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Phase I and pharmacokinetic study of vinblastine and high-dose megestrol acetate

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Abstract *Purpose*: Preclinical data indicate that progestational agents (progesterone, medroxyprogesterone acetate and megestrol acetate) interact with p-glycoprotein (P-gp) and reverse P-gp-associated resistance to vinca alkaloids and other natural products. Based on these data, we performed a phase I study of high-dose oral megestrol acetate and vinblastine to evaluate the safety of this regimen. *Patients and methods*: Enrolled in the study were 61 patients with advanced solid tumors, refractory to standard therapy. Cohorts of patients received megestrol acetate according to the following escalation scheme (loading dose/maintenance dose, twice daily for 7 days): 750 mg/250 mg, 750 mg/375 mg, 1000 mg/500 mg, 1500 mg/1000 mg, 3000 mg/2000 mg, 4500 mg/3000 mg, 6000 mg/4000 mg, and 7500 mg/5000 mg. They also received 1.5 mg/m^2 per day of vinblastine by continuous infusion for 5 days (days 2 to 6). *Results*: Of the 61 patients, 59 were evaluable for toxicity. A maximum tolerated dose (MTD) was not reached. The regimen was well tolerated. Of the 59 patients, 10 (17%) experienced grade 4 leukopenia. All of these cases were at dose levels 3 to 8. There was an increase in the steady-state concentration (Css) of megestrol acetate with increasing dose up to the sixth dose level. Further increases in the dose produced no change in the megestrol acetate Css. Only 2.4% of megestrol acetate was free in the plasma as compared to 65.6% in RPMI culture medium. Megestrol acetate administration was associated with profound suppression of ACTH and cortisol levels. *Conclusions*: The combination of vinblastine and megestrol acetate was well tolerated. An MTD for this combination was not achieved as a result of the saturable absorption of megestrol acetate. Although potentially therapeutic serum concentrations of megestrol acetate were achieved, it is unlikely that MDR was reversed given the high protein-binding of the drug. Profound suppression of the pituitary-adrenal axis was also observed during the administration of megestrol acetate.

Keywords Vinblastine · Megestrol acetate · P-gp · MDR

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Introduction

Tumor cells often become resistant to anticancer drugs, and this is a major obstacle to the successful treatment of human malignancies [7, 24]. One pattern of resistance is manifested as multidrug resistance (MDR), where cancer cells demonstrating MDR develop cross-resis-

tance to multiple, seemingly unrelated agents. This is due, at least in part, to expression of the *MDR-1* gene [7, 9, 24]. *MDR-1* encodes a 170-kDa membrane glycoprotein (p-glycoprotein, P-gp) that is expressed in a variety of human malignancies and normal tissues. P-gp is a transmembrane efflux pump that decreases intracellular accumulation of natural product-derived cytotoxic agents such as anthracyclines, epipodophyllotoxins, *Vinca* alkaloids, and taxanes [6, 7, 12].

Normal tissues expressing P-gp include secretory epithelial cells of the gastrointestinal tract and kidney, and vascular endothelial cells of the placenta, testis, and central nervous system. In these locations P-gp is believed to decrease exposure to potentially toxic substances. Elevated P-gp levels are often observed at diagnosis in tumors such as gastrointestinal, renal cell, hepatocellular, and adrenal carcinomas, while breast and ovarian carcinomas and hematopoietic malignancies rarely express increased P-gp levels at diagnosis [7, 24]. P-gp expression is common in these tumor types following treatment with natural product chemotherapeutic agents [6, 9, 12]. P-gp belongs to the adenosine triphosphate-binding cassette (ABC) superfamily of proteins which perform a wide variety of functions, ranging from maintaining cellular homeostasis to pumping out potentially damaging chemicals [8]. Other pumps in this family, such as the MDR protein (MRP) and the breast cancer resistance protein (BCRP), are also involved in resistance to chemotherapy [10, 16, 18, 26].

Because of the potential clinical importance of P-gp-mediated drug resistance in vivo, there is considerable interest in developing pharmacologic strategies for MDR reversal through P-gp antagonism [9, 12, 24]. A variety of agents block P-gp-mediated drug efflux in vitro and in vivo. Among these agents are verapamil, tamoxifen, toremifene, quinidine, trifluoperazine, cyclosporine A, and valspodar (PSC833) [1, 2, 3, 5, 25]. However, translating the in vitro work into clinical practice is problematic. No pharmacologic agent has been found that is capable of inhibiting the efflux pump without causing significant side effects or excessively perturbing the pharmacokinetics of the antineoplastic agent with which it is combined [2, 25].

