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# Assessment of Systemic Toxicity in Children Receiving Chemotherapy With Cyclosporine for Sarcoma

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***Background.*** Overexpression of P-glycoprotein in malignant tumors has been associated with poor responses to chemotherapy. It appears biologically plausible that addition of the P-glycoprotein inhibitor cyclosporine (CsA) to standard chemotherapy may improve the outcome. The protective functions of P-glycoprotein in healthy tissues, however, have not been fully elucidated. Addition of CsA may lead to increased systemic chemotherapy toxicity, so we compared the rate and severity of chemotherapy-associated systemic toxicity in the presence and absence of CsA. ***Procedure.*** Standard chemotherapy consisted of etoposide/ifosfamide (VP16/IFOS) cycles, alternating with vincristine/dactinomycin/cyclophosphamide (VAC) cycles. CsA was given at a median dose of 20 mg/kg with unaltered doses of the antineoplastic drugs. The analysis of toxicity was performed by comparing clinically significant toxicity events recorded during and after chemotherapy cycles with and without CsA. ***Results.*** Toxicity-related hospital admissions occurred after 93% of VAC cycles with CsA compared to 40% of the cycles without CsA ($P < 0.0001$); 29% of VP16/IFOS cycles with CsA led to admissions vs. 12% with non-CsA cycles ($P = 0.04$). Infections or fever and neutropenia were the main reasons for these admissions. Thirty-seven percent of the VAC cycles with CsA were complicated by culture-proved sepsis, which did not occur in cycles without CsA ($P < 0.0001$). Requirements for blood and platelet transfusions were greatly increased after VAC cycles with CsA compared to VAC cycles without CsA. ***Conclusions.*** The chemosensitizer CsA increases the systemic toxicity of VAC chemotherapy in patients with sarcomas. Future trials of chemotherapy with chemosensitizers will have to take into account a potential increase in systemic toxicity. Careful monitoring of chemotherapy-related toxicity becomes mandatory in such studies. Med. Pediatr. Oncol. 34:242–249, 2000. 

**Key words:** cyclosporine; P-glycoprotein; antineoplastic agents; combined clinical trials; sarcoma; children

## INTRODUCTION

Resistance of tumor cells to drugs is a major problem in antineoplastic therapy. One such form of drug resistance occurs with a protein encoded by the *mdr1* gene, P-glycoprotein [1,2], which actively transports multiple classes of natural products out of resistant cancer cells [3–5]. Overexpression of P-glycoprotein in some neoplasms correlates with poor outcome [6–10]. It is possible, therefore, that inhibition of the function of P-glycoprotein by chemosensitizers might improve the outcome of antineoplastic chemotherapy in patients with P-glycoprotein–overexpressing neoplasms. Cyclosporine A (CsA) has been shown to reverse multidrug resistance in cell cultures and animal experiments [11] and is tolerated by cancer patients in doses that effectively inhibit P-glycoprotein in vitro [12–18].

P-glycoprotein is also expressed in normal tissues such as the kidney, liver, adrenal cortex, bowel, hema



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Part of this work was presented as an oral communication at the 2nd Congress of the European Association for Clinical Pharmacology and Therapeutics [see Theis JGW, Chan HSL, Greenberg ML, et al. Chemotherapy combined with the p-glycoprotein inhibitor cyclosporin (CsA) in children: assessment of the systemic toxicity. Eur J Clin Pharmacol 1997;52(Suppl):A102] and one of its satellite symposia [see Theis JGW, Chan HSL, Greenberg ML, et al. Increased systemic toxicity of sarcoma chemotherapy due to combination with the P-glycoprotein inhibitor cyclosporine (extended abstract). Int J Clin Pharmacol Ther 1998;36:61–64].

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topoietic cells, and blood–brain barrier [19–21]. Inhibition of the P-glycoprotein drug efflux pump might, therefore, enhance the toxicity of chemotherapy in these tissues. Furthermore, concomitant therapy with CsA may reduce the clearance of certain drugs, including cytotoxins, resulting in higher tissue exposure and drug toxicity [14–16,22].

