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Research Paper

Modification of the P-Glycoprotein Dependent Pharmacokinetics of Digoxin in Rats by Human Recombinant Interferon-α

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Purpose. This study was conducted to investigate *in vivo* the impact of interferon-alpha (IFN)-α on P-glycoprotein (P-gp) activity in rats by studying how its administration modifies the bioavailability of digoxin, a fairly pure P-gp substrate.
Methods. Human recombinant IFN-α was given to rats (n = 5–7 per group) daily for 8 days at different doses (IntronA® 10^6, 2.10^6, or 4.10^6 IU kg^{-1}, s.c.), whereas pegylated-IFN-α (ViraferonPeg®, 29 μg kg^{-1}) was given s.c. three times a week. Rats were then given digoxin (32 μg kg^{-1}) i.v. or orally. The pharmacokinetics of digoxin was studied. Intestinal P-gp expression was also examined.
Results. The pharmacokinetics of i.v. administered digoxin was not modified by IFN-α, but a dose-dependent increase in areas under the curve (AUCs) was observed in the orally administered digoxin parameters in rats (AUCs: 392 ± 83 min μg L^{-1}, $p < 0.01$ and 550 ± 97 min μg L^{-1}, $p < 0.001$, respectively, vs. 286 ± 111 min μg L^{-1} for control). A decrease in P-gp expression in the ileum (relative intensities: 0.70 ± 0.19 for 4 Million International Unit (MIU) kg^{-1} IFN-α-treated animals *vs.* 1.00 ± 0.13 for controls, $p < 0.05$) and mainly in the jejunum (relative intensities: 0.46 ± 0.13 for 4 MIU kg^{-1} IFN-α-treated animals *vs.* 1.00 ± 0.08 for controls, $p < 0.001$) was observed.
Conclusion. IFN-α induces *in vivo* a significant dose-dependent inhibitory effect on intestinal P-gp activity related to a local decrease in its expression, thereby predicting important clinical consequences when IFN-α and other P-gp substrates are associated.

KEY WORDS: bioavailability; digoxin; interferon alpha; P-glycoprotein; pharmacokinetics; Sprague–Dawley rats.

INTRODUCTION

The multidrug-resistant transporter MDR1 [P-glycoprotein (P-gp)], a product of the ABCB1 gene, is a drug transporter member of the ABC protein superfamily of 170 kDa widely distributed in tissues (1). Rats possess three multidrug resistance (*mdr*) genes: *mdr1a*, *mdr1b*, and *mdr2* (2). *Mdr1a* and *mdr1b* products, which present 85.6 and 87.6% homology of amino sequence with human MDR1 product, respectively (3), act as drug transporters and *mdr2* functions as a phospholipid translocator (4). In humans, P-gp is implicated in cross-resistance to cancer chemotherapy (5,6) and in the transport of a wide variety of compounds such as doxorubicin, etoposide, verapamil, cyclosporine, and digoxin. The latter, a cardiac heteroside, is a fairly pure P-gp substrate (7): P-gp affects its intestinal absorption (8), biliary excretion (9), and renal elimination (10). Digoxin can spontaneously degrade in biological fluids, especially under acidic conditions (11), and although digoxin is slightly metabolized and excreted unchanged in humans (12), it is extensively metabolized in rats (up to 60% of an i.v. dose) (13) specially by CYP 3A family (14).

A number of molecular mechanisms regulate P-gp transcription, including several members of the steroid family of orphan receptors and pregnane X receptor (PXR/SXR), which has been shown to play a major role in P-gp induction (15,16). These receptors are inducible by xenobiotics and function as xenosensors that mediate drug-induced activation of the detoxifying transport of P-gp (15). Many cytokines such as interleukin-6 and interleukin-1β have been shown to modulate these orphan receptors (17), and in a general manner, drug transporters. The specific *in vivo* effect of some cytokines on P-gp has been already studied. Interleukin-2 reduces P-gp functionality in mice by increasing digoxin bioavailability (18). Interferon-gamma, a type 2 interferon, also modulates P-gp (19): in an *in vivo* P-gp mouse model, IFN-γ reduced P-gp tissue levels in liver, kidney, and intestine, and reduced (3H)digoxin excretion in urine and bile. Interferon-alpha (IFN-α), a member of the type 1 interferon family and which acts through different signaling pathways than IFN-γ, is a widely used cytokine in antiviral and cancer therapeutics (20). In humans, IFN-α has been shown to interfere with

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ABBREVIATIONS: AUC, area under the curve; CYP, cytochromes P450; IFN, interferon; P-gp, P-glycoprotein; rhIFN, human recombinant interferon.

the metabolism of many drugs by acting as a potent CYP inhibitor (21), whereas in rats it had no inhibitory effect on CYP activity (22,23) The impact of IFN-α on P-gp is not yet clear. *In vitro*, IFN-α increases sensitivity to doxorubicin in multidrug-resistant Chinese hamster ovary cells with an increase in P-gp expression and mdr-1 mRNA synthesis (24). The increase in sensitivity to doxorubicin of a HCT-15 cell line after IFN-α treatment was reported not to be induced by a downmodulation of P-gp (25). On the contrary, the rise in sensitivity to cisplatin of human hepatoma cell lines observed after IFN-α treatment was associated in other studies to a reduction of P-gp expression (26); similar observations were made with MDR osteocarcinoma cell exposed to doxorubicin (27).

