REVIEW

The role of iron in cancer

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Numerous laboratory and clinical investigations over the past few decades have observed that one of the dangers of iron is its ability to favour neoplastic cell growth. The metal is carcinogenic due to its catalytic effect on the formation of hydroxyl radicals, suppression of the activity of host defence cells and promotion of cancer cell multiplication. In both animals and humans, primary neoplasms develop at body sites of excessive iron deposits. The invaded host attempts to withhold iron from the cancer cells via sequestration of the metal in newly formed ferritin. The host also endeavours to withdraw the metal from cancer cells via macrophage synthesis of nitric oxide. Quantitative evaluation of body iron and of iron-withholding proteins has prognostic value in cancer patients. Procedures associated with lowering host iron intake and inducing host cell iron efflux can assist in prevention and management of neoplastic diseases. Pharmaceutical methods for depriving neoplastic cells of iron are being developed in experimental and clinical protocols.

Key words: Cancer, iron, iron chelation, iron overload, iron withholding.

Introduction

“One might worry about the iron-injectable compounds which are being tested and used. One could almost guess that someone is going to find iron dextran carcinogenic.” Furst (1960)

Awareness of the role of iron in carcinogenesis began to develop in the middle decades of the twentieth century. During the latter third of the century, a cascade of observations in human and animal hosts have provided strong evidence that one of the dangers of iron is its ability to favour neoplastic cell growth. Summaries of the extensive literature on this topic include those of Weinberg (1981, 1984, 1992a), Cazzola et al (1990), Stevens (1990, 1992), Sussman (1992), Sahu (1992) and Strain (1994).

The present review is focussed on developments in five subsets of the topic: I, carcinogenic mechanisms of iron; II, association of iron with neoplasia in animals; III, association of iron with neoplasia in humans; IV, prognostic use of iron values in human cancer patients; and V, methods for withholding and withdrawing iron from cancer cells.

Carcinogenic mechanisms of iron

“Playing with iron is almost literally playing with fire.” Stevens (1992)

Iron can be carcinogenic in three ways. First, ferric ions are reduced by superoxide and the ferrous product is reoxidized by peroxide to regenerate ferric ions and to yield hydroxyl radicals (McCord, 1992). Reactive oxygen species such as hydroxyl radicals, when generated in close proximity to DNA, can alter normal cells by causing point mutations, DNA crosslinking, and DNA strand breaks (Reid and Loeb, 1992; Sahu, 1992; Hardy and Aust, 1995). As a corollary, iron mediated-production of single- and double-strand breaks in DNA can be inhibited by...
either iron chelators or free radical scavengers (Toyokuni and Sagripanti, 1992). Oxygen free radicals also can cause conformational changes in nuclear and cytoplasmic membranes by inducing lipid peroxidation (Bacon and Britton, 1990). Such conformational changes can modulate signal transduction and can activate protein kinases and growth factors and their receptors, as well as oncogenes (Sahu, 1992).

Secondly, in addition to the initiation of the cancer process, iron can bolster the growth of neoplastic cells by suppressing host defences. In excess, the metal inhibits activity of CD4 lymphocytes (de Sousa, 1989; Djeha and Brock, 1992). Furthermore, the tumoricidal action of macrophages is suppressed by phagocytosed erythrocytes, erythrocyte lysate, haemoglobin, iron dextran or iron salts, but not by erythrocyte ghost membranes, latex spheres, myoglobin or dextran (Weinberg and Hibbs, 1977). In a confirming study, tumoricidal activity of macrophages was found to be nullified by iron dextran, carbonyl iron and ferrated ferritin, but not by plain dextran, carbon particles, or apoferritin (Green et al., 1988).

The third mechanism whereby iron can be carcinogenic is that of functioning as an essential nutrient for unrestricted tumour cell multiplication. Although normal and neoplastic cells have a similar qualitative requirement for iron, the unrestricted proliferation of tumour cells would be expected to require an enhanced, and probably diversified, supply of the metal. In 1972, Holley proposed that "the crucial change in a malignant cell is an alteration in the cell surface membrane that results in internal concentrations of nutrients that regulate cell growth". He noted that the widely differing growth rates of different cancers correspond to changes in concentrations of intracellular nutrients and that it should be possible to arrest growth of malignant cells in the G1 phase of the cell cycle by limiting uptake of a critical nutrient.

In a succession of subsequent studies, iron has been identified as one of the critical nutrients. For example, in normal human B lymphocytes, expression of transferrin (Tf) receptors is a multistep, tightly regulated process that requires antigen or mitogen stimulation, interleukin-2 and interleukin-2 receptors. In contrast, malignant B lymphocytes express Tf receptors constitutively (Neckers, 1984). Similarly, Tf receptors were observed to be expressed in greater amounts on leukaemia progenitor cells than on normal ones (Cazzola et al., 1990). Likewise, in cells of myogenic lineage, transformed cells were found to have more total and expressed Tf receptors and higher rates of Tf and iron endocytosis than did normal cells proliferating at the same rate (Sorokin et al., 1989). Human melanoma cells produce an iron-binding 97 kDa glycoprotein that is structurally related to Tf, is associated with the cell membrane, and which apparently functions in iron translocation (Brown et al., 1982).

Moreover, small cell lung cancer cells can synthesize their own Tf, an ability that may account for the very short doubling time of this neoplasm, even in areas that are not well vascularized (Vostrejs et al., 1988). Additionally, in the absence of Tf, some, but not all, leukaemia cell lines can obtain iron for DNA synthesis and growth from 1–10 μM inorganic iron salts (Basset et al., 1986; Neumannova et al., 1995).

Normal and neoplastic cells may differ not only in mechanism of iron acquisition but also in their intracellular deposition of the metal. Normal cells apparently convey a higher percentage of acquired iron into ferritin deposition, whereas neoplastic cells consign a greater proportion to metabolic tasks (Zhou et al., 1987; Shterman et al., 1991).

Association of iron with neoplasia in animals

"Animals (and humans) burdened with excess iron appear to have a greater than normal risk of developing one or more primary neoplasms. The latter tend to be associated with the site of deposition of the metal". Weinberg (1981)

Substantiation of Furst's prediction (made at a meeting in 1959) that iron might be carcinogenic began to be reported later the same year. In the initial study in rats (Richmond, 1959), 16 of 23 animals injected s.c. weekly with 20 mg iron complexed with dextran developed tumours in 11–16 months at the site of inoculations. In subsequent reports, the observation was corroborated and extended (Hadow and Hornung, 1960; Lundin, 1961; Hadow et al., 1964; Roe et al., 1964; Langvaal, 1968; Carter, 1969; Magnusson et al., 1977). In one study, doses as low as 4 mg iron per week induced sarcomas in 26 of 32 rats (Magnusson et al., 1977).

Iron in low molecular mass complexes was consistently found to be inactive, presumably because the metal could be withdrawn from the subcutaneous site of deposition and safely sequestered by components of the iron-withholding defense system. Likewise, plain dextran consistently is non-carcinogenic. Sarcomas could be induced by iron dextran not only in rats but also in mice and rabbits. However, tumours did not develop in dogs. Apparently the
canine macrophages were able to safely remove the metal before it could initiate or promote growth of sarcomatous cells.

