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Commentary

Multidrug Resistance Reversal in Childhood Malignancies—Potential for a Real Step Forward?

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WITH FEW exceptions, the 5-year survival and probable cure rate for cancers in childhood have plateaued over the past decade. The last major advance in terms of new chemotherapy came with the introduction of cisplatin and the epipodophyllotoxins. The impact of these agents on cure rates in paediatric germ cell tumours [1], hepatoblastoma [2] and neuroblastoma [3] have been clear, but the basic combinations of alkylating agent with vincristine, actinomycin and anthracyclines remain the cornerstone of most solid tumour chemotherapy. The introduction of new analogues with differing spectra of toxicity may have influenced early and late toxicity, but have had limited impact on survival rates.

The main cause of treatment failure is either initial failure to respond (comparatively unusual in paediatric tumours), incomplete initial response with an inability to achieve local control using surgery or radiotherapy, or recurrence of disease after an initial complete clinical response. It remains unclear to what extent the chemoresistant clone, which causes incomplete response or results in disease recurrence, is a primary phenomenon or is due to induction of resistance following drug exposure.

Drug resistance is a multifactorial phenomenon, and it is unlikely that any one single strategy to circumvent drug resistance will be adequate in all cases. Over the last few years, multidrug resistance (MDR) has emerged as a major identifiable cause of drug resistance in a wide range of cell lines and tumour types, and for once it appears to be a mechanism which is amenable to effective manipulation both *in vitro* and *in vivo*.

There are now data indicating that the MDR phenotype, as defined by either overexpression of the *MDR-1* gene or the presence of p-glycoprotein, may be of relevance in childhood cancer. Demonstration of MDR in leukaemia, rhabdomyosarcoma, neuroblastoma, retinoblastoma and Ewing's sarcoma has been associated with adverse prognosis [4–7]. Unfortunately, the data for some of these tumours are somewhat conflicting, at least in part, owing to differences in the method of determination of MDR. In particular, studies in rhabdomyosarcoma and neuroblastoma have produced differing conclusions [10, 11].

Classical MDR involves a 170 kDa molecular weight transmembrane p-glycoprotein pump (Pgp), which causes drug efflux from the cell. The demonstration that this efflux could be reversed by a variety of compounds led to interest in their clinical potential [12]. The most widely studied reversal agents are verapamil and its isomer, dexverapamil, cyclosporin A and its analogue PSC833. A wide range of other agents have also been shown to inhibit drug efflux as shown in Table 1. The mechanism by which efflux is affected may be different for the various agents. Competitive inhibition for the energy-dependent drug transport mechanism may be involved with both verapamil and the cyclosporins. It is probable, however,

Table 1. Drugs with ability to reverse multidrug resistance (MDR) in vitro

Calcium channel blockers
Verapamil
Diltiazem
Nifedipine
Nicardipine
Cardiovascular drugs
Diyridamole
Quinidine
Steroids
Tamoxifen
Progesterone
Antibiotics
Cefoperazone
Ceftriaxone
Cyclosporines
Cyclosporin A
PSC833
Calmodulin inhibitors
Fluphenazine
Trifuoperazine
Antimalarials
Quinicrine
Quinine

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that a direct cell membrane effect by cyclosporin influences one of several mechanisms for drug translocation via Pgp. The so-called "flippase" theory involves direct transfer of drug across the lipid bilayer with lateral efflux via Pgp [13]. The first part of the mechanism could be altered by the membrane effect of cyclosporin. *In vitro*, PSC833 is approximately 10-fold more effective than cyclosporin A and, as discussed later, its lesser toxicity makes it an attractive compound for investigations *in vivo*.

Most clinical studies evaluating the potential of MDR reversal have involved adults with myeloma. Both verapamil and cyclosporin A have been shown to reverse resistance to VAD chemotherapy [14, 15]. Recently, dexverapamil has been evaluated in renal carcinoma [16] and lymphoma [17]. In paediatric practice, early studies showed that a combination of verapamil and vinblastine was efficacious [18], and a recent study using verapamil in combination with etoposide has also indicated a beneficial effect in etoposide-resistant or refractory tumours [19]. Provisional results using cyclosporin A in sarcomas and retinoblastoma have been encouraging [20, 21], although to date no major studies have been published.