In this work, we present results of *in vivo* experiments studying the effects of IFN-α on P-gp expression in rat bowel on one hand, and its activity in rats on the other hand by studying the pharmacokinetics of digoxin orally and i.v. administered to rats, after repeated pretreatment with different doses of interferon-α. Although many *in vivo* models are proposed for the study of P-gp activity, the digoxin pharmacokinetics model is one of the most widely used because of digoxin's reliability and rodents' tolerance for digoxin (28–30).

MATERIALS AND METHODS

Chemicals

Digoxin Nativelle® was purchased from Procter & Gamble Pharmaceuticals (Neuilly-Sur-Seine, France). Pegylated and nonpegylated recombinant human IFN-α2b (ViraferonPeg® and IntronA®, respectively) were purchased from Schering-Plough (Hérouville-Saint-Clair, France). The pegylated IFN-α2b (ViraferonPeg®), an antiviral molecule used in hepatitis C treatment, is a covalent conjugate of recombinant IFN-α2b with monomethoxypolyethylene glycol (PEG). The average molecular weight of the PEG portion of the molecule is 12 kDa and the average molecular weight of the pegylated molecule is approximately 31 kDa. Its specific activity is approximately 0.7×10^8 IU mg^{-1} protein.

Animals

Male adult Sprague–Dawley rats weighing 225–250 g were obtained from Charles River Company (L'Arbresle, France). Rats were fed on standard laboratory food *ad libitum* during 1 week prior to the digoxin pharmacokinetics study. Animal handling and experimentation were performed in accordance with the guidelines issued by the European Economic Community, as published in the official *Journal of the European Community* (December 18, 1986; authorization L3600).

Digoxin Pharmacokinetics Studies

Animal Treatment

Rats treated with nonpegylated IFN-α (IntronA®) received a single daily subcutaneous (s.c.) dose of IFN-α for 8 days. For each pharmacokinetics study (i.v. and *per os*), the rats were divided into five groups ($n = 5–7$), with each group receiving a different dose of IFN-α: 1 Million International Unit (MIU) kg^{-1} (10^6 IU kg^{-1}), 2 MIU kg^{-1}, 4 MIU kg^{-1}, Peg-IFNα, or phosphate saline solution (control group). Peg-IFNα is usually administered once a week in humans (20). As pegylated IFN-α is eliminated more rapidly in rats than in humans (31), rats were s.c. treated three times a week (days 1, 4, and 7), with a 29 μg kg^{-1} ViraferonPeg® dose which is equal to 2 MIU kg^{-1} IFN nonpegylated dose (32). The plasma half-life of IFN-α2b in rat varies from 47 to 68 min (33), whereas human interferon-α pegylation half-life increases up to 10 h in rat (34).

Doses used in interferon treatments were calculated in accordance with the FDA animal dosage calculations (http://www.fda.gov/cder/cancer/animalframe.htm): IFN-α human doses can reach 18 MIU/dose (men with 1.8 m^2 estimated body surface, which corresponds to about 2.3 MIU kg^{-1} in rat. Hence, the doses used in this study were chosen to enclose the calculated dose. Toxicity of IFN-α was taken in consideration when the doses were calculated: in rats, IFN-α LD_{50} is $>24.10^8$ IU kg^{-1} dose. At this dose (which is up to 600-fold of the doses currently used), no clinical abnormalities were observed (35).

Pharmacokinetics Study

Pharmacokinetics studies were performed on day 8. To avoid possible interference of food with digoxin pharmacokinetics, animals were fasted overnight with free access to water prior to the experiments. Rats were fed 8 h (480 min) later.

Rats received a single dose of digoxin (32 μg kg^{-1}) either orally or i.v. bolus. Whole-blood samples were collected using jugular cannulation according to the Upton technique (36) in heparinated vials at 0, 20, 40, 60, 120, 240, 360, 480, and 1440 min. Blood was immediately centrifuged and plasma frozen at −15°C until analysis.

Digoxin Assay

An automated analytical method was used. Plasma digoxin concentrations were measured via homogeneous enzyme immunoassay (Emit® 2000, Dade Behring, Paris-La Defence, France; linearity range: 0.2–5.0 ng mL^{-1}, limit of quantification: 0.2 ng mL^{-1}, minimum required volume: 50 μL) and performed with a Cobas Mira 2000® (ABx Diagnostics, Montpellier, France), after appropriate dilution in HEPES buffer to a concentration range of 0.2–5 ng mL^{-1}. Each sample was quantified twice. Concentrations are expressed as mean ± SD. The absence of a cross-reactivity of IFN-α with this technique was examined using a 4 MIU mL^{-1} IFN-α solution, which showed no significant measurable values.

