Anti-Cancer Drugs 1995, **6**, pp. 736–743

Cardiotoxicity in the SCID mouse following administration of doxorubicin and cyclosporin A

WT Bellamy, YM Peng, A Odeleye, L Ellsworth, MJ Xu, TM Grogan and RS Weinstein

Department of Pathology and Arizona Cancer Center, University of Arizona, 1501N Campbell Avenue, Tucson, AZ 85724, USA. Tel: (+1) 520 626-6032; Fax: (+1) 520 626-1027.

Multiple myeloma is a plasma cell malignancy which is generally incurable in spite of a high initial response to chemotherapy. Relapsing disease commonly heralds an increase in the incidence of drug resistance which is often mediated by the product of the *MDR*-1 gene, P-glycoprotein (Pgp). One approach to modulating drug resistance due to Pgp overexpression has involved the use of agents known as chemomodulators which inhibit its function. We have developed a human xenograft model of multiple myeloma using the SCID mouse to evaluate the efficacy and toxicities of new MDR-1 chemomodulators. Cyclosporin A (CsA) is a widely used immunosuppressant which has been demonstrated to be a potent inhibitor of Pgp *in vitro* at concentrations which are clinically achievable. Preliminary studies revealed an acute toxicity in our SCID model which was associated with the combination of CsA and doxorubicin, and which was not observed with either drug alone, nor with cremaphor, the vehicle for CsA. In the current study, non-tumor bearing SCID mice were dosed with doxorubicin or the combination of doxorubicin with cremaphor, verapamil or CsA. Animals were sacrificed and tissues harvested for morphologic examination and for HPLC analysis of doxorubicin levels. In all tissues examined, there was a marked increase in tissue levels of doxorubicin when combined with CsA. Results also revealed a higher incidence and severity of myocardial damage in those animals receiving the combination of doxorubicin and CsA than in those receiving other combinations. The elevations in tissue levels observed with doxorubicin and CsA may contribute to the acute toxicities observed in the SCID mouse model.

Key words: MDR, multiple myeloma, SCID mouse.

Introduction

Multiple myeloma is a plasma cell malignancy which is generally incurable in spite of a high initial response to chemotherapy. Relapsing disease commonly heralds an increase in the incidence of drug resistance. Prior studies indicate that terminal drug resistant myeloma commonly expresses P-glycoprotein (Pgp).[1–4] The Pgp is a putative cellular efflux pump encoded by the *MDR*-1 gene and is frequently overexpressed in chemotherapy-refractory cancers in the clinic.[5] One approach to modulating drug resistance due to Pgp overexpression has involved the use of agents known as chemomodulators which inhibit its function. *In vitro* and *in vivo* studies have demonstrated the efficacy of compounds such as verapamil and cyclosporin A (CsA) to reverse Pgp-mediated multidrug resistance when combined with antineoplastic agents.[6,7] Such findings have led to the initiation of clinical trials, particularly in hematological malignancies, utilizing such an approach.[8–12] Initial results have revealed the need, however, for more potent chemomodulators. Confounding the development of new chemomodulators is the observation that Pgp is expressed in normal tissues including the kidney, adrenal gland, liver and endothelial cells lining the capillaries of the brain.[13–15] The effects of new chemomodulators on these tissues is of great importance because of the possibility of increased toxicities as a consequence of enhanced tissue uptake or altered tissue distributions of either the chemomodulator or the chemotherapeutic agents.[16–20]

We have established a reproducible *in vivo* model of human multiple myeloma in the SCID mouse to assess new chemomodulators.[21,22] When we examined the combination of doxorubicin and CsA in this model, we observed an acute toxicity which was characterized by death of the animals within 24–48 h following the second dose of a planned three-dose regimen.[23] This toxicity was associated only with the combination of CsA and doxorubicin, and was not observed with either drug alone nor with other combinations studied. The current study was undertaken to determine if the acute toxicity observed in the SCID mice was the result of elevated tissue levels of doxorubicin.

This work was supported in part by National Cancer Institute grants CA57228, CA23074, ES06694 and The Phoenix Friends of the Arizona Cancer Center.

