Induction of cell death by HBI-8000, an HDAC inhibitor, in adult T-cell leukemia/lymphoma (ATL) is associated with activation of Bim and NLRP3

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ABSTRACT

Adult T-cell leukemia/lymphoma (ATL) is an aggressive T-cell malignancy caused by human T-cell lymphotropic virus 1 (HTLV-1). Treatment options for acute ATL patients include chemotherapy, stem cell transplantation, and most recently the anti-CCR4 antibody mogamulizumab, though most patients still have a poor prognosis and there is a clear need for additional options. HBI-8000 is a novel oral histone deacetylase inhibitor (HDACi) with proven efficacy for treatment of T-cell lymphomas that recently received marketing approval in China. In the present study, we evaluated the effects of HBI-8000 on ATL-derived cell lines and primary ATL cells obtained from Japanese ATL patients. In most cases HBI-8000 induced apoptosis in both primary ATL cells and ATL cell lines. In addition, findings obtained with DNA and apoptosis-related antibody microarrays revealed the contributions of NFκB inhibition, activation of Bim, and, interestingly, the NLRP3 inflammasome pathway in HBI-8000-induced ATL cell death. Further investigations using siRNAs confirmed that Bim contributes to HBI-8000 induced apoptosis. Our results provide a rationale for a clinical investigation of the efficacy of HBI-8000 in patients with ATL. Although the role of NLRP3 inflammasome activation in ATL cell death remains to be verified, HBI-8000 may be part of a novel therapeutic strategy for cancer based on the NLRP3 pathway.

METHODS

Primary ATL cells and ATL-derived cell lines

11 PBMC samples were obtained from 10 patients with aggressive type ATL, based on Shimoyama's diagnostic criteria, and contained more than 85% leukemic cells. The ATL-derived lines KOB, KK1, ST1, SO4, LMY1, LMWT5 and LMY2 were established from aggressive type ATL patients. KOB, LMY1, LMY2, and ST1 cells have wild-type p53.

DNA microarray analysis

Total RNA was extracted and biotinylated cRNA was fragmented and hybridized to Agilent SurePrint G3 Human Gene Expression 8x60K v2.0. The arrays were scanned using the Gene Array Scanner and analyzed using the DNA-Chip Analyzer.

Quantitative real-time RT-PCR

mRNA levels were measured using a LightCycler480 PCR System.

PathScan stress and apoptosis signaling array

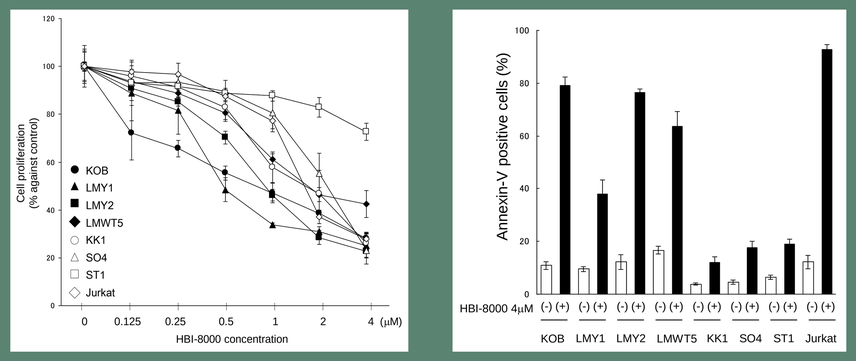
This assay allows for simultaneous detection of 19 different signaling molecules that are involved in stress response and apoptosis. Capture antibodies were spotted onto nitrocellulose-coated glass slides, whole-cell lysates were incubated on the slides, followed by a biotinylated detection antibody cocktail. Slide images were captured with a LAS3000 image analyzer and spot signals were quantified.

Transfection and small interfering RNA experiments

Two different small interfering RNAs (siRNAs) were used against each target. For Bim Silencer Select Validated siRNA s195011 (#1) and Silencer Select siRNA s195012 (#2), were used, and for NLRP3 Silencer Select siRNA s41555 (#1), s41556 (#2) and control siRNA (Silencer negative control #1) were used, respectively. The effect of each siRNA was by monitoring target mRNA and protein (data not shown).

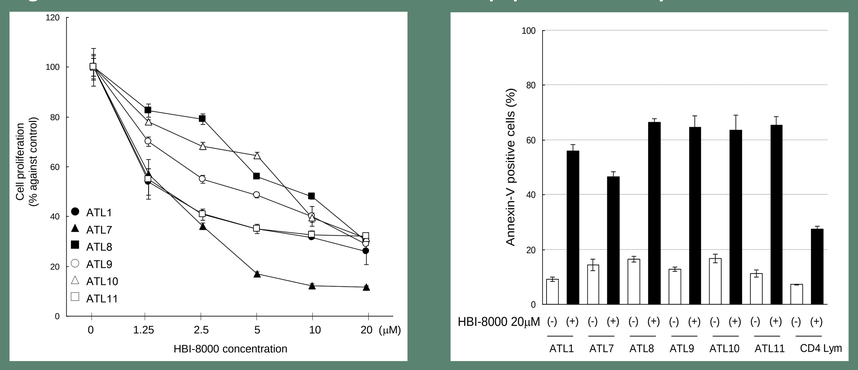
RESULTS

Figure 1. HBI-8000 Induced Growth Inhibition and Apoptosis an ATL-derived Cell Lines.



