A comparative proteomic study of secretomes in kaempferitrin-treated CTX TNA2 astrocytic cells

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kaempferitrin is extracted in significant high quantity from the leaves of Cinnamomum osmophloeum and from Bauhinia forficata leaves, both are used as antidiabetic herbal remedy in China and Brazil. Commercial product using dry Cinnamomum osmophloeum leaves have been sold locally in Taiwan. Oral administration of KAEMPFERITRIN reduced blood sugar in diabetic rats. Though previously demonstrated to activate the classical insulin signaling pathways, mechanism of action for kaempferitrin is still not fully understood. Further, as references for kaempferitrin on immune related cells have been inconclusive, and people consuming extract containing kaempferitrin often happen to be of high risk for diabetes and neurodegenerative diseases. For kaempferitrin to be used every day, a comprehensive study is needed. Astrocytic cell line was used as a model to test the differentially regulated secretomes, to test kaempferitrin effect on CNS glia, on pro-inflammatory cytokines, and to test how different the mechanism of kaempferitrin is from that of insulin. CTX TNA2 astrocytic cells were differentially treated with and without 10 μM kaempferitrin for 24 hrs, and the conditioned medium was collected. For proteomic study, protein in conditioned medium was trypsin digested, and resulting peptides in kaempferitrin/non-treated sample pair were differentially dimethyl labeled. The labeled peptides were further fractionated by StageTip-based strong-exchange method before LC-MS/MS analyses. IL-6 and TNF-alpha level were confirmed using astrocyte conditioned medium by ELISA. C.O. leaf crude extract treated samples were included for a comparison of effects of purified kaempferitrin vs kaempferitrin containing crude extract. Results and Conclusions: It is found that no pro-inflammatory cytokines or inhibitory ECM were elevated upon kaempferitrin treatment, nor cytokines that would induce alteration of immune state. This suggested that chronic use of kaempferitrin containing herbs may do not inflict alteration of immune state or hinder nerve regeneration. LDL-R trafficking between the cell membrane and the extracellular niche was regulated by kaempferitrin toward reduced secretion. Our proteomic study also demonstrated that molecules related to plasma membrane recycling were regulated by kaempferitrin. However, these proteins were differently regulated when cells were treated with crude extract.