Steroid hormones and related compounds, such as progesterone, tamoxifen and megestrol acetate, are substrates for P-gp and reverse P-gp-mediated MDR. Moreover this reversal appears to occur at clinically achievable concentrations [4, 15, 21, 23, 28, 34, 35]. The combination of high-dose oral tamoxifen and vinblastine has been shown to be safe in a phase I study [32]. However, this combination was not successful in reversing drug resistance in a clinical trial [27]. Megestrol acetate was first used more than two decades ago as treatment for metastatic breast cancer. In subsequent studies with megestrol acetate, the utility and toxicities of doses larger than those conventionally used to treat breast cancer were investigated [29, 31]. Those studies demonstrated weight gain to be a significant side effect associated with high doses of megestrol acetate, and this pharmacodynamic effect of high-dose megestrol acetate is now routinely exploited therapeutically in patients with cachexia [13, 14, 22, 30, 33]. There are data to support the potential for megestrol acetate to modulate MDR [34]. Pharmacokinetic studies in patients taking megestrol acetate to combat cachexia have demonstrated that a daily dose of 800 mg can produce total plasma megestrol acetate concentrations of approximately 2 μM, which is in the range of concentrations that modulate P-gp in vitro. Therefore, efforts have been made to evaluate higher doses of megestrol acetate than those used to enhance appetite.

We performed a phase I study to evaluate the safety of high-dose oral megestrol acetate in combination with vinblastine in patients with refractory solid tumors. In our study, we carefully evaluated megestrol acetate pharmacokinetics so that we could determine whether we achieved concentrations in plasma similar to those that have been shown to modulate MDR in vitro. We also compared the protein-bound fraction of megestrol acetate in plasma to that present in the in vitro conditions under which P-gp modulation has been studied. As megestrol acetate had been previously reported to suppress the pituitary-adrenal axis [11, 20], we decided to evaluate ACTH and cortisol levels before and during treatment with megestrol acetate.

Methods

Patient eligibility

Patients were eligible if they met the following criteria: (1) histologically confirmed malignancy that was refractory to standard therapy; (2) aged 18 years or older; (3) measurable or evaluable disease; (4) ECOG performance status 0-2; (5) life expectancy >12 weeks; (6) WBC $>3500/\mu l$; (7) platelet count $>90{,}000/\mu l$; (8) serum bilirubin <2.0 mg/dl; (9) AST less than six times the upper limit of normal; and (10) serum creatinine <2.0 mg/dl. Recovery from prior anticancer treatment was required. No chemotherapy or radiation treatment was permitted for at least 4 weeks before enrollment, and in the case of nitrosoureas and mitomycin-C, that period was 6 weeks. Patients could have previously received treatment with either megestrol acetate or vinblastine. Exclusion criteria included metastases to the central nervous system, symptomatic neuropathy and pregnancy. Women of childbearing age were required to have a negative pregnancy test at study entry. Patients with a history of deep venous thrombosis or pulmonary embolus were also excluded. Concurrent use of other progestins or any calcium channel blocker was not permitted.

Prior to treatment, all patients gave written informed consent indicating the investigational nature of the treatment and its potential risks. The consent form was approved by the University of Pittsburgh Institutional Review Board.

Study design and treatment plan

Megestrol acetate and vinblastine were administered every 4 weeks. Vinblastine was given as a 5-day continuous i.v. infusion on days 2 to 6 [32]. The vinblastine dose of 1.5 mg/m^2 per day was administered by a portable pump and through tunneled external venous devices, such as Hickman catheters, or subcutaneous devices, such

as Infusaports. The first 4–6 h of the infusion were observed in the outpatient setting. Megestrol acetate was supplied as 250-mg tablets or a 40-mg/ml oral suspension and was administered as a loading dose on day 1, followed by twice-daily maintenance dosing. The first of the twice-daily doses was begun 6 h after the loading dose, and twice-daily dosing was continued for 7 days thereafter. This schedule was designed to establish megestrol acetate concentrations rapidly on the day prior to initiating treatment with vinblastine and to maintain those concentrations during therapy. The dose-escalation scheme for megestrol acetate is shown in Table 1. Patients did not receive low-dose coumadin as prophylaxis for deep venous thrombosis.

