Bioanalysis, Pharmacokinetics, and Pharmacodynamics of the Novel Anticancer Drug Paclitaxel (Taxol)

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Several high-performance liquid chromatographic assays have been reported for the analysis of paclitaxel (Taxol; Bristol-Myers Squibb Company, Princeton, NJ) in biologic matrices. The recently developed method of using solid-phase extraction as a sample pretreatment is preferred, as it is the most sensitive assay and is also capable of detecting metabolites in the plasma of treated patients. The pharmacokinetics of paclitaxel (Taxol; Bristol-Myers Squibb Company, Princeton, NJ), administered in different doses and schedules, has been studied using this method. After cessation of the infusion, a three-phasic decay of plasma concentrations has been found. There are indications for nonlinear pharmacokinetics when paclitaxel is administered as a short infusion and at higher doses. Different metabolic products of paclitaxel have been detected in the plasma of treated patients. Three hydroxylated metabolites have been identified so far. Pharmacokinetics have been related with pharmacodynamics. Neuropathy, mucositis, and leukopenia correlate with pharmacokinetic parameters such as area under the plasma concentration-time curve and steady-state paclitaxel levels. The hematologic toxicity of paclitaxel also has been modelled with a sigmoidal maximum effect equation with the time spent above the biologically active threshold concentration of 0.1 μmol/L as a pharmacokinetic parameter.

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Based on the clinical results obtained so far with the novel antitumor drug paclitaxel (Taxol; Bristol-Myers Squibb Company, Princeton, NJ) (Fig 1), there is every indication that this agent will have a major impact on cancer chemotherapy in forthcoming years. In particular, its efficacy in otherwise refractory solid tumors (eg, platinum-resistant ovarian carcinoma and breast carcinoma) has been impressive. Its unlikely history, including its natural origin, problems associated with its pharmaceutical formulation, its unique mechanism of action, and concern about the initial high incidence of allergic reactions associated with its administration have been adequately reviewed elsewhere. Although its place in cancer therapy is evolving rapidly, a lot of pharmacologic questions concerning, for example, drug interactions and metabolism remain unanswered. More insight into the pharmacokinetics of paclitaxel, in particular with relation to its pharmacodynamics, may help efforts to find its optimum dose and schedule. This paper addresses issues in this field, including bioanalysis, pharmacokinetics, and pharmacodynamics.

**BIOANALYSIS**

It is clear that before a pharmacokinetic study with a new chemical entity can commence a reliable and validated method must be available with sufficient sensitivity to quantitate low levels of the agent and potential metabolites in different biologic matrices. During the development of an assay for paclitaxel, its chemical instability and unfavorable UV absorbance characteristics hampered efforts to obtain an appropriate method for clinical pharmacologic research. So far, high-performance liquid chromatography (HPLC) has been the methodology of first choice. Protein precipitation followed by solid-phase extraction (SPE), liquid-liquid extraction, also followed by SPE, and SPE alone have been used as sample pretreatment procedures. These procedures are relatively comprehensive, which is necessary to obtain sufficiently clean extracts for injection into the chromatograph with UV detection at a low wavelength (approximately 230 nm). Reversed-phase chromatography using C8 or C18 columns and methanol/acetonitrile/buffer mixtures as mobile phase have become standard in paclitaxel bioanalysis. The UV spectrum of the drug displays a maximum wavelength of approximately 230 nm, with no absorptivities of any analytical importance at higher wavelengths; the analyte has no fluorescence or electrochemical properties that can be exploited for HPLC detection. The assays developed so far, with the exception of the method designed by Lognecker et al, do not use an internal standard. The precision and accuracy validation results are, however,

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within accepted limits and do not require the use of an internal standard.

