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In vitro effects and clinical evaluation of a human chorionic gonadotrophin preparation in acute leukemia

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Commercial human chorionic gonadotrophin (HCG) preparations decrease the tumorigenicity of human tumors in immunodeficient mice and induce apoptotic cell death in animal tumor models. Preliminary studies in humans have demonstrated tumor regression in patients with Kaposi's sarcoma given intralesional injections of HCG. To further evaluate HCG's antitumor activity we conducted *in vitro* and clinical evaluations of HCG in acute myeloid leukemia (AML). In HL-60 leukemic cell lines, a 20–40% inhibition of cell density was demonstrated by trypan blue exclusion method at low concentrations of an HCG preparation (2×10^{-3}–2×10^{-2}). Similar concentrations also resulted in a reduction in the proportion of cells in G_2M phase of the cell cycle, as well as enhanced differentiation compared to control cells. Fifteen patients with advanced AML with marrow blast counts >30%, and five with marrow blast counts between 10 and 26% were given daily subcutaneous injections of HCG 2–4 IU and oral levamisole 50 mg weekly. Five patients with absolute blast counts in the blood ranging from 0 to 3500/μl and percent blasts in the marrow ranging from 16 to 81% were observed to have no progressive increase in either marrow or peripheral blast counts for 70–121 days. One patient with a pretreatment blast count of 10% in the marrow, no circulating blasts and minor cytopenias had a decrease in marrow blasts to less than 5% which has persisted at 550 days. No significant improvement from baseline levels of neutrophils, hemoglobin or platelets were observed in any of the patients treated. Increases in apoptotic cell death were observed in over 50% of patients' cells with some demonstrating peak levels similar to experiences in patients treated with DNA-damaging chemotherapy. A decreased expression of bcl-2 was seen in the majority of patients ranging from 6 to 62%. These new observations suggest that HCG preparations may inhibit leukemic cell growth through enhancement of cell death mechanisms and could be used in judicious combinations with other approaches. The results confirm the pro-apoptotic effects of HCG preparations reported in patients with Kaposi's sarcoma. Identification of the active component of HCG preparations and further understanding of its growth modulatory action will be important in its development as a clinically useful agent.

Keywords: chorionic gonadotrophin; acute leukemia

Introduction

Human chorionic gonadotropin (HCG) is a glycoprotein hormone normally produced and secreted by trophoblastic cells of the placenta during pregnancy. HCG has also been shown to be expressed on tumor cells of both trophoblastic and non-trophoblastic origin.[1,2] Although its integral role in fetal implantation and development is well established,[3] its function in the growth and dissemination of tumors is unclear.

Some studies have suggested that HCG increases tumorigenicity. For example, Acevedo *et al*[4,5] demonstrated that cultured human cancer cells expressed HCG on the cell membrane and suggested a direct correlation between the extent of membrane-associated HCG and the metastatic potential of the cells. Kellen *et al*[6] demonstrated that adenocarcinoma cells have significantly diminished tumorigenicity when inoculated into mice previously exposed to HCG conjugated to a tetanus toxoid intended to prophylactically stimulate an immune response against HCG-like substances.

Other observations, however, have demonstrated a direct growth inhibitory effect on tumors when exposed to HCG. Lunardi-Iskandar *et al*[7] observed that Kaposi's sarcoma tumors could not be established in immunodeficient mice during pregnancy or after treatment with commercial HCG preparations. In addition, in Kaposi's sarcoma cell lines, exposure to several of these preparations resulted in growth inhibition and induction of apoptotic cell death. In initial therapeutic trials, Gill *et al*[8,9] observed that intralesional injections and subcutaneous administration of HCG preparations in patients with Kaposi's sarcoma resulted in regression of tumors and morphologic evidence of apoptotic cell death within the treated lesions.

In addition to these observations in Kaposi's sarcoma, administration of an HCG preparation has also been evaluated in animals with spontaneous occurring malignant tumors. McMichael[10] treated several animals with subcutaneous injections of HCG and demonstrated anti-tumor activity in several tumor types including both solid tumors in horses and dogs as well as feline leukemia. In this experience with systemic administration of HCG, two initial animals treated developed a febrile illness and sudden death following administration of HCG which was attributed to a Herxheimer-type reaction caused by sloughing of necrotic tissue. Subsequent animals treated concomitantly with levamisole to enhance cell-mediated immunity and phagocytosis, did not experience this reaction. Based on these observations, and in order to further assess the effects of HCG in human cancer cells, we evaluated the *in vitro* effects and systemic clinical activity of an HCG preparation in acute leukemia.

