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Pilot study of megestrol acetate and hydralazine in gynecologic adenocarcinomas

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Summary

Background: DNA promoter methylation serves as an alternative to gene mutation in silencing genes. The progesterone receptor, a target for cancer therapy, has been shown to be silenced in this manner. The object of the study was to determine if patients taking hydralazine as a global demethylating agent would have tumor stabilization or response from using sequential megestrol acetate and secondarily whether the tumor would show changes consistent with the demethylation. *Materials and Methods:* Heavily pretreated women with gynecologic adenocarcinomas were enrolled on an institutional review approved study. *Results:* Five patients were enrolled on this initial study. Three patients continued therapy beyond the first four week cycle. These patients had progression free intervals of six, seven, and nine months, respectively. All of the evaluable patients had negative staining for progesterone receptor prior to study. All three patients who finished at least one cycle of therapy had repeat biopsies that demonstrated their tumors stained positively for progesterone receptor. *Conclusions:* In this pilot study, three patients had progression free intervals of ≥ six months and their tumors which previously stained negatively for progesterone receptor stained positively for progesterone receptor after treatment with hydralazine.

Key words: Adenocarcinomas; Demethylation; Epigenetic; Progesterone.

Introduction

Silencing tumor suppressor genes by promoter methylation may serve as an alternative to gene mutation. As with the two-hit hypothesis of Knudson, one wild-type gene could be silenced by promoter methylation and the other gene could be lost or mutated resulting in two hits, a tumorigenic phenotype. Silencing could also occur by methylation of both alleles [1, 2].

For many years, megestrol acetate has been known to have activity in women with certain gynecologic malignancies [3]. Yang *et al.* elegantly demonstrated that in advanced cases of endometrial adenocarcinomas, progesterone receptor silencing was caused at the epigenetic level by promoter methylation [4]. This silencing helps explain some of the failures of progestin therapy in women with gynecologic adenocarcinomas. In an astrocytoma cell line, Hansberg-Pastor *et al.* demonstrated that dacarbazine could demethylate the progesterone receptor β, increase its expression, and then progesterone could be used to decrease the number of cells [5].

Hydralazine is a direct smooth muscle dilator that has been show to have effects on DNA methyltransferase causing demethylation. Hydralazine reactivates genes that have been previously silenced by promoter methylation. In myelodysplastic syndrome, it has been studied as a safe and effective anti-cancer treatment in conjunction with the magnesium valproate [6].

The goal of this study was to determine whether women with heavily pretreated gynecologic adenocarcinomas could benefit from sequential hydralazine and megestrol acetate. A secondary goal was to see if progesterone receptors that were not present by immunohistochemistry (IHC) initially developed after therapy with hydralazine.

Materials and Methods

This was a phase II pilot study to see if there was any activity of the sequential combination of hydralazine and megestrol acetate in women with heavily pretreated gynecologic adenocarcinomas. This was an institutional review board approved study. To be part of the study, patients had to have had at least three different chemotherapy regimens (ie., repeating carboplatin would count as one regimen); not have been on hormones for 30 days; an ECOG performance status of 0, 1, 2; measurable disease by imaging or physical exam; and disease that was able to be biopsied. Initial starting dose of megestrol acetate was 25 mg by mouth three times a day for days 1-14. Megestrol acetate was dosed at 80 mg by mouth twice a day for days 15-28. A four week period was considered one cycle. Biopsies were performed within one month of starting the hydralazine and during the megestrol acetate portion of the third cycle to determine the presence or absence of progesterone receptor staining by IHC.

Results

In this pilot study, five patients with gynecologic adenocarcinomas were enrolled. Table 1 shows the clinical

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Table 1. — Clinical characteristics of the patients included in the study.

Cancer	Clinical information					
	Number of previous regimens of chemo-therapy	Radiation therapy	Initial progesterone receptor status	Progesterone receptor status after hydralazine	Previous progression free interval (months)	Progression free interval (months)
Cervical adenocarcinoma	3	Yes	−	+	3	6
Cervical adenocarcinoma	4	Yes	−	+	2	7
Ovarian serous grade 3	4	No	−	+	3	9
Ovarian undifferentiated	4	No	−	NA	4	NA
Uterine papillary serous carcinoma	5	No	+	NA	3	NA

"–" = negative staining for progesterone receptors; "+" = positive staining for progesterone receptors.

characteristics of all five patients. Two of the patients had cervical adenocarcinomas, two had high-grade ovarian cancer and one had high-grade endometrial cancer. All of the patients were heavily pretreated with a minimum of three previous different chemotherapy regimens (mean four, median four, range three to five). Four of the five patients' tumors were negative for progesterone receptor staining prior to therapy. Of those that were evaluable (because of completing four weeks of therapy), all three had biopsies showing receptor positivity after treatment with hydralazine and megestrol acetate.

No grade 3 or 4 toxicities were encountered. Patient 4 stopped therapy during the first 14 days because hydralazine had made her dizzy (no evidence of orthostatic hypotension). The fifth patient signed consent and then withdrew from the study before starting therapy but after the initial biopsy.

In the three evaluable patients, a mean progression free interval (PFI) of > seven months was found (median seven, range six to nine months). All three patients had a longer PFI on the current regimen than they had had with their most recent chemotherapy regimen.

Discussion

For over three decades, progesterone analogues have been used to treat women with gynecologic adenocarcinomas [3]. Despite continued use, less than 25% show response and another approximately 20% show stable disease [7]. In a trial of alternating megestrol acetate and tamoxifen, higher grade disease was inversely correlated with both response and stable disease [8]. This point corresponds with the fact that hormone receptor expression decreases as histologic grade increases [9]. Only in the past decade has it been demonstrated that progesterone and estrogen receptor silencing is caused by epigenetic promoter methylation [4, 10]. This epigenetic phenomenon is a potential therapeutic target.

Hydralazine has been shown to be involved in global demethylation [6]. This was used first therapeutically in myelodysplastic syndrome [6]. In the current study, hydralazine was used sequentially to demethylate the progesterone receptor. This allowed it to then be a target for megestrol acetate which was used in the latter half of each cycle.

Many shortcomings are apparent in this study. The first and most obvious one is the size of the study. The fact that three of five patients had benefit from this regimen could be a false positive result. A larger study is now ongoing since this pilot study had positive results. Another shortcoming in this study is the unknown best regimen for hydralazine to cause global demethylation. When treating myelodysplastic syndrome, hydralazine was used as a continuous treatment. Although hydralazine is safe for short-term use as evidenced by its use in pregnancy, using it long term continuously can lead to a lupus-like syndrome. That is why it is used for two weeks at a time in this study. Potential benefit of this sequential use can be seen by the fact that three tumors that were negative for progesterone receptor became positive for progesterone receptor after global demethylation with hydralazine.

Despite multiple shortcomings, the study is potentially exciting for two reasons. First, three patients with progesterone receptor negative tumors had PFI of > six months when treated with megestrol acetate. Each of these responses was longer than the respective patient's response with the latest chemotherapy they received. These responses make more sense when coupled with the information gathered from the biopsies after being treated with hydralazine.

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