In more recent studies of parenteral iron, mice were injected with a single dose of iron dextran (15 mg/animal) and then fed 0.01% hexachlorobenzene (HCB) for 18 months (Smith et al., 1989) or 0.01% polychlorinated biphenyls (PCBs) for 12 months (Smith et al., 1990). With HCB or iron alone, mice remained free of neoplasia. With HCB plus iron, nine of 10 mice developed hepatocellular carcinomas. With PCBs alone, the carcinoma appeared in one of 16 mice; with iron alone, in none of 16. In contrast, in the set of 18 mice that received PCBs plus iron, 15 developed hepatocellular adenomas and seven acquired hepatocellular carcinomas (four rats had both lesions).

In rats given 10 weekly injections of 1,2-dimethylhydrazine (DMH), 73% formed a mean of 1.4 colorectal tumours per animal (Nelson et al., 1989). In rats given 10 doses of DMH plus five weekly injections of iron dextran (4 mg iron/animal/week), 90% developed a mean of 2.6 colorectal tumours (P = 0.14).

In a different study (Okada et al., 1989), 19 rats were injected i.p. daily for 3 months with ferric oxide (1 mg iron/animal/day). Nine of the animals produced abdominal and peritoneal mesothelomas. Controls without iron remained free of the malignancy. In an earlier study in the same laboratory (Li et al., 1987), the ferric complex of nitrosotrinitroacetic acid (Fe-NTA) was injected i.p. in 25 male and 21 female mice (2 mg iron/kg body wt/day for 12 weeks). Renal tubular cell carcinoma developed in 15 of the male and in one of the female animals. Mice injected with NTA remained cancer-free.

An alternative route whereby iron can enter the body is that of inhalation. Although much concern exists over human inhalation of iron (see below), few animal model studies are available. In an early report, mice exposed to iron oxide dust developed 3.5 times as many lung tumours as did control animals (Campbell, 1940). In later studies, the incidence of neoplasms in the lower respiratory tract of hamsters who inhaled benzo(α)pyrene (Saffiotti et al., 1972) or diethylnitrosamine (Nettesheim et al., 1975) was markedly elevated by concurrent inhalation of iron oxide.

A third route of acquisition of iron is that of ingestion. Since 1988, a number of laboratories have reported that excessive dietary iron enhances development of neoplasia in animals that (a) produce spontaneous tumours, (b) are inoculated with tumour cells or (c) are exposed to chemical carcinogens.

In a murine strain that is congenitally infected with mammalian tumour virus, spontaneous development of tumours occurs between the ages of 7.2 and 9.2 months. Animals fed 5 mg iron/kg food, an amount of the metal that was sufficient to maintain haematocrit of 40% with normal body weight and behaviour, developed mammary tumours at an average rate of growth of 62% per week. In mice fed 26 times as much iron, the rate of growth of the tumours was 112% per week (P = 0.02) (Hann et al., 1991).

In an earlier study from the same laboratory (Hann et al., 1988), three non-tumorigenic strains of mice were fed 5 mg iron/kg food. Again, behaviour remained normal in all three strains. Body weight was 19% lower in one strain and 16% and 12% higher in the other two strains as compared with animals who received 62 times more iron. Mean haematocrits were 27.9, 28.8 and 36.9% in the mice fed low iron and were 51.5, 48.0 and 51.2% in the animals stressed with the excessive amount of the metal. The strains of mice were then inoculated, respectively, with colon adenocarcinoma cells, hepatoma cells and mammary adenocarcinoma cells. In all three strains, tumour sizes were larger (P ≤ 0.05) in the animals burdened with excessive iron.

Several research groups have studied the efficacy of various carcinogens in rats fed diets with differing quantities of iron. In animals given excessive amounts (> 200 mg iron/kg food), tumour growth was significantly greater than in controls that were fed adequate quantities of the metal. Examples include a three-fold greater incidence of colorectal tumours in iron-loaded rats injected with DMH (Siegers et al., 1988, 1991; Nelson et al., 1989) and a 2.25 times greater incidence of hepatic preneoplastic foci in iron-loaded animals injected with diethylnitrosamine (Yoshiji et al., 1991). Similarly, iron-loaded rats injected with 1-methyl-1-nitrosourea developed approximately twice as many mammary cancers as did controls on a normal diet (Thompson et al., 1991b). In addition, appearance of the tumours required 9 weeks in contrast to 13 weeks in controls (Singh et al., 1994).

When animals that are exposed to chemical carcinogens are fed less than adequate amounts of iron (generally < 5 mg iron/kg food) observations vary. In three laboratories, the quantity of breast cancer was similar to that of control animals fed an adequate iron diet (Webster, 1981; Thompson et al., 1991b; Spear and Sherman, 1992). However, in one of the studies (Spear and Sherman, 1992), a low-moderate concentration of iron (15 mg/kg food)
resulted in an enhanced amount of neoplasia. The authors proposed that both the low–moderate and the iron-deficient diets suppressed natural killer cell defence but, in the deficient set, the cancer cells were growth-restricted by insufficient iron.

In a different laboratory, iron-deficient rats fed DMH had a greater amount of colonic and a much greater amount of duodenal tumours than did controls fed adequate iron (Jagadeesan et al., 1994). In another study (Prime et al., 1983), 4-nitro-quinoline-N-oxide was painted on rat palates. Animals fed a low moderate amount (12 mg iron/kg food) initiated tumour development at 183 days, in contrast to rats fed 140 mg iron/kg food who began to show tumours at 229 days. The low iron group had more neoplastic growth on palates whereas the iron-loaded set had more tongue tumours.

**Association of iron with neoplasia in humans**

"Iron doping of healthy individuals to improve performance may well have dire health consequences not less severe than anabolic steroids". Nelson (1992)

**Introduction of iron by parenteral routes**

As had been reported in rodents and rabbits, injections of iron complexes in humans have been observed to result in sarcomas at the sites of deposition. In a study of 90 cases of sarcoma arising in the buttocks, sarcomata occurred in four patients in whom, in earlier years, iron preparations had been injected (Greenberg, 1976). Unfortunately, the drug histories of another 106 cases had been "lost or destroyed". Moreover, the percentage of persons in the population who had been given iron injections was unavailable; thus the risk of acquiring cancer by this route could not be calculated. A fifth case was subsequently reported, in which, after 14 years, a large fibrosarcoma had developed in the gluteal region at the site of three injections of iron preparations (Robertson and Dick, 1977). Fortunately, the total number of recorded cases has remained small.

Persons who walk barefoot over volcanic clay that is rich in iron oxide in such regions as the East African Rift System, the West African Benue Trough, Corsica, Sardinia and Peleponese are at risk of developing an aggressive spindle-cell sarcoma (Ziegler, 1993). The neoplasia, termed lubambo in Zaire and endemic Kaposi sarcoma in the medical literature, comprises ≤20% of adult malignancies in specific geographic areas of Cameroon, Zaire, Rwanda, Burundi and Uganda. Development of the condition apparently occurs in the presence of iron as a cocarcinogen plus a specific herpes virus. A suppressor of cell mediated immunity such as human immunodeficiency virus can enhance the risk of development of Kaposi sarcoma, but it is not a requisite for the neoplasia (Moore and Chang, 1995; Boshoff et al., 1995). The herpes virus was observed in 39 of 40 Kaposi sarcoma cases, irrespective of association with HIV, and was absent in 11 patients with other types of skin lesions (Boshoff et al., 1995).

