Pharmacodynamic Evaluation of RWJ-270201, a Novel Neuraminidase Inhibitor, in a Lethal Murine Model of Influenza Predicts Efficacy for Once-Daily Dosing

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Received 21 November 2000/Returned for modification 1 April 2001/Accepted 24 April 2001

We examined RWJ-270201 in a lethal model of influenza in BALB/c mice. The aim was to delineate the pharmacodynamically linked variable for the drug. Challenge was performed with influenza virus A/Shongdong/09/93 (H3N2). Treatment was administered by gavage. Five doses (1 to 10 mg/kg of body weight) and three schedules (every 24, 12, and 8 h) were evaluated with 10 mice per group. There were 39 placebo-treated mice. Drug exposure was evaluated for infected mice. Exposures were calculated after population modeling of all the plasma concentration-time data simultaneously using the NPEM3 program. Evaluation of dose and schedule with Kaplan-Meier analysis and Cox proportional hazards modeling demonstrated that schedule offered no explanatory power relative to dose alone. Evaluation of peak concentration, trough concentration, and area under the concentration-time curve (AUC) by the same methods revealed that AUC was the dynamically linked variable. Again, schedule offered no further explanatory power when included in the model with AUC. This indicates that AUC is the linked variable and that the anti-influenza effect of RWJ-270201 is independent of schedule. We conclude that once-daily dosing of RWJ-270201 should be evaluated in clinical trials of influenza therapy.

Influenza virus infections caused by both A and B strains continue to be a cause of morbidity and mortality, particularly for patients who are elderly or otherwise immunocompromised (2, 9).

The viral neuraminidase is a logical target for antiviral chemotherapy, as it is readily accessible and is a necessary site for ongoing spread of viral infection. In addition, this enzyme is appealing as a target for chemotherapy because it would be expected to affect clinical outcome, even after infection initiation, because of its ability to interrupt viral spread. Further, it differs from older agents like amantadine and rimantadine in having a lower probability of viral emergence of resistance (7).

RWJ-270201 is a new, potent member of the neuraminidase inhibitor class of influenza antiviral agents. Other members of this class currently available to the physician have been well reviewed (1). Previous studies with mice have demonstrated a pharmacokinetic profile that produced sufficient exposure to conclude that this agent would be useful for the therapy of influenza infection (data on file, R. W. Johnson Pharmaceutical Research Institute, Raritan, N.J.).

Experience with other classes of anti-infectives has demonstrated that animal model experiments that delineate which pharmacodynamic variable (peak concentration/50% inhibitory concentration [IC50], area under the concentration-time curve [AUC]/IC50, or time > IC50) is most closely linked to outcome have been highly predictive of the results seen for humans (3–5, 8).

The ability to delineate which aspect of the concentration-time curve affects outcome is extremely valuable, as it allows the choice of the proper dosing schedule and facilitates the setting of a therapeutic goal. For these reasons, we decided to investigate RWJ-270201 in a lethal murine model of influenza infection and attempt to delineate the pharmacodynamic variable most closely linked to outcome.

MATERIALS AND METHODS

Virus. Influenza virus A/Shongdong/09/93 (H3N2) was used for the challenges and was kindly provided by Helen Regnery at the Centers for Disease Control and Prevention (Atlanta, Ga.). The virus had been passaged seven times through weanling mice and used as a pretted cell culture preparation seeded from a seventh-passage cell homogenate. The challenge titer was 107.0 cell culture 50% infective doses (CCID50/ml). Virus was administered intranasally to mice anesthetized by intraperitoneal injection of 100 mg of ketamine (Ft. Dodge Animal Health, Fort Dodge, Iowa) per kg of body weight. A volume of 90 µl of virus was administered. The IC50 of RWJ-270201 for the challenge virus was 0.83 nM.

Mice. Female BALB/c mice, weighing 18 to 21 g, were obtained from B and K International (Fremont, Calif.). Once infected with influenza, the animals were given oxytetracycline (0.006%) in their drinking water to control possible secondary bacterial infections.

