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Vitamin B-2와 (또는) Vitamin B-6 결핍이 흰쥐의 Hematologic Profile에 미치는 영향

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The Effect of Vitamin B-2 and (or) Vitamin B-6 Deficiency on Hematologic Profile in Rats

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국 문 초 록

Vitamin B-2와 Vitamin B-6 결핍이 hematologic profile에 미치는 영향을 in vivo계에서 알아보고자 하였다. 흰쥐에게 Vitamin B-2 결핍 (-B2)식이, Vitamin B-6결핍(-B6)식이, Vitamin B-2와 Vitamin B-6복합 결핍 (-B2-B6)식이 또는 통제 식이를 6주 동안 공급한 후 희생시켜 Hematocrit (Ht), Hemoglobin (Hb), 혈장철 (PI), Transferrin포화도 (TS), 간장철 (LI)를 비교하였다.

통제군에 비하여 -B2군에서는 PI, TS, LI가 유의하게 낮았으나 Ht와 Hb은 유의한 차이가 없으며, -B6군에서는 Ht와 Hb는 유의하게 낮았으나 LI는 유의하게 높았다. 통제군에 비하여 -B2-B6군에서는 Hb, PI, TS, LI가 -B2군과 -B6군의 중간수준이었으며 Ht는 낮은 수준을 나타냈다.

2주간의 보충식이 후에는 -B2군과 -B6군에서 모든 혈액지표와 LI가 개선된 것으로 나타났다. 본 연구의 결과는 Vitamin B-2와 Vitamin B-6의 섭취가 영양적 빈혈군에서 혈액 지표를 개선시켜 빈혈의 예방 또는 치료에 중요하다는 것을 시사한다.

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INTRODUCTION

Vitamin B-2 deficiency in animal and man has been associated with several disturbances of iron metabolism including a reduction in hemoglobin concentration and variable degrees of anemia[1,2]. This involvement may be via a reduction in iron absorption[3,4] or an increase in iron loss after absorption[5]. It has been suggested that vitamin B-6 may play some part in hemoglobin synthesis. Pyridoxal 5'-phosphate(PLP) is a cofactor for aminolevulinate synthetase (EC 2,3,1,37)(ALS), the first and rate limiting enzyme of heme synthesis[6]. The activity of ALS was markedly reduced in erythroblasts of a patient with sideroblastic anemia and vitamin B-6 treatment resulted in reconstitution of erythroblastic ALS activity with concomitant disappearance of all hematological abnormalities [7,8,9]. Also, in rat liver, pyridoxamin (pyridoxin) 5'-phosphate oxidase (EC 1,4,3,5) (PPO) activity is sensitive to riboflavin depletion[10]. This flavin mononucleotide dependent enzyme catalyzes the conversion of pyridoxamine 5'-phosphate and pyridoxine 5'-phosphate to PLP. Therefore, vitamin B-2 and vitamin B-6 may play some part in erythropoiesis directly or indirectly. The objectives of this study were (a) to investigate the effect of vitamin B-2 and (or) vitamin B-6 deficiency on hematologic status in vivo and (b) to determine the effect of vitamin B-2 and (or) vitamin B-6 deficiency on the iron content of liver which indicate the economy of storage iron.

MATERIALS and METHODS

Animals & diets

Table 1. Composition of diet

Components	percent
Casein-Vitamin-free[1]	20.0
DL Methionine	0.3
Cornstarch	15.0
Sucrose	50.0
Cellulose	5.0
Corn Oil	5.0
Choline bitartrate[2]	0.2
AIN mineral mix[3,5]	3.5
AIN vitamin mix without riboflavin and pyridoxine[4,5,6]	1.0

1. Difco lab. Detroit, Michigan.
2. Aakash Chemical Co. Maywood, Illinois.
3. Ishizu Pharmaceutical Co. Osaka, Japan.
4. Supported by F. Hoffmann-La Roche & Co. Ltd. 4002, Basle, Switzerland.
5. Mix was formulated as described(Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies(1977) J. Nutr. 107 : 1340-1348).
6. Based on the AIN-76 mouse-rat diet with the exception of vitamin B-2 and vitamin B-6. Supplemented with either 600mg of riboflavin or 700 mg of pyridoxine per Kg vitamin mixture for control diet or experimental diet as described in the text.

