A Preclinical Study on the Rescue of Normal Tissue by Nicotinic Acid in High-Dose Treatment with APO866, a Specific Nicotinamide Phosphoribosyltransferase Inhibitor

Uffe Høgh Olesen1,2, Annemette V. Thougaard2, Peter Buhl Jensen2, and Maxwell Sehested1,2

Abstract

Inhibitor of nicotinamide phosphoribosyltransferase APO866 is a promising cancer drug currently in phase II clinical trials in oncology. Here, we present a strategy for increasing the therapeutic potential of APO866 through the rescue of normal tissues by coadministration of nicotinic acid (Vitamin B3). We examined the toxicity profile of APO866 in B6D2F1 mice and the effect of oral administration of nicotinic acid on tissue toxicity. Nicotinic acid (50 mg/kg) protects mice from death and severe toxicity from an APO866 dose (60 mg/kg) four times the monotherapy maximum tolerated dose (15 mg/kg). In a panel of six cancer cell lines, we find that three (including ML-2 cells) are protected by nicotinic acid in vitro, whereas the cytotoxicity of APO866 remains unaffected in the remaining three (including A2780 cells). A selective biomarker for the protection by nicotinic acid was subsequently identified by quantitative RT-PCR. The expression of nicotinic acid phosphoribosyltransferase is low in the cell lines not rescued from APO866 by nicotinic acid compared with protected cell lines. The findings in cell lines translated into xenograft models in which the combination of 50 mg/kg nicotinic acid and 50 mg/kg APO866 in mouse xenografts of A2780 cells increased life span by >3-fold compared with standard treatment of 15 mg/kg, and the effect of APO866 was clearly decreased when using the same treatment paradigm in ML-2 xenografts. In conclusion, the combination of high doses of APO866 with rescue by nicotinic acid may significantly increase the therapeutic potential in a subset of cancers with low expression of nicotinamide phosphoribosyltransferase. Mol Cancer Ther; 9(6); 1609–17. ©2010 AACR.

Introduction

Historically, the administration of leucovorin (folic acid) in high-dose methotrexate therapy regimen has established the concept of rescuing normal tissue cells from toxicities of cancer treatment. Methotrexate functions as an irreversible, competitive inhibitor of dihydrofolate reductase, which is part of tetrahydrofolate synthesis from folic acid, blocking DNA synthesis. Leucovorin is readily converted to tetrahydrofolate in a dihydrofolate reductase–independent manner (1), thus effectively bypassing the inhibitory effect of methotrexate. When administered at appropriate timing and dosage, a protective effect is seen in rescuing normal, rapidly dividing cells without diminishing the anticancer effect of methotrexate. This was successfully taken to the clinic in the late 1960s (2, 3) and is now a part of methotrexate treatment regimen in several cancers (4, 5).

Nicotinamide phosphoribosyltransferase (Nampt) is a promising target for cancer treatment. It is the rate-limiting enzyme for the primary salvage pathway in mammals for the synthesis of NAD (Fig. 1; refs. 6, 7), an important cofactor for numerous cellular processes and a substrate for sirtuins and poly(ADP-ribose) polymerases (8). The inhibition of Nampt leads to the depletion of cellular NAD levels due to its continuous turnover. This is followed by ATP depletion (9, 10) and eventually cell death through apoptosis or necrosis (11, 12), although the precise mechanism of cell death remains to be elucidated. Cancer cells have a higher basal turnover of NAD, possibly due to an increased activity of poly(ADP-ribose) polymerase, and they also display higher energy requirements compared with nonmalignant cells (9, 13). Cancer cells typically generate ATP through glycolysis, a characteristic known as the “Warburg effect” (14, 15). Glycolysis produces fewer ATP molecules per NAD/NADH redox reaction; thus, malignant cells may be more dependent on NAD in energy production compared with nontransformed cells. APO866 (FK-866) and CHS-828 are two known small-molecule inhibitors of Nampt of different chemical classes (9, 10). APO866 is currently in phase II and I/II clinical trials in advanced melanoma, cutaneous T-cell lymphoma, and B-chronic lymphocytic leukemia. CHS-828 is, as the prodrug EB1627/GMX1777, in phase I...
clinical trials in solid tumors and lymphomas. Thrombocytopenia is a dose-limiting toxicity for APO866, CHS-828, and GMX1777 (16-18). Lymphopenia is observed in treatment with GMX1777 (18), and mild cases of this adverse reaction have also been reported for APO866 (16). APO866 is a competitive inhibitor of Nampt, and high concentrations of nicotinamide can protect against APO866-mediated toxicity and death in vivo (19), serving as a potential antidote. However, this also inhibits the effect of APO866 in cancer cells, negating the antiproliferative effect. This also applies for CHS-828 treatment (10).