Cells (2-5×10^5 /mL) were treated with either the vehicle or indicated concentrations of HBI-8000 for 72 hours, then cell proliferation (MTS) or apoptosis (Annexin-V) were evaluated. All experiments were performed in triplicate and the results are expressed as the mean ± SD.

Figure 2. HBI-8000 Induced Growth Inhibition and Apoptosis in Primary ATL Cells



Cells (2-5×10^5 /mL) were treated with either the vehicle or indicated concentrations of HBI-8000 for cell proliferation (72 hours; MTS) or apoptosis (48 hrs; Annexin-V). All experiments were performed in triplicate and the results are expressed as the mean ± SD.

Figure 3. HBI-8000 Induced Changes in Histone Acetylation, Tumor Suppressor (p21, p53) and NFκB-related Protein Expression in ATL Cell Lines (a, b, c) and Primary ATL Cells (d, e, f)

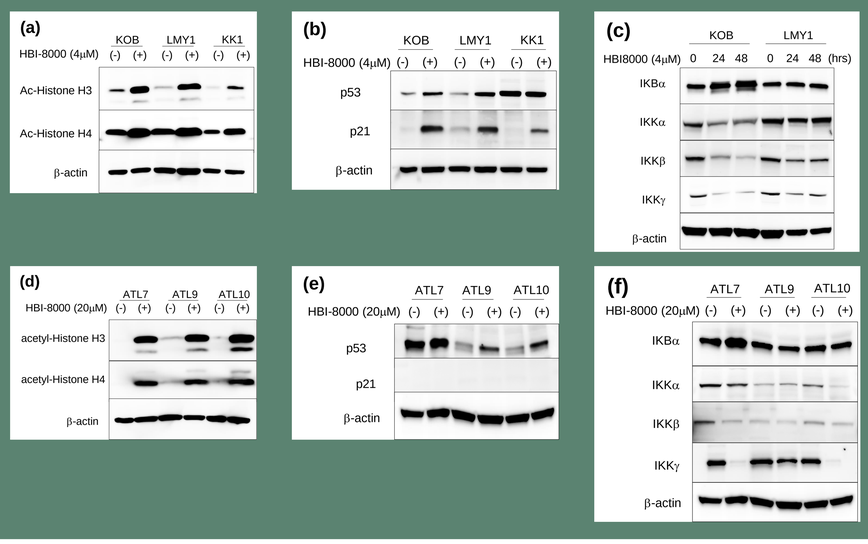


Figure 4. HBI-8000 Induced Changes in Apoptosis-related Protein Expression and Bim mRNA in ATL-derived Cell Lines (a, c) and Primary ATL Cells (b, c)

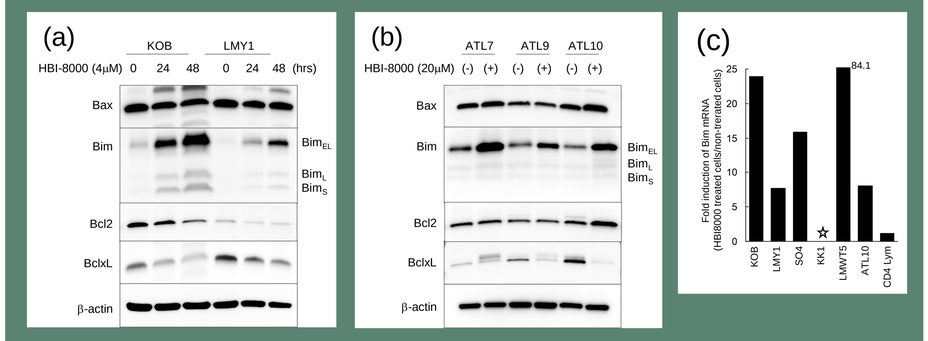


Figure 5. HBI-8000 Induced Changes in Intrinsic Apoptotic and NLRP3 Pathway-related Proteins (a, b) and mRNA (c)

