Effect of Ascorbate Supplementation on Iron Storage and Hematopoiesis of Vitamin A Deficient Rats

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Introduction

Role of vitamin A in iron metabolism has been demonstrated in several previous studies[1-10]. Vitamin A deficiency leads to anaemia with a concomitant accumulation of iron in liver, spleen and other organs[4,5,8]. It is suggested that in absence of vitamin A, iron is somehow arrested in liver storage resulting in anaemia for want of iron. Mobilization of stored iron in liver occupies an important area in intracellular iron-metabolism. A smooth mobilization of stored iron is a pre-condition for its proper utilization in the synthesis of hemoglobin and other iron containing compounds. Amongst several other factors, ascorbic acid has been implicated to the mobilization of stored iron[11-12]. Level of ascorbic acid has been reported to be decreased in rats as a result of vitamin A deficiency, though the decrease was not enough to produce the signs of ascorbate deficiency[13]. Since ascorbicacid is active in the mobilization of stored iron, it was assumed that anaemia as well as larger deposition of liver iron in vitamin A deficient rats might be due to some reason (s) secondary to the immobilization of stored-iron in absence of adequate ascorbic acid. Thus, the present study was undertaken where attempts were made to investigate the effect of ascorbate supplementation on (i) hematopoietic parameters as well as on (ii) tissue iron accumulation in vitamin A deficient rats with lower level of ascorbic acid.

Materials and Methods

Twenty four post weaning rats of Long-Evans strain weighing 75.25 ± 5.32 (mean ±S. D) g were made deficient in vitamin A by feeding them vitamin A deficient diet (table-1). Control rats (six) were provided with same diet supplemented with vitamin A (8000 I. U/kg. diet). Vitamin A deficient and control diets were not supplemented with ascorbic acid, considering that rats can synthesize their own ascorbic acid. Rats fed

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vitamin A deficient diet developed vitamin A deficiency after a period of 8 weeks, as was indicated by an initial weight plateau amongst them. After depletion, hemoglobin and hematocrit of all rats were determined by collecting blood from tail tips. Control rats and a representative group of deficient rats (six) were then sacrificed under mild chloroform anesthesia by decapitation. Serum samples and organs such as liver, spleen and brain were harvested and preserved at -18°C in a deep freeze for biochemical analysis. This was done as a base line study. Rest eighteen vitamin A deficient rats, in three groups, were then repleted with (i) vitamin A, (ii) ascorbic acid and (iii) vitamin A plus ascorbic acid. Repletion was continued for a period of 3 weeks. Dosage of vitamin A and ascorbic acid were (8000 I.U/kg. diet) and (1 g/kg. diet) respectively. Dosage of ascorbic acid was selected according to Gruber et al[20]. After supplementation, hemoglobin and hematocrit of rats were determined by collecting blood from tail-tips and then sacrificed. Serum samples and organs were harvested and preserved, as in baseline study, for biochemical analysis.

Analytical Methods- Hemoglobin was determind by cyanmethemoglobin method[14] using commercial kit (Sigma). Hematocrit was determined by microcentrifugal method[15]. Vitamin A in serum was estimated by HPLC[16]. Liver vitamin A was determined colorimetrically by using TCA[17]. Ascorbic acid was estimated colorimetrically by using 2,4-dinitrophenylhydrazine as chromogenic reagent[18]. Serum iron was determined colorimetrically by using orthophenanthroline as chromogenic reagent[19].

Statistical Analysis- Difference between two means was determined by Student's t-test.

Results

Table-2 shows the effect of vitamin A deficiency on the level of ascorbic acid, hematopoietic parameters and tissue iron storage of rats. Rats fed vitamin A deficient diet have been found to have significantly lower level of vitamin A in liver and serum as compared to control. Vitamin A deficient rats have been found to have lower concentrations of ascorbic acid in liver, spleen and serum as compared to cntrol. Brain ascorbic acid, however, remained unaffected. Vitamin A deficient rats showed significant decrease in hemoglobin, hematocrit and serum iron as compared to the control. Moreover, vitamin A deficient rats have been found to have larger deposition of iron in liver and spleen. As depicted in Table-3, Vitamin A deficient rats upon repletion with vitamin A or vitamin A plus ascorbic acid or

ascorbic acid alone exhibited significant restoration of ascorbic acid in liver, spleen and serum. Ascorbic acid concentration was restored to control level.

Vitamin A deficient rats supplemented with vitamin A plus ascorbic acid showed greater concentrations of ascorbic acid in liver, spleen, brain and serum as compared to those supplemented with either vitamin A or ascorbic acid alone. Thus supplementation of either ascorbic acid or vitamin A were found to restore the normal concentration of ascorbic acid in liver, spleen and serum of vitamin A deficient rats. Moreover, vitamin A deficient rats upon repletion with vitamin A or vitamin A plus ascorbic acid showed greater concentrations of ascorbic acid in liver, spleen, brain and serum as compared to those supplemented with either vitamin A or ascorbic acid alone. Thus supplementation of either ascorbic acid or vitamin A were found to restore the normal concentration of ascorbic acid in liver, spleen and serum of vitamin A deficient rats.

