LONG TERM ORAL ETOPOSIDE AS SECOND-LINE THERAPY IN RECURRENT EPITHELIAL CARCINOMA OF THE OVARY

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ABSTRACT

Background: The activity and toxicity of etoposide in women with recurrent ovarian cancer was evaluated in a case series of women with recurrent ovarian cancer who had measurable disease.

Methods: All patients had prior platinum-based chemotherapy and developed progressive disease. Etoposide was given as 50mg/day for 21 days every 4 weeks until progression of disease or prohibitive toxicity. Between December 1999 and January 2004, 32 patients were enrolled in this study.

Results: 30 patients received a total of 133 cycles of etoposide. Median age was 49 years (range, 19 to 75). The median number of etoposide cycles was 4 (range, 1 to 12). There were 5 partial responses (16.6%). The mean response duration was 4.8 months (range, 3.5 to 6), median progression-free interval (PFI) was 7 months (range, 3 to 13), and median survival time was 12.5 months (range, 1.3 to 36).

Conclusion: The major toxicity was leukopenia. One patient required red blood cell transfusions, and the main non-hematologic toxicity was nausea and vomiting. There were no treatment-related mortalities. Although etoposide appears to exhibit modest activity in recurrent ovarian cancer after platinum-based therapy, response and survival durations are short.

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INTRODUCTION

The current standard of care for optimally debulked ovarian cancer patients consists of a platinum compound (cisplatinum or carboplatin) and paclitaxel.[1,2]

Despite the high incidence of remission following initial therapy, the majority of cancers ultimately recur. The approach to patients with recurrent disease depends, in large part, on the treatment-free interval between the time of the initial therapy and initiation of second-line therapy.[3] Patients defined as platinum resistant, who relapse within 6 months of completing plati-

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num-based therapy, have a poor prognosis with limited response to second line chemotherapy.[4] Patients who relapse after 6 months are defined as platinum-sensitive and have a better prognosis.[5]

There is a continuing need to identify new agents that are active in ovarian cancer.

A variety of second-line agents with various response rates are available, including topotecan (14% to 23%), vinorelbine (22%), gemcitabine (29%), paclitaxel (19 to 40%), and liposomal doxorubicin (26%).[6,7,8,9,10,11]

Because these second-line agents have produced similar response rates and median survival duration, physicians can consider other factors, such as patient's quality of life, patient satisfaction, simplicity of the regimen, toxicity, and cost, in selection of second-line treatment. Clearly, oral agents are preferable in terms of ease of administration and cost, and are least disruptive to the patients quality of life.

Etoposide is a derivative of the plant alkaloid epipodophyllotoxin. It interacts with DNA topoisomerase II, an enzyme which is active during the late S and early G2 phases of the cell cycle, and produces a transient double strand break in DNA. Etoposide stabilizes the formation of the DNA-topoisomerase II complex, which results in inhibition of rejoining and increased DNA scission.[12] The interaction of etoposide with topoisomerase II is reversible and allows DNA annealing following withdrawal of the drug. This mechanism of action is consistent with the schedule dependency of etoposide, which has been demonstrated in both preclinical and clinical studies.[13,14] There is a theoretical advantage to prolonged administration. Indeed, clinical studies have substantiated that multiple drug dosing is superior to single dose administration.[15]

The availability of etoposide in oral preparation allows prolonged administration by the oral route. A comparison between studies using intravenously administered etoposide to those using prolonged oral etoposide concluded improved efficacy in several malignancies for prolonged oral administration and stimulated renewed interest in this agent.[16] In addition oral etoposide is appealing in that it is easy to administer. This report describes the results of a prospective phase II study using a 21-day oral schedule of etoposide to assess the activity and toxicity in women with recurrent epithelial ovarian cancer who had prior platinum-based chemotherapy.

PATIENTS AND METHODS

Inclusion criteria

All patients had histologically confirmed epithelial ovarian cancer with radiological and/or clinical evidence of disease progression. Patients were eligible if they had not previously received etoposide. They were required to have bi-dimensional tumor measurable by physical examination and radiographic study. The patients were required to have at least one m^2 body surface area, adequate intestinal function, no history of other malignancy, GOG performance status <2, and to have had at least 3 weeks elapse since any prior therapy. Pretreatment laboratory eligibility requirements included: leukocyte count >3000/mm^3, platelet count >100,000/mm^3, and granulocyte count >1500/mm^3, creatinine <2 mg%, billirubin <1.5× and SGOT and alkaline phosphatase <3× upper limit of institutional normal and signed informed consent.