Correspondence to WT Bellamy

Materials and methods

Animals

BALBc/C.17 mice homozygous for the SCID defect (*scid/scid*) were bred and maintained in a dedicated facility at the Arizona Health Science Center. Principles of animal care as set forth in NIH publication no. 85-23, rev. 1985, were followed through out. Adult 6–8 week old (20–22 g) male and female animals were used for our studies. The animals were housed in microisolator cages under specific pathogen-free conditions and were handled in a laminar flow food. The animals were fed LM45 5% fat, autoclavable pellets (Tekland Premier, Madison, WI) and given autoclaved water supplemented with antibiotics. Mice were screened at regular intervals for the presence of bacteria, Sendai virus, mouse hepatitis virus and mycoplasma. All mice were evaluated for the presence of mouse IgG by ELISA assay and only those animals with 1 mg/l or below of mouse Ig were used.

Drugs

Doxorubicin, cremaphore and verapamil were obtained from Sigma (St Louis, MO), while CsA (Sandimmune) was obtained from Sandoz (East Hanover, NJ). With the exception of CsA and cremaphor, all drugs were dissolved in sterile water for injection. Drug concentrations were adjusted with 0.9% saline so that a maximum volume of 0.2 ml was injected per animal.

Drug administration

SCID mice were dosed with the combination of doxorubicin (1.5 mg/kg, i.p.) and either CsA (50 mg/kg, i.m.), cremaphor (50 mg/kg, i.m.), verapamil (40 mg/kg, i.m.) or saline (i.m.). The doses of doxorubicin and verapamil were previously established as the MTD for SCID mice.[21,22] All animals received an identical injection volume of 0.2 ml. CsA, verapamil or cremaphor were administered as bolus intramuscular injections 1 h prior to each dose of doxorubicin. Following drug administration, the animals received food and water *ad libitum*, and were monitored for weight loss and other signs of toxicities. Deaths, if any, were recorded daily. Tissues were harvested from the animals 24 h following the completion of dosing.

HPLC assay

Doxorubicin tissue levels were measured by reverse-phase HPLC. Following excision of the tissues from the mice, they were rinsed in iced PBS, weighed and homogenized in a Brinkman polytron at 4°C. The homogenate was then extracted with 2-propanol and dried under N_2. To each sample, 4-deoxydoxorubicin was added as an internal standard prior to HPLC analysis. The HPLC analysis was performed on a Waters Associates Model 6000A solvent delivery system and a C_{18}-μ Bondapak reverse-phase column using a procedure previously described.[24] Compounds were eluted isocratically with a mobile phase consisting of 30% acetonitrile and 70% 0.1% ammonium formate, pH 4.0. Solvent flow rate was 2.0 ml/min. Fluorescence measurements of doxorubicin were made with a Perkin-Elmer LS-1 fluorescence detector (excitation wavelength 252 nm, emission wavelength 550 nm). Results are presented as ng doxorubicin/g tissue wet weight.

Histopathology

At the appropriate time interval, tissues were removed and fixed in freshly made 10% neutral buffered formalin and processed in an automated tissue processor (Model MVP-II; RMC, Tucson, AZ) and embedded in paraffin. Sections were cut (4–5 μm) and stained with hematoxylin & eosin for evaluation by light microscopy.

Statistics

Differences in tissue doxorubicin levels between different experimental groups were statistically analyzed with Student's *t*-test.

Results

Previous studies carried out in tumor-bearing SCID mice revealed an acute toxicity when doxorubicin and CsA were administered together.[23] In the current study, carried out in non-tumor bearing SCID mice, the toxicity was observed to be independent of the presence of tumor. The administration of either drug alone did not result in observable toxicities (data not shown). No deaths were observed in animals receiving verapamil or cremaphor treatment either alone or in combination with doxorubicin (Figure 1). In contrast, 100% of the mice given

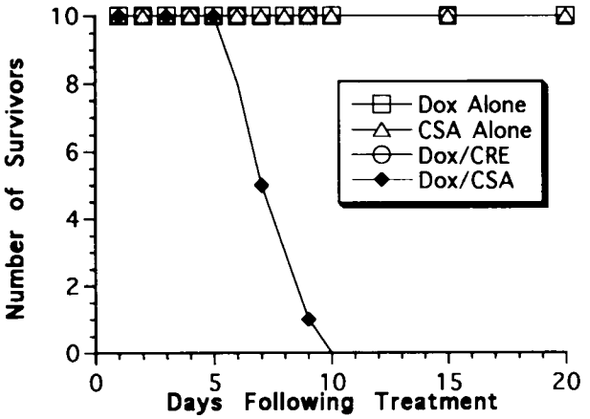

Figure 1. Effects of cyclosporin A on doxorubicin-induced lethality in SCID mice. At time 0, SCID mice were administered doxorubicin (1.5 mg/kg, i.p.) and CsA, or cremaphor. No deaths were observed in groups 2–4 but 100% of the animals in group 1 died within 10 days of the initiating of dosing.

