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Apoptotic Effect of Boron Derivatives on HL-60 Acute Promyelocytic Leukemia Cell Line †

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Abstract: Acute myeloid leukemia (AML) is the most common form of acute leukemia and the overall 5-year survival in AML patients is 40–45% in young patients and less than 10% in elderly patients. Therefore, there is still a need to improve therapeutic approaches. After development of boron-based anticancer drugs (such as Bortezomib), boron compounds have gained attention with possible anti-leukemic feature. In this study, we evaluated anti-tumoral effects of borax pentahydrate (BP) and disodium pentaborate decahydrate (DPD) on leukemic cell line (HL-60). Cell viability was assessed by MTT assay and apoptotic effect of the compounds were evaluated by flow cytometry and cleaved PARP level detection by western blot. We observed that BP (24 mM) and DPD (6 mM) exhibit anti-leukemic effect via induction of apoptosis. Our study suggest that BP and DPD have potential anti-leukemic effect, which needs to be confirmed by further wide-spectrum studies.

Keywords: acute myeloid leukemia; apoptosis; borax pentahydrate; disodium pentaborate decahydrate

1. Introduction

The cause of leukemia are explained by abnormal cell regulation, excessive, uncontrolled proliferation of hematopoietic stem cells in the bone marrow and it is one of the main causes of cancer related death in world-wide population [1]. AML is the most common form of acute leukemia and the 5-year survival of young patients is 40–45% and less than 10% for elderly patients [2]. Therefore, finding of new therapeutic approaches causing apoptotic cell death of leukemia cells have gain importance nowadays. Apoptosis is a well-documented programmed cell death in many cellular systems and it plays an important role in hematopoietic system regulation, therefore triggering of apoptosis is important for anti-leukemic therapeutic approaches [3]. Boron is natural trace element in human diet and according to the current studies, boron and its derivatives may have anticancer feature through multiple directions which include effecting of cell signalization and biochemical processes [4]. Previous studies have only revealed that DPD has greater capacity to induce cell death on solid cancer cell line (DU145), but there is no study pointing out its effects on leukemia. On the other hand, the limited studies with BP have shown its anti-leukemic effects but further studies are needed [5–7]. Therefore, the aim of this study was to evaluate the anti-proliferative effect of DPD and BP on AML cells and to find out whether the inhibition of cell viability was related to apoptosis. The apoptotic effects of DPD and BP on HL-60 cell line were assessed by flow cytometry analysis and the

detection of cleaved PARP levels by western blot. Our results showed that DPD and BP had anti-proliferative and apoptotic effects on HL-60 cell line. The study also revealed the comparison of the two boron compounds with their IC_{50} values and apoptotic effects on HL-60 cell line for the first time. Our results imply that DPD and BP have anti-leukemic potential on AML and can be used in the development of anti-AML drugs which is need further research.

2. Materials and Methods

2.1. Cell Culture

HL-60 cells were obtained from ATCC (CCL-240) and cultured in RPMI-1640 at 37 °C in 5% CO_2 incubator. Viability of cells were confirmed by using the trypan blue exclusion.

2.2. Cell Viability

The viability and IC_{50} values were determined by a colorimetric assay that measures the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,4,diphenyltetrazolium bromide (MTT) by metabolic active cells. Boron compounds were dissolved in sterile distilled water, and cytotoxic effects of the compounds were studied (1.5 mM, 3 mM, 6 mM, 12 mM, 24 mM BP and DPD) in concentration dependent manner according to the kit instructions. The absorbance of the culture plates were measured at 570 nm with Biotek ELX800 plate reader (Winooski, VT, USA).

2.3. Western Blotting

Cells were seeded in T25 flasks (2×10^6 cells). Protein was extracted from BP or DPD-treated cells with lysis buffer and protein concentration was determined with BCA assay (Pierce Chemical, Rockford, IL, USA) and 30 μg of total protein was loaded to each well. After performing SDS-PAGE, proteins were transferred to PVDF membranes by wet-transfer. Membranes were blocked and then blotted overnight at 4 °C with primary antibody (anti-PARP, anti-actin). After 1 h incubation with HRP-linked secondary antibody, detection was performed using the West ECL chemiluminescent substrate kit (Thermo Scientific, Rockford, IL, USA).

2.4. Flow Cytometry

Apoptotic and/or necrotic cell death was determined by flow cytometric analysis using an assay kit from BD PharMingen (San Diego, CA, USA) with Navios Flow Cytometer (Beckman Coulter, Miami, FL, USA).

