Growth Inhibitory Effects of Aromatic Fatty Acids on Ovarian Tumor Cell Lines

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ABSTRACT

Epithelial ovarian cancer is a major cause of cancer-related mortality in women, making the search for new treatment modalities essential. Sodium phenylacetate (NaPa), a phenylalanine derivative, has been shown to induce cytostasis and differentiation by inhibiting protein isoprenylation. Similar effects have been observed with phenylbutyrate, a phenylacetate congener. We examined in parallel the growth inhibitory activity against human ovarian carcinoma cell lines of phenylacetate, phenylbutyric acid (PB), and certain related compounds, and comparisons were made with lovastatin. On a molar basis, hydroxykynurenine and kynurenine showed the highest activity followed by PB and NaPa. Ovarian carcinoma cell lines were also sensitive to lovastatin in micromolar concentrations. Additive effects were observed when PB was combined with cisplatin or when NaPa or PB were combined with lovastatin. NaPa and PB, but not kynurenine, inhibited incorporation of [3H]mevalonate into ovarian carcinoma cells. An immune modulatory role might also be suggested for PB because it resulted in increased ovarian tumor cell expression of human leukocyte antigen class I and the cluster of differentiation molecule CD58, whereas transforming growth factor-β2 expression was decreased. Phenylbutyrate, which is the ester form of PB, has shown acceptable pharmacological properties and clinical responses in patients with other malignancies, and might be considered for evaluation in ovarian cancer.

INTRODUCTION

The search for novel drugs for the treatment of ovarian malignancies has included less toxic agents to which these tumors may be sensitive. Attention has been focused recently on NaPa (1–3), due to its in vitro activity against a variety of human tumor cell lines, including ovarian carcinoma (3), and clinical responses associated with the absence of dose-limiting toxicity in Phase I clinical trials (4, 5). In other experiments, phenylbutyrate, which is the ester form of PB and is a NaPa congener, and synthetic derivatives from substitutions on the aromatic ring or aliphatic side chains of phenylacetic acid, have demonstrated even higher tumor inhibitory activity (6, 7). NaPa is produced from phenylalanine by human colonic bacteria and relatively high quantities of phenylacetic acid are excreted in the urine of healthy subjects (8). Whereas little is known about the natural occurrence of PB, structurally similar molecules with substitutions on the aromatic ring and aliphatic side chains (like KYN and hydroxykynurenine) have been detected in human body fluids (9, 10). These molecules, in addition to PCA, are metabolites of tryptophan (Fig. 1).

These agents could have some similarity in their mechanisms of action to lovastatin, an inhibitor of hydroxy-methyl-glutaryl CoA reductase commonly used in the treatment of hypercholesterolemia (11) and recently shown to exert tumor growth inhibitory activity in vitro (12, 13) and in a Phase I clinical trial (14). It has been suggested that tumor growth inhibitory effects exerted by lovastatin could occur by the inhibition of the biosynthesis of mevalonate, the substrate for biosynthesis of lipid moieties involved in protein prenylation (1, 14), thus indicating the possibility of common mechanisms of action for lovastatin and aromatic fatty acids. Results of experiments conducted by others suggested that the growth inhibitory activity of NaPa, phenylbutyrate, and their synthetic derivatives might be associated with the interference with the protein prenylation pathway, although several additional or alternative mechanisms (like glutamine starvation, inhibition of histone deacetylase, and also the interaction with the human peroxisome proliferator-activated receptor, which is a transcription factor for enzymes regulating lipid metabolism) have also been reported (7, 15–19).

We have recently shown (3) that NaPa is able to exert a powerful growth inhibitory effect on ovarian cancer cell lines and on cells from malignant ascites of chemotherapy treated ovarian cancer patients, possibly by recruiting cells in the qui-
escent phases of cell cycle. Moreover, combination of NaPa with cisplatin was demonstrated to potentiate cisplatin cytotoxicity, suggesting that NaPa could be a prototype for a novel class of compounds with possible chemotherapeutic potential in ovarian carcinoma.

The aim of our experiments was to investigate several NaPa-related compounds and to conduct a comparison of the growth inhibitory and certain immunomodulatory effects of these compounds in ovarian tumor cells in tissue culture with our previously reported results with NaPa (3). In addition, we examined the growth inhibitory effects of combining PB with cisplatin and paclitaxel, which are commonly used in the treatment of ovarian cancer, and with lovastatin, which could have mechanisms of action that are common to PB and related molecules.

