Abstract. Background: The differential sensitivity of some tumors to paclitaxel and docetaxel raises questions regarding the specific mechanisms responsible for the discrepant sensitivity to these taxanes. Materials and Methods: Docetaxel and paclitaxel were evaluated and compared at maximum tolerated doses (MTD) and 0.5 MTDs against the human pediatric tumor xenograft models SK-N-MC and IMR32 (neuroblastoma), RH1 and RH30 (rhabdomyosarcoma) and KHOS/NP (osteosarcoma), with 8-10 animals per group. The drug effects on the expression of the β-tubulin isotypes, Bcl-2, Bax, Bcl-XL and proteomic profiles were evaluated by immunobloting and SELDI mass spectrometry in tumor xenografts dosed at 0.5 MTDs. Results: At MTDs, docetaxel was superior in neuroblastoma and osteosarcoma, while paclitaxel was more active in the rhabdomyosarcoma models. Docetaxel showed remarkable efficacy in KHOS/NP even at 0.5 MTD. The drugs had significantly different, yet highly heterogeneous effects on the tumor levels of βT-tubulin (RH30), βMT-tubulin (IMR32, KHOS/NP, RH1), Bax (IMR32, SK-N-MC) and Bcl-XL (KHOS/NP). In contrast, six protein species identified by proteomic profiling were consistently and differentially regulated by docetaxel and paclitaxel in all KHOS/NP xenografts. Conclusion: Anticancer activity showed no apparent correlation with drug effects on β-tubulin isotypes and apoptotic markers. The mass spectrometry approach has potential for the discovery of proteomic biomarkers for drug sensitivity.

The taxanes paclitaxel and docetaxel belong to one the most powerful classes of anticancer agents. Although their activity is attributed in part to the shared ability to stabilize microtubules, significant differences in efficacy have also been reported. Docetaxel compared to paclitaxel used as monotherapy or in combination with other chemotherapeutic agents demonstrated a more favorable benefit-to-risk ratio. Some of the differences are apparently due to specific effects on cell cycle phases, liver metabolism by cytochrome P-450 forms, affinity for microtubules, induction of Bcl-2 phosphorylation, retention time in tumor cells and pharmacokinetics (1).

Little is known about predictive biomarkers for tumor sensitivity to docetaxel and paclitaxel. Some reports from clinical studies linked β-tubulin isotype expression with docetaxel-resistant phenotypes (2), while others argued against it (3). Bcl-2 levels were shown to correlate with tumor response to combination chemotherapy with docetaxel (4, 5) and, taken together with Bax, have prognostic implications. While a decrease in Bcl-2 may indicate a pathological complete response, no relationship was found between the Bax levels and response to therapy (4). Bcl-2 and Bax expressions did not predict response to docetaxel in combination with other drugs (6). Bcl-XL expression was linked with resistance to paclitaxel (7).

The discovery of predictive biomarkers for drug sensitivity in preclinical and clinical studies increasingly relies on methods compatible with screening of entire tumor or host proteomes (8-10). Despite considerable progress in the application of proteomics to early disease detection, drug-induced changes in tumor proteome have not been extensively explored as biomarkers for drug response. The goal of this study was to evaluate the efficacy of docetaxel and paclitaxel in a head-to-head comparison against human pediatric tumor models in vivo and to identify predictive biomarkers for docetaxel and paclitaxel sensitivity in tumor xenografts. An attempt was made to correlate preclinical end-points of drug efficacy, such as tumor growth inhibition, with drug effects on the expression of known proteins.

Key Words: Docetaxel, paclitaxel, xenograft, pediatric cancer, biomarker.
implicated as potential biomarkers for taxane activity, as well as to search for novel biomarkers in tumor tissue that might be predictive of drug response.

Materials and Methods

Maximum tolerated dose determination. Female nude mice (nu/nu) at 5–6 weeks of age, weighing approximately 20 g (Harlan, Indianapolis, IN, USA), were randomized into seven groups of five mice. Initial doses were given on Day 1 and continued once weekly for 4 weeks. Docetaxel (Aventis Pharmaceuticals, Schiltigheim, France) was administered intravenously (i.v.) at doses of 12.5, 25 and 50 mg/kg. Paclitaxel (Zenith Goldline, Miami, FL, USA) was administered intraperitoneally (i.p.) at doses of 20, 30, 40 and 60 mg/kg. The mice were weighed twice weekly and observed daily for viability.

Drug treatment. Female nude mice (nu/nu) as above were implanted subcutaneously (s.c.) by trocar using human pediatric cell lines: SK-N-MC and IMR-32 neuroblastoma and KHOS/NP osteosarcoma (American Type Culture Collection, Manassas, VA, USA), and RH1 and RH30 rhabdomyosarcoma (a gift from Peter Houghton at St. Jude’s Hospital, Memphis, TN, USA). When the tumors had reached approximately 66 mg (SK-N-MC, RH30), 60 mg (KHOS/NP), 63 mg (RH1), or 78 mg (IMR32), the mice were pair-matched into treatment and control groups (all eight to ten animals), ear-tagged (Day 1) and were followed individually throughout the experiment. Initial doses were given on Day 1 with saline control. The drug and docetaxel were administered i.v. at 10 mg/kg and 20 mg/kg, respectively, once a week for 4 weeks (or 3 weeks for IMR32). Paclitaxel (UDL Laboratories, Rockford, IL, USA) was administered i.p. at 15 mg/kg and 30 mg/kg once a week for 4 weeks (or 3 weeks for IMR32). The experiment was terminated on Day 28 when the control group tumor size had reached an average of 1 g. The mice were weighed, sacrificed and their tumors were excised, weighed and then snap-frozen for further biomarker analysis. The mean tumor weights per group were calculated to determine tumor growth inhibition (TGI) for each group (11).

