THE ANTI-CANCER DRUG DABRAFENIB IS A POTENT ACTIVATOR OF THE HUMAN PREGNANE X RECEPTOR

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ABSTRACT

The human pregnane X receptor (hPXR) is activated by a large set of endogenous and exogenous compounds and play a critical role in the control of detoxifying enzymes and transporters regulating liver and gastrointestinal drug metabolism and clearance. hPXR is also involved in both the development of multidrug resistance and enhanced cancer cells aggressiveness. Moreover, its unintentional activation by pharmaceutical drugs can mediate drug-drug interactions and cause severe adverse events. In that context, the potential of the anticancer BRAF inhibitor dabrafenib suspected to activate PXR has not been thoroughly investigated yet. Using different reporter cellular assays, we demonstrate that dabrafenib can activate hPXR as efficiently as its reference agonist SR12813 whereas it does not activate mouse or zebrafish PXR. We also showed that dabrafenib binds to recombinant PXR, induces the expression of PXR responsive genes in colon LS174T cancer cells overexpressing hPXR and human hepatocytes and finally increase the proliferation in LS174T-hPXR cells. Our study reveal that using a panel of different cellular techniques it is possible to improve the assessment of PXR agonist activity for new developed drugs.

Keywords : dabrafenib, hPXR, colon and liver cancer cells, proliferation.

1. INTRODUCTION

Because cancer patients are usually treated with a combination of medications, a critical issue of the drug development process is the potential existence of drug-drug interactions (DDIs). DDIs can directly impact drugs' pharmacokinetics and result in reduced efficacy of the treatment or in the occurrence of severe adverse events. In accordance with FDA (Food and Drug Administration) and EMA (European Medicines Agency) guidelines, such a risk can be assessed by evaluating the potential of any new drug to act as inducer of clinically relevant metabolizing enzymes such as cytochrome P450 or UGT enzymes (1).

Since the human nuclear receptor pregnane X receptor (hPXR) and constitutive androstane receptor (CAR) are considered as master regulators of most of these metabolizing enzymes or transporters at the transcriptional level, they could indirectly modulate the activity of various drugs and be responsible for DDIs when they are co-administered (2). This is particularly true for pharmaceuticals and anticancer drugs such as paclitaxel, flutamide or tamoxifen which can activate hPXR and hCAR (3-5). Chemoresistance to these agents was often associated with an activation of hPXR and hCAR and the expression of its target genes in various cancer cell types such as ovary, prostate, liver, intestinal and colon (6-13). Other compounds that are routinely co-administrated with anticancer agents such as corticosteroids, anticoagulants, analgesics, antibiotics, antiemetics, anticonvulsants or antiepileptics were also shown to activate hPXR and hCAR (4, 14). It is thus critical to take into account the potential of hPXR and hCAR activation in the rational development of anticancer drug combinations to limit DDIs and reduce the risk of adverse events. Although the mechanism is not fully elucidated, hPXR and hCAR may also be involved in the modulation of tumour progression. Indeed, the ectopic expression of hPXR stimulates cell proliferation of HepG2 liver cancer cells (15). Furthermore, *in vitro* and *in*

vivo activation of endogenous hPXR in colon cancer cells (16, 17) also results in enhanced cell growth.

Dabrafenib is a reversible, highly potent ATP-competitive inhibitor of the BRAF serine/threonine kinase involved in the regulation of the mitogen-activated protein kinase (MAPK) pathway. Dabrafenib was developed to specifically target the V600E activating mutation of BRAF ($BRAF^{V600E}$) that is observed in more than 40% of melanomas and probably contributes to the progression of the disease. In vitro studies reported IC_{50} values of 0.65 nM for $BRAF^{V600E}$ (18).

Dabrafenib is currently approved alone or in combination with the MEK inhibitor trametinib for the treatment of metastatic melanoma (19). Interestingly, the prescription notice of dabrafenib indicates that it is metabolized by CYP2C8 and CYP3A4, while its metabolites hydroxy-dabrafenib and desmethyl-dabrafenib are CYP3A4 substrates (20). In addition, dabrafenib is described as an inducer of CYP3A4, CYP2C9, CYP2C8, CYP2C19 and of UGT enzymes that could lead to a decrease in the intracellular concentrations of their respective substrates (16, 20). While these effects may be due to hPXR or hCAR mediated transcriptional effects, the possibility that dabrafenib could directly affect the activity of these nuclear receptors has not been fully investigated yet.