MATERIALS AND METHODS

Cell Lines. Experiments were conducted using eight human ovarian cancer cell lines. SKOV3, CAOV3, and OVCAR3 were obtained from American Type Culture Collection (Manassas, VA). A2780 and its drug-resistant variant ADDP (20) were obtained from Dr. W. Plunkett (The University of Texas, M. D. Anderson Cancer Center). The ovarian cancer cell lines OVA420, OVCA433, and HEY were obtained from Dr. R. Bast (M. D. Anderson Cancer Center), and cell line 107 was obtained from Dr. C. Verschraegen. Ovarian cancer cell line 2774 was established in the laboratory of J. Sinkovics (St. Joseph’s Hospital, Tampa, FL) (21). All cell lines were grown in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 10% FCS (Sigma Chemical Co., St. Louis, MO) at 37°C and 5% CO₂.

Amino Acid Derivatives and Biological Agents. NaPa was obtained from Elan Pharmaceutical Research Corp. (Gainesville, GA) and supplied through the Cancer Therapy Evaluation Program at the National Cancer Institute (NSC# 3039). PB was obtained from Sigma-Aldrich (St. Louis, MO).

l-KYN, mevalonic acid lactone, and 3-hydroxy-DL-kynurenine were purchased from Sigma Chemical Co. Lovastatin was a generous gift of Merck Research Laboratories (Rahway, NJ). cDDP and paclitaxel were obtained from Bristol-Myers Squibb (Princeton, NJ). All chemical reagents tested negative for endotoxin with E-Toxate (Sigma Chemical Co.).

Reagents Used for Chemical Assays. MTT and NP40 were purchased from Sigma Chemical Co. SDS was obtained from Life Technologies, Inc. (Gaithersburg, MD). Sodium chloride was supplied by Curtin Mathesonic Scientific, Inc. (Houston, TX). Tris(hydroxymethyl)aminomethane (Tris base) was purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN).

Cytostasis. Cytostasis was determined by the MTT and thymidine incorporation assays. For the MTT assay, 2000 tumor cells/well were seeded in 96-well plates (Becton Dickinson Labware, Lincoln Park, NJ) and left to adhere overnight at 37°C. After adding the amino acid derivatives or biological agents, the cells were incubated for 96 h. Fifty microliters of MTT (2.5 g/liters) was then added to each well, and incubation was prolonged for another 4 h. After removing the supernatants, the newly formed formazan product was solubilized in 100 µl of DMSO (Sigma Chemical Co.), and absorbance was read at 570 nm on a microplate reader. Tests were performed in quadruplicate, and cytostasis was calculated according to the formula: percentage of cytostasis = absorbance control wells – absorbance-treated wells/absorbance control wells.

To determine thymidine incorporation, tumor cells were seeded and incubated with the agents of interest as in the MTT assay. After 96 h the cells were pulsed with 10 µCi/ml of methyl[3H]-thymidine (Amersham Corp., Arlington Heights, IL) and incubated overnight. The plates were then processed using a Titerakit cell harvester (ICN Radiochemicals, Costa Mesa, CA), and the radioactivity (cpm) was determined on a Beckman LS 3801 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, CA). Cytostasis was calculated by the formula: percentage of cytostasis = cpm control – cpm-treated cells/cpm control.

Analysis of the Effects of Drug Combinations. The growth inhibitory effects from drug combinations that included cDDP or paclitaxel with NaPa, PB, or lovastatin were analyzed by median effect analysis (22). Serial dilutions were prepared with each agent alone or in combination. The IC₅₀ values were calculated for each drug alone (denominator), and the concentration of drug combination was determined that would result in 50% inhibition (numerator). Results were expressed as the CI: CI = (concentration of agent “a” in combination/concentration of agent “a” alone) + (concentration of agent “b” in combination/concentration of agent “b” alone). Synergism, additive, or antagonistic effects were indicated, respectively, as CI <1, CI = 1, or CI >1.