Materials and methods

In vitro studies

Human leukemia cell line HL-60 cells were seeded at 2.5×10^{-5} cells/ml in RPMI-1640 medium supplemented with 15% heat inactivated fetal calf serum and antibiotics. HCG in saline (Schein Pharmaceutical, Baxter, NJ, USA) was added at various concentrations to HL-60 cells. Cell cultures supplemented with saline in place of HCG were employed as controls. Culture medium was changed every 2–4 days. Cell density, calculated as number of cells/ml, and viability were determined by the Trypan blue exclusion method. Cell cycle phase distribution was quantified after DNA was stained with propidium iodide and analyzed by a Coulter Profile II cyto-

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meter (Coulter, Miami, FL, USA) according to described procedures. The presence of cell membrane complement receptor was quantified by a rosette procedure previously described.[11]

To assess *in vitro* effects on expression of bcl-2, Jurkat and U937 human leukemia/lymphoma cell lines (American Type Culture collection, Rockville, MD, USA) were maintained in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum 2 mm l-glutamine and antibiotics. The suspension cultures were passaged by dilution to a cell concentration 2×10^{-5} cells/ml, and the experiments were performed on asynchronous exponentially growing cells. The triplicate cultures were exposed to HCG at concentrations of 0.2 and 0.002 IU/ml. After treatment for 48 h cells were fixed and expression of bcl-2 was detected in individual cells immunocytochemically and measured by flow cytometry (see below).

Peripheral blood and/or bone marrow aspirates were obtained from patients entering this study to assess the degree of spontaneous and therapy-induced apoptotic cell death. Heparinized peripheral blood was obtained on the first 3 days of treatment with HCG and then weekly thereafter until treatment was discontinued.

Detection of apoptotic cells by DNA strand break labeling

Mononuclear cells were isolated from the heparinized blood and bone marrow by density gradient centrifugation on Histopaque-1077 (Sigma Chemical, St Louis, MO, USA). The mononuclear cells were then fixed in 1% formaldehyde in phosphate-buffered saline (PBS, pH 7.4) for 15 min on ice. After washing in PBS, cells were resuspended in 70% cold (–20°C) ethanol and transferred to the freezer for storage up to 14 days. After rehydrating in PBS, the cells were resuspended in 50 μl of a solution containing 5 U terminal deoxynucleotidyl transferase, 2.5 mm $COCl_2$, 0.2 m potassium cacodylate, 2.5 mm Tris-HCL, 0.25 mg/ml bovine serum albumin (Boehringer Mannheim, Indianapolis, IN, USA) and 0.5 nm of digoxygenin-labeled dUTP (Oncor, Gaithersburg, MD, USA). The cells were incubated in this solution at 37°C for 30 min, then rinsed in PBS and resuspended in 100 μl of the staining solution which contained 2.5 μg/ml fluoresceinated-labeled antidigoxygenin (Oncor), 4 × concentrated saline citrate buffer (Sigma), 0.1% Triton X-100, and 15% (w\v) non-fat dry milk. Cells were incubated in this solution for 30 min at room temperature in the dark.

Cell staining and fluorescence measurements

Following incubation in the staining solution, the cells were rinsed in PBS containing 0.1% Triton X-100 and resuspended in 1 ml PBS containing 5 μg/ml propidium iodide (PI; Molecular Probes, Eugene, OR, USA), and 0.1% RNAse (Sigma). Green (d-UTP) and red PI-DNA) fluorescence of individual cells was measured with a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA). The data from 10^4 cells was collected and stored using LYSYS II software. Apoptotic cells were distinguished based on their green fluorescence representing labeled DNA strand breaks as described previously.[12]

