Background: Micronutrients are cofactors and substrates of several DNA repair enzymes. Among them, zinc (Zn) is particularly relevant in the regulation of oxidative stress, apoptosis, cell cycle, DNA synthesis and DNA damage response (DDR). DDR comprises a variety of mechanisms that protect genome integrity and repair damaged DNA. Acute myeloid leukemia (AML) is a clonal malignant disease of hematopoietic stem and/or progenitor cells characterized by a block in myeloid differentiation and increased proliferation, which displays DDR defects. Additionally, patients usually present decreased levels of serum Zn, which impact in the DDR is not completely understood. Further, studies have shown a role for Zn in the modulation of anticancer therapy in some malignancies. However, its effects in the modulation of therapeutic compounds in AML is not well understood.

Aims:

The aim of this work was to investigate and characterize the potential of zinc sulfate (ZnSO₄) in the modulation of genotoxic compounds (cytarabine; ara-C) and DDR inhibitors (olaparib; Ola) in cell lines of several AML subtypes.

Methods:

The AML cell lines, HEL, NB-4, and K-562 cells, were incubated in the absence and presence of ara-C and Ola in monotherapy and in combination with ZnSO₄ (IC25) for 72h. Cell density and viability were evaluated by trypan blue exclusion test at each 24h. Cell death was assessed by flow cytometry (FC), using the Annexin V and 7-AAD double staining, and by optical microscopy through May-Grünwald-Giemsa staining. Cell cycle was analyzed by FC using PI/RNAse. Results were statistical analyzed, considering a significance level of 95%.

Results:

Ola in monotherapy decreased cell density in a dose- and time-dependent manner (p<0.05), while viability varied depending on the cell line. HEL cells were the most sensitive to Ola. The estimated IC₅₀ of Ola at 48h were of 51μM, 173μM, and 180μM for HEL, K-562, and NB-4, respectively. The effect of ara-C in monotherapy was also cell line dependent. The most sensitive cell lines were HEL and NB-4, and the least were K-562 cells (IC₅₀ at 48h: 2μM, 2μM, and 23μM, respectively). ZnSO₄ increased the cytostatic and cytotoxic effects of Ola and ara-C in all cell lines (p<0.05). Combination of Ola and ZnSO₄ reduced 2.9-fold, 1.6-fold, and 7.5-fold the IC₅₀ in HEL, NB-4, and K-562 cell lines, respectively, after 48h. Similarly, combination of ara-C and ZnSO₄ also decreased the IC₅₀ 4.8-fold, 19-fold, and 19.2-fold the IC₅₀ in HEL, NB-4, and K-562 cell lines (after 48h). Combinations with ZnSO₄ increased the percentage of cells in apoptosis, comparatively to monotherapy (p<0.05). The morphological analysis also showed that ara-C and ola in monotherapy as well as in combination with ZnSO₄ induced cell death mainly by apoptosis. Moreover, the association of Zn with ara-C increased the cytostatic effect in HEL and K-562 cells, leading to cell cycle arrest in G₀/G₁ (p<0.05). Similarly, combination of Ola with ZnSO₄ induced G₀/G₁ arrest in HEL cells (p<0.05).
Summary/Conclusion:

The results suggest that Zn synergize with ara-C and Ola in AML cells, increasing the cytostatic and cytotoxic effects of these compounds. The considerable reduction of the IC₅₀ of the ara-C and Ola in the presence of Zn may result in more efficient therapeutic responses.