Chapter 4

Short-Chain Fatty Acids Are Antineoplastic Agents

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Additional information is available at the end of the chapter

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Abstract

Human diet contains a mixture of saturated and unsaturated fatty acids. These are either long, medium or short chain fatty acids. As commonly believed, all fatty acids are not detrimental to human health. In addition to energy reserves, long chain fatty acids are known as acylating agents for many biomolecules such as cholesterol, terpenoids as well as steroid hormones. They are also involved in acylation of polyphenols such as flavonoids making them palatable for better absorption and biological activities. Polyunsaturated fatty acids (PUFAs) are known for their numerous beneficial health effects including cancer and inflammation. PUFA, particularly ω3 fatty acids, have attracted attention as anticancer agents and particularly for colorectal cancer. PUFAs exhibit immunomodulatory activities controlling inflammosome and are used as adjuvants together with standard anticancer drugs. A reciprocal interaction of short chain fatty acids with PUFAs has been suggested for their anticancer activities. Thus, in colon cancer cells, sodium butyrate (NaB) interacts with docosahexaenoic acid inducing cell differentiation or catalyze apoptosis. These results encouraged us to investigate NaB, a C4 acid, as an adjuvant to standard proteasome inhibitors. Our results show that NaB sensitizes colon cancer cell lines for treatment with proteasome inhibitors.

Keywords:histone deacylating agents, proteasome inhibitors, short-chain fatty acids, sodium butyrate, polyunsaturated fatty acids

1. Background

Cancer appears when the cellular growth network is disturbed and tumor cells resist apoptosis, resulting in uncontrolled growth and progression of tumor cells. Nucleosome acylation

and deacylation of histones play a critical role in the tumorigenesis progression by regulating chromatin structure and function. The histone acetyltransferases (HATs) and deacylases (HDACs) create a fine equilibrium between acylation and deacylation of histones (**Figure 1**). Once this equilibrium is disturbed, it leads to cancer promotion and progression. A number of synthetic compounds, such as cyclic tetrapeptides, benzamides, suberoylanilide hydroxamic acid, and associated branched hydroxamic acid derivatives, are expended as inhibitors of HDAC. Short-chain fatty acids, cogitated as novel drugs, have also been used as HDAC inhibitors. These compounds lead to an accumulation of acylated histones in healthy and tumor cells, arresting the cell cycle in the G1 and/or G2 phases, and provoking apoptosis in cancer cells. Therefore, HDAC inhibitors in controlled doses are recognized as innovative antitumor and anti-inflammatory drugs, and sodium butyrate and sodium valproate, a C8 FA, have been used as HDAC inhibitors.

Fatty acids, contingent to the number of carbons, can be classified into three groups:

(a) C2:0–C6:0, short-chain fatty acids (SCFAs) are dietetic and colonic fermentation products. SCFAs have promise as antitumor agents in numerous types of cancer cells.

(b) C8:0–C14:0, intermediate chain fatty acids (ICFAs) have antimicrobial physiognomies, but some reports have also recognized them as antineoplastic mediators.

(c) C16:0–C24:0, classified as long-chain fatty acids (LCFA), provoke oxidative stress commanding apoptosis in tumor cells.

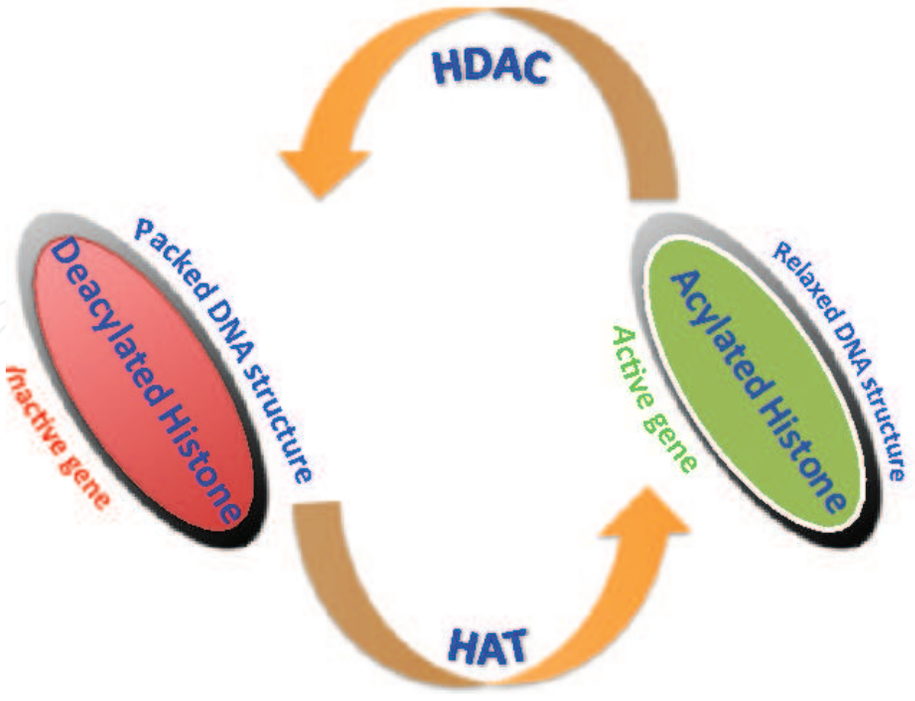

Figure 1. Acylation and deacylation of histones.

