Growth Inhibitory Effects of Sodium Phenylacetate (NSC 3039) on Ovarian Carcinoma Cells in Vitro

Gabriella Ferrandina, Bohuslav Melichar, Amy Loercher, Claire F. Verschraegen, Andrzej P. Kudelka, Creighton L. Edwards, Giovanni Scambia, John J. Kavanagh, James L. Abbruzzese, and Ralph S. Freedman

ABSTRACT

The aim of this study was to determine the antiproliferative activity of sodium phenylacetate (NaPa) against ovarian carcinoma cell lines. NaPa induced a dose-dependent inhibition (IC_{50} from 12 μM to >20 μM) of all ovarian carcinoma cell lines, although the sensitivity of individual lines to NaPa varied. Both cisplatin-sensitive and -resistant cell lines responded to NaPa, and growth-inhibitory activity was also detected against cells freshly isolated from malignant ascites of previously treated patients. The growth inhibitory effects that were produced by NaPa were time dependent, showing a maximum effect at 72 h, and were not associated with cytotoxic action. Growth inhibitory effects of NaPa were also reversible. After 48- and 72-h exposures to NaPa, a reduction in the percentage of cells in the S-phase was detected, with a concomitant recruitment of cells in the G_0-G_1 phase. Treatment with NaPa after different exposure times did not significantly increase the proportion of cells undergoing apoptosis. NaPa also produced a significant reduction in the percentage of cyclin-D1- and p21/ras-positive cells and in the percentage of cells positive for bcl-2, whereas the percentages of bax/p21-positive cells increased. NaPa produced minimal, if any, alterations of expression of HLA class I and transforming growth factor β antigens. In contrast, the percentage of transforming growth factor β2-positive cells decreased after exposure to NaPa. The combination of NaPa with cisplatin resulted in an additive inhibitory effect. Our results show, for the first time, that NaPa inhibits the growth of ovarian carcinoma cell lines and the cells from malignant ascites of chemotherapy-treated patients with ovarian carcinoma. The growth-inhibitory properties of NaPa suggest that this molecule could represent a prototype of a new class of compounds with possible therapeutic potential in patients with ovarian carcinoma.

INTRODUCTION

Ovarian cancer is the third most common gynecological malignancy and the fifth most common cause of cancer-related death in women (1). Even among patients who have undergone surgical confirmation of primary chemotherapy, most will ultimately develop tumor recurrence (2). Efforts are therefore continuing to develop new and less toxic therapeutic agents for the treatment of chemotherapy-refractory tumors.

Small molecules such as aromatic fatty acids were recently proposed as a new class of tumor growth inhibitory compounds. NaPa, a physiological product of phenylalanine metabolism, is present in micromolar concentrations in human plasma (3) and has been shown to induce antiproliferative effects against several human cancer cell lines at millimolar concentrations (4—8). NaPa also exhibits antitumor activity against breast cancer xenografts in nude mice (9) and in a rat model of malignant gliosarcoma (10). Moreover, NaPa has shown antitumor activity in Phase I clinical trials conducted in patients with prostate cancer and high-grade gliomas (11, 12). The mechanisms by which NaPa can affect cell growth and differentiation patterns are not completely understood. At first, glutamine starvation was proposed as an important mechanism underlying NaPa’s growth inhibitory activity (13). The inhibition of DNA protein prenylation, which plays a critical role in the cellular functions of molecules like p21/ras, may also contribute to NaPa-related antiproliferative activity (14—17). Tumor cytostasis that is induced by NaPa and its analogue may affect cell cycle events and molecules such as cyclin D1 (18), and it has been linked to the activation of the human peroxisome proliferator-activated receptor, which functions as a transcription factor for enzymes involved in lipid metabolism (19). NaPa may also produce immunomodulatory effects on prostate carcinoma and leukemic cells through the enhancement of MHC antigens and decreased production and secretion of TGF-β (4, 20). The effects of NaPa on human ovarian cancer cells, however, have thus far received little attention (21).

