The Cytotoxicity of a Short Chain Fatty Acid Histone Deacetylase Inhibitor on HCT116 Human Colorectal Carcinoma Cell Line

Abstract

Colorectal cancer metastases result in a significant number of cancer related deaths; Histone deacetylase (HDAC) inhibitors induce growth arrest and apoptosis in a variety of human cancer cells. Sodium butyrate (SB) is a short chain fatty acid, belongs to HDAC inhibitors which is released in the colonic lumen as a consequence of fiber fermentation. In this study we are about to assess the effect of sodium butyrate on HCT116 human colorectal carcinoma cell line. The viability of cells was measured by Microscopic Morphologic study and MTT assay. After 48 hours treatments more than 10 mM lead to cell injury in HCT116 by increasing cell granulation and decreasing cell adhesion ($p>0.05$). After 72 hours treatments at 10 mM and more lead to significant cell injury ($p<0.05$). Our results may suggest that the gene expression which is contributed in cell proliferation and apoptosis has been changed under pressure of HDAC inhibition.

Keywords: Colorectal cancer; Cytotoxicity; MTT; Sodium butyrate

Short Communication

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Abbreviations: SB: Sodium Butyrate; HDAC: Histone Deacetylase; CRC: Colorectal Cancer; HNPCC: Hereditary non Polyposis Colon Cancer; UC: Ulcerative Colitis; SCFA: Short Chain Fatty Acid; FAP: Familial Adenomatous Polyposis; IBD: Inflammatory Bowel Diseases

Introduction

Colorectal cancer (CRC) is uncontrolled cell growth in the colon or rectum . In 2012 it resulted 1.4 million new cases and caused about 50% deaths [1]. CRC is a significant cause of morbidity and mortality worldwide, especially in Europe, North America and in some regions of Asia. More than half of the disease accompany by an inappropriate diet and lifestyle. CRC is the fourth leading cause of cancer death worldwide [2]. Risk factors for CRC include environmental risks (>75-95%) that may cause increased mutations and epigenetic alterations, genetic risks (<5%) such as Hereditary non Polyposis Colon Cancer (HNPCC) (<3%) and Familial Adenomatous Polyposis (FAP) (<1%) and Inflammatory Bowel Diseases (IBD) include Crohn's disease and Ulcerative Colitis (US) [1,3].

Sodium butyrate (SB), a short chain fatty acid (SCFA), is an HDAC inhibitor (HDI) produced in the colonic lumen as a consequence of anaerobic bacteria fermentation of dietary fiber and undigested starch and proteins. SB serves as an energy source of colonic epithelium and plays a role in the maintenance of colonic homeostasis. In-addition HDIs induce growth arrest and apoptosis in a variety of human cancer cells [4]. Chromatin remodeling agents by diverse histone modification have a key role in gene regulation and tumorigenesis [2]. Previous studies indicate that SB can inhibits the growth of various carcinoma cell lines, The growth arrest induced by the SCFA was characterized by an increase in the expression of the p21 cell-cycle inhibitor and down-regulation of cyclin B1 (CB1)[2,4]. In this study we are about to assess the effect of SB (in different concentrations) on the cellular proliferation of a colorectal carcinoma cell line (HCT116) *In-vitro*.

Materials and Methods

Cell culture

Human colon cancer HCT116 cell line obtained from the National Cell Bank of Iran- Pasteur Institute of Iran, was cultured in RPMI 1640 medium supplemented with 10% FBS, 100 units/mL of penicillin, and 100 µg/mL streptomycin at 37°C in a humidified, CO_2 (5%) incubator. The Adherent HCT116 cells were cultured in 25 cm2 flask, 6-well and 96-well plates (SPL, Korea).

Morphologic study

The Adherent HCT116 cells in the exponential phase of growth were harvested and seeded in 6-well plates by 700000 cells per well. SB (0, 1.0, 2.5, 5.0, 10, 20 mM) was added and incubated for 12, 24, 48, and 72 hours at 37°C with 5% CO_2. Cell morphology was assessed by invert Microscope (Euromex, Holland).

MTT Assay

The Adherent HCT116 cells seeded in 96-well plates 7000 cells per well, SB (0, 1.0, 2.5, 5.0, 10, 20 mM) was added and incubated for 12, 24, 48, and 72 hours at 37°C with 5% CO_2. MTT solution was added to each well. After 4 hours the supernatant was discarded and dimethyl sulfoxide was added to dissolve the purple insoluble MTT formazan produced by mitochondrial succinate dehydrogenase. The absorbance was measured at 550 nm in a micro spectrophotometer; all treatments were performed in triplicate.

