Combining Cyclosporin with Chemotherapy Controls Intraocular Retinoblastoma without Requiring Radiation[1]

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ABSTRACT

Chemotherapy without radiation has not controlled most intraocular retinoblastoma, perhaps because of the common high expression of multidrug resistance P-glycoprotein that we found in retinoblastoma. Cyclosporin blocks P-glycoprotein-induced efflux of vincristine and teniposide *in vitro*, and possibly modulates responses to carboplatin. To avoid eye irradiation in bilateral retinoblastoma patients with RB1 germline mutations, which incurs a high second malignancy rate, we added cyclosporin A to a vincristine-teniposide-carboplatin protocol and consolidated chemotherapy responses with focal therapy. We scored patients requiring irradiation, enucleation, or focal ablation of central vision as failures. In 21 study patients, the overall relapse-free rate at a median follow-up of 3.3 years was 76%, with a rate of 92% for newly diagnosed and 50% for previously treated, relapsed retinoblastoma. Our results for the most unfavorable tumors with vitreous seeds (86% at 3.5 years) are better than published success rates of irradiation for similar tumors, or irradiation with the same chemotherapy without cyclosporin (45% at 2.6 years). These results also exceeded our historic success rate with similar chemotherapy without cyclosporin, focal therapy, and/or radiation in 19 equivalently poor-risk patients (relapse-free rate 37% at a median follow-up of 5.6 years, $P = 0.032$), 16 of whom were previously untreated (relapse-free rate also 37%, $P = 0.012$). A better outcome occurred with higher cyclosporin blood levels and projected tissue exposure. Cyclosporin did not enhance the usual chemotoxicity. This clinical study suggests that cyclosporin improves the long-term response of retinoblastoma to chemotherapy, possibly by more than one mechanism.

INTRODUCTION

The cure of most intraocular retinoblastoma, especially large, centrally or anteriorly located tumors or those with vitreous seeds, presently requires external-beam eye irradiation (1, 2). Only small tumors are controllable with focal laser and cryotherapy (3, 4), and only medium-sized, localized tumors away from the macula and optic nerve are treatable with focal plaque radiotherapy (5). However, irradiation of bilateral retinoblastoma patients with germline RB1 mutations causes severe long-term consequences, incurring a 35% risk of secondary bone or soft-tissue sarcoma, brain tumor, or malignant melanoma by 30 years (6), and a 90% risk of orbital deformities (7).

No published study shows successful cure of intraocular retinoblastoma by chemotherapy without radiation (8). The common high expression of the multidrug-resistance P-glycoprotein that we observed in 30% of untreated retinoblastoma and in all tumors tested at failure of treatment may account for this poor response to chemotherapy (9–11). P-glycoprotein promotes efflux of chemotherapy drugs from resistant tumor cells (12). Many studies conclude that increased P-glycoprotein correlates with therapeutic failure in neuroblastoma, rhabdomyosarcoma, leukemia, myeloma, and lymphoma, whereas undetectable P-glycoprotein correlates with lasting remission (13–19). Inhibitors of P-glycoprotein function, such as cyclosporin A and verapamil, reverse multidrug resistance in experimental animals and tumor cell lines (20, 21), including retinoblastoma (22). Furthermore, cyclosporin suppresses cisplatin induction of oncogenes (c-*fos*, c-*myc*) and genes involved in the repair of drug-induced DNA damage (23). Therefore, we added cyclo-

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sporin to a vincristine-teniposide-carboplatin protocol to block the efflux of vincristine and teniposide by P-glycoprotein and modulate the response to carboplatin, and consolidated the response to chemotherapy with focal therapy, to determine whether we could cure intraocular retinoblastoma without radiation. We report an excellent success rate in treating newly diagnosed patients without radiation and significant success in salvaging previously treated, relapsed patients. This therapeutic approach constitutes a major conceptual advance in retinoblastoma therapy and suggests that it is feasible to modulate chemotherapy efficacy with cyclosporin.

PATIENTS AND METHODS

Patient Selection. We recruited 21 consecutive bilateral retinoblastoma patients into a Phase I/II single-arm trial (1991–95) using cyclosporin to block P-glycoprotein and modulate carboplatin response in a vincristine-teniposide-carboplatin protocol consolidated by focal therapy but with no radiation. We did not test P-glycoprotein prospectively, because intraocular retinoblastoma cannot be biopsied safely; nor can we presume that the tumor P-glycoprotein level in the enucleated eye is indicative of levels in the separate tumors in the remaining treated eye (10, 11). We confirmed the diagnosis of retinoblastoma in one enucleated eye of eligible patients or by family history and ophthalmoscopic findings, if enucleation was unnecessary. Standard therapy for these retained eyes with potentially useful vision is external beam radiation, because they contained tumors with vitreous seeds, tumors too large or too anterior to the ora serrata for focal therapy or radioactive plaques, or tumors located centrally near the optic nerve or macula where focal modalities would destroy vision. Blood counts and renal and liver functions were adequate (blood urea <20 mg/dl, creatinine <0.7 mg/dl, alanine aminotransferase <35 units/dl, aspartate aminotransferase <67 units/dl, alkaline phosphatase <415 units/dl, bilirubin <1 mg/dl), Karnofsky performance status was $\geq 90\%$ (24), and extraocular disease was absent by standard testing (lumbar puncture, bone marrow, head and orbit computerized tomography, eye ultrasonography). The Hospital for Sick Children Research Ethics Board approved this study, and parents of patients provided informed consent. We treated 26 eyes in 21 bilateral retinoblastoma patients, aged 1 month to 5.3 years, for control of intraocular tumors. In 16 patients without family history who already had one eye enucleated, we treated the other eye. In four patients, three with a family history of retinoblastoma, we treated both eyes simultaneously. In one patient with separate relapses 1.9 years apart in different eyes, we treated the eyes asynchronously, and considered results in each eye separately. Eleven eyes in 6 patients had central tumors small enough for focal therapy, but that would have severely damaged their vision. Three eyes (three patients) had medium-sized tumors with a moderate likelihood of being saved by radiation (25, 26). Twelve eyes (12 patients) contained large tumors (4 also involving the ora serrata) and/or vitreous seeds, with a poor likelihood of cure by radiation (25, 26). Ten eyes in nine patients had poor prognosis because of relapse from previous chemotherapy (8), radiation (1), or radiation with chemotherapy (1).

