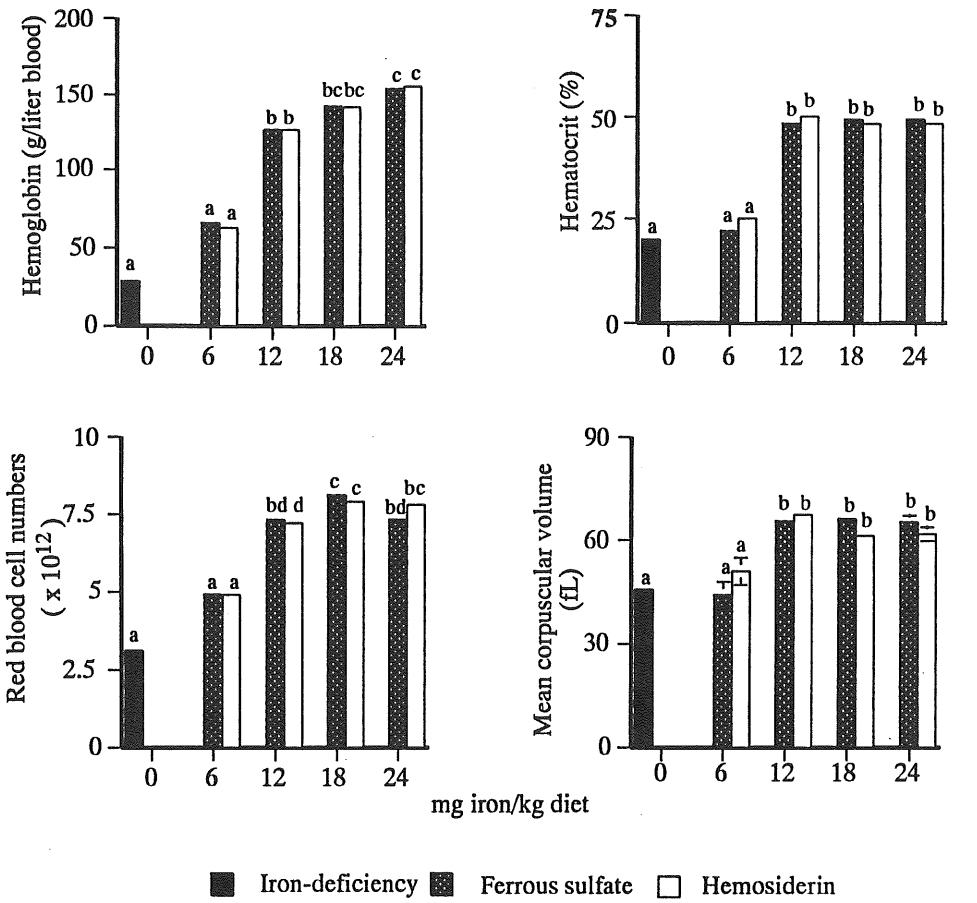

Fig. 1 Hematological response in anemic rats fed with or without ferrous sulfate and hemosiderin. Values represent the means for six animals and the standard bars error. Values with different letters are significantly different at $p < 0.05$.

