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Sodium Butyrate Enhances the Activities of Membranal Enzymes and Increases Drug Sensitivity in a Cell Line from Ascitic Fluid of an Ovarian Carcinoma Patient

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Abstract—*The effect of sodium butyrate was examined on the growth and phenotypic expression of a cell line derived from the ascitic fluid of an untreated patient with ovarian carcinoma. The chemical inducer of differentiation, sodium butyrate, markedly enhances the activity of the membrane-bound glycoprotein enzymes, alkaline phosphatase and γ-glutamyl transpeptidase. The alkaline phosphatase corresponds to placental Regan type. Sodium butyrate* (1 mM) *alone has only a small inhibitory effect on cell growth. However, it was shown to potentiate the anti-proliferative effect of Adriamycin® and to render the cells sensitive to* cis-*platinum.*

INTRODUCTION

SODIUM BUTYRATE, a known inducer of cell differentiation in hematopoietic cell lines [1], was shown to inhibit proliferation and to induce differentiated features in solid tumor cell lines [2, 3]. The phenotypic alterations induced by sodium butyrate included altered cell morphology, changes in enzyme activities, altered expression of receptors and cell surface antigens and decreased deacetylation of histones [2–10]. We have extensively studied the effects of sodium butyrate on melanona and breast cancer cell lines [9–12]. In both cancer cell types, sodium butyrate induced a marked increase in the activity of the plasma membrane-bound enzyme γ-glutamyl transpeptidase [11, 12]. Another enzyme activity that was also found to be enhanced in breast cancer and in human melanoma cancer cell line as well as in colon and rectal cancer cells was alkaline phosphatase [9, 12–14].

It has been suggested that differentiating agents, including sodium butyrate, might potentiate the action of other therapeutic modalities, such as irradiation and chemotherapy [3, 15].

In the present study we examined the effect of sodium butyrate on the growth and enzyme activities of a new cell line derived from the ascitic fluid of an untreated ovarian cancer patient. Sodium butyrate, which is only slightly inhibitory itself, increased the sensitivity of this cell line to two widely used chemotherapeutic drugs, *cis*-platinum and Adriamycin®.

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Adriamycin® and *cis*-platinum were provided as a gift from Abic, Israel.

MATERIALS AND METHODS

Sodium butyrate was obtained from Merck. Adriamycin® and *cis*-platinum were donated as a gift by Abic, Israel.

Cell line

Recently, a clone has been derived from the ascitic fluid of an untreated ovarian cancer patient. The cells (GZL-8) grow in monolayer, show an abnormal karyotype, are CA125 negative, possess estradiol binding sites and lipid droplets (submitted for publication). Cells are grown in a mixture of DMEM and F12 (1:1), supplemented with 10% fetal calf serum and antibiotics. Cells are transfered twice weekly.

Extraction and determination of enzyme activities

Cells (10^6) were incubated in growth medium in the presence or absence of sodium butyrate (1 mM) for 72 h. Then, cells were scraped with a rubber policeman and extracted for determination of enzyme activities. Alkaline phosphatase activity

was extracted and measured spectrophotometrically as previously described [12]. Activity was measured also in the presence of specific inhibitors and at 65°C, in order to classify the alkaline phosphatase type [4, 16]. Enzyme activity was expressed as nmoles product/mg protein/h.

γ-Glutamyl transpeptidase was measured spectrophotometrically on a whole cell preparation as previously described [11, 12]. Enzyme activity is expressed as nmoles product formed/mg protein/h.

Effect of sodium butyrate alone and in combination with cytotoxic drugs on cell growth

Cells (10^5) were incubated in 0.5 ml growth medium in 24-well plates in different concentrations of Adriamycin® and *cis*-platinum alone or in combination with 1 mM sodium butyrate for 72 h. Cells were detached with EDTA (1 mM) and counted in a Coulter counter.

RESULTS

GZL-8 cells were incubated with sodium butyrate (1 mM) for 72 h. This treatment resulted in a significant increase in the activities of two membrane enzymes; alkaline phosphatase and γ-glutamyl transpeptidase (Table 1). Both enzymes were previously shown to be increased following treatment of various cancer cell types with sodium butyrate [11, 12]. In Table 2 we examined the activity of alkaline phosphatase in the presence of specific inhibitors. The results suggest that the enzyme activities of untreated as well as sodium butyrate-treated cells correspond to that of placental Regan I type.

Treatment of the cells with 1 mM sodium butyrate for 72 h resulted in 19% decrease in cell number (Figs. 1 and 2). The cytotoxic agent Adriamycin® inhibited cell growth in a concentration dependent manner. Co-addition of sodium butyrate and Adriamycin® resulted in a combined inhibitory effect, especially pronounced at low concentrations (0.01–0.05 μg/ml) of Adriamycin® (Fig. 1). This cell line is found to be practically insensitive to inhibition by *cis*-platinum. Addition of sodium butyrate (1 mM) rendered the cell sensitive to inhibition by *cis*-platinum (Fig. 2).

Table 1. Effect of sodium butyrate on alkaline phosphatase and γ-glutamyl transpeptidase activities of GZL-8 ovarian cancer cell line (nmoles/mg *protein*/h)

Treatment	Alkaline phosphatase	γ-Glutamyl transpeptidase
None	48 ± 3	208 ± 40
Sodium butyrate	89 ± 12	589 ± 110
P value	0.05	0.002

Values are means ± S.E.

