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EVE/cyclosporin (etoposide, vincristine, epirubicin with high-dose cyclosporin)—chemotherapy selected for multidrug resistance modulation

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Abstract

Sixteen children and young adults were treated with high-dose cyclosporin combined with a combination of cytotoxics (epirubicin, vincristine and etoposide) (EVE) known to be influenced by *P*-glycoprotein-mediated multidrug resistance (MDR). Tumour types were neuroblastoma 3, Ewing's sarcoma 2, rhabdomyosarcoma 5, osteosarcoma 3, desmoplastic small round cell tumour 1, nephroblastoma 1, T-acute lymphoblastic leukaemia (ALL) 1. All had progressed or relapsed following at least two of the drug types included in EVE. Acute reactions to cyclosporin and myelosuppression were the major toxicities documented. Renal and hepatic toxicity was rarely severe and always transient. Partial responses (PR) were observed in 2 patients (1 rhabdomyosarcoma, 1 Ewing's sarcoma). We conclude that this combination is tolerable in heavily pretreated patients and may be suitable for further evaluation in untreated poor risk tumours.

Keywords: Multi-drug resistance; Reversal; Cyclosporin; Epirubicin; Vincristine; Etoposide

1. Introduction

Despite striking improvements in the outcome of certain childhood tumours, for example, non-metastatic Ewing's sarcoma and non-Hodgkin's lymphoma, overall increases in survival rates have generally been modest over the past decade. This is particularly the case with the commoner metastatic tumours such as neuroblastoma, rhabdomyosarcoma and osteosarcoma, where a 5-year event-free survival of around 20% remains little changed. This is despite new strategies that have been successful in localised disease, such as the introduction of cisplatin and etoposide, dose escalation and improved radiotherapy and surgery. Even very high-dose therapy with haematopoietic stem cell rescue has only had a minor impact on progression-free survival and little effect on cure rates [1].

Unfortunately, in the last decade there have been few novel chemotherapy agents that have shown particular activity in these difficult tumours. The taxanes have a limited role and although the initial data are encouraging it is too early to predict the value of topoisomerase 1 inhibitors such as topotecan or irinotecan [2]. As a consequence, much effort has been applied to make the most of the existing agents by trying to optimise dose route and schedule [3–5]. Another strategy for increasing cytotoxic effect is the modification of drug resistance mechanisms. There is now a considerable body of *in vitro* data suggesting that multidrug resistance due to P-glycoprotein (Pg*P*) or multidrug resistance protein (MRP) can be effectively modulated by a range of drugs. *In vitro* studies with neuroblastoma and rhabdomyosarcoma cell lines have shown effective chemosensitisation with cyclosporin, verapamil, PSC833 and VX710 at levels that can be achieved *in vivo* [6–8].

Preliminary clinical studies with both verapamil and high-dose cyclosporin have demonstrated responses in primary refractory and relapsed tumours suggesting that the strategy may be effective *in vivo* [9–12]. Interpretation

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of response is complicated by a number of factors, such as standardisation of drug dose, time to relapse, effects of drug pharmacokinetics and tumour blood flow [13]. Studies in myeloma and lymphoma have, however, demonstrated that disease refractory to chemotherapy can respond to the same drugs when combined with modulators [14–16]. Randomised studies in lung cancer have been disappointing [17], but the results of such studies with newer, more effective, modulators such as PSC 833 in acute myeloid leukaemia (AML) and ovarian cancer are awaited [18–20].

In some studies, the modulator is given with Pg*P* substrates combined with drugs such as AraC, cyclophosphamide/ifosfamide or cisplatin, which are not MDR modulated, thus complicating interpretation. The present study was designed to evaluate the tolerability of a novel regimen which combines high-dose cyclosporin A in a dose schedule known to produce blood levels that achieve modulation *in vitro*, with three drugs, each of which has been shown *in vitro* to be effluxed by Pg*P*. This approach is designed to optimise any potential beneficial effect from modulation.

2. Patients and methods

The study was approved by the Committee for Clinical Research and the Royal Marsden NHS Trust Ethics Committee. Written consent was obtained either from the parents and/or from the patients themselves if they were old enough.

Patients with relapsed or refractory primary or metastatic malignancy between the ages of 6 months and 20 years were eligible for the study, providing their Lansky performance scale was more than 30 and their life expectancy was more than 12 weeks. A history of cyclosporin hypersensitivity would have excluded patients from entry into the study.

