Dose-finding study of high-dose simvastatin combined with standard chemotherapy in patients with relapsed or refractory myeloma or lymphoma

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In vitro statins induce apoptosis in myeloma and lymphoma cells in a dose-and time-dependent way. In combination with dexamethasone and doxorubicin, statins have a chemo-sensitizing effect. Twenty-eight patients with relapsed myeloma or lymphoma were treated with a dose-escalating regimen of simvastatin for 7 days followed by VAD in myeloma patients and CHOP in lymphoma patients. The maximum tolerated dose was 15 mg/kg/day simvastatin. The most frequently reported side-effects were fatigue, gastrointestinal CTC grade 1-2 and neutropenic fever. The dose-limiting toxicity was neutropenic sepsis and grade 3 gastrointestinal side effects. High-dose simvastatin given immediately prior to chemotherapy is safe and tolerable up to a dose of 15 mg/kg/day.

Key words: myeloma, statins, lymphoma, chemotherapy, phase I.

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HMG-CoA reductase inhibitors such as lovastatin and simvastatin are widely used for the treatment of hypercholesterolemia.[1] HMG-CoA reductase is the rate-limiting enzyme of the mevalonate pathway and catalyses the reduction of HMG-CoA to mevalonate, which is an essential product for the synthesis of various compounds, including cholesterol and isoprenoids such as farnesylpyrophosphate and geranylgeranylpyrophosphate.[2] Isoprenoids are essential for prenylation of certain proteins, such as RAS and Rho,which are both important proteins in myeloma and lymphoma. Prenylation is necessary for membrane localization and for the participation of these proteins in various signaling pathways.[3]

In vitro, HMG-CoA reductase inhibitors induced apoptosis[4,5] and inhibited proliferation[6,7] in myeloma and lymphoma cells in a dose-and time-dependent way. Statins sensitized myeloma and lymphoma tumur cells to various chemotherapeutic agents[6,8,9] and succeeded in overcoming cell adhesion-mediated drug resistance in myeloma cells, probably due to the inhibition of the geranylgeranylation of Rho.[10] Incubation of myeloma tumor cells with lovastatin resulted in the down-regulation of Mcl-1, an important anti-apoptotic protein in myeloma.[7,11] Phase I and II studies on high-dose HMG-CoA reductase inhibitors as single agent anticancer therapy have been performed on solid tumors[12] and separately on astrocytoma,[13] and gastric cancer.[14] Lovastatin could be given safely at a dose of up to 25 mg/kg/day. The objectives of this study were to determine the maximum tolerated dose (MTD) of simvastatin, which is twice as potent as lovastatin, administered prior to standard chemotherapy for myeloma and lymphoma and to establish whether *in vivo* blockade of the mevalonate pathway was achieved by the MTD.

Design and Methods

Patients under the age of 75 years old with multiple myeloma or non-Hodgkin's lymphoma who had been treated with at least two lines of chemotherapy, including treatment with anthracyclines, and World Health Organization (WHO) performance status 0-2 were eligible for enrollment. Adequate hepatic and renal function (clearance of ≥40 mL/min) was required. The study was conducted according to the Declaration of Helsinki and was approved by the University Medical Center Utrecht institutional review board (01/051-E). All patients gave written informed consent.

The study was an open phase I dose escalation study. The initial dosage was simvastatin 5 mg/kg/day orally for 7 consecutive days, divided in two doses. The last dose of simvastatin was given on the morning of day 7, before the start of chemotherapy. Patients with myeloma received a shortened, full dose of vincristine 1.6 mg/m^2, adriamycin 36 mg/m^2, over 2 days on days 7 and 8 and dexamethasone 40 mg on days 7-10 (VAD). The lymphoma patients were treated with CHOP (cyclophosphamide 750 mg/m^2, vincristine 2000 μg, doxorubicin 50 mg/m^2 and prednisone 100 mg over 5 days) on day 7. Patients who responded or whose disease was stable, could receive a maximum of three cycles of chemotherapy.

