JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 401–408

Received July 21, 2006
Accepted November 2, 2006
DOI 10.1211/jpp.59.3.0010
ISSN 0022-3573

Bioavailability and tissular distribution of docetaxel, a P-glycoprotein substrate, are modified by interferon-α in rats

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Abstract

Interferon-α (IFN-α) inhibits intestinal P-glycoprotein (P-gp) expression in rats. In the present study, the effects of repeated pre-treatment with recombinant human INF-α (rhIFN-α) on oral and intravenous pharmacokinetics of a P-gp substrate, docetaxel (DTX; Taxotere) were investigated in a rat model. The bioavailability and distribution in different organs were also studied. Sprague-Dawley rats were subcutaneously pre-treated with either rhIFN-α for 8 days (4 MIU kg^{-1}, once daily) or with pegylated-IFN-α (ViraferonPeg; 60 μg kg^{-1}, Days 1, 4 and 7). The rats were then distributed into subgroups (n = 5–6) according to the pre-treatment type, and received one dose of [^{14}C]DTX (20 mg kg^{-1}) either orally or intravenously. Pharmacokinetics studies were then performed over 240 min, at the end of which tissues (intestine, liver, kidneys, lung, heart and brain) were immediately removed for radioactivity quantitation. Non-pegylated and pegylated IFN-α both increased DTX oral bioavailability parameters: C_{max} (17.0 ± 4.0 μg L^{-1} ($P < 0.02$) and 18 ± 5.5 μg L^{-1} ($P < 0.05$), respectively, vs 7.4 ± 2.5 μg L^{-1} for the control) and AUC (0.036 ± 0.010 μg h mL^{-1} ($P < 0.01$) and 0.033 ± 0.009 μg h mL^{-1} ($P < 0.01$), respectively, versus 0.012 ± 0.004 μg h mL^{-1} for the control). IFN-α also delayed DTX absorption from 60 min in controls to about 95 min and 80 min in non-pegylated and pegylated treated animals, respectively. However, IFN-α did not affect intravenous DTX pharmacokinetics and it had a limited effect on tissue distribution at 240 min. [^{14}C]DTX was decreased in intestine and enhanced in brain in both pre-treated groups. rhIFN-α modified the P-gp-dependent pharmacokinetics of DTX, limited its intestinal efflux and markedly enhanced its oral bioavailability.

Introduction

Docetaxel (DTX, Taxotere) is a semi-synthetic compound isolated from the needles of the European yew *Taxus baccata*. DTX belongs to the taxanes family, anti cancer agents that bind to β-tubulin, thereby stabilizing microtubules and inducing cell-cycle arrest, apoptosis and acute cytotoxicity (Pellegrini & Budman 2005). DTX is widely indicated in various solid malignancies, including gastrointestinal, lung, breast, prostate and ovarian cancers (Montero et al 2005). It is commonly administrated intravenously since its oral absorption is low: about 3.6% in mice (Bardelmeijer et al 2002) and 8% in humans (Malingre et al 2001). In fact, the oral route exposes DTX to extensive intestinal metabolism due to cytochrome P450 3A subfamily isoenzymes (CYP 3A) (Gaillard et al 1994; Marre et al 1996). The lack of efficiency of the oral route is additionally linked to the effect of P-glycoprotein (P-gp) (Wils et al 1994). P-gp or ABCB1 protein, the MDR1 multidrug-resistant transporter, is a 170-kDa membrane protein product of the ABCB1 gene. It is present in the apical surface of epithelial cells of the gastrointestinal tract and acts as an efflux protein, pumping DTX into the gut lumen and hence limiting its absorption (Wils et al 1994; van Zuylen et al 2000; Malingre et al 2001). Besides the gastrointestinal tract, P-gp is physiologically widely distributed in tissues (Thiebaut et al 1987) and acts as a detoxifying pump, extruding toxic endogenous compounds and xenobiotics from cells (Lin & Yamazaki 2003). P-gp is also an inducible protein: cell exposure to various xenobiotics increases its tissular expression, enhancing their cellular clearance and provoking resistance to these compounds. In cancer therapy, the overexpression of P-gp is responsible for cross-resistance between numerous antitumour P-gp substrates, such as *vinca* derivates, etoposide, anthracyclines and taxanes

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Acknowledgments: The authors are grateful to Paul Chavalier (Sanofi-Aventis Pharma) for kindly providing [^{14}C]docetaxel. We thank Hélène Chacun (University of Paris-Sud, Faculty of Pharmacy, UMR 8612) for technical advice on radioactive sample treatment and Simone Orbach-Arbouys for helpful discussions on the manuscript.