For docetaxel (Taxotere, Rhone-Poulec Rorer, Antony Cedex, France), two methods have been reported that use paclitaxel as the internal standard after SPE as sample pretreatment. In principle, these assays also can be used for paclitaxel analysis, exploiting docetaxel as the internal standard, although they have not been validated for this purpose. It is important to note that the reported assays for paclitaxel differ substantially in terms of sensitivity, ranging from 6 ng/mL to 85 ng/mL, and capability to detect metabolites in plasma. While Rizzo et al. found one extra peak in the chromatograms of a plasma and urine sample of a treated patient, no metabolic products could be detected with the other assays. The most sensitive assay, reported recently by Willey et al., has a detection limit of 6 ng/mL (7 nmol/L) using only a 0.5 mL sample. The strength of the assay is that it can also detect metabolites in patients’ plasma samples. Huizing et al. used this assay and reported 11 potential metabolic products in a pharmacokinetic study in which patients were given paclitaxel in 3- or 24-hour infusions and doses of 135 and 175 mg/m². In summary, the assay involves SPE with Cyano Bond Elut (Berton Scientific, Rotterdam, The Netherlands) columns (1 mL), first conditioned with methanol and 0.01 mol/L ammonium acetate buffer pH 5.0. The sample (0.5 mL plasma diluted with 0.5 mL 0.2 mol/L ammonium acetate buffer pH 5.0) is applied onto the SPE column. After washing, in consecutive order with 2 mL 0.01 mol ammonium acetate pH 5.0, 2 mL methanol-0.01 mol/L ammonium acetate pH 5.0 (2:8, v/v), and 1 mL hexane, the analytes are eluted with 2 mL of a mixture of acetonitrile-triethylamine (1:00:1, v/v). After evaporation of this solvent and reconstitution of the residue with the mobile phase, an aliquot is injected into the HPLC system. In Fig 2, a typical HPLC chromatogram is depicted from a plasma sample of a patient treated with a 3-hour infusion of 175 mg/m² paclitaxel. In this case, four metabolites appeared in the chromatogram. These peaks were absent in both the blanks and in the pharmaceutical product. They were further identified as taxanes by the use of on-line photo diode array detection, in which all the peaks exhibited the typical taxane UV characteristics with a maximum wavelength of around 230 nm.

PHARMACOKINETICS

Most pharmacokinetic data on paclitaxel have been gathered during phase I clinical trials in which the drug was given as a 1-hour infusion (dose range, 15 to 30 mg/m²), 6-hour infusion (dose range, 15 to 275 mg/m²), or 24-hour infusion (dose range, 110 to 390 mg/m²). Peak plasma concentrations are achieved at the end of infusion and are generally above the levels (0.05 to 0.1 μmol/L) that have been shown to affect microtubule metabolism. After cessation of the infusion, plasma disappearance of paclitaxel was generally biphasic, indicating both a distribution and elimination phase. However, by using a more
sensitive assay, an extra disposition phase appeared and a three exponential decay gave a better description of the postinfusion curve than did a bi-phasic description. The half-lives of these three phases were $T\frac{1}{2}(a)$: 0.2 hour; $T\frac{1}{2}(b)$: 1.9 hour; and $T\frac{1}{2}(c)$: 20.7 hour, indicating that the drug circulates for a prolonged period of time. Early phase I studies with paclitaxel indicated a linear pharmacokinetic behavior of the drug with the clearance independent of dose and, consequently, a linear relationship between area under the plasma concentration time curve (AUC) and dose. The current interest in other schedules and doses, however, has shed more light on the pharmacology of the drug with indications for nonlinear pharmacokinetics or saturable elimination processes. When paclitaxel is given as a 24-hour infusion, a linear relationship between dose and AUC is indicative for linear kinetics. Area under the curve values derived from five different studies have been combined in Fig 3, whereby a straight line is obtained ($r = 0.88$) with slope $1/CL$, yielding a clearance rate of 20.0 l/hr/m$^2$. Combining data from three different studies in which paclitaxel was given as a 6-hour infusion at different doses, nonlinear pharmacokinetics is evident at doses higher than 250 mg/m$^2$ (Fig 4). Data from two studies with the drug administered by 3-hour infusion have been tabulated in Table 1, from which it can be seen that the clearance of paclitaxel decreases when the dose increases, which is typical for nonlinear/saturation kinetics. This phenomenon also appears from the maximal plasma concentration ($C_{max}$) values, which increase more than proportional with dose. It is not yet fully clear whether saturation in liver metabolism and/or distribution from the central into the peripheral compartment lead to this pharmacokinetic behavior. When saturation occurs at the level of tissue distribution leading to nonlinear pharmacokinetics, this should, theoretically, be accompanied by irreversible tissue, protein, or tubulin binding. Paclitaxel, however, binds reversibly to microtubules and after administration of radiolabeled drug to rats all the radioactivity is excreted in 6 days, which also points to reversible binding. On the other hand, Sonnichsen et al. found a two-compartment pharmacokinetic model with saturable tissue distribution/binding to provide the best fits to the measured values in pediatric solid tumor patients with 24-hour infusions of 200 to 420 mg/m$^2$. More research is clearly warranted for better insight into the phenomenon of nonlinear pharmacokinetics, which may have important clinical consequences as this kinetic behavior may lead to dramatic changes in drug exposure when changing dose and/or schedule.