The dose of simvastatin was escalated by 2.5 mg/kg performed unless grade III or IV non-hematologic toxicity was observed, excluding neutropenic fever grade 3. Three patients were studied at each dose level. If one out of the three patients developed dose-limiting toxicity (DLT), three more patients were enrolled at the same dose level. If two patients

at one dose level developed DLT, the subsequent cohort was enrolled at the previous dose level. If no grade III or IV toxicity was detected, this level was considered the MTD. Once the MTD had been determined, additional patients were included at this dose level to confirm feasibility and to determine the *in vivo* effects of simvastatin on the mevalonate pathway and the anti-apoptotic protein Mcl-1.

The patients underwent standard pretreatment evaluation. Cardiac function was evaluated by MUGA scan. Blood chemistry tests and peripheral blood counts were repeated on days 7 and 21 to check for toxicity, especially for rhabdomyolysis. In five patients bone marrow aspiration was repeated on day 7 to evaluate the *in vivo* effects of simvastatin. In six patients cholesterol levels were measured before and after 7 days of treatment with simvastatin.

Responses were evaluated using the EBMT myeloma response criteria and according to the recommendations of the Non-Hodgkin's Lymphoma International Working Group.[15,16] Adverse events were evaluated according to Common Toxicity Criteria version 3.0 (CTCAE v 3.0).

Plasma cells were purified by magnetic activated cell sorting on the basis of CD138 expression.[6] Mononuclear cells were analyzed by flow cytometry as described previously.[17] Western blots were performed as described elsewhere.[6]

The two-tailed Student's t-test and the Mann Whitney U test were used for the statistical analyses. p values <0.05 were considered statistically significant.

Results and Discussion

A total of 28 patients were enrolled in the study between August 2002 and February 2005. The baseline characteristics of these patients are shown in Table 1. All patients had been heavily pretreated and had received a median of four previous chemotherapy regimens (range 2-7). All patients could be assessed for toxicity.

Dose escalation

The initial dosage was 5 mg/kg followed by chemotherapy. At the dose level of 17.5 mg/kg two patients experienced DLT. We subsequently treated ten more patients at the dose level of 15 mg/kg to confirm DLT and MTD.

Toxicity

Non-hematologic toxic effects are shown in Table 2A. The most frequently reported side-effect was fatigue (57%), but this did not exceed grade 2 toxicity. Treatment with a dosage of 12.5 mg/kg and under mainly resulted in gastrointestinal complaints and fatigue. Three patients also developed neutropenic fever, necessitating hospitalization in two cases. At the dose level of 15 mg/kg one patient, with a history of depression, developed severe depression and attempted suicide. One patient who received 17.5 mg/kg simvastatin died 2 days after chemotherapy due to overwhelming neutropenic sepsis. Another patient receiving this dose developed grade 2 diarrhea and stopped taking simvastatin on day 4; the other patient had grade 3 nausea and grade 3 diarrhea and simvastatin had to be stopped on day 5. According to the dose-finding protocol ten patients were then treated with 15 mg/kg to confirm the MTD. One patient developed neutropenic fever and had to be admitted to hospital. Another patient at this dose level was hospitalized after 4 days of simvastatin therapy with neutropenic fever and severe dehydration. This patient had already been suffering from diarrhea before the start of therapy, and this worsened severely with simvastatin. She developed septicemic shock, complicated by acute tubular necrosis. She died 3 months later as a consequence of the progression of multiple myeloma. The other patients treated with 15 mg/kg mainly experienced fatigue and gastrointestinal problems. Liver enzymes did not increase in any of the patients, except in the woman with septicemic shock. No rhabdomyolysis occurred. There was no clear cumulative toxicity: the first cycle was a good predictor of how the next cycles would be experienced.

Hematologic toxicity is shown in Table 2B. Neutropenia grade ≥3 occurred in 38% of the cycles. The nadir white cell count occurred a mean of 11.5 days after chemotherapy. Altogether six out of 28 patients developed neutropenic fever. Other grade ≥3 hematologic toxic effects occurred less frequently (leukopenia 29%, thrombocytopenia 17% and anemia 2% in all cycles at all dose levels).

