Simvastatin-dependent apoptosis in Hodgkin’s lymphoma cells and growth impairment of human Hodgkin’s tumors in vivo

Bastian von Tresckow, Elke Pogge von Strandmann, Stephanie Sasse, Samir Tawadros, Andreas Engert, Hinrich P. Hansen

From the Laboratory of Immunotherapy, Clinic I of Internal Medicine, University Hospital Cologne, Cologne, Germany, Center for Molecular Medicine, University Hospital Cologne, Cologne, Germany.

Acknowledgments: we would like to thank Mrs. Anne Kuressmann and Mr. Hermann Straub for their expert technical assistance.

Funding: supported by grants from the Koeln Fortune Program/Faculty of Medicine, University of Cologne (102/2005) (BvT) and the Deutsche Krebshilfe (106473) (HPH).

Statins are used to treat hypercholesterolemia and seem to have a preventive effect against cancer through pleiotropic effects including prenylation-inhibition. So far nothing is known about the activity of statins or more specific prenylation-inhibitors in Hodgkin’s lymphoma (HL). We, therefore, evaluated the anti-HL activity of simvastatin and specific prenylation-inhibitors. Two µM Simvastatin induced caspase-related apoptosis via depletion of prenylation-substrates in several HL-cell lines. Furthermore, it effectively impaired tumor growth in a mouse model for HL. Since the prenylation-inhibitors FTI-277 and GGTI-298 were also effective against HL-cells, we conclude that statins and specific prenylation-inhibitors should be evaluated in HL patients.

Key words: lymphoma, apoptosis, growth control, statins.

Haematologica 2007; 92:682-685
©2007 Ferrata Storti Foundation

Design and Methods

Cell lines, antibodies and reagents
HL-cell lines (DEV, KM-H2, L540, L540Cy, HD-LM2, L428) and the erythroleukemia cell line K562 have been previously described.4,5 All in vitro experiments used an initial concentration of cells of 2x10⁶/mL. Anti-poly (ADP-ribose) polymerase (PARP) antibody (#9542) was from Cell Signalling Technology. Anti-Actin antibody (sc-1616-R) was from Santa Cruz. Annexin V-FITC Apoptosis Detection Kit I was from BD Biosciences. Suicide-Track™ DNA Ladder Isolation Kit, simvastatin (sodium salt), FTI-277 and GGTI-298 were from Calbiochem. Complete protease

ABSTRACT

Statins are used to treat hypercholesterolemia and seem to have a preventive effect against cancer through pleiotropic effects including prenylation-inhibition. So far nothing is known about the activity of statins or more specific prenylation-inhibitors in Hodgkin’s lymphoma (HL). We report how simvastatin promotes caspase-related apoptosis in HL-cells via the isoprenoid pathway and exhibits high anti-tumor activity in a xenograft model for human HL. Both statins and prenylation-inhibitors could therefore have preventive or therapeutic potential for the treatment of HL.

Today, most patients with Hodgkin’s lymphoma (HL) can be cured, but the treatment of patients with progress or relapse needs to be improved. In addition, late treatment-related toxicities are seriously affecting up to 30% of patients. Hence, new therapies should be established. There is growing evidence for a preventive effect of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA)-reductase-inhibitors, known as statins, in a wide range of cancers.1 Recently, the EPILYMPH study indicated a reduced risk of lymphoma, including HL, in statin-treated patients.2 Statins are normally used to treat hypercholesterolemia and prevent cardiovascular disease. They block the conversion of HMG-CoA to mevalonate and thus the synthesis of downstream mevalonate products like farnesyl-transferase (FTase) substrate farnesyl-pyrophosphate and geranylgeranyl-transferase (GGTase) substrate geranylgeranyl-pyrophosphate. By depleting these isoprenoids, statins inhibit prenylation of anti-apoptotic proteins such as ras and ras-related molecules and induce apoptosis in several malignancies. More specific prenylation-inhibitors are currently being evaluated in clinical trials.3 So far nothing is known about the therapeutic action of statins or specific prenylation-inhibitors in HL. We report how simvastatin promotes caspase-related apoptosis in HL-cells via the isoprenoid pathway and exhibits high anti-tumor activity in a xenograft model for human HL. Both statins and prenylation-inhibitors could therefore have preventive or therapeutic potential for the treatment of HL.