Because no Phase I/II cyclosporin-chemotherapy data exist for retinoblastoma, and a single-institution, randomized Phase III study is not feasible for this rare tumor, we compared present results with our historic success rate for equivalently poor-risk retinoblastoma treated with focal therapy, similar chemotherapy without cyclosporin and/or radiation. Because there are inherent weaknesses in historic comparisons, we additionally compared present results with published success rates for the most unfavorable tumors with vitreous seeds irradiated at other centers over a similar period, or irradiated and given the same chemotherapy without cyclosporin.

Chemotherapy Protocol. We treated study patients with vincristine (0.05 mg/kg) and teniposide (230 mg/m^2) every 10 days (6 patients), or with vincristine-teniposide and carboplatin (560 mg/m^2), every 21 days (14 patients). The patient treated twice for two separate relapses in different eyes received each of these therapies. Infants weighing <10 kg or <1 year old conventionally received less teniposide (7.7 mg/kg) and less carboplatin (18.7 mg/kg). We administered cyclosporin in the clinic by 3-h infusions (1 h before and 2 h after days 1 and 2 chemotherapy), with 30-min infusions of carboplatin (day 1) and teniposide (day 2), and we administered vincristine by bolus injection (day 2), with antiemetics (metoclopramide, dimenhydrinate, and ondansetron), antiallergic therapy (hydrocortisone and diphenhydramine), and hydration. We started cyclosporin at 4 mg/kg/day and escalated intrapatient doses by 1-mg/kg increments in cohorts of three patients each, according to European Organization for Research on Treatment of Cancer Guidelines, to establish the maximum tolerated dose (27). Mean cyclosporin doses in patients ranged from 8 to 39 mg/kg/day, with a median of 33 mg/kg/day. Treatment continued until 3 months after there was no active-appearing tumor (see "End Points"); on average, less severely affected patients received 3 months, and more severely affected patients received 12 months of chemotherapy. We treated tumors with highly refractory vitreous seeds for 1 year, because they cannot be treated by focal therapy and do not respond well to radiation. We substituted vinblastine (4 mg/m^2) for vincristine neuropathy, and etoposide (230 mg/m^2) for teniposide allergy.

Focal Therapy Protocol. After observing a significant chemotherapy response, we applied focal therapy (cryotherapy and/or transpupillary 532- or 1064-nm laser) under anesthesia before every second or third chemotherapy. Only if patients failed chemotherapy did we use external beam radiation or radioactive plaques.

End Points. We considered the initial chemotherapy response before focal therapy favorable if tumors shrank considerably and appeared inactive, avascular, and calcified (cottage cheese-like), and if free-floating tumor "seedings" in the vitreous disappeared completely or cleared significantly, leaving only a few calcified or waxy-looking seeds. Tumors showing little shrinkage or calcification, that remained vascular or translucent (fish flesh-like), with unchanged vitreous seeds, were considered to have an unfavorable response. Long-term outcome was considered the response to chemotherapy consolidated by focal therapy. Failure was identified when tumor progression required radiation, enucleation (if previously irradiated), or macula-destroying focal therapy. Relapse-free duration was measured in January 1996 from diagnosis to relapse or

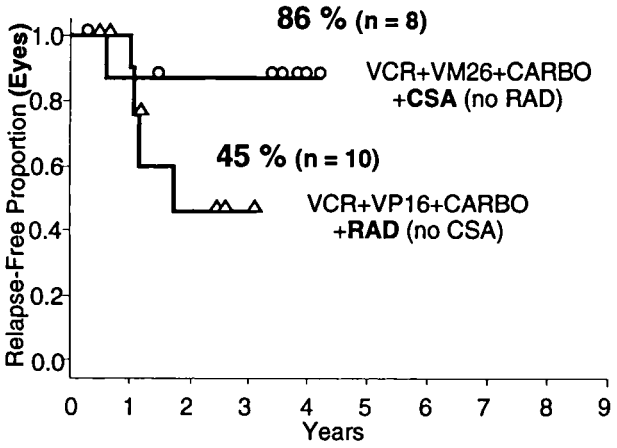

Fig. 1 Relapse-free proportion of eyes with vitreous seeds in study patients treated with the current protocol including cyclosporin but without radiation (○) *versus* equivalent chemotherapy with radiation but no cyclosporin at another center (△). *VCR*, vincristine; *VM26*, teniposide; *CSA*, cyclosporin; *CARBO*, carboplatin; *RAD*, radiation therapy.

last follow-up. We assessed vision by preferential looking, or visual evoked potential in younger children.