Exclusion criteria

Patients were excluded for any of the following: (1) prior treatment with etoposide, (2) history of another malignancy, (3) no measurable disease or (4) GOG performance status >3.

Pretreatment and follow-up evaluation

A complete history, physical examination including a pelvic examination, laboratory studies, and assessment of performance status and chest X-rays were performed prior to beginning treatment and every 4 weeks after, with the exception of the chest radiograph (unless pulmonary metastases were presented). CT scan was performed every 3 months, or sooner in the event of clinical deterioration.

A complete blood count and differential was performed weekly. All patients were followed for at least 30 days after the final dose of drug or until resolution of any drug treated toxicity.

Treatment

Etoposide was administered at a dosage of 50mg/day (one capsule) as a single daily dose on days 1-21 every 4 weeks. Although food has not been shown to interfere with etoposide absorption,[17] patients were instructed to take the entire daily dose each morning before eating. Antiemetics were not routinely used. During treatment, a CBC, differential, and platelet count were obtained weekly. Etoposide was discontinued, if leukocyte count fell below 2000/μL and/or platelets fell below 50000/μL. At the end of each 21-day cycle, etoposide was discontinued and patients underwent an evaluation on day 28. Patients who demonstrated an objective response or stable disease were given another cycle of oral etoposide. However, therapy was not initiated until counts were adequately recovered (ie, leukocytes>3000/μL, platelets>100000/μL). When the counts recovered sufficiently to resume therapy, the next cycle was started at a lower dose. Etoposide was continued until patients demonstrated evidence of tumor progression or experienced

unacceptable toxicity. Toxicity evaluations were based upon standard GOG criteria.[17] Patients who received one or more courses of drug were evaluable for toxicity, regardless of subsequent response or survival.

Response criteria

Patients were considered evaluable for response if they completed one course of therapy and lived at least 3 weeks. Tumor response was assessed after 2 cycles of treatment. Standard GOG response criteria were used.[17] Responses were determined using the products of the longest perpendicular diameters of all measurable lesions. Complete response (CR) was defined as the total disappearance of all evaluable disease without the development of any new lesions. Partial response (PR) was defined as at least a 50% reduction in the product obtained from all measurable lesions, without the progression of any lesion and without the appearance of any new lesions. Both CR and PR had to be documented on two measurement assessments at least 4 weeks apart. Progressive disease was defined as a 50% increase in the product obtained from measurement of any lesion or the appearance of new lesions. Stable disease was defined as any patient who failed to qualify for CR, PR, or progressive disease on two evaluations at least 4 weeks apart.

Response duration was defined as the time from first documentation of objective response until progression. Duration of stable disease was measured from the start of the study. Survival was measured from the time of study entry until death. Survival was analyzed by the method of Kaplan and Meier.

RESULTS

Between December 1999 and January 2004, 32 patients were entered in this study. Two were excluded; one for never receiving therapy, and one was not assessable. The median age of patients was 49 years (range, 19 to 75). The median body surface area was 1.3(range 1-1.8). The median of performance status was 1 (0 to 2). Histology was 26 serous and 4 mucinous adenocarcinomas. One patient had prior whole pelvic radiation.

Patients received a total of 133 courses of etoposide, with a median of 4 and range of 1-12 courses. Other patients' characteristics are shown in Table I.

There were 5 partial responses (16.7 %).4 in patients with platinum-sensitive, and one in a patient with platinum-resistant disease. The median time to recurrence of disease in platinum-sensitive responders was 10 months (7.5 to 13 months) and 6 months in platinum resistance responders. The mean response duration was 4.8 months (range, 3.5 to 6).We observed stable disease in 12 patients. Progression of disease was observed after 1 or 8 cycles in 13 patients. The median progression free interval (PFI) was 7 months (range, 3 to 13). The median survival of the whole group was 12.5 months (range, 1.3 to 36).