The aim of this study was to investigate the effects of NaPa on the growth activity of a large panel of ovarian carcinoma cell lines and also of freshly obtained tumor cells from patients with ovarian cancer. We determined the following effects of NaPa on ovarian carcinoma cells: (a) cell cycle-specific events; (b) modulation of p21/ras and apoptosis-related genes, including bcl-2 and bax/p21; (c) modulation of MHC expression and TGF-β1 and -β2; and (d) the growth-inhibitory effects of the combination of NaPa and CDDP in vitro.

MATERIALS AND METHODS

Chemicals and Cell Lines. NaPa (NSC 3039), dissolved in sterile water, was provided by Elan Pharmaceutical Research Corporation (Gainesville, GA) through the Cancer Therapy Evaluation Program at the National Cancer Institute. SRB was purchased from Sigma Chemical Co. (St. Louis, MO). CDDP was purchased from Bristol-Myers Squibb Co. (Princeton, NJ). Monoclonal antibodies anti-bcl-2 (used at a 1:50 dilution), anti-cyclin D1 (used at a 1:200 dilution), and anti-pan-ras (working dilution: 1:50) were purchased from Oncogene Science, Inc. (Cambridge, MA). Anti-TGF-β1 monoclonal antibody (used at a 1:100 dilution) and anti-bax/p21 polyclonal antibody (used at a 1:200 dilution) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Appropriate isotype-matched monoclonal antibodies were used as controls.

Twelve established human epithelial ovarian cancer cell lines were used. The 2774 ovarian carcinoma cells (22), SKOV3, CaOV3, OVI1225, and OVCAR-3, obtained from American Type Culture Collection, were routinely cultured in RPMI 1640 (Life Technologies, Inc., Grand Island, NY), supplemented with 10% heat-inactivated FBS, and incubated at 37°C in a humidified atmosphere containing 5% CO_2. HEY, OVCA 420, and OVCA 433 (kindly provided by Dr. R. Bast, Jr., M. D. Anderson Cancer Center) were routinely grown in MEM (Life Technologies) plus 10% FBS and 2 μM glutamine. Sodium pyruvate (1%) and nonessential fatty acids (1%) were added to the culture medium of OVCA 420 and OVCA 433. A2780 and 2008 ovarian cancer cells and their CDDP-resistant variants, ADDP and C13-2008, respectively (kindly provided by Dr. V. Ruiz Van Hage, M. D. Anderson Cancer Center) were routinely grown in RPMI 1640 plus 5% FBS.
Tissue samples were obtained from the ascitic fluid of patients with advanced ovarian cancer. Tumor cells were isolated from the ascitic fluid using a Ficoll-Hypaque gradient, and the cells that were collected from the interface were washed twice in PBS. The tumor cells were then resuspended in RPMI 1640 plus 10% FBS and routinely grown in the same medium. Patient ages ranged from 39 to 67 years. Tumors were staged according to International Federation of Gynecology and Obstetrics criteria (23) and histologically graded as well differentiated (G1), moderately differentiated (G2), or poorly differentiated (G3; Table 1).

**Growth Inhibition Experiments.** Growth rates were analyzed by the SRB assay (24) and thymidine incorporation. In the SRB tumor assay, tumor cells were plated in 96-well plates at optimal cellular density, as determined from preliminary studies conducted on each cell line, to obtain a logarithmic growth rate during exposure to the drugs. After 24 h, the medium was replaced with fresh medium containing the compounds to be tested. At the conclusion of the experiment, the cells were precipitated with 50 μl of ice-cold 50% (w/v) trichloroacetic acid and incubated for 60 min at 4°C. After five washings with tap water, the plates were air-dried, and then the cells were stained for 15 mm with 0.4% SRB, prepared with 1% acetic acid (50 μl/well). Finally, the plates were washed with 1% acetic acid and air-dried, and the protein stain was solubilized with 150 μl/well of 10 mM unbuffered Tris base. The optical density was read at 540 or 510 nm.