Distribution

The volumes of distribution for paclitaxel found in different studies are relatively large and exceed...
total body water despite high protein binding of the drug. Equilibrium dialysis\textsuperscript{9} studies and ultracentrifugation\textsuperscript{10} studies have indicated a serum protein binding of 93\% to 98\%. Reported volumes of distribution are in the order of magnitude of 50 to 100 L/m\textsuperscript{2} when the drug is given as a 3- or 6-hour infusion,\textsuperscript{6,9,15} with higher values (150 to 650 L/m\textsuperscript{2}) occurring when it is given as a 24-hour infusion.\textsuperscript{12,21,22} The values indicate that paclitaxel is extensively distributed and bound to tissues and extravascular proteins, including tubulins.

Distribution studies in tumor-bearing mice showed high paclitaxel concentrations in the liver and tumor 24 hours after administration.\textsuperscript{26}
Klecker et al\textsuperscript{22} dosed rats with $^3$H-paclitaxel (2 mg/kg) to study the distribution of the agent. Six hours after dosing they found paclitaxel levels to be negligible in brain and testes, while liver, spleen, lung, and kidneys had the greatest tissue to plasma ratios (approximately 25:1). Interestingly, while the testis appeared to be a sanctuary site for paclitaxel in studies with radiolabeled paclitaxel,\textsuperscript{24,28} other investigators found high levels 24 hours after administration in S.180-bearing mice.\textsuperscript{26} This discrepancy requires further investigation.

**Elimination**

Elimination studies in animals indicate that biliary excretion is likely the major route of paclitaxel elimination, with 98% of the radioactivity excreted in feces within 6 days.\textsuperscript{24} In humans renal elimination contributes only marginally to the overall elimination of the drug, with approximately 5% to 10% of the administered dose excreted as unchanged drug in the urine.\textsuperscript{8-10} Urinary metabolites have not yet been reported. Although there are no data available for paclitaxel pharmacokinetics in patients with renal impairment, it can be expected that dose adjustment is not necessary in these cases.

**Metabolism**

The metabolism of paclitaxel in rats has been described extensively by Monsarrat et al,\textsuperscript{29} who found nine metabolites in rat bile. The chemical structures of two metabolites were elucidated by mass spectrometry and $^3$H-nuclear magnetic resonance spectroscopy. Both metabolites appeared to be hydroxylated compounds; one metabolite was para-hydroxylated on the phenyl group at C3' and the other metabolite was hydroxylated in the meta-position on the benzoyl moiety at C2. Baccatin III, which lacks the C13 side chain, accounted for only 1% of the administered paclitaxel. Over a 24-hour period 40% of the injected drug was eliminated in bile. Glucuroconjugates and sulfate conjugates were not detected in bile.\textsuperscript{29} As the bioavailability of paclitaxel after oral administration is low (<1% in mice),\textsuperscript{30} significant enterohepatic cycling of the parent drug should not be expected. Radioactive metabolites were not found in plasma or most tissues of rats except for the liver.\textsuperscript{27} Data on human metabolism of paclitaxel are scanty. Until 1992, no metabolites were reported to occur in the plasma of paclitaxel-treated patients,\textsuperscript{30} with the exception of one unidentified peak in the HPLC chromatogram mentioned in the analytical paper of Rizzo et al.\textsuperscript{7} In human bile the major metabolite was found to be 6-hydroxy-paclitaxel (data from one patient), and the appearance of metabolic products was different from that found in rats.\textsuperscript{31} By using a highly sensitive and selective HPLC assay, Huizing et al\textsuperscript{32} reported 11 potential metabolites in a group of 30 patients with ovarian carcinoma treated with paclitaxel. So far, only hydroxylated metabolites have been identified, which suggests that cytochrome P-450 mixed oxidases play a pivotal role in paclitaxel metabolism. This may have important clinical implications when the drug is used in combination with other agents with inducing or inhibitory effects on paclitaxel’s metabolizing (iso)enzymes. Quantitative information, as well as data on biologic activity of the metabolites, are lacking.