A third parenteral route of iron penetration is that of i.v. injection of whole blood or packed erythrocytes. Each transfusion unit of whole blood contains about 200 mg iron. Thus, perioperative transfusion of multiple units of whole blood in patients undergoing cancer surgery, with no evidence of metastatic disease, might be associated with enhanced risk of recurrence. A survey (Tartter, 1989) of published reports on this matter indicated that 14 groups of investigators found an increased risk; 13 groups did not. In the absence of multi-institutional prospective studies of ≥600 patients (controlling for operative trauma and disease stage), the relationship between blood transfusion and cancer recurrence remains undetermined (Tartter, 1989).

**Introduction of iron by inhalation**

Known sources of inhaled iron include (a) industrial ferrous materials, (b) iron-containing varieties of asbestos and (c) tobacco smoke (Weinberg, 1993). The association between inhalation of industrial sources of iron and development of respiratory tract neoplasias is well established. For instance, lung cancer occurred in young adults who, as children working at home, had applied iron oxide polishing powder to clock screws (Dreyfus, 1936). Underground haematite miners in Cumberland (UK) were twice as likely to die of lung cancer as were persons who worked at the surface of the mines or who were coal miners (Boyd et al., 1970). In eastern Slovakia, the relative risk of lung cancer in iron ore miners, as compared with non-miners, was 2.81 (95% CI 1.69–5.21) in one district and 4.01 (95% CI 2.07–9.06) in a second district ($P < 0.001$) (Icso et al., 1994). A 5–16-fold increase in lung cancer mortality among underground iron ore miners as compared with non-miners was observed in Sweden (Edling, 1982) and a 5–12-fold increase in Lorraine (Antoine et al., 1979).

In Sheffield (UK) in 1926–35, mortality due to cancers of the larynx, bronchi, lungs and mediastinum was increased 2.4-fold among steel foundry and furnace workers, 2.0-fold among metal grinders, glaziers and buffers and 1.8-fold among steel machinists and turners as compared with these causes of death in males in other
occupations (Turner and Grace, 1938). Among foundry workers in Denmark in 1967–74, the incidence of lung cancer was elevated by 1.72 over workers in other occupations (Sherson et al., 1991).

In eastern Pennsylvania, USA, long-term steel industry workers, especially those employed in foundry occupations, had an excess of lung cancer (adjusted for cigarette smoking) of 1.8 ($P = 0.01$) (Blot et al., 1983). In Liege, Belgium, 61 blast furnace workers had a mean increase of alveolar macrophage (AM) iron of 4.4-fold (24 non-smokers) as compared with 19 unexposed non-smokers ($P < 0.001$) (Corday et al., 1992). Iron has been found to suppress tumoricidal macrophage activity at a quantity of 1.3 pg per cell (Green et al., 1988). In the study of blast furnace workers, unexposed non-smokers had a mean of 0.33 pg per cell. The mean amount of iron per alveolar macrophage in exposed non-smokers was 1.45 pg and in exposed smokers 2.00 pg. In a different study (Laires et al., 1982), urine of 17 persons occupationally exposed to iron oxide was higher ($P = 0.002$) in mutagenicity as determined in the Salmonella/mammalian microsome assay than was that in 16 industrial workers who were unexposed to the metal.

Inhalation of iron-containing varieties of asbestos (amosite, crocidolite, tremolite) can increase the rate of respiratory tract neoplasia. Chrysotile, a silicate of magnesium rather than of iron, is far less dangerous unless it is contaminated with tremolite (Weimbarg, 1989). In rural areas of Greece, Turkey and Melanesia, tremolite has been employed as a whitewash for indoor and outdoor walls of houses. In persons who inhale dust from the whitewash, the risk of malignant pulmonary mesothelioma is increased $\geq 300$ fold (Luce et al., 1994).

In human cell culture studies, amosite and attapulgite (a clay mineral that contains 8% iron) were found to be strongly cytotoxic (Garcia et al., 1989). Chrysotile was much less injurious, and fibreglass (physically similar to amosite but devoid of iron) was not toxic. Cytotoxicity was prevented by the iron chelator deferroxamine (DF) as well as by scavengers of hydroxyl radicals. The iron chelator phytic acid diminished the ability of amosite to damage cultured human pulmonary epithelial cells (Kamp et al., 1995).

In a study on isolated DNA (Faux et al., 1994), crocidolite but not chrysotile significantly elevated levels of 8-OH-deoxyguanosine, a biomarker of hydroxyl radical-mediated DNA damage. Moreover, crocidolite enhanced background revertants of Salmonella typhimurium TA 102. The DNA damage and mutagenicity of crocidolite was suppressed by DF (Faux et al., 1994).

Mobilization of asbestos iron by ascorbate or citrate greatly enhanced DNA destruction (Lund and Aust, 1992). Furthermore, the metal could be leached from crocidolite or amosite by iron-binding agents contained in tobacco smoke (Qian and Eaton, 1989). The synergism of iron-containing asbestos and cigarette smoke in causing DNA damage by radical-mediated processes is well documented (Lund and Aust, 1991; Kamp et al., 1992).

Cigarette smoke also contains high quantities of polynuclear benzenes that can reduce and mobilize iron from ferritin (Moreno et al., 1992). Additionally, tobacco contains 440–1150 $\mu$g iron/g and cigarette paper 420 $\mu$g iron/g (Mussalo-Rahammas et al., 1986). Approximately 0.1% of cigarette iron is contained in the mainstream smoke. Thus a one pack/day smoker might inhale 1.12 $\mu$g iron/day (Thompson et al., 1991a). Not surprisingly, AM of light smokers yielded a mean of 1.75 times more iron and of heavy smokers 2.42 times than did AM of non-smokers (Wesselius et al., 1994). In another study, the iron burden of AM of persons who smoked 50 pack-years was increased threefold and of those who smoked 100 pack-years 5.4-fold over that of non-smokers (McGown and Henly, 1988). Smokers with chronic airway obstruction had a mean of 2.26 times the AM iron load of smokers without chronic airway obstruction (Wesselius et al., 1992).

The iron burden in AM of smokers considerably exceeds the amount of the metal that permits tumoricidal activity. Accordingly, the rate of respiratory tract neoplasias would be expected to be increased markedly by cigarette smoking. In the 1982–88 Cancer Prevention Study II, which involved > 1.2 million US persons $\geq 35$ years of age, the relative risk of lung cancer in smokers in males and females, respectively, was increased by means of 22.36 and 11.94 as compared with non-smokers. Correspondingly increased risks for male and female smokers, respectively, for cancers of lip, oral cavity and pharynx were 27.48 and 5.59; of the oesophagus, 7.60 and 10.25; and of the larynx, 10.48 and 17.98 (Carbone, 1992). The degree of risk was correlated with the duration of smoking, number of cigarettes smoked and extent of inhalation.

Introduction of iron by ingestion

A variety of factors can contribute to intestinal absorption of excessive amounts of iron. Among these are (a) suppression of duodenal mucosal and/or
pancreatic secretion of bicarbonate ions, (b) enhancement of hydrochloric acid secretion by ethanol, (c) impairment, in inherited haemochromatosis, of the intestinal mucosal block and (d) excessive consumption of iron supplements, foods rich in haem, and/or foods adulterated with inorganic iron.