Efficacy

Twenty-three patients were evaluated after completing at least one cycle. The overall response rate was 30%. One patient with a diffuse large B-cell lymphoma treated with 5 mg/kg achieved a complete response which is still ongoing following a subsequent non-myeloablative allogeneic stem-cell transplantation. Three patients (two with

Table 1. Patients' characteristics.

Patient characteristics	*No.(%)**
Total no. of patients	28
Sex	
Female	12 (43)
Male	16 (57)
Age, years	
Mean	56
Range	27-72
Disease	
Multiple myeloma	19 (68)
Non-Hodgkin's lymphoma	9 (32)
Previous lines of chemotherapy	
Median	4
Range	2-7
Previous treatment for MM	
Anthracyclines	19 (100)
Thalidomide	18 (95)
Bortezomib	4 (21)
Stem cell transplantation	13 (68)
Previous treatment for NHL	
Anthracylines	9 (100)
Autologous stem cell transplantation	5 (56)
Refractory or relapsed patients	
Relapse	15(54)
Refractory	13(46)
Hematologic values at start	
Well cell count less than 3.0×10^9/L	3
Hemoglobin at least grade 2	6
Platelet count less than 75×10^9/L	4

**Values in this column are number of patients, and values in paretheses are percentages, except when indicated otherwise in the row headings.*

Table 2A. Non-hematologic toxicity.

	Simvastatin dose level, mg/kg													
	5		7.5		10		12.5		15		17.5			
No.of patients	3		3		3		3		13		3		Total	
Grade	1-2	≥3	1-2	≥3	1-2	≥3	1-2	≥3	1-2	≥3	1-2	≥3	1-2	≥3
Fatigue	1	–	3	–	1	–	1	–	7	–	3	–	16	–
Nausea	–	–	–	–	2	–	1	–	6	–	2	1	10	1
Diarrhea	–	–	2	–	2	–	–	–	3	1	1	1	8	2
Anorexia	–	–	1	–	2	–	–	–	5	–	1	–	9	–
Fever	1	–	–	–	–	–	1	–	–	–	1	–	3	–
Febrile neutropenia	NA	–	NA	2	NA	1	NA	–	NA	2	NA	1	NA	6
Renal	–	–	–	–	–	–	–	–	–	1	–	–	–	1
Mucositis	–	–	–	–	–	–	–	–	1	–	–	–	1	–
Myalgia	–	–	–	–	–	–	1	–	1	–	–	–	2	–
Depression	–	–	–	–	–	–	–	–	2	1	–	–	2	1
Muscle weakness	–	–	–	–	–	–	1	–	1	–	–	–	2	–

NA: not applicable .

Table 2B. Hematologic toxicity.

	Simvastatin dose level, mg/kg													
	5		7.5		10		12.5		15		17.5		Total	
Cycles	9		7		8		5		22		1		52	
Grade	1-2	≥3	1-2	≥3	1-2	≥3	0-2	≥3	0-2	≥3	0-2	≥3	0-2	≥3
Anemia	7	–	6	v	4	–	2	–	16	1	1	–	36	1
Thrombocytopenia	5	–	3	3	2	1	1	1	7	4	–	–	18	9
Leukopenia	3	2	–	4	1	2	3	–	12	7	–	1	19	15
Neutropenia	3	–	–	4	1	2	1	1	1	12	–	1	6	20

multiple myeloma and one with non-Hodgkin's lymphoma) had a partial response. Three patients had a minimal response. Five had stable disease. The mean time to progression was 2 months. There was no correlation between dose level and response rate. (p=0.522).

As a surrogate pharmacodynamic end-point of *in vivo* simvastatin activity, cholesterol levels were measured in six patients who received simvastatin 15 mg/kg. The mean cholesterol level at the start of treatment was 4.65 mMol/L (range 2.7-6.3). After 7 days of simvastatin therapy, the mean cholesterol level was 2.48 mMol/L (range 1.3-3.4; p=0.0033). Cholesterol levels normalized in the 21 days after simvastatin therapy had been stopped. The same decrease in cholesterol levels was seen after the second and third cycles of therapy.