We graded toxicity by Childrens Cancer Group guidelines: grade 0 (absent), grade 1 (mild), grade 2 (moderate), grade 3 (severe), and grade 4 (unacceptable); we reduced chemotherapy by 10% and cyclosporin by 1 mg/kg, for ≥grade 3 gastrointestinal toxicity, ≥grade 2 renal, liver or neurotoxicity, or grade 4 myelotoxicity delaying chemotherapy beyond 6 weeks. We assessed frequency of chemotoxicity with and without cyclosporin and compared average dose intensity and percentage of projected dose intensity (28, 29).

Cyclosporin Pharmacokinetics. We measured on day 1 the peak and the 2-, 12-, and 24-h postpeak concentrations of cyclosporin, according to a limited blood-sampling strategy (30), by high-performance liquid chromatography that distinguishes parent drug from metabolites (31). We analyzed concentration-*versus*-time data by compartmental model equations, using a modified ADAPT II nonlinear fitting program (32). An observer "blinded" to patient parameters performed curve fittings and obtained optimal fits for the data sets by the minimized sum of squares, employing unity weighing. This identified a two-compartment model, central (blood) and peripheral (tissue), as optimal for cyclosporin, according to the F test and Akaike criterion (33, 34). In 133 of 218 treatment cycles, we estimated the AUC[3] (the area under the central and the peripheral concentration-time curve), the Cmax, and the peripheral maximum amount for day 1 of cyclosporin, and calculated mean and cumulative values. We did not determine pharmacokinetics of chemotherapy drugs, because observations that cyclosporin can alter drug clearance were not yet available at inception of this study (35–37). Determining pharmacokinetics of several chemotherapy drugs requires multiple blood samples in young children, which presents an ethical problem (30).

Statistical Analysis. We compared response rates by Fisher's exact test, and estimated actuarial relapse-free rates with Kaplan-Meier life tables (38). Because of possible bias when comparing the success rate of study patients with historic outcome, we evaluated associated prognostic factors and different therapies for uniformity of distribution, and stratified patients by log-rank analysis according to each factor and therapy (39). We determined whether each factor or therapy significantly affected relapse, before and after stratifying for the effect of cyclosporin. Similarly, we assessed the effect of cyclosporin directly and with stratification for each factor or therapy. Using the Kaplan-Meier method, we compared outcome of the most unfavorable tumors (vitreous seeds) in study patients to equivalent tumors irradiated at other centers or irradiated and treated with the same chemotherapy without cyclosporin.

To determine whether there is a dose response to cyclosporin, we compared response and relapse-free rates for higher-than-median total (≥457 mg/kg; range, 47–1360 mg/kg) or mean doses (≥32 mg/kg; range, 8–39 mg/kg) with lower doses and with no cyclosporin, by χ^2 and log-rank tests for trend. We similarly compared different pharmacokinetic parameters above the median for the entire group to below the median, and to no cyclosporin, to assess whether differences in cyclosporin clearance and metabolism (blood levels, tissue concentrations) affected outcome. We projected tissue exposure to cyclosporin from pharmacokinetic fits of blood concentration-*versus*-time data and not from direct measurements.

Because we had no concurrent noncyclosporin control group, we compared the frequency of vincristine-teniposide toxicity with cyclosporin, dose intensity, and percentage of projected dose intensity of study patients to values for a historic group (Fisher's exact, Student's *t* test), to determine whether cyclosporin increased chemotoxicity (28, 29). No noncyclosporin group was available for a similar comparison with vincristine-teniposide-carboplatin. All statistical tests were two-sided.

RESULTS

Chemotherapy with cyclosporin produced excellent results. A favorable initial response to chemotherapy occurred in overall 17 of 21 study patients (20 of 26 eyes): 12 of 13 newly diagnosed patients (15 of 16 eyes), and 5 of 8 previously treated, relapsed patients (5 of 10 eyes). Overall relapse-free rate at median follow-up 3.3 years was 76% for patients (80% for eyes), 92% for newly diagnosed patients (94% for eyes), and 50% for previously treated, relapsed patients (56% for eyes). Even newly diagnosed patients with the poorest-risk eyes did well; the relapse-free rate was 83% for vitreous seeds, 86% for tumors larger than 1.5 cm, 100% for central macular region tumors, and 67% for anterior tumors involving the ora serrata. No tumors extended extraocularly. One study patient in remission died of unexplained seizures with cerebral edema (0.1 years), after receiving hydrocortisone and furosemide, but before cyclosporin or chemotherapy.

To assess the efficacy of the present therapy, we compared our results to those from other centers, because we had no

[3] The abbreviations used are: AUC, area under curve; Cmax, maximum concentration.