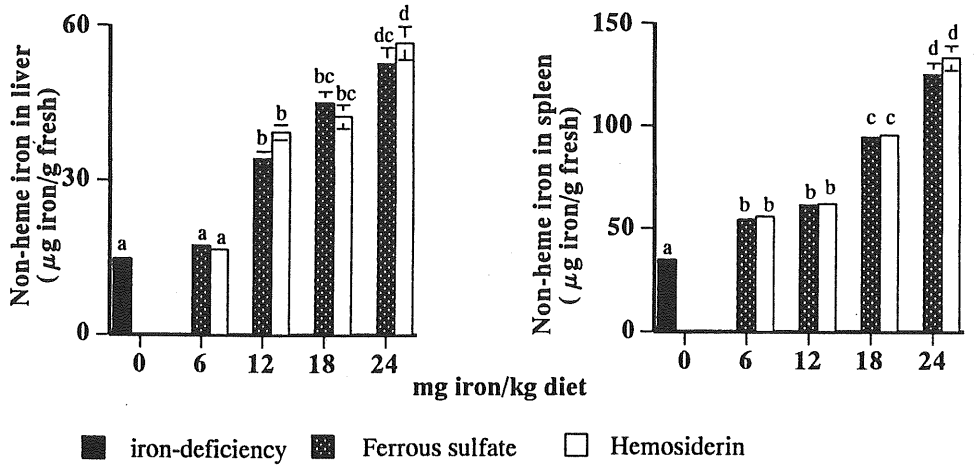

Fig. 2 Non-heme iron values in anemic rats fed with or without ferrous sulfate and hemosiderin. Values represent the means for six rats and the standard bars error. Values with different letters are significantly different at $p < 0.05$.

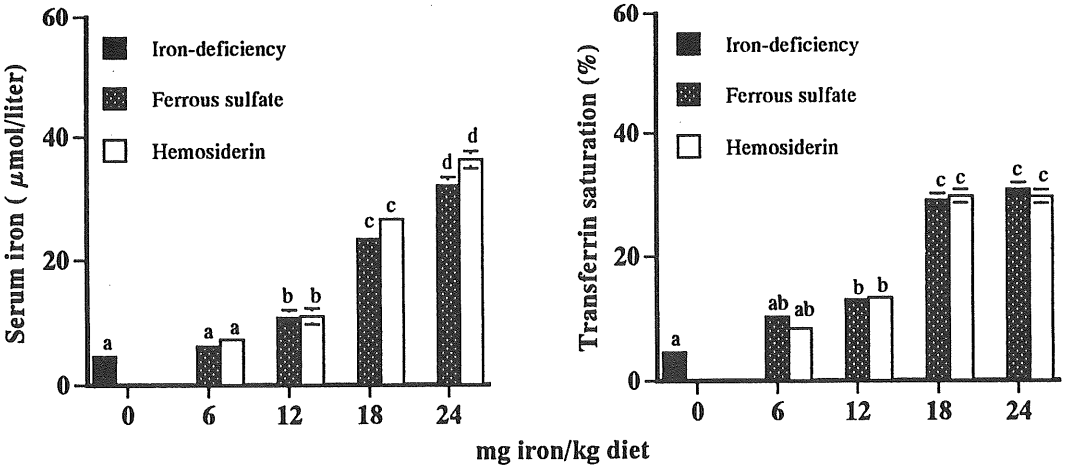

Fig. 3 Serum iron and transferrin saturation (%) values in rats fed with or without ferrous sulfate and hemosiderin. Values represent the means for six animals and the standard bars error. Values with different letters are significantly different at $p < 0.05$.

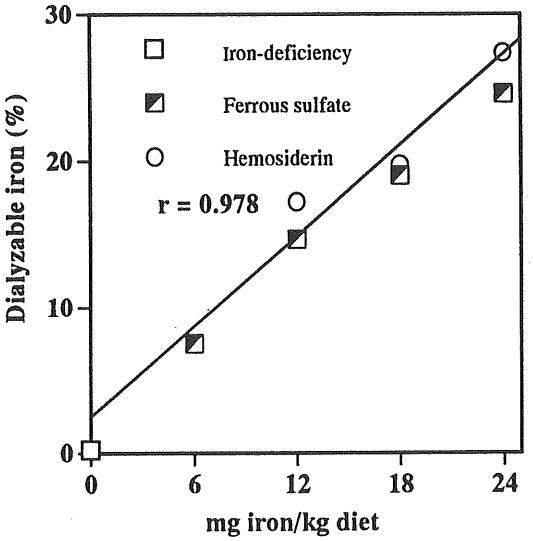

Fig. 4 Dialyzable of iron in experimental diet. Each point is a mean of duplicates.

siderin in diet increases iron absorption in animal and enhances or stimulates iron in diet; Which is supported by our finding of iron diffusion (dialyzable iron) from that diet into dialysate tube. The ability of the hemosiderin iron to enhance iron absorption or diffusibility (dialyzable iron) probably reflects the amino acid is present in hemosiderin.

The mechanism of stimulation of iron absorption by amino acid present in hemosiderin iron need further for studies. However, the data from animal experiments and in vitro studies can be encouraged that hemosiderin is a good source of iron for nutrition.

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