Antineuroblastoma Activity of Desferoxamine in Human Cell Lines

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ABSTRACT

That ferritin, an iron storage protein, may be present in increased amounts in the serum of patients with neuroblastoma (1). High concentrations predict a poor outcome in patients with advanced disease (2). Studies have indicated that serum ferritin in these patients is at least in part tumor derived (3). In an attempt to understand why ferritin levels correlate with prognosis, studies have been done in which biological effects of ferritin have been examined. These have focused on the ability of ferritin to inhibit host immune responses and thereby enhance tumor growth (4–6). We postulate that in addition to these effects, ferritin, or the ferric ions it carries, is a growth factor for neuroblastoma and directly contributes to tumor growth. To explore this possibility, we studied the effects of desferoxamine, an iron chelator which has a high affinity for ferritin-bound iron (7), on viability of human neuroblastoma cells in vitro and on their ability to express receptors for the iron-binding glycoprotein, transferrin.

INTRODUCTION

Ferritin, an iron storage protein, may be present in increased amounts in the serum of patients with neuroblastoma (1). High concentrations predict a poor outcome in patients with advanced disease (2). Studies have indicated that serum ferritin in these patients is at least in part tumor derived (3). In an attempt to understand why ferritin levels correlate with prognosis, studies have been done in which biological effects of ferritin have been examined. These have focused on the ability of ferritin to inhibit host immune responses and thereby enhance tumor growth (4–6). We postulate that in addition to these effects, ferritin, or the ferric ions it carries, is a growth factor for neuroblastoma and directly contributes to tumor growth. To explore this possibility, we studied the effects of desferoxamine, an iron chelator which has a high affinity for ferritin-bound iron (7), on viability of human neuroblastoma cells in vitro and on their ability to express receptors for the iron-binding glycoprotein, transferrin.

MATERIALS AND METHODS

CHP 126 and CHP 100, two previously characterized neuroblastoma cell lines (8) (gift from G. Johnson, Pediatric Oncology Branch, National Cancer Institute, Bethesda, MD), were maintained in tissue culture in RPMI 1640 (M.A. Bioproducts, Walkersville, MD) with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (“complete medium”). Cells from one or the other were plated into 16-mm tissue culture dishes (5 × 10⁴/dish) in 2 ml complete medium or into 96-well microtiter plates (5 × 10⁴/dish in 0.2 ml complete medium). Graded amounts (5–60 μM, final concentration) of desferoxamine mesylate (gift from C. Brownley, Jr., CIBA-Geigy, Summit, NJ), or ferrioxamine, an iron-saturated analogue of desferoxamine (gift from Drs. H. H. Peter and K. Scheibli, CIBA-Geigy, Basel, Switzerland) were added to triplicate dishes. Additional dishes contained desferoxamine as well as 60, 120, or 180 μM ferric citrate (Sigma Chemical Co., St. Louis MO). Ferric citrate (60 μM) is stoichiometric for desferoxamine. Control samples contained none of these additives, ferric citrate alone, or desferoxamine in combination with citric acid (120 μM). Dishes or plates were incubated at 37°C in 5% CO₂/95% air. Cultures were examined after four hours to ensure that cell attachment had taken place. At serial times, cells were removed from plates by pipeting without prior trypsin digestion, and viable cell counts were done in a hemocytometer using trypan blue exclusion. Cell counts also were done for cells growing in microtiter plates using a colorimetric assay which depends upon the reduction of a tetrazolium dye by viable cells (9). All experiments were repeated at least three times.

In order to examine the specificity of desferoxamine for neuroblastoma, four well-characterized, non-neuroblastoma cell lines also were studied as summarized above. These were HEp-2, a cell line derived from a human epidermoid carcinoma of the larynx; WI-38, a human diploid cell line derived from normal embryonic lungs; MRHF, a human diploid cell line derived from newborn foreskin (all from M.A. Bioproducts and a gift from Mr. Richard DeBiasio, Virology Laboratory, Children's Hospital of Pittsburgh); and ATCC CRL1427, a cell line derived from a human osteosarcoma (American Type Culture Collection).

Because transferrin appears to be the major carrier for transport of Fe⁺³ ions to cells, and because it has previously been documented that the presence of transferrin receptors correlates with proliferative status (10), we examined the effect of desferoxamine on expression of such receptors by neuroblastoma cells. After incubation of cells for 24 h with or without 60 μM desferoxamine as noted above, cells were washed in phosphate-buffered saline and incubated with transferrin receptor mouse OK-T9 monoclonal antibody (Ortho Pharmaceutical Corp., Raritan, NJ). Cellular localization of OK-T9 antibody was visualized by further incubation with goat anti-mouse immunoglobulin and immunoglobulin staining according to a previously published method (11). Control specimens were incubated with immunoglobulin stain without immunoglobulin. Cytosplasm preparations of cells processed in this manner were stained with Wright-Giemsa and examined with the oil immersion lens of a light microscope.

RESULTS

As shown in Table 1, 72 h after addition of desferoxamine (final concentration 60 μM) to either of the neuroblastoma cell lines, greater than 80% of the cells were dead. This effect was dose dependent and was not seen with ferrioxamine. Although ferric citrate alone had no effect on growth, its presence largely prevented the cytotoxic effects of desferoxamine. The protection was not due to the citrate anion, as it was not seen with citric acid.

The effect of desferoxamine (60 μM) on viability of CHP 126 and CHP 100 cells over time is shown in Table 2. Cell death was noted as early as 24 h after initial drug exposure. By 48 h, 60–70% of cells were dead and the remaining viable cells showed increased granularity of the cytoplasm. Cell death appeared to be maximal by 96–120 h. Cytotoxicity could be reversed and cell growth resumed if desferoxamine-containing medium was removed within 24 h, but not more than 48 h after plating and replaced with complete medium.

In comparison with its effect on the two neuroblastoma cell lines, desferoxamine had relatively little effect on viability of WI-38 or MRHF. After 72-h exposure to 60 μM desferoxamine, both of these lines exhibited 100% viability compared with...
The presence of ferric ions. Moreover, desferoxamine upgrades the antineuroblastoma activity in vitro. This activity appears to be a result of its chelating ability since it was not seen with ferrioxamine, a "pre-chelated" form of the drug. Presumably, desferoxamine works by specifically chelating ferric ions since its affinity for that metal is particularly high and since the effect of desferoxamine was largely abrogated by the simultaneous expression of receptors for transferrin, an iron transport protein.

Desferoxamine does exhibit some specificity for growing neuroblastoma cells. Changes in viability of two other cell lines could not be demonstrated at the concentration tested and cytotoxicity did not appear to be as profound in two additional cell lines. Whether this cytotoxicity is a function of growth rate is not yet clear and is the subject of further investigation.

Desferoxamine is a compound with which there has been ample experience in patients with iron overload (12), particularly in the pediatric age range where neuroblastoma is most common. Although a method for measuring serum levels of desferoxamine is not available, in patients with thalassemia major whose serum ferritin levels are comparable to those which might be seen in children with neuroblastoma, levels of desferoxamine are achievable which are sufficient to chelate iron. On the basis of this experience, it seems likely that drug levels sufficient to chelate ferritin-bound iron in children with neuroblastoma can be achieved with tolerable toxicity in vivo. Neuroblastoma is a pediatric cancer which often has a dismal prognosis. It will be of interest to study the effects of desferoxamine on neuroblastoma xenografts in mice and perhaps ultimately in children with neuroblastoma refractory to conventional chemotherapy.

REFERENCES

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