Immunocytochemical detection of BCL-2

Cells were fixed in 1% formaldehyde for 15 min on ice, washed twice and post-fixed in ice cold 70% ethanol overnight. Following rehydration, the cells ($1–2 \times 10^6$) were stained for bcl-2 using a 1:100 dilution of the mouse anti-human bcl-2 antibody (type 124; Dako, Carpinteria, CA, USA) in 1% BSA in PBS overnight at 4°C. The cells were then centrifuged and the pellet resuspended in 100 μl of 1% BSA in PBS containing goat anti-mouse FITC-labeled antibody (Dako; FITC-conjugated $(Fab')_2$ fragment) at 1:30 titer. The cells were incubated for 30 min at room temperature in the dark, then counterstained for DNA by addition of 1 ml of PI solution (final PI concentration 100 μg/ml) containing 10 μg/ml of RNAse A. The bivariate green FITC (bcl-2) *vs* red PI (DNA) fluorescence was measured on a FACScan flow cytometer (Becton Dickinson).

Clinical methods

Adults with chemotherapy-resistant acute myeloid leukemia (AML) were eligible for treatment. Initially patients were required to have greater than 30% blasts in their blood or marrow prior to entry but this was subsequently changed so that patients with low burden AML (5–30% blasts) were included.

Treatment consisted of daily subcutaneous injections of HCG (Schein Pharmaceutical, Baxter, NJ, USA) at a dose of 2 IU daily. Patients also received oral levamisole 50 mg weekly.

Peripheral blood counts were monitored weekly and bone marrow aspirates and biopsies were performed every 2 weeks to assess response to therapy. Patients with progressive disease in the blood or marrow were taken off study prior to day 28 and were given alternative treatment. Patients with responding disease at day 28 were continued on HCG at the initial dose of 2 IU per day until progressive disease was documented. Patients with stable disease at day 28 were given a second daily dose of HCG (total daily dose 4 IU) and were continued for an additional 4 weeks. Patients with stable or responding disease at the end of the second 28 day period could continue therapy until disease progression.

A complete remission (CR) was defined as the achievement of a cellular marrow with less than 5% blast cells, an absolute neutrophil count of 1500, and a platelet count of 100 000 for 1 month. A partial remission (PR) was defined as a 50% decrease in peripheral and bone marrow blast counts for 1 month duration. Stable disease required no progressive increase in peripheral and bone marrow blast counts for 1 month duration.

Results

In vitro studies

In vitro the effect of various concentrations of HCG on human HL-60 promyelocytic leukemia cells was analyzed. Compared to control cultures supplemented with saline, cultures treated with HCG showed a moderate reduction in cell density after 5–7 days of incubation (Figure 1a). In response to a relatively low range of concentrations of HCG, (2×10^{-3}–2×10^{-2} IU/ml) a 20–40% reduction in cell density was detected. At higher HCG concentrations (greater than