NAD can be synthesized from other precursors including nicotinic acid, tryptophan, and nicotinamide riboside (Fig. 1; ref. 20). Nicotinic acid phosphoribosyltransferase (NAPRT) is the first step in the salvage of nicotinic acid (21). Nicotinic acid is normally only supplied from diet (as part of vitamin B3), and it is likely to be converted to other precursors in the liver, leaving low-circulating concentrations of nicotinic acid (22). NAPRT activity is found in numerous tissues in mice (21), and in vivo experiments show an increase of NAD in the bone marrow in response to treatment with nicotinic acid (22). In cancer cells, nicotinic acid also can stimulate NAD production sufficiently to protect cells from cell death from Nampt inhibitors (9). However, one cell line, HepG2, is unable to use nicotinic acid (9). This has later been shown to be due to a lack of NAPRT expression, and nicotinic acid utilization can be reactivated by reintroduction of NAPRT expression (21). Nicotinic acid has been validated for use in cardiovascular disease, and it has an advantageous safety profile (23). Nicotinic acid is typically administered pharmacologically in doses of 1 to 3 g daily (24). In addition, supplementation of nicotinic acid has been suggested to ameliorate side effects from chemotherapy (25) because cancer patients frequently are deficient in nicotinic acid due to dietary status or treatment (26-28).

Here, we show that nicotinic acid can be used as an antidote to protect mice from toxicity and death from high doses of APO866. Furthermore, we find that many malignant cell lines cannot be rescued from Nampt inhibitor-mediated cell death by the addition of nicotinic acid in vitro. In vivo, this enables treating xenograft tumors with a combination of nicotinic acid and high-dose APO866 to increase the efficacy of the treatment. The expression of NAPRT is thus a marker for identifying cancer cell lines suitable for the combination treatment.

Materials and Methods

Cell lines
HCT-116 (colon cancer), A431 (epithelial carcinoma), PC-3 (prostate cancer), and A2780 (ovarian cancer) were obtained from the American Type Culture Collection. ML-2 (myeloid leukemia) was a gift from Professor Marc Dupuis, University Hospital of Lausanne, Lausanne, Switzerland. NYH (small cell lung cancer), also called GLC-2, is previously described (29). The cell lines have not been tested and authenticated.

Mouse studies
All mouse experiments were done in accordance with the national and European Union legislation, and a permit to perform the specific type of experiments was obtained from the Experimental Animal Inspectorate, Danish Ministry of Justice. The mice in the study had free access
to laboratory mouse diet Altromin 1234 (Brogaarden, DK). In toxicity and xenograft studies, B6D2F1 and NMRI nude mice (Taconic), respectively, were treated once daily by mouth with 0.5% hydroxypropyl methylcellulose in water or nicotinic acid in the same vehicle combined with two daily intraperitoneal (i.p.) injections of APO866 in PBS/saline with 3% hydroxypropyl β-cyclodextrin/48% propylene glycol. The mice were treated in weekly 4-day cycles.