The study was a single-institution, open-label, non-randomized, phase I, dose-escalation study, in which megestrol acetate dose escalations were initially administered to groups of three patients at each level. Escalation proceeded to the next dose level if those three patients were followed for at least 3 weeks and did not experience grade 2 or greater nonhematologic toxicity or grade 3 hematologic toxicity lasting 7 days or longer. If grade 4 hematologic toxicity or grade 3 or greater toxicity of any other organ system occurred in one or more patients, then at least three additional patients were treated at the same dose level to define the nature and frequency of the toxicity. If grade 3 or greater toxicity occurred in three or more of six patients at a given dose level, then dose escalations would be terminated. If fewer than three of six patients experienced reversible dose-limiting toxicities then escalations were continued to the next level. If three of six patients treated at a particular dose developed grade 3 or greater hematologic toxicity lasting for 7 days or longer, or developed febrile neutropenia, then dose escalation ceased. The dose below this was defined as the maximum tolerated dose (MTD). Toxicities were graded according to the NCI Common Toxicity Criteria.

If a patient at any given level developed irreversible or life-threatening toxicity, then dose escalations were terminated. Additional patients (a maximum of three) were to be entered at the dose level below that at which life-threatening toxicity was observed. The MTD was defined as the dose below that at which the significant toxicity occurred.

Patients had a complete history and physical examination before each cycle of therapy. Monitoring consisted of a weekly CBC, creatinine, alkaline phosphatase, AST, bilirubin, calcium, phosphorus and uric acid. Radiographic response was assessed every 8–12 weeks, and tumor measurements were recorded whenever measurable disease was present. ACTH and cortisol levels were determined before initiation of megestrol acetate treatment and afterwards on days 4–5 for evaluation of suppression of the pituitary-adrenal axis.

The dose of vinblastine was not modified for less than grade 4 nausea, vomiting, diarrhea or headache. However, if a preceding cycle was associated with grade 2 or greater reversible mucosal toxicity, grade 3 or greater nonhematologic or nonmucosal toxicity, or grade 4 hematologic toxicity, then the dose of vinblastine was reduced by 25% in all subsequent cycles. Any patient who developed hepatic toxicity, defined as an AST more than six times normal or bilirubin >2.0 mg/dl, could not receive any further treatments until that toxicity had resolved (AST less than six times normal and bilirubin <2.0 mg/dl). A patient who developed a serum creatinine >2.0 mg/dl, did not receive further treatment until the creatinine fell to <2.0 mg/dl. Patients in whom reversible grade 3 or greater toxicity attributable to megestrol acetate occurred could receive further treatment but with a 50% dose reduction of the megestrol acetate in all subsequent cycles.

Criteria for removal from the study were: progressive disease, decision of the patient to withdraw from the study, physician discretion, and changes in the patient's condition that rendered the patient unsuitable for further treatment in the judgment of the investigator.

Standard ECOG response criteria for measurable (by physical examination or radiographic evaluation) disease were used in this trial.

Megestrol acetate pharmacokinetics

Blood (10-ml samples) was collected into heparinized tubes before initiation of megestrol acetate therapy, at 4 h after administration of the loading dose, and then between 8 and 10 a.m. on days 3, 4, 5, 6, and 7. The exact time of sampling was documented as was the time of ingestion of the previous dose of megestrol acetate. Plasma was separated by centrifugation at approximately 1000 *g* and was stored at –20°C until analyzed for megestrol acetate.

Concentrations of megestrol acetate in patient plasma were quantified with a validated HPLC method. Briefly, 1 ml plasma was placed into a 16×125-mm glass screw-capped tube with a Teflon-lined cap (Fisher Scientific, Fairlawn, N.J.). To this was added 10 µl of a 60 µg/ml solution of 2,3-diphenyl-1-indenone (Aldrich Chemicals, Milwaukee, Wis.) in methanol, followed by 7 ml HPLC-grade hexane (Fisher Scientific). Caps were secured on the tubes, and the tubes were placed onto a rotating mixer (ARB rotator, Marion Scientific, Kansas City, Mo.) for 10 min at approximately 40 rpm. After mixing, the tubes were centrifuged for 7 min at approximately 1000 *g* to separate the aqueous and organic phases. A 5-ml aliquot of each organic layer was transferred to clean, 12×75-mm glass tubes and evaporated to dryness under nitrogen in an N-evap (Organomation Associates, Shrewsbury, Mass.). The dried residues were reconstituted in 250 µl mobile phase and mixed thoroughly on a vortex mixer. The reconstituted residues were then transferred to microcentrifuge tube inserts, and 200 µl was injected onto the HPLC system.

