Serum Ferritin as a Guide to Therapy in Neuroblastoma

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ABSTRACT

Elevated serum ferritin levels without a corresponding increase in tissue iron storage have been observed in patients with certain cancers. Increased synthesis of ferritin by cancer cells has also been reported. In order to see whether similar phenomena occurred in patients with neuroblastoma, we have screened serum ferritin levels in 58 children with neuroblastoma by counterelectrophoresis using antibody to human ferritin. Increased ferritin levels in serum, positive by counterelectrophoresis (≥400 ng/ml), correlated well with the presence of active disease; a return of ferritin levels to the normal ranges coincided with remission. Primary neuroblastoma tumors and cells from neuroblastoma cell lines contained ferritins with the electrophoretic characteristics different from normal liver ferritin. Supernatant fluids from six neuroblastoma cell lines grown in culture also contained ferritin. These findings suggest that the increased ferritin in the serum of patients is derived from the tumor. The serum ferritin level could be used as indicator of disease activity and as a guide to therapy.

INTRODUCTION

Ferritin is the major tissue iron-binding protein. Small amounts of ferritin circulate in human serum and in normal and pathological states (15, 19), usually in proportion to the quantity of tissue iron stores (11, 14). However, in patients with certain cancers such as Hodgkin’s disease (2, 3, 8, 12), leukemia (12, 18), breast cancer (4), and others (9, 13, 17), grossly elevated levels of serum ferritin have been observed without a corresponding increase in iron storage. Leukemia cells have been shown to contain a comparatively large amount of ferritin (24) and to synthesize ferritin at a much higher rate than do normal leukocytes (23).

We attempted to see whether similar phenomena would occur in neuroblastoma and investigated the potential use of ferritin as a tumor marker in patients with neuroblastoma.

MATERIALS AND METHODS

Sera from 58 children with neuroblastoma and from 31 normal children were tested for ferritin. As another control group for neuroblastoma, 22 patients with Wilms’ tumor were also included. Six primary neuroblastoma tumors, cells from 2 established neuroblastoma cell lines, CHP-100 and CHP-134 (20), and HeLa cells were studied. Supernatants from 6 different neuroblastoma cell lines grown in culture, the culture medium itself (Roswell Park Memorial Institute Tissue Culture Medium 1640 with 10% fetal calf serum), and the supernatant from a human fibroblast cell line (IMR-90) (16) were also tested.

Ferritin was isolated from neuroblastoma tumors and purified according to the method described by Arosio et al. (1). Tumors were homogenized in 4 volumes of water and heated to 75° for 10 min to precipitate heat-labile proteins. The supernatant fraction was then adjusted to pH 4.6 to remove other contaminants, and ferritin was subsequently recovered from the supernatant and precipitated in 50% ammonium sulfate. Using the same methods, ferritin was also extracted from neuroblastoma cells (CHP-100 and CHP-134) and from HeLa cells grown in tissue culture. The purity of the ferritins was confirmed by polyacrylamide gel electrophoresis (1). The subunit composition of ferritins was analyzed by SDS gradient-pore gel electrophoresis as described by Arosio et al. (1). Purified human liver ferritin, kindly provided by Dr. James W. Drysdale (Tufts University School of Medicine, Boston, Mass.), was used as one reference standard, and the ferritin extracted from HeLa cells was used as the other standard.

RESULTS

The anti-human placental ferritin used in these studies reacted equally well with isoferritins extracted from human liver or HeLa cells, indicating a broad specificity of this reagent.

Sera from 31 normal children 2 to 14 years old were tested by CEP, and all were negative (i.e., levels of ferritin <400 ng/ml). All 22 patients with active Wilms’ tumor were negative. Fifty-eight children with neuroblastoma at various stages and activity of disease were tested for the presence of elevated serum ferritin. Results are shown in Table 1. Among the 9 patients with Stage I neuroblastoma, one had active disease and was positive for ferritin. The other 8 were free of disease, and all were found to be negative. In Stage II, 3 of 4 patients with active disease were positive for ferritin, and one was negative. In Stage III, 4 had active disease. Two of these patients were positive, while the other 2 were negative.
the 2 negative patients was tested 3 times while her disease progressed, and the ferritin results remained negative. In Stage IV, all 19 patients had active disease, and all but 3 patients were positive for ferritin. In Stage IV-S, the association between active disease and positive ferritin was not apparent. Four of 6 patients with disease were negative. The correlation between active disease and positive ferritin and disease-free state and negative ferritin among a total of 58 patients is significant at $p < 0.001$ by Fisher’s exact 2 × 2 test (21).

In 34 of the 58 patients more than one serum sample was available, and many were tested serially. The specimens had been collected during the course of disease and stored at $-70\,\text{°C}$. The findings are depicted in Table 2. Ten patients were disease free when first tested and remained so; all 21 specimens were negative. In 20 patients, the disease activity fluctuated; in 17 patients, the ferritin correlated with the patient’s status; 43 specimens were positive when disease was active; and 28 specimens were negative when in remission. In 2 of the 20 patients, elevated ferritin levels lagged behind the clinical evidence of remission (measured by clearing of the bone marrow), and in one patient there was obvious disease progression before the ferritin level rose (the rest became positive 3 months later. It was 3 months after relapse when the first sample was available for testing.) Four additional patients with clinical evidence of disease (one each with Stage II and Stage III and 2 with Stage IV-S) were repeatedly negative (12 samples).

