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Evaluation of Sodium Butyrate-Induced Cellular and Ultrastructural Changes in K562 Cells Using Scanning and Transmission Electron Microscopy

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ABSTRACT
Sodium butyrate (NaB) is an established inducer of erythroid differentiation and fetal hemoglobin production in K562 cells. However, its cellular and ultrastructural effects across different concentrations and exposure periods require further characterization. This study evaluated the cellular and ultrastructural effects of NaB on human erythroleukemia K562 cells using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). K562 cells were exposed to 0.01, 0.1, and 1.0 mM NaB for 1, 3, and 7 days. Untreated cells served as controls, while 1 μM hydroxyurea (HU) and a combination of 1 μM HU with 0.1 mM NaB were included for comparison. SEM assessed cell shape, size, microvilli, surface folds, flattening, and surface irregularities, whereas TEM evaluated plasma and nuclear membrane integrity, chromatin organization, mitochondria, cytoplasmic organization, and vacuolation. NaB produced concentration- and time-dependent morphological alterations. Exposure to 0.01 mM NaB produced relatively limited surface changes, although cytoplasmic vacuolation increased with prolonged exposure. At 0.1 mM, cells demonstrated progressive flattening, loss of microvilli and surface folds, increased cytoplasmic vacuolation, and alterations in nuclear organization. The most pronounced abnormalities occurred at 1.0 mM NaB, including cellular flattening and distortion, loss of microvilli, surface irregularities, plasma membrane disruption, severe cytoplasmic vacuolation, and loss of nuclear membrane integrity. HU and the NaB-HU combination also produced morphological changes, although intracellular structures were relatively preserved during early exposure periods. The findings demonstrate that NaB induces concentration- and exposure-dependent cellular and ultrastructural changes in K562 cells, with the highest concentration showing the clearest evidence of cellular injury. These findings provide morphological evidence for distinguishing differentiation-associated responses from concentration-dependent cytotoxic effects of NaB.
Keywords: Sodium Butyrate; K562 Cells; Erythroid Differentiation; Fetal Hemoglobin; Scanning Electron Microscopy; Transmission Electron Microscopy; Ultrastructure; Cellular Morphology; Hydroxyurea; Cytotoxicity.
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INTRODUCTION

Sickle cell disease (SCD) is a hereditary hemoglobinopathy caused primarily by a mutation in the β-globin gene that results in the production of abnormal hemoglobin S (HbS), which polymerizes under deoxygenated conditions and promotes red blood cell sickling, hemolysis, vaso-occlusion, recurrent pain, and progressive organ damage (Embury, 1986; Ware et al., 2017). The clinical severity of SCD is influenced by several factors, including the amount of fetal hemoglobin (HbF) produced by affected individuals. HbF contains γ-globin rather than β-globin and interferes with the polymerization of HbS, such that higher HbF levels are generally associated with reduced disease severity (Bunn, 1993; Ware et al., 2017). The regulation of γ-globin expression and the developmental transition from embryonic to fetal and adult hemoglobin production, commonly referred to as hemoglobin switching, therefore represent important biological processes in the study of β-hemoglobin disorders (Stamatoyannopoulos & Nienhuis, 1987). The induction of HbF remains an important therapeutic and research strategy for the management of SCD and other β-hemoglobin disorders (Dover et al., 1986; Ware et al., 2017). The biological importance of HbF induction has stimulated considerable interest in pharmacological agents capable of reactivating γ-globin expression in erythroid cells.

Hydroxyurea is an established HbF-inducing agent and has been shown to increase HbF production in individuals with SCD, although its effects are related to dose, cellular proliferation, and cytotoxicity (Dover et al., 1986). Clinical use of hydroxyurea therefore requires continued monitoring, particularly because its therapeutic effects occur in the context of its effects on proliferating hematopoietic cells. Other agents, including butyrate and butyrate derivatives, have consequently been investigated as potential differentiation-inducing and

HbF-inducing compounds. Early experimental and clinical studies demonstrated that sodium butyrate and related compounds could increase HbF production in erythroid progenitors and individuals with hemoglobin disorders (Constantoulakis et al., 1989; Dover et al., 1992; Perrine et al., 1993; Stamatoyannopoulos et al., 1994). Constantoulakis et al. (1989), for example, demonstrated that sodium butyrate increased the frequency of HbF-positive erythroid colonies and stimulated γ-globin expression in erythroid progenitors. The investigators also provided evidence that the response involved activation of globin gene expression rather than simply an increase in the number of erythroid progenitor cells. Clinical investigations subsequently demonstrated that butyrate treatment could increase fetal-globin synthesis in patients with β-globin disorders, providing further evidence that butyrate can activate fetal-globin expression in vivo (Perrine et al., 1993). Related studies also demonstrated increased HbF among patients receiving sodium 4-phenylbutyrate, supporting continued interest in butyrate-derived compounds as potential HbF-inducing agents (Dover et al., 1992). Stamatoyannopoulos et al. (1994) further demonstrated that acetate, a metabolite of butyrate, could induce HbF, suggesting that metabolic products of butyrate may contribute to its globin-inducing activity.

The K562 human erythroleukemia cell line provides an important experimental model for investigating erythroid differentiation and hemoglobin production. K562 cells exhibit erythroid-associated characteristics and can be induced to differentiate toward an erythroid phenotype following exposure to several chemical agents (Benz et al., 1980; Rutherford et al., 1981). Rutherford et al. (1981) demonstrated embryonic erythroid differentiation and embryonic hemoglobin production in K562 cells, establishing the usefulness of this cell line for studying developmental regulation of erythroid differentiation. Benz et al. (1980) similarly characterized K562 cells as possessing embryonic-fetal erythroid properties, further supporting their use as an experimental model for investigating hemoglobin expression and erythroid maturation. Villeval et al. (1983) subsequently demonstrated that K562 cells express erythroid-associated characteristics, including embryonic and fetal hemoglobins, glycophorin A, spectrin, and acetylcholinesterase. Their study also showed that butyrate increased acetylcholinesterase activity and slightly enhanced hemoglobin production, although butyrate did not produce complete terminal differentiation of K562 cells. Sodium butyrate is particularly relevant to erythroid differentiation because its biological effects extend beyond hemoglobin production. Butyrate can alter cellular proliferation, differentiation, intracellular signaling, and cellular morphology. Davis et al. (2000) demonstrated that sodium butyrate increased Giα2 expression during erythroblastic differentiation of K562 cells and that disruption of Giα2 signaling substantially reduced hemoglobin accumulation. These findings suggest that Giα2-associated signaling contributes to the differentiation response induced by sodium butyrate. Witt et al. (2000) further demonstrated that butyrate-induced erythroid differentiation in K562 cells was associated with inhibition of ERK and activation of p38 MAP kinase pathways. Inhibition of p38 activity abolished the hemoglobin-inducing effect of butyrate, providing evidence that p38 signaling is an important component of the differentiation response. The combined findings from these studies indicate that NaB-induced erythroid differentiation involves coordinated effects on intracellular signaling, cell-cycle regulation, and hemoglobin-associated cellular maturation (Davis et al., 2000; Witt et al., 2000).

The biological effects of sodium butyrate must also be considered in relation to its pharmacological properties and potential cellular toxicity. Butyrate exposure can inhibit cellular proliferation while promoting differentiation, creating an important balance between the desired biological response and loss of viable cells. Clinical pharmacological investigations of sodium butyrate have demonstrated that treatment produces measurable systemic and cellular effects, supporting the importance of exposure conditions when evaluating its biological activity (Miller et al., 1987). Similarly, the relationship between cytotoxicity and HbF induction observed with hydroxyurea demonstrates that an increase in HbF cannot be interpreted independently of its effects on cellular proliferation and viability (Dover et al., 1986). Consequently, concentration and duration of exposure are important considerations when evaluating NaB-induced erythroid responses. While previous studies have established that butyrate can stimulate fetal-globin expression and erythroid differentiation (Constantoulakis et al., 1989; Perrine et al., 1993; Davis et al., 2000; Witt et al., 2000; Stamatoyannopoulos et al., 1994), important questions remain concerning the relationship between NaB concentration, duration of exposure, cellular proliferation, hemoglobin accumulation, and cellular structural integrity. A higher concentration of NaB may produce a more pronounced differentiation-associated response but may simultaneously suppress cellular proliferation or induce cellular injury. Conversely, a lower or intermediate concentration may maintain greater cellular growth while producing a delayed but potentially sustained differentiation response. These considerations are particularly important because the biological effectiveness of a differentiation-inducing agent depends not only on its ability to increase hemoglobin-associated markers but also on its effects on cellular viability and morphology.

Although biochemical and molecular studies have provided substantial evidence of NaB-induced erythroid differentiation, less attention has been given to the ultrastructural consequences of NaB exposure in K562 cells. Changes in cell-surface morphology, plasma membrane integrity, nuclear morphology, chromatin organization, mitochondrial characteristics, and cytoplasmic vacuolation can provide important complementary information about cellular differentiation, injury, and toxicity. Scanning electron microscopy (SEM) is particularly useful for examining external cellular morphology, including surface projections, microvilli, membrane folds, flattening, and membrane-associated abnormalities. Transmission electron microscopy (TEM), in contrast, permits examination of intracellular structures, including the plasma and nuclear membranes, mitochondria, chromatin, cytoplasmic organization, and vacuolation. Therefore, ultrastructural analysis can complement biochemical measurements by demonstrating whether changes associated with NaB exposure represent potentially favorable differentiation-related alterations or evidence of cellular stress and injury. The distinction between differentiation-associated morphological changes and cellular toxicity is particularly important when evaluating sodium butyrate. Butyrate can inhibit proliferation while promoting erythroid differentiation, but increasing exposure may also produce cellular stress and structural injury. The present findings demonstrate changes in K562 cell-surface morphology following exposure to 0.01, 0.1, and 1.0 mM NaB and show progressively more pronounced ultrastructural abnormalities at higher concentrations. At 1.0 mM, SEM demonstrated marked flattening, loss of microvilli, dark surface abnormalities, and reduced cell size, while TEM demonstrated plasma membrane disruption, severe cytoplasmic vacuolation, loss of nuclear membrane integrity, and displacement of nuclear contents. These observations provide morphological evidence that the cellular response to NaB is concentration- and time-dependent. This study therefore evaluates sodium butyrate-induced cellular and ultrastructural changes in K562 cells using SEM and TEM. Specifically, the study compares untreated control cells with cells exposed to 0.01, 0.1, and 1.0 mM NaB and examines the effects of 1 µM hydroxyurea and a combination of 1 µM hydroxyurea with 0.1 mM NaB. The study focuses on changes occurring over 1, 3, and 7 days of exposure. By combining surface and intracellular ultrastructural observations with measures of cellular growth and hemoglobin-associated responses, the study provides additional evidence concerning the cellular effects, potential differentiation-associated changes, and concentration-dependent toxicity of sodium butyrate in K562 cells.

LITERATURE REVIEW

K562 Cells as a Model of Erythroid Differentiation

The K562 cell line is a human chronic myelogenous leukemia-derived cell line that has been widely used as an experimental model for studying erythroid differentiation. Its usefulness is related to its capacity to express erythroid characteristics and to respond to chemical differentiation agents. Early investigations demonstrated that K562 cells exhibit embryonic and fetal erythroid characteristics and can express hemoglobin and other erythroid-associated markers (Benz et al., 1980; Weatherall et al., 1981).

Villeval et al. (1983) provided important evidence concerning the erythroid phenotype of K562 cells. Their investigation showed that K562 cells produce embryonic and fetal hemoglobins as well as glycophorin A, spectrin, and acetylcholinesterase. Exposure to hemin increased hemoglobin accumulation, whereas butyrate markedly increased acetylcholinesterase activity and modestly increased hemoglobin production. Importantly, the investigators concluded that butyrate and hemin did not induce complete terminal differentiation of K562 cells. These findings established K562 cells as a useful system for investigating the cellular and biochemical processes involved in erythroid differentiation.