When first introduced into clinical practice, it was clear that the cardiac toxicity of verapamil would be a major limiting factor in its general use. Levels *in vitro* which were necessary for effective MDR reversal were many times higher than those used in cardiac practice [22, 23]. Doses administered to achieve concentrations >1000 ng/ml in adults necessitated admission to an intensive care unit and required careful monitoring, with a need for cardiac supportive drugs in many cases. However, it is apparent from some studies that effective MDR reversal can be achieved with much lower levels of verapamil. In the Royal Marsden paediatric verapamil trial, a dose which was predicted to achieve levels around 1000 ng or less was used (0.1 mg/kg loading dose, 0.15 mg/kg/h for 3 days). The children required only limited monitoring with regular blood pressure and heart rate measurement. In the event of bradycardia or demonstrable heart block, the drug was stopped. Rapid recovery of heart rate was apparent in all cases and in no child was inotrope support required. In this study, no correlation was evident between the levels of verapamil achieved and efficacy [19].

In adult studies with cyclosporin A, target drug levels have also been based on *in vitro* data, which suggest that around 2000–3000 ng/ml are necessary. This is associated with significant hepatic and renal toxicity, although in the majority of cases this is reversible. The latter toxicity appears to be more marked where a prolonged continuous infusion schedule is used. Provisional data from Chan and associates indicate that a short 3-h infusion with very high levels for a short period is better tolerated [20]. Results at the Royal Marsden have confirmed this, and the incidence of liver and renal toxicity appear to be less marked with a 3-h infusion of 30 mg/kg daily × 3 compared to the more standard loading dose of 5 mg/kg followed by a continuous infusion of 15 mg/kg/day × 3. However, the short infusion may lead to earlier and more frequent sensitivity reactions, in part, related to the cremaphor diluent of cyclosporin. This may necessitate prolongation of the infusion to 6 h.

The demonstration that the cyclosporin analogue, PSC833, is considerably more effective at reversing MDR *in vitro* [24] has led to phase I and II studies using this compound [25–27]. The absence of renal toxicity and less liver toxicity has been confirmed in dose ranging studies in adults. The dose limiting toxicity of this agent appears to involve the central nervous system, and acute cerebellar signs have been observed. A dose ranging study with this compound in paediatric tumours, combined with etoposide, will be initiated in the near future.

An additional complication with MDR reversal agents is the effect on the pharmacokinetics of the chemotherapy agent with which it is combined [28]. There are conflicting data on the effect of verapamil on anthracycline kinetics [29], but the effect of cyclosporin on both anthracycline [30, 31] and etoposide [32] kinetics is clear. The mechanism of the latter may be due to direct inhibition of drug elimination from the liver via the biliary excretion mechansim. Bile transport is Pgp mediated, and, therefore, an effective degree of reversal might be expected to reduce hepatic clearance and increase blood levels. Moreover, the direct hepatotoxic effect of high doses of cyclosporin could also reduce hepatic clearance of the cytotoxic agent. Studies with daunorubicin and etoposide have shown a significant elevation in the area under the drug concentration curve (AUC) when combined with high dose cyclosporin. Animal data indicate that other drugs, such as vincristine, are also affected [33], and there is clinical experience indicating a similar phenomenon is observed with vincristine and actinomycin D, where clinical toxicity is increased, although detailed pharmacokinetics have not been described [34]. In most adult studies, involving either daunorubicin or etoposide, the dose of these drugs has been reduced by 50% when combined with high dose cyclosporin. It is probably wise that the same dose reduction is made with vincristine and actinomycin D. Verapamil may also influence tumour blood flow which further complicates evaluation of efficiacy.