(Kim 2002). Thus, overcoming P-gp and limiting its activity should increase DTX absorption, tissular penetration and thus its anti-tumour efficiency (Malingre et al 2001; Kemper et al 2004). Several reports have described the impact of P-gp inhibitor association on DTX pharmacokinetics.

Experiments performed in wild mice, showed that co-administration of P-gp inhibitors, cyclosporine A, valspodar (PSC833) and elacidar (GF120918), increases DTX (33 mg kg^{-1}) concentrations in brain by up to 38%, 56% and 59%, respectively (Kemper et al 2004). Co-administration of cyclosporine A (15 mg kg^{-1}) with DTX (75 mg m^{-2}) in patients with solid tumours increases its bioavailability from 8 ± 6% to 90 ± 44% (Malingre et al 2001). Recently, OC144-93, a P-gp blocker, was tested in patients presenting various resistant malignancies. Combined with orally administrated DTX (100 mg/dose), this P-gp inhibitor increased the oral bioavailability of DTX to 26% compared with 8% in the reference population (Kuppens et al 2005). Thus, the use of P-gp inhibitors appears to be a useful way to increase DTX oral absorption and improve systemic exposure to this drug. We have previously demonstrated that some cytokines have an inhibitory effect on P-gp. We showed that interleukin-2 (IL-2) reduced P-gp functionality in mice and enhanced the oral bioavailability of digoxin (a P-gp substrate) up to 61% compared with 43% for wild mice (Castagne et al 2004). The improved digoxin absorption after IL-2 pre-treatment was associated with a reduction in P-gp intestinal expression (Bonhomme-Faivre et al 2002). More recently, we investigated the effect of another cytokine, recombinant human interferon-α (rhIFN-α), on P-gp expression (Ben Reguiga et al 2005). IFN-α, a member of the type 1 interferon family, is a widely used cytokine in antiviral and cancer therapy. We showed in Sprague-Dawley rats that rhIFN-α pre-treatment (IntronA 10^6 (MIU), 2 MIU or 4 MIU kg^{-1} and pegylated-rhIFN-α (ViraferonPeg) 29 mg kg^{-1}) decreased intestinal P-gp expression associated with a dose-dependent improvement of the bioavailability of digoxin, a compound transported essentially by the MDR1 protein. These results formed the rationale for exploring the effect of rhIFN-α administration on the pharmacokinetics of other P-gp substrates, particularly those with clinical relevance. We report here the influence of pegylated and non-pegylated rhIFN-α repeated pre-treatment on the oral and intravenous pharmacokinetics of [^{14}C]DTX in a rat model, and on its bioavailability and distribution in several organs.

Materials and Methods

Chemicals

Taxotere (40 mg mL^{-1} of DTX solubilized in polysorbate 80) was purchased from Aventis Pharma (Vitry sur Seine, France). [^{14}C]DTX (1.5 GBq $mmol^{-1}$, 96.5% high-performance liquid chromatography purity) was a kind gift from the same company. Taxotere was added to the radiolabelled compound and appropriately diluted in ethanol (European Pharmaceutical Grade; Fluka, Mulhouse, France) to obtain a final solution of 10 mg mL^{-1} and 750 kBq mL^{-1}. The specific activity of the final solution was 75 kBq mg^{-1} of [^{14}C]DTX.

Pegylated and non-pegylated recombinant human IFN-α2b (ViraferonPeg and IntronA, respectively) were purchased from Schering-Plough (Hérouville-Saint-Clair, France). The specific activity of the pegylated rhIFN-α used in the experiment was 7×10^7 IU (mg protein)$^{-1}$ (Peg-Inton (Peg-interferon α-2b): product information from Schering Corporation, Kenilworth, NJ, USA). The molecular weight of the pegylated molecule was approximately 31 kDa, including 12 kDa for the PEG moiety.

Animals

Female adult Sprague-Dawley rats (180–200 g) were obtained from Charles River Company (L'Arbresle, France). They were fed on standard laboratory food ad libitum during the 2 weeks before the pharmacokinetics study. Animal handling and experimentation were performed in accordance with the good practice guidelines for the administration of substances and removal of blood in laboratory animals issued by the European Economic Community and detailed by Diehl et al (2001) (Ethical Committee of Paris-XI University approval – Authorization L1800, Hauts-de-Seine Department of Veterinary Services).