In this work and using a panel of binding, reporter and proliferation cellular assays and RT-qPCR experiments in LS174T-PXR and primary human hepatocytes. we were able to characterize the PXR activity of dabrafenib

2. MATERIAL AND METHODS

a. Reagents and chemicals

6-(4-Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl) oxime (CITCO), clotrimazol, Pregnenolone 16 alpha-carbonitrile (PCN) and SR12813, were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France).

Dabrafenib was purchased from Euromedex (Souffelweyersheim, France). SPA70 was purchased from Axon (Groningen, The Nederlands). Drugs were solubilized in DMSO at 10 mM and stored at -20°C.

b. PXR and CAR reporter cell lines

The different reporter cell lines used in this study are summarized in the supplementary table 1. HG5LN GAL4-m/zfPXR cells were obtained as previously reported for HG5LN GAL4-hPXR cells (21, 22). Briefly, Hela cells were stably transfected with the GAL4RE$_5$-βGlob-Luc-SVNeo plasmid alone or together with the pSG5-GAL4(DBD)-h/m/zfPXR(LBD)-puro plasmids leading to the HG5LN and HG5LN-h/m/zfPXR cell lines, respectively. HG5LN GAL4-hCAR and GAL4-hCAR-APYLT cells were obtained in the same way. The LS174T-hPXR and HepG2-hPXR reporter cell lines were previously described (22). These cells were obtained through stable co-transfection of HepG2-C3 and LS174T cells with the pcDNA3.1-hPXR (1-434)-neomycin plasmid and the XREM-CYP3A4-luciferase-hygromycin reporter plasmid. The prostate cancer 22RV1-hPXR cell line was obtained using the same protocol.

Cells were cultured at 37°C under humidified 5% CO2 atmosphere. HeLa cells were cultured in phenol red (DMEM)-F12 medium (Thermofisher, Villebon sur Yvette, France) supplemented with 5% fetal calf serum (FCS), 1% penicillin/streptomycin (100 U/mL), 0.5 µg/ml puromycin and 1 mg/ml G418. LS174T and HepG2 cells were cultured in phenol red (DMEM)-F12 mediumsupplemented with 5% FCS, 1% penicillin/streptomycin (100 U/mL), 1 mg/ml G418 and 0.25 mg/ml hygromycin. For HepG2 cells culture medium was supplemented with sodium pyruvate (1%), hepes (1%) and non-essential amino acid (1%). 22RV-1 cells were cultured in RPMI medium supplemented with 5% FCS, 1% penicillin/streptomycin, 0.5 µg/ml puromycin and 1 mg/ml G418.

The induction of luciferase activity by PXR ligands was stable for more than 3 months for the different reporter cell lines in culture which corresponds to 10-15 passages. During these passages, we did not observed significant variability in luciferase induction in both absolute luminescence and fold induction.

c. Transactivation assays

Cells were seeded in 96-well white opaque flat bottom plates at 40,000 cells per well in 150 µL of DMEM-F12 without phenol red supplemented with penicillin/streptomycin (1%) and dextran-coated charcoal-treated foetal calf serum (DCC-FCS) (5%) (test medium). Compounds were added 24h later using automated workstation (Biomek 3000, Beckman Coulter, Villepinte, Paris) and cells were incubated at 37°C for 16h. Then, medium was removed and 50 µL of test medium containing luciferin at 0.3 mM were added per well. Luciferase activity was measured for 2s in intact living cells after 10 min stabilization using a MicroBetaWallac luminometer (PerkinElmer). Each compound was tested at various concentrations in at least three independent experiments. For each experiment, tests were performed in quadruplicates for each concentration, and data are expressed as means values with standard deviations. Individual agonist dose-response curves were fitted using the sigmoid dose-response function of GraphPad Prism (version 5.0). The equation used by Graphpad was :

Y=Bottom +(Top-Bottom)/(1+10^((LogEC50-X)*HillSlope)).

X is the log of concentration. Y is the response.