Currently, the studies in paediatric tumours remain preliminary. In the Royal Marsden verapamil trial, a standard dose of verapamil and etoposide were used irrespective of prior chemotherapy. Although patients had previously received etoposide, not all responders had progressed or failed to respond to etoposide given at the same dose or schedule. A similar reservation must be applied to the earlier data by Cairo and colleagues, where a combination of etoposide and vinblastine was used. Details of Chan and associates' data with cyclosporin remain unpublished, and it is unclear whether patients were refractory to the agents subsequently combined with cyclosporin.

There is, however, sufficient evidence supporting the potential impact of MDR reversal, and it is important to proceed to randomised phase III trials as soon as possible. There has been a tendency to hold back from this until the ideal MDR reversal agent becomes available. The toxicity of both verapamil and cyclosporin A made these unattractive contenders. Once the planned dose finding paediatric study of PSC833 (an Anglo-French collaboration) is completed, it would be appropriate to proceed to such phase III studies. Detailed pharmacokinetic studies of etoposide with and without PSC833 will be performed and, where possible, MDR status will be documented in any tumour.

Ideally, any study addressing the issue of MDR reversal should also include documentation of MDR in the tumour. This issue has been bedevilled by the difficulty in standardising methods of MDR estimation. Pgp may be detected using a range of antibodies directed at its internal and external epitopes. The ability to use these antibodies in fixed tissue remains controversial, and frozen tissue from original biopsies is less readily available. The latter also restricts the use of

molecular methods, such as Northern analysis for mRNA, RT-PCR for low levels of mRNA or mRNA *in situ* hybridisation. Moreover, the most appropriate method of assessing Pgp may be functional assays evaluating efflux of marker drugs, such as rhodamine or the inherently fluorescent anthracyclines. Whilst this may be suitable for cell lines, studies in solid tumours are more difficult.

The small number of randomised studies in adults of MDR reversal have been disappointing. In small cell lung cancer and myeloma, combinations of chemotherapy with verapamil have failed to show convincing benefit [35, 36]. A single study in non-small cell lung cancer has shown a significantly higher response and progression-free survival in patients given verapamil [37]. The inherently greater chemosensitivity of many paediatric tumours has led to a benefit from dose escalation, more marked than seen in adult cancers, and this may result in a greater benefit from MDR reversal strategies.

A range of novel techniques to influence Pgp function are currently under evaluation. Antisense RNA or ribozyme may prevent *MDR1* gene expression and a direct effect of anti-Pgp antibody has been demonstrated. Finally, it may be possible to harness Pgp as a target for prodrug therapy in which the activating enzyme for the prodrug would be targeted using monoclonal antibody.

With the exception of reversible jaundice, there is little evidence that the level of Pgp in normal tissues results in significant toxicity, whereas inhibition of Pgp could increase intracellular drug concentrations in the tumour several fold. In acute myeloid leukaemia, Ewing's sarcoma and node positive rhabdomyosarcoma, a 2- to 3-fold dose escalation has produced improvements in outcome. We await the impact of the new molecular therapies for childhood cancers, but, until that time, approximately 40% of children with cancer will fail treatment. MDR reversal may allow 'tumour directed' dose escalation to levels not attainable by other means, and we should not be slow to attempt to exploit it.

