Poster

[P26-9] P26-9: Oncologic drugs (5): Pharmacokinetics, TDM practice

Chair: Kiyoshi Mihara, Japan

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[P26-9-8] Synergistic apoptotic effect of Norcantharidin combined with sorafenib in human RCC cells in vitro and in vivo

Shu-Ching Hsieh[1], Hsieh Shu-Ching[2], Hsieh Yi-Hsien[3] (1.Chung Shan Medical University, 2.Chung Shan Medical University, 3.Chung Shan Medical University)

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Background

Norcantharidin (NCTD) is the demethylated form of cantharidin that exhibits anticancer potential in many cancer cell types. However, the antitumor effects of NCTD in human renal cancer cells (RCC) are not known.

Methods

Cell viability and cell-cycle distribution were examined and analyzed by MTT assay and flow cytometric assays. Flow cytometry was also used to measure the levels of endoplasmic reticulum (ER) stress and mitochondrial membrane potential (Ψm). Immunoblotting assays were used to examine the effect of norcantharidin on apoptosis related proteins, mitochondria-related proteins and endoplasmic reticulum–related proteins. *In vivo* xenograft experiments to determine the effects of NCTD on tumor growth.

Results

We found that NCTD treatment had a cytotoxic effect on 786-O, Achn, CaKi and A-498 RCC cells in a dose-dependent manner. NCTD treatment resulted in sub G1 arrest in 786-O and A-498 cells. In addition, apoptotic cells were observed in an Annexin V-FITC/ propidium iodide double-stained assay. The activities of caspase-3, -8, -6, -7, -9, and poly (ADP)-ribose polymerase (PARP) were increased in NCTD-treated 786-O and A-498 cells. NCTD induced RCC cell apoptosis, which was associated with mitochondria dysfunction. Western blot analysis revealed that the levels of Bax were significantly increased in NCTD-treated 786-O cells. The expressions of Bcl-2, and Mcl-1 were decreased in 786-O cells after NCTD treatment. Furthermore, endoplasmic reticulum (ER) stress was observed in cells treated with NCTD, we found that upregulation of glucose-regulated protein (GRP) 78 and CHOP, phosphorylated eukaryotic initiation factor 2α (p-eIF2α) and activating transcription factor 4 (ATF4) was observed with western blotting, suggesting that NCTD-induced apoptosis is involved in the ER stress response. Mechanistic investigations suggested that NCTD modulated the Akt signaling. Overexpression of AKT suppresses induced apoptosis and mitochondria related protein expression by treated with NCTD. *In vivo* xenograft analysis revealed that NCTD signi cantly reduced tumor growth in mice with 786-O tumor xenografts. Moreover, NCTD markedly enhanced the anti-tumor effects of sorafenib in RCC cells in vitro and in vivo.

Conclusions

These findings provide insight into the NCTD combined with sorafenib may be a promising therapeutic strategy for the treatment of RCC.