EC_{50} (effective concentration for half-maximal luciferase activity) and IC_{50} (half-maximal inhibitory concentration) values were calculated. To analyse significances, we compared individual compound treatments with controls using one-way analysis of variance (ANOVA) using the GraphPad Prism software.

d. LS174T-hPXR proliferation assay (P-SCREEN)

We developed a new proliferation test that we called the P-screen to measure the effects of compounds with PXR activity on the growth of LS174T-hPXR cells. This test uses the same principle as that for E-screen that was used for measuring the effects of estrogens on the proliferation of MCF-7 breast cancer cells (23). Briefly, LS174T-hPXR cells were seeded in 96-well plates (2000 cells per well) in test medium containing the PXR antagonist SPA70 at 100 nM for 24 hours. This pre-treatment with SPA70 was performed in order to reduce the basal proliferation of the cells, thus allowing a more sensitive detection of any PXR agonist activity that would result in growth induction. The medium is then removed, and cells are treated with increasing concentrations of the different compounds for 7 days. Medium is removed and 0.1 mL of MTT solution (0.5 mg/mL) was added to each well. The MTT-containing medium was removed 4h after incubation and DMSO was added to each well. After shaking, the plates were read in absorbance at 540 nm. Results were expressed as percentage of proliferation with respect to the hormone-free control (100%). Data were obtained by dose–response curves plotted as percentage of proliferation as a function of concentrations.

The activation of the proliferation by PXR ligands was stable for more than 3 months for the LS174T-PXR cell line in culture which corresponds to 10-15 passages. During these passages, we did not observed significant variability in proliferation induction.

e. ***Lanthascreen TR-FRET PXR competitive binding assay*.**

GST-hPXR-LBD (10 nM) was incubated with different concentrations (10–30 μM) of dabrafenib and SR12813 in the presence of Fluormone PXR ligand (40 nM) and Lanthascreen terbium-anti-GST antibody (10 nM). To read a LanthaScreen TR-FRET assay, the fluorimeter (PHERAstar FS; BMG LABTECH) is configured to excite the terbium donor around 340 nm, and to separately read the terbium emission peak that is centered at B490 nm, and the fluorescein emission that is centered at B520 nm. Results are expressed as the signal from the fluorescein emission divided by the terbium signal to provide a TR-FRET emission ratio. Fluorescence ratio data were fitted using a sigmoidal dose–response model using GraphPad Prism (GraphPad Software Inc.).

f. Isolation and primary culture of human hepatocytes

Liver samples (supplementary table 2) were obtained from the Biologic Resource Center of Montpellier University Hospital (CRB-CHUM; http://www.chumontpellier.fr; Biobank ID: BB-0033-00031), and this study benefitted from the expertise of Dr Benjamin Rivière (hepatogastroenterology sample collection) and Dr Edouard Tuaillon (CRB-CHUM manager). They were obtained from liver of adult patients who underwent liver resections for medical reasons unrelated to our research program, or from anonymous donors when the liver was considered unsuitable for organ transplantation. The use of human specimens for scientific purposes was approved by the French National Ethics Committee. Written or oral informed consent was obtained from each patient or family prior to surgery. The clinical characteristics of the liver donors are presented in Supplementary Table 1. Hepatocytes were isolated by using a two-step perfusion protocol and cultured as described previously (24).

g. Real Time-Quantitative Polymerase Chain Reaction (RT-QPCR)

Total RNA was isolated and purified using "Zymo Research - Quick RNA mini prep" kit for LS174T-PXR cells and using Trizol reagent (Life Technologies) for primary human hepatocytes and HepaRG according to the manufacturer's protocol. The concentration and the purity of isolated RNA were measured using a spectrophotometer NanoVue (GE health care life sciences, Velizy-Villacoublay, France). Reverse transcription was performed with the qScript cDNA SuperMix (Quantabio) for LS174-PXR and HepaRG cells and using a random hexaprimer and the MMLV Reverse Transcriptase Kit (Life Technologies) for primary human hepatocytes. Quantitative polymerase chain reactions were performed using SYBR green (Qiagen or Roche)) and specific primers (Supplementary Table 3) with the LightCycler®-480 real time PCR system (Roche). The relative amount of RNA was calculated with the $2^{\Delta\Delta}$CT method and gene expression was normalized using GAPDH or RPLP0. In Figure 5A and B, the level of expression was compared with the mean level of the corresponding gene expression in DMSO treated cells and expressed as n-fold ratio.