In healthy persons, bicarbonate ions assist in prevention of excessive iron uptake by lowering solubility of the metal. Cigarette smoking has been observed to reduce by 78% the increase in duodenal mucosal bicarbonate secretion that would normally occur in response to luminal acidification (Ainsworth et al., 1993). Smoking also decreases pancreatic secretion of bicarbonate ions (Bochenek and Koronczewski, 1973). The relative risk of pancreatic and urinary tract cancers, leukaemias, and myelomas (Carbone, 1992) and of colonic adenomas (Kikendall et al., 1989; Nelson et al., 1994) is increased in smokers 2–3-fold over that of non-smokers.

Elevated levels of plasma iron and of transferrin iron saturation are associated with enhanced risk of pancreatic cancer (Friedman and Van den Eeden, 1993). Pancreatic secretion of bicarbonate ions is depressed in cystic fibrosis (Tonz et al., 1965). In patients with this disease, an increase of 2.3-fold in urinary 8-OH-deoxyguanosine has been observed (Brown et al., 1995). Patients with cystic fibrosis who survive beyond age 25 have a heightened risk of pancreatic, testicular and intestinal neoplasms (Neglia et al., 1991; Brown et al., 1995).

The ability of ethanol to enhance intestinal iron absorption has often been demonstrated. For instance, a challenge dose of 10 mg iron fed to healthy persons without ethanol resulted in absorption of 2–5%; with ethanol, 10% (Crosby, 1987). In a non-selected alcoholic population, 29% had excessive hepatic iron burdens (Chapman et al., 1982). Patients with alcoholic cirrhosis had up to 2.5 times as much hepatic iron sequestration as did non-alcoholics (Chapman et al., 1982). Serum iron levels were normal in persons with primary biliary cirrhosis but were elevated twofold in those with alcoholic cirrhosis (Ritland and Aaseth, 1987). Ethanol-stimulated gastric acid secretion helps to maintain ferric ions in solution as they reach the duodenum (Jacobs et al., 1964). The solvent also suppresses the ability of enterocytes to withhold the metal from absorption (Mazzanti et al., 1987). Furthermore, immoderate consumption of ethanol may result in formation of desialylated TF which can enhance hepatic siderosis (Mihas and Tavassoli, 1991).

Several research groups have noted possible associations between ingestion of ethanol and increased risk of neoplasms. Regrettably, these reports failed to include measurements of body iron. As compared with non-drinkers, persons who consumed >1 drink/day had a relative risk of mammary cancer of 1.46 (95% CI 1.00–2.3) (Roham and McMichael, 1988), of colonic adenomas of 2.81 (95% CI 1.10–7.17) (Kikendall et al., 1989), and of colorectal cancer of 1.6 (95% CI 1.1–2.3) (Wu et al., 1987). In persons who ingested ≥3 drinks/day, the relative risk for colon cancer was 1.71 (95% CI 0.92–3.19) and for rectal cancer 3.17 (95% CI 1.05–9.57) (Klatsky et al., 1988). Similarly, the risk of development of colonic adenomatous polyps in persons who consumed >1 drink/day was 3.04 (95% CI 1.14–8.15) (Cope et al., 1991); in this study, the relative risk for non-drinkers who smoked cigarettes was 2.12 (95% CI 0.54–8.29) and for persons who both drank and smoked, 12.70 (95% CI 3.02–53.42) (P < 0.01).

Persons with hereditary haemochromatosis (HH) comprise about 0.3–0.8% of Caucasian populations. These persons appear to have a defect in expression of iron-regulated proteins in cells of the duodenal mucosa. Thus, the patients can absorb 2–4 mg iron/day instead of the normal quantity of 1–2 mg. The hazardous oversupply cannot be excreted; it is initially sequestered in parenchymal cells of liver, heart and selected endocrine glands. At the time of diagnosis of the clinical disease, the total amount of body iron usually has been increased from a normal value of 4–5 g to about 20 g (Ritland and Aaseth, 1987). In undiagnosed and/or untreated patients, iron eventually can increase to 50 g and it overflows into macrophages, synovial linings, and plasma. Clinical manifestations of the disease include cardiomyopathy, arthropathy, diabetes mellitus, hypogonadism, and, not least, markedly enhanced susceptibility to neoplasia and infection (Abbott et al., 1985; Bradbear et al., 1985; Adams and Gregor, 1990).

A frequent site of neoplasia in HH is the liver. Even 7–9 years after diagnosed persons have been deironed by phlebotomy, hepatic carcinomas may occur. Apparently the iron will have participated in the initiation and early promotion of the neoplasms 15–20 years earlier (Kew, 1990). Patients who have the highest mobilizable iron levels are most likely eventually to develop hepatic carcinomas. The incidence of the neoplasm in patients who have liver cirrhosis due to HH is greater than that in persons who have other causes of cirrhosis (Kew, 1990). Although copper, like iron, catalyses formation of hydroxyl radicals, patients with cirrhosis due to excessive copper rarely develop hepatic carcinoma (Ritland and Aaseth, 1987). Unlike iron, copper
cannot function as an essential nutrient for the ribonucleotide reductase of the tumour cells. Persons without cirrhosis who develop hepatocellular carcinoma often have elevated parenchymal iron (Turlin et al., 1995).

In HH, the risk of hepatic carcinoma is at least 200 times greater than in normal persons (Bradbear et al., 1985; Niederau et al., 1985). However, other sites of neoplasia also are observed. In one study, hepatomas occurred in 18 and extra-hepatic carcinomas in 10 of 111 HH patients (Bomford and Williams, 1976). In another report of 36 HH patients, hepatoma developed in five, lung cancer in four and thyroid cancer in one (Ammann et al., 1980). Of the 10 cancer patients, eight were heavy smokers; four of these also had a history of alcohol abuse. In a set of 101 male haemochromatotics followed for an average of 4.1 years (Hsing et al., 1995), the standardized incidence ratio (compared with the general population) of primary liver cancer, oesophageal cancer and skin melanoma, respectively, was 92.9, 42.9 and 27.8.

Heterozygote carriers of the HH gene have on average a moderately increased iron burden as compared with non-carriers. For instance, the mean serum ferritin value in a set of 255 heterozygotes was 140 ± 10.2 ng/ml, in 81 normal persons 87 ± 8.5 ng/ml (P < 0.05) (Adams, 1994). The corresponding values for percent of iron saturation of Tf were 38 ± 0.88 and 29 ± 1.11 (P < 0.05). In a study of 1,950 heterozygous carriers and 1,656 non-carriers (Nelson et al., 1995), the relative risk in male carriers for colorectal cancer was 1.28 (95% CI 1.07–1.53) and for haematologic malignancies was 1.30 (95% CI 1.03–1.63). In female carriers, the relative risk for colon adenoma was 1.29 (95% CI 1.08–1.53) and for abdominal cancers 1.37 (95% CI 1.04–1.79). In this population, carriers did not have an increased risk for cancers of lung, breast or cervix.

Non-HH siderosis occurs frequently in Africa in native populations who have an inherited predisposition to absorb excessive iron and who consume iron-contaminated cereals and beverages. In a study of 601 autopsies of adults from seven countries of central and southern Africa, 20% of the males and 15% of the females had very marked liver siderosis (Gordeuk et al., 1993). These persons had a 17.8-fold increase (95% CI 1.8–179.2) in death from hepatoma as compared with those whose livers showed a slight or absent siderosis. Persons who had a marked increase in hepatic iron had a 5.7-fold increase (95% CI 1.6–20.9) over the slight-absent iron group.