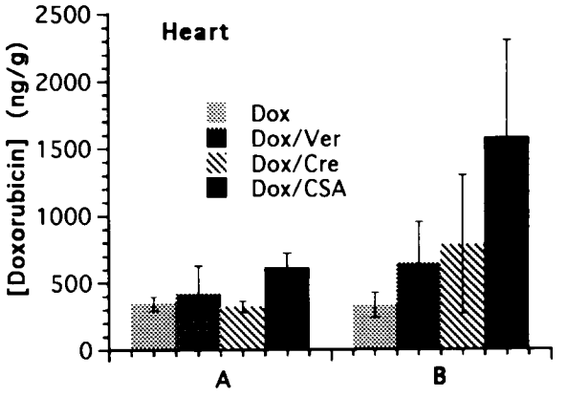

Figure 2. Doxorubicin levels in the hearts of SCID mice 24 h following administration of doxorubicin with or without concurrent chemomodulator. (A) tissue levels following a single treatment course. (B) Tissue levels following administration of two courses. Results are presented as ng dox/g tissue wet weight. N = 3–5 animals per group.

doxorubicin and CsA died within 24–48 h following the administration of a second course of treatment (Figure 1). Thus, the toxicity was specific for the combination of doxorubicin and CsA.

For tissue studies, SCID mice were dosed with either doxorubicin alone (1.5 mg/kg) or doxorubicin in combination with CsA (50 mg/kg, i.m.), cremaphor (50 mg/kg, i.m.) or verapamil (40 mg/kg, i.m.). Animals were sacrificed 24 h following drug administration and tissues harvested for analysis of doxorubicin levels by HPLC.

In all tissues studied, doxorubicin levels were higher following treatment with CsA (Table 1). In those animals receiving doxorubicin alone, tissue levels were not elevated following administration of two doses of doxorubicin and saline. At 24 h following a single administration of drug, doxorubicin levels in the kidney were not elevated relative to the doxorubicin/saline animals; however, 24 h after addition of a second dose, doxorubicin levels in the kidney were elevated in both the doxorubicin/CsA and doxorubicin/verapamil groups, although statistical significance was not reached. Following two courses of treatment, these animals receiving doxorubicin and saline had tissue levels of 1615 ± 264 ng doxorubicin/g tissue while those animals receiving doxorubicin and verapamil had levels of 2446 ± 253 ng/g and those receiving doxorubicin and CsA had 3488 ± 948 ng/g.

In contrast to the kidney, doxorubicin levels in

Table 1. Tissue levels of doxorubicin

Group	Tissue			
	Kidney	Liver	Heart	Brain
(A) Levels 24 h following a single administration[a]				
doxorubicin/saline	1871 ± 144[b]	642 ± 95	344 ± 25	55 ± 7
doxorubicin/CsA	1807 ± 353	1088 ± 138	611 ± 65**	56 ± 3
doxorubicin/cremaphor	1077[c]	671 ± 27	321 ± 24	59 ± 5
doxorubicin/verapamil	1698 ± 362	595 ± 55	417 ± 87	75 ± 6
(B) Levels 24 h following two administrations				
doxorubicin/saline	1615 ± 264	599 ± 79	336 ± 46	65 ± 10
doxorubicin/CsA	3488 ± 948	1719 ± 515	1586 ± 418**	273 ± 51**
doxorubicin/cremaphor	1804 ± 329	669 ± 65	785 ± 299	160 ± 33
doxorubicin/verapamil	2446 ± 253	1039 ± 216	644 ± 128	185 ± 48

[a]Determined by HPLC as described in text.
[b]ng doxorubicin/g tissue; data presented as mean ± SE; n = 3–7 animals per group.
[c]$n = 1$.
**$p < 0.01$.