Sodium Butyrate and Erythroid Differentiation

Sodium butyrate is a short-chain fatty acid that has been investigated extensively as an inducer of cellular differentiation. Its biological effects include changes in gene expression, cell proliferation, chromatin regulation, and cellular differentiation. The ability of butyrate to influence erythroid differentiation has made it particularly relevant to research involving HbF induction. Constantoulakis et al. (1989) demonstrated that sodium butyrate increased HbF-positive erythroid colonies in experimental systems and stimulated γ-globin expression. In bone marrow cultures, NaB increased the frequency of HbF-positive erythroid progenitor colonies without producing a corresponding increase in the overall number of progenitor cells. Their findings suggested that butyrate can influence the expression of the fetal globin program rather than simply increasing the number of erythroid progenitors.

The relationship between butyrate and HbF induction has also been investigated through its metabolites. Stamatoyannopoulos et al. (1994) reported that acetate, a product of butyrate catabolism, could itself stimulate γ-globin expression. In erythroid progenitor cultures from healthy individuals and individuals with sickle cell disease, acetate increased γ-globin protein and γ-globin mRNA, providing evidence that short-chain fatty acid metabolism may contribute to HbF induction. Clinical observations have provided additional support for the potential of butyrate-related compounds to influence fetal hemoglobin production. Dover et al. (1992) reported increased HbF in patients receiving sodium 4-phenylbutyrate. The authors noted the potential importance of differentiation-inducing agents as alternatives to conventional cytotoxic HbF-inducing approaches. However, the clinical application of butyrate-based approaches has been influenced by treatment duration, administration requirements, pharmacological limitations, and the need to establish effective and safe dosing strategies.

Cellular Signaling Mechanisms of Sodium Butyrate

The cellular effects of sodium butyrate appear to involve multiple intracellular signaling mechanisms. Witt et al. (2000) investigated the MAP kinase pathways involved in NaB-induced erythroid differentiation of K562 cells. Their results demonstrated that butyrate decreased ERK and JNK phosphorylation while increasing p38 phosphorylation. Importantly, inhibition of p38 abolished the hemoglobin-inducing effect of butyrate, whereas inhibition of ERK enhanced erythroid differentiation and acted synergistically with butyrate. These findings suggest that sodium butyrate does not simply act as a nonspecific cellular toxin. Rather, its effects can involve regulated changes in intracellular signaling that influence proliferation and differentiation. Nevertheless, the degree to which these signaling responses remain beneficial may depend on concentration and duration of exposure. Davis et al. (2000) provided additional evidence for a signaling component in NaB-induced erythroid differentiation. Their study showed a substantial increase in Giα2 protein and mRNA following sodium butyrate treatment. Disruption of Giα2 signaling reduced NaB-induced hemoglobin accumulation, indicating that Giα2 contributes to the erythroblastic differentiation process.

Sodium Butyrate and Cellular Morphology

Morphological changes are an important component of cellular differentiation studies because biochemical changes may occur simultaneously with alterations in cellular shape and organization. In K562 cells, differentiation can involve changes in cell size, surface characteristics, cytoplasmic organization, and expression of erythroid-associated structures. The previous literature primarily describes NaB-induced differentiation using measurements such as hemoglobin accumulation, γ-globin expression, acetylcholinesterase activity, cell proliferation, and intracellular signaling. Villeval et al. (1983), for example, demonstrated biochemical evidence of erythroid characteristics following butyrate exposure but did not establish complete terminal differentiation. Witt et al. (2000) similarly evaluated differentiation primarily through hemoglobin synthesis, proliferation, and MAP kinase signaling. Consequently, electron microscopy provides a valuable complementary approach. SEM permits detailed assessment of the external surface of K562 cells, whereas TEM permits examination of internal structures. In the present study, these techniques reveal progressive changes in microvilli, surface folds, cell flattening, membrane integrity, nuclear organization, and cytoplasmic vacuolation following NaB exposure.

Sodium Butyrate, Concentration, and Potential Cytotoxicity

An important consideration in the use of NaB as a differentiation-inducing compound is the distinction between desired differentiation effects and undesirable cellular injury. A concentration that promotes erythroid-associated changes without extensive cellular damage may be more useful experimentally than a higher concentration that produces rapid structural deterioration. The current findings are consistent with this concept. At 0.01 mM NaB, cells generally retained recognizable morphology, although changes in microvilli, surface folds, and cytoplasmic vacuolation were observed with prolonged exposure. At 0.1 mM NaB, cells became progressively flattened and developed reduced surface complexity, while TEM demonstrated increasing cytoplasmic vacuolation after prolonged exposure. At 1.0 mM NaB, the abnormalities were substantially more severe, including membrane disruption, extensive vacuolation, nuclear membrane loss, and apparent leakage of cellular contents. These observations suggest that the structural response to NaB is strongly dependent on concentration and duration of exposure. The literature therefore supports examining both differentiation and toxicity when evaluating NaB. The earlier studies establish that butyrate can induce erythroid-associated biochemical changes, while the present ultrastructural analysis extends this evidence by documenting the physical cellular changes accompanying NaB exposure.

METHODOLOGY

This study employed an experimental laboratory design to evaluate the cellular and ultrastructural effects of sodium butyrate (NaB) on human erythroleukemia K562 cells using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The experimental design incorporated sodium butyrate concentration and exposure duration as the principal experimental factors. Sodium butyrate was evaluated at concentrations of 0.01, 0.1, and 1.0 mM, with cellular morphology examined following exposure for 1, 3, and 7 days. Untreated K562 cells maintained in cell culture medium served as the control group. In addition, 1 μM hydroxyurea (HU) and a combination of 1 μM HU with 0.1 mM NaB were evaluated to provide comparative information regarding the ultrastructural effects of NaB, HU, and their combined treatment. The use of K562 cells was based on their established erythroid characteristics and their capacity to respond to differentiation-inducing agents, including sodium butyrate (Villeval et al., 1983; Davis et al., 2000; Witt et al., 2000).

Experimental Cell Model: Human erythroleukemia K562 cells were used as the experimental cell model. K562 cells are an appropriate model for investigating erythroid differentiation because they exhibit erythroid-associated characteristics and can respond to chemical inducers through changes in cellular morphology and hemoglobin production (Villeval et al., 1983; Davis et al., 2000). The cells were maintained under standard cell-culture conditions appropriate for K562 cell growth. Untreated cells maintained in cell culture medium served as the control condition, whereas experimental cells were exposed to sodium butyrate, hydroxyurea, or the NaB-HU combination according to the experimental treatment groups.

Experimental Treatment Groups: The experimental groups were organized according to treatment condition and exposure duration. The control group consisted of K562 cells maintained in cell culture medium without drug treatment. The sodium butyrate treatment groups consisted of K562 cells exposed to 0.01, 0.1, or 1.0 mM NaB. An additional group was exposed to 1 μM hydroxyurea, while the combination-treatment group received 1 μM hydroxyurea and 0.1 mM sodium butyrate. The three NaB concentrations were selected to permit evaluation of cellular responses across low, intermediate, and high concentrations. The 0.1 mM NaB concentration was also selected for combination with hydroxyurea to examine the ultrastructural response to combined treatment.

Sodium Butyrate and Hydroxyurea Exposure: Sodium butyrate was administered to K562 cell cultures at final concentrations of 0.01, 0.1, and 1.0 mM, while hydroxyurea was administered at 1 μM. For the combination treatment, K562 cells received 0.1 mM NaB and 1 μM HU simultaneously. Cells were evaluated following designated exposure periods of 1, 3, and 7 days. The use of multiple exposure periods permitted assessment of both early and prolonged morphological responses to the experimental treatments. The experimental observations were subsequently compared with untreated control cells at the corresponding culture periods.

Scanning Electron Microscopy

Sample Preparation: Following the designated exposure periods, control and treated K562 cells were prepared for scanning electron microscopy to evaluate changes in external cellular morphology. The cells were initially pre-fixed in a solution containing 1.25% glutaraldehyde and 2% formaldehyde in 0.1 mol/L sodium cacodylate buffer at pH 7.4 for 1 hour. Following primary fixation, the cells were rinsed three times with 0.1 M sodium cacodylate containing 5% sucrose. The cells were subsequently post-fixed with 1% osmium tetroxide in 0.1 mol/L sodium cacodylate buffer at pH 7.4, followed by three additional rinses with 0.1 M sodium cacodylate/5% sucrose buffer. For mounting, plastic microscope coverslips were trimmed into small squares and placed in a six-well multiwell culture dish. Each coverslip was treated with 10 μL of poly-L-lysine to facilitate attachment of the K562 cells to the coverslip surface. Cells from the different experimental exposure groups were placed on individual coverslips. The samples were then dehydrated through a graded ethanol series consisting of 30%, 50%, and 70% ethanol for 5 minutes each; 80% and 95% ethanol twice for 10 minutes each; and 100% ethanol three times for 15 minutes each. Following dehydration, filter paper was folded and labeled according to the corresponding coverslip and experimental condition. The filter papers containing the coverslips were placed in a Polaron E3000 critical point dryer for drying. The dried coverslips were subsequently mounted onto metal specimen stubs and coated with a thin electrically conductive layer of gold using a Cressington Auto 108 sputter coater. These preparation procedures followed established electron microscopy procedures described by Bozzola et al. (1992). The prepared specimens were examined using a Zeiss 940A scanning electron microscope.

SEM Evaluation: SEM was used to evaluate the external morphology of the K562 cells following exposure to the experimental treatments. Particular attention was given to cell shape and size, surface microvilli, surface folds, cell flattening, cellular distortion, dark or irregular surface structures, and evidence of surface membrane abnormalities. The morphology of treated cells was compared with that of untreated control cells to identify treatment-associated changes. The SEM micrographs were evaluated qualitatively according to treatment concentration and exposure duration. Control cells were examined after 1, 3, and 7 days, while NaB-treated cells were evaluated at 0.01, 0.1, and 1.0 mM. Hydroxyurea-treated cells and cells receiving the HU-NaB combination were also examined. The SEM findings were presented in Figures 10–15.

Transmission Electron Microscopy

Sample Preparation: Following the designated treatment periods, K562 cells were prepared for transmission electron microscopy to evaluate intracellular ultrastructural changes. The cells were initially pre-fixed in a fixative containing 1.25% glutaraldehyde and 2% formaldehyde in 0.1 mol/L sodium cacodylate buffer at pH 7.4 for 1 hour. Following fixation, the cells were rinsed three times using 0.1 M sodium cacodylate/5% sucrose buffer. The cells were then post-fixed with 1% osmium tetroxide in 0.1 mol/L sodium cacodylate buffer at pH 7.4, followed by three additional rinses with 0.1 M sodium cacodylate/5% sucrose buffer. Following fixation, the cells were suspended in 2% agar to facilitate subsequent processing. The agar-embedded cells were washed twice with distilled water and subsequently resuspended in 5% uranyl acetate stain for 3 hours. The cells were then washed three times with distilled water and stored overnight in 0.1 M sodium cacodylate/5% sucrose buffer at 4°C. For dehydration, the buffer was removed and the cells were exposed to a graded ethanol series consisting of 30%, 50%, and 70% ethanol for 5 minutes each; 80% and 95% ethanol twice for 10 minutes each; and 100% ethanol three times for 15 minutes each. Following dehydration, propylene oxide was used as a transitional solvent to facilitate infiltration of the cells with epoxy resin. Infiltration was performed sequentially using 3 parts propylene oxide to 1 part epoxy resin for 1 hour, 2 parts propylene oxide to 2 parts epoxy resin for 2 hours, 1 part propylene oxide to 3 parts epoxy resin for 1 hour, straight epoxy resin for 2 hours, and a final replacement with straight epoxy resin for 4 hours (Bozzola et al., 1992). The straight epoxy was subsequently removed and replaced with pure epoxy containing DMP-30 as the curing agent. The cells were embedded in beam capsules containing fresh DMP-30 and epoxy resin. The beam capsules were placed in a 60°C oven and cured overnight. Following polymerization, the resin blocks were trimmed and sectioned using an RMC-MT2C ultramicrotome. Ultrathin sections were collected on 300-mesh grids and subsequently stained with uranyl acetate followed by lead citrate according to established electron microscopy procedures (Bozzola et al., 1992). The prepared grids were examined using a Hitachi 7000 transmission electron microscope.