Fourty male weanling Sprague-Dawley rats (Seoul Nat. Univ. Exp. Animal Lab, Korea) were devided into 4 groups of 10 each. Each group was fed control diet or diets deficient in either vitamin B-2 (-B2) or vitamin B-6 (-B6) or both vitamin B-2 and vitamin B-6 ie, double deficient (-B2-B6). Animals were housed individually in wire bottomed stainless steel cages. Control group received a vitamin-free, casein-based semisynthetic diet which met AIN-76 recommendations (Table I). The composition of experimental diet was the same as that of.

Table 2. Effect of vitamin B-2 and (or) vitamin B-6 deficiency on body weight(BW) and food efficiency ratio(FER).[1,2]

		Group			
		Control	B2	B6	B2 B6
BW (g)	Initial[4]	65.2±16.5[a]	67.2±11.7[a]	64.8±7.4[a]	71.0±11.8[a]
	Deficiency[5]	143.6±12.0[a]	106.4±19.4[b]	136.2±10.1[a]	95.9±16.2[b]
	Repletion[6]	234.2± 8.0[ab]	236.0±21.2[ab]	243.3±15.7[a]	220.7±16.4[b]
FER	Deficiency	0.280±0.053[a]	0.180±0.037[b]	0.255±0.012[a]	0.128±0.015[c]
	Repletion	0.390±0.035[a]	0.504±0.032[bc]	0.459±0.041[c]	0.521±0.018[b]

1. Values are mean ±SEM, n=5
2. Within a given row, those values with different superscripts are significantly different ($P<0.5$).
3. B2 : vitamin B-2
   B6 : vitamin B-6
   B2 B6 : vitamin B-2 and B-6
4. Before feeding the deficient diets.
5. After feeding the deficient diets for 5 weeks.
6. After repletion with the control diet for 2 weeks.

control diet except that vitamin B-2 and (or) vitamin B-6 were not added to the vitamin mixture. The control diet was fed ad libitum for 1 week to adapt the animals to the diet and feeding schedule and to facilitate a similar metabolic status. At the end of the adaptation period, the rats were fed either control or one of the experimental diets for 5 weeks. Each group was pair-fed against the intake of the -B6 rats. At the end of week 6, from each group, 5 animals were sacrificed and the other 5 animals were repleted with the control diet for 2 weeks and sacrificed by decapitation under light ether anesthesia. Immediately following decapitation, blood was collected in heparinized tubes and liver was removed, blotted dry and weighed. The heparinized blood sample was used for hematocrit and whole blood hemoglobin concentration determination and was centrifuged to seperate plasma. Plasma and liver were stored at -20℃ until analyzed.

Biochemical analyses

Microhematocrit was determined by reading the percent red cell after centrifuge. Whole blood hemoglobin concentration was determined by cyanmethemoglobin method[11]. Plasma iron concentration and transferrin saturation were determined by a modification of the colorimetric method[12]. For measurement of total iron concentration in liver, liver laid in ashes at 500℃ for 24 hours. The ash was dissolved in a concentrated nitric acid and then diluted with 5% nitric acid to appropriate volums. Total iron concentration was measured by using atomic absorption spectrophotometer (Perkin-Elmer 2380, U.S.A.).

