Effect of 5'-fluoro-2'-deoxycytidine, 5-azacytidine, and 5-aza-2'-deoxycytidine on DNA Methyltransferase 1, CIP/KIP Family, and INK4a/ARF in Colon Cancer HCT-116 Cell Line

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Abstract:

Background: Cyclin-dependent kinase inhibitors (CKIs) are the negative regulator of cell cycle progression, which inhibits cyclin-cdk complexes, resulting in cell cycle arrest. Recently, we evaluated the effect of 5-Aza-CdR on DNMT1 gene expression in the WCH-17 hepatocellular carcinoma (HCC) cell line.

Objectives: The current study was designed to analyze the effects of 5-aza-2'–deoxycytidine (5-Aza-CdR, decitabine), 5-azacytidine (5-AzaC, vidaza), and 5'-fluoro-2'-deoxycytidine (FdCyd) on INK4a/ARF, CIP/KIP, and DNA methyltransferase 1 gene expression, apoptosis induction, and cell growth inhibition in colon cancer HCT-116 cell line.

Methods: The colon cancer HCT-116 cell line was treated with 5-azaC, 5-Aza-CdR, and FdCyd at 24 and 48h. To determine colon cancer HCT-116 cell viability, cell apoptosis, and the relative expression level of the INK4a/ARF, CIP/KIP, and DNA methyltransferase 1 genes, MTT assay, flow cytometry, and qRT-PCR were done, respectively.

Results: 5-azaC, 5-Aza-CdR, and FdCyd significantly inhibited colon cancer HCT-116 cell growth and induced apoptosis. Besides, they significantly increased CIP/KIP (p21CIP1, p27KIP1, and p57KIP2) and INK4 (p14ARF, p15INK4b, and p16INK4a) and decreased DNMT1 gene expression. Besides, minimal and maximal apoptosis were seen in the groups treated with FdCyd and 5-Aza-CdR, respectively. The IC50 for CAF for FdCyd was 1.72 ± 0.23 and 1.63 ± 0.21μM at 24 and 48h, respectively. The IC50 for CAF for 5-AzaC was 2.18 ± 0.33 and 1.98 ± 0.29 μM at 24 and 48h, respectively. The IC50 for CAF for 5-Aza-CdR was 4.08 ± 0.61 and 3.18 ± 0.50 μM at 24 and 48h, respectively.

Conclusions: The 5-azac, 5-Aza-CdR, and FdCyd can reactivate the INK4a/ARF and CIP/KIP families through inhibition of DNMT1 activity.

Keywords: Colonic Neoplasms, Cyclin-dependent Kinases, DNA (Cytosine-5-)-Methyltransferases