3. RESULTS

a. Transactivation of PXR by dabrafenib in human cancer cell lines

We first evaluated the potential transactivation of PXR by dabrafenib in several reporter cell lines. Since PXR ligands are usually characterized by marked cross-species differences, the ability of dabrafenib to activate several PXR orthologs was assessed in HG5LN control cells and in HG5LN cells stably expressing human, mouse or zebrafish GAL4(DBD)-PXR(LBD). Dabrafenib (3 μM) was compared to known PXR agonists such as SR12813 (3 μM), PCN (3 μM) or clotrimazol (1 μM). The results show that dabrafenib is a strong and specific agonist of human PXR with a 5-fold increase in luciferase expression (Fig. 1B). Conversely, dabrafenib could not or slightly activate the zebrafish or the mouse PXR LBD (Fig. 1C and 1D). Specific agonist effects were also evidenced for SR12813 on

the human PXR LBD and for PCN on the mouse PXR LBD, while clotrimazole strongly activate the zebrafish PXR (5-fold) and has only a moderate (2-fold) effect on the human PXR LBD (Fig. 1). The ability of dabrafenib to modulate the activity of hCAR was also assessed in HeLa cells stably expressing human CAR WT or the mutant CAR (+APYLT) that displays a reduced basal activity. No agonist or antagonist activity of dabrafenib on CAR was observed (Fig. 1E and 1F).

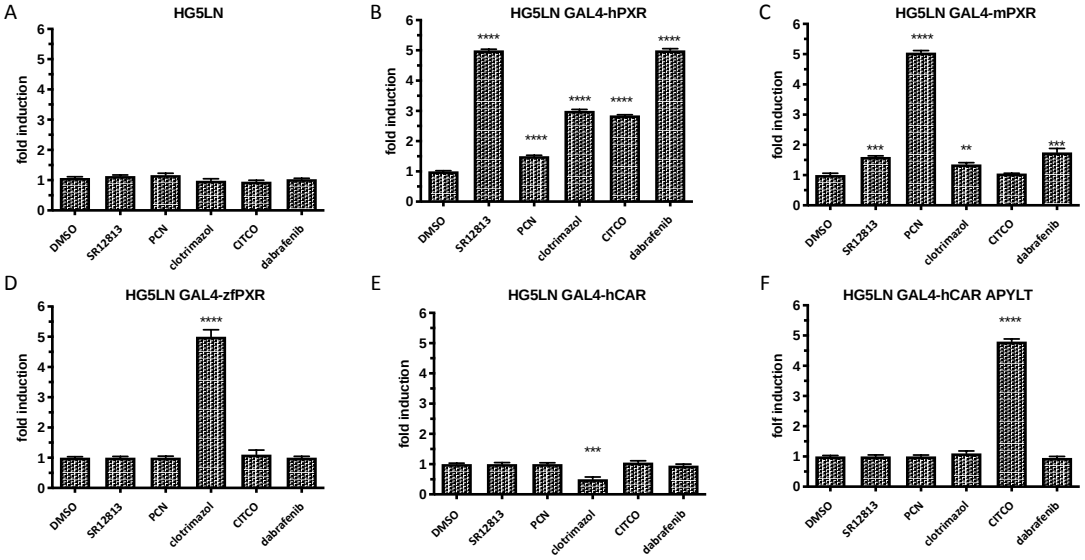


Fig. 1

Figure 1: Effects of dabrafenib on the activation of human, mouse, zebrafish PXR and human CAR nuclear receptors as measured by luciferase reporter assay. Activation has been measured in (A) HG5LN control cells or HG5LN cells expressing GAL4 fusion with (B) human (hPXR), (C) mouse (mPXR) or (D) zebrafish (zfPXR) PXR or human (E) wild type (hCAR) or (F) mutated (hCAR APYLT) hCAR LBD treated by DMSO (0,1%), SR12813 3 μM, PCN 3 μM, clotrimazol 1 μM, CITCO 1 μM and dabrafenib 3 μM. Results are expressed as fold induction as compared to control. Data are expressed as mean ± sd of three independent experiments, **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$ (Student's t-test) compared with DMSO treated cells.