Several studies have shown that a combined treatment with MCFA and SCFA is more effective for inducing cell death through apoptosis. Additionally, numerous studies have reported the use of sodium butyrate and propionate as antineoplastic agents. In this chapter, the anticancer effect of water soluble NaB and its conceivable potential to augment the anticancer effect of proteasome inhibitors, as well as the principal mechanism of action of butyrate and/or proteasome inhibitors on human colorectal tumor cells, will be discussed.

The human diet is commonly deficient in ω3-fatty acids that are common components of fish and fish products. Fish oil, high in ω3-fatty acids, is known to have anticancer activities through apoptosis of cancer cells, and numerous reports have appeared to support this claim [1–8]. Thus, docosahexaenoic (22:6, *n*-3) and eicosapentaenoic (20:5, *n*-3) acids are effective antitumor adjuvants that provoke apoptosis in several types of tumor cells without an injury to natural cells [9–11]. However, most unsaturated fatty acids can undergo oxidative stress (OS), which has been implicated in many pathological conditions. OS modifies many biological molecules/pathways, often resulting in serious consequences. Lipid peroxidation, in addition to DNA and protein oxidation, is one such modification of lipids that involves unsaturated fatty acids, resulting in the formation of fatal peroxyl radicals and activating many transcription factors. These include NF-κB, AP-1, p53, HIF-1α, PPAR-γ, β-catenin/Wnt, and Nrf2, which lead to cancer progression. In turn, a stimulation of these transcription factors activates over 500 genes, including cytokines and growth factor. This generates a strong relationship between OS, inflammation, and tumorigenesis. The fatty acid peroxyl radicals are a source of reactive hydroxyl-aldehydes, such as 4-hydroxynonenal, 4-oxononenal, and malondialdehyde. These radicals elaborate Millard reactions with proteins and other N-biomolecules, triggering mutations with an outbreak of cancer. In many ways, the metabolism of tumor cells differs from normal cells. One of the main differences between tumor cells and normal cells is the lower level of natural antioxidants desirable for defense from OS. Certain saturated fatty acids, such as odd carbon and branched carbon fatty acids, are also known to have anticancer activities [12].

Tumor cells adjust to an array of nutrient stocks for their subsistence. During an impaired glucose metabolism, tumor cells, for nutrients, switch to lipid metabolism. Cancer cells with their multiple metabolic compartments, depletion of sugars, amino acids, and lipids are considerably higher compared with their counterpart normal cells. Channeling their biosynthetic nutrients between and within cells, tumor cells contribute to their endurance and evolution. Therefore, one of the therapeutic targets to control the growth of cancer cells is to focus on the metabolic modifications between tumor and normal cells [13].

The involvement of phospholipase D1 (PLD1) in controlling the plasticity of cancer cell has been reported [14]. Cai et al. have reported that oxidation of fatty acids is the main basis of energy metabolism and inhibition of PLD1 results in a downregulation of lipid energy metabolism in tumor cells [14]. Numerous other inhibitors of lipid synthesis have also yielded encouraging results in limiting the proliferation of cancer cells. In this context, 5-(tetradecyloxy)-2-furoic acid, an inhibitor of acetyl-CoA carboxylase, giving malonyl-CoA, which is an intermediary in the synthesis of fatty acids, has offered promising results for inhibiting

cancer cell upturn and proliferation. Other lipid synthesis inhibitors, such as fatty acid synthase (FASN), cerulenin, and irgasan, suppress the proliferation of MiaPaCa-2 and AsPC-1 cells through depletion of fatty acids and apoptosis of cancer cells [15]. Leucine deficit also inhibits FASN in breast cancer cells [16]. An overexpression of FASN in neoplasms is widely reported in the literature, as its inhibition by certain synthetic imides, such as N-phenylmaleimides [17].

Histone deacylase (HDAC) promotes deacylation by hydrolyzing histone lysine residues and plays a significant role in the regulation of gene expression. However, HDAC is overexpressed in several forms of cancer and is a target for several anticancer drugs. HDAC inhibitors prohibit the deacylation of not only histone but also nonhistone proteins and promote cell survival and anticancer activity. The inhibitors of HADC, presently approved by the FDA, include vorinostat, romidepsin, belinostat, and panobinostat, and several other HDAC inhibitors are already in clinical trial. Manal et al. have reported that SCFAs are novel HDAC inhibitors with advanced anticancer characteristics [18]. However, carnitine palmitoyl transferase 1 (CPT1), which is involved in the transport of long-chain fatty acids for β-oxidation, has been reported to be a specific target for anticancer therapies that is more selective than HDAC [19].