Statistical analyses

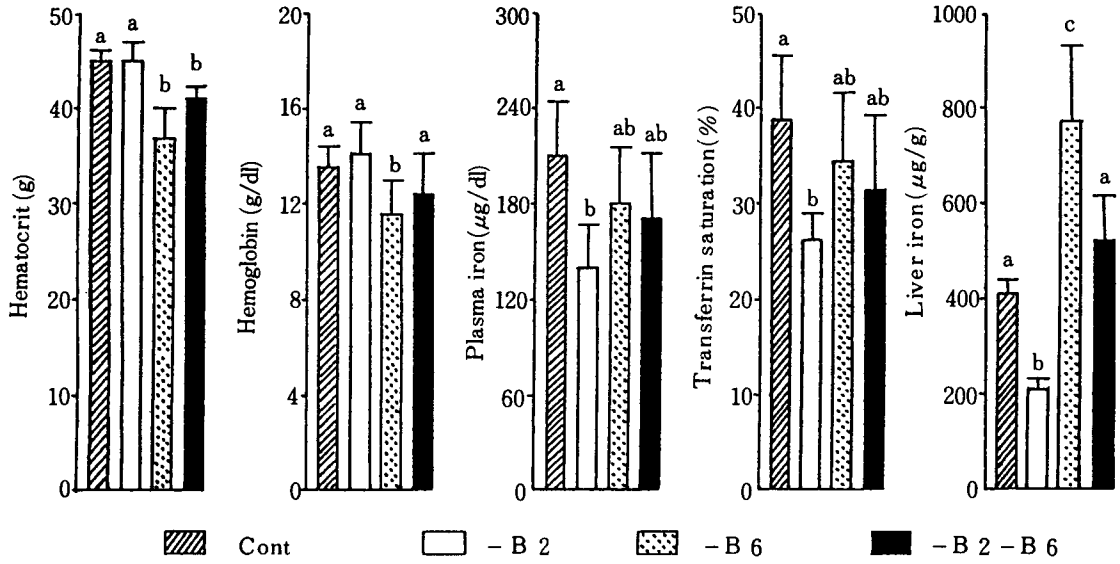

Fig. 1. Effect of vitamin B-2 and (or) vitamin B-6 deficiency on hematologic indices and liver iron concentration. (Each bar with different letters is significantly different($P<.05$).)
Cont=control group, −B2=vitamin B-2 deficient group, −B6=vitamin B-6 deficient group, −B2 −B6=vitamin B-2 and vitamin B-6 deficient group.

All data were evaluated by analysis of variance and test for significant differences by the least significant difference test[13]. A P value<0.05 was considered to be significant.

RESULTS

The effect of vitamin B-2 and (or) vitamin B-6 deficiency on body weight and food efficiency is shown in Table Ⅱ. The mean body weight and food efficiency (ratio of body weight change to food intake) of -B2 group and -B2-B6 group were significantly lower than those of control. The mean body weight and food efficiency of -B6 group were slightly lower than those of control. After -B2, -B6, and -B2-B6 groups were repleted with control diet for 2 weeks, the body weights of all groups except previous -B2-B6 group were essentially identical. The mean body weight of previous -B2-B6 rats increased but was significantly lower than the body weight observed in the control rats. Vitamin B-2 and vitamin B-6 status of rats were not measured directly because of the insufficient amount of plasma in deficient animals. The effect of vitamin B-2 and (or) vitmain B-6 deficiency on hematologic profile and iron concentration in liver is shown in Fig 1. In -B2 rats, plasma iron and transferrin saturation were lower than those of control. In both -B6 rats and -B2-B6 rats, hematocrit and hemoglobin were lower than those of control rats. Although the mean plasma iron concentration of the -B6 rats was 15% lower than that of