Animal pre-treatment

Thirty-six rats were randomized into three groups according to the nature of their pre-treatment: non-pegylated rhIFN-α (IntronA), pegylated rhIFN-α (ViraferonPeg) or daily phosphate saline solution. Each group of rats was divided into two subgroups (n = 6 per subgroup) according to the route of administration for the pharmacokinetics studies (intravenous and oral routes). Rats treated with IntronA received over 8 days a single daily subcutaneous dose of 4 MIU kg^{-1} rhIFN-α. Since ViraferonPeg is usually administered once a week in humans (Baker 2003) and since pegylated rhIFN-α clearance is greater in rats than in humans (Lave et al 1995), rats receiving pegylated rhIFN-α were subcutaneously treated three times a week (Days 1, 4 and 7) with a 60 μg kg^{-1} dose, which was equivalent to 4 MIU kg^{-1} of non-pegylated rhIFN-α (Schering Corporation 2003). The interferon doses were formerly defined in reference to the toxicity of rhIFN-α in rats (LD50 is $>24 \times 10^8$ IU kg^{-1}) (Ben Reguiga et al 2005) and to the usual dose given for cancer therapy in humans (an 18 MIU/dose for a man with 1.8 m^2 body surface area corresponds to a 2.3 MIU kg^{-1} dose in a rat; FDA Oncology Tools dose calculator; http://www.fda.gov/cder/cancer/index.htm).

Pharmacokinetics study

Pharmacokinetics studies were performed on Day 8. To avoid interference of food with DTX pharmacokinetics, animals were fasted overnight with free access to water prior to the experiments.

Rats received a single dose of DTX (20 mg kg^{-1}) either orally or as an intravenous bolus injection. Blood samples (300 μL) were collected by jugular cannulation (Tinsley et al 1983) in heparinized vials at 0, 20, 40, 60, 90, 120, 180 and 240 min. Blood was immediately centrifuged and the plasma was frozen at −15°C until analysis.

Immediately after the last sampling time, rats were killed by carbon monoxide and jejunum, liver, kidney, lungs, heart and brain were rapidly removed, washed three times in saline solution baths and then stored at −15°C.

Sample treatment

Plasma and tissues samples were treated with the same procedure. Plasma (100 μL) and carefully weighed samples (80–100 μg for various organs, 250–300 μg for brain) were mixed with 900 μL Soluene 350 (Perkin Elmer, MA, USA) and incubated overnight at 60°C. The samples were then left for 1 h at room temperature to cool and 300 μL 30% hydrogen peroxide was added to reduce quenching. Gentle agitation allowed the foaming reaction to subside during 30 min at room temperature. Vials were finally incubated for 1 h at 60°C and then cooled to room temperature before the addition of 10 mL of Hionic-Fluor (Perkin Elmer, MA, USA) scintillation cocktail. Radioactivity was counted (during 20 min per sample) in a liquid scintillation counter (LS6000TA; Beckman, Fullerton, CA, USA). Radioactivity was expressed in disintegrations per min (dpm). The limit of detection of the method was 60 dpm. Since DTX metabolites are not reabsorbed in plasma (Marlard et al 1993; Bissery et al 1995), the radioactivity found in plasma corresponds almost exclusively to [^{14}C]DTX. This allows the conversion of plasma radioactivity values into DTX plasma concentration, taking into account the specific activity of the administrated solution. The linearity study performed on the plasma dilution of [^{14}C]DTX showed a linearity range from 50 ng mL^{-1} to 10^5 ng mL^{-1} and a limit of quantification of 50 ng mL^{-1}. Plasma results were expressed as mean ± s.d. in μg L^{-1} and tissues results were expressed as mean ± s.d. of counts (g tissue)$^{-1}$.