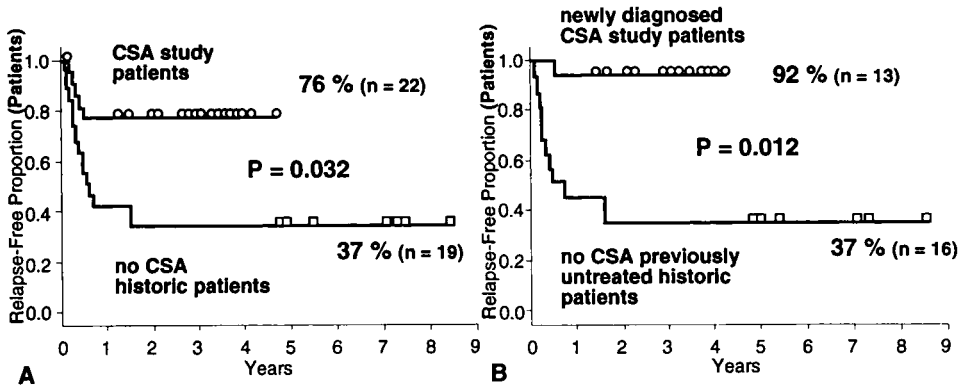

Fig. 2 Relapse-free proportion of patients treated with chemotherapy-focal therapy with cyclosporin (study patients) or without cyclosporin (historic patients). *A*, all patients; *B*, previously untreated patients. *CSA*, cyclosporin.

concurrent controls without cyclosporin. Results of this study were better than success rates for unfavorable tumors irradiated at other centers, or irradiated and given equivalent chemotherapy without cyclosporin. In the radiation studies, eyes requiring enucleation were identified as failures. Relapse-free rate with our vincristine-teniposide-carboplatin-cyclosporin protocol (no radiation) was 86% (8 vitreous-seeded eyes treated in 1991–95, median follow-up 3.5 years) *versus* 45% (10 vitreous-seeded eyes treated in 1990–93, median follow-up 2.6 years) with the London St. Bartholomew's Hospital vincristine-etoposide-carboplatin-radiation protocol without cyclosporin[4] (Fig. 1). For further comparison, a small radiation-only series at the University of California, San Francisco[5] showed a relapse-free rate of 80% (6 vitreous-seeded eyes treated in 1986–94, median follow-up 3 years), but a larger and older radiation-only series at New York Hospital-Cornell Medical Center reported a relapse-free rate of 40% (51 eyes with vitreous seeds or tumors larger than half the retina followed for 6 years; Ref. 1).

As an additional scale of reference, we compared present results with our historic success rate with chemotherapy without cyclosporin (1981–91) in 19 bilateral retinoblastoma patients, aged 6 days to 5.2 years, 16 with one eye treated and the other eye enucleated, and 3 with both eyes treated. These eyes were equivalently poor risk; 9 (6 patients) had small central tumors in which focal therapy would have severely damaged vision; 2 (2 patients) had medium-sized tumors with a moderate chance of cure by radiation; and 11 (11 patients) had large tumors (none at the ora serrata) and/or vitreous seeds poorly controllable by radiation. Only 3 (3 patients) had relapsed after prior radiation (2) or radiation with chemotherapy (1). Fourteen historic patients received vincristine and teniposide, and five received vincristine-teniposide and doxorubicin, cyclophosphamide or ifosfamide, and/or carboplatin or cisplatin (four were also irradiated concurrently); responses were consolidated with similar focal therapy. Results of the present study were better than our historic success rate [7 of 19 favorable initial response (P = 0.012), overall relapse-free rate 37% at a median follow-up of 5.6 years (P = 0.032), and relapse-free rate 37% for the 16 previously untreated patients (P = 0.012); Fig. 2, *A* and *B*]. We achieved lasting tumor control and avoided external beam radiation or enucleation in 17 of 21 study patients (12 of 13 new and 5 of 8 relapsed), but in only 5 of 19 historic patients [4 of 16 new and 1 of 3 relapsed (P = 0.0017)]. One historic patient died of radiation-induced glioblastoma multiforme at 6.5 years.

We salvaged useful vision for most study patients as follows: normal to near normal (6/6 to 6/9) in 6, moderate (6/12 to 6/30) in 5, poor (6/60 to light perception) in 6, none in the 3 enucleated; and unavailable in the patient who died. Responding macular region tumors in 11 eyes (8 patients) resulted in significant recovery of central vision. These results were comparable to published radiation results (40), but better than our historic series (normal in one, moderate in seven, poor in four, none in the three enucleated, and untested before failure in four).

To avoid possible bias due to associated risk factors when comparing the present success rates with historic outcomes, we stratified patients according to their distribution of prognostic factors. Tumors of study patients had equivalent or more poor-risk factors than tumors of historic patients: previous relapses (41 *versus* 16%), vitreous seeds (41 *versus* 32%), larger than 1.5 cm (45 *versus* 47%), central location (64 *versus* 58%), and involvement anterior to ora serrata (18 *versus* 0%; all percentages for study *versus* historic patients, respectively). Furthermore, each prognostic factor did not account for the significantly improved outcome with cyclosporin before (P range, 0.25–0.98) or after stratifying for the effect of cyclosporin (P range, 0.22–0.96). Conversely, the significantly better relative relapse rate with cyclosporin (P = 0.032) remained better after stratifying for each prognostic factor (P = 0.023 for previous relapses, 0.039 for vitreous seeds, 0.046 for tumor larger than 1.5 cm, 0.041 for central location, and 0.020 for ora serrata involvement).