The effect of *in vivo* simvastatin treatment on Mcl-1 protein expression in myeloma plasma cells was analyzed using flow cytometry of bone marrow nuclear cells collected at the start and after 7 days of simvastatin, before the administration of chemotherapy. In all patients investigated by flow cytometry for this parameter (n=5) simvastatin induced a significant down-regulation of Mcl-1 in bone marrow myeloma plasma cells (p=0.0207) (Figure 1). This down-regulation was confirmed by western blot analysis in the purified plasma cells of two patients. There were no significant effects on Mcl-1 expression in monocytes, T cells and B cells as determined by flow cytometry (*data not shown*).

Western blot analysis demonstrated inhibition of prenylation in two patients. In one patient partial inhibition of farnesylation of Dna-J was seen in purified plasma cells

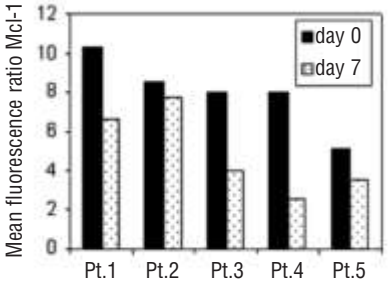

Figure 1. Mean fluorescence ratio of Mcl-1 measured in CD38/138 cells from five patients before and after therapy with 15 mg/kg simvastatin for 7 consecutive days.

and in the second patient partial inhibition of geranylgeranylation of Rap-1A was observed in bone marrow mononuclear cells (*data not shown*).

In vitro data show that inhibition of the mevalonate pathway by HMG-CoA inhibitors results in apoptosis and sensitization of tumor cells to chemotherapeutic agents by inhibition of prenylation leading to down-regulation of anti-apoptotic proteins.[4] This prompted us to evaluate the safety and tolerability of statins followed by conventional chemotherapy in end-stage myeloma and lymphoma. The rationale for performing a dose-escalation study was the dose- and time-dependent effects of statins demonstrated *in vitro*. The rationale for the sequential administration of chemotherapy was based on previous preclinical and phase I studies. Preclinical studies had shown that the chemo-sensitization of myeloma plasma cells by statins requires several days of pre-incubation.[6,8] A phase I study showed that the expected maximum peak levels of serum simvastatin could synergize effectively with doxorubicin

and dexamethasone but could not induce spontaneous apoptosis in myeloma and lymphoma tumor cells.[12] No dexamethasone pulse was given in between since simvastatin has a short half-life, such that the effect of simvastatin on anti-apoptotic proteins is transitory. We determined that the MTD of simvastatin was 15 mg/kg/day for 7 days in association with systemic chemotherapy. The most frequently reported non-hematologic side-effects were fatigue, nausea and diarrhea. The dose-limiting toxicities were gastrointestinal complaints and neutropenic sepsis. Although the high incidence of neutropenia (38% grade ≥3) in this heavily pretreated group of patients may not be unusual after chemotherapy, it cannot be excluded that simvastatin followed immediately by chemotherapy enhances bone marrow suppression. The last cohort of patients received prophylactic antibiotics. We did not find any correlation between cholesterol levels and the development of toxicity. A significant down-regulation of Mcl-1 in myeloma tumor cells was seen. In addition, inhibition of farnesylation and geranylgeranylation was observed.

Seven out of the 23 patients (30%) who could be evaluated responded. It cannot, however, be concluded from this study that simvastatin pre-treatment contributed to the response and disease stabilization. Patients previously treated with anthracyclines and prednisone/dexamethasone may respond again to regimens containing these agents. It is, however, worth noting that three out of the seven patients who responded were refractory to CHOP or VAD. We conclude that simvastatin can be administered safely with acceptable toxicity at a dose of 15 mg/kg for 7 days followed by standard chemotherapy and that at this dose level *in vivo* down-regulation of Mcl-1 and inhibition of prenylation is achieved. Based on the current study a phase 2 trial on the use of statins in patients with refractory multiple myeloma has been started.

EvdS: executive investigator; HL: primary responsibility for the study, AB and NvdD participated in the design and execution of the study including the in vitro studies; LB: primary responsibility for the execution of the in vitro studies; RvdG, MK, OdW, HL, SW treated and evaluated the patients; EvdS, HL: wrote the paper, all authors checked the final version of the manuscript.

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