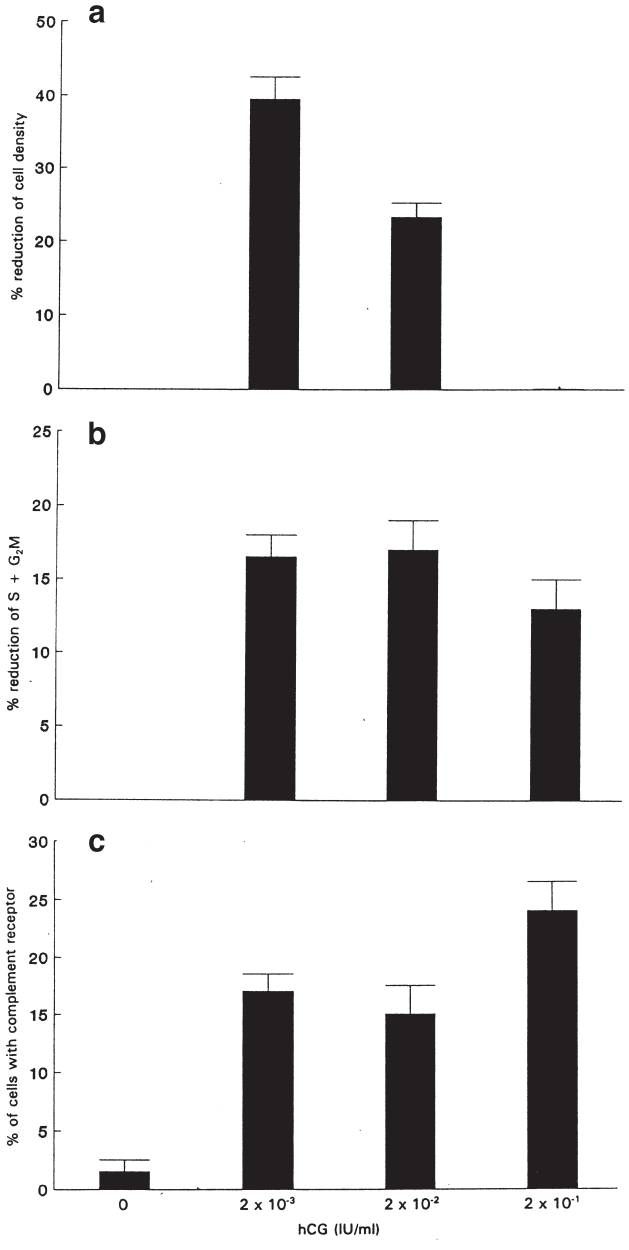

Figure 1 Modulation of proliferative and differentiation of HL-60 cells with HCG. (a) A dose-related reduction in cell density (cells/ml) of HL-60 cells was observed with HCG supplemented cultures compared to control cultures without HCG. Percent reduction was calculated as:

$$\frac{\text{Reduced cell no./ml in HCG cultures}}{\times 100 \text{ Cell No./ml in control cells}}$$

(b) The % reduction of the proliferating cell populations ($S+G_2M$) in HCG supplemented cultures compared to control cultures without HCG. Percent reduction was calculated as:

$$\frac{\text{Reduced } S+G_2M \text{ proportion in HCG cultures}}{S + G_2M \text{ proportion in control cultures}} \times 100$$

(c) Induction of membrane receptor for complement components in HL 60 cells exposed to HCG.

2×10^{-1} IU/ml), however, the reduction was not apparent. The viability in the latter cultures remained at approximately 98% throughout the culture period. Cell proliferation was further analyzed with cell cycle phase determination. In these experiments the most significant reduction in the proportion of S and G2M cells, approximately 15–17%, was only detected at the same range of HCG concentration (2×10^{-3}–2×10^{-2} IU/ml) (Figure 1b). The effect of HCG on cell differentiation was assessed by determining the development of cell membrane complement receptor after exposure to HCG. Cells with complement receptors (15–23%) were developed also in the same HCG range (2×10^{-3}–2×10^{-1} IU/ml) that showed cell growth reduction (Figure 1c). These cells were detected after exposure to HCG for 8 days. Cells with mature-appearing morphology and the presence of apoptotic cells, however, were not detected during this period.

Induction of apoptosis

The percentage of cells undergoing apoptosis (spontaneous apoptosis) was assessed in the bone marrow and/or peripheral blood of 16 patients prior to the start of therapy. Apoptotic cells were determined based on the incorporation of the digoxygenin-labeled dUTP (d-DUTP) into DNA strand breaks arising as a result of apoptosis-induced endonuclease activity. Such cells were detected when the fluorescence associated with the monoclonal antibody bound to d-dUTP was in excess of that observed with an isotype-specific control. An example from an individual patient (patient No. 4) is shown in Figure 2. The mean pretreatment percentage of apoptotic cells in the bone marrow was 1.9 ± 1.8 (range 0.4–6.2) in eight samples. The peripheral blood prior to treatment had a slightly lower level of spontaneous apoptosis: the mean was 1.1 ± 0.9 (range 0.1–2.7) for the nine samples analyzed.

Treatment with HCG led to increased levels of apoptosis in a portion of patients in this study. Thus, although the peak value (ie the highest value recorded among the samples obtained for each patient during treatment) had a mean of 6.0 ± 5.0, it ranged from a value of 1.0 to 16.0. As shown in Figure 3, the peak percentage of apoptotic cells detected during treatment was approximately that of the pretreatment samples (2.0% or less) in five patients, and was between 2.1 and 4.0 in three patients. However, there were eight patients in whom the peak percentage of apoptotic cells exceeded 4.0%, and three patients in whom peak apoptosis exceeded 10.0%. The length of time which transpired between the onset of therapy and the peak percentage apoptotic cells varied dramatically from 1 to 2 days in 7/16 cases to 20–52 days in 4/16 cases.