Flow Cytometry. The expression of MHC class I antigen and adhesion molecules was studied by flow cytometry. The SKOV3 cell line was chosen for these experiments because it expresses MHC class I antigen and adhesion molecules. Cells (0.5–1 × 10⁶) were seeded in 75-cm² flasks (Falcon; Becton Dickinson) and incubated for 48 h with the respective agent. The cells were then removed with a cell scraper, washed in PBS, and stained with monoclonal antibodies that are reactive with MHC
class I the adhesion molecules CD44 (Caltag Laboratories, Inc., Burlingame, CA) and CD58 (Becton Dickinson), and the respective isotype control antibodies. Intracellular staining for TGF-β2 was performed using an indirect procedure where cells were fixed in 2% paraformaldehyde, permeabilized with a 0.1% saponin solution, and then labeled with rabbit-anti-TGF-β2 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Goat-antirabbit FITC conjugate (Santa Cruz Biotechnology, Inc.) was used to detect labeled cells. The cells were then analyzed on a FACSScan flow cytometer (Becton Dickinson). Five thousand events were collected and analyzed by Consort30 software (Becton Dickinson). The results were expressed as MFIs after subtracting the fluorescence intensity of the isotype control antibody or as mean channel fluorescence.

**Determination of Mevalonate Incorporation.** Ovarian carcinoma cells (2 × 10^6) were seeded into 24-well plates and left to adhere for 4–6 h. The medium was then replaced with medium containing 15 μM lovastatin. After overnight incubation, amino acid derivatives in medium containing lovastatin (15 μM) and 50 μCi/ml of [3^H]mevalonolactone were added, and
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Table 1 IC₅₀" values for NaPa, PB, KYN, OH-KYN, PCA, and lovastatin

<table>
<thead>
<tr>
<th>Tumor cell lines</th>
<th>NaPa (mM)</th>
<th>PB (mM)</th>
<th>KYN (mM)</th>
<th>OH-KYN (μM)</th>
<th>PCA (mM)</th>
<th>Lovastatin (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2774</td>
<td>18.8 ± 1.5</td>
<td>4 ± 1.4</td>
<td>2.5 ± 1.0</td>
<td>152 ± 50</td>
<td>1.7 ± 0.2</td>
<td>18.0 ± 3.9</td>
</tr>
<tr>
<td>SKOV3</td>
<td>&gt;20</td>
<td>6.2 ± 1.5</td>
<td>2.2 ± 0.8</td>
<td>346 ± 39</td>
<td>&gt;5</td>
<td>7.1 ± 3.3</td>
</tr>
<tr>
<td>CAOV3</td>
<td>&gt;20</td>
<td>5.8 ± 1.9</td>
<td>1.7 ± 0.3</td>
<td>226 ± 155</td>
<td>2.5 ± 0.8</td>
<td>24.3 ± 4.9</td>
</tr>
<tr>
<td>OVCAR3</td>
<td>&gt;20</td>
<td>7.7 ± 0.6</td>
<td>1.8 ± 0.5</td>
<td>463 ± 197</td>
<td>&gt;5</td>
<td>4.2 ± 2.6</td>
</tr>
<tr>
<td>A2780</td>
<td>12.0 ± 1.8</td>
<td>1.6 ± 0.3</td>
<td>1.2 ± 0.1</td>
<td>38 ± 4³</td>
<td>1.4 ± 0.5</td>
<td>2.3 ± 0.9</td>
</tr>
<tr>
<td>OVCA433</td>
<td>14.1 ± 5.3</td>
<td>3.6 ± 1.9</td>
<td>1.7 ± 0.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>OVCA420</td>
<td>17.3 ± 3.6</td>
<td>4.8 ± 1.7</td>
<td>2.1 ± 0.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HEY</td>
<td>&gt;20</td>
<td>5.2 ± 0.6</td>
<td>2.9 ± 0.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ADDP</td>
<td>9.8 ± 2.3</td>
<td>1.9 ± 0.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.0 ± 0.3</td>
</tr>
</tbody>
</table>

" IC₅₀ was determined as described in “Material and Methods.” Each value represents mean ± SD of three to six independent experiments.
³ ND, no data.