For analysis of thymidine incorporation, the cells were labeled with 1 μCi/ml [3H]thymidine (specific activity, 82.0 Ci/mmol; Amersham Life Science Inc., Cleveland, OH) for 18 h and then harvested. The radioactivity of the filters was measured using a liquid scintillation counter (mode) LS 3801; Beckman Instruments Inc., Fullerton, CA).

For the glutamine starvation experiments, RPMI not containing glutamine was used, and the FBS was stored at 4°C for 6 weeks before use to reduce glutamine levels to less than 0.06 mM in the culture medium (4, 5). The drug interaction was evaluated and compared with the dose-response curves for single agents. The characteristics of the effects of the combined treatment were then analyzed by the isobole method (25) for combinations of drugs A and B, from the equation \( \frac{A}{A_c} + \frac{B}{B_c} = D \), where \( A_c \) and \( B_c \) correspond to concentrations of drugs used in the combination treatment, and \( A_c \) and \( B_c \) correspond to the concentrations of drugs able to produce the same magnitude of effect if used individually. If \( D \) (combination index) < 1, the effects of combination were synergistic, whereas if \( D = 1 \) or \( D > 1 \), the respective effects were additive or antagonistic, respectively. Each experiment was performed in triplicate, thus allowing a calculation of statistical significance (P) of the combination indices compared with the additive combination index of \( D = 1 \) by the one-sided Student’s t test.

**Cell Cycle Analysis and Flow Cytometric Staining.** For cell cycle analysis, the cells were plated at the initial density of 50,000 cells/ml. The culture medium was replaced, and NaPa was added after 24 h. After 12, 24, 48, or 72 h of treatment, the cells were removed from the culture plates and centrifuged at 1500 rpm for 5 min. The cell pellets were then suspended in PBS, fixed in 70% ethanol for 1 h, and, after two washes in PBS, stained with a PBS solution containing RNase A (100 μg/ml; Boehringer Mannheim, Mannheim, Germany) and propidium iodide (25 μg/ml; Sigma) at room temperature for 1 h in the dark. DNA content was analyzed by using an Epics Profile Analyzer (Coulter Corp., Hialeah, FL). Assessment of the specific staining for HLA class I, TGF-β1, TGF-β2, and certain intracellular antigens, including cyclin D1, bcl-2, and bax/p21 w343, was carried out by means of indirect immunofluorescence. Briefly, the cells were plated at an initial density of 50,000 cells/ml, and after 24 h, the medium was removed and replaced by fresh medium and drug solution. After treatment, the cells were removed from the plates by using a disposable cell scraper and centrifuged at 1500 rpm for 5 min at 4°C. Fixation and permeabilization were carried out by incubating cell pellets for 15 min at 4°C with a solution containing 2% v/v paraformaldehyde and PBS-S (Sigma) and incubated for 30 min at 4°C with the specific antibody and the respective isotype-matched control antibody. The cell pellets were washed in PBS-S, and incubated for 30 min at 4°C in the dark with a 1:150 dilution of goat antimmunoglobulin FITC (Sigma) or a 1:200 dilution of goat antirabbit FITC (Oncogene Research Products) secondary antibody. After incubation, the cells were washed twice in PBS-S and then suspended in 2% paraformaldehyde at 4°C in the dark. Analysis was performed by using the Epics Profile Analyzer (Coulter). Fluorescence intensity was expressed as mean channel fluorescence after the fluorescence intensity of the isotype-matched control antibody was subtracted. Membrane antigens were assessed using the same protocol with the following minor modifications: the cells were gently detached with EDTA (0.5 μM) and fixed after incubation with the specific antibody and the FITC-conjugated secondary antibody.

**RESULTS**

**Effects of NaPa on Cell Proliferation.** The effects of increasing concentrations of NaPa on the growth of 12 established ovarian cancer cell lines are shown in Fig. 1. NaPa induced a dose-dependent inhibition.