Gonadotropin-releasing hormone-III, when acylated with butyric acid at lysine position four, forms the bioconjugate, GnRH-III(4)Lys(Bu), and is reported to have significant benefits over free daunorubicin as an antitumor agent [20].

2. Butyrate

Butyrate is a short 4-carbon fatty acid and is one of the three observed in the mammalian colonic lumen [21]. It is known that anaerobic fermentation of carbohydrates and proteins in the lumen produces butyric acid [22]. Biological reaction modification, resulting in gene activation and growth control, by butyrate and its water-soluble sodium salt has been reported [23]. An inhibition of DNA synthesis may be responsible for arresting the proliferating cells and an induction of cell differentiation [24]. These results have led to contemplate that a short-chain fatty acid, such as butyrate, may be a useful agent with antiproliferative and antineoplastic significance for typical mucosal epithelial cells [25]. Since butyrate is a dietary short-chain fatty acid with low toxicity and growth inhibitory consequences, we decided to investigate its potential to augment the anticancer effect of a collection of proteasome inhibitors (MG115, MG132, PSI-1, PSI-2, and epoxomicin) on colorectal cancer cells [26].

For ubiquitin-dependence of cellular proteins, a proteasome of multicatalytic nature and a degradation of over 80% intracellular proteins have been proposed [27]. For the regulation of protein synthesis during cellular stress, including apoptosis, impaired DNA, hypoxia, signal transduction, and so on, ubiquitin is recognized to play a dynamic role [28]. In oncology, validation of proteasome as a clinical agent has been provided by the use of bortezomib, which is

a boronic acid dipeptide [28] and is an effective agent for treating multiple myeloma and certain types of non-Hodgkin's lymphoma [29, 30]. Nevertheless, many patients do not respond to bortezomib. This is despite the fact that a regular use of bortezomib has many serious consequences including cardiac problems, excruciating neuropathy as well as thrombocytopenia [31–34]. For proteasome recovery, the treatment with bortezomib has been restricted biweekly [35]. Furthermore, in tumorigenesis, drug resistance to proteasome inhibitors [36] is a challenge.

Numerous types of tumors have been treated by an induction of apoptosis that can be triggered by several drugs and proteasome inhibitors. Again, drug toxicity and cell resistance are the cost that the patients have to bear [37]. The dietary sodium butyrate offers a valuable treatment with minor toxicity but a high degree of apoptic strength [38] making it a substance of choice for treating various types of tumors. We hypothesized that the anticancer characteristics of the proteasome inhibitors MG115, MG132, PSI-1, PSI-2 and epoxomicin in human colorectal carcinoma could be potentiated by NaB [26].

Human colorectal cancer cell line SW837 treated with NaB, MG115, and a combination of the two, for 24 h, showed a minor growth inhibition of the tumor cells (mean, 6 ± 0.4%) (**Figure 2A**). A distinct inhibition of SW837 cells (mean, 85 +_ 2%), with an increase in the treatment time to 72 h, was observed. While a modest inhibition (mean 31 ± 4%) was detected after a single treatment with MG115, in 72 hrs. A combination of two of the therapies NaB and MG115 had a vivid inhibitory effect on the growth of SW837 tumor cells (mean, 85 ± 2%) and it was comparable with NaB when applied alone. However, the combination treatment for 72 h produced a statistically significant ($P \leq 0.004$) inhibition of SW837 tumor cells compared with a single treatment with MG115. Increasing the treatment time to 120 h with the combination therapy, SW837, exhibited an inhibition of growth (mean, 90 ± 2%) compared with a sole action of NaB (mean, 87 ± 2%). Contrarily, after 120 h of treatment, MG115 alone showed only a humble inhibition (mean, 31 ± 5%). The growth inhibition of SW837 was statistically significant ($P \leq 0.002$) in a combination therapy of NaB and MG115 for 120 h compared with MG115 alone (**Figure 2A**).

Next, we tested the efficacy of MG132 and NaB on the growth of SW837 for 24 h. It was found that the growth of SW837 was nonsignificantly affected by the individual two therapies (**Figure 2B**). However, after 72 h of treatment, a combination of the two therapies significantly constrained the growth of SW837 (mean, 86 ± 2%). A growth inhibition (mean, 84 ± 2%) comparable to the combination treatment was observed with NaB. A solitary treatment with MG132 for 72 h resulted in nonsignificant inhibition of SW837 (mean, 40 ± 4%). The inhibition change in SW837, after a combination treatment of NaB/MG132 and a sole treatment with MG132, was statistically significant ($P \leq 0.022$). With a prolonged treatment of SW837 for 120 h, the combination of NaB/MG132 resulted in a distinctive reticence in the cell growth (mean, 89 ± 2%). However, a treatment with MG132 alone for 120 h resulted in a minuscule growth inhibition (mean, 47 ± 5%). The change in inhibition of SW837 after a combination treatment of NaB/MG132 and a sole treatment with MG132 was statistically significant ($P \leq 0.037$) (**Figure 2B**).