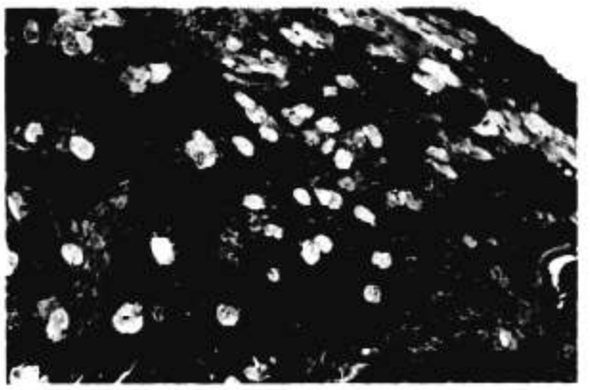

Figure 3. Cardiac tissue from a SCID mouse treated with the combination of doxorubicin and CsA as described in methods. Tissue reveals numerous areas of myocytolysis (hematoxylin & eosin, × 40).

the liver were elevated in the doxorubicin/CsA group following both doses of drug. The administration of doxorubicin and verapamil also resulted in an increase but only following the administration of two courses and then to a much lower magnitude. Tissue levels in those animals receiving doxorubicin and saline were 599 ± 79 ng/g tissue following the administration of two courses of therapy, which was compared with 1795 ± 515 ng/g tissue in animals receiving the combination of doxorubicin and CsA and 1039 ± 216 ng/g tissue in those animals receiving doxorubicin and verapamil. Animals receiving cremaphor in combination with doxorubicin revealed no increase in tissue levels compared with the control group.

The levels of doxorubicinol in tissues of SCID mice given doxorubicin alone or after administration of any of the chemomodulators were not significantly different from each other, thus the CsA-induced changes in doxorubicin tissue levels were not related to an alteration of doxorubicin metabolism (data not shown).

Clinically, doxorubicin therapy is compromised by the development of a dose-limiting and potentially lethal cardiotoxicity.[25] Since combination therapy with CsA and doxorubicin resulted in severe toxic effects, it was important to determine if CsA altered doxorubicin levels in the heart. As can be observed in Figure 2, doxorubicin levels in the heart were elevated approximately 2-fold ($p < 0.01$) following a single administration of doxorubicin and CsA. Following the second administration, there was a 4 to 5-fold elevation ($p < 0.01$) in the doxorubicin concentration of cardiac tissue in those mice receiving doxorubicin and CsA. Both the doxorubicin and verapamil and the doxorubicin and cremaphor treated animals displayed an elevation in doxorubicin levels in the heart, but to a much lower magnitude than observed in the CsA treated group. Although mice treated with doxorubicin in conjunction with verapamil or cremaphor had elevated tissue levels, their survival rate was not altered compared with those receiving doxorubicin and saline.

In those animals receiving doxorubicin and CsA, histological examination of the heart revealed multifocal myocytolysis with myofibrillar disruption which in some areas coalesced to include larger groups of myocytes (Figures 3 and 4). Those animals treated with doxorubicin and verapamil or cre-