Prior to treatment, patients underwent assessment of haematological, biochemical, hepatic, renal and cardiac function. The study entry requirements were that the total White Blood Cell count (WBC) was greater than 1.5×10^9/l, total granulocyte count greater than 1×10^9/l and platelet count greater than 100×10^9/l, unless there was known to be bone marrow involvement. Renal function as assessed by ethylene diamine tetra acetic acid (EDTA) clearance was required to be greater than 30 ml/min/1.73 m^2 and serum bilirubin and liver transaminases less than 2.5 times greater than the institutional upper limit of normal. Normal cardiac function assessed by echo-cardiography had to be documented.

2.1. Patient details

16 patients aged between 5 and 20 years were enrolled onto the study. There were 12 boys and four girls. All were heavily pretreated on national protocols of chemotherapy. 6 patients had presented at first relapse with metastatic disease, 1 on treatment, 7 patients had progressive or refractory disease on treatment at the time of entry into the study. 3 patients were treated at the time of second or subsequent relapses. Patients were recruited from six different United Kingdom Children's Cancer Study Group (UKCCSG) centres. All had received at least one MDR modulated drug (Table 1).

2.2. Treatment protocol

EVE/cyclosporin consists of etoposide, vincristine and epirubicin chemotherapy given concurrently with cyclosporin. The treatment was given over three consecutive days at 3-weekly intervals. Patients were admitted as in-patients and were kept under close observation. Cyclosporin was given as a 30 mg/kg/day infusion over 6 h, each day for 3 days. The chemotherapy infusions were commenced 1 h after the commencement of the cyclosporin infusion. The doses and scheduling were as follows: etoposide 75 mg/m^2 over 2 h daily on days 1–3, vincristine 0.25 mg/m^2 given as an intravenous (i.v.) bolus daily on days 1–3 and epirubicin 12.5 mg/m^2 as a 4-h infusion on days 1–3. Cyclosporin is known to have a significant effect on etoposide and anthracycline pharmacokinetics, causing a doubling of the area under concentration curve (AUC) [14], and therefore the drug doses were reduced to 50% of the standard doses employed in paediatric oncology practice. Because of increased toxicity observed with full dose vincristine [15] combined with Cyclosporin A this drug was also reduced to half dose. As many patients were likely to have received high doses of anthracycline, the epirubicin dose was further reduced to 25% standard dose. This was to limit the cumulative dose and to avoid having to omit this drug from subsequent courses of EVE in responding patients, due to cardiac toxicity.

Unless there was clear clinical evidence of disease progression after the first course, a second course of EVE/cyclosporin was given after 21 days, providing haematological recovery had occurred and the first course had been well tolerated. Patients were formally evaluated for response after two courses.

Toxicity monitoring included weekly laboratory tests of liver function, renal function, serum magnesium and calcium and full blood count. The EDTA clearance was repeated prior to each course of EVE/cyclosporin. Cardiac echocardiogram was performed prior to each course of EVE/cyclosporin. Toxicity was graded from 0 to IV according to standard WHO toxicity criteria.

Disease assessment was performed prior to study using the most appropriate imaging modality (ultrasound, computerised tomography scanning (CT) or magnetic resonance imaging (MRI)) for the individual tumour. A two-dimensional (2-D) measure of disease

Table 1
Details of diagnosis, nature of relapse, prior chemotherapy and response to EVE-cyclosporin

No.	Sex	Age (years/months)	Disease	Status	Previous MDR modulated treatment	No. of courses of EVE cyclosporin	Response
1	M	7 y 5 m	Neuroblastoma	Refractory disease	Etoposide Vincristine	2	Stable disease
2	F	14 y 4 m	Ewing's sarcoma	First relapse	Doxorubicin Vincristine	4	Stable disease
3	M	9 y 5 m	Alveolar rhabdomyosarcoma	First relapse	Vincristine	2	Progressive disease
4	M	6 y	Embryonal rhabdomyosarcoma	First relapse	Vincristine	3	Stable disease
5	M	20 y	Alveolar rhabdomyosarcoma	First relapse	Doxorubicin Vincristine Etoposide	2	Progressive disease
6	M	6 y 10 m	Neuroblastoma	First relapse	Vincristine Etoposide	1	Progressive disease
7	M	9 y 11 m	Osteosarcoma	First pulmonary relapse (on treatment)	Doxorubicin Etoposide	1	Progressive disease
8	M	14 y 10 m	Ewing's sarcoma	Progressive disease on treatment for first relapse	Epirubicin Vincristine Etoposide	8	Partial response
9	M	16 y 4 m	Desmoplastic small round cell tumour	Progressive disease on treatment	Etoposide Vincristine Etoposide	2	Stable disease
10	M	8 y 1 m	Embryonal rhabdomyosarcoma	Seventh local relapse	Etoposide Vincristine	2	Stable disease
11	M	5 y 8 m	Neuroblastoma	Refractory	Epirubicin Vincristine Etoposide	2	Stable disease
12	M	16 y 6 m	Alveolar rhabdomyosarcoma	Second relapse	Doxorubicin Vincristine Etoposide	3	Partial response
13	F	5 y 4 m	Wilms' tumour	Refractory	Doxorubicin Vincristine Etoposide	2	Mixed response
14	F	19 y 11 m	Osteosarcoma	Third pulmonary relapse	Vincristine Etoposide Doxorubicin	1	Progressive disease
15	M	16 y 8 m	T-ALL	First relapse on treatment	Etoposide Doxorubicin	1	Progressive disease
16	F	16 y 6 m	Osteosarcoma	Progressive disease on treatment	Doxorubicin Vincristine Etoposide	1	Withdrew

