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Renal tubular cell death induced by the novel MCL-1 inhibitor, S63845.

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Objectives: S63845 is a new selective MCL-1 inhibitor and its derivative is in clinical trials for hematopoietic cancers. Myeloid leukemia 1 (MCL-1) is an antiapoptotic protein which belongs to the BCL-2 family of proteins. In this study, we investigated the effect of S63845 on the death of human kidney tubular cell line, HK-2 cells.

Methods: Short interfering RNAs (siRNA) to silence the MCL-1 gene was introduced into HK-2 cells using Lipofectamine RNAiMAX. The type of cell death was determined using flow cytometry. mRNA expression was quantified using quantitative real time PCR (qPCR). Protein expression was measured using western blot analysis.

Results: The flow cytometry analysis showed that high concentrations of S63845 (1 & 10 μ M) increased necrotic and late apoptotic cells while decreasing early apoptotic cells, leading to decreased cell survival. Surprisingly, cell death from S63845 was partially inhibited along with decreased necrotic process by knock-down of MCL-1 using MCL-1 siRNA. However, MCL-1 knock-down converted the mode of cell death from apoptosis to necrosis resulting in decreased cell survival when cell death was induced by cyclosporine A (CsA). qPCR showed that S63845 significantly increased MCL-1 mRNA expression. Accordingly, western blot showed that S63845 increased phospho-MCL-1 protein expression. Furthermore, S63845 augmented CsA-induced phospho-MCL-1 protein upregulation. S63845 not only increased cleaved-caspase 3 and -7 protein expression but also augmented their expression induced by CsA. MCL-1 knock-down was found to inhibit the upregulation of cleaved-caspase 3 and -7 induced by CsA and S63845.

Conclusions: To summarize, S63845 paradoxically increased the expression of MCL-1 as well as induced the activation of caspase 3 and -7 leading to increased renal tubular cell death. Intriguingly, knock-down of MCL-1 and its resultant downregulation of caspase 3 and -7 also increased renal tubular cell death by converting the death mode to necrosis.