Abstract No: BP-21 **Biological Sciences**

Silibinin sensitizes TRAIL-mediated apoptosis by upregulating DR5 through reactive oxygen species-mediated endoplasmic reticulum stress-Ca^{2+}-CaMKII-Sp1 pathway

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The cytotoxic effect of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) makes TRAIL a promising candidate for the treatment of various cancers. However, some cancers are resistant to the effects of TRAIL. Therefore, in this study, we addressed how silibinin enhances TRAIL-mediated apoptosis in various cancer cells. A549, U937 and HCT116 cells were plated at a density of 1×10^5 cells/well for overnight and treated with 75 ng/ml TRAIL either in the absence or presence of silibinin for 24 h. MTT assay was done to check the cell viability. Apoptotic cells were determined by the annexin-V^+ staining (R&D Systems). Whole-cell lysates were prepared by PRO-PREP protein extraction solution (iNtRON Biotechnology, Sungnam, Republic of Korea). Cytoplasmic and nuclear protein extracts were prepared using NE-PER nuclear and cytosolic extraction reagents (Pierce, Rockford, IL). Combined treatment with silibinin and TRAIL (silibinin/TRAIL) induced apoptosis accompanied by the activation of caspase-3, caspase-8, caspase-9, and Bax and cytosolic accumulation of cytochrome c Anti-apoptotic proteins such as Bcl-2, IAP-1, and IAP-2 were inhibited as well. Silibinin triggered TRAIL-induced apoptosis in A549 cells through upregulation of death receptor 5 (DR5). Pretreatment with DR5/Fc chimeric protein and *DR5*-targeted small interfering RNA (siRNA) significantly blocked silibinin/TRAIL-mediated apoptosis in A549 cells. Furthermore, silibinin increased the production of reactive oxygen species (ROS), which led to the induction of TRAIL-mediated apoptosis through DR5 upregulation. 5mM concentration of *N*-acetyl-L-cysteine (NAC) and glutathione (GSH) reversed the apoptosis-inducing effects of TRAIL which are known antioxidants. Silibinin further induced endoplasmic reticulum (ER) stress as was indicated by the increase in ER marker proteins such as PERK, eIF2α, and ATF-4, which stimulate the expression of CCAAT/enhancer binding protein homologous protein (CHOP). *CHOP*-targeted siRNA eliminated the induction of DR5 and resulted in a significant decrease in silibinin/TRAIL-mediated apoptosis. We also found that silibinin/TRAIL-induced apoptosis was accompanied with intracellular influx of Ca^{2+}, which was stimulated by ER stress and the Ca^{2+} chelator, ethylene glycol tetraacetic acid (EGTA). Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) inhibitor, K252a, blocked silibinin/TRAIL-induced DR5 expression along with TRAIL-mediated apoptosis. Accordingly, it was demonstrated that ROS/ER stress-mediated CaMKII regulated Sp1, which is an important transcription factor for DR5 expression. Our results revealed that silibinin enhanced TRAIL-induced apoptosis by upregulating DR5 expression through the ROS-ER stress-CaMKII-Sp1 axis.

Keywords: Tumor necrosis factor-related apoptosis-inducing ligand, Silibinin, Endoplasmic reticulum