Table I. Patients' characteristics.

Age		
	Median	49
	Range	19-75
Stage		
	IIIA	2
	IIIB	3
	IIIC	16
	IV	9
Histology		
	Serous	26
	Mucinous	4
Prior chemotherapy (courses)		30 (3-13) median=9, mean (±SD) =8.5±3.15
Platinum resistance		16
Platinum sensitive		14
Courses (etoposide)		
	Median	4 (range = 1-12)

Table II. Adverse effects.

Adverse effects	Grade			
	1	2	3	4
Leukopenia	12	6	0	0
Granulocytopenia	6	5	0	0
Anemia	7	6	1	0
Thrombocytopenia	0	0	0	0
Nausea/vomiting	7	1	0	0
Diarrhea	2	1	0	0
SGOT, SGPT↑	1	0	0	0
Alk-P↑	1	1	0	0
Mucositis	2	0	0	0
Hypokalemia	2	0	0	0

Toxicities are shown in Table II. They were primarily hematologic. Grade 1 and 2 leukopenia occurred in 12 and 6 patients respectively. One patient required RBC transfusion. Nausea and/or vomiting was the most common non-hematologic toxicity occurring in 7 patients. SGOT, SGPT elevation (grade 1) was seen in one patient. One woman reported hyperpigmentation and hypokalemia occurred in two. Mild mucositis (two women), and blue-colored nail-beds were also reported by one patient. There was alopecia in 10 patients, and no treatment-related mortalities.

Table III. Oral etoposide in ovarian carcinoma.

Author/(year)	Dose	No. of patients	Response rate%	CR	PR	Duration (months)
Markkman (1992)	50 mg/d×21	18	6	1	1	11
Garrow (1992)	50 mg/m²/d×21	17		18	0	3 2,4,6
Marzola (1993)	50 mg/m²/d×21	17	6	0	1	9
Dewit (1994)	50 mg/m²/d×21	28	16	0	4	4,4,7,10
Hoskin (1994)	100 mg/m²/d×14	31	26*	1	7	2-9
Kavanagh (1995)	50 mg/m²/d×21	14	0	0	0	
GOG (1998)	50 mg/m²/d×21	41	34.1** 41	6 26.8*	8 3	1.3-8.7 8 1.9-14.4

**Platinum-sensitive
*Platinum-resistant

DISCUSSION

Patients who have progression after platinum-based therapy may be offered second-line agents. A variety of second-line agents is available for the treatment of recurrent or persistent ovarian cancer.

Numerous factors can influence the response to second-line treatments. Because of selection bias, limited numbers of patients in some studies, and differences in response assessment, it is not possible to directly compare response rates in phase II trials. What is apparent is that there is no clear-cut drug of choice that should be used in patients who have recurrent ovarian cancer.

However, cure with chemotherapy for these patients is almost never achieved. Agents with a favorable therapeutic index are more acceptable to patients easier to administer, and are less expensive.

Etoposide is a semi-synthetic podophyllotoxin derivative which interacts with the topoisomerase II-DNA complex and cause DNA strand breakage.[16] The role of prolonged oral etoposide in cancer therapy is still evolving. Its value in small cell carcinoma of the lung (SCLC) has been well established, with response rates as high as 80% in selected patients.[19] The anti-tumor activity of oral etoposide is schedule and dose dependent with prolonged oral administration, although responses were initially seen with doses as low as 25mg/m², subsequent studies in both lung and ovarian cancer utilizing daily doses less than 50mg/m² have had poor response rates.[20,21,22] However Yasumiza and Kato[23] reported activity with the prolonged oral etoposide regimen (25mg/d for 21 days, repeated every 4 weeks) in refractory ovarian cancer with a response rate of 42.8%.