Toxicity studies
For the quantification of mRNA, samples of retina, brain (right hemisphere), left testicle, thymus, spleen, liver, kidney, and popliteal lymph node; a sample of *musculus quadriiceps femoris* and heart; and samples of jejunum mucosa and muscularis, colon mucosa and muscularis, skin, and left caudal lung lobe were excised and snap frozen in liquid N2. A blood sample was also taken upon sacrifice for the purification of peripheral lymphocytes: erythrocytes were lysed using Hoffmans buffer (Bie & Berntsen), and the remaining cells were pelleted by spinning at 3,000 rpm and were resuspended in PBS. Lymphocytes and granulocytes were separated using Ficoll-Paque PLUS (GE Healthcare), spinning 30 minutes at 2,000 rpm. Following APO866 treatment, blood samples were taken on the last treatment day for hematology and biochemistry analysis. Hematology analysis was done using blood sampling and apparatus as previously described (30), and differential WBC counts were made manually with microscopy of Hemacolor-stained blood smears (Merck). Plasma biochemistry values for alkaline phosphatase, alanine aminotransferase, creatinin, and urea were measured using Reflotron sprint (Roche Diagnostics). The same tissues as examined in mRNA analysis were excised and placed in phosphate-buffered formaldehyde (4%; pH 7) for at least a week. The samples were then embedded in paraffin for sectioning and H&E staining.

Xenograft studies
ML-2 (myeloid leukemia; 1 × 10⁷) or A2780 (ovarian carcinoma; 1 × 10⁷) cancer cells were injected subcutaneously in a mixture of PBS/Matrigel in female NMRI athymic mice. The mice were observed daily until tumors started to grow, and treatment with nicotinic acid and APO866 as described above was initiated when the tumor volumes were ~100 mm³.

Messenger RNA quantification
Messenger RNA from cells was purified using a Trizol (Invitrogen) standard protocol and cDNA was produced by a High Capacity cDNA Archive kit (Applied Biosystem). Expression was analyzed on a 7500 RT-PCR system (Applied Biosystem) using species-specific probes for Actin, Nampt and NAPRT, and Taqman Universal PCR Master Mix (Applied Biosystem). The data were analyzed by a method described by Peirson and colleagues (31).

Clonogenic assay
Cells were incubated with APO866 at different concentrations with or without 100 μmol/L nicotinic acid and seeded out on semisolid agar matrix with sheep RBC and growth medium. Following a 3-week incubation period, the colonies were counted and percent survival relative to control (nontreated) cells was calculated. IC₅₀ values were calculated on the basis of survival at different concentrations of APO866.

Statistical analyses
Statistical analysis and the graphical presentation were done using the software GraphPad Prism v. 4.0 (GraphPad Software). Comparison of the different biochemical and hematologic parameters in the different treatment groups were done using Student's t test. The outcome of xenografts was quantified as the number of days used by each individual mouse to grow its tumor to a size of 800 mm³, expressed as survival days. The survival in each treatment group was compared using log-rank analysis. The level of significance was set to a P value of 0.05.

Results

High-dose APO866 toxicity in mouse tissues
To investigate the tissue toxicity encountered with the treatment of APO866, male mice B6D2F1 were given 60 mg/kg APO866 twice daily for four consecutive days. On the fourth day, mice were sacrificed 4 to 6 hours following the first daily dose. We found histologic signs of toxicity in testis and spleen (Fig. 2C) as well as in the thymus and popliteal lymph node, showing decreased cellularity, but not in the retina, lung, brain, heart, skin, or skeletal muscle (data not shown). In the liver, slight cytoplasmatic vacuolization was observed, and in the kidneys, the glomeruli seemed slightly dilated and there was occasional bleeding in descending ducts and collecting ducts. The plasma, kidney, or liver biochemical markers were, however, not changed compared with control mice, suggesting that the organ damage was not extensive. In the small intestine (jejunum), necroses were observed in the bottom of the crypts, and in the large intestine (colon), there was a highly increased occurrence of mucus-producing cells and some apoptotic epithelial cells were also observed. The mice showed decreased activity, but no signs suggestive of an adverse intestinal effect (diarrhea).