TEM Evaluation: TEM was used to evaluate the internal ultrastructure of K562 cells and to identify changes that could not be adequately characterized by SEM. The examination focused on the integrity of the plasma membrane and nuclear membrane, nuclear morphology, chromatin distribution, mitochondria, cytoplasmic organization, cytoplasmic vacuolation, and evidence of cellular disintegration. Control cells were examined after 1 and 7 days of incubation in culture medium. Experimental specimens were examined to identify treatment-associated ultrastructural alterations. Particular attention was given to membrane integrity, changes in nuclear organization, chromatin accumulation, mitochondrial characteristics, cytoplasmic vacuolation, and evidence of disruption or leakage of intracellular components. The TEM findings were presented in Figures 16–20.

Assessment of Cellular Ultrastructural Changes

The ultrastructural assessment focused on morphological characteristics that could reflect either cellular adaptation associated with differentiation or cellular injury associated with excessive treatment exposure. Preserved plasma and nuclear membranes, recognizable mitochondria and other organelles, and relatively organized cytoplasmic structures were considered indicators of preserved cellular integrity. In contrast, extensive cytoplasmic vacuolation, plasma or nuclear membrane disruption, loss of nuclear membrane definition, displacement of nuclear contents, and openings in the plasma membrane were considered evidence of substantial cellular injury. The interpretation of these changes was made in relation to both the concentration and duration of NaB exposure. This distinction was important because sodium butyrate has been reported to induce erythroid differentiation while also affecting cellular proliferation and intracellular signaling in K562 cells (Davis et al., 2000; Witt et al., 2000). Consequently, morphological changes were not interpreted automatically as evidence of toxicity; rather, the extent and pattern of structural alterations were considered in relation to the experimental concentration and exposure period.

Comparative Evaluation of Treatment Groups

The SEM and TEM findings were compared across the control, NaB, HU, and combined HU-NaB treatment groups. The comparisons considered changes in cell-surface microvilli and folds, cell size and shape, flattening and distortion, plasma membrane integrity, nuclear membrane integrity, chromatin distribution, cytoplasmic vacuolation, mitochondrial characteristics, and evidence of progressive cellular damage. Particular emphasis was placed on the concentration-dependent effects of NaB. The morphological observations were compared across 0.01, 0.1, and 1.0 mM NaB to determine whether increasing concentration was associated with increasing ultrastructural abnormalities. The effects of prolonged exposure were also evaluated by comparing observations at 1, 3, and 7 days. Finally, the morphology of cells exposed to 1 μM hydroxyurea and the HU-NaB combination was compared with that of cells receiving NaB alone.

Data Analysis

The SEM and TEM findings were analyzed using qualitative descriptive analysis because the electron microscopy results consisted primarily of visual observations of cellular morphology and ultrastructure. Micrographs were examined systematically according to treatment group and exposure duration, and the observed morphological characteristics were documented and compared across experimental conditions. The analysis focused on three primary dimensions. First, concentration-dependent changes were evaluated by comparing the morphology of cells exposed to 0.01, 0.1, and 1.0 mM NaB. Second, time-dependent changes were evaluated by comparing cellular morphology following 1, 3, and 7 days of exposure. Third, treatment-associated differences were assessed by comparing NaB-treated cells with untreated controls, hydroxyurea-treated cells, and cells receiving the combined HU-NaB treatment. The resulting SEM and TEM observations were interpreted in relation to the established biological effects of sodium butyrate on K562 cells, particularly its reported ability to influence erythroid differentiation, hemoglobin production, cellular proliferation, and intracellular signaling (Villeval et al., 1983; Davis et al., 2000; Witt et al., 2000). The electron microscopy procedures, including fixation, dehydration, critical-point drying, embedding, sectioning, and staining, were based on established electron microscopy methodology described by Bozzola et al. (1992).

RESULTS AND ANALYSIS

Scanning and Transmission Electron Microscopy

Scanning Electron Microscopy

The scanning electron microscopy findings demonstrated clear differences in the surface morphology of K562 cells according to sodium butyrate (NaB) concentration, duration of exposure, and treatment condition. The control cells generally maintained their characteristic cellular shape and surface organization, whereas NaB-treated cells demonstrated progressive alterations in cell shape, surface microvilli, surface folds, flattening, and the appearance of dark or irregular surface structures. The magnitude of these changes was generally more pronounced at the higher NaB concentrations.

Figure 10

Scanning Electron Micrograph of Control K562 Cells in Cell Culture Media for 1, 3, and 7 Days

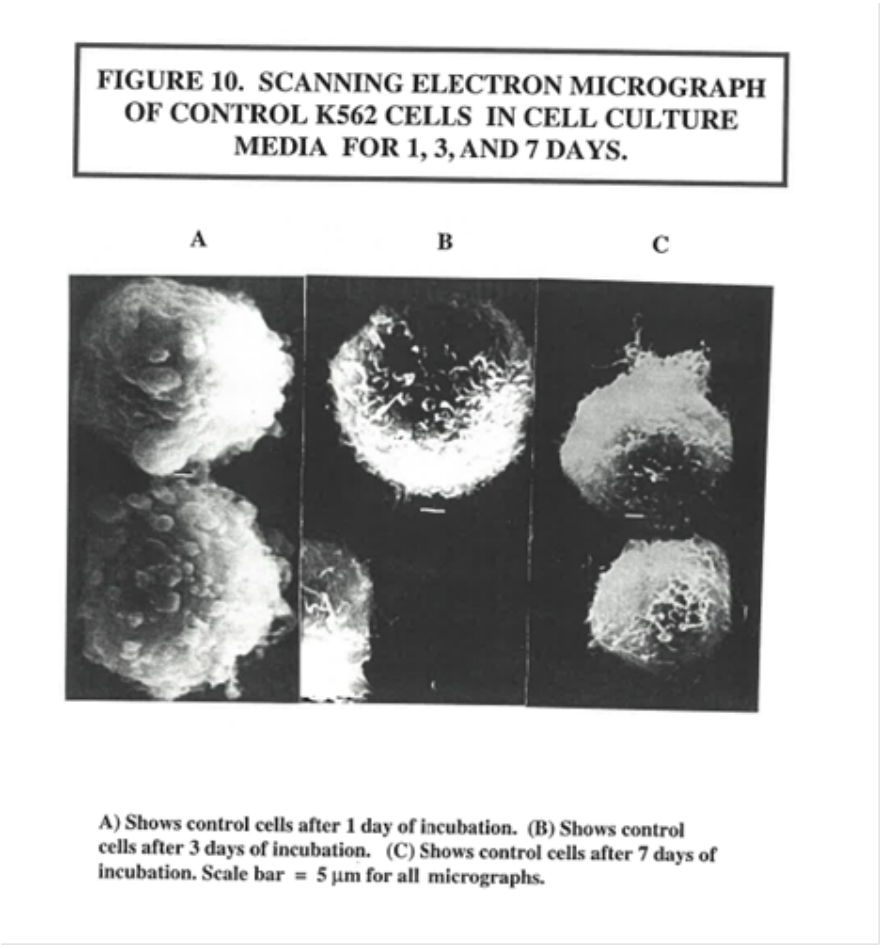


Figure 10 presents the SEM appearance of untreated K562 cells maintained in culture medium for 1, 3, and 7 days. After 1 day of incubation (Micrograph A), the control cells exhibited a relatively normal morphology, characterized by a recognizable rounded cellular form with numerous surface microvilli and well-defined surface folds. The preservation of these surface structures indicated relatively intact cellular surface organization during the initial culture period. After 3 days (Micrograph B), the control cells continued to maintain a generally normal shape. However, some changes in surface morphology were evident, including the appearance of dark spots and a reduction in the number of surface microvilli. Despite these changes, the overall cellular shape remained relatively well preserved, suggesting that the cells retained substantial surface integrity during the intermediate culture period. By 7 days (Micrograph C), the control cells remained generally normal in overall shape, but a greater reduction in surface structures was apparent. Many of the microvilli and surface folds observed at Day 1 were no longer prominent, and the cells appeared smaller in size. These findings indicate that some changes in surface morphology occurred with prolonged culture even in the absence of drug treatment. Therefore, the control observations provide an important baseline for distinguishing culture-duration effects from treatment-associated morphological alterations in the experimental groups.

Figure 11

Scanning Electron Micrographs of K562 Cells Exposed to 0.01 mM Sodium Butyrate for 1, 3, and 7 Days

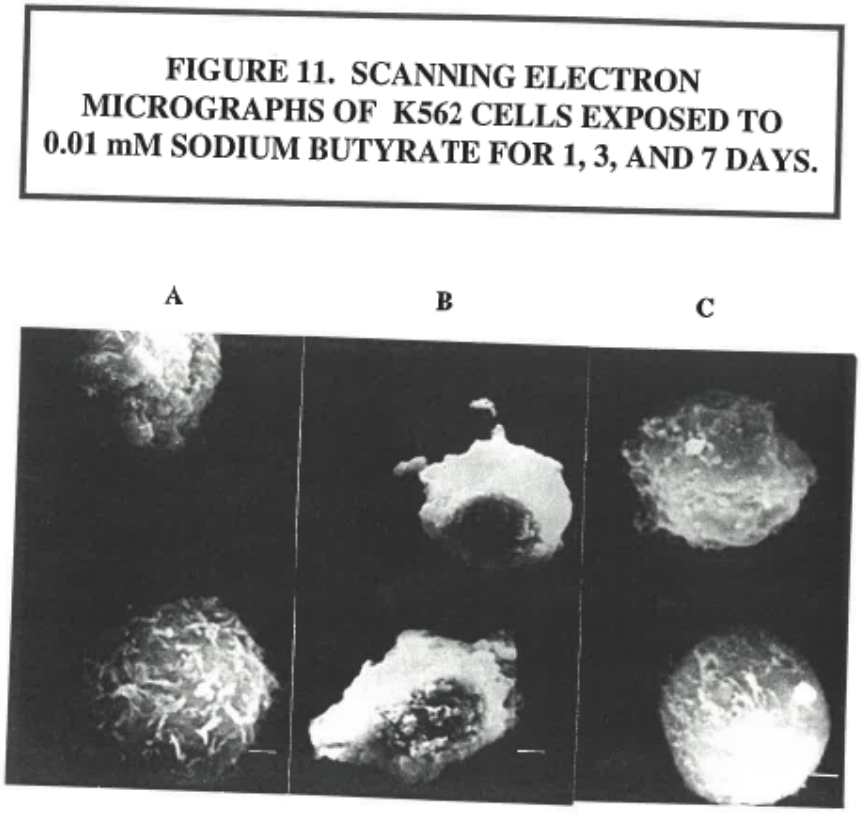
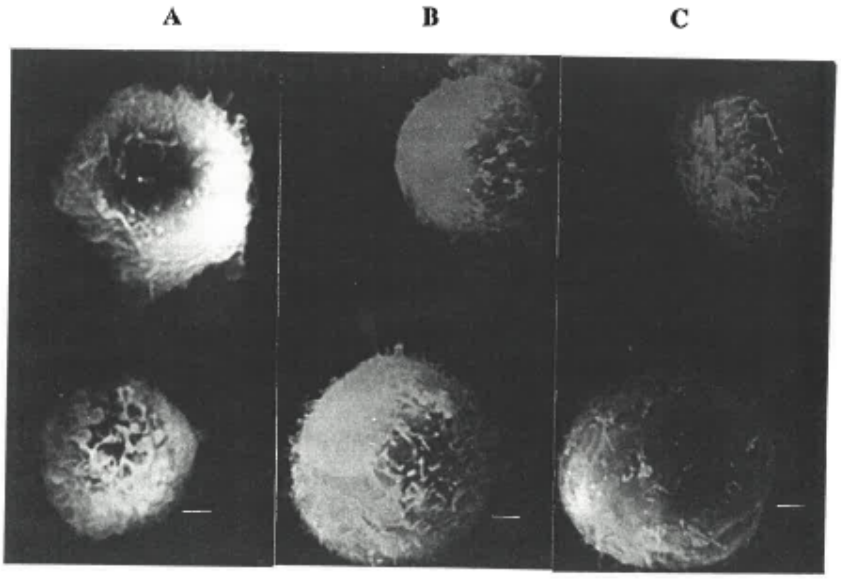


Figure 11 demonstrates the SEM morphology of K562 cells exposed to the lowest NaB concentration, 0.01 mM, for 1, 3, and 7 days. At Day 1 (Micrograph A), the cells retained a generally normal appearance and recognizable cellular shape. However, some loss of surface microvilli was observed when compared with the control cells. The relatively preserved shape and remaining surface structures indicate that exposure to 0.01 mM NaB produced only limited surface morphological alterations during the initial exposure period. At Day 3 (Micrograph B), more pronounced changes were observed. The cells appeared somewhat distorted, with fewer visible microvilli and surface folds. Relatively large dark spots were also present on the cell surface. These findings represented a greater degree of surface alteration than that observed at Day 1 and suggested that continued exposure to 0.01 mM NaB influenced the external morphology of the K562 cells. Interestingly, by Day 7 (Micrograph C), the cells appeared relatively normal in both shape and size. Surface microvilli were again visible, although relatively few surface folds were present. Thus, the SEM findings at 0.01 mM NaB did not demonstrate a simple progressive deterioration over time. Rather, the cells exhibited early and intermediate surface alterations followed by a comparatively preserved morphology at Day 7. This observation suggests that the lowest NaB concentration may have produced a relatively limited or potentially adaptive morphological response rather than severe surface injury.

