Short Communication

EFFECT OF RECOMBINANT INTERLEUKIN-2 PRETREATMENT ON ORAL AND INTRAVENOUS DIGOXIN PHARMACOKINETICS AND P-GLYCOPROTEIN ACTIVITY IN MICE

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ABSTRACT:

P-glycoprotein (P-gp) is an ATP-dependent efflux membrane transporter involved in many drug pharmacokinetics in humans. Decreasing its expression could enhance the bioavailability of substrates as digoxin. We have recently found that human recombinant interleukin-2 (rIL2) in vivo decreases P-gp expression in intestine and brain of mice and modifies oral digoxin pharmacokinetics. The aim of the study was to evaluate the involvement of bioavailability in the rIL2 pretreatment effect on digoxin pharmacokinetics by comparing oral and i.v. digoxin pharmacokinetics before and after rIL2 pretreatment (10 μg/kg). We also tried to show the possible effect of a low rIL2 dose (1 μg/kg) pretreatment on oral digoxin pharmacokinetics. First, adult Swiss mice received a single oral or i.v. dose of digoxin (0.03 mg/kg). Two weeks later, the same animals were treated by rIL2 i.p. twice a day (10 μg/kg) for 4 days and received digoxin again at day 5. As well, another group received oral digoxin (0.03 mg/kg) with a 1 μg/kg rIL2 pretreatment. Blood was collected after digoxin administration with and without rIL2 pretreatment. Digoxin pharmacokinetics were described by a one-compartment model. The 10 μg/kg rIL2 pre-treatment did not modify i.v. digoxin pharmacokinetics, whereas oral digoxin pharmacokinetics were significantly modified by the 10 μg/kg rIL2 pretreatment and not by the 1 μg/kg rIL2 pretreatment. The decrease of P-gp activity, caused by rIL2 (10 μg/kg), increased digoxin bioavailability. An increase in exposure and intracellular level of drugs is expected from rIL2 pretreatment.

P-gp1 is a membranous ubiquitous protein, playing a major role in cellular ATP-dependent efflux, allowing active extrusion of a wide range of hydrophobic drugs from the cell (Bellamy, 1996). In many healthy tissues, P-gp is found with a high expression level, particularly kidneys and liver (clearing tissues), gastrointestinal tract (absorption site), and blood-brain barrier (distribution). Therefore, a high P-gp activity in these tissues should lead to a decrease of absorption from gastrointestinal tract, an increase of elimination in urine and bile, and a decrease of distribution in central nervous system (Fromm, 2000). The P-gp expression is also very high in many cancer cells (Cordon-Cardo et al., 1990), decreasing the cytotoxic efficient intracellular drug concentration and leading to the multidrug resistance phenomenon. A drug-induced decrease in P-gp activity in cancer cells should then reduce anticancer drug efflux, restoring the antitumoral activity.

rIL2 is a human recombinant cytokine used in therapy for its immunomodulation and antineoplastic properties (Le Cesne et al., 1999). rIL2 can modulate gene expressions, including those coding for P-gp. It has been shown that rIL2 decreases cytochrome P450 activity.

1 Abbreviations used are: P-gp, P-glycoprotein; rIL2, human recombinant interleukin-2; AUC, area under the curve.

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Materials and Methods

Chemicals. Oral solution and pediatric i.v. solution at 50 μg/ml of Digoxin Nativelle were purchased from Procter and Gamble Pharmaceuticals (Neuilly sur Seine, France). Proleukine, aldesleukine at 18 · 10^6 IU/1.1 mg, obtained from Chiron (Suresnes, France), was used as rIL2.
were analyzed according to a one-compartment model with first-order absorption and elimination. Typical parameters of the model were the distribution volume (V/F), and the rate constants for renal elimination (k\text{ren}) and absorption (K\text{a}); F denotes the bioavailability fraction.

**Statistical Analysis.** Two plasma concentrations were available per mouse, one concentration was for each pharmacokinetic (period 1 and period 2). By using several mice at the different time points of the pharmacokinetics, we were only able to draw a "mean" pharmacokinetics profile and to estimate "mean" pharmacokinetics parameters, but we were not able to estimate the corresponding variances. To estimate the standard error of AUC in each treatment group, the Bailer method (Bailer, 1988) was applied, based on the variability of the concentrations at each sampling time. From the estimated mean AUC and the corresponding standard errors, pairwise comparisons of AUC between the four treatment groups were performed using a Z-test with an experiment-wise error of 0.05 (Bailer, 1988). Serum creatinine concentration measures were analyzed according to the Student paired t test with an experiment-wise error of 0.05.

**Results and Discussion**

Figure 1 depicts the plasma digoxin concentration-time courses observed before and after rIL2 pretreatment at 10 µg/kg, after i.v. digoxin administration. Figure 2 depicts the plasma digoxin concentration-time courses observed before and after rIL2 pretreatment after oral digoxin administration at 1 and 10 µg/kg.