Active digoxin metabolites are recognized by the assay technique (cross-reactivity from 50 to 80%) (37,38), but they are equally transported by P-gp (39) and are submitted to the same course as nonmetabolized digoxin.

Pharmacokinetics Modeling

Pharmacokinetics calculations were performed via WinNonLin Professional 4.1 software (Pharsight Corporation, Mountain View, CA, USA). Plasma concentration profiles of digoxin were analyzed by noncompartmental analysis

and computed by the linear trapezoidal rule with mono-exponential extrapolation to infinite time to calculate AUC (Area Under the Curve, from the time of dosing to the last measurable concentration), F (relative bioavailability, i.e. the ratio AUC *per os*/AUC IV), Cl (total body clearance), and V_D (apparent volume of distribution). C_0 (initial concentration, assimilated to the first observed concentration after i.v. bolus) was estimated by back-extrapolating from the first two concentration values. Parameter values were expressed as mean ± SD.

Semiquantitative Determination of P-gp Expression by Western Blot Analysis

P-gp expression was measured in control and IFN-α-treated (4 MIU kg^{-1}) animals. Rats were killed immediately after the last sampling, then segments of intestine (duodenum, jejunum, and ileum), liver, and kidney were rapidly and separately removed and washed with ice-cold isotonic saline containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and 20 μg mL^{-1} aprotinine as protease inhibitors. Intestinal mucosae were scraped on ice with a slide glass. Obtained mucosae, liver, and kidney fragments were homogenized using a glass Teflon potter (10 strokes) in a buffer containing 250 mM sucrose, 50 mM Tris–HCl pH 7.4, 1 mM PMSF, and appropriate dilution of Protease Inhibitor Cocktail (product P8340; Sigma-Aldrich, St. Louis, MO, USA). Homogenates were centrifuged 10 min at 3000 *g*, and the supernatant was again centrifuged for 30 min at 15,000 *g*. The pellets containing the crude membranes were resuspended in 0.5 ml of a buffer containing 50 mM mannitol, 50 mM Tris pH 7.4, 1 mM PMSF, and 20 μg mL^{-1} aprotinin and stored at −80°C until use. Protein concentrations were determined using the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, USA) with bovine serum albumin (BSA; Sigma-Aldrich) as standard.

Crude membrane suspensions were diluted to a final protein concentration of 0.5 μg μL^{-1} in 1% Triton and placed at 4°C during 30 min for P-gp solubilization. A 10-μL volume of loading buffer (240 mM Tris–HCl pH 6.8, 8% SDS, 40% glycerol, 0.1% bromphenol blue, and 20% 2-mercaptoethanol) was added to 40 μL of each solubilized sample. Fifteen micrograms (liver, ileum and jejunum extracts) or 30 μg (kidneys and duodenum extracts) of proteins were separated through a 7.5% SDS-polyacrylamide gel. After electrophoresis, the proteins were electrophoretically transferred to a nitrocellulose membrane. The membrane was blocked in phosphate-buffered saline containing 0.1% Tween 20 (PBST) and 10% skim dried