Moreover, vitamin A deficient rats upon repletion with vitamin A or vitamin A plus ascorbic acid showed a complete restoration of hematopoiesis with normal levels of hemoglobin, hematocrit and serum iron. Liver iron and splenic iron also decreased significantly with the supplementation of either vitamin A or vitamin A plus ascorbic acid. Administration of ascorbic acid alone failed to bring about a significant increase in hemoglobin, hematocrit and serum iron of vitamin A deficient rats. Accumulation of iron in liver and spleen of vitamin A deficient rats did not decline following the supplementation of ascorbic acid alone, instead liver iron and splenic iron were found to be on increase, though not significantly.

Table 1 : Composition of vitamin A deficient diet[a].

Ingredients	Percentage
Rice flour	50
Skimmed milk powder[b]	35
Soybean oil	5
Vitamin A free vitamin mixture[c]	6
Salt mixture[d]	4

a Vitamin A deficient basal diet is a semisynthetic diet rich in all nutrients except vitain A and carotene. It was prepared according to Barua et al (21).

b Skimmed milk powder was procured from local market and was washed with hot ethanol.

c Vitamina A free vitamin mixture was prepared according to Embree et al[22]. Vitamins were premixed with a bulk of rice flour to provide the required amount of vitamins per kg diet when mixed in 6% combination.

d Salt mixture was prepared according to the recommendation of American Institute of Nutrition[23].

Discussion

In present experiment, ascorbate concentration in tissue and serum of vitamin A deficient rats decreased remarkably over those of control (Table-2), though the level of ascorbic acid was not low enough to produce the signs of deficiency. This finding is in accordance with the previous observations[24-26]. Sure et al[24] reported decreased concentration of ascorbic acid in vitamin A deficient rats. Tissue and urinary levels of ascorbic acid were found to be significantly decreased in vitamin A deficient rats by Mohanram et al[25], which fact they attributed to decreased storage of ascorbic acid or reduction in L-gulono - gamma - lactone oxidase activity. Thus, it is now considered that ascorbate biosynthesis is impaired by vitamin A deficiency, resulting in its lower concentration in tissue and serum. Moreover, vitamin A deficiency in rats (table-2) caused anaemia with lower levels of hemoglobin, hematocrit and serum iron, accompanied with a larger deposition of iron in liver and spleen. It is in agreement with previous observations[4-10] which also demonstrated anaemia during vitamin A deficiency. Hodges et al[10] demonstrated lower levels of hemoglobin and liver ascorbic acid in vitamin A deficient rats. Notably serum ascorbic acid was not reduced to deficient level.

Ascorbic acid is very often regarded as compound involved in iron metabolism in various capacities especially in reductive absorption and reductive release of iron from its stores. In ascorbutic guineapigs[12], reticuloendothelial release of iron is defective. This defect is rapidly corrected when ascorbic acid is administered. The sudden rise in plasma iron concentration when human subjects with scurvy are given ascorbic acid[27], indicates the existence of similar situation in man. Another feature of ascorbic acid deficiency in guineapigs was found to be increased hemosiderin : ferritin ratio in liver and spleen[28]. The ratio is rapidly reversed to normal by the supplementation of ascorbic acid. Since minimum level of tissue ascorbic acid required for the mobilization of stored iron is not yet confirmed, it was assumed that decrease in tissue ascorbic acid, whatever is caused by vitamin A deficiency, might be related to immobilization of iron in vitamin A deficient rats. In present study (table-3), ascorbate supplementation alone could neither correct anaemia nor could reduce iron accumulation caused by vitamin A deficiency, though the ascorbate concentration in tissue was restored to normal levels. On the other hand, under similar condition, administration of either vitamin A or vitamin A plus ascorbate

were able to restore hematopoiesis to normal level with a concomitant decrease of iron in tissue. Thus, presence of vitamin A itself made the difference. Though ascorbic acid concentration was not low enough to produce ascorbutic condition in rats, the decrease in tissue ascorbic acid, whatever is caused by the deficiency of vitamin A, appears to be unrelated to anaemia and iron accumulation in vitamin A deficient rats.

Summary

Effect of ascorbate supplementation on hematopoiesis and tissue iron accumulation of vitamin A deficient rats was investigated in present study. For the purpose, weaning rats of Long Evans strain were made deficient in vitamin A by feeding them vitamin A deficient diet for a period of 8 weeks until they showed weight plateau. Vitamin A deficient rats, in three groups, were then supplemented with (i) vitamin A (ii) ascorbic acid and (iii) vitamin A plus ascorbic acid for a period 3 weeks. Vitamin A deficient rats had significantly ($p<0.001$) lower levels of serum ascorbic acid, tissue ascorbic acid, hemoglobin,

Table 2. Effect of vitamin A deficiency on tissue ascorbic acid, hematopoiesis and tissue iron storage in rats.