2.5. Statistical Analysis

All experiments were carried out for three times, results were expressed as the mean ± SD. Statistical significance was determined by Oneway-ANOVA tests, with $p < 0.05$.

3. Results

3.1. MTT (Cell Viability)

24 mM BP and 6 mM DPD treatment decreased cell viability approximately 50% at 24h and 48h and 24h was chosen to analyze the first response of the compounds (Figure 1).

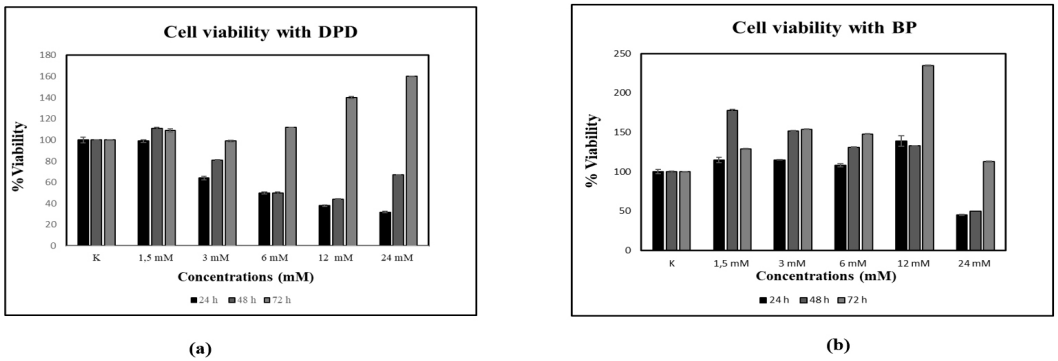


Figure 1. Effects of DPD (**a**) and BP (**b**) on cell viability. The results are expressed as mean ± SD.

3.2. Flow Cytometry

Flow cytometric analyses showed that BP (24 mM) and DPD (6 mM) treatment significantly increased apoptotic cell death in HL-60 cells compared to non-treated control cells. These results also indicate that BP (24 mM) induced apoptotic cell death rate is significantly higher than DPD (6 mM) induced apoptotic cell death rate (Figure 2).

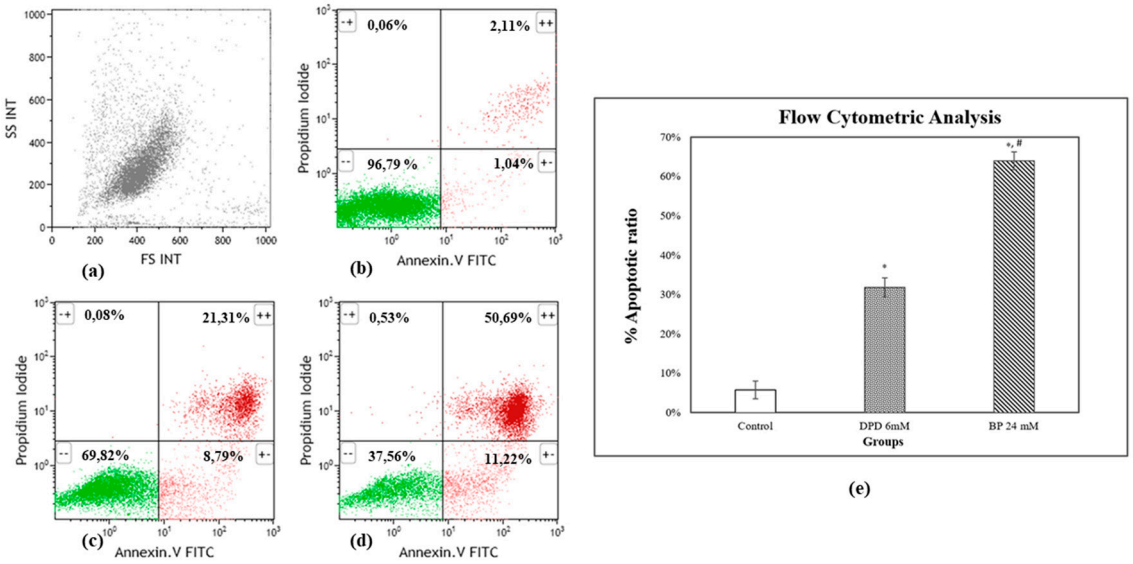
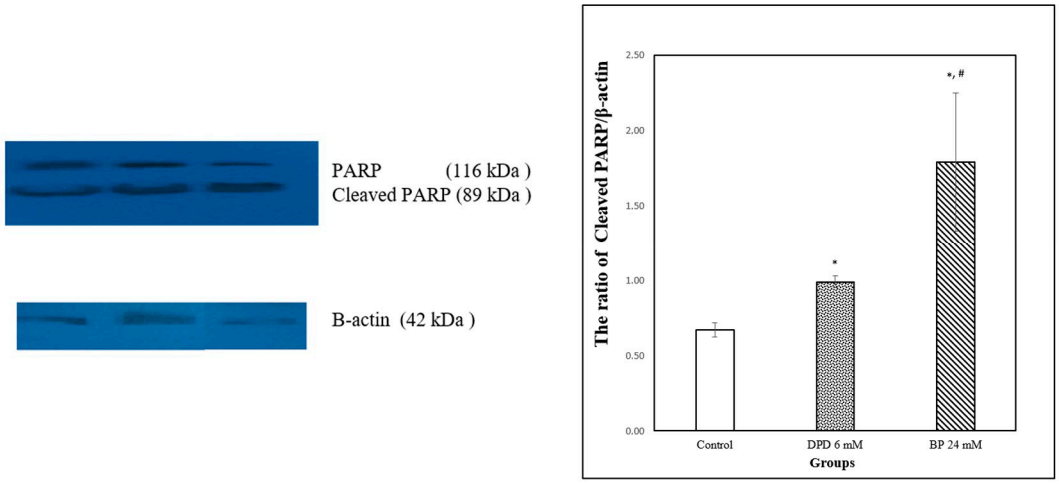