In the hematology experiment, a slightly lower dose of APO866 (40 mg/kg ×2/d) was included in addition to the 60 mg/kg ×2/d dose to avoid early toxic death and enable blood sampling on the last treatment day. A reduction in the thrombocyte count compared with vehicle-treated mice was found in the mice treated with 60 mg/kg ×2/d (61%; Fig. 2B) and a severe drop in lymphocyte counts of 80 and 98%, respectively, at the 40 and 60 mg/kg dose was also observed (Table 1).
We examined the mRNA expression of the rate-limiting enzymes in the NAD synthetic pathways using nicotinamide and nicotinic acid as precursors in a range of tissues (Fig. 3A). We found that the more sensitive tissues, testis and lymphatic tissues (peripheral lymphocytes, spleen, thymus, and popliteal lymph node) have a relatively low expression of NAPRT compared with Nampt (Fig. 3B), which indicates dependency on Nampt activity for NAD production. A similar expression pattern was observed in rats (data not shown). However, a low NAPRT/Nampt ratio is not in itself an indicator for toxicity reactions as can be seen from striated muscle (heart and skeletal muscle), retina, brain, and lung tissue in which no toxicity is observed despite relatively high Nampt expression compared with NAPRT. In addition, tissues such as the mucosa of jejunum having a relatively high expression of Nampt were affected by a supralethal dose of APO866, which was evident in the proliferating jejunal crypt cells.

Effect of nicotinic acid on the maximally tolerated dose of APO866 in mice

We speculated that an oral supplement of nicotinic acid could protect against APO866 toxicity related to the mechanism of action of the drug and drug-induced death. The maximally tolerated dose (MTD) of APO866 in B6D2F1 mice is 15 mg/kg twice daily (data not shown). We treated mice with 60 mg/kg twice daily on 4 consecutive days combined with vehicle or 50 mg/kg/d nicotinic acid orally, a dose well below the corresponding levels used in clinical settings in cardiovascular disease in humans. Six of seven (86%) of the mice in the APO866 monotherapy group died on day 3 or 4. In comparison, all mice receiving the combination of APO866 and nicotinic acid survived until day 27 in which the experiment was terminated (Fig. 2A). In a second study in which a lower (40 mg/kg ×2/d) dose of APO866 was also tested, we examined hematology parameters: The thrombocyte count was significantly decreased in mice given APO866 alone; right column, mice given APO866 + nicotinic acid. Cotreatment with nicotinic acid resulted in a normalization of tissue architecture with less APO866-induced dead spermatocytes/disturbed spermiogenesis and less connective tissue in the spleen, which also had an amelioration of spleen follicle atrophy in tissues of combination-treated mice. Bars, 140 μm.
treated with 60 mg/kg ×2/d and cotreatment with nicotinic acid reversed toxicity and increased thrombocyte counts to a level comparable with control mice (Fig. 2B). The blood lymphocyte count significantly decreased in all treated groups compared with control mice but increased when nicotinic acid was supplied orally compared with animals receiving APO866 monotherapy (Table 1). The blood neutrophil counts were not affected by APO866 treatment and combination with nicotinic acid did not affect the counts either (the mean neutrophil count was 0.9 × 10⁸/L in the control group and ranged from 0.8–1.8 × 10⁸ cells/L in the four APO866-treated groups, showing no difference in levels; data not shown). Amelioration of histologic signs of toxicity in testis and lymphoid tissues (spleen) was found in mice cotreated with nicotinic acid (Fig. 2C), and in the slightly affected tissues of liver, kidney, and large and small intestinal mucosa described in the previous section, the signs of tissue affection were not found in groups treated with nicotinic acid together with APO866.

Protection of cells by nicotinic acid against APO866-induced death in vitro

To determine whether the HepG2 cell line is unique with respect to its lack of nicotinic acid use, we examined whether nicotinic acid could rescue cells from APO866-mediated cell death in vitro in a panel of cancer cell lines of diverse origins. All the cell lines were sensitive to APO866 with LD₅₀ values of 1 to 13 nmol/L in clonogenic assay with continuous treatment of APO866 (Table 2). A2780, NYH, and PC-3 were not protected from cell death by the presence of 100 μmol/L nicotinic acid in the media. In contrast, ML-2, HCT-116, and A431 displayed an increase of LD₅₀ values of >40- to 104-fold when nicotinic acid was added to the media compared with control (Table 2). Studies of cotreatment with nicotinic acid and CHS-828 showed similar results (data not shown). Addition of 100 μmol/L nicotinic acid to the medium had in itself no effect on the survival of the cells. The overexpression of Nampt has previously been shown to induce resistance toward standard chemotherapeutics such as alkylating drugs and topoisomerase inhibitors (32). As this effect could be due to increased NAD synthesis, one concern could be that nicotinic acid supplement would impair the effect of standard chemotherapeutics. However, we observed no increase in the LD₅₀ values of etoposide, a topoisomerase II inhibitor, or cisplatin, an alkylating drug, in treatment of either ML-2 or A2780 cells upon addition of 100 μmol/L nicotinic acid (data not shown).