We then performed dose-response experiments in different hPXR reporter cell lines to compare the agonist activity of dabrafenib to the activity of SR12813 and EC_{50} values were calculated (Fig. 2). In HG5LN GAL4-hPXR cells, the EC_{50} value for dabrafenib (82 nM) was approximately 3-fold lower than that of SR12813 (239 nM), confirming the strong agonist potency of dabrafenib for hPXR (Fig. 2A and supplementary table 2). Similar results were obtained in the human colon LS174T (EC_{50} of 110 nM), liver HepG2 (EC_{50} of 97.6 nM) and prostate 22RV1 (EC_{50} of 98.4 nM) carcinoma cell lines stably overexpressing hPXR (Fig. 2B-D and supplementary table 4). SR12813-induced hPXR transactivation was also slightly different for all tested cell lines with EC_{50}s ranging from 125 to 434 nM (supplementary table 4). Similar results were obtained in terms of efficacy as dabrafenib behaved as a full agonist in all reporter cell lines. We observed a decrease of transactivation at concentrations higher than 3 μM that may be attributed to its toxicity which was more pronounced in HepG2 cells than in the other cell lines (Fig. 2). No induction of luciferase expression was observed in HG5LN control cells (supplementary Fig 1). Furthermore, the PXR antagonist SPA70 (24) could abrogate dabrafenib-induced luciferase expression in HG5LN PXR cells (supplementary Fig 1). Together, these results demonstrate that dabrafenib is a strong activator of the transcriptional activity of human PXR. One should also note that the PXR antagonist SPA70 decreased the basal luciferase expression in HeLa cells (supplementary Fig 1), indicating that PXR is partially activated in its apo form.

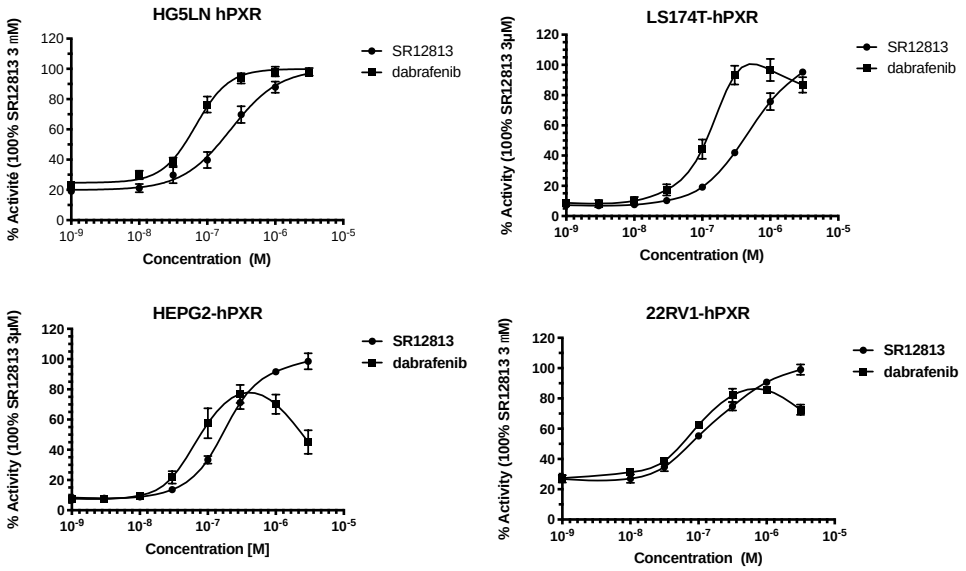


Fig. 2

Figure 2: Dabrafenib is a potent activator of human PXR in different cell lines. Results of luciferase assays showing dose-response curves for SR12813 and dabrafenib in (A) HG5LN Gal4-hPXR, (B) LS174T-hPXR, (C) HepG2-PXR and (D) 22RV1-PXR reporter cell lines. Results are expressed as a percentage of the maximal response obtained in the presence of 3 µM SR12813. Data are the mean ± sd of three to five independent experiments.

b. ***Time-resolved fluorescence energy transfer experiments***.

We next assessed the binding characteristics of dabrafenib to PXR using competitive binding assays with time resolved fluorescence resonance energy transfer between a fluorescent PXR ligand and purified hPXR-LBD (LanthaScreen TR-FRET PXR Competitive Binding Assay). The results confirmed that dabrafenib directly binds to hPXR with an affinity that is slightly lower than that of SR121813 (Fig. 3). The fact that in transactivation experiments, dabrafenib is slightly more potent than SR12813 could indicate a potentially better bioavailability compared to SR12813.