Excessive consumption of haem iron and/or inorganic iron contained in foods or supplements predictably should increase cancer risk in persons with HH or African siderosis and, perhaps also, to some extent in normal persons. However, it has been difficult to quantitate dietary iron because of lack of accurate long-term recall by subjects, as well as lack of recognition by some investigators of iron content of food items. Nevertheless, in a few reports, fragmentary information is available. Additional evidence concerning the association of African iron overload with hepatocellular carcinoma has recently been reviewed (Gangaidzo and Gordeuk, 1995).

In a survey of 3,287 males in 1971–88, 25 developed colon cancer and 354 had cancers at other sites (Stevens et al., 1988). As compared with subjects who remained free of cancer, dietary iron was higher (P < 0.05) in persons with colonic but not with other cancers. In a study of 277 case–control pairs of males and 145 case–control pairs of females, risk of rectal cancer in males who were placed in the highest quartile of iron ingestion was increased 2.15-fold (P < 0.01) but the risk in females was not altered by dietary iron (Freudenheim et al., 1990). In a 6-year study of 88,751 women, the risk of colon cancer was increased 2.49-fold in subjects who consumed mammalian sources of meat daily as compared with those who ate these forms of meat less than once per month (Willett et al., 1990). In a similar survey of 47,949 men, the increase in colon cancer in meat consumers was 3.57 (P = 0.01) (Giovanucci et al., 1992).

A group of 200 pre-menopausal women with breast cancer was compared with 420 matched controls (Lee et al., 1991). High intake of mammalian meat was associated with an increased risk for breast cancer of 3.99 (95% CI 1.87–8.51) (P < 0.001). Mammalian meats contain only 1–3.5 mg iron per serving, but unfortunately the metal mainly is incorporated in haem (Lauffer, 1992). The percentage of haem iron absorbed is 5–10 times greater than that of non-haem iron. Moreover, the amount of haem iron absorbed does not decrease with increasing meat content in the diet. Several studies have shown that meat intake by normal persons increases their hoard of body iron (Lauffer, 1992). However, in the three studies cited above (Willett et al., 1990; Giovanucci et al., 1992; Lee et al., 1991), body iron values were not determined.

Increased iron burden by decompartmentalization
Iron can be released from its normal body compartments by haemolysis, bleeding or destruction of cells that contain iron deposits. Thus, in a patient with spherocytosis, iron from destroyed erythrocytes accumulated in the liver with subsequent hepatic
carcinoma (Kew, 1990). Persons with intestinal mucosal bleeding due to untreated ulcerative colitis or Crohn's disease have, respectively, a 10–20-fold or a 4–7-fold increased risk of developing colorectal cancer as compared with the general population (Levin, 1988). Patients with haematuria due to infection with Schistosoma haematobium have an elevated risk of bladder cancer (El Boulhany et al., 1972); additional carcinogenic mechanisms of schistosomiasis have been proposed (Badawi et al., 1995).

Chronic hepatitis B virus (HBV) infection produces a sustained hepatonecrosis with concurrent regenerative hyperplasia (Berman, 1988). Serum iron levels rise during the acute HBV infection and continue to do so during the carrier stage (Blumberg et al., 1981). Moreover, continually elevated body iron predisposes to persistence of HBV infection (Israel et al., 1989). In one study, carriers had an increased risk of 94-fold of developing hepatocellular carcinoma as compared with non-carriers (Bisegli et al., 1988). Among the chronic carriers, increased iron is associated with both enhanced risk of initiation of primary liver cancer and of promotion of growth of the tumour (Israel et al., 1989).

**Increased iron burden by unspecified routes**

Four epidemiological studies have reported a positive association of increased available body iron burden, derived from unspecified sources, with an increased risk of various kinds of neoplasms. Two epidemiological studies have reported a negative association. A seventh study observed that elevated total iron-binding capacity (TIBC) was associated with reduced risk of cancer in women but not in men.

In the initial report based on the First National Health and Nutrition Examination Survey in the US (NHANES), 232 men who developed cancers over a 10-year period had a mean Tf iron saturation value of 33.1% at least 4 years before diagnosis, whereas 3,113 men who remained free of cancer had a mean Tf iron saturation value of 30.7% (P = 0.002) (Stevens et al., 1988). In the same study, in 5,228 women with at least 6 years of follow-up (during which time 149 developed cancer), the relative risk of neoplasia associated with a Tf iron saturation value ≥ 36.8% was 1.5 (95% CI 1.0–2.2).

In a second report on the NHANES population, the subjects were divided into quintiles according to baseline Tf iron saturation. For men, the relative risk of neoplasia in the highest quintile (> 60%) as compared with the lowest (≤ 30%) was 1.69 (95% CI 1.02–2.77; P = 0.03); for women, 2.09 (95% CI 1.05–4.18; P = 0.10) (Stevens et al., 1994).

A third report described a cohort of 41,276 women and men followed for 14 years; 2,469 developed cancers. Among persons whose initial Tf iron saturation value was > 60%, the relative risk (as compared with those with levels ≤ 60%) for all cancers, lung cancer, and colorectal cancer, respectively, was 1.43, 1.51 and 3.04 (Knekt et al., 1994). In the fourth study, a group of 140 controls and 131 colonic adenoma patients were divided into quartiles on the basis of their serum ferritin values (ng/ml): 8–43, 44–83, 84–156 and 157–399. The odds ratio of developing colonic adenomas for persons in the respective quartiles were 1.0, 1.6, 2.8 and 4.3 (Nelson et al., 1994).

It should be noted that when synthesized to sequester hazardous quantities of the metal, ferritin is a useful estimate of body iron status. However, production of the protein also is a component of the iron-withholding defence against such inducers of inflammation as microbial and neoplastic cell invaders. Thus, in future clinical studies, it would be desirable to include one or more additional measures of body iron such as Tf iron saturation and serum concentration of Tf receptors (Leduc and Craig, 1995).

In the initial study that found a negative association between iron burden and neoplasia, 233 patients with stomach cancer were examined. Their mean serum ferritin value was 49 ng/ml as compared with a mean value of 69.2 ng/ml in 350 controls (P < 0.05) (Akiba et al., 1991). The authors proposed that the inverse correlation between serum ferritin concentration and stomach cancer risk may at least partially be explained by an involvement of hypochlorhydria or achlorhydria which would reduce the absorption of ingested non-haem iron.

The second negative study followed a population of 38,538 persons for an average of 17.7 years (Herrinton et al., 1995). The Tf iron saturation (TS) values had been determined in 1969–71. In men, TS was inversely associated with risk of colorectal carcinoma [≥ 40.7% compared with ≤ 26.0%; colon, RR = 0.62 (95% CI 0.35–1.1); rectum, RR = 0.30 (95% CI 0.08–1.10)] and with non-Hodgkin's lymphoma [32.1–40.6% compared with ≤ 26.0%; RR = 0.31 (95% CI 0.11–0.88)]. In women, TS was inversely associated with risk of epithelial cancers of rectum, kidney and thyroid. Paradoxically, a positive association of TS with risk of stomach carcinoma was found in women [≥ 34.5% compared with ≤ 20.3%; RR = 3.5 (95% CI 0.98–12)]. The
authors noted that although their results provided no support for the hypothesis that elevated TS is associated positively with cancer development, further investigation is warranted. They recommended that a series of determinations of body iron, rather than a single initial observation, be made during the lengthy follow-up periods. They suggested also that TS may not be the most appropriate measure of body iron burden.