Effects on bcl-2 expression

Treatment with HCG also appeared to effect the expression of the anti-apoptotic protein bcl-2. *In vitro,* the mean expression of bcl-2-positive cells (determined by comparing cells with antibody specific for bcl-2 with an isotype control) decreased by $19 \pm 8\%$ and $10 \pm 5\%$, respectively, in Jurkat cells treated with 0.2 and 0.002 IU/ml of HCG. In U937 cells the decreases in bcl-2 expression following 48 h of incubation with HCG, 0.2 and 0.002 IU/ml concentrations were $11 \pm 10\%$ and $19 \pm 5\%$, respectively.

In patient-derived cells, the percentage of cells expressing bcl-2 levels greater than the isotypic control were calculated

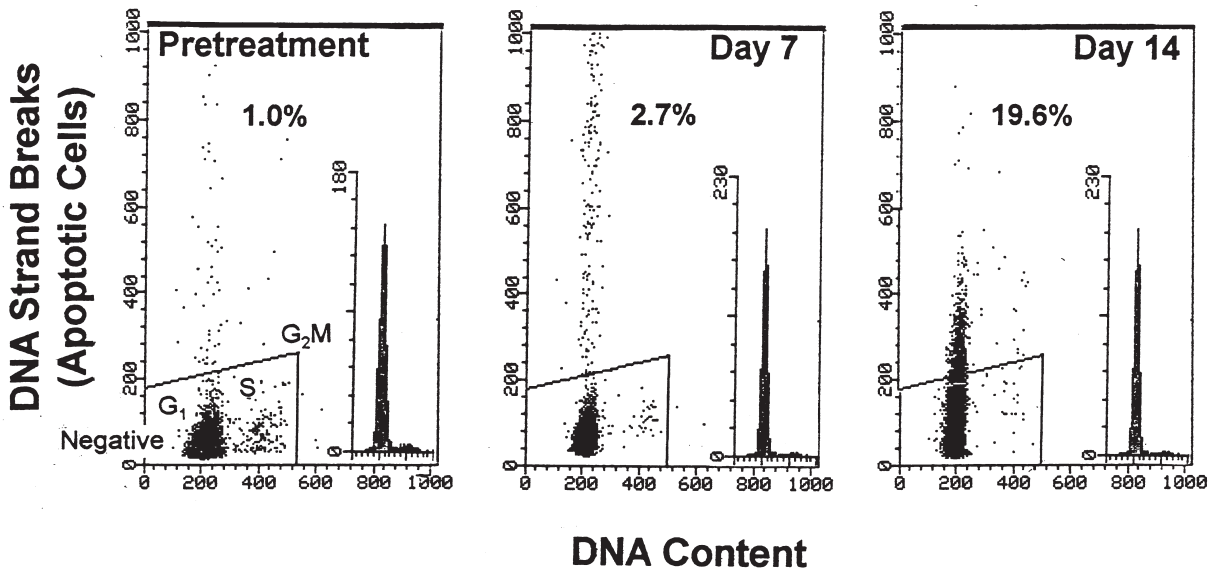

Figure 2 DNA content *vs* DNA strand breaks (apoptosis) in a patient treated with HCG. DNA content was determined by measurement of red (propidium iodide) fluorescence while the extent of DNA damage was determined by measuring the green (FITC) fluorescence of the anti-BrdU antibody bound to BrdUTP incorporated into DNA strand breaks caused by endonucleases released during apoptosis. The trapezoid delineates the extent of fluorescence exhibited by the cells processed in the absence of terminal deoxynucleotidyl transferase; cells above the trapezoid are considered positive for DNA strand breaks. The pretreatment sample contained slightly more than 1% apoptotic cells which increased to 2.7% on day 5 and jumped dramatically to 19.6% on day 6 (patient No. 4).