Tables 1 and 2 report digoxin pharmacokinetics parameters calculated with a compartmental analysis for i.v. and oral administration, respectively, before and after 10 or 1 µg/kg rIL2 pretreatment. The Bailer approach (Bailer, 1988) shows that rIL2 pretreatment, only at 10 µg/kg dosage and after oral digoxin administration, increased significantly the digoxin AUC value.

Following the Student paired t test, serum creatinine concentrations were not significantly modified after rIL2 pretreatment, excluding a renal failure related to rIL2 pretreatment.

Different mechanisms, such as modification of absorption, distribution, metabolism, or elimination, may be involved in a drug interaction. In the case of oral digoxin pharmacokinetics modification after rIL2 pretreatment, shown by Bonhomme-Faivre et al. (2002) and Veuve et al. (2002), we can exclude the metabolism hypothesis. Indeed, the digoxin undergoes limited liver metabolism (<10%), and then a decrease in digoxin metabolism could not explain the observed re-
sults. A modification by rIL2 pretreatment on absorption and elimination parameters, involving efflux protein as P-gp, is expected.

Since no significant variation was observed on the elimination rate constant ($k_{10}$), the clearance/F decrease, after 10 μg/kg rIL2 pretreatment with oral digoxin administration, should be related to an increase in bioavailability, F. This conclusion is also supported by the absence of rIL2 effect on intravenous digoxin pharmacokinetics. Indeed, the drug interaction between digoxin and rIL2 only occurs with digoxin administered orally. It means that rIL2 pretreatment modifies one or several digoxin pharmacokinetics parameters specific to the oral route: the bioavailability parameters.

Western blot measurements of P-gp expression indicate that 10 μg/kg rIL2 pretreatment strongly decreased P-gp expression in intestinal tissue but not in renal tissue (Bonhomme-Faivre et al., 2002) and support the hypothesis, also proposed by Sababi et al. (2001), of an increased net digoxin influx from the gastrointestinal tract.

Moreover, a dose-dependent relationship for this rIL2 effect is strongly suggested by the relationship observed between the digoxin bioavailability increase (4.1 and 41% for 1 and 10 μg/kg, respectively) and the rIL2 dose. Indeed, no significant modification was observed on oral digoxin pharmacokinetics after 1 μg/kg rIL2 pretreatment in our study.

In conclusion, our observations show that rIL2 administration modified oral digoxin pharmacokinetics, resulting in a dose-dependent increase in plasma digoxin AUC. These modifications are related to a decrease in the intestinal P-gp activity. It could have clinical interest

![Fig. 2. Oral digoxin plasma concentration-time courses before (●) and after (▲) 1 μg/kg rIL2 pretreatment, and before (●) and after (×) 10 μg/kg rIL2 pretreatment.](image)

Each concentration point is the mean ± S.E.M. of eight measurements.

### TABLE 1

*Effect of a 10 μg/kg rIL2 pretreatment on i.v. and oral digoxin pharmacokinetics parameters*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Digoxin Administration Route</th>
<th>Control (No rIL2)</th>
<th>Effect of rIL2 Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V$ (ml)</td>
<td>i.v.</td>
<td>34.4</td>
<td>37.2</td>
</tr>
<tr>
<td>$V/F$ (ml)</td>
<td>p.o.</td>
<td>16.0</td>
<td>12.8</td>
</tr>
<tr>
<td>$k_{10}$ (h$^{-1}$)</td>
<td>i.v.</td>
<td>0.17</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>0.31</td>
<td>0.28</td>
</tr>
<tr>
<td>AUC ($\mu g \cdot h^{-1}$) from $t = 0$ to 24 h</td>
<td>i.v.</td>
<td>156 (19.0)$^a$</td>
<td>132 (8.5)$^a$</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>67.6 (4.7)$^a$</td>
<td>95.1 (6.4)$^a$</td>
</tr>
</tbody>
</table>

$^a$ Standard deviation of the AUC, as estimated with the Bailer method (Bailer, 1988).

### TABLE 2

*Effect of 1 and 10 μg/kg rIL2 pretreatments on oral digoxin pharmacokinetics parameters*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>rIL2 Dose</th>
<th>Control (No rIL2)</th>
<th>Effect of rIL2 Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{10}$ (h$^{-1}$)</td>
<td>μg/kg</td>
<td>1</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.31</td>
</tr>
<tr>
<td>$k_a$ (h$^{-1}$)</td>
<td>1</td>
<td>4.00</td>
<td>5.53</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.12</td>
<td>3.66</td>
</tr>
<tr>
<td>AUC ($\mu g \cdot h^{-1}$) from $t = 0$ to 24 h</td>
<td>1</td>
<td>56.0 (7.0)$^a$</td>
<td>58.3 (4.4)$^a$</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>67.6 (4.7)$^a$</td>
<td>95.1 (6.4)$^a$</td>
</tr>
<tr>
<td>F</td>
<td>μg/kg</td>
<td>1</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.43</td>
<td>0.61</td>
</tr>
</tbody>
</table>

$^a$ Standard deviation of the AUC, as estimated with the Bailer method (Bailer, 1988).
for other poorly metabolized P-gp substrates, orally used in association with rIL2 as anticancer or anti-human immunodeficiency virus drugs.


References


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