We also investigated the effect of another proteasome PSI-1 alone and in combination with NaB. A 24 h treatment of SW837 tumor cells resulted in (mean, 12 ± 1.0%; 1.3 ± 0.4% and 3.0 ± 0.4%) for NaB, PSI-1, and combination of the two agents, respectively (**Figure 2C**). A striking difference (mean, 96.0 ± 4.0%) was observed when SW837 was treated with a combination of NaB and PSI-1, compared with PSI-1 alone (mean, 54.0 ± 2.0%) for 72 h. The change in inhibition of SW837 after a combination treatment of NaB/PSI-1 and a sole treatment with PSI-1 was statistically significant ($P \leq 0.001$). The change in inhibition of SW837 cells after a combination treatment of NaB/PSI-1 and a sole treatment with PSI-1 was statistically nonsignificant ($P \leq 0.32$). A prolonged treatment of SW837 cells for 120 h produced comparable results **Figure 2C**. The combination action showed a higher inhibition for SW837 cells, compared

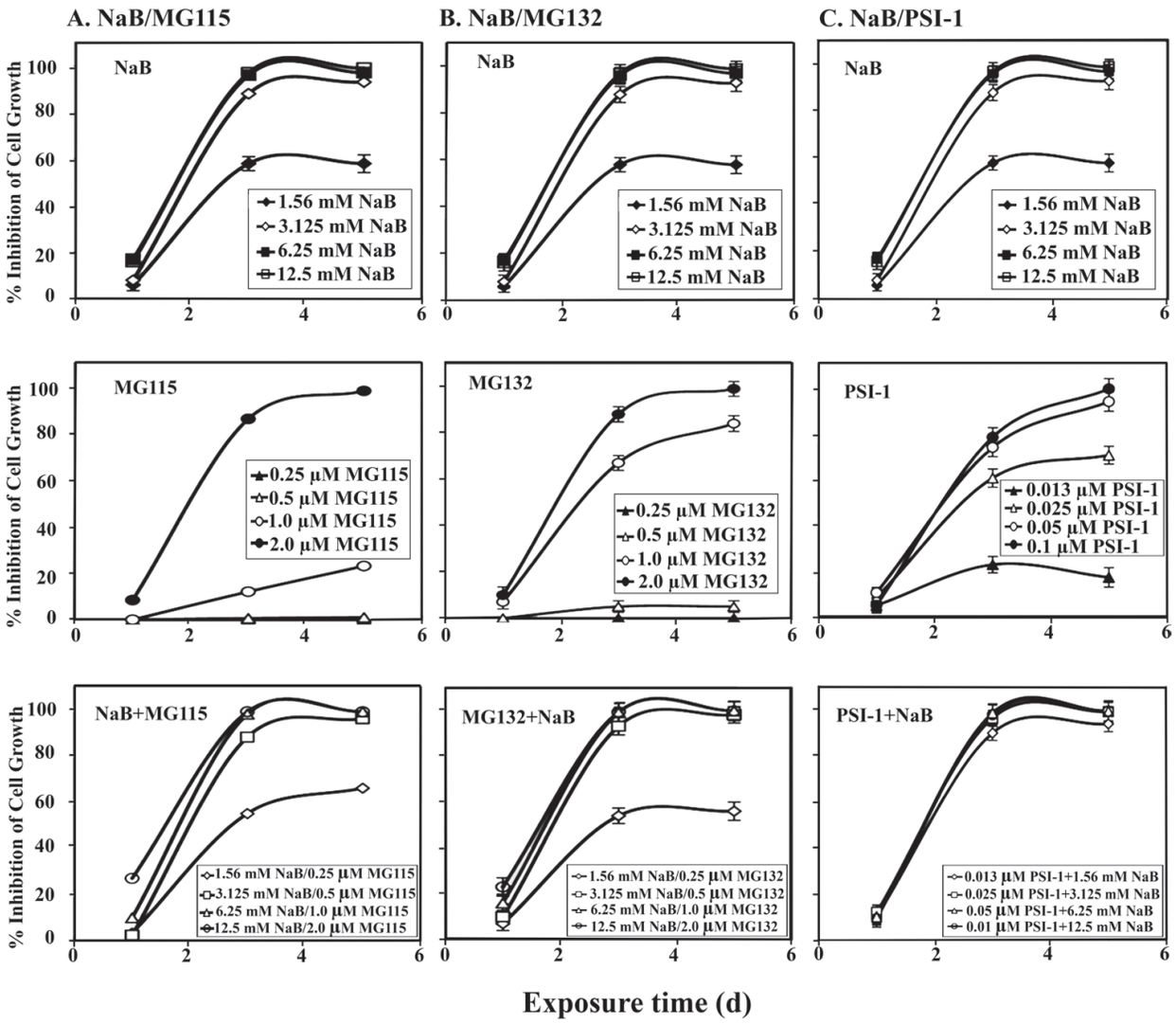

Figure 2. Enhancement of the anticancer effect of proteasome inhibitors MG115, MG132, and PSI-1 with NaB on human colorectal cancer SW837 cells. SW837 cells were plated (27×10^3 cells/well) into 96-well plates and incubated at 37°C in a non-CO_2 incubator. After 18 h, the cells were treated with NaB (1.56–12.5 mM), MG115 (0.25–2.0 μM), MG132 (0.25–2 μM), PSI-1 (0.013–0.1 μM), and the combinations of NaB and MG115 (A), MG132 (B), or PSI-1 (C) starting 18 h after seeding the cells in culture. Control cells were left untreated or treated with vehicle (DMSO) at a final concentration (0.1%). Cell growth was monitored by MTT assay.

with a usage of NaB alone (mean, 87.0 ± 2.0%). The other results of SW837 cells inhibition with NaB, PSI-2, epoxomicin, and their combinations are shown in **Figure 3**.