Table 1. Blood lymphocyte counts in mice dosed with 40 or 60 mg/kg APO866 i.p. ×2/d for 4 d

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mean (SD)</th>
<th>t tests</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicles</td>
<td>6</td>
<td>5.99 (0.72)</td>
<td>vs</td>
<td></td>
</tr>
<tr>
<td>APO866 40 × 2 + vehicle</td>
<td>7</td>
<td>1.25 (0.50)</td>
<td>† vs</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>APO866 40 × 2 + NA 50</td>
<td>7</td>
<td>4.37 (0.85)</td>
<td>† vs</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>APO866 60 × 2 + vehicle</td>
<td>6</td>
<td>0.11 (0.12)</td>
<td>† vs</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>APO866 60 × 2 + NA 50</td>
<td>7</td>
<td>0.51 (0.19)</td>
<td>† vs</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

NOTE: Treatment with APO866 decreased lymphocyte counts in all groups compared with the vehicle group (P < 0.001, t tests). However, combining with nicotinic acid rescued a fraction of the lymphocytes at both APO866 dose levels (P < 0.005), although lymphocyte counts were very low in mice dosed with 60 mg/kg ×2/d. Abbreviations: SD, standard deviation; NA, nicotinic acid. *P < 0.0001. †P < 0.005.
Effects of the combination of nicotinic acid and APO866 in the treatment of A2780 and ML-2 xenografts

We investigated the in vivo effect of combination treatment with nicotinic acid and high-dose APO866 on nude mice with xenograft tumors of A2780 and ML-2 cells. APO866 was given twice daily in weekly 4-day cycles for 2 weeks, starting when tumors had reached a size of 100 mm³, and the time used by each mouse to grow its tumor to a size of 800 mm³ was recorded. Treatment of A2780 xenografts with the MTD dose of 15 mg/kg APO866 i.p. gave a significant increase of life span (% ILS) of 50% compared with the mice treated with nicotinic acid alone (P = 0.009; Fig. 4A). The survival in mice treated with nicotinic acid alone was not different from the survival in vehicle-treated control mice in previous studies with the two xenografts, and thus, this group was used as the control group in both xenograft experiments. Cotreatment of 50 mg/kg APO866 i.p. and 50 mg/kg nicotinic acid orally resulted in an increase in ILS of 180%, a significant improvement compared with standard treatment with the MTD (P = 0.0002). We did not include a control group treated with 50 mg/mL APO866 monotherapy as this would result in severe toxicity or death in the xenografted mice. Interestingly, although A2780 cells are unable to be rescued from APO866-induced death in vitro, cotreatment with nicotinic acid negates the antiproliferative effect of standard dose 15 mg/kg APO866 treatment in vivo. Treatment of ML-2 xenografts with APO866 alone is very effective with complete elimination of the tumors before day 10, resulting in 100% survival for >60 days (Fig. 4B). If nicotinic acid is coadministered, the antiproliferative effect of the same APO866 dose is partially

Table 2. In vitro protection from APO866 by nicotinic acid

<table>
<thead>
<tr>
<th>Cell line</th>
<th>APO866 LD₅₀ in CA (nmol/L)</th>
<th>NA rescue effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML-2</td>
<td>4.8 ± 1.0</td>
<td>+ (&gt;500)</td>
</tr>
<tr>
<td>HCT-116</td>
<td>9.0 ± 3.2</td>
<td>+ (&gt;500)</td>
</tr>
<tr>
<td>A431</td>
<td>11.6 ± 5.5</td>
<td>+ (&gt;500)</td>
</tr>
<tr>
<td>NYH</td>
<td>1.8 ± 0.2</td>
<td>(1.5 ± 0.1)</td>
</tr>
<tr>
<td>PC-3</td>
<td>4.8 ± 3.0</td>
<td>(5.5 ± 1.4)</td>
</tr>
<tr>
<td>A2780</td>
<td>3.3 ± 1.7</td>
<td>(5.34 ± 0.2)</td>
</tr>
</tbody>
</table>

NOTE: Rescue effect defined as ≥40-fold increase of LD₅₀ to APO866 treatment. No rescue effect defined as <2-fold increase in LD₅₀. LD₅₀ values for combination treatment with nicotinic acid are shown in nmol/L in parentheses. All LD₅₀ values are displayed with SDs.