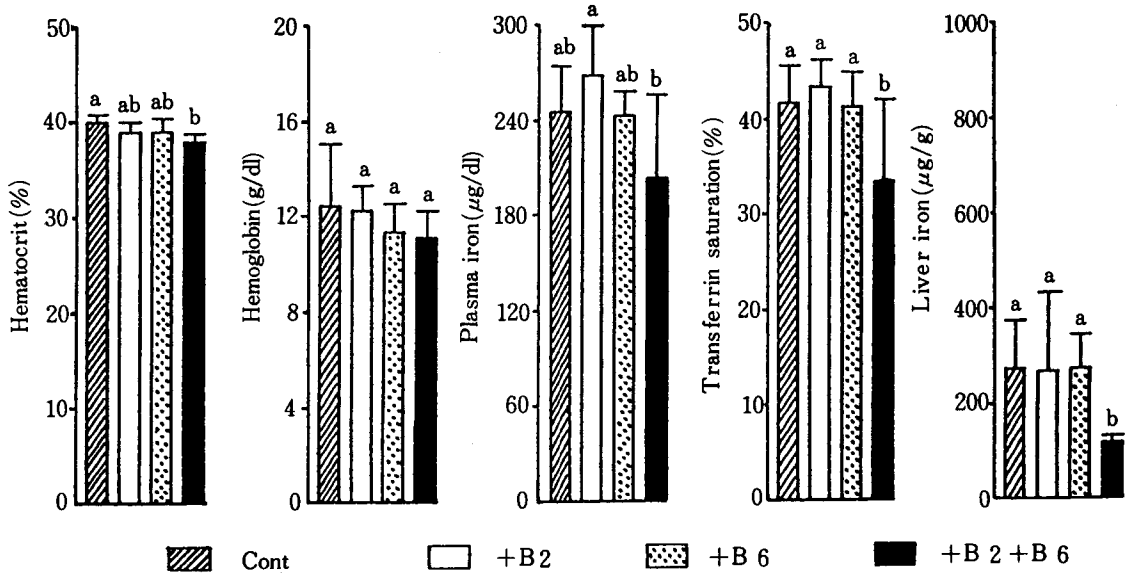

Fig. 2. Effect or vitamin B-2 and (or) vitamin B-6 repletion on hematologic indices and liver iron concentration. (Cont=control group, +B2=vitamin B-2 repleted group, +B6=vitamin B-6 repleted group, +B2+B6=vitamin B-2 and vitamin B-6 repleted group.

control rats, this difference was not significant because of the large standard deviation of both groups. In liver, -B2 had an effect in reducing iron concentration while -B6 had the effect in increasing iron concentration. A deficiency of both nutrients had an intermediate effect on liver iron. Fig. 2 shows hematologic indices and the iron concentration in liver of repleted rats. After vitamin B-2 and (or) vitamin B-6 repletion for 2 weeks on rats previously fed deficient diets for 5 weeks, all the hematologic indices and iron concentration in liver were similar among groups except previous -B2-B6 rats. In previous -B2-B6 rats, hematocrit and liver iron concentration were still significantly lower than those observed in control rats.

DISCUSSION

This study demonstrates that vitamin B-2 and (or) vitamin B-6 deficiency resulted in the changes in hematological indices in vivo.

The growth retardation was observed in rats fed vitamin B-2 and (or) vitamin B-6 deficient diet, although they were pair-fed. Following repletion with control diet, body weight of previously deficient rats was similar to that of control animals. This growth retardation was reported in vitamin B-2 and vitamin B-6 deficient animals[4][14][15]. Also, the other symptoms of vitamin B-2 or vitamin B-6 deficiency was observed in -B2 or -B6 rats. Thus, these rats were considered as vitamin B-2 or vitamin B-6 deficient animals by 6 weeks.

Although -B2 had no effect on depressing hemoglobin and hematocrit, plasma iron and transferrin saturation were significantly lower

than those of control. Because plasma iron and transferrin saturation are more specific indicators of early iron status than hemoglobin and hematocrit[16], -B2 rats might have some disturbances of iron metabolism. Another evidence of an impaired iron metabolism in -B2 rats was that the iron concentration in the liver of −B2 rats was significantly lower than that of control. Because liver is the main site of iron storage as either ferritin or hemosiderin[17], further vitamin B-2 deficiency may lead a lack of iron storage for hemopoietic system. These evidences may partly support the study in Gambia showing additional benefits on hematological status of treating anemia with riboflavin and iron compared with iron alone[19]. The impairement of iron metabolism in the liver of -B2 rats may be due to the reduced activity of NADH-FMN oxidoreductase (EC 1,6,8,1), the enzyme plays a role in the mobilization of iron from storage compartments[19] or may be associated with both a reduced absorption of iron and an elevated postabsorption loss[3][4][5].