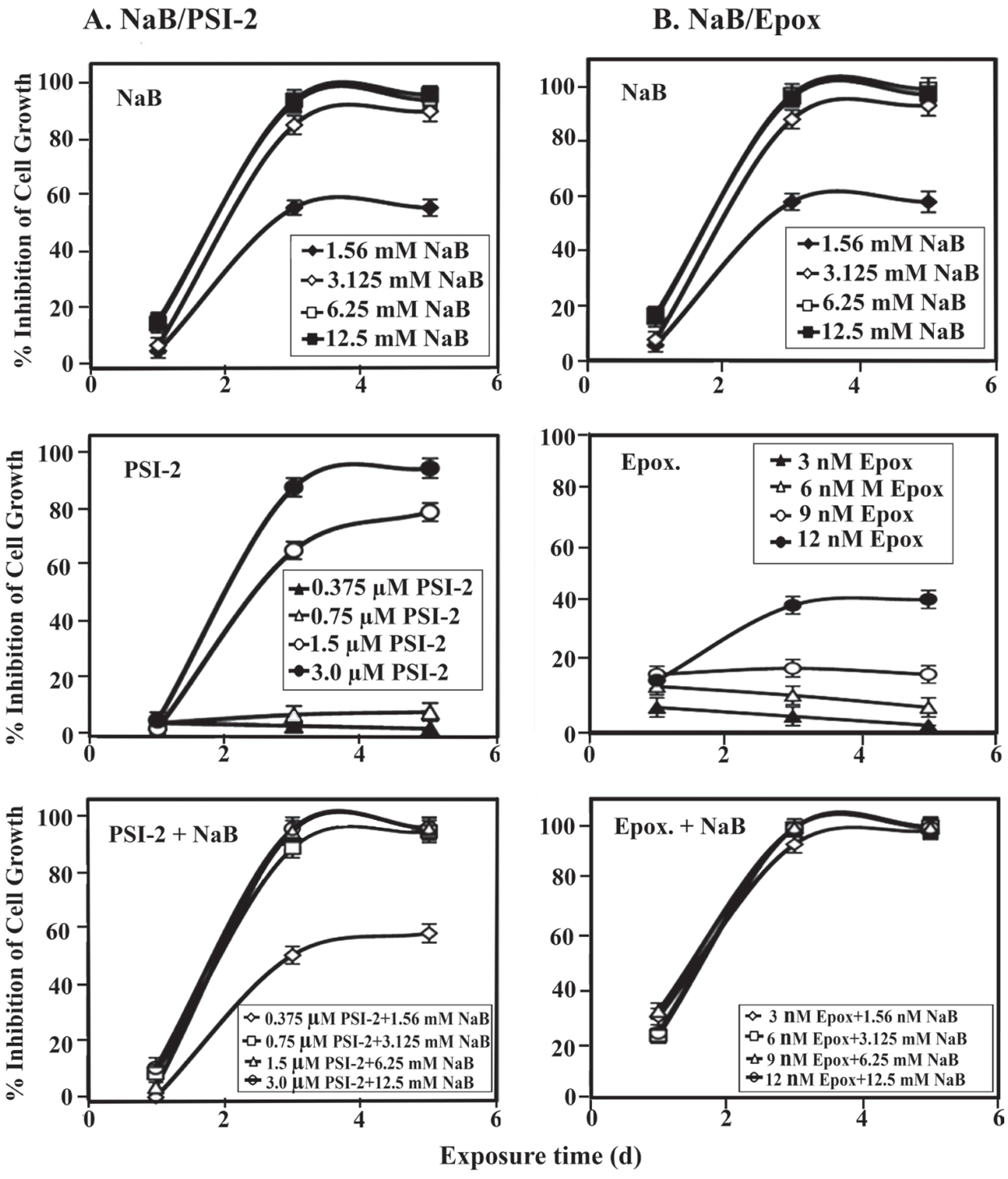

Figure 3. Enhancement of the anticancer effect of proteasome inhibitors PSI-2 and epoxomicin with NaB on human colorectal cancer SW837 cells. SW837 cells were plated (27×10^3 cells/well) into 96-well plates and incubated at 37°C in a non-CO_2 incubator. After 18 h, the cells were treated with NaB (1.56–12.5 mM), PSI-2 (0.375–3.0 µM), epoxomicin (3.0–12 nM), and the combinations of NaB and PSI-2 (A) or epoxomicin (B) starting 18 h after seeding the cells in culture. Control cells were left untreated or treated with vehicle (DMSO) at a final concentration (0.1%). Cell growth was monitored by MTT assay.

An analysis of our investigations has established that human colorectal cancer cell line, SW837, when treated with 3 mM NaB caused amassing of cells in the G1-phase (82.7%), and a corresponding reduction in the number of cells in G2/M- (2.61%) and S- (14.7%) phases (**Figure 4**). In addition, treatment with 1.0 μM MG115, 0.1 μM MG132, 0.1 μM PSI-1, 1.5 μM PSI-2, or 12 nM epoxomicin followed a buildup of cells in the S-phase (55.5, 33.7, 41.5, 42.7, and 32.2%, respectively) and G2-phase (12.2, 36.1, 44.3, 29.9, and 45.7%, respectively) with a consequent reduction in the total cells in the G1-phase (29.0, 29.7, 14.1, 27.4 and 22.3%, respectively).

A combination of NaB at 3 mM and MG115 or MG132 at 1.0 μM concentration caused the colorectal cancer cells arrest in the G1-phase (79.8 or 75.5%, respectively) and the G2-phase (6.73 or 14.4%, respectively). The upturn in the G1-phase complemented by an equivalent reduction in the S-phase of the cells (13.5 or 10%, respectively) (**Figure 4**). A combination treatment with NaB (3 mM) and PSI-1 (1.0 μM), PSI-2 (1.5 μM), or epoxomicin (12 nM) resulted in an increase in the number of cells in the G2-phase (45.2, 92.7, and 88.3%, respectively). This was accompanied by a parallel reduction in the quantity of cells in the G1-phase (55.4, 7.29, or 12.3%, respectively) and the S-phase (0.0%) (**Figure 4**).