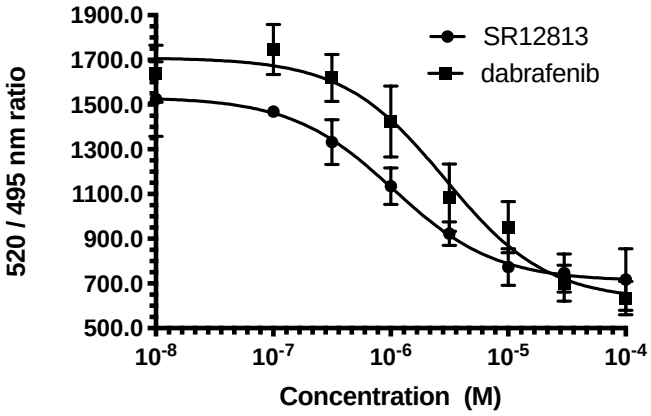


Fig 3.

Figure 3: Dabarafenib binds to hPXR. Inhibition of FRET between fluorescein-labelled PXR ligand and recombinant GST-PXR by SR12813, TNC and EE2, alone or in combination. Results are expressed as the signal from the fluorescein emission divided by the terbium signal to provide a TR-FRET emission ratio. Data are the mean (± SEM) from three independent experiments.

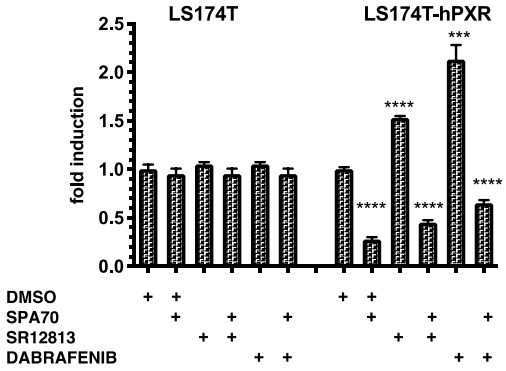
c. Induction of hPXR-mediated proliferation by dabrafenib

Since PXR activation is known to stimulate proliferation of human colon cancer cell lines (16), we investigated whether dabrafenib could affect the growth of LS174T-PXR cells. To that purpose, proliferation of LS174T control cells and LS174T-hPXR cells were compared in the absence or in the presence of the PXR antagonist SPA70, SR12813 or dabrafenib using MTT assays (Fig. 4 A). As shown, in LS174T control cells, the

proliferation was not affected by PXR ligands. On the contrary, the basal proliferation of LS174T-hPXR cells was significantly reduced by the PXR antagonist SPA70 indicating that PXR is partially activated on proliferation in its apo form. Proliferation was also significantly increased by SR12813 and dabrafenib. Curiously, the efficacy of dabrafenib was higher than that of SR1813 (2.2-fold vs 1.5-fold). Both activation of proliferation was abrogated in presence of SPA70 confirming that this proliferation is mediated through hPXR.

We developed a new test that we referred to as the P-screen test to specifically assess compounds with PXR activity that could induce cell proliferation (see Materials and Methods). The novelty of this test resides in the fact that LS174T-hPXR cells were pretreated 24h with SPA70 100 nM and then treated with the potential PXR agonist. The P-Screen was performed using increasing concentrations of dabrafenib and SR12813 (1 nM-10 μM). We confirmed that dabrafenib was more efficient than SR12813 at inhibiting the proliferation of LS174T-hPXR cells (4.56-fold vs 3.2-fold). Conversely, the potency of both PXR agonists was very similar (24 and 38 nM for dabrafenib and SR12813, respectively), which is in accordance with their EC50 for luciferase expression (82 and 98 nM for dabrafenib and SR12813, respectively).

A



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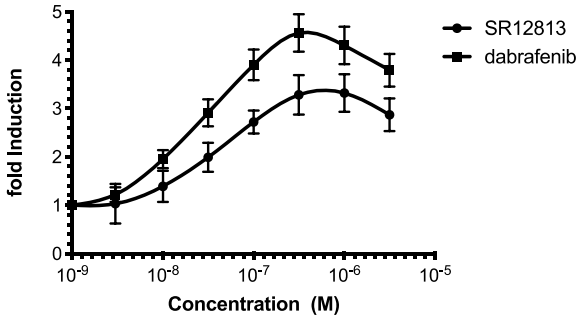

FIG 4.