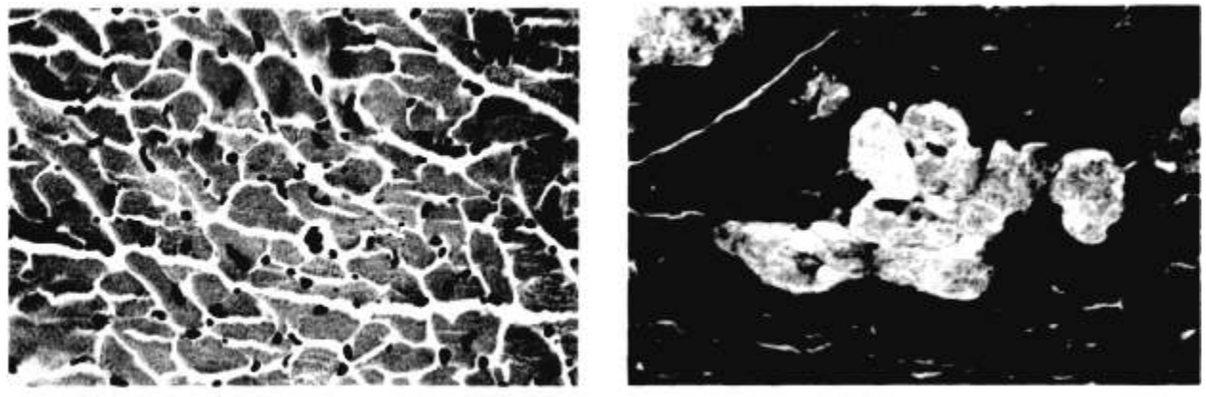

Figure 4. Histopathologic examination of cardiac tissues from mice treated with doxorubicin alone or with the combination of doxorubicin and CsA. Cardiac tissue from mice treated with doxorubicin alone appears normal following two doses of drug (A) while tissue from those animals treated with the combination of doxorubicin and CsA revealed myocytolosis and myofibrillar disruption (B) (hematoxylin & eosin, × 400).

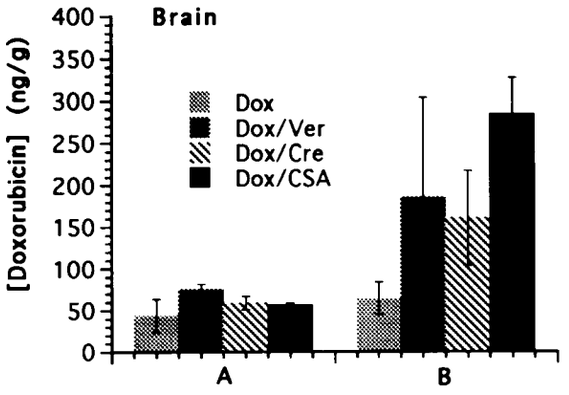

Figure 5. Doxorubicin levels in the brains of SCID mice 24 h following administration of doxorubicin with or without concurrent chemomodulator. (A) Tissue levels following a single treatment course. (B) Tissue levels following administration of two courses. Results are presented as ng dox/g tissue wet weight. $N = 3$–5 animals per group.

maphor showed only rare instances of myocardial damage. Animals treated with doxorubicin or CsA alone displayed no evidence of morphological damage.

Doxorubicin levels in the brain were not found to be elevated following administration of a single course of any of the drug combinations. Following administration of a second course, however, a statistically significant elevation in doxorubicin levels was observed in the mice receiving doxorubicin in combination with CsA (273 ± 51 ng/g tissue compared with 65 ± 10 ng/g tissue; $p < 0.01$) (Figure 5). Tissue levels of doxorubicin in those animals receiving verapamil or cremaphor in conjunction with doxorubicin were also elevated, but to a much lower magnitude (185 ± 48 ng/g tissue and 160 ± 33 ng/g tissue, respectively). This increase may reflect the inhibition of Pgp located in the endothelial cells lining the capillaries of the brain.

Discussion

Both *in vitro* and clinical trials have shown that chemomodulators such as verapamil and CsA may be beneficial.[7,8,10] The clinical results obtained to date have been disappointing, however, and in an effort to improve the duration and number of responses, new chemomodulators are being developed which will be more potent and less toxic than the initial compounds. *In vitro* studies have established that a multitude of agents are capable of altering the function of Pgp.[5,7] Clinical trials of these agents have focused primarily on agents such as verapamil and CsA. Although the use of chemomodulators combined with drugs such as doxorubicin can partially restore drug sensitivity in cancer patients refractory to chemotherapy, this therapeutic advantage may potentially be offset by increased toxicities. With the demonstration of Pgp expression in a wide variety of normal tissues,[13–15] it becomes imperative to evaluate these compounds in an *in vivo* model in order to examine the possibility of adverse effects due to the combination of antineoplastic agent and chemomodulator. We have utilized a SCID mouse model to evaluate chemomodulators directed against MDR-1. This model was used initially to evaluate the combination of verapamil and doxorubicin, and demonstrated both a decrease in tumor burden and an increase in survival of those animals bearing the multidrug resistant tumor when administered this combination.[22]