Figure 2. (**a**) Flow cytometric analyses of HL-60 cells Morphological analysis (**b**) Diagram of Annexin V-FITC/PI of control cells. (**c**) Diagram of Annexin V-FITC/PI DPD-treated HL-60 cells. (**d**) Diagram of Annexin V-FITC/PI BP-treated HL-60 cells. (**e**) Apoptotic cell ratios among the groups. Comparison between control and DPD or BP. The results are expressed as mean ± SD., * $p < 0.05$. Comparison between DPD and BP, # $p < 0.05$.

3.3. Western Blot Analysis

Our results showed that PARP cleavege is increased with DPD (6 mM) or BP (24 mM) treatment in HL-60 cells compared to control cells. Results also indicate that BP induced PARP cleavege rate is higher than DPD induced PARP cleavege rate (Figure 3).



(**a**) (**b**)

Figure 3. (**a**) Western blot analysis of PARP, cleaved PARP levels on control, DPD (6 mM) and BP (24 mM) treated HL-60 cells, respectively. (**b**) Comparison of the cleaved PARP/β-actin ratio (mean ± SD) among the groups, $p < 0.05$.

4. Discussion

As having an important role in the synthesis of biologically active compounds and involving in pharmaceutical agents, studies of boronic compounds have gained acceleration. Korkmaz et al. demonstrated that the treatment of DU145 cells with 7 mM DPD triggered apoptosis at 24, 48 and 72 h (8%, 14% and 41%, respectively) [5]. Despite the valuable effect of DPD on prostate cancer, there is no research that reveal the cell death triggering effect of DPD on leukemia and no article that involves the evaluation and comparison of DPD and BP with IC_{50} values on acute myeloid leukemia in aspect of apoptosis. Our results indicate that both BP and DPD have cytotoxic effects on HL-60 cells through apoptosis. However, BP had greater capacity (63.95%). Cantürk et al. [6] have studied the anti-leukemic effect of boric acid and sodium tetraborate in HL-60 cells and their results indicate that boric acid have greater potential to inhibit leukemic cells than sodium tetraborate with 8.8% and 4.9% apoptotic ratios. Although some sources indicate that sodium tetraborate and BP have the same chemical formulas, the apoptotic rate of sodium tetraborate was lower than our apoptotic rate of BP. We thought that the difference may be related with the structural difference or used concentrations of the compounds. Overall, our results pointed out that BP and DPD can be used for development of a new anti-leukemic agent but it needs further in vitro and clinical studies.

5. Conclusions

Boron compounds have valuable candidate for anti-leukemic drug research and we believe that particularly BP (24 mM) and DPD (6 mM) may meet the need in this area through further in vivo and in vitro research.

Author Contributions: S.K., T.E. and B.S. conceived the hypothesis of the study and designed the experiments; T.E., B.S., H.A. performed the experiments and analyzed the data; M.K. provided the boron compounds, T.E., S.K. wrote the paper.

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Conflicts of Interest: The authors declare that there are no conflicts of interest in the design of the study; collection, analyses, or interpretation of data; writing of the manuscript, and decision to publish the results.

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