The HPLC system consisted of a Hewlett-Packard 1090L high-performance liquid chromatograph. The mobile phase, consisting of acetonitrile/water/acetic acid (66:33:1, v/v/v), was pumped at 1.8 ml/min. Megestrol acetate and internal standard were separated on a Waters (Milford, Mass.) µ-Bondapak C18 (10 µm) (3.9×300 mm) column protected with a Brownlee NewGuard RP-18 guard column (3.2×15 mm) (Alltech, Deerfield, Ill.). Column eluate was monitored at 280 nm with a Waters 440 detector, and detector output was integrated to give the areas under the megestrol acetate and internal standard peaks. Under these conditions, megestrol acetate eluted at approximately 3 min, internal standard eluted at approximately 6 min, and there were no endogenous materials that interfered with their measurement. Megestrol acetate concentrations were determined by calculating the ratio of the area under the megestrol acetate peak to that of the respective internal standard peak in each sample and comparing that ratio to a concomitantly performed standard curve. Concentrations used in the

Table 1. Dose escalation scheme for megestrol acetate with number of treatment cycles received

Level	No. of patients	Loading dose, mg (ml)	Maintenance dose, mg (ml) twice daily	Median cycles (range)
1	6	750 (19)	250 (6)	2 (1–12)
2	6	750 (19)	375 (9)	2 (2–6)
3	12	1000 (25)	500 (13)	3 (1–11)
4	5	1500 (38)	1000 (25)	2 (1–5)
5	8	3000 (75)	2000 (50)	2 (1–8)
6	7	4500 (113)	3000 (75)	2 (1–3)
7	6	6000 (150)	4000 (100)	2 (1–4)
8	9	7500 (188)	5000 (125)	1 (1–5)

standard curve included 0.01, 0.03, 0.1, 0.3, 1, and 3 μg/ml. Quality control samples were also run with each assay and included concentrations of 0.01, 0.5, and 1.5 μg/ml. Megestrol acetate concentration versus time data were assessed visually.

The protein binding of megestrol acetate was also assessed in tissue culture medium and control human plasma. In these studies, 2 μg/ml (5 μ*M*) solutions of megestrol acetate were prepared in RPMI 1640 medium (Bio-Whittaker, Walkersville, Md.) containing 15% heat-inactivated fetal bovine serum (FBS; Biofluids Division, Biosource International, Rockville, Md.) and in citrate-anticoagulated human plasma (Central Blood Bank, Pittsburgh, Pa.). Quadruplicate 1-ml portions of each solution were then placed into the upper chambers of Amicon Centrifree ultrafiltration devices (Millipore Corporation, Bedford, Mass.). Protein-free ultrafiltrates were prepared by centrifuging the Centrifree devices at 2000 *g* for 20 min at 4°C. Megestrol acetate concentrations in the resulting ultrafiltrates and in the starting tissue culture medium and plasma solutions were then measured with the HPLC assay system described above.

Results

Of the 61 patients entered into the study at eight dose levels, 59 were evaluable (Table 1). One patient did not receive any treatment due to rapidly progressive disease and another declined treatment. The median age of the study population was 57 years (range 27–78 years). Of the 59 patients, 48 had an ECOG performance status of 0 or 1, and 11 had a performance status of 2. The number of patients actually enrolled at each dose was greater than three due to the occurrence of toxicities in each set of patients (Table 1). Also shown in Table 1 is the median number of treatment cycles that the patients at each dose level were able to tolerate. The largest group by tumor type was colorectal (18 patients) with a wide distribution of other tumor types. There were 34 male and 25 female patients. Ten patients (17%) had received only chemotherapy previously, 10 patients (17%) had received chemotherapy as well as biotherapy, and 16 (27%) had received chemotherapy and radiation treatments. The other 17 patients had received different combinations of the above three treatment modalities. Only six patients (10%) had received no treatment for their malignancy prior to enrollment in this study.