Next, we turned to analyze the effect of antineoplastic agents on the DNA of treated cells by agarose gel electrophoresis. The DNA was extracted from the untreated and treated human colorectal cancer cells that displayed a discrete ladder pattern, displaying apoptosis. These consequences obviously displayed that the action of NaB, proteasome inhibitors, or their combination triggered the apoptotic trail. The magnitude of apoptosis of cancer cells, treated with a combination of NaB and proteasome inhibitors was prominent compared with the NaB or proteasome inhibitors alone (**Figure 5**).

The regulation of gene expression and inhibition of histone deacylases are regulated by NaB [39]. The hyperacetylation of histones and an amelioration of the availability of the transcription factors to nucleosomal DNA are due to inhibition of histone deacylases [40]. Hyperacetylation of nonhistone proteins, modification of DNA methylation, careful inhibition of histone phosphorylation, and alteration of intracellular kinase signaling may be the other cellular targets of NaB [39]. This multistage mechanism of butyrate explains the gene expression regulation and its impact on the crucial regulators of apoptosis and the cell cycle.

The synergistic apoptotic consequences of NaB and proteasome inhibitors may offer new opportunities in research to develop therapeutic strategies to contain human colorectal cancer. The proteasome inhibitors seem to act as apoptotic agents only in the rapidly dividing cells while shielding quiescent cells from apoptosis that may be activated by many diverse compounds [30]. For this specific action, proteasome inhibitors may be used as a substitute in the treatment of some proliferative disorders. Moreover, treatment with a combination of proteasome inhibitors, effective apoptotic agents, such as NaB, and other short-chain fatty acids may be a valuable therapeutic strategy for the treatment of proliferative diseases such as colorectal cancer. Thus, further research in this area may be very rewarding and offer hope to the suffering patients around the globe.

