A Phase III Randomized Study of Oral Verapamil as a Chemosensitizer to Reverse Drug Resistance in Patients with Refractory Myeloma

A Southwest Oncology Group Study

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Background. **Multiple myeloma is considered to be a drug responsive disease; however, there is no cure for this disease and virtually all patients will develop drug resistance. One form of drug resistance that has been documented is the multidrug resistance phenotype or MDR.**

Methods. **A randomized trial of the combination of vincristine, doxorubicin, and dexamethasone (VAD) and VAD plus oral verapamil (VAD/v) in drug refractory multiple myeloma patients was performed by the Southwestern Oncology Group. Verapamil was used as a chemosensitizing agent to attempt to overcome or prevent MDR and improve the therapeutic outcome.**

Results. **Response rates between the two treatment arms were similar with an overall response rate of 41% for the VAD alone arm and 36% for the VAD/v arm. Overall survival of patients was also similar with a median survival of 10 months for the VAD arm and 13 months for the VAD/v arm. The toxicity profile was also similar for both treatments, with myelosuppression being the dose-limiting toxicity. No significant correlation was observed between expression of P-glycoprotein, serum verapamil levels, and response to therapy.**

Conclusions. **No beneficial effect was observed from the addition of oral verapamil to the VAD chemotherapy regimen for the treatment of drug-resistant myeloma patients. More effective and less toxic chemosensitizers are needed to study the role of chemosensitizers in reversing MDR in the clinic. *Cancer* 1995;75:815–20.**

Key words: multidrug resistance, verapamil, chemosensitizing agent, multiple myeloma.

Multiple myeloma generally is considered to be a drug responsive disease; however, many patients experience drug resistance and eventually die of their disease. Approximately 60% of patients will respond to initial chemotherapy, and go on to have a median survival of approximately 4 years.[1] Patients who experience disease progression while receiving therapy have a median survival of only 15 months. One approach to improving therapeutic response is by adding different drugs to the therapeutic regimen. Alkylating agents and corticosteroids remain the mainstay of therapy for multiple myeloma, but the use of natural products, especially vincristine and doxorubicin, also has proven effective. VAD chemotherapy, the 4-day continuous infusion of vincristine and doxorubicin plus oral dexamethasone, is considered to be the best treatment for patients experience relapse after treatment with alkylating agents.[2] Unfortunately, the use of combination chemotherapy has not improved the therapeutic outcome for most patients and the development of drug resistance remains the most common obstacle to curing this disease.[3]

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At least one form of drug resistance that has been documented to occur in multiple myeloma is the multidrug resistance phenotype or MDR.[4–7] This form of drug resistance is caused by the overexpression of P-glycoprotein (P-gp) on the surface of cells, which results in decreased intracellular accumulation of cytotoxic drugs, primarily natural product agents such as doxorubicin and vincristine.[4,8,9] Several clinical studies have demonstrated that P-gp expression occurs in patients with myeloma and that the level of expression increases as patients experience resistance to vincristine and doxorubicin.[6,10] Interest in the MDR phenotype and its role in clinical drug resistance has emerged because of the possibility of overcoming resistance by using agents that inhibit P-gp function.[11,12] This approach stemmed from the seminal observation of Tsuruo et al., who reported that the calcium channel blocker, verapamil, was capable of overcoming MDR in mouse lymphocytic leukemia cells.[13] Soon thereafter, it was reported that verapamil could reverse MDR in vitro in human myeloma cells known to overexpress P-gp.[4,14] Based on these in vitro laboratory reports, clinical studies using verapamil as a chemosensitizer were conducted. Dalton et al.[15] reported the results of a clinical pilot study in which seven patients were treated with high dose continuous infusion verapamil and VAD chemotherapy. Two of seven patients who were resistant to VAD alone and whose myeloma cells expressed P-gp had a response when verapamil was added to the regimen. In a follow-up study by Salmon et al.,[16] 5 of 22 patients had a response to high dose intravenous verapamil infusion plus VAD after VAD alone failed. These pilot studies serve as the basis for a randomized trial conducted by the Southwestern Oncology Group (SWOG 8900) of VAD and VAD plus oral verapamil for refractory multiple myeloma.