Drug. Drug solutions were made once daily in sterile physiologic saline. Drug RWJ-270201 was provided by the R. W. Johnson Pharmaceutical Research Institute.

Pharmacokinetic studies. Infected mice were used for the development of RWJ-270201 pharmacokinetics. Animals were dosed with one of the following regimens: (i) 20 mg/kg once by gavage, (ii) 10 mg/kg once by gavage, (iii) 5 mg/kg once by gavage, or (iv) 3.33 mg/kg once by gavage. Plasma was obtained on 10 occasions over 24 h. from six animals at each time point for each regimen (240 animals). Plasma samples were assayed for RWJ-270201 by a sensitive and specific liquid chromatography-mass spectroscopy procedure. The interrun accuracy (measured as percent bias from the expected concentration) and precision...
TABLE 1. Dose versus schedule

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Log-likelihood</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>−432.472</td>
<td>0.000000001</td>
</tr>
<tr>
<td>Schedule</td>
<td>−450.414</td>
<td>NS*</td>
</tr>
<tr>
<td>Dose + schedule</td>
<td>−432.201</td>
<td>NS b</td>
</tr>
<tr>
<td>Schedule (as stratification variable)</td>
<td>NS c</td>
<td></td>
</tr>
<tr>
<td>Dose + schedule (schedule as stratification variable)</td>
<td>NS*</td>
<td></td>
</tr>
</tbody>
</table>

NS, not significant.

a NS, dose plus schedule, both as covariates, does not differ from dose alone.
b NS, schedule is not significant alone as a stratification variable (log-rank test), nor in combination with dose as a covariate (log-rank—Tarone-Ware method).

TABLE 2. Ranges of pharmacodynamic parameters

<table>
<thead>
<tr>
<th>Schedule</th>
<th>Dose (mg/kg)</th>
<th>Peak (nM)</th>
<th>AUC (nM·h)</th>
<th>Time &gt; IC50 (24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Every 24 h</td>
<td>10</td>
<td>1,232</td>
<td>1,532</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>123</td>
<td>153</td>
<td>17</td>
</tr>
<tr>
<td>Every 8 h</td>
<td>3.333</td>
<td>410</td>
<td>1,532</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>0.333</td>
<td>41</td>
<td>153</td>
<td>42</td>
</tr>
</tbody>
</table>

a 24-h AUC.

TABLE 3. Pharmacodynamic parameter selection

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Log-likelihood</th>
<th>Final model</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak</td>
<td>−628.406</td>
<td>0.00000813</td>
<td></td>
</tr>
<tr>
<td>Trough</td>
<td>−636.625</td>
<td>0.00476622</td>
<td></td>
</tr>
<tr>
<td>AUC</td>
<td>−617.168</td>
<td>−0.00388*</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

95% confidence interval, −0.00505 (lower) to −0.00271 (upper).

RESULTS

Challenge study outcome. We initially evaluated the effect of schedule of administration by examining dose and schedule (schedule as both a stratification variable and a covariate) in both Kaplan-Meier and Cox proportional hazards analyses. The outcomes are displayed in Table 1.

The log cumulative hazard plot (data not shown) demonstrated that schedule should be treated as a covariate and not as a stratification variable. Dose + schedule (both as covariates) was not different from dose alone when examined by a likelihood ratio test. Further, backwards stepping resulted only in dose remaining in the final model. One can then conclude that schedule of administration has no influence on the time to death in this lethal model of murine influenza infection.

The pharmacokinetic experiments (carried out with infected mice) were population modeled, and the results are as follows (values are means ± standard deviations): volume/F, 0.288 ± 0.0741 liter; Ka, 4.262 ± 0.594 h−1; clearance/F, 0.398 ± 0.0548 liter/h; half-life, 0.5 h (estimate of half-life is directly calculated from clearance/F and volume/F). Clearly, the drug has a short half-life in these mice. The drug is rapidly absorbed after gavage, as is evidenced by the relatively large Kp. The parameters allow us to calculate drug exposures for the dosing groups and allow examination of different measures of exposure as covariates to ascertain whether they significantly shift the hazard function.

