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POSTER ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Probiotic-Derived Heptelidic Acid Demonstrates Therapeutic Efficacy Against Pediatric B-Cell Acute Lymphoblastic Leukemia

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Introduction

Pediatric B-cell acute lymphoblastic leukemia (B-ALL) is known to be sensitive to chemotherapy. However, approximately 15-20% of pediatric B-ALL patients experience relapse, and survival rates after relapse can drop to 5-10%. Therefore, new drugs need to be developed. Probiotics are live microorganisms with potential health benefits. Heptelidic acid (HA) from the probiotic *Aspergillus oryzae* has been shown to exert antitumor effects against pancreatic cancer, lung cancer, thyroid cancer, breast cancer and melanoma *in vivo* as well as *in vitro*. In this study, we assessed the antitumor effects of HA in B-ALL, which is the most common type of pediatric cancer.

Methods

Two B-ALL cell lines (JM1 and Reh), four CD34 $^{+}$ hematopoietic stem cells (HSCs) and three high-risk patient-derived B-ALL cells (s90: age < 1 year old, s96: age > 10 years old, s98: relapse) collected from the spleen or bone marrow of a patient-derived xenograft (PDX) model were used in this study. Cytotoxicity of HA was assessed by MTS assay. GAPDH activity and intracellular ATP contents were measured, as HA binds to cysteine 152 of GAPDH, a crucial region for its enzymatic activity and ATP production in glycolysis. The synergistic effects of HA with therapeutics, vincristine (VCR), doxorubicin (DXR) and L-asparaginase (L-Asp), were evaluated. GAPDH is involved not only in glucose metabolism but also in amino acid and fatty acids for energy production. Therefore, we analyzed whether HA exerted cytotoxicity under glucose, amino acid or fatty acid-deprived conditions in Reh cells. To analyze the underlying mechanism for HA's cytotoxicity against B-ALL, cell cycle analysis by flowcytometry, western blotting of cleaved PARP, caspase assays, and inhibitory assays using specific inhibitors for PCD mechanisms, such as RIPK-1 inhibitor, MLKL inhibitor, and autophagy inhibitor, were conducted. *In vivo* studies were done using a B-ALL PDX model. Oral administration of 0.5mg/kg HA was carried out for 21 days, while intraperitoneal injection of 0.15mg/kg VCR was performed once per week for 3 cycles in the HA-VCR combinational therapeutic efficacy study.

Results

HA exhibited significant cytotoxicity in JM1 (IC50: 169 ng/ml), Reh (IC50: 126.5 ng/ml), and three samples obtained from PDX mice (s90 IC50: 66.6 ng/ml, s96 IC50: 89.9 ng/ml, and s98 IC50: 275.6 ng/ml), while sparing CD34 $^{+}$ HSCs. GAPDH activity of CD34+ HSCs was significantly lower than both B-ALL cell lines and patient-derived cells. The cytotoxic effect of HA was associated with the reduction of GAPDH activity and ATP contents, suggesting glycolysis inhibition. Glucose deprivation induced significant reduction of cell viability, and HA showed the cytotoxicity in amino acid and fatty acid deprived media in Reh cells. Oral daily administration of HA prolonged the survival of the B-ALL PDX mice (34.5 days, n=8) compared to control mice (28 days, n=7) without causing body weight loss ($p<0.05$) (Figure 1A). The effects of over 0.5mg/kg of HA was confirmed by the reduction in GAPDH activity observed in leukemia cells isolated from the bone marrow. HA demonstrated synergistic effects when combined with chemotherapeutic agents, especially VCR. The combination therapy of HA and VCR resulted in improved survival outcomes in the B-ALL PDX model (49 days, n=5) compared to single-agent treatments (HA: 28 days, n=5, VCR: 40 days, n=5) and control groups (26 days, n=5) ($p<0.05$) (Figure 1B). Mechanistic studies revealed that HA induced PCD in B-ALL cells. HA treatment caused G2/M arrest (Control: 18.8%, HA: 27.6% in JM1; Control: 13.3%, HA: 18.3% in Reh), and this was accompanied by increased cleavage of PARP, a hallmark of PCD, in both cell lines. Moreover, the cytotoxicity of HA was attenuated by RIPK1 inhibition in JM1 and Reh.

Conclusion

We have demonstrated that B-ALL cells utilize glucose for their survival and HA is cytotoxic in B-ALL cells by disrupting glycolysis through the inhibition of GAPDH. The therapeutic efficacy of HA is further enhanced when combined with chemotherapeutic agents. The underlying mechanisms of HA involve the induction of RIPK-1 mediated PCD. This is the first study to demonstrate HA as a novel therapeutic agent for B-ALL.

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Disclosures No relevant conflicts of interest to declare.

Figure 1

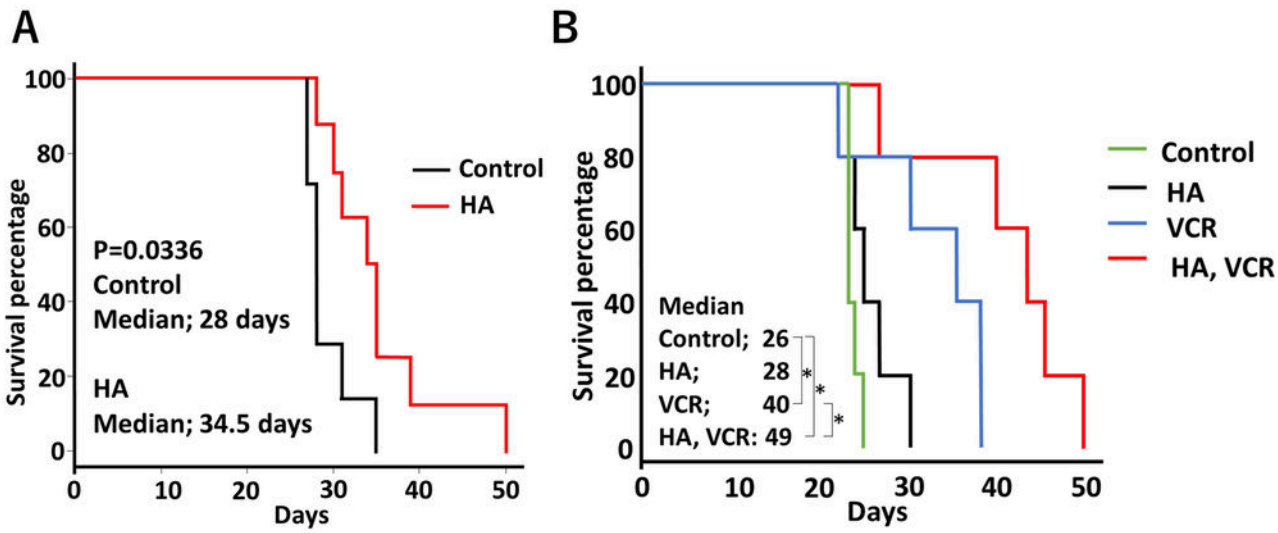

Figure 1

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