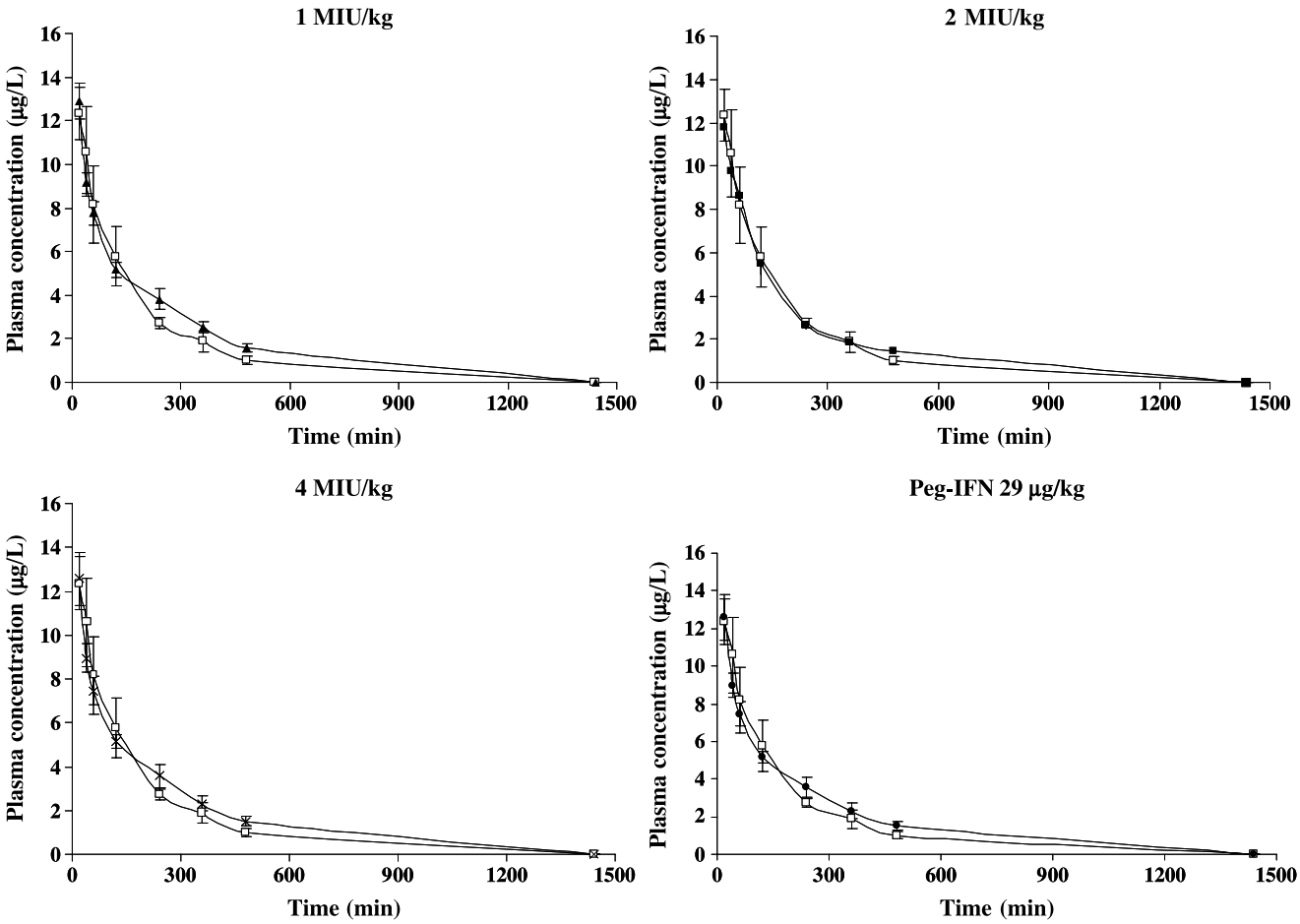

Fig. 1. Plasma time–concentration curves of digoxin in i.v. injected rats (unfitted data). Rats were s.c. pretreated with saline solution (□, control group) or rhIFN-α: 1 MIU kg^{-1} (▲), 2 MIU kg^{-1} (■), 4 MIU kg^{-1} (×), and Pegylated IFN-α 29 μg kg^{-1} (●). Digoxin was i.v. injected at a dose of 32 μg kg^{-1}. The values represent the means ± SD.

Table I. Pharmacokinetics Parameters After i.v. Digoxin Administration[a]

Parameters[b]	Control	1 MIU kg^{-1}	2 MIU kg^{-1}	4 MIU kg^{-1}	Peg-IFN-α
C_0 (μg L^{-1})	14.1 ± 3.2	12.9 ± 1.7	14.4 ± 3.0	16.3 ± 4.4	18.0 ± 5.9
AUC (min μg L^{-1})	2016 ± 338	2068 ± 254	2087 ± 422	2390 ± 369	2169 ± 369
Cl (mL min^{-1} kg^{-1})	14.7 ± 2.2	12.9 ± 1.7	13.7 ± 2.4	12.3 ± 2.0	12.7 ± 2.4
V_D (L kg^{-1})	3.0 ± 0.6	3.6 ± 0.6	3.3 ± 0.8	2.7 ± 0.5	3.5 ± 0.7

[a] Rats were given i.v. 32 μg kg^{-1} digoxin, once.
[b] AUC, Cl, and V_D values are not significantly different in pretreated and control groups.

milk for 2 h at room temperature, and washed twice for 5 min in PBST and incubated overnight at 4°C with primary monoclonal antibody C219 (Dako, Glostrup, Denmark) diluted 100-fold in PBST–1% BSA. After washes in PBST buffer, the membrane was incubated for 2 h at room temperature with a secondary mouse anti-IgG polyclonal antibody conjugated to peroxidase (Biosys, Boussens, France) and diluted 1000-fold in PBST–1% BSA. P-gp protein in crude membranes was detected by chemiluminescence using Enhanced Chemiluminescence Western Blot reagents (Perkin-Elmer Life Sciences, Boston, MA, USA). The membrane was scanned and the intensities of the bands were estimated with Scion Imaging Software (Scion Corporation, Frederick, MD, USA). Two blots were done for each organ. Results were expressed as relative band intensities (treated/control ratio) ± SD.

Statistical Calculations

As plasma concentrations were normalized to body weight, the homogeneity of animal weight was checked with a one-way ANOVA test.