Figure 12

Scanning Electron Micrographs of K562 Cells Exposed to 0.1 mM Sodium Butyrate for 1, 3, and 7 Days

FIGURE 12. SCANNING ELECTRON MICROGRAPHS OF K562 CELLS EXPOSED TO 0.1 mM SODIUM BUTYRATE FOR 1, 3, AND 7 DAYS.



(A) Shows K562 cells after 1 day of exposure to 0.1 mM sodium butyrate. (B) Shows K562 cells after 3 days of exposure to 0.1 mM sodium butyrate. (C) Shows K562 cells after 7 days exposure to 0.1 mM sodium butyrate. Scale bar = 5 μm for all micrographs.

Figure 12 shows the surface morphology of K562 cells exposed to 0.1 mM NaB. The cells exhibited substantially greater morphological alterations than those observed at 0.01 mM NaB. After 1 day (Micrograph A), the cells were somewhat distorted and flattened. Although microvilli remained visible, several surface folds contained dark spots, and there was evidence of reduction in the normal organization of the cell surface. Compared with the control cells, the presence of flattening and distortion indicated an early response to the intermediate NaB concentration. After 3 days (Micrograph B), the morphological changes became more pronounced. The cells appeared considerably flattened and distorted, with little or no visible microvilli. Only a limited number of surface folds remained, and small dark spots were observed on the cell surface. The reduction in microvilli and folds represented a substantial alteration of the normal surface architecture. By Day 7 (Micrograph C), the cells appeared smooth and markedly flattened. Little or no microvilli or surface folds were visible. Thus, prolonged exposure to 0.1 mM NaB was associated with sustained loss of the surface structures observed in untreated cells. The progressive flattening and disappearance of microvilli and folds indicate that 0.1 mM NaB produced a stronger and more persistent surface morphological response than the 0.01 mM treatment.

Figure 13

Scanning Electron Micrographs of K562 Cells Exposed to 1.0 mM Sodium Butyrate for 1, 3, and 7 Days

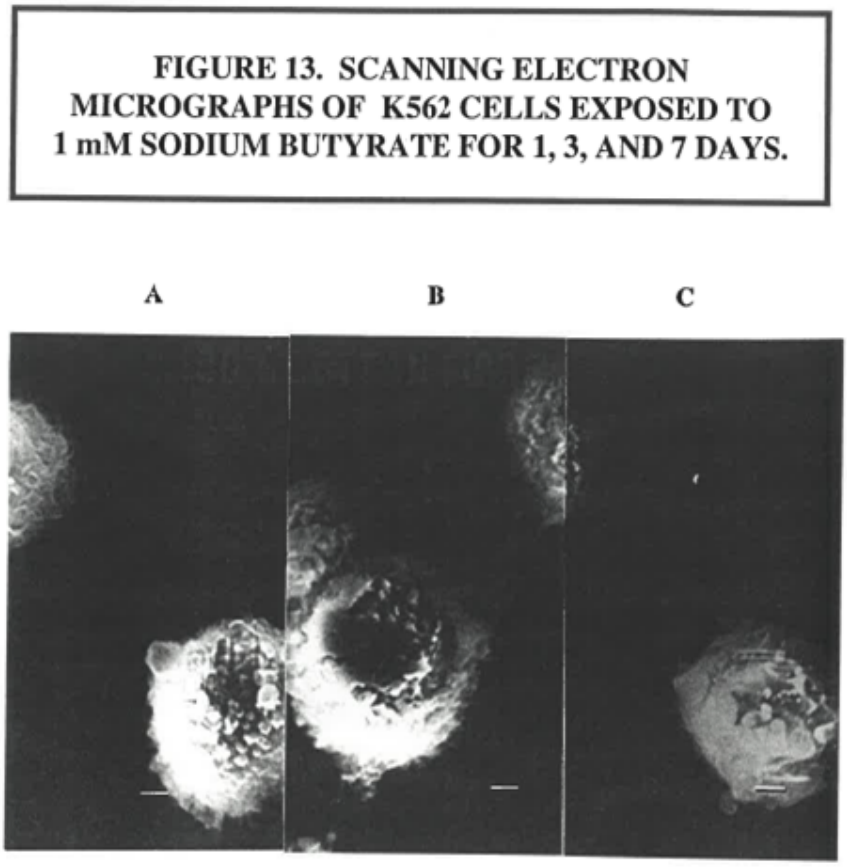
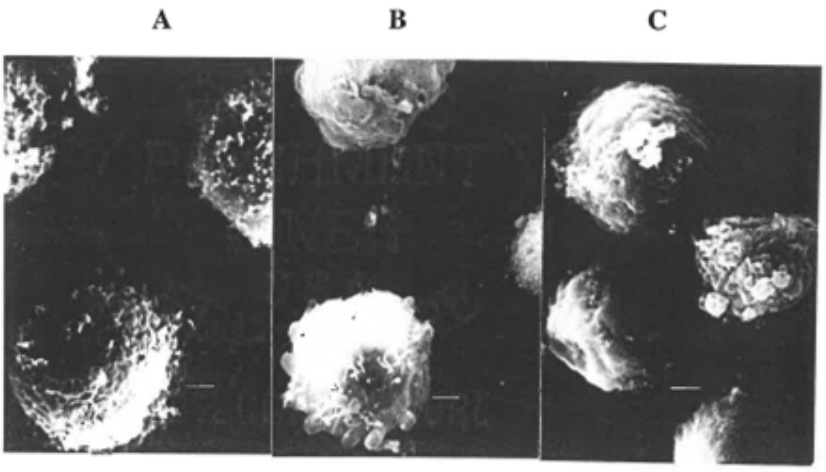


Figure 13 demonstrates the most pronounced SEM alterations among the NaB treatment groups. K562 cells exposed to 1.0 mM NaB showed substantial changes in cellular shape and surface architecture beginning at the earliest observation period. After 1 day (Micrograph A), the cells were somewhat flattened and distorted, with most of the surface microvilli lost. Large dark spots were also clearly visible on the cell surface. These changes represented a more extensive surface response than that observed with either 0.01 or 0.1 mM NaB at the corresponding early exposure period. At Day 3 (Micrograph B), the cells remained distorted and flattened. Large dark spots continued to be visible, while only a few microvilli remained. The persistence of these abnormalities indicated that the effects observed at Day 1 were not transient at this concentration. After 7 days (Micrograph C), the cells were considerably smaller and very flat. Although some surface microvilli and folds were again visible, the overall cellular morphology remained substantially altered. The combination of reduced cell size and marked flattening indicated considerable morphological stress associated with prolonged exposure to 1.0 mM NaB. The SEM findings for 1.0 mM NaB demonstrated the greatest degree of surface alteration among the three NaB concentrations. The findings were characterized by flattening, distortion, loss of microvilli, abnormal surface structures, and reduction in cell size. Within the context of the present experiment, these observations provide morphological evidence consistent with a pronounced adverse effect of the highest NaB concentration on K562 cells.

Figure 14

Scanning Electron Micrographs of K562 Cells Exposed to 1 µM Hydroxyurea for 1, 3, and 7 Days

FIGURE 14. SCANNING ELECTRON MICROGRAPHS OF K562 CELLS EXPOSED TO 1 µM HYDROXYUREA FOR 1, 3, AND 7 DAYS.



(A) Shows K562 cells exposed to 1 µM hydroxyurea for 1 day.
(B) Shows K562 cells exposed to 1 µM hydroxyurea for 3 days.
(C) Shows K562 cells exposed to 1 µM hydroxyurea for 7 days.
Scale bar = 5 µm for all micrographs.

Figure 14 presents the SEM morphology of K562 cells exposed to 1 µM hydroxyurea (HU). At Day 1 (Micrograph A), the cells displayed an abnormal surface morphology characterized by altered cell shape, abnormal microvilli and folds, and prominent dark spots on the surface. These changes indicated an early morphological response to hydroxyurea. At Day 3 (Micrograph B), the appearance of the cells was improved relative to Day 1. More recognizable surface folds and some microvilli were present, although the cells appeared smaller and small dark spots remained visible on the surface. This finding indicates that the initial surface abnormalities associated with HU exposure were less pronounced at the intermediate observation point. By Day 7 (Micrograph C), however, the cells demonstrated substantial morphological abnormalities. The cells were highly distorted, with abnormal surface folds and microvilli, and their size was reduced. Thus, the HU group demonstrated a time-dependent pattern characterized by pronounced early abnormalities, partial morphological preservation at Day 3, and renewed or increased surface abnormalities by Day 7.

Figure 15

Scanning Electron Micrographs of K562 Cells Exposed to 1 μM Hydroxyurea and 0.1 mM Sodium Butyrate for 1,3, and 7 Days.