Correspondence:
Bastian von Tresckow, M.D., University Hospital Cologne, Laboratory of Immunotherapy, Department of Internal Medicine I, LFI-Eb.4, room 703, Kerpener Str. 62, 50924 Cologne, Germany.
E-mail: bastian.von-tresckow@uk-koeln.de
inhibitor tablets were from Roche. All other reagents were from Sigma. Dimethylsulfoxid (DMSO) was used to prepare stock solutions of simvastatin (5 mg/mL), FTI-277 (4.46 mg/mL) and GGTTI-298 (4.46 mg/mL). Ethanol was used to prepare stock solutions of Farnesol (50 mg/mL) and Geranylgeraniol (50 mg/mL).

**XTT assay**

XTT assay was performed as reported. After incubation of 200 µL cell aliquots in 96-well plates, 50 µL of culture medium with tetrazolium compound and electron-coupling reagent was added. Absorbance was measured after 20 h.

**Apoptosis assays**

Annexin V-binding assay, DNA ladder isolation and immunoblotting of PARP were performed according to the manufacturers’ instructions.

**Xenograft model of human HL**

The subcutaneous xenograft model of human HL has been previously described. Simvastatin tablets (ZOCOR, MSD) were mixed with food and pressed into pellets by Altromin GmbH at a ratio of 1.67 mg of simvastatin per 1 g of food. Untreated animals received pellets without simvastatin. Simvastatin-pellets were mixed in different ratios with untreated food, and uptake was measured by weighing the remaining simvastatin-pellets twice weekly. The Institutional and national guides for the care and use of laboratory animals were observed.

**Results and Discussion**

**Anti-tumor activity of simvastatin in HL-cells via caspase-related apoptosis**

Cellular viability was analyzed after simvastatin treatment. Four out of six HL-cell lines were sensitive to simvastatin in the XTT assay (IC50 < 2 µM) and the Annexin V-binding assay (Figures 1A-B). Apoptosis induction was verified by DNA ladderning in L540 cells (Figure 1C). Increased DNA laddering after simvastatin treatment was also detectable in L540Cy cells but very weak in DEV and KM-H2 cells (data not shown). Whereas L540 and L540Cy cells displayed constitutive DNA ladderning dependent on cell number (Figure 1C, lane 2). These apoptotic DNA fragments were barely detectable in DEV and KM-H2 cells even when large amounts were analyzed. Therefore, DNA laddering might not be a suitable assay for detection of simvastatin-related apoptosis in the latter cell lines. Consequently, a PARP cleavage immunoblot was performed (Figure 1D). The increase in the 89 kD cleaved PARP fragment in simvastatin treated KM-H2 cells confirmed caspase-related apoptosis to be the major mechanism of simvastatin-induced cell death.

Simvastatin treatment also increased PARP cleavage in L540 cells. However, high constitutive PARP cleavage in untreated L540Cy and DEV cells meant this test was not suitable to use for these cells (data not shown).

Statins have previously been reported to have cyto-
induction of apoptosis by statins has This could be because effective statin Hence, high dose simvastatin before M were of the mevalonate pre-

Figure 2. Prenylation-dependent cytotoxicity of simvastatin in HL-cells. (A-B) XTT assays after 5 days exposure of L540Cy cells to 2 mM simvastatin (Simva) ± 100 µM mevalonolactone (Mev) (A), 10 µM farnesol (FOH) and/or 10 µM geranylgeraniol (GGOH) (B). Mean (% untreated) ± SD of at least three independent experiments. (C-D) XTT assays after 48 hours exposure of L540 (C) or KM-H2 (D) cells to FTI-277 (R) or GGTI-298 (L) with specification of vehicle (DMSO, ∆) concentration as indicated. Mean (% untreated) ± SD of at least three independent experiments.

mals in the treatment group (2/7). Only by day 29 had all mice in the treatment group developed tumors. Measurement of established tumors beginning at day 26 revealed that the average tumor-volume of control mice increased four-fold in 6 days, while there was no increase in the tumor-volume of treated mice.