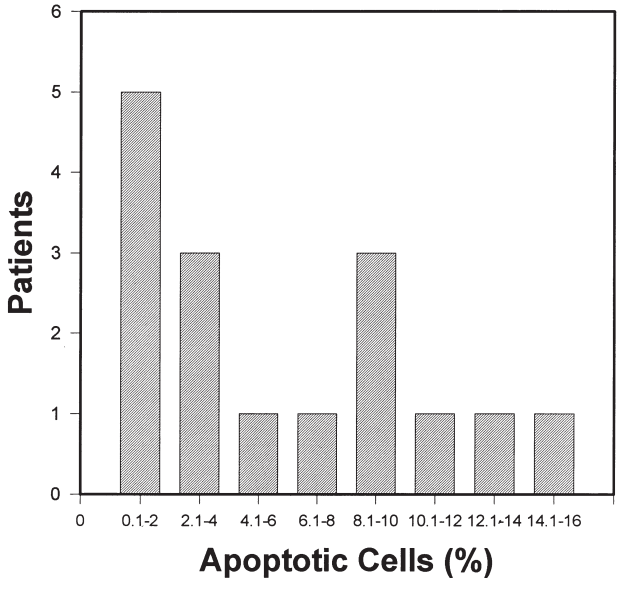

Figure 3 Peak apoptotic responses of patients treated with HCG. The proportion of cells with increased DNA strand breaks (apoptotic cells) was determined. The maximum response was recorded for each patient and then the number of patients with various levels of apoptosis plotted. Half (8/16) of the patients studied had peak apoptotic responses of 4% or less while the remaining half (8/16) had responses from >4 to <16%.

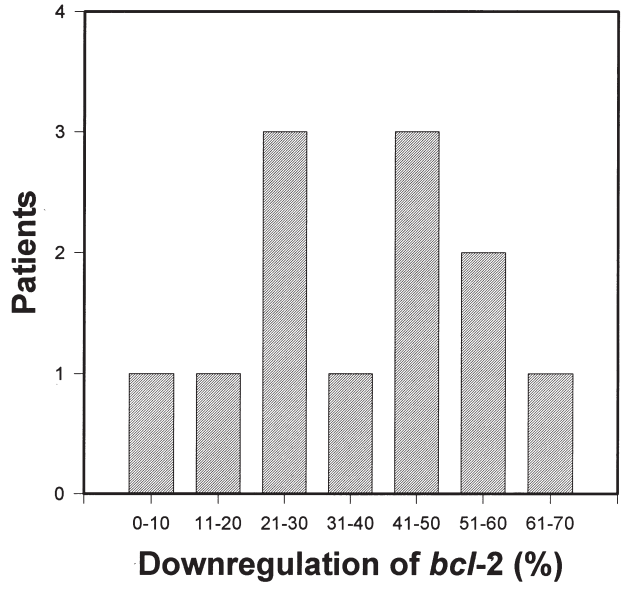

Figure 4 The effect of HCG on the expression of bcl-2 in patients with acute myeloid leukemia. Mononuclear cells were stained for DNA (propidium iodide) and for bcl-2 with a FITC-labeled monoclonal antibody. Cells from each patient were also stained with an isotypic control for bcl-2. The percentage of cells with FITC fluorescence (bcl-2) above control was calculated for each sample from each patient. The percentage change from pretreatment levels to the lowest level measured during treatment (see Table 1) was determined for each patient and plotted above.

for each sample. Prior to treatment, the percentage of cells expressing high levels of bcl-2 varied between 13 and 95%. In nearly every instance, the percentage of bcl-2 positive cells fell at some point during treatment, often within the first 2–3 days. The percentage change in cells expressing high levels of bcl-2 tended to be substantial with 10 of 12 patients showing a decrease of 20% or more and with six of 12 patients showing a decrease of 40% or more (Figure 4). Peak apoptotic percentage, as well as maximal fold increase from baseline, was plotted against maximal decrease in expression of bcl-2. No significant correlation between these parameters was found with the available data.

Clinical results

Twenty patients with a median age of 61 years (range 35–85) were treated with HCG. All patients had received at least one prior induction regimen for AML and the majority had failed two or more regimens (Table 1). The initial 15 patients treated had more than 30% blasts in the marrow. Subsequently, five patients were treated at relapse with low burden leukemia (blast count between 10 and 26%).

All patients tolerated therapy extremely well with no discontinuation of treatment due to toxicity. Other than symptoms related to active leukemia no serious adverse reactions were observed. Two patients did note increased irritability while on study.