**Fig. 1. Effects of increasing concentrations of NaPa on different established ovarian cancer cell lines. Data points, means of at least five experiments performed in quintuplicate; SD less than 5%.**

**Fig. 2. Effects of increasing concentrations of NaPa on cells isolated from the ascitic fluid of patients with ovarian cancer. Numbers 1–5, patients listed in Table 1. Data points, means of four experiments performed in quintuplicate; SD less than 5%.**

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**Table 1 Patient characteristics**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Histology</th>
<th>Stage</th>
<th>Grade</th>
<th>Previous treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Serous</td>
<td>IIIc</td>
<td>G2</td>
<td>Platinum, cyclophosphamide</td>
</tr>
<tr>
<td>2</td>
<td>Undifferentiated</td>
<td>IIIC</td>
<td>G2</td>
<td>Platinum, paclitaxel</td>
</tr>
<tr>
<td>3</td>
<td>Serous</td>
<td>IIIc</td>
<td>G3</td>
<td>Platinum, paclitaxel, melphanol</td>
</tr>
<tr>
<td>4</td>
<td>Serous</td>
<td>IIIc</td>
<td>G3</td>
<td>Platinum, doxorubicin, paclitaxel</td>
</tr>
<tr>
<td>5</td>
<td>Serous</td>
<td>IIIc</td>
<td>G3</td>
<td>Platinum, cyclophosphamide</td>
</tr>
</tbody>
</table>

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4310
bition of all cell lines examined, although there was variability in the
responses of different cell lines. A2780 cells were the most sensitive
to NaPa, whereas SKOV3 and HEY were the least sensitive. Interest-
ingly, NaPa was equally effective in both CDDP-sensitive and
-resistant cell lines. The growth-inhibitory activity exerted by NaPa
was also observed in cells freshly isolated from the ascitic fluid of
previously treated patients (Fig. 2). The decline in proliferation was
associated with comparable inhibition of DNA synthesis (data not
shown). Time-dependent analyses of NaPa in Fig. 3 showed that the
antiproliferative effect started at 24 h after the addition of the drug in
2774 ovarian cancer cells. In initial experiments, no significant
changes were seen following a shorter exposure of the cells to Pa (data
not shown).

The inhibitory effect of NaPa was not due to a nonspecific cyto-
toxic action because cell viability, as assessed by trypan blue exclu-
sion, was about 95% and did not differ between control and treated
cells. Moreover, the inhibitory effects of NaPa were reversible; in
wash-out experiments, the recovery of cell growth was observed after
the drug was removed and replaced with fresh medium (Fig. 4).

The effects of NaPa on cycle phase distribution of 2774 cells after
NaPa treatment are summarized in Table 2. After 48- and 72-h
exposures to NaPa, the percentage of cells in the S-phase of the cell
cycle decreased with a concomitant recruitment of cells in the G0-G1
phase. In the same table, flow cytometric assessment of the percentage
of hypodiploid cells stained with propidium iodide is shown. Treat-
ment with NaPa after different times of exposure does not signifi-
cantly increase the percentage of cells undergoing apoptosis. More-
ever, by using pulsed-field gel electrophoresis of high molecular
weight DNA fragments (mostly located at 50 kb), which are consid-
ered to be more reliable and earlier markers of commitment to
apoptosis, no evidence of NaPa induction of apoptosis was observed
in the 2774 ovarian cancer cells (data not shown).

