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The Inhibitory Effect of Vitamin E on Desferrioxamine-Induced Iron Excretion in Rats[1] (39372)

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Iron overload is a major limiting factor in the life expectancy of patients with severe forms of congenital hemolytic anemia such as thalassemia major, particularly in those patients who are participating in hypertransfusion programs. Treatment with iron chelating agents has been introduced in an attempt to reduce excessive iron accumulation in these patients. The most commonly used iron chelating drug is desferrioxamine (DF), which has been shown to interact mainly with parenchymal iron deposits with subsequent excretion in urine and bile (1). Peroxidation of membrane lipids and proteins was described in thalassemic erythrocytes in association with markedly reduced serum levels of alpha tocopherol (vitamin E) a known antioxidant (2). Preliminary studies have shown that some parameters reflecting membrane peroxidation can be corrected by the administration of large doses of vitamin E (3). It is probable that both antioxidants and iron chelating drugs will be employed simultaneously in the future management of thalassemic patients. The present study was undertaken in order to evaluate the effect of vitamin E on the mobilization and excretion of iron following DF administration to normal and iron overloaded rats.

Methods. Female Wistar rats (Hadassah strain) weighing 170 to 200 g were used throughout. Injections were performed through the tail veins. Animals were sacrificed under ether anesthesia by exsanguination into heparinized syringes through the abdominal aorta. Parenteral iron overload was induced by four consecutive injections of 12 mg of iron dextran within 2 weeks. All procedures involving iron analysis were carried out in iron-free glassware. Ferritin was isolated from tissue homogenates by the method of Fulton and Ramsay (4) employing ammonium sulfate, incubated overnight in 3 *N* HCl at 37° and its iron content was measured colorimetrically. Total non-heme iron was determined by the method of Torrance and Bothwell (5). Serum vitamin E levels were determined by the method of Hashim and Schuttringer (6).

Counting techniques. The radioactivity of spleen, weighed portions of the liver, and 1-ml samples of blood were determined in an automatic well-type scintillation counter (Packard model 3003 spectrometer). Whole body counts were performed in a small animal counter (Packard model 447, Packard Instruments Co., Downers Grove, Illinois). Radioiron excretion was determined by whole body counts on the first and last days of the study, with corrections for decay and differences in geometry. Hepatic radioactivity was determined by relating sample counts to total organ weight. Residual radioactivity was defined as whole body radioactivity minus the combined activities of blood, liver, and spleen (1).

Preparation and initial processing of ^{59}Fe-ferritin. In vivo-labeled ferritin was prepared by injecting 200 μCi citrated ^{59}Fe into iron overloaded rats. The animals were sacrificed 24 hr later, and purified radioactive ferritin was prepared by the method of Bjorklid and Helgeland (7). Acrylamide gel electrophoresis of the ferritin preparation at pH 8.5 produced a single protein band, and a single precipitin line was demonstrated on immunodiffusion against antiferritin serum. The specific activity of radioferritin was 4 μCi per mg iron. The initial processing of soluble ferritin ^{59}Fe has been described in

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detail in a previous communication (1). Briefly, 89 ± 1% of the injected dose were located to the liver within 1 hr. The cellular distribution of radioactivity in the liver, determined by a quantitative autoradiographic method (8) was 0 to 3% in R.E. cells and 97 to 100% in hepatic parenchymal cells. The half time disappearance of intravenously injected soluble ferritin was 16 ± 1 min. Within 1 day of injection about 80% of the hepatic radioactivity could be recovered in the ferritin fraction of tissue homogenates, and the rest in soluble nonferritin iron.

Outline of study. Three weeks prior to labeling, 40 rats were divided into two equal groups of normal and iron-overloaded animals. In addition, half of each group was given 50 i.u. of dl-alpha-tocopherol (Hoffman La Roche, Nutley, New Jersey) by gastric tube daily for 3 weeks prior to radioiron labeling, and continued throughout the study. On the first day of study all animals were labeled by iv injection of ^{59}Fe ferritin at a dose representing 40 μg of iron per rat. Thereafter, one-half of each group was treated by injections of 10 mg of DF (Desferral, Ciba Pharmaceutical Co., Summit, N.J.) twice daily for 10 consecutive days.

Results. The mean serum vitamin E level of the normal and iron overloaded groups of rats was 0.68 ± 0.15 and 0.95 ± 0.15 mg/dl (± SD), respectively. Following vitamin E administration there was a twofold increase in serum vitamin E levels in both groups to 1.43 ± 0.29 and 1.75 ± 0.21 mg/dl, respectively. The effect of vitamin E on iron chelation in normal, and iron overloaded animals was evaluated by the measurement of total hepatic nonheme iron and ferritin iron stores (Table I). In normal controls 62 ± 4% of the total hepatic iron was found in ferritin. DF administration resulted in a significant reduction of ferritin iron in both control and vitamin E-treated groups, and a similar, but less pronounced decrease in total nonheme iron. Unlike normal animals in iron overloaded rats, DF-induced reduction in ferritin iron and total nonheme iron was completely inhibited following vitamin E administration.

From the above results, one could not distinguish between a direct effect of vitamin E and DF on hepatic iron stores, or an extracellular effect manifested indirectly in altered rates of storage iron accumulation. The former possibility was examined by employing ^{59}Fe-ferritin as a selective parenchymal radioiron probe. As shown in Table II, DF-induced radioiron excretion was restricted to hepatic parenchyma. The reduction in hepatic Fe^{59} activity induced by DF administration was almost equal to the enhancement of whole body ^{59}Fe excretion found in the same animals. No significant changes in radioactivity could be observed in any of the other organs examined. Vitamin E administration did not alter radioiron excretion following DF treatment in normal rats. However, in iron overloaded rats the effect of DF was completely abolished when vitamin E was given.

