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Bleomycin-detectable iron in serum from leukaemic patients before and after chemotherapy

Therapeutic implications for treatment with oxidant-generating drugs

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Patients with acute myeloid leukaemia show elevated plasma iron and, in 2/6 cases studied, low-molecular-mass iron complexes capable of stimulating radical reactions were present in the plasma. Shortly after the onset of chemotherapy, there is a sharp rise in transferrin saturation and all patients studied showed low-molecular-mass iron in their plasma. It is proposed that such iron could interact with oxidants generated by certain drugs (e.g. adriamycin or daunorubicin) to facilitate tissue damage, and that some of the side-effects of chemotherapy might be ameliorated by careful co-administration of small doses of desferrioxamine.

Leukemia; Iron; Bleomycin; Chemotherapy; Daunorubicin; Redox-cycling drug

1. INTRODUCTION

Hypoferraemia is a common finding in cancer patients [1], but acute leukaemia appears to be an exception. Thus of 34 patients with acute myeloid leukaemia, 21 had high levels of serum iron and in 17 the transferrin was reported to be iron-saturated [2]. Of 6 patients with acute lymphoblastic leukaemia, two had high levels of serum iron and fully saturated transferrin [2]. Similar results have been reported by others [3], and it is well established [4] that serum ferritin is elevated in acute leukaemia.

The high percentage saturation of transferrin in patients with acute leukaemia raises the possibility that non-transferrin-bound, low-molecular-mass, serum iron complexes might be present in this disease. Such iron complexes can mediate tissue damage in several disease states by catalyzing free radical reactions such as the peroxidation of membrane lipids and the formation of highly reactive hydroxyl radical ($^{\cdot}OH$) from superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) [5–7]. This is especially relevant because several of the drugs used to treat leukaemia, such as daunorubicin and adriamycin, lead to increased generation of $O_2^{\cdot-}$ and H_2O_2 in vivo [8]. Indeed, an iron(III)-adriamycin complex ('quelamycin') has proved to be much more toxic to humans than adriamycin itself [8,9].

Low-molecular-mass iron complexes, capable of stimulating free radical reactions, can be measured by a technique known as the bleomycin assay [6,10,11]. Bleomycin is an anti-tumour antibiotic that binds weakly to iron ions, and the bleomycin-iron complex degrades DNA in the presence of ascorbic acid as a reducing agent [10]. If bleomycin, ascorbate and DNA are in excess, the amount of DNA degradation is proportional to the concentration of iron ions available to bleomycin [11]. When applied to human serum samples, the bleomycin assay does not measure iron bound to ferritin or transferrin [6,11,12], but only low-

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molecular-mass iron ions in forms that can stimulate free radical reactions. The bleomycin assay has been used in studies of iron overload disease [6,11,12], rheumatoid arthritis [7,10,11], and in several other disorders [10]. In the present paper, we have applied the assay to study such catalytic iron complexes in the serum of patients with acute leukaemia, before and during chemotherapy.

2. MATERIALS AND METHODS

Blood samples were taken, with informed consent, from the antecubital veins of patients with acute or chronic leukaemia immediately before, and at intervals during chemotherapy (described in the legend to table 2). Blood was allowed to clot and the serum was immediately subjected to the bleomycin assay [6]. Determinations of transferrin iron saturation and total serum iron were as described in [13]. Diagnoses were made by an experienced haematologist (G.M.).

3. RESULTS

Initial studies on 6 patients with acute myeloid leukaemia showed that plasma iron concentrations and percentage saturations of transferrin with iron tended to be greater than normal, in agreement with previous results [2,3]. Four of these patients showed no bleomycin-detectable iron in their plasma. However, in two patients (B and F) such iron was present despite the fact that transferrin was not completely saturated with iron. Bleomycin-detectable iron is never observed in plasma from healthy human subjects [6,10,11]. As a further control, plasma samples were obtained from 14 patients with chronic myelogenous leukaemia, who do not show elevations in plasma iron [1]. None of them showed any bleomycin-detectable iron.

For four patients, further blood samples were obtained after chemotherapy had begun. Table 2 shows that, in all cases, chemotherapy caused a rise in percentage transferrin saturation. Bleomycin-detectable iron appeared in the plasma in patients A and E, and its concentration rose in patients B and F. In patient E, both percentage transferrin saturation and concentrations of bleomycin-detectable iron were beginning to fall by 48 h after onset of chemotherapy.

