Alterations in tissue ferritins in iron storage disorders

LAWRIE W. POWELL, LEONARD V. McKEERING, AND JUNE W. HALLIDAY

From the Department of Medicine, University of Queensland, Royal Brisbane Hospital, Brisbane, Australia

SUMMARY Purified tissue ferritins isolated from Bantu subjects with gross haemosiderosis, from a patient with idiopathic haemochromatosis (HC) treated by phlebotomy, and from rats with experimental iron overload were studied in order to determine the significance of the abnormality previously demonstrated in tissue isoferitins in patients with IHC. The isoferitin profile of the tissues from the Bantu subjects and the iron-loaded rats showed a similar abnormality to that previously found in patients with untreated IHC—that is, an abnormally uniform distribution of iron-containing isoferitins with an increase in the more basic isoferitins and an apparent absence of the more acidic ones. In contrast, tissues from the patient with treated IHC, who was iron depleted at the time of death, showed the normal organ-specific isoferitin distribution. These findings strongly suggest that the abnormal distribution of tissue isoferitins in IHC is an acquired phenomenon and unlikely to be related to an underlying genetic defect in ferritin or iron metabolism.

Ferritin is the major iron storage protein in mammalian tissues and is found, or may be induced by iron administration, in most organs (Granick, 1951; Munro and Drysdale, 1970). Many tissue ferritins have been resolved into multiple isoferitins by the technique of isoelectric focusing (Drysdale, 1970; Urushizaki et al., 1971; Drysdale, 1974; Powell et al., 1975). These isoferitins do not represent differences in aggregation or iron content of the protein but intrinsically different apoferritin shells (Drysdale, 1970; 1974). Each tissue appears to have a characteristic isoferitin composition (Drysdale, 1974; Powell et al., 1975). This organ-specific isoferitin profile has recently been shown to be markedly abnormal in patients with untreated primary idiopathic haemochromatosis (Powell et al., 1974). The present study was designed to determine whether the abnormality in tissue isoferitins in idiopathic haemochromatosis is directly related to the genetic defect in iron metabolism or the result of long-standing iron overload.

Methods

HUMAN TISSUES

Necropsy tissues were obtained from a normal adult male accident victim and also from a patient with classical familial idiopathic haemochromatosis whose case history and family history have been documented previously (Powell, 1965). This patient had been treated by repeated phlebotomy for 11 years and was in a state of marginal iron deficiency at the time of his death at the age of 74 years. Histological examination showed no stainable iron in any of the tissues examined. Tissues from two Bantu patients with gross haemosiderosis were kindly provided by Professor R. Charlton of Johannesburg.

ANIMAL STUDIES

Ten male Sprague Dawley rats weighing 250 to 300 g were each given 1 g/kg body weight of iron as iron-dextran by intramuscular injection over a period of two weeks. The rats were maintained on a normal diet for three months and then killed by exsanguination from the dorsal aorta under ether anaesthesia. The carcasses were then perfused with phosphate-buffered saline (PBS), pH 7.4, and the liver, spleen, heart, and kidneys were removed. Samples of tissue were taken for histological examination, and ferritin was prepared from the remainder. Organs from 60 normal rats were used to prepare normal rat tissue ferritins.

PREPARATION OF TISSUE FERRITINS

Ferritin was isolated from tissue samples by a modification of the method of Drysdale and Munro (1965) as described previously (Powell et al., 1974; 1975). Each tissue ferritin preparation was subjected to polyacrylamide gel electrophoresis as a test of purity, staining for both protein by Coomassie blue.
Blue, and iron by potassium ferrocyanide. Each preparation showed only one major protein band in the position corresponding to the ferritin monomer and one other band corresponding to the ferritin dimer. Both protein bands stained for iron.

**Gel Electrofocusing (GEF)**

This was performed in the pH range 5-7 as previously described by Righetti and Drysdale (1971) in gels containing 4% polyacrylamide and 2% ampholyte. The gels were precooled to 4°C and a current of 0.5 mA/gel was applied until the voltage reached 400 V. Samples containing 20-30 μg ferritin in 10% glycerol were then added, and the current applied for 18 h at 400 V. The gels were stained for protein with Coomassie Blue or for iron using potassium ferrocyanide.