Pediatric patients with sarcomas overexpressing P-glycoprotein appear to have a worse prognosis than those with sarcomas not overexpressing P-glycoprotein [8,23]. Consequently, a study was initiated with children whose sarcomas overexpressed P-glycoprotein. This was designed to determine the maximal tolerated dose and the therapeutic and adverse effects of the chemosensitizer CsA given in conjunction with standard chemotherapy [24,25]. The study was approved in 1990 by the Research Ethics Board of The Hospital for Sick Children in Toronto.

Our study differs from most other studies of high-dose CsA in combination with chemotherapy with respect to the patient population, the stage and prognosis of the tumor, and the doses of the conventional antineoplastics. The patient population consisted exclusively of children. Their enrollment, including those with a potential for cure, to this protocol was justified 1) because of the generally poor prognosis of patients with sarcomas that overexpress P-glycoprotein and 2) by the biological plausibility that addition of a P-glycoprotein inhibitor might increase the chances for survival. In order not to deprive these patients of maximum therapeutic benefit, the dose of the conventional antineoplastics was not reduced when CsA was added. The observation that CsA can alter the metabolic clearance of cytotoxins [14–16] was not available at the inception of the study.

A safety committee was formed to ensure the well-being of children on this novel therapeutic approach during this study. The committee members were to conduct their investigations independently of the investigators who had conceived and were conducting the trial. The objectives of the safety committee were to analyze and evaluate the incidence, rate, and severity of documented toxicities. Those that occurred during and after chemotherapy cycles given in conjunction with CsA were to be matched to similar cycles given without CsA. This report details the apparent increase in systemic toxicities associated with the addition of high dose CsA to standard doses of chemotherapy.

## MATERIALS AND METHODS

### Design of the Phase I/II Trial

The basis of this toxicity analysis was a single-arm pilot trial of CsA given in conjunction with the chemotherapy cycles described below. CsA was started at 4 mg/kg/day, and individual doses were escalated by 1 mg/kg increments in cohorts of three patients each, according to the E.O.R.T.C. Guidelines [26] to establish the maximum tolerated dose. The trial used short infusions of CsA on each day of chemotherapy rather than the prolonged continuous infusions of CsA used in most of the trials for reversal of multidrug resistance that have been reported [12–18].

### Chemotherapy Cycles

Sarcoma patients were treated with VP16/IFOS cycles consisting of 1-hr infusions of etoposide (150 mg/m$^2$/day) on days 1 and 2 and of 3-hr infusions of ifosfamide (3,000 mg/m$^2$/day) on days 1 and 2, alternating every 3 weeks with 5-day VAC cycles consisting of bolus injections of vincristine (0.05 mg/kg/day on days 1 and 5), dactinomycin (15 μg/kg/day on days 1–5) and cyclophosphamide (300 mg/m$^2$/day on days 1–5) [27,28]. Trial patients received CsA as 3-hr infusions (1 hr before until 2 hr after days 1–5 VAC) or 5-hr infusions (1 hr before and 4 hr during days 1 and 2 VP16/IFOS), with antiemetics (metoclopramide, dimenhydrinate, granisetron, dexamethasone), antiallergics (hydrocortisone, diphenhydramine), and uroprotector therapy (hydration, mesna). VP16/IFOS and VAC cycles given to sarcoma patients without cotherapy with CsA during the same time period (1991–1994) at the same institution served as controls to evaluate the impact of CsA on efficacy and systemic toxicity of the antineoplastic therapy. These cycles without CsA were given to patients who elected not to participate in the open trial or who no longer received CsA with their antineoplastic therapy (see reasons for discontinuation in Results).

### Selection of Chemotherapy Cycles for Analysis

**Inclusion criteria.** For comparison of toxicity, all evaluable cycles that were administered between 1991 and 1994 and had delivered at least 50% of the protocol doses of each component of VAC or IFOS/VP16 were included.

**Exclusion criteria.** Cycles of chemotherapy that had been given with additional cytotoxins or during concurrent radiotherapy or within 7 days of completing radiation were excluded. We did not assess those patients who had received fewer than two evaluable cycles of chemotherapy. Cycles that had to be stopped or interrupted because of the occurrence of an acute allergic reaction to CsA were also excluded.