The objectives of this trial were to: (1) estimate the response rate and response duration with chemotherapy alone (VAD) and chemotherapy plus the chemosensitizer verapamil (VAD/v) in patients for whom previous chemotherapy had failed; (2) correlate responses to the detection of P-gp; and (3) compare the toxicity of the two regimens.

Patients and Methods

Patient Population

Patients entered in the study had myeloma that was resistant (had never responded to chemotherapy) or relapsing (initially responded to chemotherapy and subsequently relapsed). Only patients with a measurable monoclonal protein were eligible. The total dose of prior doxorubicin could not have exceeded 360 mg/m^2. No patients could have received prior continuous infusion doxorubicin or vincristine. All patients had a performance status of 2 or better (SWOG grading scale). All patients had normal cardiac function as judged by pretreatment cardiac ejection fraction and electrocardiogram. All patients signed a Human Subjects Committee approved consent form before participating in this study.

Patients were stratified by (1) tumor mass: low versus intermediate versus high; (2) risk: good (no prior large volume radiotherapy to more than 20% of the bone marrow, age 70 years or younger, and creatinine less than 2.5 mg%) versus poor (prior large volume radiotherapy to more than 20% of the bone marrow, or age older than 70, or creatinine 2.5 mg% or greater); (3) presence or absence of prior treatment with vincristine or doxorubicin; and (4) response to previous therapy: primary resistant versus relapsing disease during or within 6 months of therapy versus relapsing disease after 6 months of prior therapy.

Treatments

Using the stratification factors described, patients were randomized to two treatment arms: Arm I, VAD alone (VAD); or Arm II, VAD plus oral verapamil (VAD/v). All patients, whether they were randomized to Arm I or Arm II, received the same doses of VAD chemotherapy as originally described by Barlogie et al.[2] Vincristine (0.4 mg/day) and doxorubicin (9 mg/m^2/day) were administered as a concomitant continuous infusion during a period of 4 days. Dexamethasone was given orally at 40 mg/day on days 1–4 and 9–12. Chemotherapy was repeated every 28 days. Doses of chemotherapy were adjusted by standard criteria used for administration of this regimen, and all patients received antacids and prophylactic antibiotics as previously recommended.[2]

For patients randomized to the VAD/v treatment arm, oral racemic verapamil was administered for 12 days, encompassing the 4-day continuous infusion of VAD. Verapamil, 120 mg twice a day, was administered on days 1–3 and increased to 120 mg four times a day on days 4–12. Vincristine and doxorubicin were administered by a 4-day continuous infusion beginning on day 8 of each cycle. The dexamethasone was administered in the usual 4-day pulse fashion. The regimen was repeated every 28 days.

Patients were removed from the study if there was documented progression, unacceptable toxicity, or if a patient refused to continue treatment.

Clinical response to therapy was determined in accord with standardized myeloma response criteria based on serial quantitative determinations of the patient's myeloma immunoglobulin (Ig) (M-protein) in the serum or urine by laser nephelometry or protein electrophoresis.[17] Briefly, a remission was recorded if a sus-

tained decrease in the production rate (synthetic index) of the monoclonal serum protein occurred by nephelometry to 25% or less of the pretreatment value (75% reduction) on at least two successive measurements at 3-week intervals or the urine M-component decreased to 10% or less of the initial pretreatment value. A partial remission was defined as a decline in the serum myeloma protein by less than 75%, but not less than 50% of the pretreatment level. An increase in disease was noted when there was more than a 25% increase of the baseline myeloma protein production or a definite increase in the size or number of lytic lesions was recognized on bone radiographs. The disease was considered stable when neither the criteria for response or increase in disease was satisfied.

Immunocytochemical Staining of Myeloma Cells

Marrow aspirates were separated using Ficoll-Hypaque separation media and applied to glass microscope slides as previously described.[10,18] Slides were fixed in cold acetone, air dried, and stored at −180°C until staining. A biotin-avidin, diaminobenidine immunoperoxidase detection system was optimized for P-gp detection using the monoclonal antibodies JSB-1 and C-219 as previously described.[18] A series of human myeloma cell lines, 8226/S and doxorubicin selected 8226/Dox_6 and 8226/Dox_{40}, were used as negative and positive controls for P-gp detection, respectively.[4] Additional antigens were studied using the following primary antibodies:

1. to B-cell antigens: Calla (CD 10), leu 12 (CD 19), Leu 16 (CD 20) (all from Becton Dickinson, Mountain View, CA);
2. to nuclear proliferation antigens: Ki67 (Dako, Santa Barbara, CA);
3. Ig (k, l, m, g, a) (Becton Dickinson).