The regimen of megestrol acetate and vinblastine was well tolerated. Despite escalating to the eighth dose level of 5000 mg twice daily, no dose-limiting toxicity was seen, and an MTD was not reached. The most common toxicity was hematologic. Of the 59 patients, 10 (17%) experienced grade 4 leukopenia during their first cycle of therapy; all of these occurred at dose levels 3 to 8 (Table 2). Grade 3 or 4 leukopenia occurred in 18 of the 59 patients (30%) (Table 2). Thromboembolic disease was the most significant nonhematologic toxicity (Table 3). Three patients had a pulmonary embolism (grade 4), three had upper extremity deep venous thrombosis with superior vena cava syndrome (grade 3), and three developed lower extremity deep venous thrombosis (grade 2). These were all separate patients and there was no dose effect seen. Five patients experienced grade 3/4 constipation. Two patients had grade 3/4 diarrhea, two had grade 3/4 dyspnea (related to disease-associated effusions), and one patient had a life-threatening infection (Table 3). Weight gain, which is associated with continuously dosed megestrol acetate, was only seen in one patient at a level of grade 3 (> 20% weight gain) and one patient at a level of grade 1 (5–9.9% weight gain). These patients each received three cycles of treatment. No complete or partial responses were observed.

Table 2. Effect of dose escalation on WBC toxicity

Dose level	WBC toxicity grade				
	0	1	2	3	4
1	4	1		1	
2	3	2		1	
3	6		1	2	3
4	1	1	2		1
5	3		1	2	1
6	2	1		1	2
7	1	1	2	1	1
8	4	2	1		2

Table 3. Non-hematologic toxicities

Toxicity	Grade		
	2	3	4
Thrombosis	3	3	3
Nausea	10		
Anorexia	11	1	
Diarrhea	13	1	1
Constipation	5	3	2
Neuropathy	5	1	1
Dyspnea	7	1	1
Infection	5		1

We also observed profound changes in the serum concentrations of ACTH and cortisol in patients after they started taking megestrol acetate. Of the 59 patients, 35 had both pretreatment and day 4–5 cortisol samples drawn, making them evaluable for acute changes in cortisol. Of these 35 patients, 34 (97%) had a profound drop in their cortisol levels by day 5 (classified as a normal pretreatment concentration, i.e. 7–29 μg/dl, with a drop to ≤ 5 μg/dl on day 4–5). Of 27 patients in whom ACTH concentrations were evaluated prior to treatment and on day 4–5, 22 (81%) had profound suppression of their ACTH concentrations by day 5 of treatment (classified as a normal pretreatment concentration, i.e. 9–46 pg/ml, with a drop to ≤ 5 pg/ml on day 4–5). None of these patients was noted to have any symptoms of adrenal insufficiency.

Plasma megestrol acetate concentrations were uniformly stable between days 4 and 7. Therefore, the average megestrol acetate serum concentrations obtained on days 4, 5, 6 and 7 of the first cycle was used to calculate a steady-state plasma megestrol acetate concentration (Css) for each patient. Although plasma megestrol acetate Css increased with the first six dose

escalations, there was no further increase with dose levels 7 and 8 (Fig. 1). In view of the fact that there was a plateau in the Css at maintenance doses of 3000 mg twice daily and above, the study was stopped. This decision was made despite the fact that an MTD had not yet been reached.

Assays were also performed to compare the percentage of free megestrol acetate, i.e. non-protein-bound, in human plasma and FBS-supplemented RPMI 1640, a culture medium commonly used for in vitro studies. In RPMI 1640 medium, $65.6 \pm 6.1\%$ of the megestrol acetate was free, whereas in plasma, only $2.4 \pm 0.9\%$ was free.

Discussion

Due to the potential importance of P-gp in clinical drug resistance and the fact that MDR can be reversed in vitro and in vivo by different agents [2, 5, 25], a number of phase I and II studies have been performed to evaluate this strategy clinically. Unfortunately, toxicities associated with doses of agents that pharmacokinetically modulate MDR have been a major stumbling block. The combination of high-dose megestrol acetate and paclitaxel A has been recently evaluated in a phase I trial in patients with ovarian cancer [17]. That study used megestrol acetate doses similar to those used in our study, and like our treatment regimen, was well tolerated. Of their 44 patients, four had major venous thrombosis (two had pulmonary embolus) and one had a stroke. However, our study included pharmacokinetic components not found in this other study, and the pharmacokinetic data developed provided important insights into the clinical results produced.

