Effect of Fluconazole on the Pharmacokinetics of Doxorubicin in Nonhuman Primates

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Antifungal prophylaxis in cancer patients who are undergoing chemotherapy is associated with prolonged neutropenia. We measured the effect of fluconazole on doxorubicin pharmacokinetics in nonhuman primates to determine if neutropenia is related to a pharmacokinetic interaction that delays the clearance of the chemotherapeutic agent. Fluconazole pretreatment had no effect on doxorubicin pharmacokinetics.

Dose-intensive anticancer chemotherapy regimens are associated with prolonged neutropenia and an increased risk of fungal infections. Although the routine use of antifungal prophylaxis in this patient population remains controversial, fluconazole appears to prevent mucosal and disseminated candidiasis in bone marrow transplant recipients (4, 15). The limitations of prophylactic antifungal therapy include an increased risk of resistant fungal infections and a higher incidence of bacterial infections (14). In addition, in randomized clinical trials with adult patients who received dose-intensive anticancer chemotherapy, antifungal prophylaxis with ketoconazole or fluconazole was associated with prolongation of chemotherapy-induced, severe neutropenia (9, 12). The mechanism of this prolonged neutropenia was not identified.

The triazole antifungal agent fluconazole and the imidazole ketoconazole inhibit hepatic cytochrome P450 enzymes, and these agents are known to alter the clearance of a variety of drugs (1). Doxorubicin is a myelosuppressive anticancer drug that is frequently incorporated into combination dose-intensive treatment regimens for a variety of solid tumors and acute leukemias. The pharmacokinetics of doxorubicin is characterized by an initial rapid tissue distribution phase (half-life $[t_{1/2}]_0$, 10 min), followed by a prolonged elimination phase $[t_{1/2}]_0$, 30 h) (5, 11, 13). Although the plasma doxorubicin concentration at the start of the elimination phase is only 2% of the peak plasma drug concentration, 75% of the total drug exposure is accounted for during the elimination phase (5). Doxorubicin is eliminated by hepatic metabolism and biliary excretion (11), and drugs that inhibit or induce hepatic drug-metabolizing enzyme systems, such as ranitidine (6) and phenobarbital (10), can alter the clearance of doxorubicin. We hypothesized that the prolonged neutropenia associated with fluconazole prophylaxis in patients with hematological malignancies may be due to delayed clearance of doxorubicin.

Four adult male rhesus monkeys (Macaca mulatta) ranging in weight from 7.1 to 12.7 kg were used in this study (7). The animals were group housed in accordance with the Guide for the Care and Use of Laboratory Animals (8) and received food and water ad libitum. Heparinized blood samples were drawn from a saphenous or femoral venous catheter (contralateral to the site of drug injection) prior to infusion of doxorubicin, 30 min after the start of the infusion, at the end of the infusion, and 5, 10, 15, 30, 60, and 90 min and 2, 3, 4, 6, 8, 10, 24, and 48 h after the end of the infusion. Plasma was separated immediately by centrifugation and frozen at $-70^\circ$C until assayed.

Doxorubicin (Rubex; Chiron Therapeutics, Emeryville, Calif.) at a dose of 2.0 mg/kg was administered intravenously (i.v.) over 60 min, alone and after fluconazole, using a randomized crossover design. Studies using the same animal were separated by 2 to 4.5 months. Fluconazole (10 mg/kg/day) was given i.v. over 30 min daily for the 3 days prior to doxorubicin, and a fourth dose was administered 2 h prior to doxorubicin. Fluconazole was not continued after the doxorubicin dose, because it has a long $t_{1/2}$ (25 h in nonhuman primates, $31.6 \pm 4$ h in humans), which results in prolonged drug exposure (2, 3). Complete blood counts and chemistries were performed on the animals twice weekly for at least 4 weeks after drug administration.

Doxorubicin concentrations in plasma were measured with a reverse-phase high-performance liquid chromatography method using fluorescence detection. Daunorubicin served as an internal standard. Plasma samples were prepared by solid...

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FIG. 1. Plasma concentration-time profile of doxorubicin (2 mg/kg) administered i.v. over 60 min alone (□) and after 4 days of fluconazole (●). The values shown are geometric means for four animals. The mean CV for doxorubicin alone was 33.6%; the mean CV for doxorubicin with fluconazole was 57%.
distribution at steady state was calculated from the AUC and the trapezoidal rule and extrapolated to infinity; clearance was calculated from the area under the concentration-time curve (AUC) was derived using the linear trapezoidal rule. The coefficients of variation (CV) for the assay were 10%. The lower limit of detection was 5 nM, and the lower limit of quantification was 10 nM. The interday and intraday variability was 5%. Phase extraction. The mobile phase consisted of 79:21 ammonium formate-acetonitrile at pH 4.0 with a flow rate of 2 ml/min through a μ-Bondapak phenyl column (Waters Corporation, Milford, Mass.) with a PS-GU phenyl 5 μ guard column (Thomson Instrument Company, Springfield, Va.). An excitation wavelength of 480 nm and emission wavelength of 595 nm was used for fluorescence detection. Chromatographic analysis was performed on Millennium software (Waters Corporation). The lower limit of detection was 5 nM, and the lower limit of quantification was 10 nM. The interday and intraday coefficients of variation (CV) for the assay were ≤10%.

Pharmacokinetic parameters were calculated using standard model-independent methods. The area under the plasma concentration-time curve (AUC) was derived using the linear trapezoidal rule and extrapolated to infinity; clearance was calculated by dividing the dose by the AUC, the volume of distribution at steady state was calculated from the AUC and area under the moment curve, and the terminal t<sub>1/2</sub> at β-phase was derived by least-squares regression analysis using a biexponential equation in MLAB (Civilized Software, Bethesda, Md.).

The plasma concentration-time profile of doxorubicin in nonhuman primates is shown in Fig. 1. The initial rapid decline in the plasma doxorubicin concentration after completion of the 1-h infusion, followed by the prolonged elimination phase, is similar to the profile observed in humans (11). The pharmacokinetic parameters are listed in Table 1. Pretreatment with fluconazole had no effect on the pharmacokinetics of doxorubicin.

### Table 1. Doxorubicin pharmacokinetic parameters in nonhuman primates

<table>
<thead>
<tr>
<th>Monkey</th>
<th>AUC (nM·h)</th>
<th>Clearance (liter/h/kg)</th>
<th>V&lt;sub&gt;ss&lt;/sub&gt; (liters/kg)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
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<td>R330A</td>
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<td>14</td>
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<tr>
<td>B9078</td>
<td>2.030</td>
<td>1.7</td>
<td>13</td>
<td>25</td>
</tr>
</tbody>
</table>

Mean 2.130 2.160 1.7 2.4 24 20 20 17

SD 280 670 0.2 0.6 21 4 18 2

<sup>a</sup> Doxorubicin was administered i.v. at a dose of 2 mg/kg alone and after 4 days of fluconazole. –, doxorubicin alone; +, fluconazole followed by doxorubicin.

<sup>b</sup> V<sub>ss</sub>, volume of distribution at steady state.

<sup>c</sup> t<sub>1/2</sub> at β phase.

The incidence of severe neutropenia (absolute neutrophil count of <500/μL) was higher with doxorubicin alone (three animals) than with the combination of doxorubicin and fluconazole (zero animals). The prolongation of chemotherapy-induced neutropenia associated with prophylactic fluconazole does not appear to be related to a pharmacokinetic interaction with doxorubicin.

### References