Immunoblots. Sample processing including pulverization of frozen tumor xenografts under liquid nitrogen, homogenization in osmotic lysis buffer, protein assays, gel electrophoresis, protein detection by enhanced chemiluminescence and quantitative analysis were performed as described (12). Immunoblots were probed with anti-β-actin ascites IgM (Oncogene Research Products, Boston, MA, USA), anti-β-tubulin ascites IgG1 cross-reactive with all β-tubulin subtypes (Sigma Chemical Co., St. Louis, MO, USA), anti-βII tubulin IgG2b (cross-reactive with β-tubulin I and II subtypes, anti-βIII tubulin IgG2b (InnoGenex, San Ramon, CA, USA), anti-Bcl-2 IgG1 that recognizes unphosphorylated and phosphorylated antigen, anti-Bcl-XL and anti-Bax (Dako, Carpinteria, CA, USA). The statistical analysis of protein expression in the immunoblots was assessed with Excel; p values <0.05 were considered significant.

Surface-enhanced ligand desorption-ionization (SELDI) mass spectrometry. Tumor lysates adjusted to a total protein concentration of 1 mg/mL were processed robotically, applied onto IMAC-Cu, H4, SAX, and WCX ProteinChip arrays and read in a SELDI PBS-II system (Ciphergen Biosystems, Fremont, CA, USA). The sample preparation and SELDI assays, instrument calibration, spectra collection parameters, peak clustering and data analysis followed established protocols (12).

Results and Discussion

Based on the MTD responses in non-tumored animals (data not shown), the doses of docetaxel chosen for the tumor xenograft studies were 10 and 20 mg/kg, while the doses of paclitaxel were 15 and 30 mg/kg, corresponding to 0.5 MTD and MTD, respectively. Significant end-points of these studies included final tumor weight, TGI determination, partial tumor responses (PR), complete tumor responses (CR) and mean weight loss. The animals experienced acceptable toxicity with both compounds and in most models showed no toxicity. No toxic deaths were observed with either compound. As shown in Figure 1, docetaxel demon-strated superior antitumor activity compared to paclitaxel in the SK-N-MC and KHOS/NP lines. The SK-N-MC model showed the best tumor growth inhibition (98.7%) when administered docetaxel at 20 mg/kg. There were 3/10 PR and 6/10 CR. The KHOS/NP tumor xenograft showed the best TGI (91.8%) when administered docetaxel at 20 mg/kg, however, there was only 1/9 PR and 0/9 CR in this model. Paclitaxel demonstrated superior activity compared to docetaxel in the RH1 and RH30 cell lines. The taxanes were equally active in IMR32, where all animals experienced CR in the high-dose groups. These results indicate a statistically significant, high degree of tumor growth inhibition for docetaxel. It was more or equally active than paclitaxel in the osteosarcoma and neuroblastoma models, while paclitaxel was more active against rhabdomyosarcoma.

Although major differences between docetaxel and paclitaxel were observed at MTD, many tumors regressed and no tumor tissue was available for biomarker analysis. Therefore, the drug effects were evaluated in tumor tissue extracts from the animals dosed at half MTDs. At these doses, lower non-specific toxicity and more specific biomarker effects would be expected. We investigated the drug effects on β-tubulin isotypes and the apoptotic proteins Bel-2, Bax and Bcl-XL, since these proteins are implicated both as drug targets and potential markers for taxane efficacy. The immunodetection of protein targets in individual tumors and a graphical representation of tumor sizes are presented in Figure 2. Heterogeneity in tumor sizes was evident in the controls and treatment groups. The staining for β-actin was relatively uniform and consistent with a normalized protein load per lane, but biomarker expression showed large heterogeneity reminiscent of wide ranges in tumor sizes. The baseline levels of β-tubulin isotypes were comparable in most models, except for low expression in RH1. The taxanes significantly exerted different effects on the expression of βI-tubulin in RH30 (p = 0.026) and βII-tubulin in IMR32 (p = 0.0054), KHOS/NP (p = 0.038) and RH1 (p = 0.050). The expression of Bax was comparable and
relatively uniform in all models except for RH1. Significant differences between paclitaxel and docetaxel on Bax were noted in IMR32 ($p=0.026$) and SK-N-MC ($p=0.027$), but there were no apparent differences in drug effects on Bel-2 expression. Evidence of differential regulation of Bcl-XL expression was seen in KHOS/NP ($p=0.049$).