In patients with liver tumor involvement, the clearance of paclitaxel is reduced,\textsuperscript{33} which may have clinical consequences, as found in the cisplatin/paclitaxel combination and sequence study of Rowinsky et al,\textsuperscript{17} in which decreased paclitaxel clearance induced more severe myelosuppression.

**Drug Interactions**

In view of the putative importance of hydroxylation reactions in paclitaxel metabolism, it is obvious that drug–drug interaction studies with paclitaxel are now getting high priority. The effects of concomitant medications on paclitaxel levels in human studies are limited; reported studies are summarized in Table 2.\textsuperscript{17,25,34-39} Cimetidine, used in the premedication scheme before paclitaxel administration, is a well-known modulator of the
Table 2. Effects of Concomitant Medication on Paclitaxel Levels

<table>
<thead>
<tr>
<th>Drug</th>
<th>Paclitaxel Concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cimetidine</td>
<td>0</td>
<td>34/35</td>
</tr>
<tr>
<td>Famotidine</td>
<td>↑</td>
<td>36</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>↑</td>
<td>17</td>
</tr>
<tr>
<td>Granulocyte colony-stimulating factor</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td>Anticonvulsants</td>
<td>↓</td>
<td>25</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>↑</td>
<td>38</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>↑</td>
<td>39</td>
</tr>
</tbody>
</table>

Given alone or in combination with doxorubicin by 72-hour continuous infusion. Sequence pharmacologic studies using paclitaxel in combination with cyclophosphamide in doxorubicin-resistant metastatic breast cancer patients and with carboplatin in non–small cell lung cancer patients are ongoing. In vitro studies with human liver microsomes also suggest that paclitaxel metabolism is altered by therapeutically relevant concentrations of ketoconazole and fluconazole. From the data available so far, it is difficult to predict whether a drug interaction will occur with therapeutic relevance. The nonlinear pharmacokinetic behavior of paclitaxel and the difficulty predicting outcome of a potential drug–drug interaction require that extreme caution be exercised when using paclitaxel in combination with other drugs; such combinations should only be considered in an investigational setting.

PHARMACODYNAMICS

One of the main purposes of investigating the pharmacokinetics of an anticancer drug is to explore whether relations exist between the clinical outcome (antitumor activity and/or toxicity) and any pharmacokinetic parameter, and how these can further optimize therapy. Ample evidence has shown a relationship between the dose of paclitaxel and toxicity. In 1991, Brown et al observed a rough relationship between the AUC of paclitaxel and the grade of neuropathy and leukopenia. Area under the curve values less than 23 hr·µmol/L were not related to these toxicities, while AUC values higher than 29 hr·µmol/L led to grades I to III neuropathy and leukopenia. In a phase I/II study in refractory lymphoma and breast cancer, paclitaxel steady-state plasma concentrations greater than 0.07 µmol/L during 96-hour infusions correlated significantly with grade II/III mucositis and grade IV neutropenia. Relationships between steady-state paclitaxel concentrations and principal toxicities of treatment were studied by Rowinsky et al in a phase I study with 24-hour infusions of paclitaxel (135 to 250 mg/m²) followed by cisplatin (75 mg/m²) the next day and granulocyte colony-stimulating factor support (5 µg/kg/d) commencing on day 3. Relationships, however, with poor correlation (r = 0.5; P < 0.01) were found between the grade of neurotoxicity and steady-state paclitaxel concentrations, which were nearly identically cor-
related to the dose of paclitaxel. The absolute neutrophil count could be described adequately in this dosing regimen with an $E_{\text{max}}$ model, from which the steady-state plasma concentration of paclitaxel required to yield a 50% decrease in neutrophils is 0.32 $\mu$mol/L. Neutropenia in relation to pharmacokinetics was also investigated in more detail as part of a European-Canadian trial in which paclitaxel, in doses of 135 and 175 mg/m$^2$ administered by either 3- or 24-hour infusion, was studied in relapsed ovarian cancer. The percentage decrease ($E$ [\% change]) in granulocyte or white blood cell count (calculated as 100% $\times$ [pretreatment value-nadir value]/pretreatment value) was modelled with the sigmoidal maximum effect equation

