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Effect of Administration of Iron on the Lipid Concentrations in the RBC Membrane and Plasma

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철분 투여가 적혈구막 및 혈장내의 지질함량에 미치는 영향

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초 록: 철분투여가 용혈성 빈혈에 미치는 영향을 알아보기 위하여 흰쥐의 사료내에 철분을 혼합급여 또는 근육내에 주사하면서 60일간 사육하고 체중은 10일 간격으로 측정하였으며 적혈구의 취약성 실험과 적혈구막 및 혈장내의 vitamin E 함량과 인지질의 함량을 비색방법으로 측정한 결과 다음과 같은 성적을 얻었다.

증체량 측정에서는 A군의 190g에 비하여 C군 155g, B군 142g, D군 112g으로 철분함량이 높은 군에서 낮은 증가를 보였으며 적혈구 취약성 실험 결과 5마리 암수 평균값이 각각 A군 7.63% 및 2.68%, C군 10.84% 및 16.75%, B군 25.54% 및 33.62%, D군 47.23% 및 38.35%로 철분함량에 비례하여 각 실험군 사이에 유의한 증가를 나타내었다. 또한 적혈구막의 vitamin E 및 인지질의 함량은 5마리 암수 평균값이 각각 A군에서 18.54mg/dl 및 17.43mg/dl 그리고 120.55mg/dl 및 113.94mg/dl, C군에서 14.99mg/dl 및 10.56mg/dl 그리고 108.54mg/dl 및 99.97mg/dl, B군에서 13.10mg/dl 및 11.02mg/dl 그리고 91.67mg/dl 및 87.45mg/dl, D군에서 6.86mg/dl 및 8.87mg/dl 그리고 79.78mg/dl 및 70.12mg/dl로서 철분 투여량에 비례적으로 감소하였다.

혈장내의 vitamin E 및 인지질의 함량은 A군, C군, B군, D군의 순으로 각각 함량이 2.51mg/dl 및 31mg/dl, 1.94mg/dl 및 21mg/dl, 1.37mg/dl 및 20mg/dl 그리고 1.37mg/dl 및 15mg/dl로 철분 투여량에 비례적으로 감소하는 경향을 나타내었다.

Introduction

Iron is one of the most abundant elements in the earth's crust, but the body of a normal adult weighing 70kg contains only 3-4g of iron,[10] approximately 65% is in hemoglobin, 3% in myoglobin and 30% in the form of storage compounds ferritin and hemosiderin.[10] The remainder is distributed among the heme enzymes, the nonheme enzymes and the iron transport protein transferrin (siderophilin).[9]

The most important function of iron is to combine with protoporphyrin to form heme and the heme combines with various proteins to form the heme proteins.[9] If the protein is a globin, hemoglobin and

myoglobin are formed. If protein is an apoenzyme, heme enzymes are formed. Heme enzymes are of fundamental importance in the vital respiratory processes of life, namely, in the cytochrome system, the ultimate site of O_2 utilization.[9]

Food iron is predominantly in the ferric state, tightly bound to organic molecules. In the stomach, where the pH is less than 4, Fe^{+3} can be dissociated and react with low-molecular-weight compounds such as fructose, ascorbic acid, citric acid, and amino acids to form complexes that will allow Fe^{+3} to remain soluble at the neutral pH of intestinal fluid.[10]

Feeding various combinations of polyunsaturated fatty acid composition and iron content to premature infants, Williams *et al.*[20] observed a significant hydrogen peroxide-induced hemolysis and vitamin E deficiency anemia. Melhorn and Gross[12] reported that oral iron supplement group showed significantly low mean hemoglobin and serum vitamin E concentrations with higher reticulocyte count and hydrogen peroxide fragility in an experiment with 186 premature infants.

With rats fed on a synthetic diet, Golberg and Smith[6] observed the vitamin A and E deficiency with increased ceroid pigment formation. It is reported by Marvin *et al.*[11] that lifetime of Cr^{51} tagged erythrocytes, autologously transfused, is severely shortened in monkeys maintained with vitamin E deficient diet.