1. Pinkerton CR, Pritchard J, Spitz L. High complete response rate in children with advanced germ cell tumors using cisplatin-containing combination chemotherapy. *J Clin Oncol* 1986, **4**, 194–199.
2. Perilongo G, Plaschkes J, Brown J, *et al.* Response of hepatoblastoma (HB) to pre-operative chemotherapy with cisplatin (CPDD) and doxorubicin (Doxo) PLADO in the International Society of Paediatric Oncology (SIOP) Liver Tumour Study. *Proc Am Soc Clin Oncol* 1995, **14**, 444.
3. Shafford EA, Rogers DW, Pritchard J. Advanced neuroblastoma: improved response rate using a multiagent regimen (OPEC) including sequential cisplatin and VM-26. *J Clin Oncol* 1984, **2**, 742–747.
4. Tishler DM, Weinberg KI, Sender LS, Nolta JA, Raffel C. Multidrug resistance gene expression in paediatric primitive neuroectodermal tumours of the central nervous system. *J Neurosurg* 1992, **76**, 507–512.
5. Roessner A, Ueda Y, Bockhorn-Dworniczak B, *et al.* Prognostic implication of immunodetection of P glycoprotein in Ewing's sarcoma. *J Cancer Res Clin Oncol* 1993, **119**, 185–189.
6. Chan HSL, Thorner PS, Haddad G, Ling V. Immunohistochemical detection of P glycoprotein: prognostic correlation in soft tissue sarcoma of childhood. *J Clin Oncol* 1990, **8**, 689–704.
7. Chan HSL, Haddad G, Thorner PS, *et al.* P glycoprotein expression as a predictor of the outcome of therapy for neuroblastoma. *N Engl J Med* 1991, **325**, 1608–1614.
8. Brophy NA, Marie JP, Rojas VA, *et al.* MDR 1 gene expression in childhood acute lymphoblastic leukemias and lymphomas: a critical evaluation by four techniques. *Leukemia* 1994, **8**, 327–335.
9. Bernard J, Bourhis J, de Vathaire F, *et al.* Prognostic value of MDR1 gene expression in neuroblastoma: results of a multivariate analysis. *Adv Neuroblastoma Res* 1994, **4**, 111–116.
10. Kuttesch J, Parham D, Luo X, *et al.* P glycoprotein (P-gp) expression at diagnosis is not predictive of worse outcome in pediatric rhabdomyosarcoma (RMS). *Proc Am Soc Clin Oncol* 1994, **13**, 1409.
11. Favrot M, Combaret V, Goillot E, *et al.* Expression of P glycoprotein restricted to normal cells in neuroblastoma biopsies. *Br J Cancer* 1991, **64**, 233–238.
12. Leyland-Jones B, Dalton W, Fisher G, Sikic BI. Reversal of multidrug resistance to cancer chemotherapy. *Cancer* 1993, **72**, 3484–3488.
13. Higgins CF, Gottesman MM. Is the multidrug transporter a flippase? *Trends Biochem Sci* 1992, **17**, 18–21.
14. Dalton WS, Grogan TM, Meltzer PS, *et al.* Drug resistance in multiple myeloma and non-Hodgkin's lymphoma: detection of P glycoprotein and potential circumvention by addition of verapamil to chemotherapy. *J Clin Oncol* 1989, **7**, 415–424.
15. Sonneveld P, Durie BGM, Lokhorst HM, *et al.* Modulation of multidrug resistance multiple myeloma by cyclosporin. *Lancet* 1992, **340**, 255–259.
16. Motzer RJ, Lyn P, Fischer P, *et al.* Phase I/II trial of dexverapamil plus vinblastine for patients with advanced renal cell carcinoma. *J Clin Oncol* 1995, **8**, 1958–1965.
17. Wilson WH, Bates SE, Fojo A, *et al.* Controlled trial of dexverapamil, a modulator of multidrug resistance, in lymphomas refractory to EPOCH chemotherapy. *J Clin Oncol* 1995, **13**, 1995–2004.
18. Cairo MS, Siegel S, Anas N, Sender L. Clinical trial of continuous infusion verapamil, bolus vinblastine, and continuous infusion VP-16 in drug resistant pediatric tumours. *Cancer Res* 1989, **49**, 1063–1066.