**Cycles included and excluded.** During 1991–1994, 36 patients with soft tissue sarcoma were treated in our institution. Twenty of these patients received at least one dose of intravenous CsA with standard chemotherapy. Overall, CsA was added to 118 chemotherapy cycles in these patients. Forty-three of these CsA cycles had to be excluded because the antineoplastic drugs used were different from the two standard chemotherapy regimens.

**TABLE I. Characteristics of Chemotherapy Cycles***

| | VAC cycles | | | VP16/IFOS cycles | | |
|---|---|---|---|---|---|---|
| | With CSA (n = 27) | Without CsA (n = 53) | *P* | With CSA (n = 34) | Without CsA (n = 84) | *P* |
| Cycle characteristics | | | | | | |
| Patient age (days) | 1,547 ± 1,105 | 2,935 ± 1,817 | <0.0001 | 1,623 ± 1,041 | 3,508 ± 2,075 | <0.0001 |
| Patient sex (male/female) | 8/19 | 20/33 | 0.6 | 18/16 | 22/62 | 0.01 |
| Stage (II/III/IV) | 0/11/16 | 2/41/10 | <0.0001 | 0/13/21 | 11/56/17 | <0.0001 |
| Radiotherapy prior (yes/no) | 5/22 | 25/28 | 0.02 | 7/27 | 36/48 | 0.04 |
| Time after last chemotherapy (days) | 25 ± 5 | 27 ± 11 | 0.7 | 26 ± 10 | 26 ± 8 | 0.3 |
| Time after first cycle (days) | 125 ± 77 | 178 ± 99 | 0.02 | 205 ± 314 | 246 ± 454 | 0.6 |
| Number of previous cycles | 5 ± 4 | 7 ± 4 | 0.1 | 7 ± 7 | 7 ± 6 | 0.5 |
| Basal blood counts | | | | | | |
| White blood cell count | 6.2 ± 2.2 | 5.5 ± 2.4 | 0.1 | 6.6 ± 4.1 | 6.0 ± 3.4 | 0.4 |
| Neutrophil count | 4.1 ± 1.9 | 3.4 ± 2.0 | 0.1 | 4.1 ± 3.8 | 3.6 ± 2.7 | 0.7 |
| Platelet count | 530 ± 201 | 527 ± 313 | 0.3 | 465 ± 225 | 410 ± 200 | 0.2 |
| Hemoglobin | 109 ± 10 | 109 ± 12 | 1 | 109 ± 11 | 116 ± 14 | 0.002 |
| Antineoplastic drug dose | | | | | | |
| Vincristine/kg | 0.09 ± 0.01 | 0.10 ± 0.02 | 0.3 | — | — | — |
| Dactinomycin/kg | 71 ± 5 | 70 ± 18 | 0.2 | — | — | — |
| Cyclophosphamide/$m^2$ | 1,442 ± 151 | 1,468 ± 204 | 0.3 | — | — | — |
| Etoposide/$m^2$ | — | — | — | 302 ± 8 | 301 ± 21 | 0.9 |
| Ifosfamide/$m^2$ | — | — | — | 6,016 ± 145 | 5,990 ± 378 | 0.7 |
| Cyclosporine dose (mg/kg/day) | 18 ± 6 | — | — | 19 ± 6 | — | — |

*Characteristics of the chemotherapy cycles; data are given per cycle. Numbers represent mean ± standard deviation or the absolute count.

We ultimately analyzed 34 of the 46 remaining cycles of VP16/IFOS with CsA, excluding another 10 cycles given with concomitant radiotherapy and two cycles associated with an acute hypersensitivity reaction, which we had reported previously [29]. We analyzed 27 of the 29 remaining cycles of VAC with CsA, excluding one cycle given with concomitant radiotherapy and one cycle associated with an acute hypersensitivity reaction to CsA. The median daily dose of CsA given with the evaluable VP16/IFOS and VAC cycles was 19.5 mg/kg (range 9–27) and 20 mg/kg (range 6–26), respectively. Eighty-four VP16/IFOS cycles and 53 VAC cycles given during the same period without CsA fitted the inclusion criteria and served as controls.