Pharmacokinetics data obtained for each treated group were compared to those of the control group by a two-tailed Student's t test for unpaired samples. A p value of less than

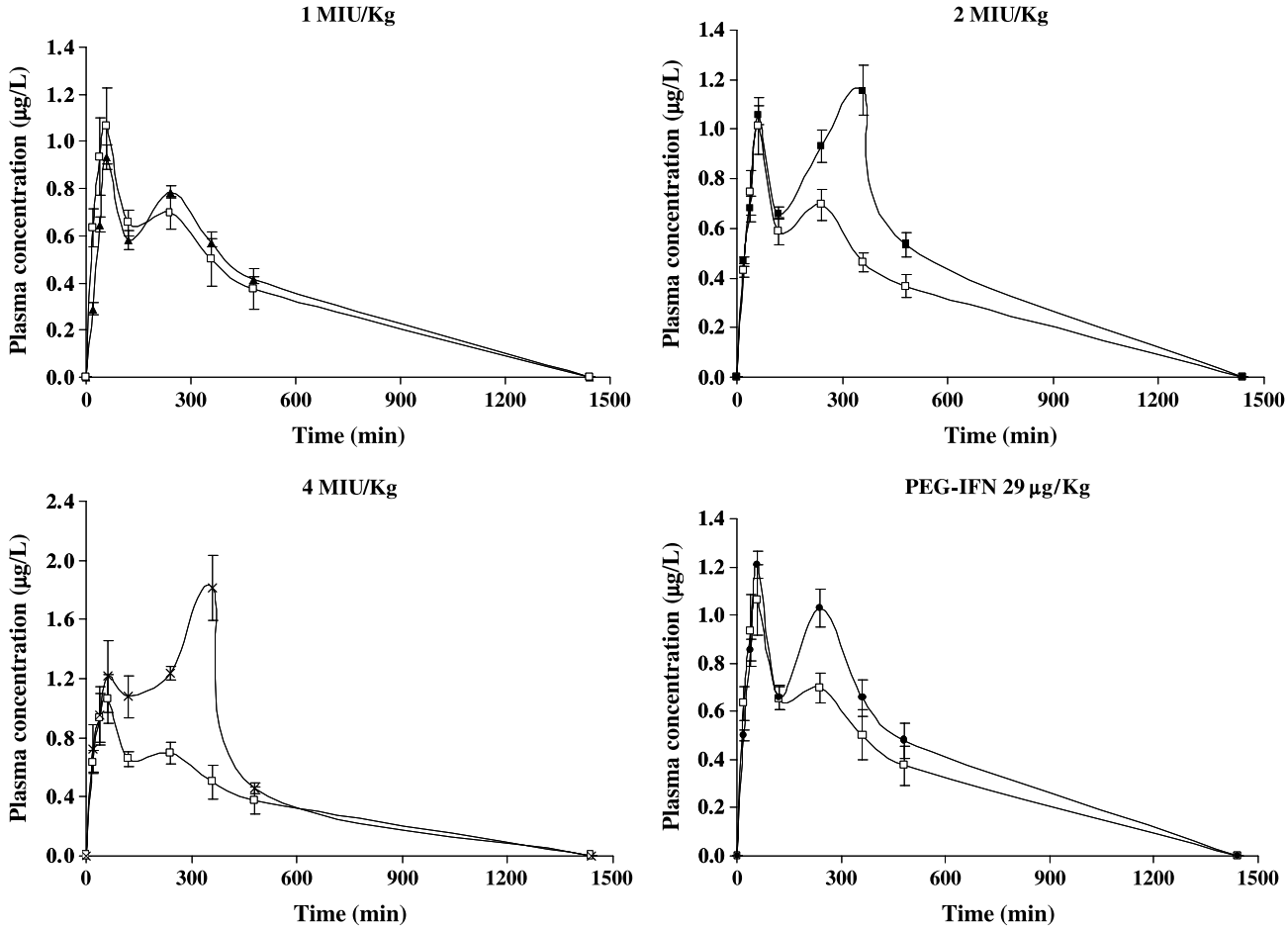

Fig. 2. Plasma time–concentration curve of digoxin in orally administrated rats (unfitted data). Rats were s.c. pretreated with saline solution (□, control group) or rhIFN-α: 1 MIU kg^{-1} (▲), 2 MIU kg^{-1} (■), 4 MIU kg^{-1} (×), and Pegylated IFN-α 29 μg kg^{-1} (●). Digoxin was orally given at a dose of 32 μg kg^{-1}. The values represent the means ± SD.