Overall, therapy was given to patients for a median of 78 days (range 42–550+). One patient discontinued treatment at her request after only one dose of HCG and was inevaluable for response. Progressive leukemia prior to or at day 28 was documented in 11 of 20 patients. At day 56, two additional patients had increased leukemic burden and were taken off study. Five patients with absolute blast counts in the blood ranging from 0 to 3500/μl and marrow blasts ranging from 16 to 81% were observed to have no progression in either marrow or peripheral blast counts at day 28 and day 56 evaluations. These patients were increased to 4 IU of HCG and continued on treatment for 70, 72, 83, 119 and 121 days, respectively, until they were taken off study due to progressive disease. Only one patient with low-burden leukemia had evidence of regression of leukemia on treatment. Starting with a blast count of 10% documented in two consecutive marrow evaluations, she achieved a remission marrow (<5%) at day 28 and has remained without progression on treatment for 550 days. No significant improvement from baseline levels of neutrophils, hemoglobin or platelets were observed in any of the patients treated.

Discussion

Based on the preclinical activity of HCG in a variety of human malignancy cell lines as well as initial clinical activity demonstrated in spontaneously occurring tumors in animals and in patients with Kaposi's sarcoma, we evaluated the antileukemic activity of HCG *in vitro* as well as in patients with refractory leukemia. The laboratory and clinical observations from these studies have suggested that HCG has modest but detectable growth modulatory activity in acute leukemia.

In HL-60 leukemia cell lines in liquid culture, supplementation with HCG resulted in decreased cell growth, a reduction in the percentage of cells entering proliferative phase, and evidence for enhanced cell differentiation compared to controls. These effects were seen best with relatively long exposure times (5–7 days) and, although some variation in the magnitude of the different effects were seen, they were consistent within the same concentration range of HCG.

In vivo assessment of apoptosis in blast cells taken from patients during treatment with HCG, indicated that significant increases in cells undergoing apoptosis were observed in some patients compared to baseline values. We have previously measured the extent of spontaneous apoptosis in over 200 patients with acute leukemia using the flow cytometric analysis used in this study. We have observed a median 2% apoptotic cells prior to treatment with a range of 0.1 to 8%.[13] In response to chemotherapy, apoptotic responses have ranged from 0.5 to 70% in relapsed patients with a mean peak response of 18%.[14] The results with HCG suggest that apoptosis is induced in some patients significantly above spontaneous levels, and in some cases approaches what was observed with DNA-damaging chemotherapeutic drugs.

Although evidence for decreased cell proliferation and differentiation was observed *in vitro*, this effect was relatively modest and no evidence of apoptotic cell death was seen under the conditions tested. The observation of apoptosis in

Table 1 Patient characteristics and response

Age/Sex	% Blasts	Cyto	No. prior regimens	Days treatment	Cumulative HCG dose (IU)	Peak apoptotic %	Day	% Decrease bcl-2	Response
81/M	81	46xy	2	119	420	1.4	52	—	SD
61/M	74	t(7;14)	3	42	84	1.3	2	—	PROG
62/F	63	–5	2	70	140	6.1	20	23	SD
60/F	51	t(5;7)	2	121	242	19.6	13	47	SD
85/M	46	+8	2	83	276	16	20	39	SD
69/F	78	46xx	3	1	2	—	—	—	INEVAL
64/F	25	55xx	2	30	60	2.2	13	56	PROG
41/M	88	t(8;21)	6	28	56	9.6	2	—	PROG
60/M	49	46xy	2	28	56	5.1	3	15	PROG
49/M	—	IM	3	18	36	10.9	2	21	PROG
6/M	73	–5, +8	1	10	20	—	—	—	PROG
61/F	36	t(1;17)	2	56	112	4.8	6	46	PROG
35/F	30	t(2;17)	2	22	44	1.3	2	6	PROG
57/M	96	46xy	3	9	18	—	—	—	PROG
74/M	33	44/45xy	2	16	32	2.5	–1	46	PROG
67/F	10	–7	2	28	56	1	2	59	PROG
67/F	26	inv 9	2	9	18	13.3	1	21	PROG
65/F	10	46xx	2	550+	1100	—	—	—	CR
60/M	16	46xy	2	72	232	8.8	35	—	SD
56/M	25	48/52xy	1	27	54	—	—	—	PROG

CR, complete remission; SD, stable disease; PROG, progressive disease; INEVAL, inevaluable response; IM, insufficient metaphases.

patients' cells sampled during therapy may reflect differences in sensitivity between cell lines *vs* patient-derived cells or the possibility that HCG *in vivo* acts indirectly to induce apoptosis by mechanisms as yet unidentified.