There were significant differences between study cycles and control cycles with regard to some of the characteristics of the cycles presented in Table I. Of note, patients who received chemotherapy with CsA tended to have more advanced disease than patients treated without CsA, perhaps reflecting that the expression of P-glycoprotein is more prevalent in patients with higher staged tumors [8]. Patients who received chemotherapy with CsA were also less likely to have received previous radiotherapy. Possibly this reflects the more disseminated disease of the P-glycoprotein–positive patients, which was not considered amenable to radiotherapy. Other differences may have occurred by chance.

## Toxicity Analysis

**Data collection.** Two data managers retrospectively collected the toxicity data from the hospital and clinic charts, laboratory results, and blood bank records. All data that were interpreted as a form of chemotherapy-related toxicity were associated with the preceding chemotherapy cycle. The data were verified by a member of the safety committee and the primary investigator of the trial. The medical records and the outpatient charts (in this order) were used as the ultimate source for any inconsistencies that were observed among the different sources of data.

**Toxicity grading.** Toxicity was graded according to the Childrens Cancer Group guidelines as grade 0 for absent, grade 1 for mild, grade 2 for moderate, grade 3 for severe, and grade 4 for unacceptable toxicity [28]. For each type of toxicity, the lowest assignable grade was assigned if the patient records contained insufficient information for more accurate grading.

**Data analysis.** VAC and VP16/IFOS cycles were separately stratified into those with and without CsA. Systemic toxicity associated with the addition of CsA was evaluated by comparing the incidence and severity of adverse events following cycles with CsA to those following cycles without CsA. Denominator for the incidences of various systemic toxic events was the number of VAC or VP16/IFOS chemotherapy cycles, not the number of patients.

The following outcomes were compared: hospital admissions necessary for treatment of chemotherapy-related complications (admission yes/no, duration of hospital stay because of toxicities, main toxicities/complications responsible for the necessity for inpatient therapy), delay in initiation of the next chemotherapy

**TABLE II. Toxicity-Related Admissions After Chemotherapy Cycles***

| | VAC cycles | | | VP16/IFOS cycles | | |
|---|---|---|---|---|---|---|
| | With CSA (n = 27) | Without CsA (n = 53) | *P* | With CSA (n = 34) | Without CsA (n = 84) | *P* |
| Admissions | | | | | | |
| Incidence | 25 (93%) | 21 (40%) | <0.0001 | 10 (29%) | 10 (12%) | 0.04 |
| Length of stay per admission (days) | 13 ± 7 | 7 ± 4 | <0.0001 | 6 ± 3 | 6 ± 4 | 0.6 |
| Reasons for admissions | | | | | | |
| Infections + fever | 25 (93%) | 21 (40%) | <0.0001 | 10 (29%) | 10 (12%) | 0.04 |
| Sepsis (culture-proved) | 10 (37%) | 0 | | 2 (6%) | 0 | |
| Pneumonia | 4 (15%) | 1 (2%) | | 0 | 2 (3%) | |
| Urinary tract infection | 4 (15%) | 1 (2%) | | 0 | 0 | |
| Cellulitis | 0 | 1 (2%) | | 0 | 1 (1%) | |
| Otitis media | 2 (7%) | 1 (2%) | | 2 (6%) | 1 (1%) | |
| Colitis clost. diff. | 0 | 1 (2%) | | 1 (3%) | 0 | |
| Liver abscess | 1 (4%) | 0 | | 0 | 0 | |
| Fever and neutropenia | 10 (37%) | 16 (30%) | | 5 (15%) | 6 (8%) | |
| Obstipation | 9 (33%) | 7 (13%) | 0.1 | 3 (9%) | 0 | 0.04 |
| Stomatitis | 24 (89%) | 17 (32%) | <0.0001 | 0 | 0 | — |
| Bleeding | 6 (22%) | 3 (6%) | 0.1 | 0 | 0 | — |
| Bone pain | 5 (19%) | 7 (13%) | 0.8 | 0 | 0 | — |

*Toxicity-related hospital admissions and reasons for these admissions. Numbers are given as totals and percentage of cycles given.

cycle of more than 28 days after initiation of the evaluated chemotherapy cycle, hematologic toxicities as recorded by blood counts and the incidence of needed platelet or red cell transfusions, and clinical toxicities as recorded in the charts. The fact that the data collection was retrospective led to concern regarding the accuracy of extracting clinical data such as paresthesia, constipation, and nausea. Particular emphasis was therefore given to definite and measurable indicators of clinically significant toxicity, such as hospital admissions for the treatment of systemic chemotherapy toxicity, documented septic episodes, delay of chemotherapy because of toxicity, or requirement for blood and platelet transfusions.