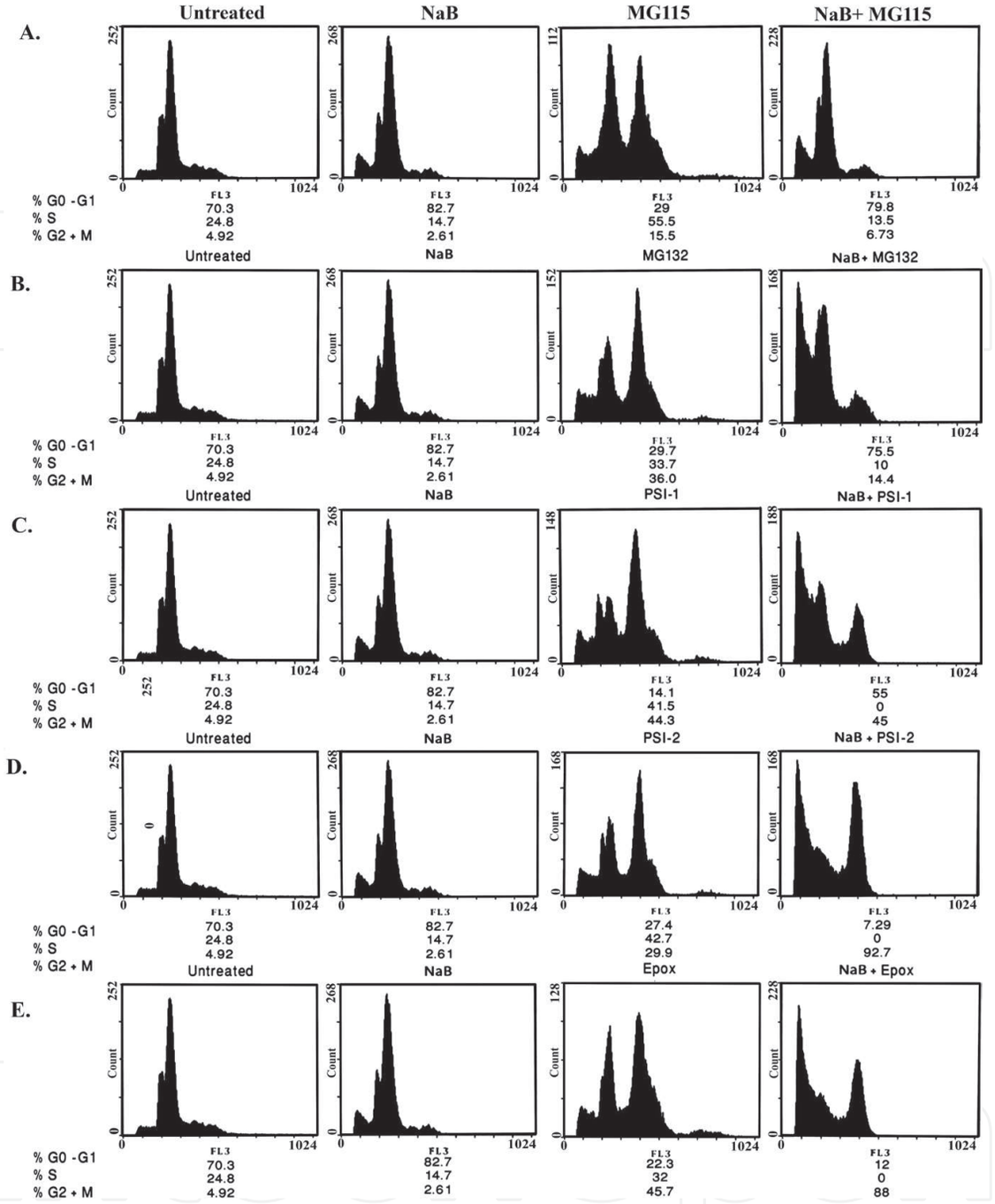

Figure 4. Cell cycle distribution of human colorectal cancer SW837 cell treated with NaB, proteasome inhibitors, and their combinations. SW837 cells were plated (5×10^5 cells/well) into 24-well plates and incubated at 37°C in a non-CO_2 incubator. After 18 h, the cells were treated individually with NaB (3.0 mM), MG115 (1.0 μM), MG132 (1.0 μM), PSI-1 (0.1 μM), PSI-2 (1.5 μM), and epoxomicin (12 nM) or treated with the combinations NaB/MG115 (3 mM/1.0 μM), NaB/MG132 (3.0 mM/1.0 μM), NaB/PSI-1 (3.0 mM/0.1 μM), NaB/PSI-2 (3.0 mM/1.5 μM), and Na/epoxomicin (3.0 mM/12 nM) for 72 h. At least duplicate samples were analyzed and 20,000 events were scored for each sample. The vertical axis represents the relative number of events and the horizontal axis represents the fluorescence intensity. The percentage of cells in different cell cycle phases was calculated using Phoenix statistical software package. A-E: Single and combined treatments with NaB and MG11S, MG132, PSI-1, PSI-2 or Epox, respectively.