Pharmacokinetics modelling

WinNonLin Professional 4.1 software (Pharsight Corporation, CA, USA) was used to determine pharmacokinetics parameters. The intravenous DTX data were analysed following a two-compartment model with bolus input and first-order output, according to the equation:

$$C(t) = A \times \exp^{(-\alpha t)} + B \times \exp^{(-\beta t)}$$

The parameters A, B (A and B are intercept constants), $t½_\alpha$ (initial half-life), $t½_\beta$ (terminal half-life) and C_0 (initial concentration, assimilated to the first observed concentration after i.v. bolus) were calculated. A non-compartmental analysis was computed by the linear trapezoidal rule. We thus determined for both intravenous and oral routes AUCs (area under the curve, from the first time of dosing to the last measurable concentration) and calculated F (the relative bioavailability, i.e. the ratio of AUC p.o./AUC i.v.) and K_T of brain (ratio: brain concentration/plasma concentration) at time 240 min. For the orally treated rats, we also determined C_{max} (maximal absorption peak concentration), T_{max} (time of maximal absorption peak), CL (total body clearance, CL = dose/AUC), and V_D (apparent volume of distribution, $V_D = (\text{dose} \times t½_\beta)/(\text{Ln2} \times \text{AUC})$. Values are expressed as mean ± s.d.

Statistical analysis

Plasma concentrations were adjusted to bodyweight, animal weight homogeneity was checked with a one-way analysis of variance test. Pharmacokinetic data and tissue concentrations were statistically analysed using a Tukey multiple test comparison. A P value less than 0.05 was considered statistically significant (SPSS software version 10; SPSS Inc. Chicago, IL, USA).

Results

Effect of rhIFN-α pre-treatment on intravenous [^{14}C]DTX pharmacokinetics

Figure 1 shows the concentration–time profile of plasma [^{14}C]DTX after intravenous injection in control, pegylated and non-pegylated IFN-α pre-treated rats. Data were fitted with a two-compartment model (Table 1). In control rats, data analysis showed a rapid elimination phase ($t½_\alpha$: 19.0 ± 5.8 min) followed by a slower one ($t½_\beta$: 207.4 ± 46.8 min), with the appearance of a little absorption peak at about 180 min. Pharmacokinetic parameters obtained in the pre-treated rats were not significantly different compared with those of the control rats.

Effect of rhIFN-α pre-treatment on intravenous [^{14}C]DTX tissue distribution

Organ radioactivity was comparable in liver, kidneys, lung and heart between all groups (Figure 2). [^{14}C]DTX was greatly diminished in intestine: $3.9 \times 10^3 \pm 1.3 \times 10^3$ counts g^{-1} and $3.2 \times 10^3 \pm 1.4 \times 10^3$ counts g^{-1} ($P < 0.01$) versus $25 \times 10^3 \pm 9.1 \times 10^3$ counts g^{-1} ($P < 0.01$) for non-pegylated, pegylated and control groups, respectively.

On the contrary, brain concentrations were higher in both pre-treated groups: $47.6 \times 10^1 \pm 1.1 \times 10^1$ counts g^{-1} ($P < 0.01$) and $51.0 \times 10^1 \pm 8 \times 10^1$ counts g^{-1} ($P < 0.01$) versus

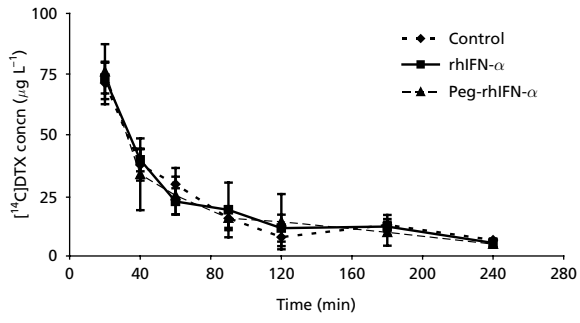

Figure 1 Plasma concentration–time curves of rats intravenously injected with 20 mg kg^{-1} [^{14}C]docetaxel ([^{14}C]DTX) (unfitted data). Rats were subcutaneously pre-treated with saline solution (control group, n = 5), 4 MIU kg^{-1} rhIFN-α (n = 6) or 60 μg kg^{-1} pegylated IFN-α (Peg-IFN-α) (n = 6). Values represent mean ± s.d.