Our study could be compared with others in the literature (Table III). Markman et al[21] found one responder of 18 patients (6% response rate with 11 months duration) treated with oral etoposide (50mg/d for 20 days, every 28 days), the treatment program was generally well tolerated, with mild neutropenia being the most common side effect. In another study,[24] a similar etoposide schedule was used in 18 ovarian cancer patients who had previously received cisplatin, and only one partial remission lasting 9 months was observed among 17 evaluable patients. The investigators concluded that oral etoposide was active in both platinum-resistant and platinum-sensitive disease and warranted further study in combination therapy.[24,25] Garrow et al.[26] used 50mg/m²/d for 21 days every 4 weeks in 17 women with refractory ovarian cancer and achieved three partial responses; the response rate was 18%. The largest study of using pro-

longed oral etoposide in ovarian carcinoma has been reported by Rose et al.[25] The response rates were 26.8% and 34.1% for platinum resistant and platinum-sensitive patients, respectively. This is similar to the result of a phase II trial of prolonged oral etoposide in platinum-resistant ovarian carcinoma using a dose of 100mg/m^2/d for 14 days every 3 weeks that reported a response rate of 26%.[27]

In other studies, data for truly platinum-resistant patients were not presented separately. So a comparison cannot be made.[25,27,29] However other trials[25,26,28] such as our study, which have small sample size are difficult to interpret because they have included a mixture of platinum-sensitive and platinum-resistant patients. These studies and ours had variable patient populations with many prior chemotherapy regimens. In the GOG study[25] patients who had previously responded to platinum based therapy and who were reinduced with their original regimens were classified as having received only one prior regimen. The importance of the extent of prior treatment is evident in the differing response rates of second-line versus fourth-line therapy (33% and 4%).[30] As a significant number of our patients had received many courses of chemotherapy, we chose a reduced starting dose (50mg/day). A response rate as low as 6% has been reported with oral etoposide at a dose of 50mg/day in a small group of heavily pretreated patients.[22] Such reduced dosing may decrease the plasma etoposide concentration to less than 1μg/mL and limit the activity of this regimen. An association between the duration of plasma levels>1μg/mL and activity has been demonstrated in clinical trials. However, oral etoposide has the advantage of home administration, the drug is largely protein bound and myelosuppression has also been related to albumin levels less than 3.5g/d, which result in increased free etoposide. Patients with abnormal renal or liver function despite a normal serum albumin or of advanced age also have decreased etoposide clearance and increased myelotoxicity.

Anemia in this regimen is common and appears cumulative. Patients who receive prolonged oral etoposide regimens must have their CBC monitored closely. Common non-hematologic toxicities included nausea, vomiting, and alopecia which are consistent with previous studies.

Although response to second-line chemotherapy is not unusual, responses tend to be brief and long-term survival is rare. Thus, the focus of treatment should aim to optimize quality of life and delay the development of further symptoms. However this regimen has the advantages of home and easy administration, less expenditure and acceptable response with no severe side effects, the value of maintenance etoposide without evaluation in a phase III trial is uncertain. This would be difficult to perform because of heterogenicity of the patients and the small number of eligible patients. Therefore clinical trials with etoposide should be continued.

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mosome spreads, which are not always available. Secondly, for prenatal diagnostic applications, diagnosis is labor-intensive and time-consuming as it depends on the culture of fetal cells and the analysis of metaphase chromosomes.[3,4]

It has been shown that fluorescence in situ hybridisation can detect the number of copies of a particular chromosome present in interphase nuclei.[5,11] The major advantage of this technique is that there is no requirement for cell culture and hence the results can be available in two days. The technique has important applications for the aneuploidy analysis of fetal chromosome abnormalities if it can be shown to be reliable in uncultured amniotic fluid cells. However chromosome 21 analysis in interphase appears to be more complicated than for many other chromosomes, as there is no reliable chromosome 21-specific repeat probe available. To overcome these problems the Alu-PCR product of a chromosome 21-specific YAC were used in this study to prenatally detect the number of chromosome 21 copies on uncultured amniocytes by FISH. The results obtained from application of the technique on 214 uncultured amniotic fluid samples revealed high detection efficiency on cell preparations.