### Statistical Analysis

Statistical analyses were performed by $\chi^2$ test and Mann-Whitney rank sum test as appropriate, utilizing Sigma Stat for Windows (Jandel Corporation, San Rafael, CA). Multivariate analyses were performed using SPSS for Windows Release 6.1.2.

## RESULTS

### Hospital Admissions for Toxicity

VAC with CsA was followed by admission of 13 ± 7 days for chemotherapy complications in 25 of the 27 cycles (93%), 9 ± 2 days after the start of chemotherapy (Table II). Conversely, only 40% of VAC cycles without CsA ($P < 0.0001$) were followed by admissions of 7 ± 4 days ($P < 0.0001$) for chemotherapy-related complications, 10 ± 4 days after the start of chemotherapy.

VP16/IFOS cycles with CsA were followed by admissions of 6 ± 3 days for chemotherapy-related complications in 29% of the cycles, 9 ± 2 days after the start of chemotherapy (Table II). Conversely, VP16/IFOS cycles without CsA were followed by admissions of 6 ± 4 days (not significant) for chemotherapy-related complications in 12% of the cycles ($P = 0.04$), 8 ± 3 days after the start of chemotherapy.

Multivariate analyses using logistic regression and models that included patient age and sex, tumor stage, previous radiotherapy, number of previous chemotherapy cycles, time elapsed since the first chemotherapy cycle, and doses of the antineoplastics and CsA adjusted for weight or surface area were separately performed for VAC and VP16/IFOS cycles to elucidate whether any of these parameters predicted the occurrence of toxicity-related admissions. For VP16/IFOS cycles, tumor stage ($P = 0.002$) and history of radiotherapy ($P = 0.009$) predicted the necessity for hospital admissions. Patient age ($P = 0.08$) and CsA ($P = 0.12$) were not statistically significant in this analysis but were statistically significant ($P < 0.05$) when the method of stepwise logistic regression analysis was chosen. This indicates that other factors, such as tumor stage, prior radiotherapy, and age, are perhaps as important as or even more important than CsA in accounting for the hospital admissions after VP16/IFOS cycles. The admission rate, however, was higher (47%) in the cycles in which CsA dosages were higher ($P = 0.06$) than the median (19.5 mg/kg/day) compared to those cycles in which CsA dosages were lower than the median (12% admissions).

In the VAC cycles, CsA was the only predicting factor for the need for toxicity-related hospital admissions (*P*

**TABLE III. Hematologic Toxicities***

| | VAC cycles | | VP16/IFOS cycles | |
|---|---|---|---|---|
| | With CSA (n = 27) | Without CsA (n = 53) | With CSA (n = 34) | Without CsA (n = 84) |
| Hematological toxicities grade 4 | | | | |
| Any | 27 (100%) | 24 (45%) | 21 (62%) | 19 (23%) |
| White blood cells <1.0 cells/nl | 23 (85%) | 13 (25%) | 13 (38%) | 12 (14%) |
| Neutrophils <0.5 cells/nl | 26 (96%) | 23 (43%) | 20 (59%) | 18 (21%) |
| Platelet count <25 cells/nl | 20 (74%) | 7 (13%) | 3 (9%) | 1 (1%) |
| Cycles followed by transfusions | | | | |
| RBC transfusions | 18 (67%) | 10 (19%) | 9 (26%) | 7 (8%) |
| Platelet transfusions | 24 (89%) | 6 (11%) | 2 (6%) | 3 (4%) |

*Hematologic toxicities observed after chemotherapy and number of cycles followed by transfusions. Indicated is the incidence of diagnosed grade 4 hematologic toxicities and the incidence of transfusions given. The actual incidence may be higher because grade 4 toxicities might have been missed (for details see text), so statistics were not performed on these data. Hemoglobin never reached grade 4 because the patients were transfused before reaching the low hemoglobin concentrations required for diagnosis of grade 4 toxicity.