In contrast to our findings with verapamil, the administration of doxorubicin and CsA resulted in an acute toxicity in the SCID mice.[23] This toxicity was characterized by death of the animals within 24–48 h following the second dose of a planned three-dose regimen and was associated only with those animals receiving the combination of CsA and doxorubicin. CsA is a cyclic endecapeptide widely used as an immunosuppressant and which has been demonstrated to be a potent inhibitor of Pgp *in vitro* at concentrations ranging from 500 to 2000 ng/ml.[26,27] These levels have been demonstrated to be clinically achievable and therefore there has been considerable interest in its use in the clinic.[10–12] Clinical studies have demonstrated that the use of CsA alters the pharmacokinetics of antineoplastic agents, including doxorubicin, resulting in a decrease in the clearance and an increase in the concentration × time product (AUC).[16,17,19,20] Such reports are of importance due to the potential of increased toxicities when chemomodulators are utilized concomitantly with chemotherapeutic agents. The current study was undertaken to determine if the acute toxicity we observed was due to increased tissue levels of doxorubicin in target organs.

CsA, in the absence of doxorubicin, did not result in animal toxicities. However, when CsA was combined with doxorubicin, we observed SCID mice

dying within 24 h of the second administration of the combination. This toxicity was independent of whether or not the animals were tumor bearing. When tissues from non-tumor bearing SCID mice administered the combination of doxorubicin and CsA were examined, we found markedly elevated levels of doxorubicin in several target organs including the kidney, heart and liver. While we did not observe statistically significant increases in doxorubicin levels in any of the tissues in the SCID mouse receiving doxorubicin and verapamil, this combination also resulted in an elevation in various tissues. The result of this study demonstrates that CsA dramatically alters the distribution of doxorubicin in SCID mice.

Histologically, we observed marked multifocal random myocytolysis with myofibrillar disruption in those animals receiving doxorubicin and CsA, a finding consistent with doxorubinic-induced cardiomyopathy. In certain areas, these lesions coalesced to include larger groups of myocytes. While we did not perform electron microscopy on these tissues, such lesions would be observed ultrastructurally as myofilament lysis, disruption of the Z-band, swollen mitochondria and vacuolization of the sarcoplasmic reticulum.[28] Those animals treated with doxorubicin and verapamil or cremaphor showed only rare instances of myocardial damage. Morphologic damage was not evident in the liver or kidney in any of the groups examined.

Of interest was the finding of elevated dox levels in the brain. Under normal circumstances, doxorubicin does not enter the CNS to an appreciable amount. Recent reports, however, have demonstrated the presence of Pgp in the endothelial cells lining the capillaries of the brain.[15,29,30] This expression appears to be functional and thus may serve to inhibit the passage of agents such as doxorubicin into the CNS. The potential exists therefore, that in the presence of MDR-1 chemomodulators, there may be an elevation in the CNS levels of various natural product antineoplastic agents resulting in untoward adverse effects. Indeed, Barbui *et al.* have described neurological complications in a patient receiving chronic CsA therapy following heart transplantation who was subsequently treated with doxorubicin for stage IV Burkitts lymphoma.[31] Additionally, studies of *mdr*-knockout mice have demonstrated increased toxicities as a consequence of elevated levels of vinblastine and ivermectin in the brains of these animals.[32]

In contrast to our findings, Colombo *et al.*[19] observed no alteration in doxorubicin levels in the brain of mice as a function of CsA administration. These investigators, however, administered lower concentrations of CsA (25 and 12.5 mg/kg compared with 50 mg/kg) and only administered a single dose). As observed in our studies (Figures 2 and 3), elevations in the brain and heart were not observed following a single administration, but were markedly elevated following the administration of a second course of the combination.

The observed increase in doxorubicin levels may be the result of at least two possibilities. First, the serum CsA levels attained with dosing regimen may have been sufficient to directly inhibit Pgp function or there may have been a cumulative effect resulting from an inhibition in the elimination of doxorubicin and thereby leading to higher tissue drug levels. While the kidney express high levels of endogenous Pgp and would be subject to both of the above mechanisms, the heart expresses low levels of Pgp and the liver expresses the MDR-2 protein thus making these tissues more susceptible to the later scenario of indirectly elevated tissue levels.

While we did observe elevations in doxorubicin levels in the heart and brains of animals receiving doxorubicin in conjunction with verapamil or cremaphor, these were of a much lower magnitude than observed with CsA and may reflect the rank order of potency of these three compounds in terms of inhibiting P-glycoprotein function. We have not assayed CsA levels and therefore we cannot exclude the possibility that the increased toxicity is at least in part a consequence of altered CsA pharmacokinetics due to subsequent treatment with doxorubicin.