The covariates of peak concentration, trough concentration, and AUC were tested in a Cox proportional hazards model. The ranges of these covariates are displayed in Table 2. The results from the Cox model are displayed in Table 3.

Clearly, all three exposure covariates significantly affect the time to death. It is also clear that AUC most strongly affects the hazard function. This shows that AUC is the pharmacodynamically linked variable for RWJ-270201. As this is an important finding, we wished to again make sure that schedule of administration did not have a significant impact on the outcome. We performed another analysis in which we attempted to add schedule, both as a covariate and as a stratification variable to AUC to determine whether this belonged in the final model. In both instances, schedule was not included in the final model. We can then conclude that schedule of administration adds no information to the AUC regarding time to death in this lethal murine influenza model. The parameters of the final model are displayed in Table 3.

In order to put this finding into perspective, we employed the parameters above to simulate the survival plots for four different dosing groups: in Fig. 1, panel A, the dose is 10 mg/kg; in panel B, the dose is 5 mg/kg; in panel C, the dose is 1 mg/kg; in panel D, the dose is 0 mg/kg (placebo). It is clear that there...
is a clear-cut exposure response, as results for 1 mg/kg/day are virtually not different from those of the placebo, whereas the 10-mg/kg/day dose (each given only to day 5) protects greater than 60% of the mice out to day 21.

**DISCUSSION**

Delineation of the aspect of the concentration-time curve that most directly affects outcome allows determination of the most effective schedule for administration. For instance, if peak is linked to outcome, this would mandate once-daily dosing to provide the greatest peak concentration. If time > IC₅₀ (or IC₉₀) were linked to outcome, then more fractionated dosing schedules would be most appropriate. In this instance, we found that, for RWJ-270201, AUC was the dynamically linked variable. This implies that the effect is independent of schedule of administration, out to the longest administration schedule examined (every 24 h in this evaluation).

The data to support this conclusion are straightforward in this evaluation. When the statistical evaluation was allowed to examine dose and schedule, the outcome was clear. Schedule of drug administration added no information to dose, irrespective of how schedule was evaluated (covariate or stratification variable).

We also performed an extensive pharmacokinetic evaluation of RWJ-270201 in influenza-infected mice. This was done to examine whether peak concentration, trough concentration, or AUC was most directly linked to outcome. It should be recognized that when only one administration schedule is employed (e.g., every 24 h [q24h] dosing), it maximizes the correlation between peak, trough, and AUC. One cannot make peak rise without making AUC larger and without increasing trough. To obviate this difficulty, we examined multiple dosing schedules (q24h, q12h, and q8h) and multiple different doses. The analysis chose AUC as the most directly linked covariate. It should be noted that this is concordant with

**FIG. 1. Simulated survival plots for animals given one of three doses of drug or placebo.** Shown are plots of survivorship as influenced by the AUC from a dose of 10 mg/kg (A), 5 mg/kg (B), or 1 mg/kg (C) and from a placebo dose (D).
choosing dose over schedule. To extend the confidence that we had found the correct variable, we then retested to determine whether schedule added any information to AUC. Again, irrespective of how schedule was treated in the Cox model (covariate versus stratification variable), it added no further information to AUC. We can conclude from this that AUC is the dynamically linked variable for RWJ-270201.

We performed this evaluation with a single isolate. Clearly, more data on this subject would be welcome. It is also important to recognize that other isolates will have significant differences in pathogenicity and susceptibility to RWJ-270201. In reality, the dynamically linked variable from our study is not AUC, but rather the AUC/IC\textsubscript{50} (or IC\textsubscript{90}) ratio.

These results provide a basis for evaluating once-daily dosing of RWJ-270201 in clinical trials. Indeed, it is our intent to test this hypothesis by examining once- versus twice-daily dosing of RWJ-270201 in a volunteer challenge model of influenza virus A infection.

REFERENCES