In -B6 rat, because the iron concentration in liver was higher than that of control while hematological indices were lower than those of control, we suggest that the release of iron from liver into the plasma might be impaired and limit the amount of iron available for hemolobin synthesis as a result of a vitamin B-6 deficiency. It was reported that PLP is a cofactor for ALS, the first and rate-limiting enzyme of heme synthesis [6]. Thus, the sinificantly lowered hemoglobin and hematocrit in -B6 rats may be a result of an impaired hemoglobin synthesis in addition to a lack of available iron for hemopoietic system. The mechanism of the impairment in stored iron release of -B6 rats was not understood.

Because PPO, the enzyme catalyzes the conversion of the other phosphorylated forms of vitamin B-6 to PLP, activity is sensitive to riboflavin depletion[10], it was expected that -B2-B6 rats would further aggravate the hematologic indices of both -B2 rats and -B6 rats. In fact, the combined deficiency had an intermediate effect on hematologic indices and liver iron concentration. Because the greatest growth retardation was observed in -B2-B6 rats, depressing growth of -B2-B6 rats might have lowered demands of iron on the hemopoietic system due to the reduced expansion of blood volume. Thus, the combined deficiencies alleviated effects of either -B2 or -B6 deficiency alone. Although the impaired absorption of iron due to vitamin B-2 deficiency reduced iron concentration in liver, the impaired iron releasing activity of liver due to vitamin B-6 deficiency resulted in an accumulation of iron in the liver. Thus, high concentration of liver iron in -B2-B6 rats could not be considered as an available iron for hemopoietic system. Therefore, the above data support the hypothesis that vitamin B-2 and (or) vitamin B-6 deficiency impairs hemopoietic system in vivo.

Further support for vitamin B-2 and (or) vitamin B-6 requirement in hemopoietic system is provided by the effect of repletion with the control diet for 2 weeks. Following repletion, the iron concentration in plasma, liver and transferrin saturation of previonsly vitamin B-2 or vitamin B-6 deficient rats returned to those observed in the rats fed the control diet for 8 weeks. After repletion on previous -B2-B6 rats, vitamin B-2 and vitamin B-6 had to be utilized for an added demand of increased growth as well as an improvement in hematological status. The increase in body mass would increase the

body's requirement for the blood volume and thereby aggravated in vitamin B-2 and vitamin B-6 deficiency. Therefore, it appeared that the rats repleted with control diet were able to satisfy vitamin B-2 and vitamin B-6 requirements for the hemopoietic system but not enough for either iron utilization or iron mobilization from liver storage.

In conclusion, the observations from this study have shown that hematoloic status and liver iron concentration are greatly influenced by vitamin B-2 or vitamin B-6 deficiency. Also, a repletion of these vitamins on deficient animals improved the hematologic status. Thus, this study suggests that the intakes of vitamin B-2 and vitamin B-6 as well as iron itself are important for preventive and therapeutic approaches to improve the hematoloical status in nutritionally anemic groups.

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SUMMARY

The purpose of this study was to determine if vitamin B-2 and vitamin B-6 deficiency affects hematologic profile in vivo. Rats were fed a vitamin B-2 deficient (-B2) diet or a vitamin B-6 deficient (-B6) diet or a combined vitamin B-2 and vitamin B-6 deficient (-B2-B6) diet or a control diet for 6 weeks.

Hematocrit (Ht), hemoglobin (Hb), plasma iron (Pl), transferrin saturation (TS) and liver iron concentration (LI) were compared. In -B2 rats vs. control rats, PI, TS and LI were significantly lower but Ht and Hb were not. In -B6 rats vs. control, Ht and Hb were significantly lower but LI was higher. The -B2-B6 rats had intermediate effects in Hb, PI, TS and LI and a decrease in Ht. The repletion with a control diet for 2 weeks resulted in significant improvements in hematologic indices and LI in both -B2 rats and -B6 rats. This study suggests that the intakes of vitamin B-2 and vitamin B-6 are important for preventive and therapeutic approaches to improve the hematologic status in nutritionally anemic groups.

KEY WORDS : Vitamin B-2 deficiency · vitamin B-6 deficiency. Hematologic status. liver iron.

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