Figure 4: Effects of dabrafenib and SR12813 on the proliferation of LS174T-HPXR cells. (A). LS174T control cells and LS174T hPXR cells were treated with DMSO (0.1%), SPA70 3 μM, SR12813 0.3 μM, SR12813 0.3 μM + SPA70 3 μM, dabrafenib 0.3 μM and dabrafenib 0.3 μM + SPA70 3 μM for 7 days. (B) LS174T hPXR cells were pretreated 24 hours with SPA70 100 nM. This pretreatment was performed in order to reduce the basal proliferation of the cells, thus allowing a more sensitive detection of any PXR agonist activity that would result in growth induction. Then medium was removed and cells were treated with increasing concentrations of SR12813 or dabrafenib for 7 days continuously and cell growth was measured using MTT assay. Data are expressed as fold change in cell growth as compared to untreated cells and expressed as mean ± sd of three to six independent experiments, **** $P < 0.0001$, *** $P < 0.001$ (Student's t-test) compared to DMSO treated cells.

d. Effects of dabrafenib on the expression of hPXR target genes

The effects of dabrafenib and SR12813 on the expression of representative PXR target genes involved in drug metabolism (CYP3A4, CYP2B6), drug transport (ABCG2) and cell proliferation (FGF19) were then measured by RT-qPCR in LS174T-hPXR and human normal hepatocytes (Fig. 5). The results show that the expression of hPXR target genes was increased in a concentration-dependent manner but was differentially modulated depending on the agonist. In LS174T-hPXR, CYP3A4 expression was increased by 190-fold with SR12813 as compared to 90-fold with dabrafenib. However, dabrafenib exhibited a higher potency as maximal induction of CYP3A4 expression was observed at 0.3 µM as compared to 1 µM for SR12813 (Fig. 5A). The increase in ABCG2 expression was also higher when cells were treated with SR12813 as compared to dabrafenib (12-fold vs 9-fold, respectively, Fig. 5A). Conversely, expression of FGF19 was drastically induced by dabrafenib (33-fold induction at 1 µM) whereas it was only increased by 4-fold with SR12813, even at the highest concentration used (3 µM). This higher efficacy of dabrafenib to induce FGF19 expression could explain the better efficacy of dabrafenib to activate the LSPXR-PXR proliferation. This is in agreement with the results of Wang et al (16) suggesting FGF19 as the main mediator of PXR in colon cancer cells proliferation. Finally, using more biologically relevant primary cultures of freshly isolated human hepatocytes from three independent donors, we confirmed that both SR12813 and dabrafenib could significantly induce the expression of CYP3A4 and CYP2B6 genes and showed a similar potency (Fig. 5B).