Results and Discussion

After 24 hours, a dose dependent cytotoxicity and morphological changes were observed which were accentuated after 48 and 72 hours. Treatments more than 10 mM, lead to HCT116 cell injury by increasing cell granulation and decreasing cell adhesion (Figure 1) MTT assay results confirmed the morphologic observations. IC_{50} for HCT116 cells was in 10 mM concentration after 72 hours ($p<0.05$). Results confirmed the anti-proliferative effect of Sodium butyrate on HCT116 cells at concentrations more than 1 mM after 48 and 72 hours (Figure 2). In previous studies it has been shown that SB can induce differentiation and inhibit the growth of H460 cells [4]. Butyrate treatment causes apoptosis in human colon cancer HT-29 cells [2]. World Academy of Science, Engineering and Technology International Journal of Medical, Health, Pharmaceutical and Biomedical Engineering 2014

a) Untreated (400x magnification)

b) SB con: 1 mM (400x)

c) SB con: 2.5 mM (400x)

d) SB con: 5 mM (400x)

e) SB con:10 mM (400x)

f) SB con:20 mM (400)

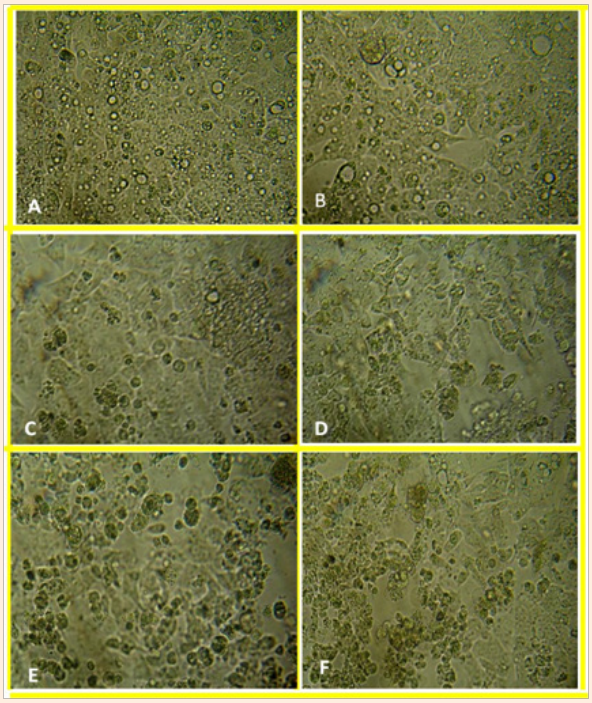

Figure 1: SB effect on cell morphology after 48 hours.

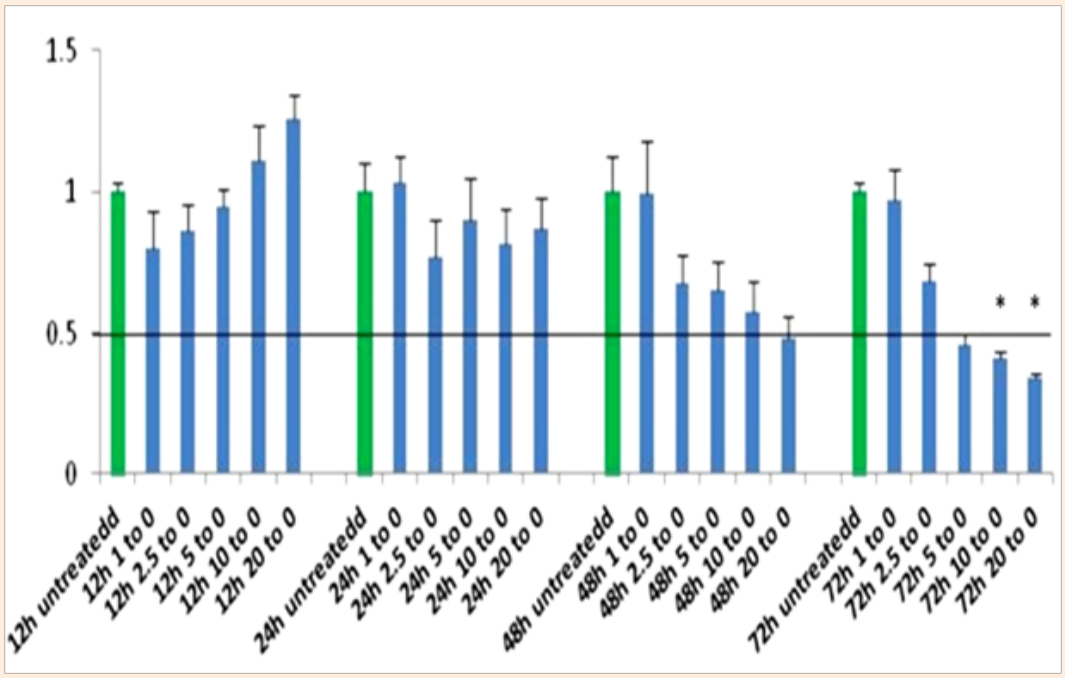

Figure 2: SB effect on cell proliferation in MTT assay.

*: $p<0.05$.

SB up-regulates metastatic suppressor Kangai 1 (KAI1) in a time-dependent manner in H460, SW620 cell lines [4]. SB treatment also increases β-catenin signaling and diminishes cell adhesion ability in Adherent metastatic cells SW620 [5]. SB significantly increased Wnt5a expression in SW620 [5]. SB is also a potent HDI that induce growth arrest, differentiation, and apoptosis of SW480 cells [6].

Conclusion

To conclude, our data show that inhibition of proliferation and cytotoxic effects by a short chain fatty acid, histon deacetylase

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inhibitor Sodium butyrate on human colorectal cancer cells (HCT116). Our results may suggest that the gene expression which is contributed in cell proliferation and apoptosis has been changed under pressure of HDAC inhibition. In addition, the naturally occurring colonic SB secondary to the microbial degradation of dietary fibers, may affect as a natural preventive factor against CRC.

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