MATERIAL AND METHODS

Sample preparation

About 17-20 mL of amniotic fluid samples was received through gynecology hospitals for each patient who was at increased risk of a Down syndrome conception. About 15 mL of each sample was assigned by a lab code number and used for amniocyte culture according to standard cytogenetic techniques. The remaining 2-5 mL of each sample was detected by a different identification number and used for uncultured amniocyte preparation as described by Klinger et al.[12] Uncultured amniocytes in PBS were dispensed on to 3-aminopropyl triethoxy silane-coated slides at 37°C (35µL vol/slide), two volumes of d. H_2O pre-warmed at 37°C were added and incubated at 37°C for 15 min. The hypotonic solution was carefully decanted and replaced by 100 µL of 30% 3:1 fix (methanol: acetic acid) and 70%, 75mM KCl for 5 min at room temperature. This solution was carefully decanted and fresh 3:1 fix was dropped on to the slide from a height of 60 cm. Excess fix was decanted and slides dried for 5 min at 60°C, dehydrated through alcohol series (50%, 70%, 90% and 100%), air dried and stored at -20°C until required.

Probe preparation

Two Alu primers: BK-33 (5'-CTGGGATTACAGGCGTGAGC-3') priming to the 5' end of the Alu consensus sequences (nt positions 15-34) and SR1 (5'-CCACTGCACTCCAGCCTGGGG-3') close to the 3' end (nt position 241-261)[13] were used to selectively amplify the chromosome 21 specific DNA sequence inside of the YAC: 831B9. The PCR assay was performed as described by Lengauer et al.[14] with small modifications. 100 ng of the primer were each at a concentration of 0.25 µM in a total volume of 50 µL PCR buffer containing 250 µM of each of the four dNTPs, and 2.5 units of Taq polymerase (perkin-Elmer/cetus). After an initial denaturation at 96°C for 5 min, 30 cycles of PCR were carried out with denaturation at 96°C for 1 min, annealing at 37°C for 30s and extension at 72°C for 6 min. A 10 min extension was performed at the end of the last cycle.

Ten-microlitre aliquots of amplified DNA sequences were fractionated by electrophoresis in 1.3% gel in 1x T.B.E. (0.9 M Tris-HCl, 0.9 M boric acid and 20 mM EDTA). PCR products were ethanol precipitated, dissolved in TE (10 mM tris-HCl, 1mM EDTA, pH 8), and used for nick translation with biotin-11-dUTP. The labelled DNA was used as a probe for FISH.

Chromosome in situ suppression hybridisation

Chromosomal in situ suppression (CISS) hybridization and probe detection with fluorescein isothiocyanate (FITC) conjugated to avidin were carried out according to Carter et al.[15] with the following modifications: For hybridization 100-150 ng of Alu-PCR amplified YAC DNA was used as probe after pre-annealing with 100 ng of human placental DNA. The signals were amplified once. Cells were counter stained with 0.4 µg/mL 4.6 diamino-2-phenylindol-dihydrochloride (DAPI) and 0.2 µg/mL propidium iodide in mounting medium AF1 (Citiflour Ltd) and were evaluated with conventional fluorescence microscope.

RESULTS

The hybridisation and detection conditions were optimized using cultured lymphocytes. Various concentrations of probe and competitor DNA were investigated to achieve intense signals specific for chromosome 21 with little background. Figure 1a demonstrates a cultured lymphocyte from a normal individual and Figure 1b a cell from an individual with trisomy 21 hybridised with probe 831B9. In all experiments strong signals were observed on both chromatids of chromosome 21 at the expected locus on the long arm (21q22).

To evaluate the detection efficiency of approach, the probe was initially hybridised to an unselected series of twenty uncultured lymphocytes and the results were rechecked by lymphocyte culture and GTG-banding for each sample. Eighteen samples were correctly scored as

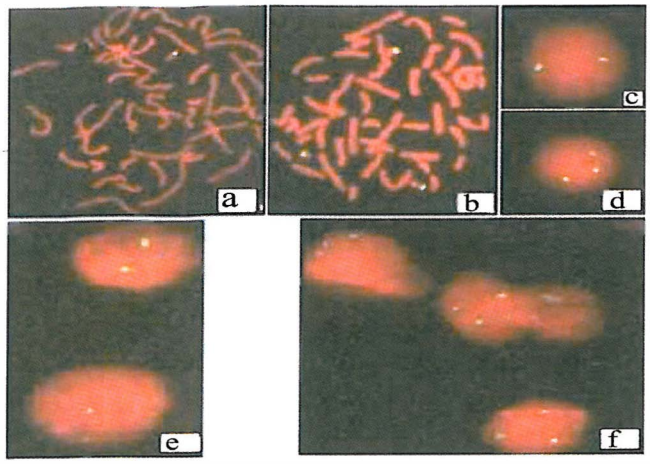

Fig. 1. Cultured and uncultured lymphocytes and uncultured amniocytes hybridised with Alu-PCR amplified YAC 831B9 probe specific for 21q22.