These data confirm that simvastatin is effective in a xenograft model of human HL. To date, there have been few reports of successful in vivo experiments with statins in xenograft models for human hematological malignancies. This could be because effective statin concentrations are difficult to obtain in vivo. In a recent study in patients with relapsed or refractory myeloma or lymphoma, high-dose simvastatin (15 mg/kg/d) given immediately prior to chemotherapy has been found to be safe and tolerable. Since mice metabolize statins more rapidly than humans, a dose of 15 mg/kg/d is comparable to 202 mg/kg/d used in our mouse model. Hence, high dose simvastatin before chemotherapy is a promising combination for the treatment of patients with HL.

Given the preventive effect of statins reported in the EPILYPH study and the results of this report, it could be interesting to evaluate simvastatin at a dosage of 80 mg/d as a preventative drug for high risk HL patients in remission. Furthermore, statins have been seen not only to increase antitumor action but also to reduce cardiotoxicity of doxorubicin in tumor models in animals. Because doxorubicin is part of most regimens used in HL patients and heart failure is one of the most common late toxicities, it would also be interesting to evaluate a potential cardio-protective effect of simvastatin in HL patients.

Anti-tumor activity of prenylation-inhibitors in HL-cells

Besides inhibiting HMG-CoA conversion to mevalonate and causing subsequent inhibition of protein prenylation, induction of apoptosis by statins has been attributed to pleiotropic effects such as inhibition of the proteasome, inhibition of surface molecule interactions, or displacement of signalling proteins from lipid rafts. The complete reversal of simvastatin-induced cell death by 100 µM of the mevalonate precursor mevalonolactone (Figure 2A) indicates that the effect of simvastatin in HL depends on HMG-CoA reductase. Since farnesol and geranylgeraniol, which are precursors of the mevalonate downstream metabolites farnesyl-pyrophosphate and geranylgeranyl-pyrophosphate respectively, both led to a partial inhibition of simvastatin-induced cell death (Figure 2B) we hypothesized that prenylation-inhibitors also have a cytotoxic effect on HL cells. Indeed, the FTase-inhibitor FTI-277 and the GGTase-inhibitor GGTI-298 induced dose-dependent cell death in two HL-cell lines (Figures 2C-D). Only inhibitor concentrations up to 50 µM were tested in L540 cells because of the significant toxicity
encountered in the vehicle DMS at >0.5%. However, in KM-H2 cells, inhibitor concentrations up to 100 µM could be tested (Figures 2C-D). In pre-clinical tests, the FTase-inhibitor FTI-277 acted on malignancies and the well-tolerated therapeutic FTase-inhibitor tipifarnib is especially effective in hematological neoplasia. It is important to note that FTI-277 in HL-cells exhibits a relatively high cytotoxicity, whereas it has little effect on the proliferation of Non-HL-cell lines. In conclusion, therapeutic FTase-inhibitors like tipifarnib in HL merit clinical evaluation. Indeed, this study provides further evidence that HL cells are sensitive not only to an FTase-inhibitor but also to a GGTase-inhibitor. Consequently, therapeutic dual FTase and GGTase-I-inhibitors could be even more effective in HL.

Authors’ Contributions

BvT designed and performed research, analyzed data and wrote the paper; EPvS, AE and HPH designed research and analyzed data; SS and ST performed research and analyzed data; and all authors checked the final version of the manuscript.

Conflict of Interest

The authors reported no potential conflicts of interest.

References