OXIDATION OF PEROXIREDOXINS BY THIOREDOXIN INACTIVATION IS MEDIATED IN ARSENIC TRIOXIDE-INDUCED REACTIVE OXYGEN SPECIES FORMATION AND ITS CYTOTOXICITY IN ACUTE PROMYELOCYTIC LEUKEMIA CELLS.

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The Arsenic trioxide (ATO) is an effective cancer therapeutic drug for acute promyelocytic leukemia (APL). ATO exerts its effect mainly raising oxidative stress. However, not only the mechanisms of reactive oxygen species (ROS) generation by ATO but involvement of redox enzymes including peroxiredoxin (PRX) and thioredoxin (TRX) remains elusive. Aim of current study is to elucidate the mechanism of redox enzymes to elevate ROS during ATO-induced apoptosis in APL-derived NB4 cells. NB4 cells were cultured with 2 μM arsenic trioxide to induce apoptosis for 16-48 hours. 2, 7-dichlorodihydro-fluorescein-diacetate (H2DCF-DA) and MitoSOX Red were used to detect cellular and mitochondrial ROS. SO2 form for PRXs was detected by western blot assay using PRX SO2 form-specific antibody. Monomer/Dimer assay for PRXs, and TRX was performed by western blot using non-reducing gel. Intracellular ROS of NB4 cells was increased significantly after 16 hour of ATO but decreased after 24 hour of ATO. Mitochondrial ROS of NB4 cells was increased significantly after 39 hour of ATO. Apoptosis of NB4 cell after ATO treatment was increased as time elapsed (24% on 16hr, 26% on 24hr, 48% on 39hr, and 60% on 48hr). Monomer, indicated active and reduced form, of peroxiredoxins was decreased and cysteine sulfenic acid (CP–SO2H) peroxiredoxins, indicated inactive and oxidized peroxiredoxins, was increased in NB4 cells after ATO treatment as time goes by. Similarly, monomer of thioredoxin-1 (active thioredoxin) was decreased and multimer of thioredoxin-1 (inactive thioredoxin) was increased in NB4 cells after ATO treatment as time elapsed. Our data showed inactivation of peroxiredoxins by oxidation was developed during ATO-induced ROS generation and APL cell apoptosis. These peroxiredoxins oxidation was probably due to increment of reduced thioredoxin in NB4 cells after ATO treatment. These findings suggest ATO-induced anti-leukemic activity is more likely due to a TRX system-mediated cellular redox changes.