normal displaying two distinct signals specific for chromosome 21 on an average of 94 per cent of the hybridised cells (Figure 1c). Two samples showed three signals on an average of 87 per cent of hybridised cells and were correctly identified as trisomy 21 (Figure 1d). Figure 3.11a and b diagrammatically illustrates the detection efficiency of probe 831B9 on uncultured normal and abnormal lymphocytes respectively.

The optimised procedure was applied to uncultured amniocytes, to detect the copy number of chromosome 21 in interphase nuclei. A total of 214 amniotic fluid samples were analysed in a blind fashion. The hybridisation signals were analysed using a conventional epifluorescence microscope and the results were compared to those obtained by traditional cytogenetic assay for each sample. One-hundred and ninety-nine samples showed two distinct signals on an average of 90.5 per cent of randomly evaluated nuclei and correctly detected as normal when compared to the results obtained from GTG-banding assay. Seven samples were revealed to be trisomic for chromosome 21 with a detection efficiency of 87 percent and confirmed by cytogenetic analysis (Figure 1e). One of the samples was shown to be normal using interphase FISH, however the cytogenetic assay revealed a Robertsonian translocation between long arms of chromosomes 14 and 21. The remaining 7 samples failed to produce a result owing to poor quality of the preparation and maternal cell contamination (Figure 1f). No false positive results was obtained in this study.

DISCUSSION

The most common chromosomal abnormality in newborns is Trisomy 21, with an incidence of 1/700. Prenatal diagnosis is routinely offered to women at increased risk of having a child with chromosomal abnormality, the most common indications being advanced maternal age or positive screening results based on biochemical marker screening and ultrasound evaluation.[16,17]

Conventional cytogenetic techniques based on banding of metaphase chromosomes are accurate and can often detect subtle rearrangements. However the time required to perform an analysis is around 2 weeks under the best circumstances. Methods that allow rapid and accurate detection of the major fetal aneuploidies are valuable, since they provide sufficient time to develop an appropriate course of action.

It had been previously shown that fluorescence in situ hybridisation is a rapid technique for detection of aneuploidies in uncultured amniocytes if it can be shown to be reliable and the detection efficiency is acceptable.[18] However in a given sample, both the percentage of cells that hybridise and the extent to which hybridisation reflects the correct genotype are products of probe design and performance, hybridisation efficiency and signal detection capability. It has been shown that subtle variations in sample fixation, cell permeability and probe size markedly influence the hybridisation/detection efficiency.

Our previous study using a small number of uncultured amniotic fluid samples had shown that the Alu-PCR amplified YACS 831B9 is more suitable for aneuploidy detection of chromosome 21 compared to the commercially available probes.[19] The present study was carried out using a large scale of samples to assess the susceptibility of the technique for prenatal diagnosis of Down syndrome.

Hybridisation of cultured and uncultured lymphocytes with biotin labelled YACs 831B9 revealed that the signals are large and intense with minimum background fluorescence. The detection efficiency of the probe in normal and trisomy 21 uncultured amniotic fluid samples was in the range of 87-94 percent and 85-89 percent respectively. The signal intensity was comparable to those of alpha satellite DNA probes. These results compare favorably with similar studies reported by others.[20,21] A false negative result was encountered in this study, which was subsequently detected as a Robertsonian translocation by GTG-Banding assay. As about 4 percent of Down's syndrome is caused by a Robertsonian translocation,[22] it is recommended that the interphase FISH be used as a parallel to standard cytogenetic techniques to avoid the undetectable chromosomal abnormalities by this method. The failure rate in this study was about 0.3 percent that is lower

than those reported for other probes in similar studies.[23] The results indicate that the prenatal diagnosis of trisomy 21 can be reliably carried out by the procedure used in this study.

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