Since serum ferritin may increase in hepatic cell damage, we attempted to evaluate whether this played a role in our findings. Liver function test results were available in the records of 16 of the 24 patients with elevated serum ferritin. Of these, 15 patients in various stages of neuroblastoma had normal serum transaminase levels; one of the 2 patients in Stage IV-S with a positive ferritin had an elevated aspartate aminotransferase of 160 units. When serial liver function tests were compared with simultaneous ferritin levels, there was no correlation.

Supernatant fluids were collected from 6 neuroblastoma cell lines grown in culture, concentrated 16 to 20 times, and tested for ferritin by CEP. All 6 were positive. The tissue culture medium, Roswell Park Memorial Institute Culture Medium 1640 with 10% fetal calf serum, and the supernatant obtained from a human fibroblast cell line, IMR-90 (16) were used as controls. Both were concentrated 20 to 25 times, and there were no detectable amounts of ferritin.

Extracted ferritins from neuroblastoma tumors, cells from neuroblastoma cell lines, purified human liver, and HeLa ferritins were characterized by subunit analysis on SDS-gel, and their subunit compositions are shown in Chart 1. The liver ferritin consists of greater than 80% L and the remainder H. The other extreme is represented by the ferritin from HeLa cells; it has 70 to 90% H with the remainder L. These findings were described previously by Drysdale et al. (7). Subunit composition of 3 preparations of neuroblastoma contained 53% H and 47% L in the cell line and 52% H and 48% L in the tumors as measured by densitometry.

**DISCUSSION**

Our study indicates that elevated serum ferritin levels detected by CEP in patients with neuroblastoma correlate well with the presence of active disease. The association between positive ferritin and active disease and negative ferritin and disease-free status is statistically significant. A longitudinal study of 34 patients done retrospectively on stored sera also showed a similar correlation (Table 2). Therefore, assaying ferritin levels during the course of illness could be of value in assessing disease activity.

With Stage IV-S disease, this correlation is not apparent (Table 1); 4 of 6 patients with active disease are negative. Patients with Stage IV-S constitute a unique group among children with neuroblastoma. They are usually infants (under 1 year of age) with a small primary tumor and metastatic spread to skin, bone marrow, or liver. Despite extensive metastases, patients with IV-S disease have a good prognosis because of a high rate of spontaneous regression or, rarely, maturation of their tumors.

A similar poor correlation was noted in patients with ganglioneuroblastoma, a more mature form of neuroblastoma. Only 2 of 8 children with active ganglioneuroblastoma had elevated...
levels of serum ferritin. These children could have slightly increased levels which are not detected by CEP, or the presence of ferritin may simply be dependent upon the portion of neuroblastoma relative to the ganglioneuroblastoma in the tumor.

Although we cannot identify with certainty the origin of increased ferritin in the serum of each patient with neuroblastoma, the elevated circulating ferritin does not seem to be due to liver damage or inefficient erythropoiesis. The presence of ferritin in cultured neuroblastoma tumor cells, as well as in the supernatant fluid and in neuroblastoma primary tumors, suggests that the increased ferritin in sera of neuroblastoma patients comes from the tumor. Ferritins extracted from the neuroblastoma tumors and cell lines showed subunit compositions different from liver ferritin on SDS-gel (Chart 1). According to the reports of Drysdale, ferritin is a spherical molecule composed of a total of 24 subunits (polypeptides) of 2 different sizes, H (M.W. 21,000) and L (M.W. 19,000). Different combinations of these 2 subunits account for the observed variation in isoferritins (5). The most commonly seen isoferritin pattern in normal adults is that of liver or spleen. These ferritins consist of greater than 80% L and the remainder H (7). The other extreme is represented by the ferritins from HeLa cells, some hepatocellular carcinomas and fetal livers. They have 70 to 90% H with the remainder L (5, 7). Ferritin isolated from heart tissue was reported as 40 to 70% H and 30 to 60% L (6, 7, 10). The composition of ferritin from normal neural tissue is unknown. Three neuroblastoma ferritins showed subunit compositions which differed from either liver or HeLa ferritin, containing an average of 53% H and 47% L and are similar to heart ferritin.

Since no normal children or patients with active Wilms’ tumor were found to have a positive CEP test for serum ferritin, the combination of an elevated serum ferritin in a child and the subunit composition of this ferritin may be sufficiently unique to permit their use as indicators of active neuroblastoma.

The results reported here suggest that the concentration of ferritin in serum could prove to be a valuable guide to therapy for children with this disease. Further research is needed to develop an assay for routine clinical use that would identify those elevated serum ferritins originating from tumor cells.

ACKNOWLEDGMENTS

The authors wish to thank Dr. James W. Drysdale and JoAnn Eccher at Tufts University School of Medicine for their instruction and assistance in the chemistry of ferritin; Dr. Gene Zwolinski, Clinical Assays, Boston, for performing radioimmunoassays; Dr. H. Schlesinger, Children’s Hospital of Philadelphia, for providing the neuroblastoma cell lines and supernatant fluids; Dr. L. Castor, The Institute for Cancer Research, Philadelphia, for culturing neuroblastoma and HeLa cells and for providing supernatant from IMR-90; Drs. J. W. Drysdale, W. T. London, and B. Werner at The Institute for Cancer Research for their review and criticism on this paper, and Maureen Welch for her excellent typing of the manuscript.

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