Regulation of the Both Anti-Inflammatory and Inflammatory Cytokines Expression by 5-Aminosalicylic Acid in Caco-2 Cells

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Aim of the study: This study has provided new clues for further studies required to be carried out to clarify the effect of 5-ASA as cytokines interaction to understand which cytokines anti-inflammatory or inflammatory.

Material and Methods: The human epithelial colorectal adenocarcinoma cells line (Caco-2) was purchased from American Tissue Culture Collection. Caco-2 cellswere grown in monolayer culture in DMEM medium containing 10% PBS, 1% penicillin/streptomycin at 37°C in a humidified atmosphere comprised of 95% air and 5% CO2. Cell viability was assessed using WST. Caco-2 cells were treated with varying concentrations of 5-ASA at mM range for 24 h at 37 °C. Total RNA was isolated using RNaesy Mini Kit (Qiagen) by the manufacturer’s standard protocol. Quantitative Real Time PCR (qRT-PCR) analysis was performed using KiloGreen qPCR Master Mix in an Exicycler 96 Real Time Quantitative Thermal Block PCR System to determine IL-1b, IL-2, IL-6, IL-10 and IL-13gene mRNA expression level.

Results: Three different concentrations of 5-ASA (10, 20, and 50 mM) were identified to measure the differential responses of Caco-2 cells. IL-6 gene mRNA expression level was decreased 1.47, 1.05, 3.02 times in Caco-2 cells as compared to controls as a result of 10, 20 and 50 mM 5-ASA treatments, respectively. Similarly, IL-2 gene mRNA expression level was decreased 1.55, 1.48 and 3.65 times in Caco-2 cells with 10, 20 and 50 mM 5-ASA applications, respectively. Moreover, IL-1b gene mRNA expression level was lowered 1.06, 1.58 and 2.08 times in Caco-2 cells as a result of 10, 20 and 50 mM concentrations of 5-ASA treatments, respectively. On the other hand, IL-13 and IL-10 genes mRNA expression levels increased 1.55, 1.47 and 12.46 times and 1.12, 1.37 and 9.06 times in Caco-2 cells with 10, 20 and 50 mM doses of 5-ASA, respectively. To summarize, mRNA expression levels of IL-6, IL-2, IL-1b were downregulated 3.02-, 3.65- and 2.00-folds, respectively; and of IL-13 and IL-10 were upregulated 12.46- and 9.06-folds with 50 mM 5-ASA-treatment, respectively. These results suggest that high doses of 5-ASA inhibit proinflammatory genes while inducing anti-inflammatory genes in Caco-2 cells.

Acknowledgments: This work supported by Pamukkale University PAUBAP-2015FBE042

Keywords: 5-aminosalicylic acid (5-ASA), Caco-2, Cytokines, Anti-inflammatory