The possibility of a direct effect of vitamin E on ferritin iron chelation was studied *in vitro* by incubating aliquots of ^{59}Fe-ferritin representing 50 μg of iron with 1 mmole of DF with or without the addition of 10 u of vitamin E, followed by overnight dialysis in Tris-HCl buffer 0.175 *M* at pH 8.4. Only 0.2 ± 0.1% of the radioactivity were removed by dialysis from the control sample

TABLE I. TOTAL HEPATIC NON-HEME IRON AND FERRITIN IRON.[a]

	Normal		Iron Overload	
Treatment	Nonheme	Ferritin	Nonheme	Ferritin
Control	1626 ± 106†	1009 ± 98	15,110 ± 658	4872 ± 206
Control + DF	1311 ± 40*	560 ± 52***	10,602 ± 1277**	3600 ± 373**
Vitamin E	1588 ± 111	733 ± 72	13,926 ± 1206	4393 ± 192
Vitamin E + DF	1313 ± 120 n.s.	551 ± 46*	15,425 ± 1066 n.s.	4540 ± 202 n.s.

[a] Micrograms of Fe per organ.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
† Mean ± 1 SE.

TABLE II. ORGAN DISTRIBUTION OF RADIOACTIVITY AT 10d (PERCENTAGE OF INJECTED ^{59}Fe-FERRITIN).

Condition	Treatment	Blood	Liver	Spleen	Residual	Excretion
Normal						
	Control	14.5 ± 1.6†	76.5 ± 2.5	0.7 ± 0.1	2.3 ± 0.2	6.0 ± 0.9
	Control + DF	13.7 ± 0.7	56.9 ± 1.3***	0.8 ± 0.1	1.6 ± 1.2	27.0 ± 0.7***
	Vitamin E	15.3 ± 1.2	72.9 ± 4.0	0.7 ± 0.1	5.4 ± 3.8	5.7 ± 1.3
	Vitamin E + DF	15.4 ± 2.6	54.2 ± 3.5***	0.7 ± 0.1	2.0 ± 2.4	27.7 ± 0.8***
Iron overload						
	Control	3.8 ± 0.5	75.9 ± 2.4	0.8 ± 0.1	12.1 ± 2.3	7.4 ± 1.9
	Control + DF	4.8 ± 0.5	59.9 ± 1.9***	0.9 ± 0.1	18.2 ± 3.1	16.2 ± 1.4***
	Vitamin E	4.1 ± 0.2	57.6 ± 8.8	1.3 ± 0.6	20.0 ± 9.5	7.0 ± 0.6
	Vitamin E + DF	5.2 ± 0.6	69.4 ± 4.1 n.s.	0.9 ± 0.1	15.5 ± 5.8	9.0 ± 2.3 n.s.

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
† Mean ± 1 SE.

without DF, as compared to 27.7 ± 0.1% with DF and 28.9 + 0.9% with DF and vitamin E.

Discussion. The mode of action of the iron chelating agent DF has been studied extensively in man and in experimental animals (9). Using selective radioiron probes of various iron compartments, it was shown that DF interacts primarily with hepatocellular ferritin (1). Chelated iron is subsequently eliminated through biliary and urinary excretion. DF-induced iron excretion is closely related to the magnitude of iron stores, and its measurement is widely used as a diagnostic tool in the assessment of iron overload (9). However, factors other than the size of iron stores might affect the amounts of iron removed by DF. Depletion of vitamin C, often associated with iron overload, was shown to reduce response to DF. Conversely, vitamin C administration results in a marked enhancement of DF-induced iron excretion (10). Very low serum vitamin E levels were found in patients with β-thalassemia major with varying degrees of iron overload (2). Thus, it was postulated that in analogy with vitamin C, the administration of vitamin E might also modify iron chelation by DF.

In the present study, DF-induced iron excretion was completely inhibited when vitamin E was administered in pharmacological doses to rats with iron overload. Theoretically, this inhibition could be the result of either (a) direct interference with ferritin–DF interaction; (b) increased breakdown of DF by microsomal drug-metabolizing systems, or (c) prevention of membrane lipid peroxidation in iron overloaded animals, resulting in reduced permeability to either DF or the DF–iron complex. The first possibility is least likely since in the present study, the addition of vitamin E did not inhibit *in vitro* ^{59}Fe-ferritin chelation by DF. Experimental evidence in support of vitamin E action on drug metabolism and membrane protection is available (11, 12), but to what extent this is related to the present findings is as yet unknown. The inability to demonstrate a similar inhibitory effect of vitamin E on DF-induced iron chelation in normal rats is probably explained by their smaller ferritin pool, for which partial inhibition of a relative excess of DF may not be a limiting factor.

Long-term clinical studies employing vitamin E and desferrioxamine in the management of thalassemic patients with transfusion-induced iron overload are ongoing in several centers. The demonstration of an inhibitory effect of vitamin E on DF-induced iron excretion may have significant practical implications in the planning of therapy for thalassemic patients with severe secondary iron overload.

Summary. The influence of vitamin E on the mobilization and excretion of storage iron induced by DF was studied in normal and iron-overloaded rats. Vitamin E administration in pharmacologic doses resulted in complete inhibition of the effect of DF on storage iron in iron-overloaded rats while no such effect could be demonstrated in rats with normal iron stores. The mechanism of the observed inhibition of DF action by vitamin E is at present unknown. Nevertheless this drug interaction has to be considered in view of ongoing therapeutic trials

where both antioxidants and iron chelating drugs are administered simultaneously to thalassemic patients with transfusion induced iron overload.

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