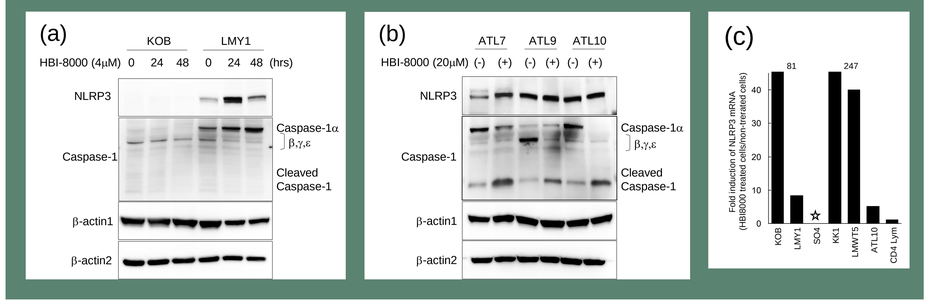
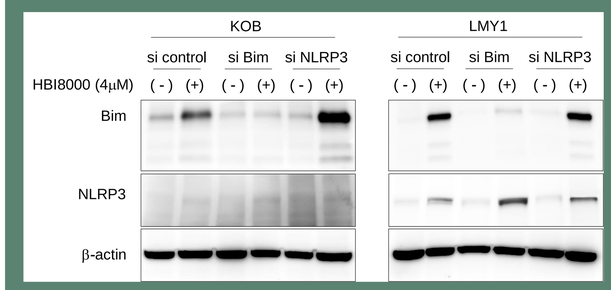


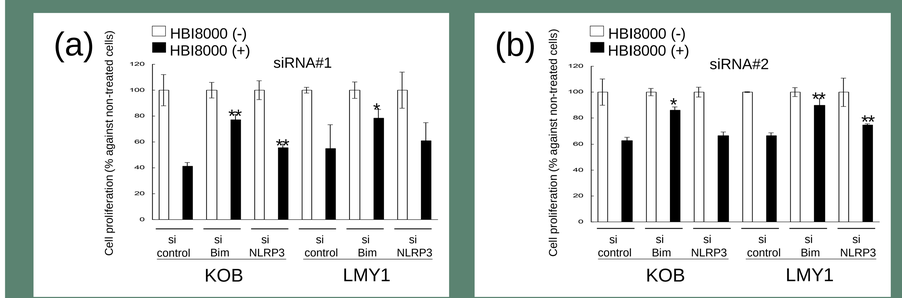
Figure 6. si RNA Knockdown Experiments to Evaluate the Role of Bim and NLRP3 in HBI-8000 Induced Growth Inhibition (a, b) and Apoptosis Induction (c , d, e, f) in ATL Cells

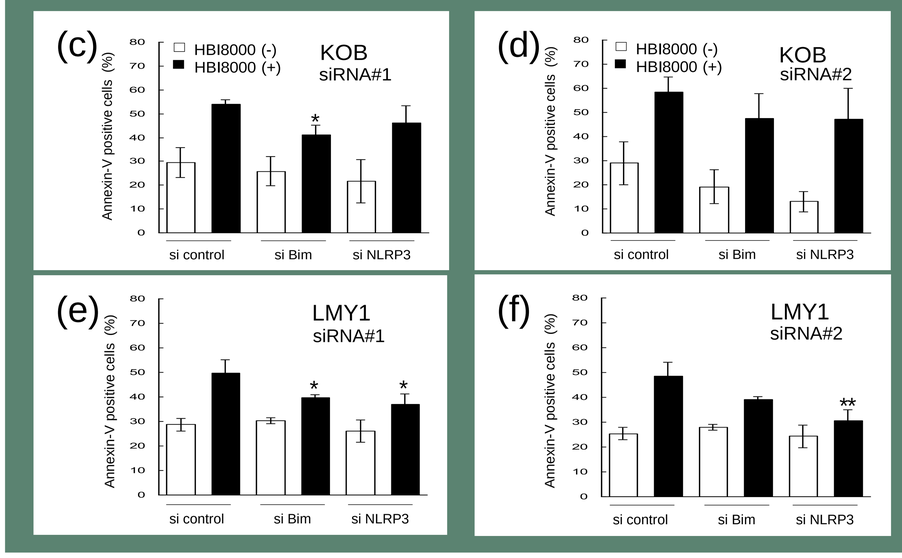


si RNA

BIM
Silencer Select siRNA s195011
Silencer Select siRNA s195012

NLRP3
Silencer Select siRNA s41555
Silencer Select siRNA s41556





Summary and Conclusions

1. The class I selective HDAC inhibitor HBI-8000 causes growth inhibition and induces apoptosis in ATL-derived cell lines and fresh primary ATL cells derived from patients
2. DNA and apoptosis-related antibody arrays demonstrate contributions of NFκB inhibition, activation of Bim and the NLRP3 inflammasome pathway in HBI-8000-induced effects in ATL cell lines and primary ATL cells
3. HBI-8000 upregulates expression of pro-apoptotic Bim and downregulates anti-apoptotic Bcl-XL; siRNA blockade of Bim prevented apoptosis induction
4. HBI-8000 demonstrates a unique ability to activate the NLRP3-caspase 1 inflammasome pathway in ATL cells, known to be activated in retrovirus infected cells, which has implications for the possible role of HBI-8000 as part of a novel therapeutic strategy for cancer and virus infection based on the NLRP3 pathway