4. DISCUSSION

Many cytotoxic drugs used in cancer chemotherapy generate oxidants such as superoxide and H_2O_2, and these oxidants have been implicated in the side-effects produced by such drugs, e.g. the cardiotoxicity of anthracyclines [8]. Iron ions stimulate generation of highly-reactive oxidants and they contribute to tissue injury in several diseases by this mechanism [5,10]. The bleomycin assay, when applied to plasma or serum, appears to measure iron ions capable of stimulating radical reactions [5–7,10–12]. Normal control subjects never show bleomycin-detectable iron; most plasma iron in healthy subjects is bound to the protein transferrin, and transferrin-bound iron will not stimulate radical reactions [5,14]. None of the patients with chronic lymphocytic leukaemia showed any bleomycin-detectable iron, nor did four of the patients with acute myeloid leukaemia (table 1). However, two of the latter patients did show the presence of such iron, even though their plasma transferrin was not saturated. The existence of bleomycin-detectable iron concurrent with incomplete transferrin saturation has also been reported in some patients with iron overload consequent upon idiopathic haemochromatosis [12,13] and possible reasons for this are discussed in [13].

The onset of chemotherapy presumably induces a massive destruction of leukaemic cells. Cytolysis would be expected to release internal iron pools from within cells [5]. The results in table 2 show that this effect was striking enough for there to be marked rises in the percentage saturation of transferrin and for bleomycin-detectable iron to appear in the plasma. The internal cellular iron pool of cells is thought to exist in a form that can stimulate free radical reactions [5,15]. Presumably some of the released iron binds to transferrin whilst the rest remains in a low-molecular-mass form, detectable in the bleomycin assay.

Cell proliferation requires a source of iron, and there have been several suggestions that strong iron chelators such as desferrioxamine could be used to halt rapid growth of leukaemia cells in vivo [16], as they do in vitro [17]. However, it would be difficult to combine this cytostatic agent [15] with cytocidal agents such as adriamycin or daunorubicin which act on replicating cells [8].

Table 1

Plasma iron parameters in patients with acute myeloid leukaemia

Patient code	Plasma iron (μmol/dm^3)	% transferrin saturation	Bleomycin-detectable iron (μmol/dm^3)
A	35.0	73	0
B	23.4	45	1.5
C	22.7	58	0
D	19.5	41	0
E	21.4	50	0
F	32.1	66	4.5

Plasma iron normal values 17.9 ± 6.7 μmol/dm^3, % transferrin saturation 29.9 ± 10.2%

Our results raise a different possibility: that many of the side-effects produced by drugs used in chemotherapy may involve release of iron ions and their exacerbation of free radical reactions. Indeed, an iron-adriamycin complex is much more toxic to humans than is adriamycin alone [8,9]. Iron irons bound to desferrioxamine do not usually participate in free radical reactions [5,15,18] and this chelator has already been shown to minimize the toxicity of some radical-generating drugs in vivo, such as paraquat and alloxan (reviewed in [15]). It is thus possible that small doses of desferrioxamine, sufficient only to bind the bleomycin-detectable iron, might diminish the radical-mediated injurious side-effects of some drugs used in cancer chemotherapy (e.g. daunorubicin or adriamycin) without affecting their action against leukaemic cells.

Table 2

Effect of chemotherapy on plasma iron parameters in patients with acute myeloid leukaemia

Patient code	Plasma sample taken in relation to start of chemotherapy	Total plasma iron (μmol/dm^3)	% transferrin saturation	Bleomycin-detectable iron (μmol/dm^3)
A	before	35.0	73	0
	24 h after	47.0	97	5.8
B	before	23.4	45	1.5
	24 h after	32.0	67	1.8
E	before	21.4	50	0
	24 h after	27.1	95	21.5
	48 h after	26.4	74	18.1
F	before	32.1	66	4.5
	24 h after	28.4	72	8.7

Chemotherapy consisted of A daunorubicin/cytosine arabinoside/6-thioguanine; B as A plus vincristine/prednisolone; E 6-thioguanine/6-mercaptopurine; F as A

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