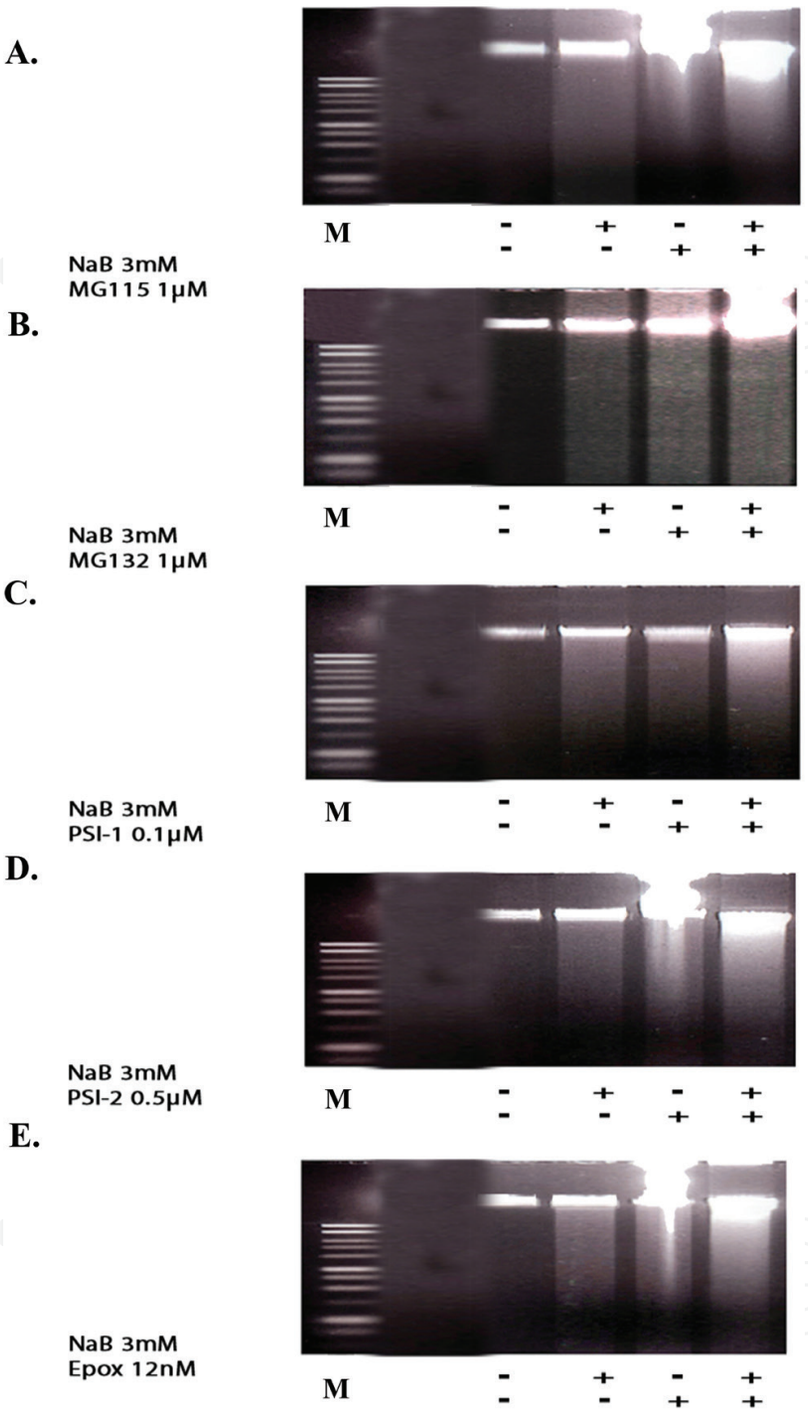

Figure 5. Assessment of apoptosis in human colorectal SW837 cells treated with NaB, proteasome inhibitors, and their combinations. SW837 cells were plated (5×10^5 cells/well) into 24-well plates and incubated at 37°C in a non-CO2 incubator. After 18 h, the cells were treated individually with NaB (3.0 mM), MG115 (1.0 μM), MG132 (1.0 μM), PSI-1 (0.1 μM), PSI-2 (1.5 μM), and epoxomicin (12 nM) or treated with the combinations NaB/MG115 (3 mM/1.0 μM), NaB/MG132 (3.0 mM/1.0 μM), NaB/PSI-1 (3.0 mM/0.1 μM), NaB/PSI-2 (3.0 mM/1.5 μM), and Na/epoxomicin (3.0 mM/ 12 nM) for 72 h. DNA fragments were extracted and analyzed on 1.0% agrose gel. A-E: Single (+) and combined (++) treatments with NaB and MG115, MG132, PSI-1, PSI-2 or Epox, respectively.

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