In the present studies we could not discern with certainty whether the acute toxicity observed was due to the elevated levels of doxorubicin in the CNS, the heart and other tissues or to a combination of these. However, given the marked cardiac lesions observed in the animals receiving doxorubicin and CsA, this certainly contributed significantly to their death. The exact mechanism of the increased toxicity resulting from the combination of doxorubicin and CsA has not been investigated in detail, and it was beyond the scope of our original aims to calculate the precise serum kinetic parameters which would have required a much larger number of animals and time points. Instead, our main focus was to investigate whether CsA treatment altered the tissue levels of doxorubicin.

This study demonstrates the need for *in vivo* models to evaluate new chemomodulators. While the SCID mouse may be a very sensitive indicator of toxicities due to drugs or agents which induce DNA damage,[33] other investigators have also demonstra-

ted increased toxicities in murine models following the administration of antineoplastic agents such as doxorubicin or vincristine and a chemomodulator; thus, this phenomena is not limited to the SCID mouse.[34,35]

The results of our studies, in combination with results observed by others, suggest caution in combining CsA and doxorubicin in clinical use without a careful phase I clinical trial designed to establish the maximum tolerated doses of the two drugs. Further studies may lead to the development of dosing regimens taking into account the pharmacokinetic alterations resulting from these combinations and resulting in an improved therapeutic index.