Table 1 Pharmacokinetics parameters for intravenously administrated [^{14}C]docetaxel (20 mg kg^{-1}) in rats

	AUC (μg h mL^{-1})	C_0 (μg L^{-1})	A (μg L^{-1})	B (μg L^{-1})	t½α (min)	t½β (min)
Control (n = 5)	0.101 ± 0.015	139.3 ± 27.8	124.3 ± 25.9	15.0 ± 6.4	19.0 ± 5.8	207.4 ± 46.8
rhIFN-α 4 MIU kg^{-1} (n = 6)	0.097 ± 0.005	153.1 ± 22.5	139.1 ± 25.0	19.7 ± 6.5	16.4 ± 2.2	160.7 ± 63.2
Pegylated rhIFN-α 60 g kg^{-1} (n = 6)	0.108 ± 0.011	159.4 ± 34.1	97.8 ± 41.0	16.8 ± 10.7	18.4 ± 2.9	152.9 ± 98.6

Values are not significantly different between the different groups.

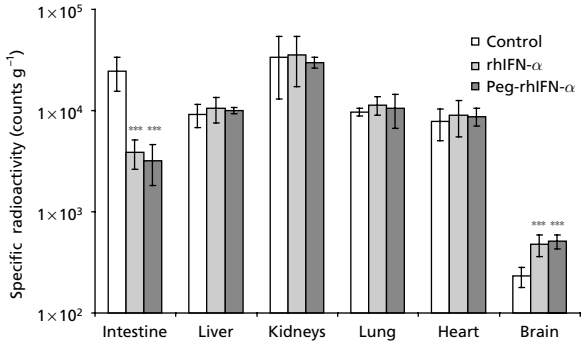

Figure 2 Tissue radioactivity in organs of rats intravenously injected with 20 mg kg^{-1} [^{14}C]docetaxel ([^{14}C]DTX) (logarithmic scaled y-axis). Rats were subcutaneously pre-treated with saline solution (control group, n = 5), 4 MIU kg^{-1} rhIFN-α (n = 6) or 60 μg kg^{-1} pegylated IFN-α (Peg-IFN-α) (n = 6). Values represent mean ± s.d. ***$P < 0.01$ significantly different compared with the control group.

$23.1 \times 10^1 \pm 4.3 \times 10^1$ counts g^{-1} for control group. The increase in brain DTX concentrations was confirmed by an increase of the brain/plasma partition values: $2.7 \times 10^{-1} \pm 4.6 \times 10^{-2}$ ($P < 0.01$) and $2.2 \times 10^{-1} \pm 4.6 \times 10^{-2}$ ($P < 0.01$) versus $7.9 \times 10^{-2} \pm 2.1 \times 10^{-2}$ for non-pegylated, pegylated and control groups, respectively (Figure 3).

Effect of rhIFN-α pre-treatment on oral [^{14}C]DTX pharmacokinetics

Figure 4 shows the concentration–time profile of plasma [^{14}C]DTX after oral administration in control, pegylated and non-pegylated IFN-α pre-treated rats. In the control group, the concentration–time profile indicated a peak 60 min after [^{14}C]DTX administration, followed by an exponential decrease. In the rhIFN-α pre-treated group, the maximum absorption time (t_{max}) appeared later (about 95.0 ± 22 min after administration), associated with a rise in absorption parameters (Table 2): C_{max} 17.0 ± 4.0 μg L^{-1} ($P < 0.02$) and AUC 2160 ± 617 min μg L^{-1} ($P < 0.02$) versus 7.4 ± 2.5 μg L^{-1} and 735.7 ± 254.5 min μg L^{-1} for the control. This led to an increase in the relative oral bioavailability (37.2% vs 10.4% for control, i.e. 3.4 ratio). Comparable results were obtained in rats pre-treated with pegylated rhIFN-α: t_{max} was delayed to 80 min ± 15.5 min ($P < 0.05$), C_{max} increased to 18 ± 5.5 μg L^{-1} ($P < 0.05$) and AUC increased to 1967 ± 551.8 min μg L^{-1}. The elimination half-lives were, however, not significantly different between the three groups.