Ritchie *et al.*[17] reported that infants had been fed commercial formulas with iron and a high content of polyunsaturated fatty acid resulted in a low ratio of vitamin E to fatty acids. When vitamin E(alpha-tocopherylacetate), 75 to 100 IU daily, was given separately orally to five infants available for treatment and study, serum tocopherol level rose, reticulocyte count fell to normal and erythrocyte survival time lengthened. This was followed by recovery from the anemia, clearing of the edema and subsidence of the thrombocytosis.

Reports mentioned above could be summarized that the development of vitamin E deficiency anemia occurs in infants and experimental animals receiving iron supplementation. However, study about the mechanism of shortented RBC life-time with vitamin E deficiency by administration of iron could hardly be found.

It was assumed that the shortened survival time of erythrocyte due to the decreased concentration of vitamin E with iron supplement might be attributed to the functions of the membrane structural substances. Therefore, this study was attempted to understand the effect of iron on the vitamin E concentrations in the RBC membranes and plasma. Phospholipids and lipid concentrations were also determined. RBC fragility test with hydrogen peroxide was performed to support the assumption.

Thus, the data from this experiment as well as other laboratories support the suggestion that excess iron supplementation induces vitamin E deficiency, lipid peroxidation and then increase in RBC fragility.

Materials and Methods

Chemical: The agent used in the present study was ferric hydroxide, $Fe(OH)_3$, which is an odorless crystal with 99% purity(Junsei Chem. Co., Japan).

Experimental animals and designs: Forty growing rats (5-week-old) of the wistar strain were used in all the trials. The animals were randomly assigned to groups of 10 (groups A through D), consisting of 5 of each sex in a group, and housed in wire cages with a wire-meshed bottom. They were acclimatized in the cages for a week by feeding a control, basal ration. $Fe(OH)_3$ was mixed with the basal ration (Woo Sung Feed Co.) at the appropriate concentrations and given to the respective the animals groups.

Experimental design and conduct: Animals in group A were fed basal ration only, that is, control group. Group B were fed basal ration and administered 3ml of 1.5g/dl $Fe(OH)_3$ intramuscularly into alternate hind limbs every week. Group C were fed 2.5% $Fe(OH)_3$ supplementedration and group D were fed 5% $Fe(OH)_3$ supplemented-ration. All experimental animals of each group were bred for 60 days.

Blood sampling and analytical methods.: Blood sample was obtained by heart puncture under light ether anesthesia after 60 days of iron-administration The blood was instantaneously mixed with EDTA (mg/ml of blood) to prevent clotting.

Body weight was measurede very 10 days with tap

loading balance. REC fragility test was conducted by the method of Gerden *et al.*[7] Vitamin E concentrations in REC membrane and plasma were determined by the method of Nair and Magar[14] and phospholipid concentrations in REC membranes and plasma by the method of Huh *et al.*[8] with use of Hitachi Model 250 double beam spectrophotomer.

Before this experiment was started, vitamin E and iron concentrations had been measured in the basal and iron supplemented-rations.

Results

Vitamin E and iron concentrations in the basal and iron supplemented-ration are shown in Table 1. In the basal ration, iron and vitamin E concentrations were 40mg/kg and 63.4mg/kg, respectively. But iron and vitamin E concentrations in the diet of group C and group D were 13,496mg/kg, 50.6mg/kg and 26,973mg/kg, 39.7mg/kg, respectively.

Table 1. Vitamin E and Iron Concentrations in Basal and Iron Supplemented Rations

	Basal ration	2.5% $Fe(OH)_3$ Supplemented ration	5% $Fe(OH)_3$ Supplemented ration
Vitamin E (mg/kg)	63.4	50.6	39.7
Iron, as Fe (mg/kg)	40	13,496	26,973

The effects of iron on the average body weight gains of growing rats are shown in Figure I. The growth rates were retarded in proportion to the iron levels supplemented to basal ration.