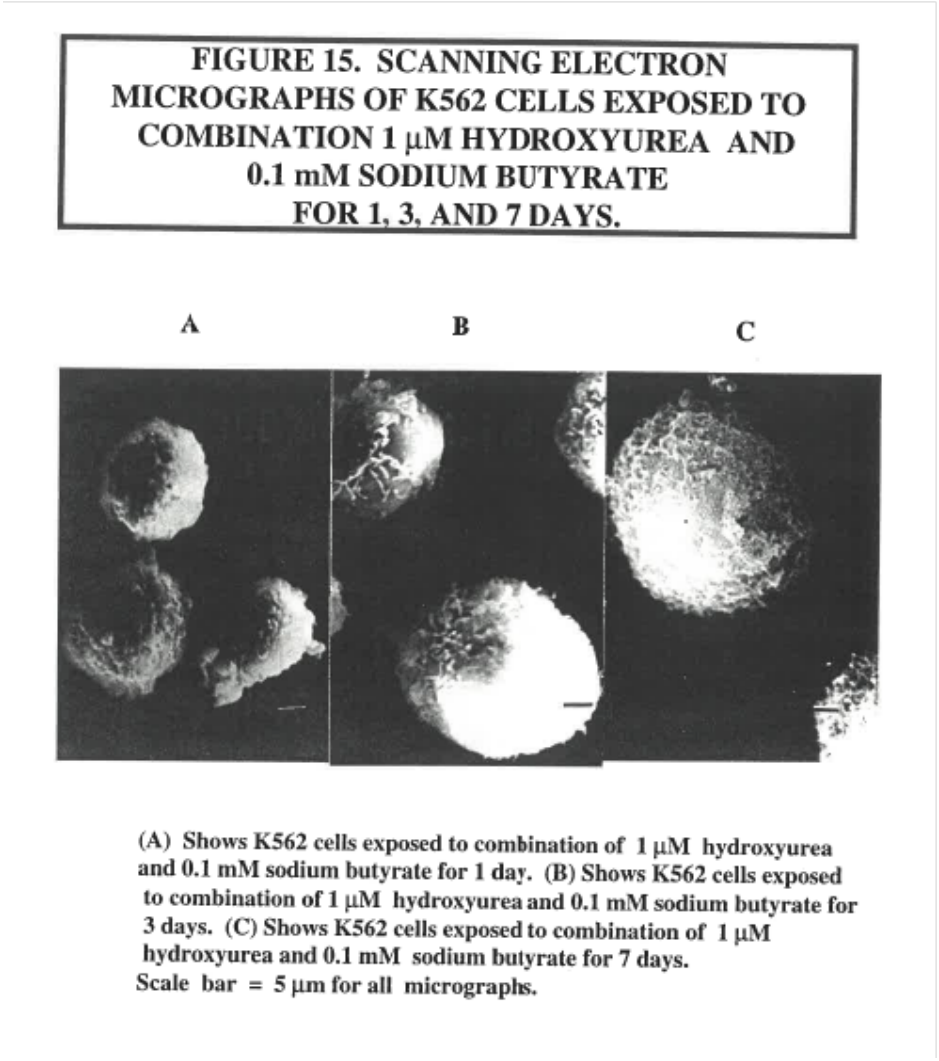


Figure 15 shows the SEM morphology of K562 cells treated simultaneously with 1 μM HU and 0.1 mM NaB. At Day 1 (Micrograph A), the combination treatment produced considerable surface alterations. The cells were very flat and distorted, with some remaining microvilli and prominent large dark spots on the surface. The appearance suggested substantial early disruption of the normal cellular surface. After 3 days (Micrograph B), the cells remained abnormal in shape and exhibited a fluffy or irregular surface. Some microvilli and a few surface folds were present; however, the overall surface appeared compromised, with evidence suggestive of surface membrane disruption. By Day 7 (Micrograph C), the surface morphology was markedly altered. No obvious microvilli or surface folds were observed. Compared with the control cells, which retained recognizable cellular organization, the combined-treatment cells exhibited substantial loss of normal surface architecture. These findings demonstrate that prolonged combined exposure to HU and NaB produced persistent alterations in the external morphology of K562 cells.

Transmission Electron Microscopy (TEM)

TEM provided additional information regarding the internal ultrastructure of K562 cells and complemented the surface-level observations obtained using SEM. The TEM findings demonstrated differences in plasma membrane integrity, nuclear membrane organization, chromatin distribution, mitochondrial appearance, and cytoplasmic vacuolation across the treatment groups. The severity of the intracellular changes generally increased with increasing NaB concentration, with the most extensive abnormalities observed at 1.0 mM NaB.

Figure 16

Transmission Electron Micrographs of Control K562 Cells After 1 and 7 Days

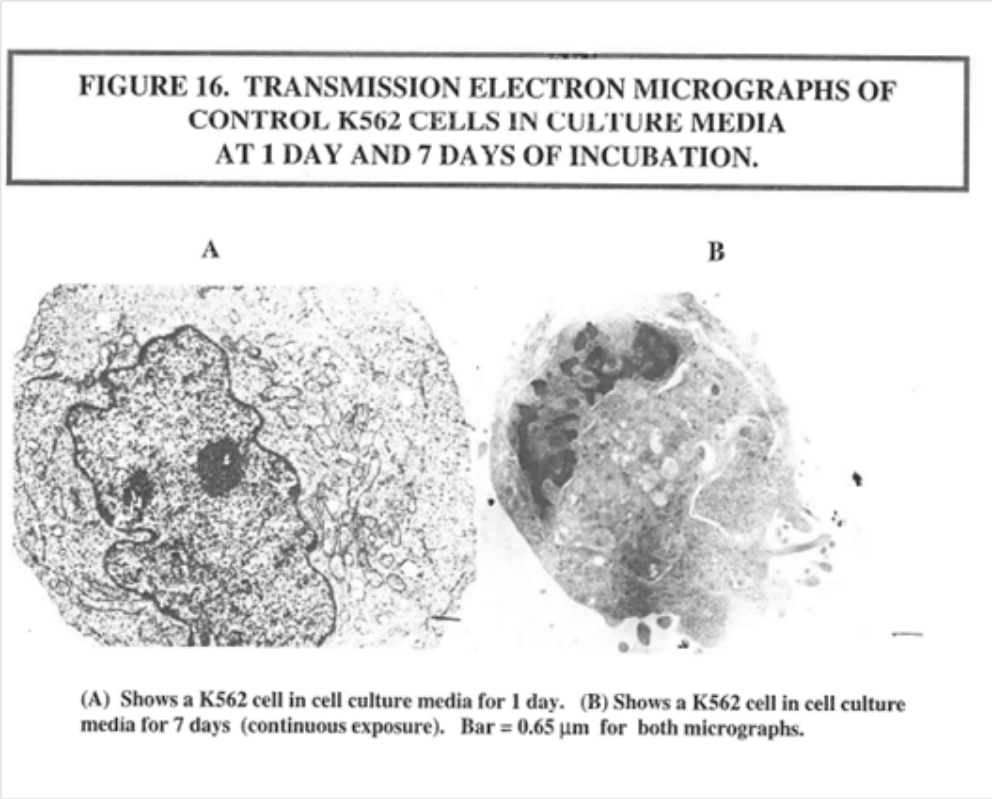


Figure 16 presents the ultrastructural appearance of untreated K562 cells following 1 and 7 days of incubation in culture medium. At Day 1 (Micrograph A), the plasma membrane and nuclear membrane were intact. The cytoplasm contained numerous mitochondria, while the nucleus contained recognizable chromatin. The preservation of the cellular membranes and intracellular organelles demonstrated relatively normal ultrastructural organization. After 7 days (Micrograph B), the plasma and nuclear membranes remained intact. The nucleus continued to contain recognizable chromatin, although some cytoplasmic vacuolation was observed. The presence of limited vacuolation after prolonged culture indicates that some intracellular changes occurred naturally during the culture period; however, the overall membrane integrity of the control cells was preserved. These findings establish the baseline ultrastructural condition against which the NaB- and HU-treated cells can be compared.

Figure 17

Transmission Electron Micrographs of K562 Cells Continuously Exposed to 0.01 mM Sodium Butyrate for 1 and 7 Days

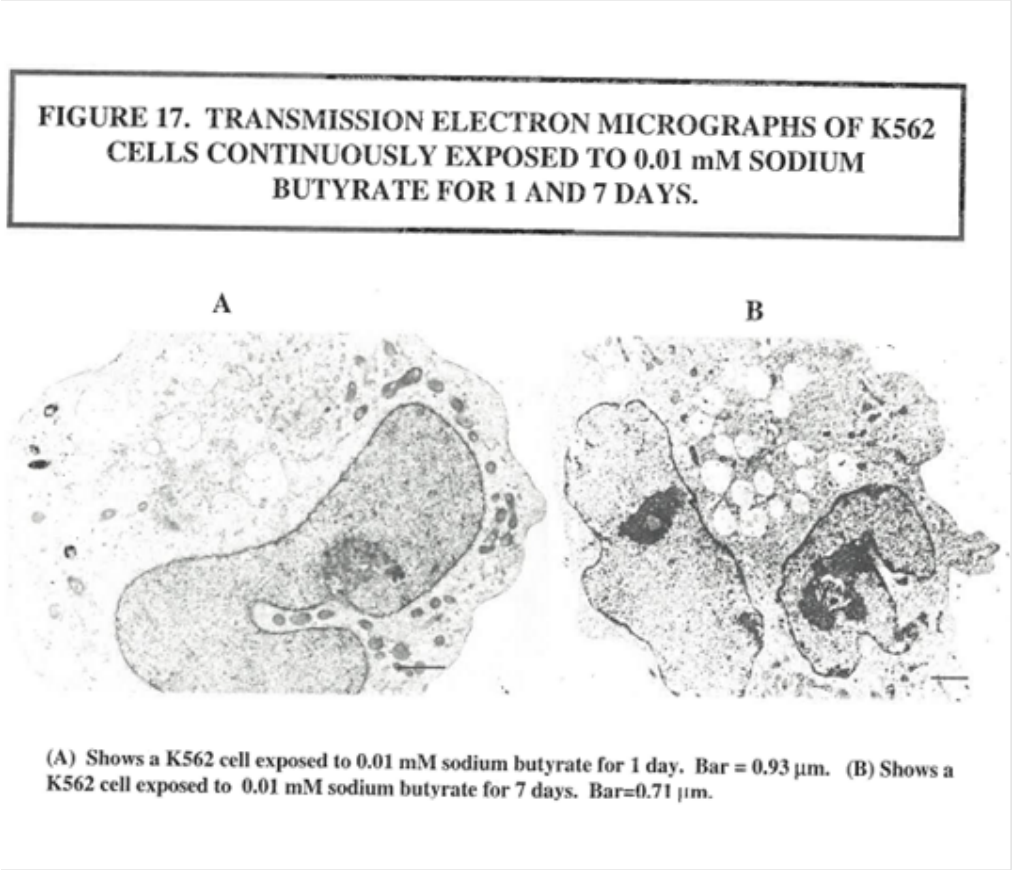


Figure 17 shows the ultrastructural characteristics of K562 cells exposed to 0.01 mM NaB for 1 and 7 days. At Day 1 (Micrograph A), both the plasma and nuclear membranes remained intact. The cytoplasm appeared relatively normal and contained identifiable mitochondria and Golgi structures. The nuclear membrane was associated with peripheral chromatin, indicating preservation of nuclear organization. After 7 days (Micrograph B), however, more noticeable intracellular changes were present. A large amount of cytoplasmic vacuolation was observed despite preservation of the plasma and nuclear membranes. The cell also contained two nuclei with substantial peripheral chromatin and additional chromatin within the nucleus. Thus, prolonged exposure to the lowest NaB concentration produced intracellular changes, particularly increased cytoplasmic vacuolation, while major membrane disruption was not observed. The findings suggest that 0.01 mM NaB produced relatively limited ultrastructural injury, particularly when compared with the more severe alterations observed at higher concentrations.

Figure 18

Transmission Electron Micrographs of K562 Cells Exposed to 0.1 mM Sodium Butyrate for 1 and 7 Days

