The Signaling pathways involved in Fluoxetine-induced apoptosis in human gastric adenocarcinoma cell line (AGS)

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The antidepressant fluoxetine (FLX) is a selective serotonin re-uptake inhibitor. It has been reported that fluoxetine can inhibit cancer cell growth and induce apoptosis in various cancer cell lines in addition to treat mental disorders. Moreover, it was described that fluoxetine can inhibit NF- κ B signaling in intestinal epithelial cells. Since the expression of NF- κ B was up-regulated in gastric adenocarcinoma samples. In this study, the apoptotic effect of fluoxetine in human gastric adenocarcinoma cell line (AGS) was investigated.

The cytotoxicity of the AGS in response to fluoxetine was determined by MTT assay. The changes in apoptotic nuclei of AGS cells were stained with DAPI and observed using fluorescence microscopy. The changes in apoptotic-related protein expression were studied by western blotting. The induction of reactive oxygen species (ROS) by fluoxetine was analyzed by using fluorescent probe DCFDA, and fluorescence intensity was detected with a fluorescence microplate reader at maximum excitation and emission wavelengths of 485 nm and 535 nm, respectively.

Fluoxetine significantly inhibited the AGS cell proliferation starting at the concentration of 20 μ M in a dose-dependent manner. The expression of NF- κ B was significantly reduced by the treatment with fluoxetine. The increase of fluorescence intensity and chromatin condensation were observed with DAPI staining. The hallmarks of apoptosis such as the elevated expression of activated caspases, cleavage of poly (ADP-ribose) polymerase (PARP) and the changes in Bcl-2/BAX ratio were seen in fluoxetine-treated cells. The decrease in the ratio of Bcl-2/BAX indicates that fluoxetine may have effect on the mitochondrial membrane potential. The phosphorylation of AKT which can induce cell proliferation was suppressed by fluoxetine treatment. Moreover, the expression of the extrinsic signaling pathway proteins such as TRAIL, DR4, DR5 and FADD were significantly increased by fluoxetine.

This study showed that fluoxetine could significantly increase the production of ROS and inhibition of ROS production by NAC and GSH could attenuate the cytotoxic effect of fluoxetine. Moreover, fluoxetine induced apoptosis by decreasing bcl-2/BAX ratio, inhibiting NF- κ B and phosphorylation of p-AKT and p-53. Taken together, the apoptotic effect induced by fluoxetine in gastric adenocarcinoma cell line (AGS) was mediated by both extrinsic and intrinsic pathways.