19. Cowie FJ, Pinkerton CR, Phillips M, *et al.* Continuous-infusion verapamil with etoposide in relapsed or resistant paediatric cancers. *Br J Cancer* 1995, **71**, 877–881.
20. Chan HSL, Koren G, Thorner PS, *et al.* Reversal of multidrug resistance (MDR) in retinoblastoma (RB) by cyclosporin A (CsA). *Proc Am Assoc Clin Oncol* 1992, **ii**, 375.
21. Chan HSL, Thorner PS, Weitzman S, *et al.* Cyclosporin A for reversal of multidrug resistance in childhood malignancies. *Proc Am Assoc Cancer Res* 1992, **33**, 478.
22. Ozols RF, Cunnion RE, Klecker RW, *et al.* Verapamil and adriamycin in the treatment of drug resistant ovarian cancer patients. *J Clin Oncol* 1987, **5**, 641–647.
23. Salmon S, Dalton WS, Grogan TM, *et al.* Multidrug resistant myeloma: laboratory and clinical effects of verapamil as a chemosensitizer. *Blood* 1991, **78**, 44–50.
24. Friche E, Jensen PB, Nissen NI. Comparison of cyclosporin A and SDZ PSC833 as multidrug-resistance modulators in a daunorubicin-resistant Ehrlich ascites tumor. *Cancer Chemother Pharmacol* 1992, **30**, 235–237.
25. Fisher GA, Hausdorff J, Lum BL, *et al.* Phase I trial of etoposide with the cyclosporin SDZ PSC 833, a modulator of multidrug resistance/MDR. *Proc Am Soc Clin Oncol* 1994, **13**, 368.
26. Fisher GA, Halsey J, Hausdorff J, *et al.* A phase I study of paclitaxal in combination with SDZ PSC 833, a potent modulator of multidrug resistance (MDR). *Anti-Cancer Drugs* 1994, **5**, 43.
27. Sonneveld P, Marie JP, Lokhorst HM, Nooter K, Schoester M. Clinical modulation of multidrug resistance in VAD refractory multiple myeloma: studies with cyclosporin and SDZ PSC833. *Anti-Cancer Drugs* 1994, **5**, 72.
28. McLeod HL. Clinical reversal of the multidrug resistance phenotype: true tumour modulation or pharmacokinetic interaction. *Eur J Cancer* 1994, **30**, 2039–2041.
29. Gigante M, Toffoli G, Boiocchi M. Pharmacokinetics of doxorubicin co-administered with high-dose verapamil. *Br J Cancer* 1995, **71**, 134–136.
30. Bartlett NL, Lum BL, Fisher GA, *et al.* Phase I trial of doxorubicin with cyclosporin as a modulator of multidrug resistance. *J Clin Oncol* 1994, **12**, 835 842.
31. Rushing DA, Raber SR, Rodvold KA, Piscitelli SC, Plank GS, Tewksbury DA. The effects of cyclosporin on the pharmacokinetics of doxorubicin in patients with small cell lung cancer. *Cancer* 1994, **74**, 834–841.
32. Yahanda AM, Adler KM, Fisher GA. Phase I trial of etoposide

with cyclopsorin as a modulator of multidrug resistance. *J Clin Oncol* 1992, **10**, 1624–1634.
33. Horton JK, Thimmaiah KN, Houghton JA, *et al.* Modulation by verapamil of vincristine pharmacokinetics and toxicity in mice bearing human tumor xenografts. *Biochem Pharmacol* 1989, **38**, 1727–1736.
34. Cowie F, Pinkerton CR. Enhanced toxicity of dactinomycin and vincristine by cyclosporin given to reverse multidrug resistance. *J Clin Oncol* 1994, **12**, 1998–1999.
35. Milroy R. A randomised clinical study of verapamil in addition to combination chemotherapy in small cell lung cancer. *Cancer* 1993, **68**, 813–818.
36. Dalton WS, Crowley JJ, Salmon SS, *et al.* A phase III randomized study of oral verapamil as a chemosensitizer to reverse drug resistance in patients with refractory myeloma. *Cancer* 1995, **75**, 815–820.
37. Millward MJ, Cantwell BMJ, Munro NC, Robinson A, Corris PA, Harris AL. Oral verapamil with chemotherapy for advanced non small cell lung cancer: a randomised study. *Br J Cancer* 1993, **67**, 1031–1035.