was recorded. In one patient with a superficial solid tumour clinical 2-D measurements were documented. In one case of leukaemia, there were blasts visible on the peripheral blood film and the peripheral blast percentage was used as a measure of disease. Bone marrow metastatic disease in solid tumours was assessed by bilateral bone marrow aspirates and trephines.

Disease reassessment was performed after two courses of EVE/cyclosporin using the same imaging modality. Response was defined according to the following criteria, partial response (PR), mixed response (MR), stable disease (SD) or progressive disease (PD).

A PR was defined as a reduction of 50% or greater in two dimensional measurements of disease, a MR as a partial response at one or more of several disease sites, but with stable disease at others, SD as a less than 50% reduction or a less than 25% increase in measurable disease and PD as a greater than 25% increase in the size of existing lesions or the development of new lesions. Complete marrow response was $<5\%$ leukaemic blasts on aspirate.

3. Results

15 patients are evaluable for response including 3 patients who developed progressive disease after the first course. One patient electively withdrew during the

first course of treatment due to drug intolerance. Thus, 11 patients received two or more courses of EVE-cyclosporin. 2 patients (4 and 11), who had a reduction in cardiac shortening fraction on echocardiography after one course of EVE-cyclosporin, received cyclosporin, with only vincristine and etoposide as a second course. They are included in the response data, but their second course of chemotherapy was not evaluated for toxicity. Details of response are shown in Table 1.

After two courses of chemotherapy, 2 patients had progressive disease, 6 had stable disease, 1 had a MR and 2 patients had a PR. Duration of stable disease was short and only 2 of these patients received more than two courses before their disease progressed. Both patients who achieved a partial response had received previous anthracycline, etoposide and vincristine chemotherapy. Patient 12 had stage IV alveolar rhabdomyosarcoma which had relapsed 2 months after primary therapy, and had then received 6 months of oral etoposide chemotherapy, to which he had had an initial response before developing local progressive disease at which stage he received EVE-cyclosporin. Patient 8 had a primary Ewing's sarcoma of the skull vault and had been treated initially according to the European Intergroup Study 92 protocol, including radiotherapy, but relapsed after only 2 months off therapy. At relapse, he received cisplatin and etoposide chemotherapy, but progressed through this, receiving EVE-cyclosporin at this stage.

3.1. Toxicity

15 patients completed 36 courses of EVE-cyclosporin. One patient with bone marrow involvement was neutropenic and thrombocytopenic on commencement of chemotherapy and was not therefore evaluable for haematological toxicity.

Transient hypertension developed acutely in four children, ranging from a transient episode not requiring therapy in one child, to more severe requiring treatment with antihypertensives in three children. One child had very severe acute reactions on commencement of cyclosporin on both occasions it was given, requiring symptomatic therapy with lorazepam, ondansetron and paracetamol. Although after two courses he had stable disease, he refused further therapy. One other child had symptoms suggestive of a reaction to cyclosporin, although these varied in severity with courses. On one occasion she developed chest pain, and on another occasion complained of dizziness and tremulousness. One patient had a single self-limiting generalised seizure four days after commencing EVE-cyclosporin.

The other major toxicity seen was myelosuppression, with neutropenia as the predominant feature. Moderate or severe infection developed after 13 courses, with resultant hospitalisation. There were no toxic deaths.

Two children suffered cardiac toxicity as measured by a reduction in fractional shortening on echocardiography. Neither of these children had received high doses of anthracycline chemotherapy prior to receiving EVE-cyclosporin. One child had been treated for a rhabdomyosarcoma according to the MMT '95 trial and received IVA (ifosfamide, vincristine, actinomycin D). At relapse, he received 75 mg/m^2 of epirubicin, following which an echocardiogram was reported as normal with a (FS) of 33%. After one course of EVE-cyclosporin fractional shortening (FS) was recorded as 29% and after two was 28%. A second child, who had been treated for a stage IV alveolar rhabdomyosarcoma, and had received in total 225 mg/m^2 of epirubicin prior to receiving EVE-cyclosporin A showed a fall in FS from 29 to 24%. Neither patient developed clinical evidence of cardiac impairment.