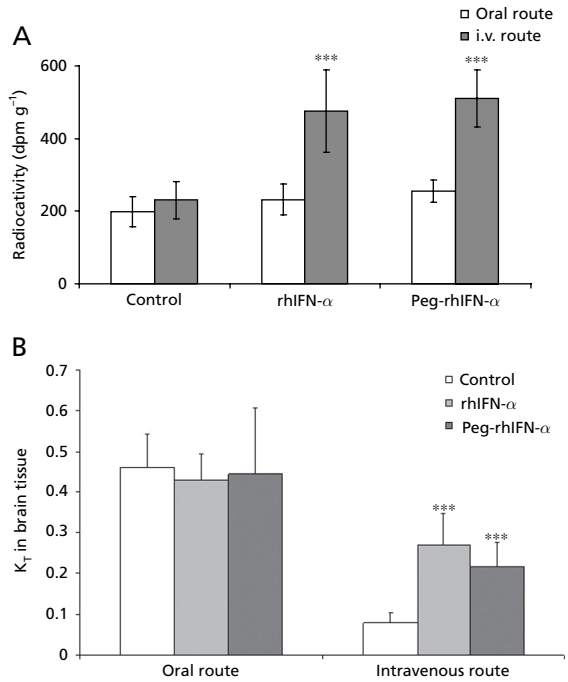

Figure 3 A. Radioactivity in the brain after oral or intravenous administration of [^{14}C]docetaxel ([^{14}C]DTX) in control, rhIFN-α (4 MIU kg^{-1}) or pegylated rhIFN-α (Peg-IFN-α) (60 μg kg^{-1}) pre-treated rats. Values represent mean ± s.d. (non-logarithmic scaled y-axis; significant data are reported in Figures 2 and 4). B. Brain/plasma radioactivity partition (K_T) after oral or intravenous administration of [^{14}C]DTX in control, rhIFN-α (4 MIU kg^{-1}) or pegylated IFN-α (Peg-IFN-α) (60 μg kg^{-1}) pre-treated rats. Values represent mean ± s.d. (logarithmic scaled y-axis). ***$P < 0.01$ significantly different compared with the control group.

Effect of rhIFN-α pre-treatment on oral [^{14}C]DTX tissue distribution

No differences were observed in the radioactivity of the different tissues (intestine, liver, kidneys, lung, heart and brain) between pre-treated and treated groups (Figure 5).

Discussion

In a previous study, we showed that IFN-α inhibits the expression and activity of intestinal P-gp, thus increasing the absorption of digoxin, a P-gp substrate (Ben Reguiga et al 2005). Since taxane antimitotics are P-gp substrates, it seemed reasonable to explore the effect of IFN-α on the

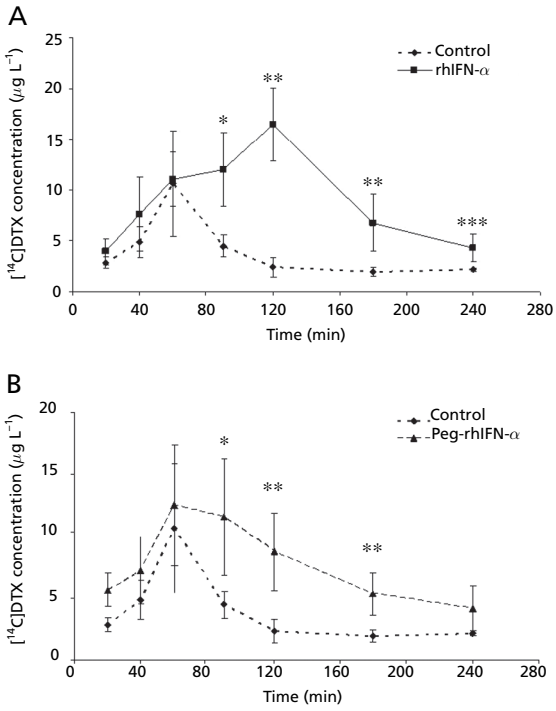

Figure 4 Plasma concentration–time curves of rats after oral administration of 20 mg kg^{-1} [^{14}C]docetaxel ([^{14}C]DTX) (unfitted data). Rats were subcutaneously pre-treated with saline solution (control group, n = 5), 4 MIU kg^{-1} rhIFN-α (n = 6) or 60 μg kg^{-1} pegylated IFN-α (Peg-IFN-α) (n = 5). Values represent mean ± s.d. *$P < 0.05$, **$P < 0.02$, ***$P < 0.01$ significantly different compared with the control group.

pharmacokinetics of DTX, to which it may be associated in anti-cancer therapy.

Although DTX is metabolized by CYP3A family isoenzymes in rats, mice and humans (Marre et al 1996; Nallani et al 2001), we choose to use the rat model in the present study because DTX metabolites are not found to a significant extent in rat plasma, contrary to what is observed in mice (Bissery et al 1995; Monsarrat et al 1997). This property limited metabolite interference in the [^{14}C]DTX radioactivity measurements. The DTX used in our experiments is formulated with polysorbate 80, a vehicle that has insignificant interactions with P-gp in-vivo (Webster et al 1997; van Tellingen et al 1999), which is not the case for Cremophor EL, the vehicle used for paclitaxel in commercial formulations.