Because of possible bias due to different therapies when comparing success rates of this study with historic outcomes, we stratified patients according to their treatment. Radiation did not affect outcome significantly before (P = 0.72) or after stratifying for the effect of cyclosporin (P = 0.58). Adding carboplatin or other drugs to vincristine-teniposide did not affect outcome significantly (P = 0.073); their effect was even less significant (P = 0.52) after stratifying for cyclosporin. Conversely, the effect of cyclosporin on outcome was significant (P = 0.032),

[4] J. E. Kingston and J. L. Hungerford, unpublished data.
[5] J. M. O'Brien, unpublished data.

Table 1 Effect of cyclosporin pharmacokinetics on response and relapse-free rate in retinoblastoma

Day 1 CSA[a] pharmacological parameters (range)	Cyclosporin-treated (N = 22) Favorable response rate, %	P value	Relapse-free rate, %	Relative relapse rate, O/E[b]	P value	$O/E_{highest\ CSA}$:$O/E_{no\ CSA}$ ratio
Central compartment AUC (mg.h/L)						
Highest						
>113 (114–169)	82		82	0.36		
<113 (34–113)	73	0.011	70	0.70	0.025	4.67
No CSA[c]	37		37	1.68		
Mean						
>96 (97–123)	91		81	0.38		
<96 (22–96)	64	0.0035	70	0.66	0.028	4.42
No CSA[c]	37		37	1.68		
Cumulative						
>1213 (1459–4062)	91		90	0.18		
<1213 (159–967)	64	0.0035	60	0.96	0.011	9.33[d]
No CSA[c]	37		37	1.68		
Central compartment Cmax (mg/L)						
Highest						
>27 (27–65)	82		82	0.36		
<27 (7–27)	73	0.011	70	0.71	0.024	4.67
No CSA	37		37	1.68		
Mean						
>21 (21–30)	82		91	0.18		
<21 (5–20)	73	0.011	60	0.91	0.012	9.33[d]
No CSA[c]	37		37	1.68		
Peripheral compartment AUC (mg.h/kg)						
Highest						
≥230 (238–4850)	82		80	0.40		
<230 (44–222)	73	0.011	73	0.62	0.031	4.20
No CSA[c]	37		37	1.68		
Mean						
≥137 (153–789)	82		81	0.38		
<137 (25–121)	73	0.011	70	0.66	0.028	4.42
No CSA[c]	37		37	1.68		
Cumulative						
≥1641 (1667–14204)	91		90	0.18		
<1641 (209–1616)	64	0.0035	60	0.96	0.011	9.33[d]
No CSA[c]	37		37	1.68		
Peripheral maximum amount (mg/kg)						
Highest						
≥8 (8–19)	100		90	0.18		
<8 (3–8)	55	0.00097	60	0.93	0.012	9.33[d]
No CSA[c]	37		37	1.68		
Mean						
≥6 (6–11)	91		81	0.38		
<6 (2–6)	64	0.0033	70	0.65	0.028	4.42
No CSA[c]	37		37	1.68		

[a] CSA, cyclosporin.

[b] O/E is ratio of observed numbers of relapses to extent of exposure to the risk of relapse; P values, overall P values for favorable response rate (χ^2 for trend) and relative relapse rate (log-rank for trend) of subgroups for each parameter.

[c] Historic patients with no CSA.

[d] Parameters with the highest $O/E_{highest\ CSA}$:$O/E_{no\ CSA}$ ratios correlated with better outcomes.

becoming even more significant after stratifying for radiation (P = 0.019) but less significant after stratifying for carboplatin or other drugs [observed relative to expected ratio of relapses was 0.65/1.29 for patients (P = 0.21) but approaching significance at 0.55/1.37 (P = 0.069) for the larger number of eyes]. Apparently, cyclosporin improved outcome most significantly with carboplatin-vincristine-teniposide (P = 0.049); the cyclosporin effect was less evident without carboplatin, suggesting a benefit from combining cyclosporin with carboplatin.

We could not simultaneously stratify patients according to their prognostic factors and therapies because of small numbers in each subgroup. Adding cyclosporin improved outcome strikingly for previously untreated patients, irrespective of their type

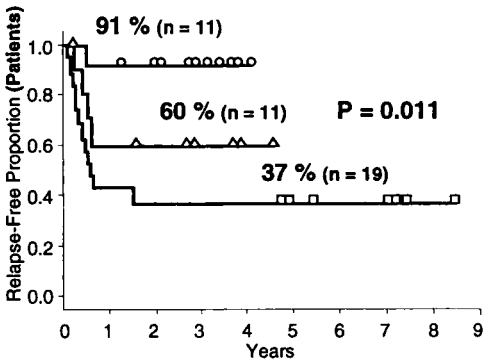

Fig. 3 Relapse-free proportion of study patients comparing high (≥1213 mg.h/L, ○) to low (<1213 mg.h/L, △) cumulative central AUC for cyclosporin and historic patients receiving no cyclosporin (□).