Irrelevant mouse Ig of identical isotype was applied instead of primary antibody as a negative control on each run.

Antibody staining was interpreted by immunopathologists who were blinded to the clinical data. To be called positive for P-gp, myeloma cells in the patient's cytospin exhibited 1+ to 3+ positivity (compared with the positive control cell lines) in at least 30% of the myeloma cells.[10]

Serum Verapamil Levels

Venous blood samples for serum verapamil levels were drawn on day 7, just before the start of VAD chemotherapy for patients enrolled in Arm II (VAD/v). High performance liquid chromatography analysis of verapamil was performed according to the method of Harapat and Kates.[19]

Table 1. Patient Characteristics

	VAD (n = 61)	VAD/verapamil (n = 59)
Age (yr)		
Median	62.0	61.0
Minimum	39	31
Maximum	78	77
Sex		
Males	33 (54)	40 (68)
Females	28 (46)	19 (32)
Race		
White	46 (75)	49 (83)
Black	14 (23)	10 (17)
Other	1 (2)	0 (0)
MASS		
High	43 (70)	45 (76)
Intermediate low	18 (30)	14 (24)
Risk		
Good	41 (67)	40 (68)
Poor	20 (33)	19 (32)
Response		
Resist	15 (25)	12 (20)
Relapse during treatment	37 (61)	37 (63)
Posttreatment relapse	9 (15)	10 (17)
VCR/ADR		
Yes	38 (62)	37 (63)
No	23 (38)	22 (37)
Previous treatment		
≤2	46 (75)	37 (63)
>2	15 (25)	22 (37)

Values are no. (%).
VAD: vincristine, Adriamycin, dexamethasone; VCR: vincristine; ADR: Adriamycin.

Statistics

The P-gp and serum verapamil levels of patients with response and those without were compared using *t* tests.

Results

Clinical Trial Results

In a 31-month period ending in December 1991, 63 patients were randomized to the VAD alone treatment arm and 64 patients in the VAD/v treatment arm, for a total of 127 patients. Four patients were ineligible because of insufficient baseline documentation of their disease. One patient randomized to the VAD alone treatment arm received verapamil, and two patients randomized to the VAD/v treatment arm refused treat-

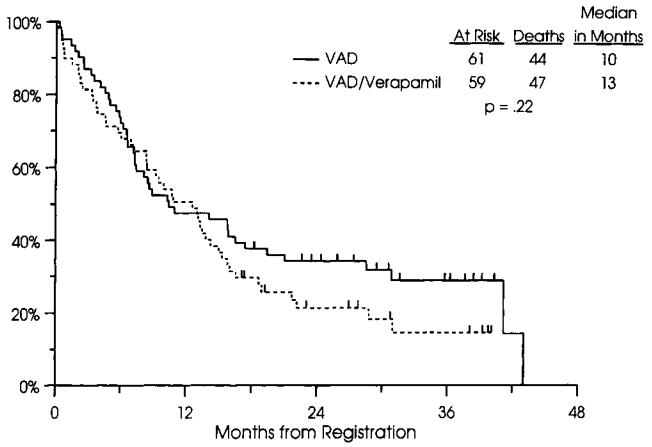

Figure 1. Overall survival of patients treated with VAD versus VAD/verapamil.

ment; none of these patients was evaluable for toxicity, response, or survival. One patient randomized to the VAD/v treatment arm had no toxicity information but was evaluable for response and survival. Table 1 shows the patient characteristics of the 120 evaluable patients. The treatment arms were balanced for all of the stratification factors listed.