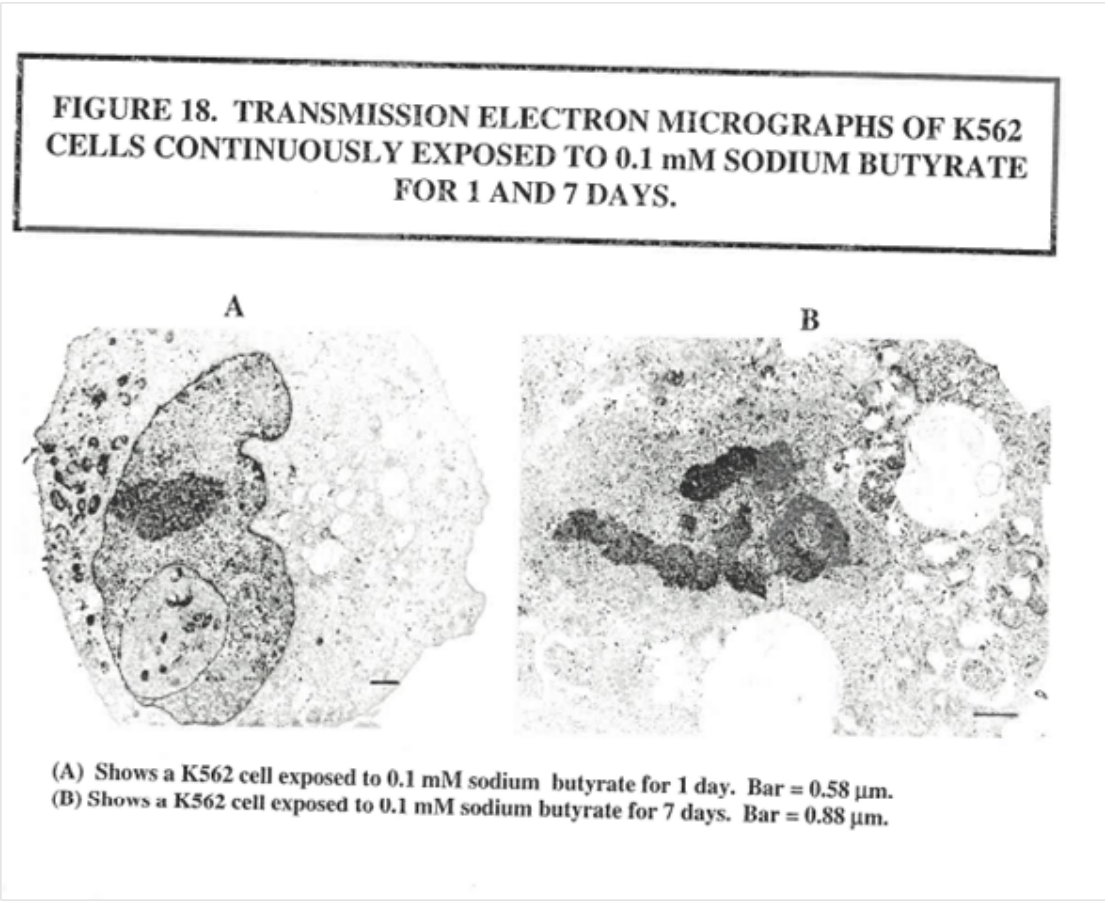


Figure 18 illustrates the ultrastructural response to 0.1 mM NaB. Following 1 day of exposure (Micrograph A), the plasma and nuclear membranes remained intact. Peripheral chromatin was associated with the nuclear membrane, while increased chromatin was present within the nucleus. Some cytoplasmic vacuolation was already evident, indicating that the intermediate NaB concentration produced an intracellular response during the initial exposure period. At 7 days (Micrograph B), the effects were more pronounced. Although the plasma membrane remained intact, the nuclear membrane was less clearly defined and peripheral chromatin was no longer prominent. The nucleus contained a substantial amount of chromatin, while the cytoplasm demonstrated extensive vacuolation. The increased vacuolation and altered nuclear membrane organization indicate greater ultrastructural stress following prolonged exposure to 0.1 mM NaB. Therefore, the TEM findings indicate that 0.1 mM NaB produced a more pronounced intracellular response than 0.01 mM NaB, particularly following prolonged exposure.

Figure 19

Transmission Electron Micrographs of K562 Cells Exposed to 1.0 mM Sodium Butyrate for 1 and 7 Days

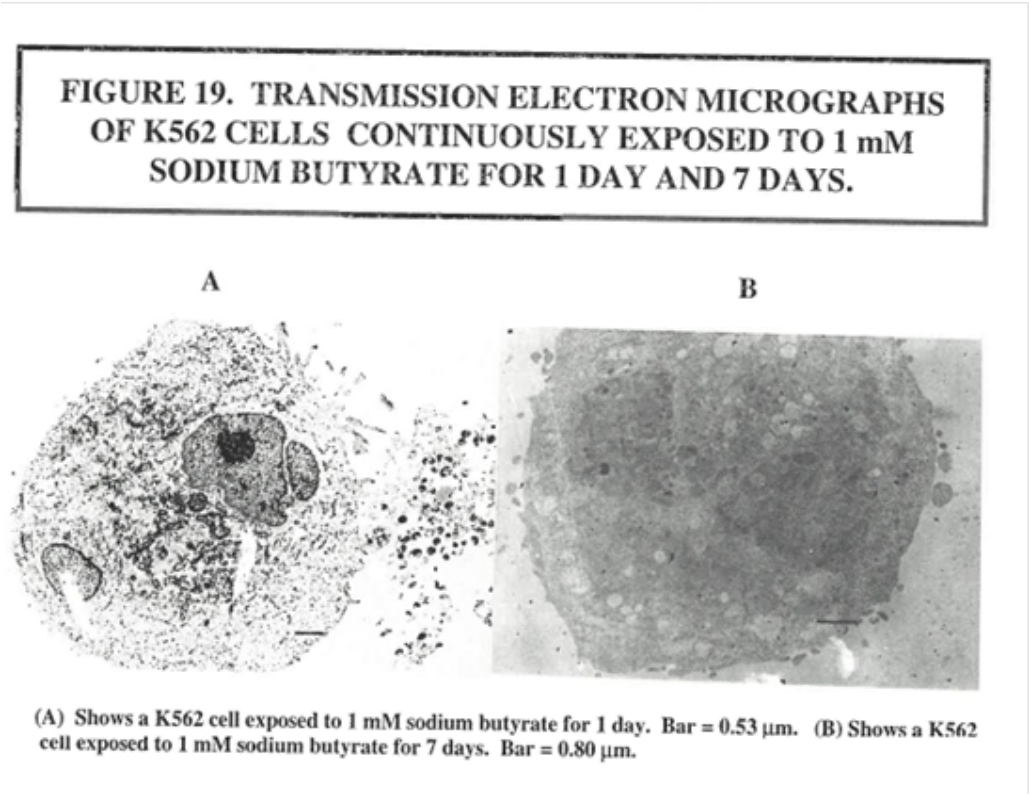


Figure 19 demonstrates the most severe ultrastructural abnormalities among the NaB treatment groups. After 1 day of exposure to 1.0 mM NaB (Micrograph A), the plasma membrane was disrupted. The cell contained multiple nuclei with substantial amounts of chromatin and peripheral chromatin, together with numerous mitochondria. In addition, some cytoplasmic components appeared to be leaving the cell. These findings indicate substantial disruption of cellular integrity at the highest NaB concentration even after the relatively short exposure period. After 7 days (Micrograph B), the ultrastructural abnormalities were markedly more severe. The cytoplasm exhibited extensive vacuolation and appeared lightly stained. The nuclear membrane was no longer clearly present, and nuclear contents appeared to have dispersed into the cytoplasm. Openings were also apparent in the plasma membrane. These findings represent substantial disruption of both nuclear and cytoplasmic organization. The TEM findings therefore demonstrate a concentration-dependent pattern of NaB-associated ultrastructural alteration. The lowest concentration primarily produced cytoplasmic changes with preserved membranes, the intermediate concentration produced increased vacuolation and changes in nuclear organization, and the highest concentration produced extensive membrane disruption, severe vacuolation, and loss of nuclear structural integrity.

Figure 20

Transmission Electron Micrographs of K562 Cells Continuously Exposed to a Combination of 0.1 mM Sodium Butyrate and 1 µM Hydroxyurea for 1 and 3 Days

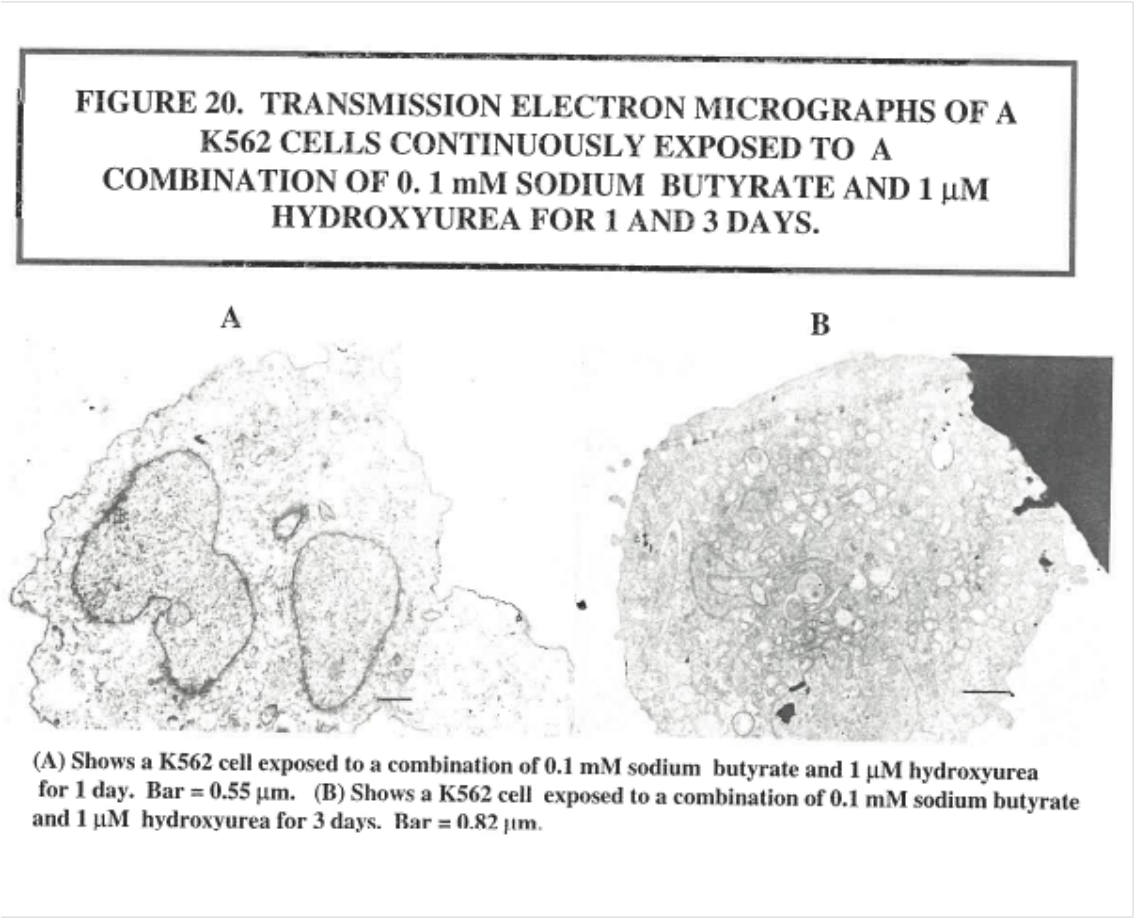


Figure 20 presents the TEM findings for K562 cells exposed to the combination of 0.1 mM NaB and 1 µM HU. After 1 day of exposure (Micrograph A), both the plasma and nuclear membranes remained intact, and no obvious cytoplasmic vacuolation was observed. Two nuclei were present, accompanied by a small amount of chromatin. The preservation of the major cellular membranes and the absence of marked vacuolation indicated relatively preserved ultrastructural integrity at this exposure period. Following 3 days of continuous exposure (Micrograph B), the cells continued to exhibit preserved plasma and nuclear membranes. No significant cytoplasmic vacuolation or obvious membrane damage was observed, and numerous mitochondria were present within the cytoplasm. Thus, unlike the pronounced ultrastructural abnormalities observed with 1.0 mM NaB, the combination of 0.1 mM NaB and 1 µM HU did not produce severe ultrastructural disruption during the 1- and 3-day observation periods.

DISCUSSION OF FINDINGS

The study examined the cellular and ultrastructural effects of sodium butyrate (NaB) on human erythroleukemia K562 cells using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The findings demonstrate that NaB exposure was associated with changes in both the external morphology and internal ultrastructure of K562 cells. Importantly, the magnitude of these changes varied according to NaB concentration and exposure duration. The general pattern showed relatively limited structural changes at 0.01 mM NaB, increasingly evident alterations at 0.1 mM, and pronounced cellular abnormalities at

1.0 mM NaB. These observations suggest that the biological effects of NaB on K562 cells are dependent not only on exposure to the compound but also on the concentration and duration of exposure. The findings are particularly relevant because K562 cells have historically been used as an experimental model for studying erythroid differentiation and the effects of chemical inducers on erythroid-associated characteristics. Previous research demonstrated that K562 cells possess erythroid properties and can undergo morphological and biochemical changes following exposure to agents such as hemin, butyrate, and other differentiation-inducing compounds (Villeval et al., 1983). Subsequent research has further demonstrated that sodium butyrate can induce erythroid differentiation through alterations in cellular signaling pathways (Davis et al., 2000; Witt et al., 2000). The present study extends these observations by demonstrating corresponding changes at the surface and ultrastructural levels.