of chemotherapy [92 *versus* 37% overall relapse-free rates (P = 0.012); 100 *versus* 33% for vincristine-teniposide; and 91 *versus* 50% for vincristine-teniposide with carboplatin or with other drugs]. The effect of cyclosporin was also evident for previously treated, relapsed patients (67 *versus* 0%) or poor-prognosis patients with vitreous seeds (73 *versus* 50%), large tumors (73 *versus* 40%), and central tumors (100 *versus* 33%) treated with vincristine-teniposide-carboplatin but not with vincristine-teniposide (data not shown). This further suggests that carboplatin may be a factor in successful therapy. However, results in study patients receiving vincristine-teniposide may be compromised by the larger number of previous relapses (6 of 7) compared to similarly treated historic patients (2 of 14). Study patients receiving vincristine-teniposide also received lower mean cyclosporin doses (<21 mg/kg), because this was the protocol during early cyclosporin dose escalation. Furthermore, three of five study patients failing initial vincristine-teniposide did achieve lasting remissions when cyclosporin was added to the same drugs. By serving as their own controls, these successfully salvaged patients strongly suggest an important role for cyclosporin in this protocol. Adding cyclosporin and carboplatin to two other patients who failed previous vincristine-teniposide also elicited stable remissions.

Higher cyclosporin doses and pharmacokinetic parameters correlated significantly with better outcome (Table 1). Response rates were better (P = 0.0035 for patients; P = 0.00045 for eyes) and relapse-free rates greater, comparing higher total (P = 0.011; P = 0.0015) or mean doses of cyclosporin (P = 0.028; P = 0.0043) with lower doses, and with no cyclosporin. Peak and 2-, 12-, and 24-h postpeak concentrations on day 1 of cyclosporin (≥32 mg/kg) were 21,736 ± 6,333 (mean ± SD), 6,537 ± 3,798, 512 ± 202, and 432 ± 162 ng/ml, and higher on day 2 (data not shown). Higher cumulative central AUC and mean Cmax (reflecting higher total doses and blood concentrations), and higher cumulative peripheral AUC and highest peripheral maximum amount (reflecting higher projected tissue exposure) also correlated with better outcome (Fig. 3).

Assessment of toxicity was difficult because there were no true controls, and we graded toxicity in historic patiens retrospectively (Table 2). For each criterion, we had to change the denominator (evaluable cycles) because of missing data. Data were missing in historic patients in whom tests were ordered only for clinical indications. Cyclosporin patients treated with vincristine-teniposide had fever tests because cycles were only 7–10 days apart. Therefore, we placed more reliance on factual data such as hospital admissions for fever and neutropenia or infections, blood and platelet transfusions, and body weight changes, and less on documentations of vincristine neuropathy, blood counts, and liver and renal function tests. Our data showed that despite cyclosprorin, vincristine-teniposide was well tolerated by young children, who gained weight and developed normally. Cyclosporin did not significantly increase hospital admissions or blood products usage, or cause more weight loss or less weight gain (Table 2).

We had no comparable non-cyclosporin group for vincristine-teniposide-carboplatin (Table 3). We followed blood counts twice a week and data were more complete in these more recent patients. Interim renal and liver funciton tests between cycles have consistently shown no abnormalities. Despite up to twelve cycles of 2 days of cyclosporin every 3 weeks, hospital admissions for fever and neutropenia or infections were only 16%, blood and platelet transfusions were 12% and 18%, respectively, and 87% of patients gained weight while 13% lost weight (Table 3). We found no ototoxicity and only rarely, transient hypertension (from intravenous hydration), mild hypomagnesemia, or hypophosphatemia. Allergic reactions to cyclosporin were absent. Our data suggest that the present retinoblastoma protocol with cyclosporin produced acceptable chemotoxicity.

Up to 5-day continuous cyclosporin was reported by others to enhance etoposide and doxorubicin chemotoxicity, from reduced clearance of these drugs (35–37) requiring reduction in chemotherapy dosages by one half or more for toxicity to become acceptable. It is evident that such a degree of dosage reduction was not required in our retinoblastoma protocols (Tables 2 and 3), in which average dose intensity and percentage of projected dose intensity of chemotherapy were good with cyclosporin (28, 29).

DISCUSSION

These results strongly suggest that cyclosporin enhances the efficacy of our chemotherapy protocol for the following reasons. (*a*) Half of the previously relapsed patients achieved lasting remissions with cyclosporin when given the same drugs that previously failed. (*b*) Results for poor-prognosis tumors with vitreous seeds were better than radiation-containing chemotherapy regimens or radiation only at other centers (1). (*c*) Despite more relapses from previous treatment and ora serrata involvement, study patients did better than historic patients. (*d*) Adding cyclosporin strikingly improved outcome for newly diagnosed patients, irrespective of the type of initial chemotherapy. (*e*) A dose response was observed with higher cyclosporin doses and blood concentrations. (*f*) The greatest projected tissue exposure to cyclosporin correlated with the best outcome. This is the first clinical study to suggest that cyclosporin improves long-term response to chemotherapy in newly diagnosed intraocular retinoblastoma and possibly salvages a proportion of previously treated, relapsed tumors. Two previous cyclosporin studies also reported prolonged chemotherapy

Table 2 Toxicity of vincristine-teniposide, according to whether cyclosporin given