Abbreviations: NA, nicotinic acid; CA, clonogenic assay.
negated and most tumors persist. The survival in mice given the combination of 15 mg/kg APO866 and nicotinic acid was not significantly different from controls \( (P = 0.14) \), and increasing the dose of APO866 to 50 mg/kg combined with nicotinic acid did not improve the effect on tumor size in a significant way compared with this group \( (P = 0.97) \). However, 50 mg/kg treatment with APO866 and nicotinic acid reduced tumor growth when compared with nontreated controls \( (P = 0.002) \). The overall body weight did not differ significantly between the study groups during the \textit{in vivo} experiments (data not shown).

**NAPRT expression is a marker for nicotinic rescue in cancer cells**

The rationale for nicotinic acid rescue from APO866 cytotoxicity is the utilization of the alternative NAD synthesis pathway with nicotinic acid as a substrate. We examined the mRNA expression of NAPRT, the enzyme catalyzing the first step of conversion of nicotinic acid, in the panel of cancer cell lines. We find that the expression of NAPRT is highest in cell lines rescued with nicotinic acid and lowest in cell lines not protected from APO866 (Fig. 5). Thus, the expression in ML-2 cells is 24-fold higher than what is found in A2780 cells. We found a similar difference in expression in tissue from xenograft tumors of A2780 and ML-2 cells (Fig. 5).

**Discussion**

A persisting challenge in cancer therapy is to achieve a sufficient therapeutic window between inducing tumor cell death and cytotoxic effects in somatic cells to obtain optimal anticancer treatment. Previously, it has been shown how leucovorin administration can increase the therapeutic potential of methotrexate through rescue of the normal, sensitive tissues. The cytotoxic effect of APO866 on tumor cells is due to its reduction of cellular NAD levels. We examined the possibility of activating an alternative NAD synthesis pathway by supplementation of nicotinic acid to protect against toxicities and overdose-related death following Nampt inhibitor treatment \textit{in vivo} in mice. We found NAPRT, the first step in NAD synthesis from nicotinic acid, is expressed at low concentrations in all studied mouse tissues with higher levels in liver, kidney, heart, and both the mucosa and muscular layers of the intestine as measured by mRNA. The expression pattern we find correlates well with what has been published (21). We also find that the NAPRT/Nampt ratio is low in tissues that are sensitive to toxicity from treatment with APO866. However, not all tissues with a low NAPRT/Nampt ratio are sensitive to APO866 toxicity. This could also be due to differences in the distribution of nicotinic acid perhaps due to sufficient blood perfusion. Notably, among tissues with a low NAPRT/Nampt ratio, skeletal muscle stands out with a surprisingly high expression of Nampt, which we have previously not observed at the protein level when comparing muscle and liver.\(^3\) We believe that this high mRNA expression may be in reserve for strenuous exercise in which glycolysis is responsible for a greater part of the ATP generation.

We find that nicotinic acid protects against death even at four times the normal MTD of APO866 in mice if administered on the same days as APO866. We also observe a general amelioration of the signs of toxicity from APO866 treatment with coadministration of nicotinic acid. Apparently, distribution of orally supplemented nicotinic acid is sufficient to protect APO866-sensitive tissues. In this respect, nicotinic acid can be used as an antidote for APO866 toxicity caused by accidental overadministration. This has recently also been found for another Nampt inhibitor (33), GMX1777, indicating that the protective effect of nicotinic acid could be effective against Nampt inhibitors in general. In addition, these results indicate that the toxicities observed with APO866 are mostly target specific. This may make APO866 suitable for combination treatments. Thus, APO866 has shown great potential for combination with treatment that induces DNA damage (34).