In terms of toxicity, bone marrow suppression was limited. A few cases of hypercoagulability were seen. It is unclear whether these were due to the megestrol acetate or the underlying malignancy. Only a few patients experienced gastrointestinal side effects such as diarrhea, constipation or nausea. Significant weight gain was not seen, possibly reflecting the intermittent nature of megestrol acetate dosing. We also did not observe any correlation between dose and frequency or severity of toxicity, except that grade 4 leukopenia was only observed at dose levels 3 and higher. An MTD was not achieved because after a certain dose, increasing the dose of megestrol acetate did not result in an increased serum concentration of megestrol acetate. Ideally, we would have assessed the effect of megestrol acetate on vinblastine pharmacokinetics, but unfortunately the fact that we did not have access to a suitable reliable assay for vinblastine precluded this.

The effects of megestrol acetate on the pituitary-adrenal axis have previously been reported [11, 20]. Although the majority of our patients had profound suppression of ACTH and cortisol synthesis, they had no clinical symptoms of adrenal insufficiency. This implies that megestrol acetate has clinically significant corticosteroid activity.

The pharmacokinetic data demonstrated that with the first six dose levels there was a steady increase in the megestrol acetate Css. However, the Css data for dose levels six to eight showed no increase despite a significant increase in the dose administered. This suggests that absorption of megestrol acetate from the gastrointestinal tract was saturated or that some saturable excretory mechanism was overcome, with the result that serum megestrol acetate concentrations could not increase further.

Preclinical in vitro models have shown that megestrol acetate concentrations of 2–5 μM (0.8–2 μg/ml) are capable of reversing P-gp-mediated resistance [4, 15, 34]. Our data show that these total concentrations can be achieved in plasma, but the proportion of free drug in plasma is considerably lower (2.4%) than that in

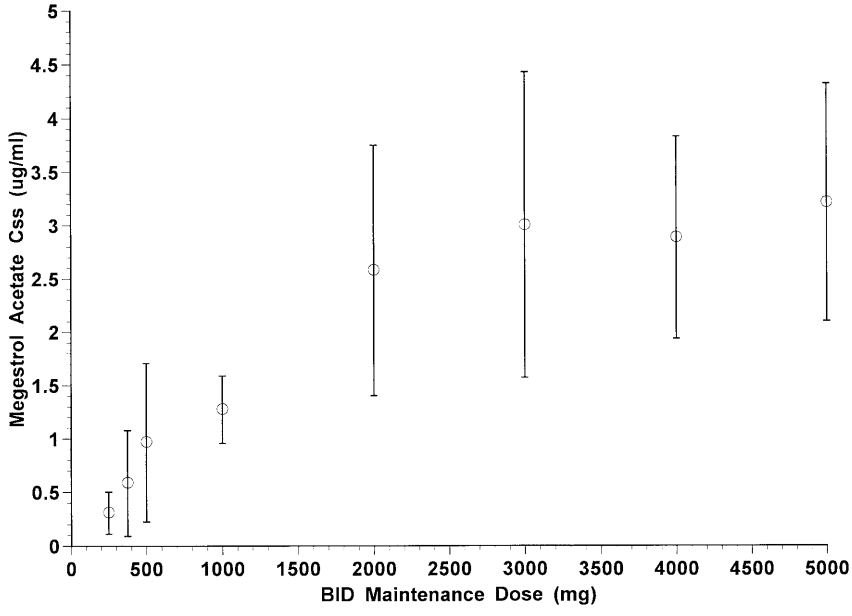

Fig. 1. Relationship of megestrol acetate dose to steady-state plasma megestrol acetate concentrations produced (*symbols* mean values, *error bars* standard deviations)

FBS-supplemented tissue culture medium (65.6%). Because it is the free fraction that is available to enter cells, we believe that direct comparison of in vivo and in vitro data can prove misleading. One such example is novobiocin, an antibiotic, which modulates resistance to etoposide in vitro but has been found to be highly protein-bound in vivo and therefore is not able to produce a similar pharmacologic effect in patients [19, 36].

In conclusion, our data show that the combination of high-dose megestrol acetate and vinblastine was well tolerated. The pharmacokinetics of megestrol acetate are saturable. Moreover, although the total plasma megestrol acetate concentrations achieved were in the range shown to reverse the resistance of cancer cells to antineoplastic agents in vitro, the extensive protein binding of megestrol acetate in plasma makes it unlikely that concentrations capable of reversing MDR are achievable in patients.

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