Response rates between the two treatment arms were similar, with an overall response rate of 41% for patients in the VAD alone treatment arm and 36% for those in the VAD/v treatment arm (see Table 2). Twenty percent of the patients in the VAD alone treatment arm had a complete remission, compared with 14% of patients in the VAD/v treatment arm. Partial responses were obtained in 21% of the VAD alone patients and 22% of the VAD/v patients. Overall survival of patients was similar, with a median survival of 10 months for patients in the VAD treatment arm, compared with 13 months for those in the VAD/v treatment arm (Fig. 1).

The toxicity profile was similar between the two treatment arms. Extent and duration of myelosuppression were similar. Grade 3 and 4 leukopenia were seen in 6 and 5, respectively, of the 61 patients evaluated on the VAD alone treatment arm; whereas, 9 and 1 patients, respectively, of the 58 evaluated on the VAD/v treatment arm had Grade 3 and 4 leukopenia. Thrombocytopenia was similar in both treatment arms, with three patients in each experiencing Grade 4 toxicity. Neurologic complications, including reports of paresthesia, were infrequent and low grade in patients in both treatment arms. Alterations in liver function, including hyperbilirubinemia, were rare in patients in both treatment arms. Two patients in the VAD/v treatment arm died of treatment related deaths; one patient died with a cardiac bundle branch block and hypotension possibly related to sepsis, and another died of a perforated colon. Serum verapamil levels were not available in these patients at the time of death, and we cannot determine the possible role of verapamil in these treatment related deaths. One patient in the VAD alone treatment arm died of an infection.

Table 2. Response

	VAD	VAD/verapamil
Remission*	12 (20)	8 (14)
Partial remission	13 (21)	13 (22)
Stable/no response	20 (33)	21 (36)
Increasing disease	6 (10)	2 (3)
Early death	2 (3)	2 (3)
Assessment inadequate	8 (13)	13 (22)
Total	61 (100)	59 (100)

Values are no. (%).
VAD: vincristine, Adriamycin, dexamethasone.
* More than 75% tumor regression.

Table 3. P-Glycoprotein Status of Patients Enrolled on SWOG 8900

	P-glycoprotein status	
Response status	*Negative*	*Positive*
VAD alone		
Responders	11	3
Nonresponders	4	0
VAD/v		
Responders	9	5
Nonresponders	5	4

VAD: vincristine, Adriamycin, dexamethasone.

Immunophenotyping

Forty-three patients had immunophenotyping data of their myeloma cells at the time of enrollment in the study. Of these 43 patients, 41 had P-gp assays performed before treatment began. Only two patients had repeat measurements at the time of disease relapse. Table 3 lists the P-gp results obtained in these 41 patients. There was an equal distribution of patients with P-gp negativity in both treatment arms. Eleven of 14 patients with response in the VAD alone treatment arm and 9 of 14 with response in the VAD/v treatment arm had P-gp negativity. Because of the limited sample size, we are unable to relate response to therapy to the presence or absence of P-gp on patient's myeloma cells. In the two patients who had consecutive P-gp measurements, one who initially had P-gp negativity, experienced a complete response on VAD alone and upon relapse became positive for P-gp. The second patient had P-gp negativ-

ity at the beginning, was treated with VAD/v, achieved a complete response, and at relapse remained negative for P-gp.

Proliferative activity of the myeloma cells was measured by the monoclonal antibody Ki67. In general, the number of cells staining positively for this nuclear proliferative antigen was higher in the patients with no response than in those with response, with mean values of 9.8 ± 3.2 versus 4.0 ± 0.8, respectively. However, the limited sample size precludes statistical significance ($P = 0.27$).

Serum Verapamil Levels

Of the 64 patients enrolled in the VAD/v treatment arm, 25 had verapamil and norverapamil levels determined. Ten of the 25 patients had a response to the VAD/v treatment. The mean serum verapamil level for those with response was 300 ± 77 ng/ml, compared with 190 ± 50 ng/ml for those with no response. Although there was a trend for patients with response to have a higher serum verapamil level than that of patients with no response, the small sample size precluded significance for this finding ($P = 0.22$). Eleven of the 25 patients also had P-gp immunostaining performed, and there was no difference in serum verapamil levels for patients whose cells were positive versus those whose cells were negative for P-gp. No difference was found for serum levels of the primary metabolite, norverapamil, for patients with response compared with those with no response.