VCR-VM26[a]	No cyclosporin		Cyclosporin[c]		
DI and toxicity[b]	14 historic patients 158 cycles	%	10 study patients 81 cycles	%	*P* value
Average VCR DI (mg/kg/wk), % projected	0.033	93	0.029	79	0.090
Average VM26 DI (mg/m^2/wk), % projected	123.7	98	121.0	98	0.86
Average % projected VCR-VM26 DI		96		88	0.34
	No. of events or pts./no. of evaluable cycles or pts.				
Weight loss (average % loss)	4/14 pts. (6.4%)	29	4/9 pts. (4.5%)	44	0.66
Weight gain (average % gain)	10/14 pts. (30.8%)	71	5/9 pts. (19.4%)	56	0.66
VCR neuropathy					
Decreased deep tendon reflexes, grade 1	5/14 pts.	36	0/10 pts.	0	0.053
Constipation, grade 4	0/158	0	1/81	1	0.34
Constipation, grade 3	7/158	4	2/81	2	0.72
Constipation, grades 1–2	8/158	5	2/81	2	0.50
Paresthesia, grade 3	4/158	2	1/81	1	0.66
Paresthesia, grades 1–2	18/158	11	2/81	2	0.024
VCR reduced/held, vinblastine substituted	15/170	9	0/81	0	0.0033
Myelosuppression					
Neutropenia, grade 4	16/16[e]	100	20/20[e]	100	1.00
Fever and neutropenia, infection admission	12/158	8	8/81	10	0.62
Anemia, grades 2–3	25/62[e]	40	19/45[e]	42	0.84
Blood transfusion	9/158	6	9/81	11	0.19
Thrombocytopenia, grade 2	1/20[e]	5	0/29[e]	0	0.41
Platelet transfusion	0/158	0	0/81	0	1.00
Renal dysfunction	0/16[e] (16)	0	0/23[e] (71)	0	1.00
Transient hypertension, grade 1[d]	0/158	0	3/81	4	0.038
Transient hypomagnesemia, grade 1	3/5[e] (4)	60	0/19[e] (71)	0	0.0049
Transient hypophosphatemia, grade 1	0/5[e] (4)	0	2/19[e] (71)	11	1.00
Liver dysfunction	0/9[e] (14)	0	0/13 (70)	0	1.00
Hyperbilirubinemia	0/7[e] (11)	0	0/14[e] (72)	0	1.00
Allergic reaction					
VM26 reaction, grades 2–3	3/158	2	1/81	1	1.00
Cyclosporin rash, grade 1			4/81	5	

[a] VCR, vincristine; VM26, teniposide; DI, dose intensity.

[b] Regimen slated for every 10 days with projected VCR DI of 0.035 mg/kg/wk for all ages and projected VM26 DI of 161 mg/m^2/wk for older children but reduced per convention to 5.4 mg/kg/wk for infants weighting <10 kg or <1 year old (seven study patients, nine historic patients); average DI and percentage of projected DI calculated according to the Hryniuk method (28, 29).

[c] VCR-VM26: seven patients having VCR-VM26 throughout and three patients having VCR-VM26 initially.

[d] Transient increased blood pressure during hydration for cyclosporin therapy.

[e] Cycles with evaluable interim observations; cylces with pretreatment studies in parentheses.

response in refractory myeloma and acute myeloid leukemia (41, 42).

For many resistant tumor types, P-glycoprotein expression correlated strongly with treatment outcome (13–19). Because we cannot safely biopsy intraocular retinoblastoma, we cannot study this correlation. However, in the extraocular retinoblastoma that we did biopsy before treatment, overexpression of P-glycoprotein correlated with ultimate failure, and undetectable P-glycoprotein correlated with lasting remission (10, 11). Although verapamil and cyclosporin render resistant cells chemosensitive by increasing intracellular drug accumulation and prolong survival of animals implanted with resistant tumors (20, 21), clinical reversal of multidrug resistance has been only modestly successful (35–37, 43, 44). Relapsed lymphoma, myeloma, and acute myeloid leukemia showed better (13, 17, 45) or more prolonged response (41, 42) but not increased cures, and high P-glycoprotein-expressing carcinoma showed no response (46). These lasting remissions in our retinoblastoma patients are exceptional when compared with the overall results of multidrug resistance reversal trials. They are by far the most optimistic data to suggest that cyclosporin enhances chemosensitivity. Our results also suggest that chemotherapy can replace and even surpass radiation in treatment of retinoblastoma.

Why does this protocol work for retinoblastoma? Although cyclosporin may modulate P-glycoprotein, direct evidence is lacking in the absence of cytotoxin pharmacokinetics. Several other factors may contribute. Retinoblastoma tumor volumes are smaller than most other tumors, systemic metastases are rare, and the eye might function as a "sanctuary" with persistent high drug concentrations (47). The much higher cyclosporin doses and blood and tissue levels than previously achieved may block the heterogeneous expression of tumor P-glycoprotein more effectively (35–37, 41–44). Importantly, treatment consolidation by focal therapy may destroy residual resistant cells. However, focal therapy cannot account for improved success rates with vitreous seeds that are not treatable with focal modalities (83 and 67% for new and relapsed patients, respectively), nor explain better results (100 and 40% for new and relapsed patients, respectively) for tumors at the center of vision minimally treated with focal therapy to avoid damaging vital structures.