Effect of iron on the REC membrane fragilities are shown in Table 2. REC fragility rates of male and female in group A were 7.62% and 2.68%, respec-

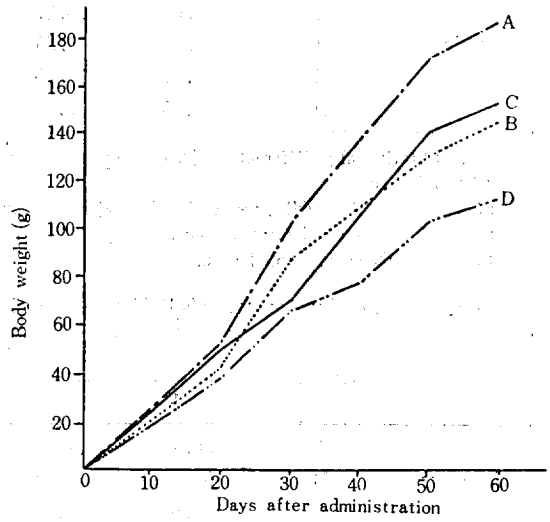

Fig. 1. Effect of administration of iron on body weight in growing rats.
A : Control group.
B : 3ml of 1.5g/dl $Fe(OH)_3$ per week I.M. injection group.
C : 2.5% $Fe(OH)_3$ supplemented group.
D : 5% $Fe(OH)_3$ supplemented group.

Table 2. Effect of Iron on the Cell Membrane Fragilities of RBC of Rats(%)

Sex	Group	Individual No.					Mean
		1	2	3	4	5	
Male	A	4.35	8.00	8.00	11.54	6.25	7.63[a]
	B	38.89	25.00	12.50	26.18	25.14	25.54[b]
	C	7.41	11.11	12.50	10.06	13.10	10.84[a]
	D	50.00	58.33	29.41	50.25	48.17	47.23[c]
Female	A	3.45	0.00	4.00	3.70	2.25	2.68[a]
	B	35.29	42.86	34.19	31.62	24.12	33.62[b]
	C	11.54	14.81	23.08	18.08	16.25	16.75[c]
	D	25.00	37.50	46.15	42.86	40.26	38.35[b]

a, b, c : Statistically different ($p<0.05$) between different superscripts within each sex-group.
A : Control group.
B : 3ml of 1.5g/dl $Fe(OH)_3$ per week I.M. injection group.
C : 2.5% $Fe(OH)_3$ supplemented group.
D : 5% $Fe(OH)_3$ supplemented group.

Table 3. Effect of Iron on Vitamin E Concentration in RBC Membrane of Rats(mg/dl)

Sex	Group	Individual No.					Mean
		1	2	3	4	5	
Male	A	18.18	19.80	18.30	18.03	18.37	18.54[a]
	B	10.53	15.31	13.31	12.33	14.01	13.10[b]
	C	13.34	16.13	14.17	15.42	15.91	14.99[c]
	D	7.62	5.53	7.90	6.92	6.31	6.86[d]
Female	A	18.07	18.37	15.03	17.63	18.03	17.43[a]
	B	10.02	9.56	11.29	12.11	12.09	11.02[b]
	C	7.41	12.75	10.20	10.29	12.17	10.56[b]
	D	15.63	9.18	7.56	5.04	6.92	8.87[b]

a, b, c, d : Statistically different ($p<0.05$) between different superscripts within each sex-group.
A : Control group.
B : 3ml of 1.5g/dl $Fe(OH)_3$ per week I.M. injection group.
C : 2.5% $Fe(OH)_3$ supplemented group.
D : 5% $Fe(OH)_3$ supplemented group.

Table 4. Effect of Iron on Phospholipid Concentrations in RBC Membrane of Rats(mg/dl)

Sex	Group	Individual No.					Mean
		1	2	3	4	5	
Male	A	130.25	102.77	124.74	125.00	119.99	120.55[a]
	B	92.44	85.54	98.12	90.09	92.16	91.67[b]
	C	112.97	109.44	106.67	108.33	105.28	108.54[c]
	D	81.59	83.89	73.75	79.38	80.29	79.78[d]
Female	A	112.73	118.99	112.00	112.02	114.03	113.94[a]
	B	80.00	94.49	88.78	84.92	89.08	87.45[b]
	C	103.66	104.00	93.69	96.51	102.02	99.97[c]
	D	62.37	98.24	66.39	60.36	63.22	70.12[d]

a, b, c, d : Statistically different ($p<0.05$) between different superscripts within each sex group.
A : Control group.
B : 3ml of 1.5g/dl $Fe(OH)_3$ per week I.M. injection group.
C : 2.5% $Fe(OH)_3$ mixed group.
D : 5% $Fe(OH)_3$ mixed group.

tively. But those of both sexes in groups B, C, and D were 25.54% and 33.62%, 10.84% and 16.75%, and 47.23% and 38.35%, respectively.