We wanted to determine if mass spectrometry protein profiling could identify protein species whose expression correlated with the differential effects of the taxanes in pediatric tumor models. Because of significant and discrepant activities of docetaxel versus paclitaxel in the SK-N-MC and KHOS/NP models, finding unique protein biomarkers for docetaxel in these xenografts was of particular interest. We looked for unique protein species that are significantly up- or down-regulated by docetaxel when compared to paclitaxel in 100% specimens in a given drug treatment group. The analysis of proteomic patterns generated with four ProteinChip arrays revealed no biomarkers in RH1, RH30 and IMR32 that would meet the above criteria. In SK-N-MC, five proteins were found to meet the requirements but the $p$ values for the differential expression were on the borderline of significance ($p=0.05$).

By far the greatest differences between docetaxel and paclitaxel were found in all KHOS/NP tumor extracts. Six protein peaks regulated in an opposite manner by the two taxanes showed significant ($p<0.05$) differences in expression, not only with respect to controls but also to each other (Table I). One protein peak was found in the H4 and IMAC-Cu arrays and two peaks were found in the SAX and WCX arrays. Respective values for mass-to-charge ratio (M/Z) standard deviations and percentage coefficient of variation (%CV) were under 7 and 0.07%. Representative trace and gel views of the M/Z 10,225 peak are presented in Figure 3. Over two-fold differences in signal intensity of this protein were measured in docetaxel versus the paclitaxel-treated KHOS/NP tumor lysates. Scatter plots further underscore

Figure 1. Antitumor activity of docetaxel and paclitaxel against human pediatric cancer models in vivo. The compounds were administered i.v. or i.p., respectively, following the schedule and per injection dose levels as indicated in Materials and Methods. Mean relative tumor weights (mg) measured through the course of drug treatment are reported for each tumor model. Filled circles; vehicle control, open squares; docetaxel 10 mg/kg, filled squares; docetaxel 20 mg/kg, open triangles; paclitaxel 15 mg/kg, filled triangles; paclitaxel 30 mg/kg.
The differential activities of docetaxel and paclitaxel observed in preclinical and clinical studies cannot be explained solely on the basis of pharmacological data. The reasons for the intrinsic resistance of some patients to taxane therapies are under intensive scrutiny and there is substantial interest in identifying prognostic biomarkers of drug efficacy for individual patients. The results of our in vivo study in human pediatric cancer models raise the question as to the specific mechanisms responsible for diverse and discrepant sensitivity between these two taxanes. The results indicate a statistically significant, high degree of tumor growth inhibition of pediatric human tumor xenografts by docetaxel and paclitaxel, with marked model-dependent differences. Docetaxel was more active in the osteosarcoma and neuroblastoma, while paclitaxel showed better activity against the rhabdomyosarcoma models. The taxanes exerted significantly different effects on the expression of the β-tubulin isotypes and apoptotic markers, but the biomarker levels showed large variation within

Table I. Biomarkers for docetaxel in KHOS/NP.

<table>
<thead>
<tr>
<th>Array</th>
<th>M/Z</th>
<th>p</th>
<th>M/Z STD</th>
<th>M/Z % CV</th>
<th>Docetaxel</th>
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<tbody>
<tr>
<td>IMAC</td>
<td>8262.6</td>
<td>0.0380</td>
<td>1.94</td>
<td>0.02</td>
<td>↑</td>
</tr>
<tr>
<td>H4</td>
<td>10225.3</td>
<td>0.0128</td>
<td>6.57</td>
<td>0.06</td>
<td>↓</td>
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<tr>
<td>SAX</td>
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<td>0.0114</td>
<td>5.82</td>
<td>0.07</td>
<td>↑</td>
</tr>
<tr>
<td>WCX</td>
<td>10380.7</td>
<td>0.0222</td>
<td>2.46</td>
<td>0.02</td>
<td>↑</td>
</tr>
<tr>
<td>H4</td>
<td>3660.7</td>
<td>0.0412</td>
<td>1.18</td>
<td>0.03</td>
<td>↑</td>
</tr>
<tr>
<td>WCX</td>
<td>4211.8</td>
<td>0.0412</td>
<td>1.35</td>
<td>0.03</td>
<td>↓</td>
</tr>
</tbody>
</table>

M/Z, mass-to-charge ratio; % CV, percentage coefficient of variation.
treatment groups and did not apparently correlate with model-dependent differences in drug activities. In contrast, proteomic profiling identified protein species to be consistently and differentially regulated by the taxanes in all xenografts in tumor models that showed substantial response to docetaxel at half MTD. Our current efforts are devoted to the identification of these proteins and the validation of the findings in clinical specimens.

To date, biomarker discovery has primarily been focused on disease markers (13-15), while less attention has been devoted to predictive biomarkers of drug response, especially in tumor tissue. Pharmacoproteomic approaches utilizing mass spectrometry are considered of value in correlating chemosensitivity with specific drug effects on cellular proteome. In vivo models offer a unique advantage for discovery, identification and characterization of pharmacoproteomic biomarkers. Validation of such biomarkers in clinical studies should bring us closer to the concept of personalized therapy.

References


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