= 0.006). This result was confirmed by stepwise linear regression analysis, which resulted in the exclusive selection of CsA as a statistically significant predictor for the necessity for hospital admissions.

The main causes for hospital admissions were fever combined with neutropenia and documented infection (Table II). Ten of the twenty-seven VAC cycles with CsA (37%) compared to none of the VAC cycles without CsA were complicated by culture-proved sepsis ($P < 0.0001$). Two of the VP16/IFOS cycles with CsA were also complicated by documented sepsis but none of the non-CsA VP16/IFOS cycles. The organisms isolated included *Streptococcus* (5), *Staphylococcus epidermidis* (4), *Staphylococcus aureus* (1), and *Candida* (1) after VAC with CsA and *Staphylococcus epidermidis* (2) after VP16/IFOS with CsA. Other reasons for admission included stomatitis, ileus, bleeding, and paresthesia.

## Clinical Documentation of Toxicity

Overall, VP16/IFOS cycles with CsA were infrequently accompanied by higher grades of toxicity. Most prominent was gastrointestinal toxicity; severe gastrointestinal toxicities of grade 3 or 4 were documented in 9% of VP16/IFOS cycles with CsA and 2% of VP16/IFOS cycles without CsA (difference not significant). Severe clinical toxicities were more frequently documented in VAC cycles, predominantly in VAC cycles with CsA. Severe grade 3 or 4 gastrointestinal toxicity was documented in 33% of VAC cycles with CsA and 8% without CsA ($P = 0.008$). Severe paresthesia was observed in 11% of VAC cycles with CsA and 4% of those without CsA (not significant). Inability to administer the subsequent chemotherapy cycle is also an indicator for systemic toxicity. The subsequent chemotherapy was delayed after 22% of the VAC cycles with CsA compared to 4% of the VAC cycles without CsA ($P = 0.03$). No differences were observed in delay of the subsequent chemotherapy cycle after VP16/IFOS cycles (6% with CsA vs. 8% without CsA).

## Hematologic Toxicities

Grade 4 hematologic toxicity was recorded following 100% of the VAC cycles with CsA compared to 45% of those without CsA and after 62% of the VP16/IFOS cycles with CsA compared to 23% of those without CsA (Table III). However, few of the cycles without CsA were followed by three complete blood counts between days 7 and 18 after chemotherapy. Therefore, documentation of grade 4 hematologic toxicity is incomplete, and the true frequency of grade 4 toxicities may be underestimated. Because of this uncertainty, statistical analyses were not performed on these data. An increase in clinically significant grade 4 hematologic toxicity with CsA could be inferred from the increased requirement for blood and platelet transfusions after VAC cycles with CsA and for blood transfusions but not platelet transfusions after the VP16/IFOS cycles with CsA (Table III). It cannot be excluded that individuals of the non-CsA group may have been undertransfused; fewer blood counts that could have documented anemia were recorded from this group.

## Reasons for Discontinuation of CsA

During this trial 20 patients received chemotherapy cycles with CsA. Only four of these patients completed the intended course of chemotherapy cycles with CsA; one of these petients died of sepsis and multiorgan failure after the last VAC cycle with CsA. Six patients received either no further chemotherapy cycles with CsA or no further VAC cycles with CsA because of objective or subjective toxicity or discomfort during prior cycles with CsA. Three patients did not receive any further CsA infusions after having experienced various degrees of hypersensitivity reactions to CsA (details are presented in an earlier report [29]), and three patients did not receive

further chemotherapy because of lack of response to prior chemotherapy cycles. Four patients had a relapsed tumor at the time when chemotherapy with CsA was started. Among these patients, one died of the disease after two chemotherapy cycles, one had the chemotherapy discontinued because of poor general status, and one died of severe cardiomyopathy and multiorgan failure after the last cycle. One patient received further treatment at a different hospital.