$$E\ (%\ change) = \frac{100 \times T^H}{T_{50} + T^H}$$

in which $T$ is the time spent above 0.1 $\mu$mol/L, $T_{50}$ is the time spent above 0.1 $\mu$mol/L that results in 50% of the effect, and $H$ is the Hill constant. The threshold concentration of 0.1 $\mu$mol/L was selected as the pharmacokinetic parameter of interest, as this concentration has been shown to induce pertinent antimicrotubule effects in vitro. In Fig 5, the sigmoidal relationship between the percentage decrease in white blood cells and the time spent above 0.1 $\mu$mol/L is shown, from which it can be deduced that a 50% decrease in white blood cell count will occur when the time above 0.1 $\mu$mol/L is approximately 15 hours. In Figs 6 and 7, the pharmacokinetic curves of paclitaxel (135 mg/m$^2$) given as a 3- or 24-hour infusion, respectively, with the 0.1 $\mu$mol/L (85 ng/mL) threshold level are represented. From these graphs it is clear that the 24-hour infusion (time above 0.1 $\mu$mol/L is approximately 28 hours) is more myelosuppressive than the 3-hour infusion (time above 0.1 $\mu$mol/L is approximately 12 hours). Any relationship between antitumor activity and pharmacokinetics has not yet been reported, as far as we know.

**CONCLUSION**

The new HPLC methodologies for paclitaxel measurements in biologic matrices have provided more insight into the drug's pharmacology. While pharmacokinetics was initially described by biexponential equations, the use of a more sensitive assay has demonstrated an extra disposition phase yielding a $T^{1/2}(\gamma)$ of approximately 20 hours. Pharmacokinetic monitoring of different doses and schedules has revealed that biologically active concentrations of paclitaxel are achieved in patients with the current schedules used in most studies. At higher doses, but also during shorter infusion times, the pharmacokinetics of paclitaxel appears to be nonlinear. Saturation processes at the level of liver metabolism are most obvious. More knowledge about the metabolic fate of paclitaxel, as well as the pharmacokinetics of the

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**Fig 5. Percentage decrease in white blood cells versus time above paclitaxel concentration of 0.1 $\mu$mol/L, modelled with a sigmoidal $E_{\text{max}}$ model. (Adapted and reprinted with permission. Copyright © 1993 by the American Society of Clinical Oncology.)**
metabolic products, will be very helpful to elucidate the processes underlying the drug's nonlinear kinetic behavior. Nonlinear pharmacokinetics may lead to dramatic differences in drug exposure, in terms of $C_{\text{max}}$ and AUC, when doses and/or schedules are changed; thus, it is urgent that this area be investigated in more detail. Several metabolic products of paclitaxel have been identified, all of which are hydroxylated compounds, indicating that cytochrome P-450-mediated metabolism may play a major role in paclitaxel metabolism. Not all the metabolites or their biologic properties have been determined yet, however, which is also a subject for further research. Drug-drug interactions already have been identified, and extreme caution is emphasized.
when paclitaxel is used in combination. Pharmacokinetic-pharmacodynamic relationships for paclitaxel have been demonstrated, which may help us further optimize therapy with this interesting novel anticancer drug.

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