Surface Morphology of Control K562 Cells

The SEM observations of the untreated control cells provide an important baseline for interpreting the treatment-related changes. After 1 day in culture, the control cells exhibited relatively normal morphology, including recognizable cell shape, microvilli, and surface folds. By Day 3, some microvilli had been lost and dark spots appeared on the surface, although the overall cellular shape remained relatively normal. By Day 7, additional microvilli and surface folds had been lost, and the cells appeared somewhat smaller. These findings indicate that K562 cells can undergo some morphological changes during prolonged culture even without drug exposure. Consequently, not every alteration observed in the treated groups can be attributed exclusively to NaB. The control findings are particularly important because they demonstrate that culture duration itself may influence surface morphology. The greater degree of flattening, distortion, loss of surface structures, and membrane abnormalities observed in several NaB treatment groups therefore provides stronger evidence of treatment-associated effects when these changes exceed the alterations observed in the corresponding controls.

Effects of Low-Concentration Sodium Butyrate

The SEM and TEM findings indicate that 0.01 mM NaB produced the least extensive structural alterations among the NaB treatment groups. After 1 day, the cells maintained a relatively normal shape, although some microvilli were lost. By Day 3, greater surface distortion and loss of microvilli and folds were observed. Interestingly, the Day 7 SEM images showed relatively preserved cell shape and size, with some microvilli present and relatively few folds. The TEM findings provide additional information regarding this response. Following 1 day of exposure, the plasma and nuclear membranes remained intact, and the cytoplasm contained recognizable mitochondria and Golgi structures. After 7 days, however, considerable cytoplasmic vacuolation was observed, although the plasma and nuclear membranes remained intact. The presence of two nuclei and substantial peripheral chromatin also indicated changes in nuclear organization. These findings suggest that 0.01 mM NaB produced measurable cellular responses without the extensive membrane destruction observed at higher concentrations. The results are compatible with the broader evidence that butyrate can alter K562 cell biology and promote erythroid-associated differentiation rather than simply causing nonspecific cellular destruction (Villeval et al., 1983; Witt et al., 2000). However, because the present analysis was primarily morphological, the observed structural changes alone cannot establish whether the alterations represent differentiation, cellular adaptation, or early cellular stress.

Effects of Intermediate-Concentration Sodium Butyrate

Exposure to 0.1 mM NaB produced more substantial and persistent morphological changes than exposure to 0.01 mM NaB. SEM demonstrated cellular distortion and flattening after 1 day, accompanied by changes in microvilli and surface folds. By Day 3, the cells were substantially flattened, with little or no microvilli. After 7 days, the cells appeared smooth and very flattened, with little or no recognizable surface folds or microvilli. The TEM findings were consistent with the SEM observations. After 1 day, the plasma and nuclear membranes remained intact, although cytoplasmic vacuolation was already evident. After 7 days, the cytoplasm exhibited excessive vacuolation and the nuclear membrane became less clearly defined. The nucleus also demonstrated substantial chromatin accumulation. The combination of surface flattening and loss of microvilli observed by SEM with cytoplasmic vacuolation and altered nuclear organization observed by TEM indicates that 0.1 mM NaB produced both external and internal cellular changes. These changes were more pronounced with prolonged exposure, suggesting an exposure-duration component to the response. The findings are important because sodium butyrate has been reported to induce erythroid differentiation in K562 cells. Witt et al. (2000), for example, demonstrated that butyrate-induced erythroid differentiation involves inhibition of ERK and

activation of p38 MAP kinase pathways. Davis et al. (2000) likewise reported involvement of Gαi2 in sodium butyrate-induced erythroblastic differentiation of K562 cells. Thus, some cellular morphological changes observed following NaB exposure may reflect the biological response of K562 cells to a differentiation-inducing stimulus. Nevertheless, the substantial vacuolation observed after prolonged exposure to 0.1 mM NaB indicates that the treatment may also produce cellular stress, particularly when exposure is sustained.

Effects of High-Concentration Sodium Butyrate

The most pronounced cellular abnormalities were observed in cells exposed to 1.0 mM NaB. SEM demonstrated substantial flattening and distortion after only 1 day. Most surface microvilli were lost, and large dark spots were visible on the cell surface. Similar abnormalities remained evident after 3 days. By Day 7, the cells were smaller and markedly flattened. The TEM findings provided stronger evidence of severe cellular injury at this concentration. After only 1 day, the plasma membrane was disrupted, multiple nuclei with substantial chromatin were observed, and cytoplasmic components appeared to be leaving the cell. Following 7 days of exposure, the cytoplasm was severely vacuolated, the nuclear membrane was no longer clearly identifiable, nuclear material appeared displaced into the cytoplasm, and openings were visible in the cell membrane. These findings represent a substantial increase in severity compared with the 0.01 and 0.1 mM treatments. The results therefore provide strong morphological evidence for a concentration-dependent adverse effect of NaB, with 1.0 mM producing the greatest degree of structural damage. The distinction between differentiation-associated changes and toxicity is especially important in interpreting these findings. Sodium butyrate has recognized biological activity in erythroid cells, and previous research has demonstrated its ability to promote erythroid differentiation in K562 cells (Villeval et al., 1983; Davis et al., 2000; Witt et al., 2000). However, the severe membrane disruption, extensive vacuolation, loss of nuclear membrane integrity, and apparent leakage of cellular contents observed at 1.0 mM are more consistent with substantial cellular injury than with a simple differentiation response. Therefore, the findings suggest that the concentration of NaB is an important determinant of whether exposure is associated primarily with relatively preserved cellular responses or with marked structural injury.

Relationship Between SEM and TEM Findings

An important contribution of this study is the complementary information provided by SEM and TEM. SEM demonstrated changes occurring primarily at the cell surface, whereas TEM demonstrated changes occurring within the cellular interior. At 0.1 and 1.0 mM NaB, SEM demonstrated progressive loss of microvilli, disappearance of surface folds, cellular flattening, and distortion. TEM findings at these concentrations simultaneously demonstrated increasing cytoplasmic vacuolation and alterations in nuclear and plasma membrane integrity. The consistency between the two microscopy techniques strengthens the conclusion that NaB exposure affected K562 cellular architecture at multiple levels. The 1.0 mM treatment provides the clearest example of this relationship. The SEM findings showed substantial surface deterioration, while TEM demonstrated corresponding disruption of the plasma membrane and nuclear organization. Therefore, the surface abnormalities were accompanied by internal structural abnormalities rather than representing isolated changes in cell appearance.

Findings Related to Hydroxyurea

The hydroxyurea treatment provided a useful comparison because HU is an established pharmacological agent associated with increased fetal hemoglobin production and is used in the management of sickle cell disease. The SEM findings showed that 1 μM HU produced abnormal surface morphology after 1 day, including altered cell shape, abnormal microvilli and folds, and dark surface spots. At Day 3, the cellular appearance improved, with more recognizable folds and some microvilli. By Day 7, however, the cells again demonstrated considerable distortion, abnormal surface structures, and reduced cell size. The changing morphology across the three time points indicates that the response to HU was not uniform over time. This observation is important when comparing NaB and HU because both compounds can influence erythroid-associated cellular processes, but their effects may manifest differently depending on exposure conditions. The findings also provide evidence that morphological abnormalities are not unique to sodium butyrate. Hydroxyurea itself produced observable changes in K562 cell morphology. Therefore, the structural effects observed with the NaB-HU combination should be interpreted in relation to the effects of each treatment individually.

Findings Related to the Combined NaB-HU Treatment

The combined treatment of 0.1 mM NaB and 1 µM HU produced notable surface alterations under SEM. After 1 day, the cells were very flat and distorted, with large dark spots and some remaining microvilli. At Day 3, the cells exhibited an abnormal, fluffy surface, with limited microvilli and folds and evidence suggestive of surface membrane damage. By Day 7, microvilli and surface folds were no longer apparent. Remarkably, the TEM findings at 1 and 3 days showed a different pattern. Both the plasma and nuclear membranes remained intact, no major cytoplasmic vacuolation was observed, and numerous mitochondria remained visible. Thus, the combined treatment produced pronounced surface changes without corresponding severe internal ultrastructural damage during the early periods examined. This finding suggests that surface morphological alterations may precede detectable severe intracellular structural damage. It also demonstrates the value of using both SEM and TEM because examination with only one technique could provide an incomplete representation of the cellular response.

Concentration- and Time-Dependent Pattern of NaB Effects

One of the principal findings of the study is the apparent concentration-dependent relationship between NaB exposure and cellular structural abnormalities. The progression from 0.01 to 0.1 to 1.0 mM NaB was associated with increasing severity of morphological changes. At 0.01 mM, cellular membranes remained largely intact despite some cytoplasmic vacuolation. At 0.1 mM, greater surface flattening, loss of microvilli and folds, and extensive cytoplasmic vacuolation were observed. At 1.0 mM, these effects were accompanied by severe membrane disruption, nuclear abnormalities, and apparent loss of cellular integrity. The results also indicate that duration of exposure influenced the severity of some effects. Prolonged exposure to 0.1 mM NaB resulted in greater flattening and cytoplasmic vacuolation than shorter exposure. Similarly, 1.0 mM NaB produced severe abnormalities that became particularly evident following 7 days of exposure. However, the response was not strictly linear across every time point. For example, some surface features were relatively preserved at Day 7 following 0.01 mM exposure. Therefore, the findings support a general concentration- and duration-associated response, while also indicating that K562 cellular responses to NaB may involve periods of alteration, adaptation, or recovery.

The present findings are consistent with the established literature demonstrating that sodium butyrate can influence erythroid differentiation in K562 cells. Villeval et al. (1983) demonstrated that butyrate could alter the erythroid properties of K562 cells. Davis et al. (2000) subsequently demonstrated the involvement of Gαi2 in sodium butyrate-induced erythroblastic differentiation, while Witt et al. (2000) reported that butyrate-induced erythroid differentiation involves inhibition of ERK and activation of p38 MAP kinase pathways. These previous findings provide a biological basis for expecting cellular changes following NaB exposure. The current study adds to that literature by showing that these responses are accompanied by observable alterations in the surface architecture and internal ultrastructure of K562 cells. At lower concentrations, the cells retained substantial structural integrity, whereas high-concentration exposure resulted in pronounced abnormalities consistent with cellular injury. The findings should therefore be interpreted within a concentration-dependent framework. Sodium butyrate may produce biologically desirable erythroid-associated responses at appropriate exposure levels, but increasing concentration and prolonged exposure may produce structural abnormalities that are inconsistent with a benign differentiation response.

Above all, the results indicate that sodium butyrate has a dual morphological effect on K562 cells that depends strongly on concentration and exposure duration. Lower concentrations were associated with comparatively preserved cellular architecture, although changes in surface morphology and cytoplasmic organization were observed. Intermediate exposure produced more pronounced and persistent changes, including loss of microvilli, cellular flattening, cytoplasmic vacuolation, and changes in nuclear organization. The highest concentration, 1.0 mM, produced the most severe alterations, including plasma membrane disruption, extensive vacuolation, loss of nuclear membrane integrity, and apparent leakage of intracellular material. These findings are important because they demonstrate that the biological activity of sodium butyrate should not be interpreted solely in terms of its ability to induce erythroid differentiation. Concentration is a critical determinant of the cellular response. The results suggest that there may be a range in which NaB can alter K562 cellular characteristics while maintaining relatively preserved ultrastructural integrity, whereas higher concentrations may shift the cellular response toward substantial structural injury. The study therefore provides additional

morphological evidence supporting the need to distinguish potential differentiation-associated responses from concentration-associated cytotoxicity when evaluating sodium butyrate as an erythroid-inducing compound. Further studies incorporating quantitative measures of cell viability, apoptosis, reactive oxygen species, membrane integrity, and fetal hemoglobin production would help determine whether the morphological changes observed in this study correspond to erythroid differentiation, cellular stress, or cytotoxicity.