Effect of iron on vitamin E concentrations in RBC membrane are shown in Table 3. Vitamin E concentrations of male and female in group A were 18.54 mg/dl and 17.43mg/dl, respectively. But vitamin E concentrations of male and female in groups B, C, and D were 13.10mg/dl and 11.02mg/dl, 14.99mg/dl and 10.56mg/dl, and 6.86mg/dl and 8.87mg/dl, respectively.

Effect of iron on phospholipid concentrations in RBC membrane are shown in Table 4. Phospholipid concentrations of male and female in group A were 120.55mg/dl and 113.94mg/dl, respectively. But phospholipid concentrations of male and female in group B, C and D were 91.67mg/dl and 87.45mg/dl, 108.54mg/dl and 99.97mg/dl, and 79.78mg/dl and 70.12mg/dl, respectively.

Effect of iron on vitamin E concentrations in pooled plasms are shown in Figure 2. Vitamin E concentrations of group A, B, C and D were 2.51mg/dl, 1.37 mg/dl, 1.94mg/dl and 1.37mg/dl, respectively. In this experiment, plasma is pooled, because the volume

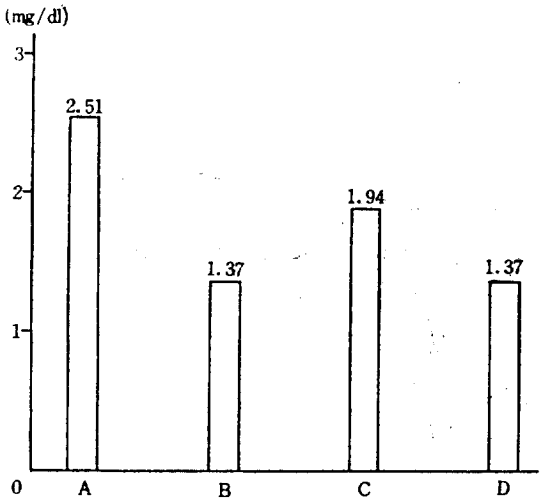

Fig. 2. Effect of iron on vitamin E concentrations in the pooled plasma of rats.

A : Control group
B : 3ml of 1.5g/dl $Fe(OH)_3$ per week I.M. injection group
C : 2.5% $Fe(OH)_3$ mixed group
D : 5% $Fe(OH)_3$ mixed group

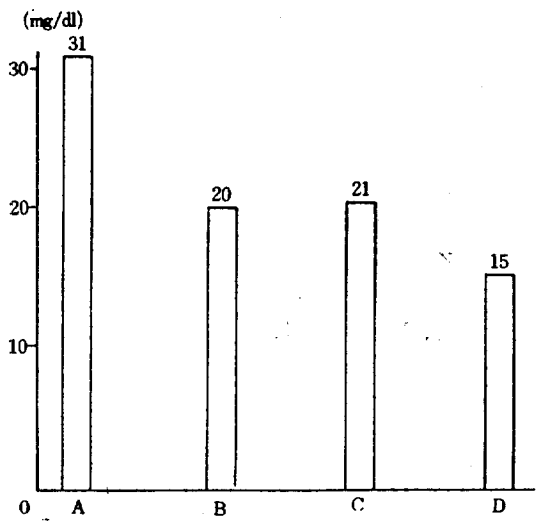

Fig. 3. Effect of iron on phospholipid concentrations in the pooled plasma of rats.

A : Control group
B : 3ml of 1.5g/dl $Fe(OH)_3$ per week I.M. injection group
C : 2.5% $Fe(OH)_3$ mixed group
D : 5% $Fe(OH)_3$ mixed group

of each blood sample was not sufficient for analysis.

Effect of iron on phospholipid concentrations in pooled plasma are shown in Figure 3. Phospholipid concentrations of group A, B, C, and D were 31mg/dl, 20mg/dl, and 15mg/dl, respectively.