Fig. 3 Results of growth inhibition experiment in which CAOV3, OVCAR3, SKOV3, and 2774 ovarian carcinoma cells were cultured in medium containing: (a) NaPa alone (10 mM), lovastatin alone (5 μM), or NaPa (10 mM) plus lovastatin (5 μM); or (b) PB alone (1 mM), lovastatin alone (5 μM), or PB (1 mM) and lovastatin (5 μM). Tumor cell growth inhibition was determined by the MTT assay.

the incubation was extended for another 40 h. Cells were then recovered by trypsin treatment, washed twice with PBS, mixed with 0.5 ml of lysis buffer (1% NP40, 50 mM Tris-base, 0.1% SDS, and 150 mM NaCl), incubated on ice for 45 min, and passed through a 20-gauge needle. The sample was then centrifuged at 4000 × g for 30 min, and the cells were recovered. Protein concentration was determined by bicinchoninic acid method with a commercial protein detection kit (Pierce Chemical Corp., Rockford, IL) according to manufacturer’s instructions. Identical quantities of protein (1–10 μg) from cells treated by different compounds were then mixed with scintillation liquid (Safety-Solve Research Products International, Mount Prospect, IL), and the radioactivity was determined on Beckman LS 3801 liquid scintillation counter (Beckman Instruments, Inc.).
RESULTS

Inhibitory Effects of Aromatic Fatty Acids on Cell Growth. Inhibition of tumor cell growth, measured by the MTT assay and by inhibition of thymidine incorporation in 2774 ovarian cancer cells, is shown in Fig. 2. The aromatic fatty acids NaPa, PB, KYN, OH-KYN, PCA, and lovastatin all produced dose-dependent growth inhibition (shown by $IC_{50}$ values determined from the MTT assay) on each of the five to eight ovarian tumor cell lines tested (Table 1 and Fig. 2), and including the cDDP-resistant clone ADDP.

### Table 2

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Posttreatment</th>
<th>Isotype antibody control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells (%)</td>
<td>MCF</td>
<td>Cells (%)</td>
</tr>
<tr>
<td>2774</td>
<td>52.1</td>
<td>12.27</td>
</tr>
<tr>
<td>433</td>
<td>57.0</td>
<td>7.29</td>
</tr>
<tr>
<td>107</td>
<td>69.5</td>
<td>14.96</td>
</tr>
</tbody>
</table>

*Cells were examined for TGF-β2 expression at 48 h.

Antitumor activity was usually detected in the millimolar range for the aromatic fatty acids: PB ($IC_{50}$ 1.6–7.7 mM) was approximately four times more active on a molar basis than NaPa ($IC_{50}$ 12 to >20 mM), whereas KYN (1.2–2.9 μM) and PCA was more active than PB (2-fold on a molar basis). OH-KYN was the only compound that exhibited significant activity at a concentration <1 mM. The $IC_{50}$ for lovastatin was also in the micromolar range (2.3–24.3 μM).

There was no difference between the $IC_{50}$ values determined after treatment of A2780 and its platinum-resistant clone ADDP with PB, NaPa, or lovastatin (Table 1). In contrast, the $IC_{50}$ values for the effects of cDDP and paclitaxel were: for cDDP, 0.29 ± 0.03 μg/ml on A2780 cells and 3.0 ± 0.4 on ADDP cells; for paclitaxel, 3.8 ± 1.2 ng/ml on A2780 cells and 5.6 ± 1.7 on ADDP cells. Differences in $IC_{50}$ values (results of three experiments) for cDDP and paclitaxel on A2780 and its drug-resistant variant ADDP were significantly different ($P <0.05$).

The effects of concentrations of NaPa, PB, and KYN corresponding to the $IC_{50}$ values were examined on the cell morphology of 2774 ovarian carcinoma cells. NaPa and PB both resulted in minor changes in the appearance of the cells. In contrast, treatment with KYN resulted in cell death over the same time period (data not shown).

The growth inhibitory effects of the combination of NaPa (10 mM) or PB (1 mM) with lovastatin (5 μM) were then...
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Cl values that were 0.95 ± 0.03 and 0.88 ± 0.09 for the ovarian cancer cells the combination of PB with lovastatin, cDDP, and paclitaxel using median effect analysis (Fig. 4). In ovarian tumor cell lines of combining PB with lovastatin, the isobole method (3). Here we examined the treatment effects on the isobolograms demonstrated an additive effect.