In the present study, IFN-α treatment showed no effect on intravenously administered DTX pharmacokinetics. The elimination parameters were comparable between treated and untreated groups. Elimination half lives in the control group (19 min and 207 min) were greater than those previously published (4 min and 42 min; Sandstrom et al 1999), values determined in a 90-min pharmacokinetics study with a 5 mg kg^{-1} dose. These differences are related to the higher dose of DTX used in our study (20 mg kg^{-1}, about 120 mg m^{-2}) and the longer duration of the pharmacokinetics study (240 min). Indeed, at these dose levels, the pharmacokinetics of DTX becomes non-linear, with a slowed elimination and a prolonged half-life (Bissery et al 1995). In addition, DTX undergoes an enterohepatic recycling (that does not exceed 13% of the total radioactivity), which appears late (about 180 min after administration), prolonging the presence of the DTX in plasma and increasing its elimination half-life (Bruno & Sanderink 1993).

The intravenous pharmacokinetics study bypasses the gastrointestinal absorption phase and only modifications of the elimination phase can be highlighted. Since intravenously administrated DTX is mainly eliminated in bile and, to a lesser extent, by the kidneys (Bissery et al 1995), the absence of modification of intravenous DTX pharmacokinetics in the treated groups agrees with our previous findings, where we showed that IFN-α (4 MIU kg^{-1}) had no effect on hepatic and renal P-gp expression (Ben Reguiga et al 2005). In addition, even if DTX is extensively metabolized by hepatic CYPs in rats compared with humans (Vaclavikova et al 2003), several studies indicate that rhIFN-α does not modify CYP3A activity in rats (Craig et al 1989; Melzer et al 1994; Goerz et al 1995).

The oral pharmacokinetic studies provided interesting results since both forms of IFN-α showed a significant increase of DTX absorption and bioavailability, from 10.4% in the control to 37.2% and 30.2% in the non-pegylated and pegylated treated groups, respectively. In addition, the maximum of the oral absorption phase was delayed to 95 min and 80 min in the non-pegylated and pegylated treated groups, respectively, compared with 60 min for the control group. This confirms the hypothesis that IFN-α improves oral absorption of P-gp substrates such as DTX and digoxin by inhibiting the P-gp present in the last parts of the bowel, especially in the jejunum.

While IFN-α influenced oral DTX pharmacokinetics, no significant differences on tissues distribution were seen 240 min after DTX administration. This is probably due to

Table 2 Pharmacokinetics parameters for orally administrated [^{14}C]docetaxel (20 mg kg^{-1}) in rats

	C_{max} (μg L^{-1})	t_{max} (min)	t½ (min)	AUC (μg h mL^{-1})	AUC treated/ AUC control	F (%)	V_D (mL)	CL (mL min^{-1})
Control (n = 5)	7.4 ± 2.5	60.0	14.48 ± 4.01	0.012 ± 0.004	–	10.4%	560.2 ± 106.6	4.5 ± 0.7
rhIFN-α 4 MIU kg^{-1} (n = 6)	17.0 ± 4.0**	95.0 ± 22.6**	11.84 ± 1.92	0.036 ± 0.010***	3.4	37.2%	256.3 ± 119.1***	2.2 ± 0.8***
Pegylated rhIFN-α 60 μg kg^{-1} (n = 5)	18.6 ± 5.5**	80.0 ± 15.5	13.37 ± 3.84	0.033 ± 0.009***	3.1	30.2%	289.5 ± 217.3***	2.3 ± 1.0***

*$P < 0.05$, **$P < 0.02$, ***$P < 0.01$, significantly different compared with respective controls.