As a result of reports in humans that NaPa is able to bind to
glutamine and deplete its levels, experiments were carried out to
determine whether the absence of glutamine in culture medium or
increasing glutamine concentrations that are sufficient to overcome
the effects of glutamine starvation of cell growth would influence the
sensitivity of the tumor cells to the growth inhibition that is exerted
by NaPa. As shown in Fig. 5, 0.2–2 mM of glutamine positively affects
the growth of 2774 ovarian cancer cells, whereas at glutamine con-
centrations 5–10-fold higher than those normally used, cell prolif-
eration is inhibited. The maximal activity of NaPa to inhibit growth is
Table 2  Effects of NaPa on the distribution of 2774 cells in the different phases of the cell cycle

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Exposure (h)</th>
<th>G0-G1</th>
<th>S</th>
<th>G2-M</th>
<th>Hypodiploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaPa (30 mM)</td>
<td>12</td>
<td>36.2</td>
<td>34.6</td>
<td>29.1</td>
<td>5.1</td>
</tr>
<tr>
<td>None</td>
<td>24</td>
<td>28.2</td>
<td>44.4</td>
<td>27.4</td>
<td>4.5</td>
</tr>
<tr>
<td>NaPa (30 mM)</td>
<td>48</td>
<td>33.2</td>
<td>38.2</td>
<td>28.6</td>
<td>7.2</td>
</tr>
<tr>
<td>None</td>
<td>72</td>
<td>36.0</td>
<td>41.7</td>
<td>22.2</td>
<td>8.6</td>
</tr>
</tbody>
</table>

* Values represent the mean of two experiments performed in duplicate; SD less than 10%. Results are expressed as the percentage of cells in each phase of the cell cycle.

Fig. 5. Effect of NaPa on the growth of 2774 ovarian cancer cells according to glutamine content in culture medium. The cells were plated at an initial density of 50,000 cells/ml (100 μl/well), and after 24 h the medium was replaced with fresh medium either without glutamine or supplemented with increasing concentrations of glutamine, 0.2, 2, 10, and 20 mM. The cells were exposed to 20 mM (△) and 30 mM (○) of NaPa for 3 days. Comparisons were made with cells cultured in the absence of NaPa (□). Data points, means of three experiments performed in quintuplicate; SD less than 5%.

Fig. 6. Effects of CDDP, alone or in combination with NaPa (5 mM) on the growth of 2774 ovarian carcinoma cells. SD, <7%.
Table 3 Effects of combination of CDDP and NaPa on established ovarian cancer cell lines

<table>
<thead>
<tr>
<th>Ovarian tumor cell line</th>
<th>CDDP (A; µg/ml)</th>
<th>NaPa (B; mM)</th>
<th>% of growth control</th>
<th>CDDP (A; µg/ml)</th>
<th>NaPa (B; mM)</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>2774</td>
<td>0.01</td>
<td>5</td>
<td>60</td>
<td>0.04</td>
<td>10</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>5</td>
<td>38</td>
<td>0.08</td>
<td>26</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>5</td>
<td>30</td>
<td>0.16</td>
<td>30</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>5</td>
<td>25</td>
<td>0.22</td>
<td>&gt;30</td>
<td>ND</td>
</tr>
<tr>
<td>OVCA 433</td>
<td>0.01</td>
<td>5</td>
<td>74</td>
<td>0.1</td>
<td>9</td>
<td>0.65b</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>5</td>
<td>63</td>
<td>0.13</td>
<td>14</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>5</td>
<td>57</td>
<td>0.2</td>
<td>17</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>5</td>
<td>31</td>
<td>&gt;0.5</td>
<td>15</td>
<td>ND</td>
</tr>
</tbody>
</table>

* A; and B; concentration of agent used in the combination treatment; A; and B; concentration of agents able to produce the same magnitude of effect if used individually; D, combination index (see "Materials and Methods"); ND, not determined.

b P value < 0.05.