Table II. Pharmacokinetics Parameters After Oral Digoxin Administration[a]

Parameters	Control	1 MIU kg^{-1}	2 MIU kg^{-1}	4 MIU kg^{-1}	Peg-IFN-α
C_{max} (μg L^{-1})	1.08 ± 0.30	0.93 ± 0.12	1.06 ± 0.0.08	1.22 ± 0.48	1.21 ± 0.11
C_2^b (μg L^{-1})	0.71 ± 0.12	0.78 ± 0.06	1.16 ± 0.23***	1.81 ± 0.44***	1.03 ± 0.16**
AUC (min μg L^{-1})	286 ± 111	286 ± 50	392 ± 83**	550 ± 97***	354 ± 101
AUC-treated/AUC	–	1.0	1.37	1.92	1.23
F (%)	14.2	13.8	18.8	23	16.3

Treated *vs*. control: $^{**}p < 0.01$, $^{***}p < 0.001$.
[a] Rats were given *per os* 32 μg kg^{-1} digoxin, once.
[b] C_2: peak corresponding to the second peak.

0.05 was considered statistically significant. Statistical calculations were made with JMP 5.1 software (SAS Institute, Cary, NC, USA).

RESULTS

Body weight was not statistically different among the different groups (one-way ANOVA test, $p = 0.45$ for the orally treated animals and $p = 0.48$ for the i.v. treated animals).

Effect of Interferon-α Pretreatment on i.v. Digoxin Pharmacokinetics

Figure 1 shows the time–concentration course of plasma digoxin after i.v. injection into control, nonpegylated, and pegylated IFN-α pretreated rats. Digoxin concentrations decreased in an exponential way with time in all groups and AUC, Cl, or V_D values were not significantly different in pretreated and control groups (Table I).

Effect of Interferon-α Pretreatment on *per os* Digoxin Pharmacokinetics

Figure 2 shows the time course of digoxin plasma concentrations after oral administration in control, pegylated, and nonpegylated IFN-α pretreated rats. In the control group, a first absorption peak was observed approximately 60 min after digoxin administration; a decrease phase was followed by a smaller peak (C_2) about 240 min after administration (since digoxin plasma concentration had increased), and finally, concentration diminished in an exponential way. The shape of the curve was the same as in the IFN-α pretreated groups. As shown in Table II, C_2 values were comparable in control and 1 MIU kg^{-1} pretreated groups (0.71 ± 0.12 and 0.78 ± 0.07 μg L^{-1}, respectively). C_2 was higher in the

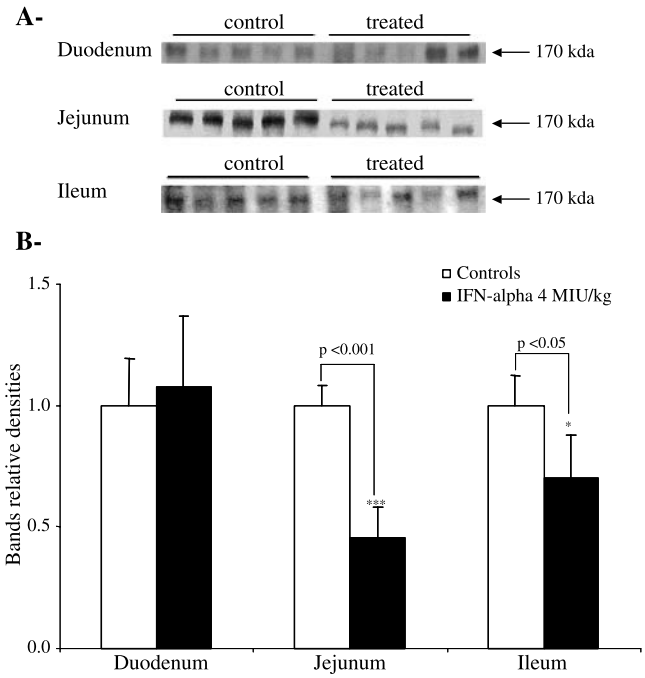

Fig. 3. (A) Western blot analysis of membrane P-gp (about 170 kDa) from duodenum, ileum, and jejunum. Rats were s.c. pretreated with saline solution (□, control group) or 4 MIU kg^{-1} of rhIFN-α (■). (B) Relative intensities are shown for each intestinal segment of control and 4 MIU kg^{-1} IFN-α-treated rats.