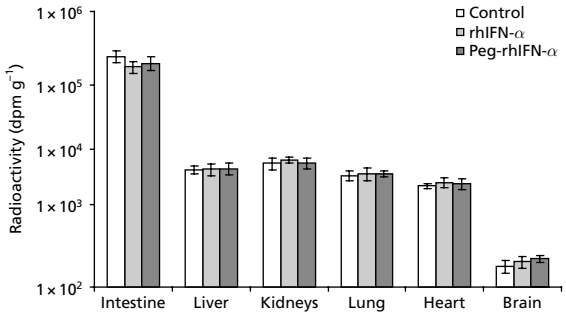

Figure 5 Tissue radioactivity in organs of rats after oral administration of 20 mg kg^{-1} [^{14}C]docetaxel ([^{14}C]DTX) (logarithmic scaled y-axis). Rats were subcutaneously pre-treated with saline solution (control group, n = 5), 4 MIU kg^{-1} rhIFN-α (n = 6) or pegylated IFN-α 60 μg kg^{-1} (Peg-IFN-α) (n = 5). Values represent mean ± s.d.

statistical considerations. Indeed, the radioactive counting error e ($e = \sqrt{N}/N$, where N = counts per min (cpm); e.g. N = 100 cpm, e = 10%) is important as the radioactivity measurements are low (Sprawls 1995). In our case, the radioactivity measurements in tissues and especially in brain were low (10^2 to 10^3 cpm). Thus, the counting errors were significant, preventing differences between tissues being highlighted.

After intravenous administration, a significant reduction in the DTX concentration in the intestines was found. The presence of radioactivity in the intestines is due to hepatobiliary elimination and the P-gp intestinal efflux of DTX into the lumen (Wils et al 1994; van Zuylen et al 2000). Since IFN-α has no effect on cytochrome metabolism (Craig et al 1989; Pageaux et al 1998) and therefore on the radioactive quantity of metabolites eliminated in bile, the reduction in radioactivity in intestines after IFN pre-treatment is attributable to the decrease in intestinal P-gp expression (Ben Reguiga et al 2005), which causes a decrease in DTX intestinal efflux. However, no differences in the intestinal radioactivity were observed in the orally treated groups, owing to the significant amount of radioactivity remaining after oral administration, which probably prevented significant results being observed.

IFN-α pre-treatment significantly increased brain DTX penetration when DTX was given intravenously but not when given orally (Figure 5). The lack of effect after oral administration could be due to the smaller amount of radioactivity present in the brain in this case, with values close to the limit of detection of the method leading to a greater variability and a lower sensitivity of the measures. However, the amelioration of the DTX cerebral diffusion after intravenous administration remained significant in spite of the low values of the measurements. Such results were also observed with Elacridar (GF120918), a P-gp inhibitor, which increased approximately 60% of the cerebral passage of intravenously administrated DTX (Kemper et al 2004). In the present study, the amelioration of the passage cannot be due only to P-gp inhibition. In fact, studies have showed that several cytokines, such as IFN-γ or tumour necrosis factor-α, are able to increase the cerebral passage of several drugs (not necessarily P-gp substrates) by modulating the permeability of the human blood–brain barrier (Wong et al 2004).

The mechanisms by which IFN-α affects P-gp expression or activity are not yet clear. Type I interferons are known to modulate ABCB1 gene transcripts in human melanoma cell lines by modulating the telomerase-associated protein and MDR1 mRNA (Miracco et al 2003), and have an additional effect on paracellular transport (Kawaguchi et al 2005). We also made the hypothesis of a direct action on nuclear transcription factors. In fact, the MDR1 gene is regulated by several factors, such as ubiquitous transcription factors NF-κB (Bentires-Alj et al 2003) and Sp-1 (Sundseth et al 1997). Several reports showed that specific inhibition of SP-1 (Allen et al 2004; Zhan et al 2005) or NF-κB (Chen et al 2005) represses P-gp expression or activity. Since IFN-α is a potent inhibitor of Sp-1 transactivation (Rosewicz et al 2004) and modulates NF-κB expression (Kramer et al 2006), our results may be explained by the P-gp inhibitory effect of IFN-α on these nuclear pathways.

Conclusion

The present study provided evidence of an in-vivo interaction between IFN-α and DTX, a P-gp substrate anti cancer agent. rhIFN-α increased DTX oral absorption in rats and therefore its bioavailability, without affecting its elimination phase. IFN-α also increased DTX brain penetration after intravenous administration, potentially amplifying its neurological side-effects. IFN-α is widely used in cancer therapy and our findings could have pertinent clinical consequences. The findings need to be confirmed in other animal species and in humans, and the toxicity of such an association must be evaluated. Thus, rhIFN-α may have potential as an anti-cancer adjuvant to increase the bioavailability of orally administered P-gp substrates such as DTX, the oral route being the more comfortable and safer route for cancer patients.

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