Table 3 Toxicity of vincristine-teniposide-carboplatin given with cyclosporin

VCR-VM26[a]-carboplatin DI and toxicity[b]	Cyclosporin 15 study patients, 137 cycles	%
Average VCR DI (mg/kg/wk), % projected	0.012	71
Average VM26 DI (mg/m^2/wk), % projected	57.1	91
Average carboplatin DI (mg/m^2/wk)	142.2	92
Average % projected VCR-VM26-carboplatin DI		86
	No. of events or pts./ no. of evaluable cycles or pts.	
Weight loss (average % loss)	2/15 pts. (1.3%)	13
Weight gain (average % gain)	13/15 pts. (18.5%)	87
VCR neuropathy		
Syndrome of inappropriate secretion anti-diuretic hormone	1/137	1
Decreased deep tendon reflexes, grade 1	3/15 pts.	20
Constipation, grade 4	0/137	0
Constipation, grade 3	2/137	1
Constipation, grades 1–2	6/137	4
Paresthesia, grade 3	1/137	1
Paresthesia, grade 1–2	8/137	6
VCR reduced/held, vinblastine substituted	2/137	1
Myelosuppression		
Neutropenia, grade 4	84/122[c]	69
Neutropenia, grade 3	31/122[c]	25
Fever-neutropenia-infection admission	22/137	16
Anemia, grades 2–3	53/110[c]	48
Blood transfusion	17/137	12
Thrombocytopenia, grade 4	22/106[c]	21
Thrombocytopenia, grade 3	13/106[c]	12
Thrombocytopenia, grade 2	14/106[c]	13
Platelet transfusion	25/137	18
Renal dysfunction	0/32[c] (131)	0
Transient hypertension, grade 1[d]	2/137	1
Transient hypomagnesemia, grades 1–2	9/24[c] (132)	37
Transient hypophosphatemia, grade 1	0/24[c] (132)	0
Liver dysfunction	0/26[c] (128)	0
Hyperbilirubinemia	0/24[c] (132)	0
Increased alkaline phosphatase	2/26[c] (132)	8
Allergic reaction		
VM26 reaction, grades 2–3	0/137	0
Cyclosporin rash, grade 1	0/137	0

[a] VCR, vincristine; VM26, teniposide; DI, dose intensity.

[b] Regimen slated for every 21 days with projected VCR DI of 0.017 mg/kg/wk for all ages and projected DI of 76.7 mg/m^2/wk for VM26 and 186.7 mg/m^2/wk for carboplatin for older children but reduced per convention to 2.6 mg/kg/wk and 6.2 mg/kg/wk, respectively, for infants <10 kg or <1 year (nine study patients); average DI and percentage of projected DI calculated according to the Hryniuk method (28, 29).

[c] Cycles with evaluable observations; cycles with pretreatment studies in parentheses.

[d] Transient increased blood pressure during hydration for cyclosporin therapy.

Benefit may also derive from combining cyclosporin with carboplatin or other drugs. Cyclosporin may enhance carboplatin efficacy by modulating non-P-glycoprotein resistance mechanisms, such as suppressing platinum-induced expression of oncogenes (*e.g.*, c-*fos* and c-*myc*) and genes required for repair of drug-induced DNA damage (23, 48). Preclinical data suggest that cyclosporin may potentiate etoposide efficacy even in nonresistant tumors (49). Cyclosporin may improve response by increasing systemic drug exposure through inhibition of the metabolism of cytotoxins (35–37). However, we saw no apparent increase in chemotoxicity with short but high-dose cyclosporin infusions, unlike previous prolonged cyclosporin infusion studies (10–26 mg/kg/day, up to 5 days; Refs. 35–37 and 41–44). Our acceptable toxicity and excellent dose intensity argue that the improved efficacy cannot be entirely due to increased systemic drug exposure from cyclosporin inhibition of drug metabolism (35–37). It is possible that shorter cyclosporin infusions decrease drug clearance only slightly to give a modest but important increase in "true" dose intensity without markedly accentuating chemotoxicity. The more common vincristine neuropathy without than with cyclosporin probably reflected the higher projected vincristine dose intensity rather than cyclosporin suppression of an immunological mechanism (Table 2). Furthermore, we saw no hyperbilirubinemia or the increased neural, renal, and myelotoxicity reported by others (35–37, 41–44) due to prolonged inhibition of drug efflux from normal tissues expressing P-glycoprotein (liver, kidney, blood-brain barrier, myeloid progenitors; Refs. 50–54). Young children have enhanced clearance of cyclo-sporin and tolerate high doses well (55). Therefore, unlike other studies, we required no chemotherapy or cyclosporin dose reduction that might compromise dose intensity and possibly curtail response.

We expect no long-term sequelae from short cyclosporin exposures. We avoided alkylating agents that may increase secondary cancers in retinoblastoma patients (56). A 2–3% leukemogenic risk was reported with intensive weekly and twice-weekly epipodophyllotoxin schedules (57). The potential tumorigenic risk of this protocol will require long-term follow-up.

Greatly improved efficacy for treatment of retinoblastoma without radiation in this protocol provides a sound basis for further evaluation of the role of chemotherapy and cyclosporin. To define the relative benefit of cyclosporin and carboplatin and examine cytotoxin pharmacokinetics with and without cyclosporin, a cooperative controlled study must be conducted.

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