Limitations

Although this study provides important evidence concerning the cellular and ultrastructural effects of sodium butyrate (NaB) on K562 cells, several limitations should be considered when interpreting the findings. First, the study was conducted using the K562 human erythroleukemia cell line, which is an established in vitro model for investigating erythroid differentiation and hemoglobin-related responses. Although K562 cells have been widely used to examine the effects of erythroid-inducing agents, they are a leukemia-derived cell line and therefore may not completely reproduce the biological behavior of normal erythroid progenitor cells or erythrocytes in humans. Consequently, the ultrastructural responses observed in K562 cells cannot be directly generalized to patients with sickle cell disease or other hemoglobin disorders without confirmation in additional experimental models. Second, the electron microscopy component of the study was primarily qualitative and descriptive. SEM and TEM provided detailed visual evidence of changes in cellular morphology and ultrastructure, including loss of microvilli, cellular flattening, cytoplasmic vacuolation, membrane disruption, and changes in nuclear organization. However, the study did not quantitatively measure the frequency or severity of these morphological changes. Consequently, the results establish clear patterns of structural alteration but do not provide statistical estimates of the magnitude of the differences among treatment groups. Third, the study evaluated a relatively limited number of sodium butyrate concentrations—0.01, 0.1, and 1.0 mM. Although these concentrations were useful for demonstrating a concentration-associated response, they do not establish the precise concentration at which beneficial erythroid-associated effects transition into cellular toxicity. In particular, the severe abnormalities observed at 1.0 mM indicate substantial cellular injury, but the study does not identify the exact threshold between a potentially tolerable concentration and a toxic concentration.

Fourth, the exposure periods were limited primarily to 1, 3, and 7 days. These time points provided information about early and prolonged exposure, but they did not permit a detailed assessment of intermediate time points or longer-term cellular recovery. The findings also suggest that some cellular responses may not progress linearly over time. For example, certain surface characteristics appeared relatively preserved at later time points at the lowest NaB concentration. Additional time points would therefore be useful for determining whether observed abnormalities represent transient cellular stress, adaptation, differentiation, or progressive injury. Fifth, the study relied primarily on SEM and TEM to characterize cellular responses. Although these techniques provide high-resolution information concerning cell-surface and intracellular morphology, morphological observations alone cannot definitively establish the biological mechanism responsible for the observed changes. For example, cytoplasmic vacuolation and membrane disruption may be associated with cellular injury, but these observations alone cannot distinguish among apoptosis, necrosis, autophagy, differentiation-associated remodeling, or other cellular processes.

Sixth, the study did not directly quantify fetal hemoglobin (HbF) production, erythroid differentiation markers, apoptosis, cell viability, or molecular signaling pathways within the electron microscopy analysis. This limitation is particularly important because sodium butyrate has been investigated as an inducer of erythroid differentiation and fetal hemoglobin production. Previous studies have demonstrated that NaB can influence erythroid differentiation in K562 cells through cellular signaling mechanisms, including ERK and p38 MAP kinase pathways (Davis et al., 2000; Witt et al., 2000). Without simultaneous biochemical and molecular measurements, it is difficult to determine whether a particular ultrastructural alteration represents a desirable differentiation-associated response or an adverse cellular effect. Seventh, the study evaluated hydroxyurea and the NaB-HU combination for comparison, but the treatment groups and exposure periods were not completely identical across all SEM and TEM observations. For example, TEM observations of the combination treatment were available for 1 and 3 days, whereas several NaB groups were examined at 1 and 7 days. This limits direct comparison of the complete time-dependent effects of all treatment conditions. Finally, the study was conducted under controlled laboratory conditions, which do not reproduce the complex physiological environment of the human body. Factors such as drug metabolism, tissue distribution, immune responses, bone marrow microenvironment, pharmacokinetics, and interactions with other blood and bone marrow cells may influence the effects of sodium butyrate in vivo. Therefore, the findings should be viewed primarily as experimental cellular evidence rather than direct evidence of clinical effectiveness or safety.

Future Studies

Future research should build upon these findings by incorporating quantitative, molecular, biochemical, and functional measures alongside electron microscopy. A major priority should be the simultaneous assessment of ultrastructural changes and fetal hemoglobin production. Measurements of HbF and related hemoglobin expression would help determine whether concentrations associated with relatively preserved cellular architecture also produce meaningful erythroid responses. Future studies should also evaluate cell viability and cytotoxicity using standardized quantitative assays. Measures such as metabolic activity, membrane integrity, apoptosis, necrosis, and caspase activation could help distinguish differentiation-associated morphological changes from cellular injury. Such analyses would be particularly valuable for determining whether the severe abnormalities observed at 1.0 mM NaB reflect irreversible toxicity. A broader concentration-response study should also be conducted using concentrations between 0.01 and 1.0 mM NaB. This would permit identification of a more precise concentration range in which erythroid-associated effects occur while cellular integrity remains relatively preserved. A dose-response model could subsequently be used to estimate effective and toxic concentration thresholds.

Future investigations should incorporate additional exposure periods, including shorter intervals such as several hours and longer periods extending beyond 7 days. Such a design would help establish the sequence of cellular events following NaB exposure and determine whether the observed changes are transient, progressive, reversible, or irreversible. Additional research should examine the molecular mechanisms underlying the observed ultrastructural alterations. Because previous research has linked NaB-induced erythroid differentiation to signaling pathways involving ERK, p38 MAP kinase, and Gαi2 (Davis et al., 2000; Witt et al., 2000), future studies could examine these pathways alongside markers of erythroid differentiation. Such work could clarify whether the morphological changes identified by SEM and TEM are directly associated with erythroid differentiation mechanisms. Future studies should also compare K562 cells with primary human erythroid progenitor cells, hematopoietic stem/progenitor cells, or other clinically relevant cellular models. Such comparisons would strengthen the translational relevance of the findings and determine whether the concentration-dependent effects observed in K562 cells occur in more physiologically representative systems.

Another important direction would be to investigate the combined effects of NaB and hydroxyurea across a wider range of concentrations and exposure periods. The present findings suggest that the combination of 0.1 mM NaB and 1 µM HU produced substantial surface changes under SEM but relatively preserved internal ultrastructure during the early periods examined by TEM. Future studies should determine whether this combination enhances HbF production while reducing the concentration of either agent required to produce an erythroid response. Such an approach could be particularly relevant to the development of combination strategies for hemoglobin disorders. Finally, future investigations should incorporate advanced imaging and quantitative image-analysis techniques to measure cell size, surface area, microvilli density, degree of flattening, vacuole number and size, nuclear morphology, and membrane integrity. Quantitative electron microscopy would strengthen the statistical interpretation of the findings and provide more objective evidence of concentration- and time-dependent structural changes.

CONCLUSION AND POLICY IMPLICATIONS

This study evaluated the cellular and ultrastructural effects of sodium butyrate on K562 cells using scanning and transmission electron microscopy. The findings demonstrate that sodium butyrate produces observable changes in both the external surface morphology and internal ultrastructure of K562 cells. These effects varied according to concentration and exposure duration. At the lowest concentration examined, 0.01 mM NaB, the cells generally retained relatively preserved surface and membrane structures, although some loss of microvilli and increased cytoplasmic vacuolation were observed with prolonged exposure. At 0.1 mM NaB, more pronounced changes occurred, including cellular flattening, loss of surface microvilli and folds, cytoplasmic vacuolation, and alterations in nuclear organization. At 1.0 mM NaB, the most severe abnormalities were observed. These included extensive surface distortion, loss of microvilli, plasma membrane disruption, severe cytoplasmic vacuolation, loss of nuclear membrane integrity, and apparent displacement of nuclear contents. The findings therefore support a concentration-associated increase in structural toxicity, particularly at the highest NaB concentration. The study also demonstrates the value of combining SEM and TEM. SEM provided evidence of changes in the external cellular surface, while TEM revealed changes in the internal

cellular architecture. The agreement between the two approaches, particularly at higher NaB concentrations, strengthens the conclusion that NaB can affect K562 cells at multiple structural levels.

The findings further indicate that sodium butyrate should not be viewed solely as an erythroid differentiation-inducing compound. Although previous research has established that butyrate can induce erythroid differentiation in K562 cells (Villeval et al., 1983; Davis et al., 2000; Witt et al., 2000), the present study demonstrates that its effects are strongly dependent on concentration and exposure duration. A concentration that produces a biological differentiation response may not necessarily be equivalent to a concentration that preserves cellular structural integrity. The severe abnormalities observed at 1.0 mM NaB emphasize the importance of identifying an appropriate therapeutic or experimental concentration range. The findings concerning the NaB-HU combination are also noteworthy. Although SEM revealed substantial surface abnormalities following combined treatment, TEM demonstrated relatively preserved plasma and nuclear membranes and limited cytoplasmic vacuolation during the early exposure periods examined. These findings suggest that combination treatment warrants further investigation because it may produce a different structural response from either treatment alone. However, the current findings are insufficient to establish therapeutic superiority or safety. The study concludes that sodium butyrate induces concentration- and time-dependent cellular and ultrastructural changes in K562 cells, with high-concentration exposure associated with substantial cellular injury. The findings support continued investigation of NaB as an erythroid-modulating compound while emphasizing the need to establish concentration thresholds that maximize potential biological benefits and minimize cellular toxicity.

Policy Implications

The findings have several potential implications for public health policy, pharmaceutical development, and the management of hemoglobin disorders. Sickle cell disease remains an important public health concern, particularly because individuals affected by the disease may experience recurrent complications associated with abnormal hemoglobin production and sickling. Fetal hemoglobin is an important therapeutic target because increased HbF can reduce the tendency of hemoglobin S-containing red blood cells to sickle. Consequently, interventions capable of safely increasing HbF remain relevant to sickle cell disease research and treatment. The findings suggest that policymakers and healthcare decision-makers should support continued research into safe and effective fetal hemoglobin-inducing therapies, including compounds such as sodium butyrate. However, the results also emphasize that potential therapeutic benefits should not be considered independently of cellular safety. The severe ultrastructural abnormalities observed at 1.0 mM NaB demonstrate why drug development and policy decisions should incorporate evidence concerning both therapeutic effectiveness and cellular toxicity.

A second policy implication concerns the importance of supporting translational research before experimental findings are incorporated into clinical practice. Findings obtained from K562 cells provide valuable mechanistic information but cannot by themselves establish clinical safety or effectiveness. Research funding and regulatory frameworks should therefore encourage sequential evaluation involving cell models, primary human cells, appropriate animal models where justified, and carefully controlled clinical investigations before therapeutic recommendations are made. Third, the findings support the importance of dose optimization in the development of HbF-inducing therapies. Rather than focusing only on whether a compound increases fetal hemoglobin, future therapeutic development should consider the concentration at which the desired biological response occurs while maintaining cellular integrity. This principle is particularly important for agents capable of producing both differentiation-associated effects and cytotoxic responses.

Fourth, the findings provide a rationale for investigating combination therapeutic approaches. The observations from the NaB-HU treatment indicate that combining erythroid-inducing agents may produce cellular responses that differ from those associated with either compound individually. If future research demonstrates that combination therapy can achieve clinically meaningful HbF induction at lower concentrations of individual agents, such strategies could potentially contribute to efforts to improve treatment effectiveness while reducing treatment-related toxicity. However, this possibility requires rigorous experimental and clinical validation. Finally, the study underscores the importance of incorporating cellular safety and ultrastructural evidence into preclinical drug evaluation. SEM and TEM can identify structural abnormalities that may not be apparent from conventional measures of cell growth or hemoglobin production. Therefore, a comprehensive drug-development framework should integrate morphological, functional, biochemical, molecular, and toxicity assessments rather than relying on a single indicator of treatment effectiveness. Toward this end, the policy relevance of this study lies in its demonstration that therapeutic promise must be balanced with cellular safety.

The findings support continued investment in research on sodium butyrate and other HbF-inducing strategies, while cautioning against assuming that increased erythroid activity automatically represents a beneficial or clinically safe response. Future policy and clinical research should prioritize evidence-based dose optimization, combination strategies, translational validation, and long-term safety assessment before sodium butyrate-based interventions can be considered for broader therapeutic application.

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