Measures	Groups of rats	
	Control	Vitamin A deficient
Liver ascorbic acid (ug/g.)	333.87± 20.83[a]	212.31 ± 13.37[b]
Splenic ascorbic acid (ug/g)	360.54 ± 28.70[a]	234.28 ± 17.58[b]
Brain ascorbic acid (ug/g)	270.89 ± 21.86[a]	255.82 ± 16.93[a]
Serum ascorbic acid (ug/dl)	818.44 ± 68.36[a]	521.06 ± 31.13[b]
Hemoglobin (g/dl)	14.42 ± 0.53[a]	10.67 ± 0.25[b]
Hematocrit (%)	42.88 ± 1.29[a]	32.67 ± 1.54[b]
Serum iron (ug/dl)	153.85 ± 11.15[a]	105.03 ± 12.47[b]
Liver iron (ug/g)	197.12 ± 13.78[a]	275.87 ± 15.20[b]
Splenic iron (ug/g)	268.16 ± 15.54[a]	373.17 ± 21.52[b]
Liver vitamin A (ug/g)	185.50 ± 10.57[a]	13.51 ± 1.25[b]
Serum vitamin A (ug/dl)	65.21 ± 5.20[a]	15.22 ± 1.55[b]

Values are mean ± S. D. ($n = 6$). Values in the same line bearing different superscripts are significantly ($p< 0.001$) different.

Table 3. Effect of supplementations of vitamin A, ascorbic acid and both vitamin A and ascorbic acid administered together on tissue ascorbic acid, hematopoiesis and tissue iron storage of vitamin A deficient rats.

Measures	Groups of rats			
	Vitamin A deficient (Base limiting)	Vitamin A deficient upon repletion with		
		Vitamin A	Vitamins A + Ascorbic acid	Ascorbic acid
Liver ascorbic acid (ug/g)	212.31 ± 14.37[a]	348.55 ± 17.29[b]	512.74 ± 20.76[c]	404.96 ± 20.68[d]
Splenic ascorbic acid (ug/g)	234.28 ± 17.58[a]	354.68 ± 14.83[b]	496.75 ± 24.85[c]	388.64 ± 18.35[b]
Brain ascorbic acid (ug/g)	255.82 ±16.93[a]	286.37 ± 13.33[a]	309.31 ± 11.68[b]	293.73 ± 15.94[b]
Serum ascorbic acid (ug/dl)	521.06 ± 31.13[a]	714.83 ± 26.61[b]	995.18 ± 36.18[c]	740.93 ± 30.58[b]
Hemoglobin(g/dl)	10.67 ± 0.25[a]	14.76 ± 0.18[b]	14.57 ± 0.33[b]	11.07 ± 0.27[a]
Hematocrit (%)	32.67 ± 1.54[a]	41.80 ± 1.15[b]	41.88 ± 1.29[b]	43.46 ± 0.64[a]
Serum iron (ug/dl)	105.03 ± 12.47[a]	160.84 ± 13.10[b]	173.84 ± 11.15[b]	115.03 ± 10.97[a]
Liver iron (ug/g)	275.87 ± 15.20[a]	197.47 ± 16.28[b]	201.92 ± 11.78[b]	308.28 ± 10.66[a]
Splenic iron (ug/g)	373.17 ± 21.52[a]	263.77 ± 17.04[b]	283.80 ± 17.09[b]	389.08 ± 15.14[a]
Liver vitamin A (ug/g)	14.51 ± 1.25[a]	170.31 ± 18.81[b]	166.46 ± 10.53[b]	12.02 ± 1.44[a]
Serm Vitamin A (ug/dl)	15.22 ± 1.55[a]	50.50 ± 5.35[b]	53.75 ± 4.32[b]	16.21 ± 1.11[a]

Values are mean ± S. D. (n = 6). Values in the same line not bearing common superscript letter are significantly (P < 0.001) different.

hematocrit and serum iron; but significantly (P < 0.001) higher storage of iron in liver and spleen. Administration of vitamin A or vitamin A plus ascorbic to vitamin A deficient rats was followed by a cmplete restoration of tissue ascorbic acid and normal hematopoiesis, with a concomitant decrease of iron in liver and spleen. Under similar condition, administration of ascorbic acid alone to vitamin A deficient rats could neither correct anaemia nor could reduce iron accumulation, though tissue ascorbic acid was restored to control level. Thus presence of vitamin A made the difference. Lower concentration of tissue ascorbic acid, whatever is caused by the deficiency of vitâmin A, appears to be unrelated to anaemia and iron accumulation in vitamin A deficient rats.

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