In the seventh study, body iron values were obtained for 260 non-institutionalized, cancer-free persons aged 64–87 years (Van Asperen et al., 1995). During the subsequent 4–17 years, mortality due to cancer was monitored. The relative risk for 131 women whose TIBC was \( \leq 63.4 \, \mu M \), 63.5–75.2 \( \mu M \), or \( > 75.2 \, \mu M \), respectively, was 1.00, 0.55 (95% CI = 0.25–1.24) and 0.05 (95% CI = 0.007–0.39) \( (P < 0.001) \). The risk of mortality due to cancer in the 129 men was not associated with the level of TIBC.

**Prognostic use of iron value in human cancer patients**

"The iron status of the host can be a critical ecological influence on neoplastic growth." de Sousa and Potaznik (1984)

A profound shift in iron metabolism occurs in patients with diseases that trigger an inflammatory defence. Beginning with observations by Locke et al. (1932) and extended by many subsequent authors, a hypoferremia is seen to develop early in the disease, to lessen as clinical symptoms wane, and to intensify as the disease worsens. The process is independent of dietary iron, nor is body iron efflux altered. In earlier years, the process was termed the 'anaemia of chronic disorders' although the haematocrit typically averages 35% and rarely falls below 30% (Lee, 1983). Later, the process was termed the 'macrophage block'.

In the macrophage block process, iron from phagocytosed senescent erythrocytes, after having been released from haemoglobin by microsomal haem oxygenases, is retained by hepatic and splenic macrophages instead of being promptly discharged to plasma Tf (Konijn and Hershko, 1989). To safely sequester the retained iron, the macrophages synthesize additional ferritin. Excessive iron can be shifted to hepatocytes which also synthesize more ferritin (Aisen, 1988). In bone tissue, excessive iron can be moved from macrophages to neighbouring normoblasts (Deiss, 1983). More recently the macrophage block has been recognized to be a feature of the inducible phase of the iron-withholding defence system and to be initiated by such cytokines as interleukin-1 and -6 and by tumour necrosis factor-\( \alpha \) (TNF-\( \alpha \)) (Weinberg, 1992b).

In as much as newly synthesized ferritin is a prominent component in the inducible phase of the withholding defence process, it is not surprising that, in a variety of neoplastic diseases, some of the protein is released from host cells and accumulates in plasma to result in a hyperferritinaemia (Table 1). As the patient's condition improves, serum ferritin values tend to return to normal; as the disease process worsens, serum ferritin values mount (Table 2). However, the correlation between high serum ferritin and disease progression is not always observed. Neoplastic conditions that have failed to show a correlation include stage IV-S neuroblastoma, ganglionuroblastoma and Wilms' tumour (Hann et al., 1980, 1981).

Ferritin molecules that appear in serum during the inflammatory process have only 2–4% iron, whereas serum ferritin molecules in non-inflamed, iron-loaded persons contain 16–20% iron (Herbert et al., 1995). The 'inflammatory' molecules presumably have been more recently synthesized whereas in persons with long-term iron overload the ferritin molecules would have been exposed earlier to excessive iron. In either case, the function of intracellular ferritin is that of safely sequestering iron that might otherwise injure host defence cells and/or stimulate growth of such invaders as tumour cells. On the other hand, extracellular ferritin is not considered to have an iron-scavenging role.

A different source of serum ferritin can be that of the tumour cells themselves. Examples of neoplastic cells that can produce the protein include neuroblastoma, Hodgkin's disease, and hepatocellular carcinoma (Hann et al., 1989). Human ferritin has been recovered from sera of nude mice who had been inoculated with human tumour cell lines and who developed the human tumours (Hann et al., 1984).

In iron-overloaded persons, a hyperferritinaemia generally is accompanied by an elevation in TS value. In contrast, in patients who have an inflammatory process, the hyperferritinaemia generally is accompanied by a reduction in TS. Additionally, in patients with inflammation associated with activation of cell mediated immunity (including malignancies), neopterin levels in serum and in urine are often elevated. Neopterin, produced by human macrophages stimulated by interferon-\( \gamma \) (IFN-\( \gamma \)) rises as the disease is intensified and falls as the patient enters remission or recovers (Wachter et al., 1989).
Table I. Examples of prognostic utility of serum values of ferritin, transferrin iron (Tf-iron) saturation and iron obtained prior to, or at the time of, diagnosis

<table>
<thead>
<tr>
<th>Population</th>
<th>Serum component</th>
<th>Observation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women with untreated breast cancer</td>
<td>Ferritin</td>
<td>Mean level (ng/ml):</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>117 normal women</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>135 patients</td>
<td>199</td>
</tr>
<tr>
<td>Women with early breast cancer</td>
<td>Ferritin</td>
<td>Mean level (ng/ml):</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>250 normal women</td>
<td>56.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>299 patients</td>
<td>96.9</td>
</tr>
<tr>
<td>(if level ≥ 201 ng/ml, there is increased probability of recurrence within 3 years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemodialyzed adults who developed hepatitis B</td>
<td>Ferritin</td>
<td>Mean log:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 patients with transient infection</td>
<td>1.62±0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29 patients with persistent infection</td>
<td>2.07±0.63</td>
</tr>
<tr>
<td>Men with primary hepatocellular carcinoma</td>
<td>Ferritin</td>
<td>Mean level (ng/ml):</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>128 normal men</td>
<td>99.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70 patients</td>
<td>121</td>
</tr>
<tr>
<td>Children with neuroblastoma:</td>
<td>Ferritin</td>
<td>Progression-free survival (30 months):</td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td></td>
<td>12 patients ≤ 75 ng/ml</td>
<td>83%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 patients 75–142 ng/ml</td>
<td>71%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19 patients &gt;142 ng/ml</td>
<td>26%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 patients ≤ 75 ng/ml</td>
<td>37%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 patients 75–142 ng/ml</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>134 patients &gt;142 ng/ml</td>
<td>3%</td>
</tr>
<tr>
<td>Stage IV</td>
<td>Ferritin</td>
<td>Progression-free survival (24 months):</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>64 patients ≤ 150 ng/ml</td>
<td>83%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>39 patients &gt;150 ng/ml</td>
<td>18%</td>
</tr>
<tr>
<td>Children with acute lymphoblastic leukaemia</td>
<td>Tf-iron</td>
<td>Survival (% alive) at 48 months:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>saturation</td>
<td>33 patients ≤ 36%</td>
<td>91%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23 patients ≥ 36%</td>
<td>65%</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>24 patients &lt;11.6 μM</td>
<td>92%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>46 patients 11.6–29.5 μM</td>
<td>67%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21 patients &gt;29.5 μM</td>
<td>48%</td>
</tr>
<tr>
<td>Children with Hodgkin’s disease</td>
<td>Ferritin</td>
<td>Progression-free survival (120 months):</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>31 patients ≤ 142 ng/ml</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19 patients &gt;142 ng/ml</td>
<td>42%</td>
</tr>
<tr>
<td>Children with HIV-1 infection</td>
<td>Ferritin</td>
<td>Clinically stable (24 months):</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 patients ≤ 100 ng</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28 patients &gt;100 ng/ml</td>
<td>7%</td>
</tr>
</tbody>
</table>

Methods for withholding and withdrawing iron from cancer cells

"The US Food and Drug Administration does not acknowledge that iron is a carcinogen and does not invoke the Delaney law that forbids adding carcinogens to food." Crosby (1988)

"Practices recommended to lower ingested and inhaled quantities of iron are beginning to show association with reduced incidence of neoplasia." Weinberg (1992c)