Table 4 Effects of NaPa on different cellular antigens in 2774 ovarian cancer cells

<table>
<thead>
<tr>
<th>Marker</th>
<th>Controla</th>
<th>NaPatreatmenta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% positive cells</td>
<td>MCFb</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>93.6 ± 3.4</td>
<td>13.4 ± 2.1</td>
</tr>
<tr>
<td>p21/ras</td>
<td>97.5 ± 3.1</td>
<td>12.6 ± 2.8</td>
</tr>
<tr>
<td>bcl-2</td>
<td>73.2 ± 6.2</td>
<td>18.2 ± 2.7</td>
</tr>
<tr>
<td>bax/p21</td>
<td>70.1 ± 4.0</td>
<td>7.2 ± 1.6</td>
</tr>
</tbody>
</table>

a Each value represents the mean ± SD of two experiments performed in duplicate.
b MCF, mean channel fluorescence.

DISCUSSION

Our results show, for the first time, that NaPa inhibits the growth of a large panel of established ovarian carcinoma cell lines as well as cells from patients with ovarian cancer who had previously been treated with platinum-based chemotherapy. This finding is in agreement with previous studies showing that NaPa in the millimolar range has an antiproliferative activity against human cancer cells in vitro (4–8). Although most studies have reported IC50 that were generally lower than those observed in our experimental models, our results are in agreement with those reported by Carducci et al. (26) in human prostate cancer cells and by Shack et al. (21) in murine fibroblasts and several human tumor cell lines (4—9). Similar to preliminary data in human osteosarcoma cells, neuroblastoma cells (27), our results showed that, even with the addition of sufficiently high concentrations of glutamine to overcome possible glutamine depletion induced by NaPa, growth inhibition still occurred, suggesting that NaPa may affect ovarian cancer cell growth through different mechanisms.

The NaPa-induced modulation of growth factors and oncogenes involved in the regulation of tumor cell proliferation has not been fully investigated. TGF-α, TGF-β1, and epidermal growth factor receptors are not modified by NaPa treatment (4, 27). In ras-transfected murine fibroblasts, NaPa treatment failed to affect p21/ras synthesis per se, but it inhibited p21/ras farnesylation (15). We have

Table 5 Effects of NaPa on HLA I antigen, TGF β1, and TGF β2 expression in established lines of ovarian cancer cells

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Cell line</th>
<th>Controla</th>
<th>MCFb</th>
<th>% positive cells</th>
<th>MCFb</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA 1</td>
<td>2774</td>
<td>0.9 ± 1.4</td>
<td>1.0 ± 0.4</td>
<td>1.5 ± 0.6</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>SKOV3</td>
<td>97 ± 0.5</td>
<td>3.7 ± 1.8</td>
<td>93 ± 3.7</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>OVCA 433</td>
<td>94 ± 4.7</td>
<td>2.9 ± 0.9</td>
<td>93 ± 0.7</td>
<td>6.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>OVCA 420</td>
<td>29 ± 0.8</td>
<td>1.0 ± 0.1</td>
<td>30 ± 4.8</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>HEY</td>
<td>80 ± 9.4</td>
<td>1.7 ± 0.2</td>
<td>86 ± 0.2</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>97 ± 2.8</td>
<td>3.7 ± 0.3</td>
<td>94 ± 1.7</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>C13.2008</td>
<td>89 ± 3.2</td>
<td>2.4 ± 0.2</td>
<td>96 ± 2.2</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>2774</td>
<td>14 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>15 ± 0.8</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>OVCA 433</td>
<td>22 ± 5.3</td>
<td>1.0 ± 0.1</td>
<td>24 ± 3.3</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>OVCA 420</td>
<td>20 ± 2.1</td>
<td>0.9 ± 0.1</td>
<td>18 ± 0.9</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>SKOV3</td>
<td>94 ± 3.2</td>
<td>1.8 ± 0.4</td>
<td>89 ± 1.8</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>TGF-β2</td>
<td>2774</td>
<td>31 ± 1.8</td>
<td>1.4 ± 0.3</td>
<td>26 ± 0.8</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>OVCA 433</td>
<td>23 ± 2.2</td>
<td>1.1 ± 0.2</td>
<td>13 ± 1.5</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>OVCA 420</td>
<td>22 ± 0.3</td>
<td>1.1 ± 0.5</td>
<td>14 ± 1.0</td>
<td>0.8 ± 0.3</td>
</tr>
</tbody>
</table>

a Each value represents the mean ± SD of three experiments performed in duplicate.
b MCF, mean channel fluorescence.

