Deferasirox Inhibit Doxorubicin-Induced Reactive Oxygen Species Generation in Both Acute Myeloid Leukemia and Normal Heart Cell While Maintain the Cytotoxicity of Doxorubicin Only on Acute Myeloid Leukemia

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Background: Deferasirox (DFX) has anti-leukemia effect both in vitro and in vivo. DFX also potently inhibits the generation of intracellular reactive oxygen species (ROS). On the other hand, the generation of ROS by Doxorubicin (DOX) is critical for the cytotoxicity on both leukemia and normal heart cells. It is not known whether combining DFX and DOX will have synergistic or antagonizing effect on leukemia cells. It is also unknown whether adding DFX to DOX will have protective effect on heart cell.

Method: Human AML cell line THP1, mice AML cell line WEHI3, & rat normal heart cell line H9C2 were treated with DOX or DFX alone with various concentration to see the effect of cytotoxicity of each drug. Besides, they were also treated with DOX 5 μM for various duration in the presence or absence of DFX pretreatment (100 nM for 10 minutes). Intracellular ROS generation was measured by the detection of 2,7-dichlorodihydrofluorescein (DCF) fluorescence intensity using flow cytometry. Viability was determined by 7-AAD staining. Apoptosis was determined by Annexin V-Propidium Iodide staining. Cytotoxicity was determined by Trypan blue exclusion assay.

Results: Not only DOX but also DFX alone induced cell death and apoptosis of THP1 AML cells (Figure 1A-D). DOX-induced ROS production was significantly reduced when THP1, WEHI3, and H9C2 cells were pretreated with DFX (Figure 2A, 2B, 3A, 4A). However, DOX-induced apoptosis and cell death of THP1 and WEHI3 AML cells were not antagonized by DFX pretreatment (Figure 2C, 3B, 4B). More importantly, DFX-pretreated H9C2 heart cells had lower ROS generation (Figure 4A) and fewer cell death (3.7%) after exposure to DOX (5 μM for 24 hours) compared to non-DFX pretreated cells (8.5%). (Figure 4C)

Conclusions: DFX induced apoptosis of AML cells. DFX also markedly reduced the ROS generation following DOX treatment. However, DFX did not negatively influence the pro-apoptotic and cytotoxic effect of DOX on these AML cell lines. Interestingly, DFX markedly reduced the DOX-induced ROS generation and DOX-induced cell death in normal rat heart cell. We are now using Balb/c-WEHI3 AML mice model to test whether DFX can protect cardiomyocytes from DOX-related damage while maintain the cytotoxic effect of DOX on AML cells.

Figure 1: DFX alone induces cell death (figure 1A/B) and apoptosis (figure 1C/D) of hAML cell line THP1 cells

Figure 2: DOX-induced ROS production was significantly reduced (figure 2A/B) while cytotoxicity (figure 2C) was maintained when THP1 cells were pretreated with DFX

Figure 3: DFX-pretreated WEHI3 mouse AML cells significantly reduces DOX-induced ROS production (figure 3A) but not reduced cytotoxicity (figure 3B/C)

Figure 4: DFX dec. DOX-induced ROS generation in THP1, WEHI3, H9C2 cells (figure 4A), DFX did not reduce the pro-apoptotic/cytotoxicity of DOX on THP1/WEHI3 AML cells (figure 4B), but may protect H9C2 from DOX induced cell death (figure 4C)