CONCISE REPORT

Nontransferrin-Bound Iron in Plasma From Hemochromatosis Patients: Effect of Phlebotomy Therapy

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Plasma from patients with iron overload resulting from idiopathic hemochromatosis contains nontransferrin-bound iron, measurable by the bleomycin assay. During venesection therapy, the concentration of bleomycin iron declines in a way highly correlated with plasma ferritin concentrations. Even when patients had been venesected to give very low total plasma iron concentrations and high transferrin iron-binding capacity, bleomycin-detectable iron was still present at low concentrations. Bleomycin-detectable iron can stimulate damaging free radical reactions, and its persistence in plasma even after prolonged venesection might contribute to the tissue damage that results from iron overload.

EVIDENCE EXISTS THAT nontransferrin-bound iron is often present in plasma from iron overloaded patients with hemochromatosis. The presence of such iron has been demonstrated by several methods, including the bleomycin assay. Bleomycin is an antibiotic that binds weakly to iron ions, and a bleomycin-iron complex degrades DNA in the presence of ascorbic acid as a reducing agent. When the bleomycin assay is applied to a biologic sample, bleomycin, ascorbate, and DNA are in excess, so that the amount of DNA degradation is proportional to the concentration of iron ions available to bleomycin. When applied to human plasma samples, the bleomycin assay does not measure iron bound to ferritin or transferrin. Studies in the authors' laboratories strongly suggest that most or all of the "bleomycin iron" detected in plasma from iron overloaded hemochromatosis patients represents complexes of iron ions with low-molecular-mass organic ligands such as citrate (Grootveld et al, submitted for publication).

Gutteridge et al studied ten hemochromatosis patients at different stages of venesection therapy and found that the concentration of nontransferrin-bound plasma iron measured by the bleomycin assay was not obviously correlated with the concentration of total plasma nonheme iron, except that bleomycin-detectable iron was only present if total plasma iron was greater than approximately 40 μmol/L. Gutteridge et al also showed that bleomycin-detectable iron can stimulate lipid peroxidation and other free radical reactions, which are suspected to be of importance in the pathology of iron overload.

Peters et al studied 14 patients with clinical iron overload, four with hemochromatosis and ten with transfusional iron overload. Six of the patients had bleomycin-detectable iron in their blood plasma, and the amount of this iron appeared to be correlated with the plasma ferritin concentrations. Four of these patients had incompletely saturated plasma transferrin, one with only 72% saturation. This raises the possibility that nontransferrin-bound, bleomycin-detectable iron can persist in the presence of transferrin iron-binding capacity.

In this report we have carried out studies on a clearly defined group of iron-overloaded hemochromatosis patients from first diagnosis through venesection therapy. The aim of the study was to investigate the proposed relationship of bleomycin-detectable iron to plasma ferritin concentrations and the possible coexistence of such iron with unsaturated transferrin iron-binding capacity.

MATERIALS AND METHODS

Plasma samples were prepared by centrifugation of blood taken during venesection therapy of patients with established iron overload due to idiopathic hemochromatosis. Diagnosis was based on grossly elevated plasma ferritin, increased plasma iron, high percentage transferrin saturation, and liver biopsies, stained for inorganic iron, showing grade four siderosis in all cases at presentation. All patients gave informed consent for their blood samples to be studied. The bleomycin method was carried out as previously described. Total nonheme iron and iron binding capacity were measured by the ferrozine assay. Ferritin concentrations were determined by an enzyme-linked immunosorbent assay (ELISA) technique using an antibody raised against human liver ferritin.

RESULTS

Studies were conducted on seven patients (A through G) presenting at the Liver Unit of King's College Hospital, London, with iron overload as a result of idiopathic hemochromatosis. Plasma samples from these patients at first presentation, and at different times during venesection therapy, were analyzed by the bleomycin method. Total iron-binding capacity, ferritin protein, and nonheme iron were also measured on each sample, allowing a calculation of the percentage of transferrin saturation with iron. In five patients (B through F), venesection therapy caused complete clearance of hepatic parenchymal iron, as shown by liver biopsy. In two patients (A and G), therapy was still continuing when the last sample was taken for analysis (Table 1).

All seven patients studied in this series had bleomycin-
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Control values: Bleomycin-detectable iron, nil; ferritin, 18 to 440 ng/mL; (healthy subjects) transferrin iron saturation, 16% to 40%; total nonheme iron, 16 to 30 μmol/dm³.

detectable iron in their plasma at first presentation, at concentrations ranging from 7.0 to 21.7 μmol/L. Plasma samples from these patients were able to stimulate the peroxidation of phospholipid liposomes (assayed as described by Gutteridge et al⁶), confirming the ability of the bleomycin-detectable iron to accelerate free radical reactions. Of 173 control plasma samples from healthy adults of similar ages, or from noniron-overloaded patients with a range of diseases, none showed any bleomycin-detectable iron. Five patients had a transferrin saturation of 100% (no plasma iron-binding capacity) at first presentation, and in two others it was close to saturation (98% in patient E and 95% in patient A).