Fig. 6  Inhibition of mevalonate incorporation by aromatic compounds. The 2774 cells were incubated with [3H]mevalonate in the presence of 15 μM lovastatin for 48 h. After washing the cells, protein was extracted and the concentration was determined by bicinchoninic acid assay. The amount of protein solution corresponding to 10 μg of protein was mixed with the scintillation liquid and counted. The results shown are representative of three separate experiments. Control represents uptake with [3H]mevalonate and lovastatin alone.

Table 3  Effects of MVA* on the growth inhibitory activity of aromatic fatty acids and lovastatin in 2774 ovarian carcinoma cells

<table>
<thead>
<tr>
<th>Test agent</th>
<th>Medium only</th>
<th>Medium + MVA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0 ± 7.7</td>
<td>-12.2 ± 16.0</td>
<td>NS</td>
</tr>
<tr>
<td>Lovastatin (25 μM)</td>
<td>64.9 ± 5.3</td>
<td>4.8 ± 10.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NaPa (20 mM)</td>
<td>75.2 ± 0.8</td>
<td>80.4 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>PB (5 mM)</td>
<td>73.4 ± 3.6</td>
<td>72.3 ± 3.2</td>
<td>NS</td>
</tr>
<tr>
<td>KYN (2.5 mM)</td>
<td>82.0 ± 10.9</td>
<td>85.1 ± 2.9</td>
<td>NS</td>
</tr>
<tr>
<td>OH-KYN (140 μM)</td>
<td>84.6 ± 1.9</td>
<td>79.7 ± 4.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

* MVA, mevalonic acid.

The 2774 ovarian carcinoma cells were cultured for 96 h in the presence or absence of MVA (100 μM). Cytostasis was then determined by the MTT assay. Results are from triplicate experiments.

experiments conducted with a different ovarian tumor cell line, OVCA433, the combination of PB with lovastatin (CI = 1.1), cDDP (CI = 0.68), and paclitaxel (CI = 0.86) were shown to be additive.

Effects of Aromatic Fatty Acids on HLA Antigen, Adhesion Molecules, and TGF Expression. We also examined the effects of the aromatic fatty acids and lovastatin on the expression of HLA class I antigen on cultured SKOV3 ovarian carcinoma cells (Fig. 5). The results shown are representative of four separate experiments and using rIFN-γ as a positive control for induction of HLA class I antigen and CD58.

PB treatment resulted in an increase in the MFI of almost 2-fold. There was no noticeable effect of NaPa and KYN on the HLA class I expression on SKOV3 cells and, in contrast, treatment of these cells with lovastatin resulted in a decrease in the expression of HLA class I antigen. Treatment with NaPa or PB also resulted in an increase in the expression of the adhesion molecule CD58 (Fig. 5). Increased CD58 antigen expression shown as MFI, was also detected on the CAOV3 ovarian tumor cells after treatment with the aromatic fatty acids as follows: NaPa, 303; PB, 427; compared with a no-treatment control of 238. No change was detected in HLA class I expression on SKOV3 cells and, in contrast, treatment of these cells with lovastatin resulted in a decrease in the expression of HLA class I antigen.

Analysis of Growth Inhibitory Effects on Ovarian Tumor Cell Lines from Combining PB with Cisplatin, Paclitaxel, or Lovastatin. We have previously shown that NaPa produced additive effects in vitro when combined with cDDP by the isobole method (3). Here we examined the treatment effects on ovarian tumor cell lines of combining PB with lovastatin, cDDP, and paclitaxel using median effect analysis (Fig. 4). In 2774 ovarian cancer cells the combination of PB with lovastatin and cDDP produced effects that seemed to be additive as shown by CI values that were 0.95 ± 0.03 and 0.88 ± 0.09 for the combination of PB with lovastatin and cDDP, respectively. The effects of combining PB with paclitaxel on 2774 cells resulted in a CI value of 1.27 ± 0.13 that trended toward antagonism. In the

Effects of Aromatic Fatty Acids on Mevalonate incorporation. Experiments were performed to determine whether aromatic fatty acids could affect the mevalonate incorporation into 2774 ovarian carcinoma cells (Fig. 6). NaPa and PB both inhibited the incorporation of mevalonate, whereas KYN seemed to have no effect.