Discussion

Multiple myeloma is known to respond to a wide variety of cytotoxic drugs, including alkylating agents and natural product type drugs, such as doxorubicin and vincristine. Despite these responses, drug resistance develops and patients ultimately die of myeloma or its complications. One type of drug resistance is the MDR phenotype mediated by the overexpression of P-gp. A number of investigators have demonstrated that P-gp expression is related to drug resistance in myeloma.[4–7] Grogan et al.,[10] recently showed that detectable P-gp in patients with myeloma was an "acquired" trait and was related to prior administration of vincristine and doxorubicin. Recent pilot studies have shown that chemosensitizers, such as verapamil and cyclosporin A, can reverse the MDR phenotype in a subset of patients with myeloma who have drug resistance.[15,16,20] These studies attempted to use the highest dose possible of chemosensitizers to reverse MDR by using continuous intravenous infusions. In vitro studies in drug resistant myeloma cells have demonstrated a dose response relationship for verapamil and drug resistance reversal; however, levels as low as 100 ng/ml had some measurable reversing effect.[14] The continuous infusion method of verapamil administration in these pilot studies produced serum verapamil levels ranging from 200 to 1000 ng/ml.[15,16]

In this randomized Phase III trial, we were unable to demonstrate a beneficial effect of adding oral verapamil to VAD chemotherapy in patients with relapsing myeloma. Several possibilities might explain this lack of effect. One possibility is that P-gp is not a major contributor to drug resistance in myeloma and that other mechanisms of resistance to natural product agents play a more important role in the overall picture of clinical drug resistance. Thus, reversing resistance to this one mechanism of resistance would not be expected to have a major impact on outcome. A second possibility is that inadequate levels of verapamil were attained in these patients to significantly reverse P-gp function. The addition of oral verapamil to VAD was reasonably well tolerated and considerably less toxic than the experience observed with continuous infusion verapamil; however, serum levels were approximately one-third the amount seen with continuous infusion verapamil.[21] Although the lower serum verapamil levels undoubtedly account for the improved tolerance, they also might explain the lack of improvement in efficacy. The use of oral verapamil also may have allowed significant drops in serum verapamil levels between doses, resulting in insufficient blockade of P-gp function during the vincristine/doxorubicin infusion. In vitro studies have demonstrated that verapamil must be continuously present during cytotoxic drug exposure to reach maximal reversing effect.[22,23] A third possibility is that verapamil is relatively ineffective as a chemosensitizing agent in the clinical situation and that more effective agents to reverse MDR are needed. Finally, the sample size in this study is adequate for detecting survival differences on the order of 75%, but smaller differences might have been missed by chance. The low percentage of patients with P-gp–positive myeloma cells (30%) may reduce the ability to detect a verapamil effect in the VAD/v treatment arm. However, it is conceivable that the use of verapamil in patients with P-gp–negative myeloma cells may prevent the emergence of the MDR phenotype. Had this occurred, an improvement in disease free survival would have been observed in the VAD/v treatment arm.

In all likelihood, a combination of these possibilities explains the lack of verapamil effect in this study. More and more evidence suggests that non–P-gp mechanisms can confer resistance to natural product agents, such as doxorubicin and vincristine.[24–26] The clinical relevance of these drug resistance mechanisms needs to be established in individual disease types. If non–P-gp mechanisms are found to be clinically relevant, means

of overcoming these types of resistance need to be found. A trend toward showing a relationship between response to verapamil plus VAD and serum verapamil levels was observed in this study; it is conceivable that if higher levels of verapamil could have been sustained, a beneficial effect of adding verapamil to VAD may have been observed. Finally, verapamil represents the first generation of chemosensitizing agents to reverse MDR in the clinic. Tolerable oral doses of verapamil are not effective in reversing resistance, and high continuous infusion doses are too toxic and only marginally effective.[16,21] Clearly, more effective and less toxic agents are needed to try to reverse MDR in the clinic. A recently developed SCID mouse model of human P-gp–positive myeloma promises to provide an in vivo screen for efficacious agents.[27] Only when these agents are developed will we be able to determine the clinical relevance of MDR in disease progression.

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