When the mechanism of action of APO866 was discovered, the lack of protective effect of nicotinic acid by HEPG2 cells was perceived as a surprising but isolated case (9). However, we find that in a panel of six cancer cell lines of varying origin, the ability to use nicotinic acid to synthesize NAD to protect against Nampt inhibitors is only found in 50% of the cell lines. This opens the possibility of exploiting the fact that when cotreating with nicotinic acid, APO866 can be used at doses four times higher than the normal MTD. Thus, we show a dramatic increase in %ILS in an

\(^3\) U.H. Olesen, unpublished results.
A2780 xenograft mouse model when cotreating with nicotinic acid and high doses of APO866. This indicates an opportunity for better treatment in tumors unable to utilize nicotinic acid. It should be noticed that at lower concentrations of APO866, nicotinic acid displays some protection although A2870 cells are unable to use it. This may be due to the local or systemic conversion of nicotinic acid to nicotinamide and nicotinamide mononucleotide, increasing the circulating concentrations of these metabolites sufficiently to interfere with the APO866 treatment. Another possibility is that nicotinic acid affects the metabolism of APO866. APO866 is metabolized by CYP3A4, which is inhibited by nicotinic acid at high concentrations (K₀ = 114 mmol/L; ref. 35). However, it is unlikely that tissue concentrations of nicotinic acid reach this level. We also show that in xenografts of ML−2, the cotreatment with nicotinic acid completely abolishes the antiproliferative effects of APO866. This is seen even at high concentrations of APO866. This emphasizes the need for a marker for protection by nicotinic acid. Logically, the ability of cells to utilize nicotinic acid could be due to the expression levels of enzymes involved in the synthesis of NAD. NAPRT is the first step of NAD synthesis from nicotinic acid, and the enzyme is not inhibited by APO866. We found the expression of NAPRT at the mRNA level to correlate with protection by nicotinic acid in cancer cell lines. Furthermore, there is no reexpression of NAPRT in an in vivo setting using xenografted tumors. We therefore propose that the expression of NAPRT in the tumor to be treated may be used as a marker for identifying cancers suitable for the combination treatment with high-dose APO866 and nicotinic acid. We are currently working on developing more robust protein-based assays, Western blotting, and immunohistochemistry, to identify tumors suitable for this combination treatment. The concept of using nicotinic acid to increase the treatment potential of Nampt inhibitors in NAPRT-deficient tumors is supported by a very recent publication (36). Here, the authors suggest a combination of nicotinic acid and Nampt inhibitors in glioblastomas, neuroblastomas, and sarcomas in which a large proportion of tumors are insensitive to rescue by nicotinic acid.

In summary, the increased dose tolerance of APO866 with nicotinic acid and the possibility of identifying tumors not protected from APO866 by nicotinic acid may increase the potential for Nampt inhibitor treatment in a subgroup of cancer patients with deficient tumor metabolism of nicotinic acid, thus enabling a high-dose with normal tissue rescue approach.

Disclosure of Potential Conflicts of Interest

All authors are fully or partially employed by TopoTarget A/S, and all authors own stock in TopoTarget. TopoTarget A/S has licensed the rights to APO866 and has provided the drugs used for this study.

Acknowledgments

We thank Annette Nielsen, Anita Johansen, and Anne Jørgensen for the technical assistance.

Grant Support

TopoTarget A/S and Rigshospitalet. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 12/07/2009; revised 03/23/2010; accepted 03/24/2010; published OnlineFirst 06/01/2010.

References

adenine dinucleotide biosynthesis inhibitor. Invest New Drugs 2008; 26:45–51.


A Preclinical Study on the Rescue of Normal Tissue by Nicotinic Acid in High-Dose Treatment with APO866, a Specific Nicotinamide Phosphoribosyltransferase Inhibitor

Uffe Høgh Olesen, Annemette V. Thougaard, Peter Buhl Jensen, et al.

*Mol Cancer Ther* 2010;9:1609-1617. Published OnlineFirst June 1, 2010.

Updated version Access the most recent version of this article at: doi:10.1158/1535-7163.MCT-09-1130

Cited articles This article cites 34 articles, 8 of which you can access for free at: http://mct.aacrjournals.org/content/9/6/1609.full#ref-list-1

Citing articles This article has been cited by 7 HighWire-hosted articles. Access the articles at: http://mct.aacrjournals.org/content/9/6/1609.full#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.