

SF508 Protects Ceramide-induced Neuronal Cell Death

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Objectives

In the present study, we examined ceramide-induced neuronal cell death and its mechanism and process using SK-N-KH cells, and investigated whether ROS was produced and related to other factors. In addition, we tried to check whether silk fibroin had protective effect for neuronal cells, and through which cell death process this protective mechanism functioned.

Materials and Methods

Materials - Cell line : Human neuroblastoma cell, SK-N-SH

Agents : C-2 ceramide is an important mediator of apoptosis in various cell type and cell permeable C2 ceramide induces apoptosis in vitro.

Silk fibroin is a hydrolyzed product of silk protein, gathered from vomited matters of the typical fiber-producing insect, Bombyx Mori

Methods - Alarblue assay (cell viability), Light microscopy; Olympus IX70(phase contrast),

Hoechst 33258 staining (nuclear morphology), DCF-DA fluorescence staining by

Tecan Genios Flow cytometry (Exc:485nm, Emi:510nm), Caspase substrate cleavage assay (fluorogenic substrate, Ac-DEVD-AMC)

Results and Discussion

Various fraction of silk fibroin obtained by unique hydrolysis and fractionation with HPLC were addressed on the study of neuro-protection against ceramide toxicity. Among the fractions, a fraction named SF508 showed significant protective effect. Pretreatment of SF508 SF508 effectively increased cell viability up to 30% against ceramide-induced cell death. This protective effect against cell death was also observed under a microscope. Interestingly, the increased ROS(Reactive Oxygen Species) level by ceramide was down-regulated by the treatment. Moreover, caspase activity, which mediates ceramide-induced neuronal cell death, was also inhibited by the SF508.

Taken together, those results above suggest that ceramide induces apoptotic cell death in neuronal cells, and SF508 protects neuronal cells by effectively inhibiting cell death. And it seems that cellular protective mechanism of SF508 against ceramide-induced neuronal cell death works because the

physiological effect of SF508 as antioxidant reduces the amount of ROS, and the activity of enzyme related to cell death, for example, caspase, is also hindered by reduced ROS.

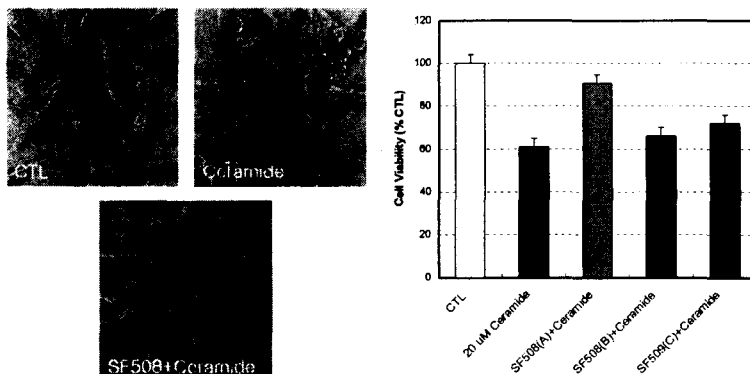


Fig. 1. C2 ceramide induced apoptotic cell death in SK-N-SH neuroblastoma cells and SF508 increased cell survival SK-N-SH cells from C2-ceramide-induced apoptosis

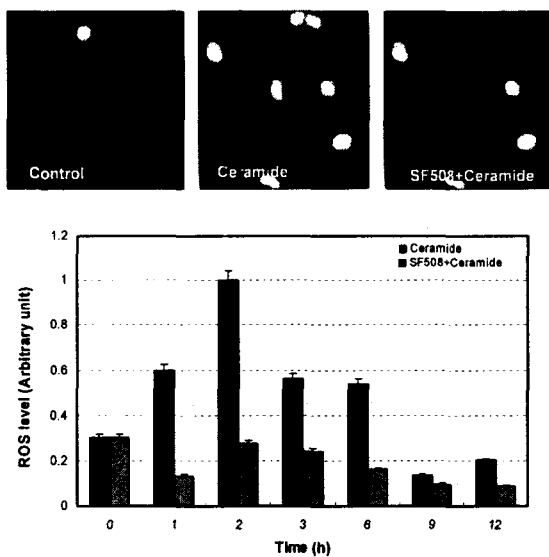


Fig. 2. ROS (reactive oxygen species) is increased by C2-ceramide affected by SF508.

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