Discussion

It has long been known that only certain portion of the introduced iron could be absorbed through gastrointestinal mucosa by active regulatory mechanism[10]; hence overloading of iron is toxic. It is a common practice in human or animal clinic that iron is administered orally or by direct injection to treat the iron deficient anemia, therefore, the treatment frequently caused hemolytic anemia.

Golberg and Smith,[6] Melhorn and Gross,[12] Williams *et al.*[20] and Ritchie *et al.*[17] reported that iron overloading in infants or young animals was the cause of hemolytic anemia and vitamin E deficiency.

This study was attempted to verify the mechanisms of RBC survival time shortened with vitamin E deficient and decrement of phospholipid concentrations by administration of iron.

The concertration of iron in basal ration (group A and group B) was 40mg/kg of feed, and the final concentrations of iron in the $Fe(OH)_3$ supplemented-rations (group C and group D) were 13,496mg/kg and 26,973 mg/kg, respectively. These values were nearly agreed with the expected ones. The concentrations of vitamin E in basal diet (group A and B) was 63.4mg/kg, and the concentrations in the $Fe(OH)_3$ supplemented-diets were 50.6mg/kg and 39.7mg/kg in group and D, respectively. This result shows that vitamin E concentrations were decreased by increasing the concentrations of iron.

These facts were nearly coincided with DeLuca's[5] report that administration of iron oxidized vitamin E. Deluca[5] reported that RBC fragility test was the indication of vitamin E deficiency.

We could see that RBC of iron treated-rats was very fragile to hydrogen peroxide compared with that of control, therefore hemolytic anemia was caused by the inactivation and subsequent decrease of vitamin E.

Accordingly, vitamin E deficiency was induced by iron adminstration. Vitamin E and phospholipid contents in the pooled plasma and RBC membranes were significantly ($p<0.05$) decreased by iron administration compared with those of control group.

Bunyan *et al.*[4] reported that vitamin E deficiency

induced peroxidation of lipids. However, the decrease in phospholipid content of RBC membrane was not investigated by them. [1~4,6,7,10,12,13,15~20])

Conclusion

The effects of iron on the concentrations of vitamin E and phospholipid in the RBC membrane and plasma and RBC fragility were investigated with use of wister rats. The rats were devided into 4 experimental groups, in which 5 animals of each sex were included:

Group A were fed basal ration, that is, control group. Group B were fed basal ration and administered 3ml of 1.5g/dl $Fe(OH)_3$ by intramuscular injection per week. Group C were fed 2.5% $Fe(OH)_3$ supplemented-ration. Group D (male: 5, female:5) were fed 5% $Fe(OH)_3$ supplemented-ration all experimental groups were maintained for 60 days with feeding on the respective ration.

The results obtained were summarized as follows:

Supplementation of $Fe(OH)_3$ by 5% in ration significantly ($p<0.05$) increased in the cell membrane fragilities of RBC (male: 48.17%, female: 40.26%) compared with that of the control group (male: 6.25%, female: 2.68%). On the other hand, vitamin E (male: 6.86mg/dl, female: 8.87mg/dl) and phospholipid concentrations (male: 79.78mg/dl, female: 70.12mg/dl) in RBC membrane were decreased compared with those of the control group (male: 18.54 mg/dl, female: 17.43mg/dl, and male: 120.55mg/dl, female: 113.94mg/dl, respectively).

Supplementation of $Fe(OH)_3$ by 2.5% in ration and injection of iron intramusculary increased in the cell membrane fragilities of RBC (male: 13.10%, 25.14%, female: 16.25%, 33.62%, respectively) compared with those of the control group while the effects were not prominent compared with those of the 5% $Fe(OH)_3$ supplemented-group. However, vitamin E and phospholipid concentrations were decreased (male: 15.91mg/dl, 14.01mg/dl female: 12.17mg/dl, 12.09mg/dl, respectively) compared wfith those of control group.

Consequences of plasma vitamin E and phospholid levels in each groups were similar to those of RBC membrane.

Therefore, the followings are the conclusions of this study:

1. Vitamin E deficiency is induced by iron administration.
2. Decrement of phospholipid concentrations are induced by iron administration.
3. Phospholipids are abnormally decreased by vitamin E deficiency.
4. Hemolytic anemia is induced by increment of H_2O_2 fragility.