Despite the growth modulatory effects of HCG observed on leukemic cells, a clinical benefit of HCG therapy could not be demonstrated in the majority of patients treated with HCG, most of whom had high leukemic burdens in the blood and marrow and were refractory to several chemotherapeutic regimens. The one clinical response observed with HCG was suggestive of the experiences with other non-chemotherapeutic agents in refractory AML such as interleukin 2 where demonstrable antileukemic activity was confined to patients with low burden disease.[15] This activity would need to be confirmed in larger numbers of patients with low burden leukemia.

The *in vitro* effects on HL-60 cells were seen with exposure to HCG alone, whereas patients in the clinical study received weekly low-dose levamisole in addition to HCG. Previous clinical studies with levamisole in AML focused primarily on its use as post-remission therapy, and limited data exist on its use in patients with active leukemia. In a study reported by Van Sloten *et al*,[16] post-remission therapy with levamisole at a dose of 90 mg/m^2 daily failed to improve disease-free survival in patients with AML compared to observation, suggesting minimal, if any, antileukemic activity. A synergistic effect of the combination of HCG and levamisole, however, cannot be ruled out as an explanation for the observed effects in patients and in patient-derived leukemic cells sampled during therapy.

Our study in AML, as well as the studies in Kaposi's sarcoma both used commercial preparations of HCG, but at significantly different dose levels. Gill *et al* reported widely varying activity against Kaposi's sarcoma cell lines with different commercial preparations and with different lots of the same preparation. In addition, they reported that a highly purified HCG heterodimer had significantly less *in vitro* inhibitory activity compared to the commercial preparations. These observations have led some to suggest that the activity seen may not be due to HCG but to degradation products or other as yet unidentified associated proteins contained in commercial HCG products.[17] Although this is suggested by the experiences of Gill *et al*, it may be that HCG has biological effects at varying concentrations which are as yet not fully understood. Recent data, for example, have indicated that HCG receptors are present on prostate carcinoma cells.[18] It is possible, therefore, that HCG's effect at low concentrations may be related to receptor binding and modulation of growth factor production and/or other cytokines associated with cell proliferation and differentiation, whereas the activity at high concentrations may be a direct cytotoxic effect as suggested by Gill *et al*. Since the beta subunit of HCG is structurally similar to that of other growth factors associated with angiogenesis, such as platelet-derived growth factor (PDGF),[19] binding of HCG fragments to receptors for PDGF and subsequent down-regulation has been proposed as an alternative explanation for HCG's antitumor activity.[17]

Preliminary assessment of bcl-2 expression in cell lines exposed to HCG, as well as in patient-derived leukemic cells before and during treatment with HCG, consistently demonstrated a decreased expression in response to treatment. No direct correlation between enhanced apoptosis and decreased expression of BCL-2 was detected, however. Although it is intriguing to suggest that down-regulation of bcl-2 is the pharmacodynamic basis for the activity of HCG, a more detailed evaluation of the effectors of apoptosis would need to be performed before this could be substantiated.

Other investigators have suggested that HCG on the surface of tumor cells alters the immune reaction between the host and malignant cell which can be modified by exposure of cells to low doses of the beta subunit of HCG conjugated to tetanus toxoid.[20] In this study we did not evaluate HCG expression in patients' leukemic cells and thus could not assess whether decreased tumor cell expression occurred in response to HCG administration. Previous observations with low-dose HCG therapy have not demonstrated detectable levels of serum HCG or the presence of anti-HCG antibodies[21] as has been reported by others.[20] Although stimulation of an immune response against malignant cells may be possible in response to HCG administration, this could not explain the *in vitro* effects seen in leukemic and Kaposi's sarcoma cells.

In addition, biopsies of Kaposi's sarcoma lesions following injection of HCG did not demonstrate a mononuclear infiltration, arguing against an *in vivo* immune response.

Our studies with AML have confirmed the observations in other human malignancies that HCG preparations can inhibit cell growth and induce apoptotic cell death. Identification of the active component of HCG preparations and further understanding of its mechanism of action will be important in its development as a clinically useful agent.

Acknowledgements

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