Renal toxicity as documented by a rise in urea and/or creatinine was observed after two courses. In 1 patient, the creatinine rose to 90 μmol/l and in the other there was more severe toxicity, where urea rose to 17 μmol/l and creatinine to 157 μmol/l. The pretreatment values had been within the normal range for the patient's age. All abnormalities subsequently returned to normal and did not cause a delay in subsequent therapy.

Hypomagnesaemia was relatively common, occurring in 3 patients, who all required supplementation.

Hyperbilirubinaemia occurred in five patients after seven courses, ranging from 25 units to a maximum recorded level of 89 International units (normal upper limit approximately 25 International units.)

Gastrointestinal disturbances were relatively common, with 10 episodes of either grade I or II nausea, vomiting or diarrhoea. More severe vomiting was recorded after two courses, in one child this was accompanied by diarrhoea and abdominal pain.

4. Discussion

The toxicity and efficacy of two different schedules of cyclosporin A, given with etoposide, have been previously studied and a short, high-dose cyclosporin A infusion (30 mg/kg/day over 3–6 h for 3 days) was found to achieve higher plasma cyclosporin A levels than 15 mg/kg over 24 h×60 h. The former schedule was associated with more acute and haematological toxicity [12]. However, because of the assumption that the high cyclosporin levels achieved are likely to be beneficial in terms of MDR modulation, and also that the shorter daily infusions are more practical in patients with single lumen i.v. access, the short high-dose infusion cyclosporin schedule was used in this study. *In vitro* studies suggest that concentrations

above 1000 ng/ml are required to achieve effective modulation. Using this schedule and dose, levels range between 2 and 4000 ng/ml for up to 8 h and 250–1500 ng/ml at 24 h [12].

Self-limiting acute toxicity was the major problem observed in the high-dose short infusion cyclosporin A arm in the previous study. In the present study, the infusion time was routinely 6 h rather than 3 h, and although acute reactions were observed in six children in total, the acute toxicity overall was more acceptable. Four children developed acute onset transient hypertension, one child had very severe generalised reactions requiring symptomatic therapy after both of the courses he received, and one other child complained of different sensations (for example, tingling and dizziness) after each of her four courses, but these were not severe enough to require therapy. Pain at the tumour site has been described in adults, but this was not notable in this study.

As expected from the previous study of etoposide alone given with high-dose short infusion cyclosporin, myelosuppression was significant, with neutrophil counts falling below 0.5×10^9/l in most patients.

The ideal scenario in which to test the clinical efficacy of MDR modulators would be to treat with EVE alone and then evaluate EVE-CyA in non-responding tumours. This approach has been used in adults with myeloma, but in the present study prior treatment with anthracyclines made it difficult to administer full-dose epirubicin prior to combination with cyclosporin. Novel MDR modulators (such as PSC 833 and VX710) have more recently been evaluated *in vitro* and in clinical practice [21]. The likelihood of these eventually being evaluated in randomised phase III trials in children may depend on their activity in adult cancer and hence their commercial future. If this does not prove possible, there is an argument to test the hypothesis that the strategy of MDR reversal is relevant to children's cancer using this combination of EVE-CyA. In such a study, endpoints would include not only response and outcome, but also evidence of prevention of MDR development following treatment [22], either by biopsy or radiolabelled sestamibi. Sestamibi uptake and efflux via the P-gp pump may act as a surrogate for chemotherapeutic agents allowing *in vivo* assessment of P-gp function.

In this study, many patients had received significant prior anthracycline chemotherapy and it was felt that using one to two courses of EVE alone initially would have made the subsequent use of EVE-cyclosporin difficult because of the total cumulative anthracycline dose. It was not, therefore, feasible to test EVE-CyA in proven EVE failures.

Cardiac toxicity was, in fact, noted in 2 of 12 patients who received more than one course of EVE. Neither patient developed symptomatic cardiomyopathy, but a significant fall in fractional shortening as assessed by echocardiography was documented. Both patients had received anthracycline-containing chemotherapy immediately prior to EVE-cyclosporin.

Although 8/11 patients who were evaluable after two courses had at least SD, most were of short duration and only two had a documented PR. However, 10 patients had disease which was either progressing on, or not responding to, active treatment, and of these 6 had stable disease or responded. The majority of patients had received etoposide, vincristine and anthracycline-containing chemotherapy prior to receiving EVE-cyclosporin.

Although severe acute toxicity occurred in only a few patients, many found the non-specific symptoms associated with high-dose cyclosporin unpleasant. A follow-on study is currently evaluating a lower dose regimen (15 mg/kg) which might be more suitable for multicentre use in a phase III trial in newly diagnosed patients.

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