Further plasma samples from each patient, obtained during venesection therapy, were analyzed, and the results are summarized in Table 1. Linear regression analysis of the combined results from all seven patients showed a weak correlation between the concentration of bleomycin-detectable iron and plasma ferritin (r = .64) and a similar weak correlation with total plasma nonheme iron (r = .55). However, when data were analyzed for each patient from whom four or more plasma samples had been obtained, it was found that the concentration of bleomycin-detectable iron was highly correlated with plasma ferritin (r = .95 to .98) but less well with plasma nonheme iron (r = -.46 to .85).

As venesection therapy continued, the concentration of bleomycin-detectable iron fell in successive plasma samples, as did plasma ferritin and total iron. Transferrin iron-binding capacity appeared in the plasma, yet concentrations of bleomycin-detectable iron did not fall to zero for any patient. For example, patient F became anemic (4.0 μmol/L nonheme iron; 7% transferrin saturation), yet 0.75 μmol/L of bleomycin-detectable iron was still present in his plasma (Table 1).

**DISCUSSION**

Previous reports⁶⁹ have shown that nontransferrin-bound iron, as measured by the bleomycin assay, can sometimes be detected in plasma from hemochromatosis patients. However, the patients studied appeared to be at several different stages in treatment⁶⁹. Our results are the first to be obtained from a well-defined group of patients, studied from first diagnosis and during therapy. All of them were highly iron-loaded at first presentation, and all showed bleomycin iron in plasma; the ability of this iron to accelerate lipid peroxidation⁶ was confirmed.
Peters et al. reported a patient with 74% transferrin saturation whose plasma still contained bleomycin-detectable iron. Our studies confirm and extend these observations. As transferrin iron-binding capacity appeared in the plasma as a result of venesection, bleomycin-iron concentrations fell, but not to zero. Hence, nontransferrin-bound iron can persist even when there is a large amount of transferrin iron-binding capacity, eg, in patient F. How can this be explained? First, it is possible that transferrin in these patients is abnormal in its iron-binding properties, although this was not evident from total iron-binding capacity determinations. Our studies (submitted for publication) strongly suggest that bleomycin-detectable iron largely exists as iron ions liganded to low-molecular-mass organic molecules such as citrate. The rate of transfer of iron ions from such ligands onto transferrin is known to be quite slow¹¹ (our unpublished data). It has been implied¹ that, in hemochromatosis, low-molecular-mass iron complexes enter the circulation from the gut. If this is so, it may be that a considerable time is necessary for iron to move from these complexes onto transferrin. Studies on rats and mice¹²,¹³ show that liver has an efficient uptake system for low-molecular-mass iron complexes. If this is also true of human liver, it follows that the amount of bleomycin-iron measured at any time would be a balance between its entry from the gut and its clearance from plasma by the liver. Our finding of bleomycin iron in venesected patients might explain previous results suggesting increased hepatic uptake of plasma iron even in treated patients with hemochromatosis.¹⁴

When all patient data (Table 1) were analyzed, only a weak correlation of bleomycin-detectable iron to ferritin concentrations was apparent. However, data for each individual patient during venesection therapy showed a much better correlation. The wide variation in ferritin concentrations seen in patients with marked iron overload (eg, Table 1) might explain why the correlation is less obvious when data from several patients are combined for analysis. The meaning of a correlation between bleomycin iron and plasma ferritin is not clear. It has been suggested¹⁵ that plasma ferritin in iron overload disease might originate from damaged hepatocytes. It is possible that a damaged liver might be less able to clear nontransferrin-bound iron from the circulation, producing a rise in bleomycin-detectable iron and a correlation with plasma ferritin. Iron bound to transferrin is not able to participate in damaging free radical reactions,⁶,¹⁶ but nontransferrin-bound iron seems to be very effective in promoting radical-dependent tissue damage to such molecules as lipids, proteins, and DNA.⁶,¹⁶,¹⁷

Our data thus confirm the presence of low-molecular-mass iron in plasma from hemochromatosis patients, even when their plasma transferrin is far from saturation. A possible interpretation of our data is that, in this disease, iron enters the plasma from the gut in a nontransferrin-bound form that is rapidly cleared by the liver and does not bind quickly to any transferrin available. Thus the concentration of bleomycin-detectable iron in plasma at any time would presumably reflect a balance between entry of iron from the gut and its clearance by the liver.

It seems likely that the concentration of bleomycin-detectable iron in the plasma of these seven patients will eventually fall to zero, since we have found several well-treated patients, on maintenance venesection for several years, whose plasma does not show bleomycin-detectable iron. Follow-up studies are being carried out on some of the patients studied here to investigate this point. Perhaps the bleomycin assay could be used to help determine the most effective regimen for venesection therapy, in view of the established ability of bleomycin-detectable iron to accelerate free radical reactions.

REFERENCES


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