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 pretreatment 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 mRNAs and proteins in cultured rat hepatocytes, and rIL2 administration may decrease total cytochrome and monoxygenase activities in humans (Elkahwaji et al., 1999). A decrease in P-gp expression, after rIL2 treatment, has been reported in vitro on human colon carcinoma HCT15 and HCT16 cells (Stein et al., 1996) and on Caco-2 cells (Belliard et al., 2002). As well, after rIL2 treatment in vivo on human colon carcinoma HCT15 and HCT16 cells (Stein et al., 1996) and on Caco-2 cells (Belliard et al., 2002), P-gp expression was decreased in brain and intestine and remained unchanged in lungs, kidneys, and liver (Bonhomme-Faivre et al., 2002).

Digoxin is an in vivo probe for studying P-gp activity in terms of tissue distribution in humans, since it undergoes limited liver metabolism (<10%) and thus has no interaction with the cytochrome P450 pathway (Kawahara et al., 1999; Hoffmeyer et al., 2000). As in humans, digoxin pharmacokinetics in mouse are mainly related to P-gp activity (excretion of digoxin by P-gp expressed in the membrane of renal and intestinal cells) and not dependent upon metabolism (Schinkel et al., 1997). Recent studies showed that a pretreatment with rIL2 (10 and 15 μg/kg twice a day for 4 days) modified the pharmacokinetics of digoxin given orally in mice (Bonhomme-Faivre et al., 2002; Veau et al., 2002).

The objective of our study was to highlight the effect, in vivo, of rIL2 (10 μg/kg) on the intestinal absorption of digoxin, comparing the effect of a 10 μg/kg rIL2 pretreatment on oral and i.v. digoxin pharmacokinetics. We also investigated the possibility of modifying oral digoxin pharmacokinetics (linked with a P-gp functionality modification) with a lower rIL2 pretreatment dose (1 μg/kg).

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 · 10⁶ IU/1.1 mg, obtained from Chiron (Suresnes, France), was used as rIL2.
Animal Experiments. Swiss NMRI mice (Ifa Credo, L’Arbresles, France), 12 weeks old and weighing 30 g, were divided into two groups, one receiving i.v. digoxin in the tail vein (group 1, 88 mice) and one receiving oral digoxin (group 2, 54 mice). In both groups, mice received the same dose of digoxin (20 μl of a solution at 50 μg/ml, i.e., 0.03 mg/kg) after 24 h of fasting with water ad libitum.

The study was divided into two periods: period 1 (digoxin alone) and period 2 (digoxin + rIL2), all mice receiving i.v. or oral digoxin twice separated by a 2-week wash-out period. In period 2, mice were pretreated i.p. twice a day for 4 days with rIL2 (10 μg/kg for i.v. administration or 1 μg/kg for oral digoxin administration).

Plasma digoxin pharmacokinetics were determined after each of the two periods. Sampling times were 5, 10, 20, 30, and 45 min, and 1, 2, 4, 6, 8, and 24 h for group 1 (i.v. administration), and 15 min, 30 min, and 1, 2, 3, 4, 6, 8, and 24 h for group 2 (oral administration). For each mouse, two blood samples were collected on heparinized tubes: one from the retro-orbital sinus after the first dose of digoxin (period 1) and one from the neck blood vessels after decapitation after the second dose of digoxin (period 2). Blood samples were centrifuged for 10 min at 13,000 rpm and plasma was separated and frozen at −20°C.

The serum creatinine concentrations of eight mice before and after rIL2 pretreatment were measured to exclude an eventual decrease of renal digoxin elimination, which would be related to adverse effects of rIL2 and not to rIL2 effects on kidneys P-gp expression.

Digoxin Assay. An automated analytical method was used. Plasma digoxin concentrations were measured with the Microparticles Enzymatic Immuno Assay method on AxSYM (Abbott Diagnostics, Rungis, France) using the Digoxin II AxSYM reagent kit. This method was linear between 0.3 and 4.0 μg/l with a limit of quantification of 0.3 μg/l. The method required a minimal 150-μl volume. If necessary, the plasma samples were diluted in the buffer solution for the Microparticles Enzymatic Immuno Assay on AxSYM. Repeatability (10 assays a day) and reproducibility (3 assays on 3 different days) were determined on a plasma sample manually diluted to the fifth (1/5). For repeatability, the average concentration was 1.19 μg/l, ranging from 1.15 to 1.22 μg/l (coefficient of variation = 3%). For reproducibility, the average concentration was 1.24 μg/l, ranging from 1.19 to 1.30 μg/l (coefficient of variation = 3%).

Pharmacokinetic Analysis. Pharmacokinetics of digoxin were first described in terms of $C_{\text{max}}$, maximal observed concentration after dosing; and $T_{\text{max}}$, time at which $C_{\text{max}}$ is observed. The linear trapezoidal rule method was then used to calculate AUC from $t$ (dosing) to $t$ (last measured concentration after dosing).

Concentration-time data were also analyzed using nonlinear curve-fitting methods implemented in the program Micropharm-K (Urien, 1995). The data 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 ($k_{\text{re}}$) 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, 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-
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 \(\mu\)g/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 \(\mu\)g/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 \(\mu\)g/kg, respectively) and the rIL2 dose. Indeed, no significant modification was observed on oral digoxin pharmacokinetics after 1 \(\mu\)g/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

**TABLE 1**

<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) (\cdot) (l^{-1})) from (t = 0) to 24 h</td>
<td>i.v.</td>
<td>156 (19.0)*</td>
<td>132 (8.5)*</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>67.6 (4.7)*</td>
<td>95.1 (6.4)*</td>
</tr>
</tbody>
</table>

* Standard deviation of the AUC, as estimated with the Bailer method (Bailer, 1988).

**TABLE 2**

<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>(\mu)g/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) (\cdot) (l^{-1})) from (t = 0) to 24 h</td>
<td>1</td>
<td>56.0 (7.0)*</td>
<td>58.3 (4.4)*</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>67.6 (4.7)*</td>
<td>95.1 (6.4)*</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>0.36</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.43</td>
<td>0.61</td>
</tr>
</tbody>
</table>

* Standard deviation of the AUC, as estimated with the Bailer method (Bailer, 1988).

![Fig. 2. Oral digoxin plasma concentration-time courses before (♦) and after (■) 1 \(\mu\)g/kg rIL2 pretreatment, and before (┼) and after (×) 10 \(\mu\)g/kg rIL2 pretreatment.](attachment:image.png)
for other poorly metabolized P-gp substrates, orally used in association with rIL2 as anticancer or anti-human immunodeficiency virus drugs.

Service Pharmacie-Pharmacologie, Hôpital Paul Brousse, Villejuif, France (V.C., L.B.F.);
Laboratoire de Pharmacologie, Faculté de Médecine, Université Paris XII, Créteil, France (S.U.); and
Service de pharmacie clinique et des biomatériaux, Hôpital Bichat Claude Bernard, Paris, France (M.B.R., R.F.)

References