## DISCUSSION

Our data indicate that the addition of the chemosensitizer CsA during sarcoma chemotherapy greatly enhances the systemic toxicity of VAC cycles but only mildly increases that of VP16/IFOS cycles. This evaluation is based mainly on quantifiable objective data such as the incidence and duration of toxicity-related hospital admissions, the incidence of documented episodes of sepsis, and hematologic data.

Hospital admissions as a marker for toxicity carry the advantage that they can be regarded as a relevant marker for clinical toxicity that is severe enough to require inpatient treatment. Additional days of hospitalization for the treatment of chemotherapy-related complications are a clear indicator of additional suffering for the child. Moreover, additional days in hospital translate into an economic burden for both the patient and society. Taking these considerations into account, the difference between an average of 12 days of hospitalization for the treatment of chemotherapy-related toxicity after each VAC cycle with CsA and only 3 days after VAC cycles without CsA becomes even more impressive.

Hospital admissions as a marker of toxicity in a nonblinded trial, however, carry the disadvantage that the threshold for admissions may be lower for study patients compared to routine (control) patients. However, all hospital admissions in this trial were necessitated by objective indications for inpatient treatment, predominantly infections and fever with neutropenia and, particularly in the case of VAC cycles, sepsis and severe stomatitis. The effect of CsA on the incidence of sepsis after VAC cycles, causing a rise from 0% to 37%, is particularly disturbing considering the fact that infections in the immunocompromised patient are life-threatening complications.

Grade 4 hematologic toxicities were documented in significantly more chemotherapy cycles with CsA than cycles without CsA, but it has to be acknowledged that grade 4 hematologic toxicity can be missed if blood counts are not performed regularly and that the number detected may underestimate the true incidence. Another way to determine clinically significant hematologic toxicity is to analyze the need for transfusions. By using this approach, the enhancement of hematological toxicity by CsA is unequivocally documented by the greatly increased need for platelet and blood transfusions in VAC cycles with CsA and the increased requirement for blood transfusions in VP16/IFOS cycles with CsA.

The noticeable differences in various baseline characteristics between the cycles with and without CsA raises the possibility that factors other than CsA may be responsible for the increased toxicity. We addressed this by conducting multivariate analyses for the most prominent outcome, the need for hospital admission. In the VP16/IFOS cycles, it became obvious that CsA cotherapy was only one predicting factor among others, such as patient age, tumor stage, and positive history of prior radiotherapy. However, CsA dose and the duration of toxicity-related hospital stay were positively correlated. Moreover, the incidence of toxicity-related admissions was greater when the CsA doses were higher than their median. This implies that CsA is at least in part responsible for the mild increase in the systemic toxicity of VP16/IFOS cycles. In contrast, for VAC cycles, the multivariate analysis left no doubt that CsA is the most important predicting factor for toxicity-related hospital admissions.

It is important to note that the true magnitude of added risks of adverse effects caused by the addition of CsA to chemotherapy may have been attenuated by selection bias, a possible confounder that cannot be addressed by multivariate analysis. Most of the patients were taken off CsA or had their VAC cycles changed to other drug combinations before the course of treatment was completed, some because of serious drug-related toxicities. This may have resulted in a selection of patients who were less susceptible to systemic toxicity receiving more CsA-containing cycles. Similarly, the patients who were more susceptible to systemic toxicities were more likely to receive cycles without CsA. This selection may have resulted in underestimation of the true differences between study and control cycles.

Another factor possibly blunting the increase in the risk of systemic toxicity brought about by high-dose CsA cotreatment is the nature of the study involving CsA dose escalation. Averaging the documented toxicities of all chemotherapy cycles with CsA regardless of the dose may underestimate the effects caused by the highest and final dose of CsA if these effects are dose-related. Such a dose-dependent effect may exist, insofar as VP16/IFOS cycles were more often followed by toxicity-related admissions at higher doses. A similar dose relationship was not observed with VAC cycles, possibly because VAC cycles with CsA almost always led to toxicity-related admissions.

Although our analysis demonstrates that CsA increases the systemic toxicity of VP16/IFOS and particularly VAC cycles, it cannot elucidate the mechanisms underlying this phenomenon. The increased incidence of severe systemic toxicities may be due to adverse effects

directly attributable to CsA itself, due to modulation of the pharmacokinetics or pharmacodynamics of the antineoplastic agents by CsA, or due to a combination of these two mechanisms.