Experiments were also carried out to determine whether the growth inhibitory activity of fatty aromatic acids, as with lovastatin, might also involve inhibition of mevalonate synthesis.

When mevalonate was added to the media, only the growth inhibition induced by lovastatin, as expected, was reversed,
in vitro effects. We selected PB for the therapeutically active agents that have additive or synergistic tumors, consideration could be given to combinations with other functions have also been enhanced by PCA (27, 28).

Both NaPa and PB resulted in increased expression of the in vivo ciliates adaptive immunity mechanisms such as the production or by decreasing TGF-β production. An increase in the expression of HLA class I was detected by flow cytometry after treatment of the SKOV3 cells with PB. A similar effect has been shown in other tumor models with NaPa and PB, but not KYN, is not reversed by the simple addition of mevalonate to the medium might also suggest that NaPa and PB could have additional or alternative mechanisms of action with respect to KYN.

The additive effects we have shown from the combination of PB with cDDP or paclitaxel are different from the recent results by Pelidis et al. (30) who demonstrated that PB did not affect the activity of cisplatin in neuroblastoma cells. The combination of PB resulted in additive effects on OVCA433 cells, whereas a trend toward an antagonistic effect was observed with PB and paclitaxel on 2774 cells. Differences observed in in vitro models are partly due to inherent differences between established cell lines. However, our results on the combination of PB with cDDP or lovastatin support an overall additive effect and seem to suggest an absence of cross-reaction between cytotoxic agents and fatty aromatic acids.

The issue of the routes of administration could be important in the possible application of these compounds to cancers that involve the peritoneal cavity such as ovarian cancer. At present, no pharmacokinetic data about fatty aromatic acid i.p. administration in humans have been reported. However, considering the demonstrated activity of this class of compounds on ovarian cancer cells and the propensity of ovarian cancer to involve the peritoneal cavity, an i.p. route of administration of these compounds might be considered. It is unknown whether there could be pharmacokinetic advantages from i.p. route of administration, although the intracellular concentrations of these drugs could possibly be due to their lipophilic properties.

We described here the results of studies on a group of compounds that have tumor cell growth inhibitory effects and other characteristics that could have useful application as possible novel approaches to the treatment of ovarian cancer.

<table>
<thead>
<tr>
<th>Table 4 Pharmacokinetic studies on NaPa, phenylbutyrate, and lovastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>NaPa</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Phenylbutyrate</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Lovastatin</td>
</tr>
</tbody>
</table>

achievable serum levels for lovastatin, PB, and NaPa are shown in Table 4 (4, 5, 14, 23–25). Peak plasma concentrations achievable after NaPa were below the IC₅₀ values determined for this compound in our experiments. In contrast, achievable peak plasma concentrations of PB or lovastatin were either in proximity to (in the case of PB) or above (in the case of lovastatin) the IC₅₀ values that were determined for these agents. The concentrations of these lipophilic agents that can be achieved within tumor tissues is unknown. Moreover, these agents might possibly mediate antitumor effects through other mechanisms such as the production in vivo of active metabolites. PB could also have a possible application for enhancing host immunological responses either by increasing antigen expression or by decreasing TGF-β production. An increase in the expression of HLA class I was detected by flow cytometry after treatment of the SKOV3 cells with PB. A similar effect has been shown in other tumor models with phenylbutyrate (6). Moreover, both NaPa and PB resulted in increased expression of the adhesion molecule CD58. It is possible that higher expression of HLA and adhesion molecules combined with a reduction in the immunosuppressive cytokine TGF-β on tumor cells, could facilitate adaptive immunity in vivo (26). Certain macrophage functions have also been enhanced by PCA (27, 28).

To enhance the in vivo effects of the aromatic fatty acids on tumors, consideration could be given to combinations with other therapeutically active agents that have additive or synergistic effects. We selected PB for the in vitro studies on drug combi-
REFERENCES


Erratum

In the article by B. Melichar et al., which appeared in the December 1998 issue of Clinical Cancer Research (pp. 3069–3076), panels b through e in Fig. 1 were incorrectly labeled. In addition, the C4 position in d should have been labeled OH. The corrected figure is reprinted below.

Fig. 1 Chemical formulas of phenylacetate (a), PB (b), KYN (c), OH-KYN (d), and PCA (e).
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