References

1. Bender, A.D., Schottelius, D.D. and Schottelius, B.A.: Effect of vitamin E deficiency on protein composition of guinea pig skeletal muscle. Proc. Soc. Exp. Biol. Med. (1959) 102:362.
2. Binder, H.J., Herting, D.C., Hurst, V., Finch, S.C. and Spiro, H.M.: Tocopherol deficiency in man. New Engl. J. Med. (1965) 273:1289.
3. Binder, H.J., Spiro, H.M. and Finch, S.C.: Autohemolysis in tocopherol deficiency secondary to steatorrhea. Am. J. Med. Sci. (1966) 90:686.
4. Bunyan, J., Green, J., Edwin, E.E. and Diplock, A.T.: Studies on vitamin E. 5. Lipid peroxidation in diapuric acid-induced hemolysis of vitamin E-deficient erythrocytes. Biochem. J. (1960) 77:47.
5. DeLuca, H.F.: Handbook of lipid research. 2. The fat-soluble vitamins, Plenum Press, New York. (1978) p.141.
6. Golberg, L. and Smth, J.P.: Vitamin A and E deficiencies in relation to iron overloding in the rat. J. Pathol. Bacteriol. (1960) 80:173.
7. Gordon, H.H., Nitowsky, H.M. and Cornblath, M.: Studies of tocopherol deficiency in infants and children. 1. Hemolysis of erythrocytes in hydrogen peroxide. Am. J. Dis. Child. (1955) 90:669.
8. Huh, R.S., Kim, S.H., Mo, K.C. and Park, H.K.: A simple and rapid colorimetric method of determining phospholipids in serum and red cell membrane. Agric. Res. Bull. Kyungpook Natl. Univ. (1984) 2:73.
9. Kaneko, J.J.: Clinical biochemistry of domestic animals, 3rd ed., Academic Press. (1980) p.649.
10. Martin, D.W., Mates, P.A. and Rodwell, V.

W.: Harper's review of biochemistry, 18th ed., Lange Medical Pub. (1983) p.559.
11. Marvin, H.N., Dinning, J.S. and Day, P.L.: Erythrocyte survival in vitamin E deficient monkeys. Proc. Soc. Exp. Biol. Med. (1960) 105: 473.
12. Melhorn, D.K. and Samuel Gross, M.A.: Vitamin E dependent anemia in the premature infant. 1. Effect of large doses of medicinal iron. J. Ped. (1971) 79:569.
13. Mengel, C.E., Kann, H.E., Heyman, A. and Metz, E.: Effect of in vivo hyperoxia on erythrocytes. 2. Hemolysis in human after exposure to oxygen under high pressure. Blood (1965) 25:822.
14. Nair, P.P. and Magar, N.G.: Determination of vitamin E in blood. J. Biol. Chem. (1956) 220: 157.
15. Nitowsky, H.M., Cornblatch, M. and Gordon, H.H.: Studies of tocopherol deficiency in infants and children. 3. Plasma tocopherol and erythrocyte hemolysis in hydrogen peroxide. Am. J. Dis. Child. (1956) 92:164.
16. Oski, F.A. and Barness, L.A.: Vitamin E deficiency: A previously unrecognized cause of hemolytic anemia in the premature infant. J. Ped. (1967) 70:211.
17. Ritchie, J.H., Frsh, M.B., McMasters, V. and Grossman, M.: Edema and hemolytic anemia in premature infants. New Engl. J. Med. (1968) 279:1185.
18. Rose, C.S. and Gyorgy, P.: Specificity of hemolytic reaction in vitamin E deficient erythrocytes. Am. J. Physiol. (1952) 168:414.
19. Rose, C.S. and Gyorgy, P.: Tocopherol requirements of rats by means of the hemolytic test. Proc. Soc. Exp. Biol. Med. (1950) 74:411.
20. Williams, M.L., Shorr, R.J., O'Neal, P.L. and Oski, F.A.: Role of dietary iron and fat on vitamin E deficiency anemia of infancy. New Engl. J. Med. (1975) 292:887.