References

1. Epstein J, Xiao HQ, Oba BK. P-glycoprotein expression in plasma-cell myeloma is associated with resistance to VAD. *Blood* 1989; **74**: 913–17.
2. Dalton WS, Grogan TM, Rybski JA, *et al.* Immunohistochemical detection and quantitation of P-glycoprotein in multiple drug-resistant human myeloma cells: association with level of drug resistance and drug accumulation. *Blood* 1989; **73**: 747–52.
3. Dalton WS, Grogan TM, Miller TP. The role of P-glycoprotein in drug-resistant hematologic malignancies. *Cancer Treat Res* 1991; **57**: 187–208.
4. Grogan TM, Spier CM, Salmon SE, *et al.* P-glycoprotein expression in human plasma cell myeloma: correlation with prior chemotherapy. *Blood* 1993; **81**: 490–95.
5. Bellamy W, Dalton W. Multidrug resistance in the laboratory and clinic. *Adv Clin Chem* 1994; **31**: 1–61.
6. Tsuruo T, Iida H, Tsukagoshi S, *et al.* Overcoming of vincristine resistance in P388 leukemia *in vivo* and *in vitro* through enhanced cytotoxicity of vincristine and vinblastine by verapamil. *Cancer Res* 1981; **41**: 1967–72.
7. Beck WT. Modulators of P-glycoprotein-associated Multidrug Resistance. In: Ozols RF, ed. *Molecular and clinical advances in anticancer drug resistance.* Boston: Kluwer 1991: 151–70.
8. 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**: 415–24.
9. Miller TP, Grogan TM, Dalton WS, *et al.* P-glycoprotein expression in malignant lymphoma and reversal of clinical drug resistance with chemotherapy plus high-dose verapamil. *J Clin Oncol* 1991; **9**: 17–24.
10. Sonneveld P, Durie BGM, Lokhorst HM *et al.* Modulation of multidrug-resistant multiple myeloma by cyclosporin. *Lancet* 1992; **340**: 255–9.
11. List AF, Spier C, Greer J, *et al.* Phase I/II trial of cyclosporine as a chemotherapy-resistance modifier in acute leukemia. *J Clin Oncol* 1993; **11**: 1652–60.
12. Yahanda AM, Adler KM, Fisher GA, *et al.* Phase I trial of etoposide with cyclosporine as a modulator of multidrug resistance. *J Clin Oncol* 1992; **10**: 1624–34.
13. Thiebaut F, Tsuruo T, Hamada H, *et al.* Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci USA* 1987; **84**: 7735–8.
14. Cordon Cardo C, O'Brien JP, Boccia J, *et al.* Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. *J Histochem Cytochem* 1990; **38**: 127712–87.
15. Cordon Cardo C, O'Brien JP, Casals D, *et al.* Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood–brain barrier sites. *Proc Natl Acad Sci USA* 1989; **86**: 695–8.
16. Lum BL, Kaubisch S, Yahanda AM, *et al.* Alteration of etoposide pharmacokinetics and pharmacodynamics by cyclosporine in a phase I trial to modulate multidrug resistance. *J Clin Oncol* 1992; **10**: 1635–42.
17. Erlichman C, Moore M, Thiessen JJ, *et al.* Phase I pharmacokinetic study of cyclosporin A combined with doxorubicin. *Cancer Res* 1993; **53**: 4837–42.
18. Kerr DJ, Graham J, Cummings J, *et al.* The effect of verapamil on the pharmacokinetics of adriamycin. *Cancer Chemother Pharmacol* 1986; **18**: 239–42.
19. Colombo T, Zucchetti M, D'Incalci M. Cyclosporin A markedly changes the distribution of doxorubicin in mice and rats. *J Pharmacol Exp Ther* 1994; **269**: 22–7.
20. Rushing DA, Raber S, Rodvold KA, *et al.* The effects of cyclosporine on the pharmacokinetics of doxorubicin in patients with small cell lung cancer. *Cancer* 1994; **74**: 834–41.
21. Bellamy WT, Odeleye A, Finley P, *et al.* An *in vivo* model of human multidrug-resistant multiple myeloma in SCID mice. *Am J Pathol* 1993; **142**: 691–98.
22. Bellamy WT, Odeleye A, Finley P, *et al.* An *in vivo* model of chemosensitization of multidrug resistant human myeloma cell line in SCID mice. *Clinical Cancer Res* 1995; in press.
23. Bellamy W, Peng Y, Odeleye A, *et al.* The addition of cyclosporin A results in markedly elevated tissue levels of doxorubicin in the SCID mouse. *Anti-Cancer Drugs* 1994; **5**: 22.
24. Peng YM, Alberts DS, Salmon SE, *et al.* A method for the simultaneous measurement of the new anthracycline derivative 4′-deoxydoxorubicin and its metabolites by reversed phase liquid chromatography. *Invest New Drugs* 1984; **2**: 277–80.
25. Olson RD, Mushlin PS. Doxorubicin cardiotoxicity: analysis of prevailing hypotheses. *FASEB J* 1990; **4**: 3076–86.
26. Slater LM, Sweet P, Stupecky M, *et al.* Cyclosporin A reverses vincristine and daunorubicin resistance in acute lymphatic leukemia *in vitro*. *J Clin Invest* 1986; **77**: 1405–8.
27. Ford JM, Hait WN. Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol Rev* 1990; **42**: 155–99.
28. Doroshow J, Gershon Y, Locker I, *et al.* Prevention of doxorubicin cardiac toxicity in the mouse by *N*-acetylcysteine *J Clin Invest* 1981; **65**: 1053–64.
29. Tatsuta T, Naito M, Sugawara I, *et al.* Functional involvement of P-glycoprotein in blood–brain barrier. *J Biol Chem* 1992; **267**: 20383–91.
30. Tsuji A, Terasaki T, Takabatake Y, *et al.* P-glycoprotein as the drug efflux pump in primary cultured bovine brain capillary endothelial cells. *Life Sci* 1992; **51**: 1427–37.
31. Barbui T, Rambaldi A, Parenzan L, *et al.* Neurological symptoms and coma associated with doxorubicin admin-

istration during chronic cyclosporin therapy. *Lancet* 1992: **339**: 1421.

32. Schinkel AH, Smit JM, O VT, *et al.* Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood–brain barrier and to increased sensitivity to drugs. *Cell* 1994; **77**: 491–502.
33. Hendrickson EA, Qin X, Bump EA, *et al.* A link between double-strand break-related repair and V(D)J recombination: the SCID mutation. *Proc Natl Acad Sci USA* 1991; **88**: 4061–5.
34. Horton J, Thimmaiah KN, Houghton JA, *et al.* Modulation by verapamil of vincristine pharmacokinetics and toxicity in mice bearing human tumor xenographs. *Biochem Pharmacol* 1989; **38**: 1727–36.
35. Sridhar R, Dwivedi C, Anderson J, *et al.* Effects of verapamil on the acute toxicity of doxorubicin *in vivo*. *J Natl Cancer Inst* 1992; **84**: 1653–60.

(Received 28 July 1995; accepted 31 July 1995)