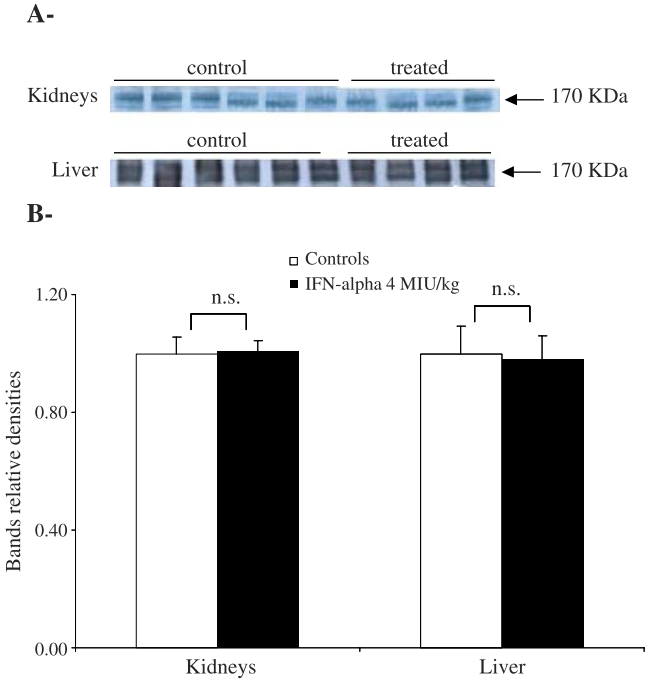

Fig. 4. (A) Western blot analysis of membrane P-gp (about 170 kDa) from kidneys and liver. Rats were s.c. pretreated with saline solution (□, control group) or 4 MIU kg^{-1} of rhIFN-α (■). (B) Relative intensities are shown for each organ of control and 4 MIU kg^{-1} IFN-α treated rats. n.s.: Not significantly different.

pegylated pretreated group (1.03 ± 0.16 μg L^{-1}). In the 2 and 4 MIU kg^{-1} pretreated rats, the peak occurred later, about 360 min after administration, and became the major peak (1.16 ± 0.2 and 1.81 ± 0.44 μg L^{-1}, respectively, compared to 0.71 ± 0.12 μg L^{-1} for the control group). No significant differences were noted between control, 1 MIU kg^{-1}, and pegylated INF-α pretreated rat AUCs (286 ± 111, 286 ± 50, and 354 ± 101 min μg L^{-1}, respectively). AUCs were significantly higher in the 2 and 4 MIU kg^{-1} pretreated groups than in control [392 ± 83 min μg L^{-1} ($p < 0.01$) and 550 ± 97 min μg L^{-1} ($p < 0.001$), respectively, compared to 286 ± 111 min μg L^{-1} for the control group]. Consequently, the relative bioavailability F increased considerably in the two last groups (18.8 and 23.0%, respectively, compared to 14.2% for control).

In addition to these data, a short-term study was carried out (1 day IFN-α 4 MIU kg^{-1} treatment) and results did not show any significant differences in the pharmacokinetics parameters (data not shown).

Western Blot Analysis

Figure 3 shows the P-gp expression in different intestine segments of control and 4 MIU kg^{-1} IFN-α injected rats. IFN-α failed to modify P-gp expression in duodenum, but was able to reduce it in the ileum (0.70 ± 0.19 for 4 MIU kg^{-1} IFN-α treated animals *vs.* 1.00 ± 0.13 for controls, $p < 0.05$) and decrease it more markedly in the jejunum (0.46 ± 0.13 for 4 MIU kg^{-1} IFN-α treated animals *vs.* 1.00 ± 0.08 for controls, $p < 0.001$).

Figure 4 shows P-gp expression in liver and kidneys obtained from treated and untreated animals. IFN-α did not significantly modify P-gp expression.

DISCUSSION

Digoxin is a classical substrate for P-gp screening activity (29) because its disposition is tightly linked to P-gp activity (14). P-gp is mainly responsible for the intestinal absorption and renal elimination phases of orally administered digoxin (8,10,40). The rat model was chosen as an *in vivo* model because human IFN-α has no effect on CYP3A expression or activity in rat; however, this does not necessarily hold true for mice, where IFN-α seems to have a complex regulatory effect on some mouse cytochrome subfamilies (41). In addition, although interferon-α is known to have a potential downregulatory effect on CYPs, this downregulation was shown to be species-dependent and particularly marked in mice but not in rats (42).

We observed that time–plasma concentration curves of orally administered digoxin presented an unexpected profile, mainly with the appearance of a second peak. This profile has already been described in the digoxin pharmacokinetics in mice (18). This particular shape of the curve can be explained by a site-selective digoxin absorption. As drug absorption in rats may vary dramatically between the different segments of the gastrointestinal tract (43), the presence of a second peak indicates a reabsorption phase, attributable to a later absorption of digoxin in the distal parts of the intestine. Digoxin is extensively absorbed in the distal part of the bowel (44), and its absorption in rat small intestine is site-dependent and linked to P-gp activity, which varies all along the intestine (increasing from duodenum to ileum) (45). Western blot studies performed in wild, untreated animals confirmed that P-glycoprotein expression increases from proximal to distal small intestine (46).

Digoxin is mainly eliminated in bile (up to 71% of an i.v. dose) (47). Hence, the hypothesis of an enterohepatic recycling could also explain the second absorption phase, as reported in humans (48) and suspected in other species such as horses (49). However, the enterohepatic recycling in rats does not significantly concern digoxin, but rather only its metabolites (50). Furthermore, the absence of a reabsorption phase in the i.v. digoxin-treated animals (Fig. 1) supports this hypothesis, because an eventual digoxin biliary excretion should have been observed both in orally and in i.v. digoxin-treated animals, and consequently, enteric reabsorption should have been observed in both groups, which was not the case in this study.