Natural methods

In hosts invaded by neoplasia, macrophages not only retain iron derived from effete erythrocytes as described above, but also serve as a crypt for the metal that is scavenged by lactoferrin (Lf) from invaded tissue sites. When the iron saturation of Lf attains 40% (Birgens, 1994), the ferrated protein can yield the metal to ferritin in macrophages that have surrounded tumour nodules. In Dunning lymphosarcoma, for instance, very little iron is contained in the nodules, whereas large quantities are seen in macrophages that surround the tumour (Price and Greenfield, 1958). In rat sarcomas induced by injection either of iron dextran or of other carcinogens, iron-stained neoplastic lesions appeared as clear zones amidst dense accumulation of siderophages (Carter, 1969). Similarly, sarcomatous cells induced by iron injection in rats contained very small amounts of the metal, whereas large quantities were present in surrounding macrophages (Magnusson et al, 1977).
### Table 2. Examples of prognostic utility of serially obtained serum values of ferritin or iron

<table>
<thead>
<tr>
<th>Population</th>
<th>Serum component</th>
<th>Observation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children with neuroblastoma</td>
<td>Ferritin</td>
<td>10 patients &lt;400 ng/ml initially and serially; all remained disease-free</td>
<td>Hann et al., 1980</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17 patients &gt;400 ng/ml when disease was active and &lt;400 ng/ml when in remission</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 patients &lt;400 ng/ml during active disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 patients: decline in ferritin lagged behind remission</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 patient: ferritin rise lagged behind relapse</td>
<td></td>
</tr>
<tr>
<td>Children with Hodgkin’s disease</td>
<td>Ferritin</td>
<td>4 patients followed 3–5 years: marked rise in ferritin preceded or accompanied progression; partial reduction in ferritin upon partial remission; marked reduction upon complete remission</td>
<td>Dörner et al., 1983</td>
</tr>
<tr>
<td>Adults with renal carcinoma</td>
<td>Iron</td>
<td>16 patients had low iron at diagnosis; after nephrectomy, 10 regained normal iron and remained well; 6 retained low iron and died with metastases</td>
<td>Loughlin and Gittes, 1986</td>
</tr>
<tr>
<td>Adults with haematogenous malignancies + bone marrow transplant</td>
<td>Ferritin</td>
<td>28 patients: ferritin increased in first 3 months due to tissue destruction; thereafter ferritin declined in those with remission and increased in those in relapse</td>
<td>Or et al., 1987</td>
</tr>
<tr>
<td>Adults with PHC</td>
<td>Ferritin</td>
<td>93 patients &lt;300 ng/ml 16% developed PHC</td>
<td>Hann et al., 1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>39 patients &gt;300 ng/ml initially and &lt;300 ng/ml subsequently; 15% PHC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 patients &gt;300 ng/ml initially and &gt;300 ng/ml subsequently; 43% PHC</td>
<td></td>
</tr>
<tr>
<td>Adults with small cell lung carcinoma</td>
<td>Ferritin</td>
<td>At discovery: 2 patients 21–40 ng/ml; 12 patients 41–300 ng/ml; 17 patients &gt;300 ng/ml</td>
<td>Milman et al., 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Near death: 1 patient &lt;300 ng/ml; 29 patients &gt;300 ng/ml</td>
<td></td>
</tr>
<tr>
<td>Children with HIV-1 infection</td>
<td>Ferritin</td>
<td>Over 2 years, ferritin decreased or remained stable at &lt;100 ng/ml in 13 patients; only 1 developed infections</td>
<td>Ellaurie and Rubinstein, 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In 7 patients, ferritin increased; 3 of these died; 4 developed infections</td>
<td></td>
</tr>
</tbody>
</table>

PHC = primary hepatocellular carcinoma

In human neuroblastoma specimens, macrophages contained three times as many ferritin particles as did undifferentiated neuroblastoma cells (Iancu, 1989). In patients with Hodgkin’s disease or other lymphomas, markedly increased amounts of haemosiderin were noted in lymph nodes whether or not the nodes were histologically involved in the disease (Dumont et al. 1976).

In addition to their important defence role as a repository for hazardous quantities of iron, macrophages activated by IFN-γ, TNF-α and products of invaders can induce a withdrawal of non-haem iron from neoplastic cells (Hibbs et al., 1984; Wharton et al., 1988). The iron-deprived cells may remain viable by maintaining anaerobic glycolysis but are unable to synthesize DNA (Lepoitre et al., 1994), express mitochondrial aconitate activity (Drapier and Hibbs, 1986; Wharton et al., 1988) or transport electrons via mitochondrial NADH:ubiquinone (complex I) and succinate:ubiquinone (complex II) oxidoreductases (Granger and Lehninger, 1982).

The iron-withdrawal factor formed by activated macrophages consists of reactive nitrogen intermediates (RNI) typified by nitric oxide, an unstable product of L-arginine catabolism. Authentic nitric oxide possesses iron-withdrawal activity (Hibbs et al., 1988). The cytotoxic compound is derived from the guanido atom(s) of L-arginine when about 30% of the amino acid is converted to L-citrulline (Hibbs et al., 1988; Marletta et al., 1988; Granger et al., 1990). In contrast, non-activated macrophages catalyze L-arginine entirely to L-ornithine.

Patients with renal cell carcinoma or malignant melanoma who were injected with interleukin-2 increased their synthesis of RNI by 10- and 8-fold, respectively (Hibbs et al., 1992). Interleukin-2, primarily formed by T lymphocytes, stimulates production of IFN-γ and TNF-α. The latter cytokines can induce creation of RNI not only by macrophages but also by endothelial fibroblasts, hepatocytes and the tumour cells themselves (Hibbs et al., 1992). In some systems, nitric oxide can be cytoidal. For example,
human blood monocytes that have matured to macrophages gain the ability to kill K562 cells via RNI (Martin and Edwards, 1993).

Iron released from non-multiplying and/or dying tumour cells presents a hazard to normal cells via its ability to catalyse formation of hydroxyl radicals. Thus, the metal must be sequestered promptly, ideally by macrophages at the tumour site. Unfortunately, the capacity of iron disposal by host defence cells is not inexhaustible. The tumoricidal activity of peritoneal macrophages, for example, is suppressed by as little as 1.3 pg iron per cell (Green et al., 1988). Moreover, mechanisms for excreting dangerous quantities of iron are not possessed by primate hosts.

Environmental and nutritional methods

Environmental and nutritional methods for prevention of iron availability to cancer cells are summarized in Table 3. For some of the methods, health benefits are associated not only with prevention of iron availability but with other factors as well. For instance, elimination of cigarette smoking results in reduction of lung pollution not only by iron but also by organic poisons. A decrease in mammalian meat consumption implies a lessening of ingestion of fat. Ingestion of unrefined grains contributes fibre as well as phytic acid. Reduced ingestion of meat and increased ingestion of fibre and phytic acid generally have been associated with decreased risk of colorectal cancer (Graf and Eaton, 1985; Nelson et al., 1989; Ullah and Shamsuddin, 1990; Freudenheim et al., 1990; Willett et al., 1990; Giovanucci et al., 1992; Neugut et al., 1993; Weinberg, 1994a).