As demonstrated by a number of recent studies (for review see Fisher and Sikic [30]), inhibition of P-glycoprotein markedly increases the area under the concentration-time curve (AUC) of those antineoplastic drugs that are substrates of P-glycoprotein. Three of the antineoplastics employed in this study, etoposide, vincristine, and dactinomycin, are known to be substrates of P-glycoprotein [11]. It has to be assumed that the AUCs of these drugs were substantially increased in the presence of CsA, thus increasing systemic exposure to these agents. Increased exposure of healthy tissues to these cytotoxins may be sufficient to explain the increased systemic toxicity of the treatment courses in the presence of the chemosensitizer CsA.

The reason for adding CsA to chemotherapy was based on its inhibition of P-glycoprotein. CsA, a substrate of the cytochrome P450 3A system [31], however, has the effect of inhibiting this enzyme [32], especially at high doses. Inhibition of cytochrome P450 3A by high-dose CsA cotherapy is likely to add to the pharmacokinetic interactions between CsA and the antineoplastics. Such an interaction leads us to the following speculation regarding the differing effects of CsA on VP16/IFOS cycles (only a mild increase in systemic toxicity) and on VAC cycles (a large increase in systemic toxicity). VP16/IFOS and VAC cycles both contain alkylating agents, ifosfamide and cyclophosphamide, that require activation through hydroxylation to become cytotoxic. Interestingly, this activation has been reported to be carried out by different cytochrome P450 enzymes, cytochrome P450 3A activating ifosfamide and cytochrome 450 2B activating cyclophosphamide 33. CsA inhibits cytochrome P450 3A but not 2B, consequently the activation of ifosfamide may be inhibited, whereas that of cyclophosphamide is not. The possibly reduced concentration of the active metabolite of ifosfamide may, therefore, clinically balance the increased AUC of etoposide resulting from P-glycoprotein inhibition. this could explain the only mild increase in toxicity after VP16/IFOS cycles. These potential mechanisms will have to be proved by future studies.

The fact that the total CsA dose was 150% higher during the VAC cycles than during the VP16/IFOS cycles might also have contributed to the severe increase in toxicity in VAC cycles vs. the only mild increase in toxicity in VP16/IFOS cycles. The difference in total CsA dose resulted from the fact that VAC cycles consisted of 5 days of chemotherapy, each accompanied by CSA infusions, whereas VP16/IFOS cycles only lasted for 2 days each accompanied by CsA.

Most of the severe adverse events observed in this study were related to infections and hematologic abnormalities. At present it remains unclear whether CsA with its immunosuppressive properties [34] and its direct effects on vascular endothelium [35] and platelets [36] might have contributed to these toxicities. In addition to CsA, hydrocortisone or dexamethasone may also have contributed to the increased risk of infections. This is because these corticosteroids were given only prior to CsA infusions to prevent the occurrence of hypersensitivity reactions. Although a single dose of corticosteroids may not be considered to have a major effect, it should be noted that the subsequent CsA infusion is likely to increase the steroid effects by interfering with their elimination [37–39].

Only future studies utilizing different chemosensitizers with the same potency for the inhibition of P-glycoprotein will be able to elucidate the true mechanisms leading to possibly improved antineoplastic effectiveness without increased systemic toxicity. Such studies would have to incorporate doses of antineoplastic drugs resulting in AUCs of the active drug or metabolite similar to those in the absence of any chemosensitizers. It is imperative that such future studies be prospectively monitored for serious systemic toxicity.

## CONCLUSIONS

Addition of the chemosensitizer CsA during sarcoma chemotherapy greatly enhances the systemic toxicity of VAC cycles but only mildly increases the systemic toxicity of VP16/IFOS cycles. Future trials involving P-glycoprotein inhibition will have to find a balance between maximum achievable antineoplastic activity and protection of the patient from excessive systemic toxicity. This can be achieved only if systemic toxicity and effectiveness are carefully and prospectively monitored and the doses of the chemotherapy and/or the P-glycoprotein inhibitor are adjusted accordingly.

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