PHENYLACETATE INHIBITION OF OVARIAN CARCINOMA
shown that NaPa treatment reduced the percentage of p21/ras-positive cells in several ovarian cancer cell lines and in cells from chemotherapy-treated patients with ovarian cancer. p21/ras isoprenylation was also significantly reduced in ovarian carcinoma cell lines by the addition of NaPa. The demonstration that NaPa and its analogues may interfere with p21/ras-promoted pathways of tumor cell growth is of utmost importance because p21 overexpression has been shown to be associated with an unfavorable prognosis in patients with ovarian cancer (28). We also reported that treatment of tumor cells with NaPa appeared to be associated with a reduction in bcl-2 positivity, as described by Adam et al. (9). Moreover, as expected by the known inverse functional relationship between bcl-2 and bax/p21 production (29), NaPa treatment caused an increase in bax/p21-positive cells. This finding suggests a common point of regulation of these two markers by NaPa. Bcl-2 family products have been identified with the apoptotic process. We were unable to observe relevant signs of apoptosis in our experimental model, and we are currently pursuing the mechanisms of bcl-2 and bax/p21 involvement in NaPa-induced growth inhibition.

MHC antigen expression showed little response to NaPa in our experimental model. This finding appears to be in contrast with the data reported by Samid et al. (4) for leukemic cells, in which a different tumor model was used. On the other hand, NaPa exposure was associated with a decrease in TGF-β expression, whereas TGF-β1 was unaffected. Similar observations were made on prostate cancer cells (20). The decrease shown in TGF-β expression could suggest a possible role for NaPa in modulating TGF-β-induced murine suppressive effects in vivo. In this respect, in vivo rejection of an experimental glioma was observed after these animals had been treated with a TGF-β antisense gene therapy approach (30).

Studies have examined the synergistic activities of NaPa in combination with differentiation agents such as retinoic acid (8, 31, 32) and agents modulating pathways of growth factors, such as suramin (20) and lovastatin (33). Preliminary studies also suggest that NaPa can potentiate the biologic effects induced by cytostatic agents (5). More recently, Shack et al. (21) reported that NaPa acts synergistically with doxorubicin in inhibiting multidrug-resistant breast and colon cancer cells.

In our model, NaPa appeared to generally have additive effects when combined with CDDP although a trend toward synergistic activity of NaPa at low concentrations of CDDP was observed. This latter finding could be explained by the demonstration that NaPa was able to recruit tumor cells in the G1–G2 phase of cell cycle, when their sensitivity to CDDP seems to be higher (34).

The growth-inhibitory properties of NaPa suggest that this molecule could represent the prototype of a new class of compounds with some therapeutic potential in ovarian cancer. The concentrations of NaPa required to inhibit ovarian cancer cells in vitro are achievable in vivo. Considering the natural history of ovarian cancer, which spreads to the peritoneal cavity, NaPa could be administered intra-tumorally, thus possibly achieving higher concentrations of the drug and limiting systemic toxicity. Finally, the possibility of identifying or synthesizing fatty acids that are structurally related to NaPa (16, 19) and that have more advantageous pharmacokinetic and pharmacodynamic features should also be considered. In this context, phenylbutyrate, which is β-oxidized in vivo to NaPa, has been shown to be more effective on a molar basis than has NaPa in inhibiting human prostate and ovarian carcinoma cells. Because phenylbutyrate does not have an unpleasant odor like NaPa, oral administration may have greater patient acceptability.

REFERENCES


Growth Inhibitory Effects of Sodium Phenylacetate (NSC 3039) on Ovarian Carcinoma Cells in Vitro

Gabriella Ferrandina, Bohuslav Melichar, Amy Loercher, et al.

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