IFN-α treatment had no effect on i.v. administered digoxin disposition. The i.v. route bypasses the oral absorption phase, and only modifications of the elimination phases could be visualized on i.v. profiles. Digoxin is mainly eliminated by kidneys and secreted by P-glycoprotein located on the luminal membrane of renal tubular epithelial cells (10). $Mdr1a^{-/-}$ mice injected with radiolabeled digoxin had 20- to 50-fold higher levels of radioactivity in their brain and 2-fold higher levels in the other tissues associated to a slower elimination than in wild-type mice, thus confirming the essential role of P-gp in digoxin renal elimination (28). Kidneys are not the only pathway for digoxin elimination. About 16% of a digoxin i.v. dose was excreted in gut lumen of mice within 90 min, whereas the expression decreased to 2% in $mdr1a^{-/-}$ mice, confirming the role of mesenteric P-gp in eliminating digoxin (40). In the current study, the absence of significant modifications in i.v. digoxin plasma curves suggests that IFN-α pretreatment with the doses used does not affect the renal or mesenteric excretion phases. These functional arguments are supported by the P-gp expression study in kidneys, which shows no significant differences between treated and untreated animals (Fig. 4).

IFN-α induced a dose-dependent increase in the bioavailability of orally administered digoxin. No changes were observed after the administration of 1 MIU kg^{-1} IFN-α, whereas AUC and relative bioavailabilities increased significantly after 2 and 4 MIU kg^{-1} (1.4- and 1.9-fold, respectively). Pegylated IFN-α given at 29 μg kg^{-1} approximately corresponds to a 2 MIU kg^{-1} activity (32). It did not significantly modify digoxin bioavailability, but considerably increased the value of the second peak.

This increase could be explained by the hypothesis of an enhancement in digoxin oxidative metabolism by human recombinant IFN-α (rhIFN-α), because digoxin is extensively metabolized in rat and the immunoenzymatic assay used is also reactive with digoxin metabolites. However, this hypothesis should be roughly excluded because several papers have yet shown that rhIFN-α has no effect on CYP 3A activity and liver metabolism in rat (23,51,52), and Western blot studies in liver has not shown any modification in P-gp expression after treatment with interferon (Fig. 4).

Digoxin bioavailability is also mainly affected by P-gp and the increase in its absorption has to be linked to a decrease in intestinal P-gp expression and/or activity in in-

testines and then digoxin excretion in the intestinal lumen. Western blot analysis has proven that the increase in digoxin absorption by IFN-α was associated to a reduction of P-gp expression, which was not observed in duodenum but was important in jejunum and, to lesser degree, in ileum. The diminution of the protein expression in the jejunal segment explains the effect of IFN-α on the second peak, which corresponds to a later phase of absorption. Therefore, IFN-α substantially inhibits P-glycoprotein expression in limited segments of the intestine: jejunum and ileum. This concept of anatomical region-dependent modification of P-gp was already proposed to account for the inhibiting effect of some drugs on intestinal P-gp—for instance, Quinidine increases ileal and jejunal digoxin absorption by inhibiting P-gp (8). Increase in digoxin disposition after ketoconazole coadministration was similarly explained (53). Besides the reduction of P-gp expression in these intestinal segments, modifications of P-gp activity should not be excluded. The shift of the second peak with higher doses of IFN-α may be attributable to an additional effect of the inhibition of P-gp activity on a large part of gut segments.

The mechanistic pathways of IFN-α interaction with P-gp are not yet clearly established. Some elements of explanation have been recently proposed by Miracco *et al.*, who demonstrated that IFN-α may modulate ABCB1 gene transcripts in human melanoma cell lines by modulating the telomerase-associated protein (TEP1) and MDR1 mRNA (54). The modifications observed in digoxin pharmacokinetics after IFN-α treatment may not be only attributable to the modulation of P-gp expression: IFN-β, another type 1 interferon, modulates Caco-2 monolayer cell's permeability through an effect on paracellular transport (55). A comparable hypothesis for IFN-α could not be excluded.

Our results could have important clinical relevance. IFN-α is widely used in cancer and antiviral therapy and could be associated to P-gp substrates such as anticancer drugs (cytarabine, doxorubicine, vincristine, etc.) or antiviral drugs (indinavir, amprenavir, efavirenz, etc.) especially in coinfected hepatitis C/HIV patients. Increase in the bioavailability of P-gp substrates associated to IFN-α may consequently lead to an increase in their efficiency and/or toxicity.

In conclusion, using an *in vivo* model, the digoxin-pharmacokinetics model, we observed a significant dose-dependent inhibitory effect of IFN-α on intestinal P-gp activity. Such results could explain the interactions between IFN-α and P-gp substrates. The digoxin absorption increase was associated with modifications in tissular P-gp expression, mainly with a decreased jejunal membrane P-gp expression. Further studies are necessary to assess the impact of IFN-α on other tissues and on other P-gp substrates to open the way toward clinical applications.

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