SHORT COMMUNICATION

The source of serum ferritin during infection. Studies with concanavalin A–Sepharose absorption

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Summary

1. Serum samples were collected from ten patients hospitalized for acute infections and from a control group of seven normal subjects. Tissue ferritin was obtained by purification of ferritin from normal human liver and from the ferritin standard of a commercially available assay kit.

2. The serum and tissue samples were incubated with concanavalin A–Sepharose, which has the ability to bind normal serum ferritin.

3. Concanavalin A, a plant lectin which binds to glucose, can be coupled to Sepharose particles and by incubation and centrifugation ferritin in normal serum can be absorbed to about 70%. The serum and tissue samples were incubated with concanavalin A–Sepharose and the ferritin content was measured before and after.

4. It was found that ferritin in the serum of patients with acute infections was absorbed to the same extent as in normal serum (about 80%), irrespective of the initial value. Only about 20% of the tissue ferritin was absorbed.

5. It is concluded that the ferritin in serum during infection is of the same glucosylated type as the ferritin normally present in serum, whereas intracellular ferritin is not glycosylated. This indicates that the elevation of serum ferritin during infection is caused by a release along the normal pathways, i.e. an augmented synthesis, not by leakage from damaged cells.

Key words: concanavalin A binding, ferritin, ferritin glucosylation, ferritin synthesis, infection.

Introduction

Acute infection as well as aseptic inflammation in man causes a rise in serum ferritin levels (Bentley & Williams, 1974; Elin, Wolff & Finch, 1977; Birgegård, Hallgren, Killander, Stromberg, Venge & Wide, 1978). The rise starts within 24–30 h and lasts for several weeks (Birgegård, Hallgren, Killander, Venge & Wide, 1978). The source of the ferritin in serum during inflammation is uncertain, but since the plasma half-disappearance time for ferritin is as short as 10–15 min (Pollock, Lipschitz & Cook, 1978), any long-standing elevation of serum ferritin must be caused by a continuous inflow of ferritin to plasma. This could be caused either by a leakage of ferritin from cells, damaged by the inflammation, or by an augmented intracellular synthesis of ferritin. Worwood, Cragg, Wagstaff & Jacobs (1979) have shown that there is a difference between normal serum ferritin and ferritin purified from liver or spleen or released from liver cells during massive cell destruction: 70% of normal serum ferritin may be absorbed by concanavalin A–Sepharose, whereas only 10–20% of intracellular ferritin is absorbed. In order to investigate the source of serum ferritin during infection, the ferritin in serum of patients with acute infections and of normal controls was absorbed with concanavalin A–Sepharose by the method of Worwood et al. (1979) and the results were compared. Concanavalin A absorption of human liver and spleen ferritin was also performed to confirm the data of Worwood et al. (1979).

Materials and methods

Subjects

Serum samples were collected from ten patients hospitalized for acute viral and bacterial infec-
tions. All had fever and leucocytosis. Samples were collected after the second and before the seventh day at the hospital. Serum samples from seven healthy subjects with normal routine haematological data were also collected.

Ferritin was measured with a Pharmacia Phadebas Prist kit. Normal range for men is 15–250 μg/l, and for women is 10–180 μg/l of serum (lower detection limit 0.5 μg/l). The method uses antibodies to human spleen ferritin raised in rabbits.

Tissue ferritin was purified from 250 g of liver tissue from a healthy 31-year-old male who died in a car accident. The material was removed 4 h post mortem and deep frozen. The purification procedure was a modification of the method used by Penders, DeRooij-Dijr & Leijuse (1968), with a series of ultracentrifugations, ultrafiltration and two final gel chromatographies on Sepharose 6B. The final product gave a single peak on gel chromatography and a single band on gel electrophoresis. A dilution curve parallel to the standard curve of the ferritin assay was obtained.

Commercially available spleen ferritin (cadmium-crystallized human spleen ferritin, Ferriron kit, ferritin standard preparation, Ramco) was also tested. Two different dilutions of each preparation were used, 2000 and 200 μg/l of the spleen ferritin, 600 and 60 μg/l of the liver ferritin.

Concanavalin A–Sepharose absorption

Concanavalin A–Sepharose 4B was bought from Pharmacia Fine Chemicals, Uppsala, Sweden. It was washed three times in five times its volume in barbitone buffer (50 mmol/l, pH 8-0). After washing, the volume was adjusted to twice the volume of the packed particles by addition of the same barbitone buffer. Of this suspension, 1.5 ml was incubated with 200 μl of test sample during slow rotation for 2 h. The mixture was centrifuged at 3000 rev./min for 15 min and the supernatant sucked off with a pipette. The ferritin concentration was estimated in the supernatant and after correction for the dilution compared with the ferritin concentration in untreated serum.

Results

The results of Worwood et al. (1979) were confirmed: in normal serum 20.8 ± 7.5% (SD) of the initial ferritin concentration remained after concanavalin A absorption, whereas 81.8 ± 12.4% of tissue ferritin was left. There was no difference between liver and spleen ferritin in this respect, and the percentage left after absorption was not influenced by the initial ferritin concentration.

Fig. 1. Ferritin concentration before and after concanavalin A–Sepharose (con A) absorption. Ferritin in normal serum (n = 7) and in serum from patients with infection (n = 10) was absorbed to the same extent, about 80%, whereas only about 20% of tissue ferritin was absorbed.

The serum ferritin levels in the patients with infection were elevated above the upper normal limit of 240 μg/l in seven of the ten cases. Irrespective of the initial level, 24.6 ± 6.2% remained after concanavalin A absorption (Fig. 1), which is not significantly different from the results with normal serum.

Discussion

The source of serum ferritin during infection is uncertain. Serum ferritin is an extremely small fraction compared with the large amounts of intracellular ferritin. Therefore several mechanisms can be suspected to cause changes in serum ferritin levels: a change in synthesis rate, elimination rate or in the balance between intra- and extra-cellular ferritin (for instance by leakage from damaged cells).

There is some direct evidence for a stimulation of ferritin synthesis during inflammation. An increase in liver ferritin synthesis in the rat has been found after experimental inflammation (Konijn & Hershko, 1977). These authors first reported such an increase of short duration, preceding the fall in serum iron seen during inflammation, but have later found a second wave of ferritin synthesis of longer duration (A. M. Konijn & C. Hershko, personal communication).

The plant lectin concanavalin A has the specific ability to bind glucose. The fact that
normal serum ferritin is absorbed by concana-valin A-Sepharose but intracellular ferritin is not, led Worwood et al. (1979) to conclude that ferritin is glucosylated during its passage to plasma.

The demonstration in the present studies that ferritin in serum during infection could be absorbed by concanavalin A-Sepharose to the same extent as normal serum ferritin indicates that this ferritin is glucosylated. This points to an augmented ferritin synthesis rather than cell damage as the explanation for the elevated serum ferritin levels during infection.

References