**Results**

The organ-specific distribution and differing iron content of the various isoferritins previously demonstrated in normal human tissues (Powell et al., 1975) was also confirmed in this study (Fig. 1a). In contrast, ferritins isolated from each of three tissues from the Bantu subjects with gross haemosiderosis showed the same abnormality as was found previously in patients with untreated haemochromatosis (Powell et al., 1974)—that is, a uniform distribution of iron-containing isoferritins with an increase in the more basic isoferritins and an apparent absence of the more acidic isoferritins, especially in heart (Fig. 1b). A similar pattern was observed in the tissue ferritins prepared from rats with experimental iron overload, although in these animals the more acidic isoferritins were still detectable in heart ferritin (Fig. 2). Histological examination of the rat tissues revealed gross haemosiderosis of liver, spleen, and kidney but only minimal iron staining in the heart, mainly in macrophages lying between the muscle bundles. In both the Bantu and the rat tissue ferritins the iron content of the more basic isoferritins was increased (Figs. 1, 2). In the normal rats there was an organ-specific variation in tissue
study has shown that the abnormal tissue isoferititin distribution previously demonstrated in patients with idiopathic haemochromatosis is also found in Bantu subjects with iron overload. This finding strongly suggests that the abnormal distribution of tissue isoferititins in haemochromatosis is an acquired phenomenon and unlikely to be related to an underlying genetic defect in ferritin or iron metabolism. This conclusion was supported by the finding of a similar abnormality in tissue ferititins of iron-loaded rats and further by the finding of a normal organ-specific variation in tissue isoferititin profile in a patient with treated haemochromatosis, who was iron-depleted at the time of death. In both the chronic iron overloaded human tissues and in the iron-loaded rats, the more basic tissue isoferititins were prominent in all organs examined. These more basic isoferititins also had a higher iron content than the acidic isomers (Powell et al., 1974; Drysdale, 1974). This increase in iron content of the protein is not, however, the cause of the abnormality demonstrated, since both ferritin and apoferritin exhibit the same microheterogeneity (Drysdale, 1970; Urushizaki et al., 1971).

The abnormality in tissue ferritin microheterogeneity in patients with haemochromatosis (Powell et al., 1974) would thus appear to be the result of long-standing iron overload and not the expression of a genetic defect in iron metabolism. However, the elucidation of the structural basis for this abnormality would provide evidence for possible metabolic interrelationships of the various tissue isoferititins. Since a normal organ-specific profile of tissue isoferititins has been demonstrated in a patient with acute transfusional iron overload (Powell et al., 1974) it would seem that long-standing chronic iron overload of considerable extent is necessary to produce the observed changes in isoferititin profile in haemochromatosis and Bantu haemosiderosis. Linder-Horowitz et al. (1970) showed that massive doses of iron in the rat caused an increase in ferritin concentration of the same magnitude in liver, heart, and kidney and both heart isoferititins detectable by polyacrylamide gel electrophoresis were induced to an equal extent. It is well documented that the presence of inorganic iron results in an increase in the rate of ferritin synthesis (Drysdale and Munro, 1966; Drysdale, 1968; Cumming et al., 1970; Linder et al., 1974) and a decrease in the rate of degradation of ferritin (Linder et al., 1974). Recent evidence suggests that all ferititins contain at least two subunits which differ with respect to either size (Konijn et al., 1973; Linder et al., 1974) or charge (Adelman et al., 1975) or both. Further, it has been demonstrated that subunits of differing molecular weight are made on

isoferititins as was observed in tissues from normal human subjects (Fig. 2).

In the patient with treated haemochromatosis the state of iron depletion was reflected in a very low yield of ferritin from all tissues, so that it proved impossible to isolate sufficient kidney and pancreatic ferritin for analysis. It can be seen, however, that the normal organ-specific variation in isoferitin profile was present in liver, spleen, and heart ferititins (Fig. 3) in contrast with the abnormality demonstrated in patients with untreated haemochromatosis (Powell et al., 1974) and in the Bantu subjects with gross haemosiderosis.

Discussion

Idiopathic haemochromatosis is now generally considered to result from an inherited abnormality in iron metabolism (Grace and Powell, 1974), whereas haemosiderosis in the Bantu is probably an acquired disorder (Charlton and Bothwell, 1966). The present

Fig. 3 Gel electrofocusing in 4% polyacrylamide containing 2% ampholytes, pH range 5-7 of purified ferritin isolated from liver (L), spleen (S), and heart (H) in a normal subject (a) and a patient with idiopathic familial haemochromatosis (b) who had been treated by venesection therapy and was iron depleted at the time of death.
different polyribosome populations (Konijn et al., 1973), the smaller being preferentially synthesized on free ribosomes and the larger on bound polyribosomes. The smaller subunits are preferentially increased by iron administration (Linder et al., 1974). Adelman et al. (1975) have proposed that the various tissue ferritins represent hybrid molecules composed of different proportions of at least three discrete subunits. They suggested that heart and liver each contain two subunits one of which is common to both (HL). If this hypothesis is correct, then under the influence of iron a subunit of the 'L' type which predominates in the more basic liver isoferritins may be synthesized at the expense of the 'H' type subunit characteristic of heart ferritin. The work of Linder et al. (1972) lends support to this hypothesis, since they demonstrated that liver ferritin subunits in weanling rats differed from those of adult rat liver, but treatment with iron induced the formation of the adult liver ferritin type. The evidence of Konijn et al. (1973) would indicate that the 'L' type subunits are those synthesized on free ribosomes.

The results presented in this study support the hypothesis that the synthesis of those subunits predominating in the more basic isoferritins is stimulated by gross long-standing iron overload and that this effect is reversible by removal of the excess storage iron. Thus, the abnormally uniform distribution of tissue isoferritins which we have demonstrated in haemochromatosis, Bantu haemosiderosis, and experimental haemosiderosis in the rat could result from the preferential synthesis of the 'L' type subunit and the more basic isoferritins leading to the generalized deposition of a 'storage' ferritin normally present predominantly in liver and spleen.

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References