Medical and pharmaceutical methods

The most obvious medical method for lowering the body burden of excessive iron in normal persons is that of routine blood donations. In a study of 37,795 blood donors, the relative risk of developing cancer (all sites combined) as compared with non-donors was 0.79 (P < 0.001) (Merk et al., 1990). The authors suggested that “blood donors may lead a more healthy life than the rest of the population” but they acknowledged that reduction in body iron could be responsible for their observations. Unfortunately, body iron values were not determined.

An alternative medical method for lowering the iron hazard is that of routine ingestion of aspirin. For example, alternate day intake of 1.3 g aspirin can cause intestinal blood loss approximately equivalent to donation of a unit of blood every 6 months (Meyer et al., 1992). In a 6-year study of 662,424 adults, the relative risk of death from colon cancer among men and women who ingested unspecified amounts of aspirin at least 16 times/month (as compared with non-users) was 0.60 (95% CI 0.40–0.89) and 0.58 (95% CI 0.37–0.90), respectively (Thun et al., 1991). Regrettably, data on body iron values were not collected.

Three kinds of pharmaceutical methods to selectively suppress the supply of growth-essential iron to neoplastic cells are being developed: (a) design and testing of intracellular iron-chelating agents for withdrawal of the metal, (b) use of gallium salts to interfere with iron uptake and utilisation and (c) use of monoclonal antibodies to transferrin receptors to block uptake of iron.

The iron-chelating agents DF and deferiprone are routinely used to elute the metal from iron-loaded patients such as thalassaemics who cannot be phlebotomized (Kontogiorgos and Weinberg, 1995). Increasingly, these and other iron chelators are being tested in animal models or in patients who have conditions such as infection or neoplasia in which excessive iron contributes to the pathologic process (Weinberg, 1994b).

The ability of DF to inhibit growth of tumour cells in culture, and to some extent in hosts, has been reported by several groups. In a study of cell viability (Hann et al., 1990), 15 μM DF killed 20–70% of cultured cells of three human hepatoma lines, but < 2% of a human diploid cell line. In a different in vitro study, the ratio between the anti-neuroblastomic effect and the haematopoietic toxicity of DF was

<table>
<thead>
<tr>
<th>Table 3. Environmental and nutritional methods for prevention of iron loading</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reduction in amount of parenteral iron</strong></td>
</tr>
<tr>
<td>1. Wear shoes when walking on volcanic soil rich in iron oxide</td>
</tr>
<tr>
<td>2. Eliminate parenteral iron medications (unless person has a correctly diagnosed iron deficiency)</td>
</tr>
<tr>
<td>3. Eliminate transfusions of whole blood and/or erythrocytes unless an unequivocal medical justification is provided</td>
</tr>
<tr>
<td><strong>Reduction in amount of inhaled iron</strong></td>
</tr>
<tr>
<td>1. Eliminate cigarette smoking</td>
</tr>
<tr>
<td>2. Eliminate use of asbestos, crocidolite and tremolite</td>
</tr>
<tr>
<td>3. Enhance protective equipment for workers in ferriferous industries</td>
</tr>
<tr>
<td><strong>Reduction in amount of ingested iron</strong></td>
</tr>
<tr>
<td>1. Reduce consumption of mammalian meats, ethanol, ascorbic acid and iron supplements (unless person has a correctly diagnosed iron deficiency)</td>
</tr>
<tr>
<td>2. Avoid processed foods that have been adulterated with inorganic iron or with blood</td>
</tr>
<tr>
<td>3. Increase consumption of tea (which contains iron-binding tannins) and of unrefined grains (which often contain iron-binding phytic acid)</td>
</tr>
</tbody>
</table>
found to be satisfactory for use of the drug in therapy (Timeus et al., 1994).

The growth rate of human hepatocellular carcinoma cells injected s.c. into athymic nude mice was 2.6–10-fold greater in control animals than in mice protected with DF (Hann et al., 1992). In some of the protected animals, the tumour disappeared. Nine neuroblastoma patients were treated with 150 mg DF/kg/day for 9 days (Donfrancesco et al., 1990). In seven of the patients, bone marrow infiltration was decreased by >50% and in one patient a reduction in tumour mass was observed. A patient with lymphoid blast crisis of chronic myelogenous leukaemia, treated with DF, showed a marked reduction in circulating blast count (Cazzola et al., 1990).

Gallium resembles iron with respect to Tf binding, cellular uptake via transferrin receptors and incorporation into ferritin. The metal blocks the cellular uptake of iron and inhibits cell growth by specifically limiting the availability of iron for activation of the M2 subunit of ribonucleotide reductase (Chitambar and Narasimhan, 1991). Sequential exposure of human leukaemia HL60 cells (Lundberg and Chitamber, 1990) and of human bladder cancer cells (Seligman et al., 1993) to DF plus gallium nitrate resulted in significant potentiation of the growth inhibitory effects of gallium.

Several research groups have attempted to deprive tumour cells of iron by employing monoclonal anti-transferrin receptor antibodies (ATRAs). Both multivalent (IgM or IgA) and bivalent (IgG) ATRAs exhibit antitumour activity. The multivalent antibodies tend to crosslink the receptors at the cell surface, whereas the bivalent antibodies are endocytosed with the receptors and decrease the cycling efficacy of the latter (Kemp et al., 1992). In one study, combined iron-depletion with an IgA ATRA plus either an iron chelator (parabactin) or gallium nitrate showed enhanced cytotoxicity against HL60 leukaemia cells but not against KB epithelial carcinoma cells (Taetle et al., 1989). In another study, an IgG ATRA was synergistic with DF in inhibiting culture growth of five murine lymphoid tumour lines (Kemp et al., 1990). IgG antibodies may be clinically more attractive reagents than IgA or IgM ATRAs because of better access to extravascular tissue spaces. Some of the anti-neoplastic drugs in current use such as the Vinca rosea alkaloids (Sethi et al., 1984), cisplatinum (Kletter et al., 1988) and hydroxyurea (Cazzola et al., 1990) function, in part, by withholding iron. The vinca alkaloids (Morgan and Iacopetta, 1987) and cisplatinum (Kletter et al., 1988) apparently suppress endocytosis of Tf–Fe, and hydroxyurea inactivates ribonucleotide reductase (Yarbro, 1992). Synergistic cytotoxicity of hydroxyurea with gallium (Chitambar and Narasimhan, 1991) and with DF (Cazzola et al., 1990) has been observed. The clinical use of iron-depletion therapy must be carefully monitored to avoid, as much as possible, iron starvation of proliferating normal marrow cells. Nevertheless, “control of tumour cell growth through perturbation of cellular iron metabolism is a potentially important strategy in the treatment of cancer and thus, warrants continued investigation” (Lundberg and Chitamber, 1990).

Conclusions

Numerous laboratory and clinical investigations over the past few decades have observed that one of the dangers of iron is its ability to favour neoplastic cell growth. The metal exerts its carcinogenic effects by catalysing formation of hydroxyl radicals, suppressing activity of host defence cells and promoting cancer cell multiplication. In both animals and humans, primary neoplasms develop at body sites of excessive iron deposits. The invaded host attempts to withhold iron from the cancer cells via sequestration of the metal in newly formed ferritin. The host also endeavours to withdraw the metal from cancer cells via macrophage synthesis of nitric oxide.

Quantitative evaluation of body iron and of iron-withholding proteins has prognostic value in cancer patients. Procedures associated with lowering host iron intake and inducing host iron efflux can assist in prevention of and management of neoplastic diseases. Pharmaceutical methods for depriving neoplastic cells of iron are being developed in experimental and clinical protocols.

References


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