Table 2. Sensitivity of alkaline phosphatase activity to heat and specific inhibitors in untreated and sodium butyrate-treated GZL-8 cells

Inhibitor	Enzyme activity (%) Untreated	Sodium butyrate-treated
None	100	100
Heat (65°C, 15 min)	130	105
L-Phenylalanine (10 mM)	61	59
L-Homoarginine (2 mM)	116	94
L-Leucine (5 mM)	100	90
EDTA (2 mM)	100	103
Levamisole (1 mM)	103	90

100% activity corresponds to 48 nmoles product formed/mg protein/h for untreated cells and 89 for sodium butyrate-treated cells.

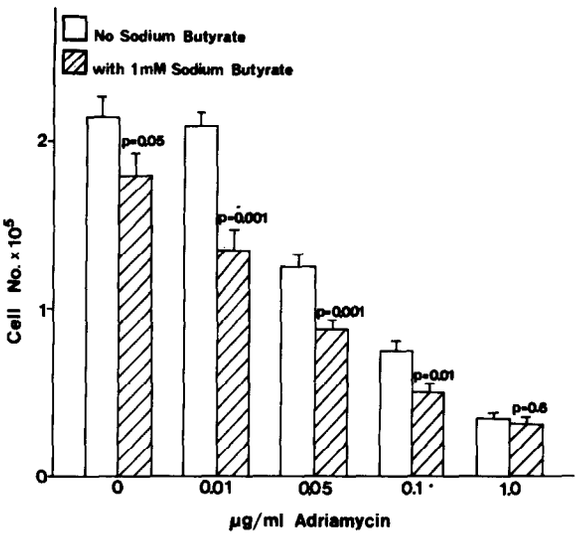

Fig. 1. The effect of Adriamycin® with and without sodium butyrate (1 mM) *on GZL-8 cell number following 3 days incubation. Each bar represents mean ± S.E. of eight replicates of two independent experiments. P values were calculated for Adriamycin® and sodium butyrate vs. Adriamycin® alone.*

DISCUSSION

In the present study we show that sodium butyrate induces phenotypic alterations in this cell line, such as enhanced activities of γ-glutamyl transpeptidase and alkaline phosphatase, which might reflect differentiated characteristics. We have previously shown that sodium butyrate induced differentiated features in melanoma and breast cancer cell lines, including enhanced activities of γ-glutamyl transpeptidase and alkaline phosphatase [9–12]. γ-Glutamyl transpeptidase has been previously shown to be associated with cell differentiation in lymphoblastoid and leukemic cells [17].

Untreated as well as sodium butyrate treated cells express alkaline phosphatase activity that seems to correspond to placental Regan I type enzyme. Placental alkaline phosphatase of Regan and Nagao

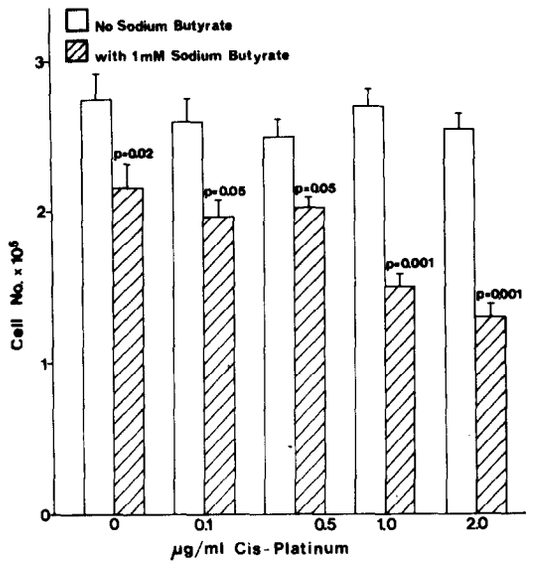

Fig. 2. The effect of cis-*platinum with or without sodium butyrate* (1 mM) *on GZL-8 cell number following 3 days incubation. Each bar represents mean ± S.E. of eight replicates.* P *values were calculated for* cis-*platinum and sodium butyrate vs.* cis-*platinum alone.*

types have been shown to appear frequently in ovarian cancer and have also been detected in serum of patients [18]. It has been suggested as a marker for reduced proliferation and differentiation in ovarian cancer cell lines and in tumor tissue [18, 19]. Lines derived from well differentiated serous adenocarcinoma showed high histochemical staining for alkaline phosphatase as compared to lines derived from poorly differentiated adenocarcinoma [19, 20]. Using monoclonal antibodies to placental type alkaline phosphatase it has also been reported that poorly differentiated and anaplastic ovarian tumor cells showed a higher proportion of negative areas [18].

We show that sodium butyrate potentiates the efficacy of widely used chemotherapeutics, although it exerts only a minor effect on cell growth by itself.

Combined treatment of cancer with chemotherapeutics and differentiating agents has been previously suggested [3, 15]. Sodium butyrate has been reported to inhibit in a synergistic manner the growth of neuroblastoma cell lines with Adriamycin®, 5-fluorouracil, irradiation, papaverine and vincristine [3]. Our present findings show that sodium butyrate potentiated the anti-proliferative effect of Adriamycin® in the ovarian cancer cell line and also rendered the initially irresponsive cells sensitive to *cis*-platinum.

Since sodium butyrate may be administered *in vivo* [21], combined treatment with these cytotoxic agents might be considered.

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