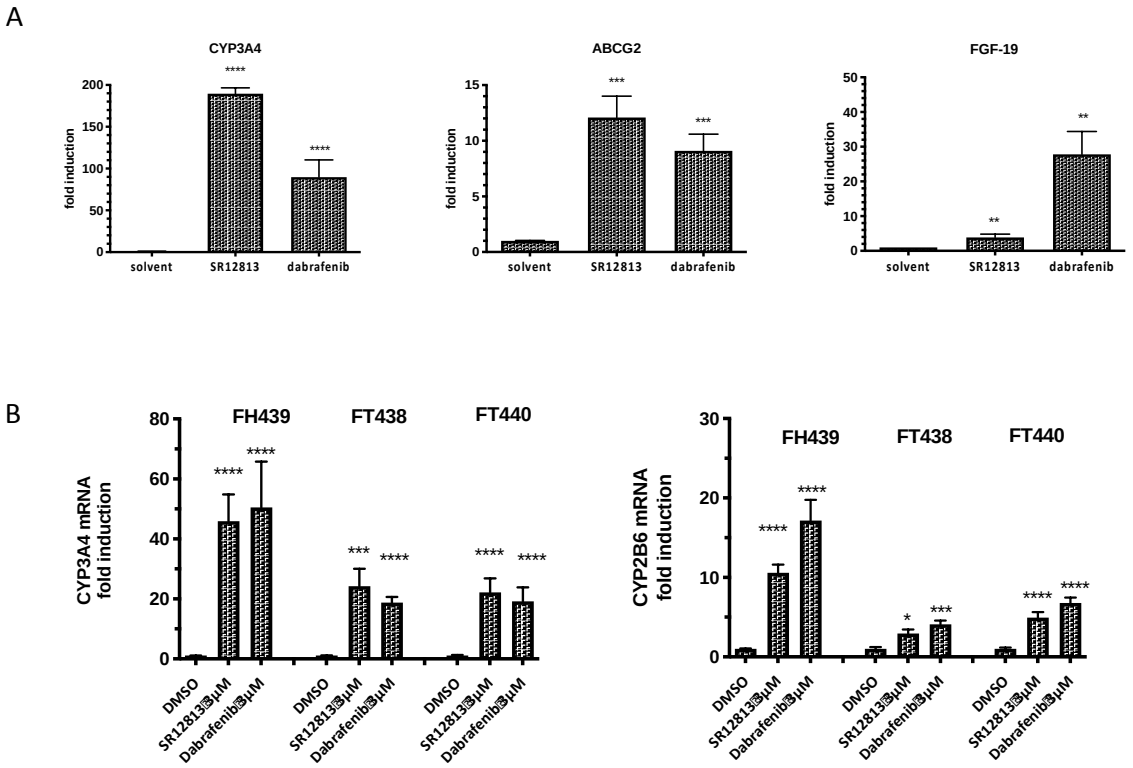

FIG 5.

Figure 5: Effects of dabrafenib on PXR-target genes expression in LS174T PXR cells and in primary human hepatocytes. (A) RT-qPCR of CYP34A, ABCG2 and FGF19 mRNA expression were performed in LS174T PXR cells treated with 3 µM SR12813 or dabrafenib for 24h. Results were obtained from three independent experiments performed in duplicate. Data are expressed as mean ± sd and compared with control cells treated with DMSO (0.1%). (B) RT-qPCR of CYP34A and CYP2B6 mRNA expression in primary cultures of human hepatocytes (three independent donors: FT438, FH439 and FH440) following a 24h treatment with the indicated concentrations of ligand. The relative gene expression levels were normalized using GAPDH content for LS174T-PXR cells and for RFLO for hepatocytes. mRNA expressions are expressed as mean ± sd of three independent experiments and compared with DMSO-treated LS174T-PXRcells or hepatocytes, **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$ (Student's t-test).

4. DISCUSSION

Dabrafenib is predominantly metabolized by oxidation leading to hydroxy-dabrafenib and subsequently to carboxy-dabrafenib that undergoes a decarboxylation to generate desmethyl-dabrafenib, an active derivative that is further transformed in minor oxidative products (26). CYP3A4 and CYP2C8 are known to play a major role in these metabolic steps and dabrafenib was shown to induce CYP3A4 and CYP2D6 expression *in vitro* and *in vivo* (20). These studies have suggested that master regulators of genes involved in drug metabolism such as the nuclear receptors PXR or CAR, could mediate cell response to dabrafenib and influence the effects of other associated medications. However, there was no study characterizing the PXR agonist activity of dabrafenib. The results of our study demonstrate for the first time that similarly to the reference PXR agonist SR12813, dabrafenib is a selective activator of the human PXR in various cell lines overexpressing this nuclear receptor. For both dabrafenib and SR12813, discrepancies in hPXR transactivation could be noticed with regards to cell type. This could be attributable to a difference in metabolic capacity of HepG2, LS174T, 22RV-1 and HeLa cells. We further demonstrated that dabrafenib-mediated PXR activation was associated with enhanced expression of several PXR-target genes, which is in accordance with increased expression of CYP2B6 and CYP3A4 previously observed in hepatocytes (20). Together, these results confirm that dabrafenib could regulate the expression of key enzymes involved in the metabolism of xenobiotics and demonstrate that this effect is linked to the PXR agonist property of dabrafenib. These results are of clinical importance as dabrafenib could directly impact its own metabolism or the metabolism of other drugs or medications, in particular MEK inhibitors such as trametinib that is approved in combination with dabrafenib for the treatment of metastatic melanoma. Future work could consist to confirm that in PXR expressing cells, dabrafenib increase the activity of metabolic enzymes and cause drug-drug interactions.

While dabrafenib usually inhibits the proliferation of cancer cells by targeting $BRAF^{V600E}$ (26), it was also shown to induce the proliferation of tumor cell lines expressing wild-type BRAF and mutant RAS (19). In vitro studies reported half-inhibition concentrations (IC_{50}) values of 0.65 nM for $BRAF^{V600E}$ (18). Here, we showed that dabrafenib could stimulate the growth of LS174T cells with an EC_{50} of 30 nM. This effect was only observed when hPXR was stably overexpressed, strongly suggesting that this effect was, at least in part, mediated by PXR and could also be attributable to an increase in FGF19 expression, a PXR target gene known to be involved in cell proliferation (28) and that is significantly increased in LS174T-hPXR cells treated with dabrafenib. Interestingly, the PXR ligand rifampicin was also shown to induce FGF19-mediated proliferation of PXR-transfected LS174T cells (16).

These results indicate that dabrafenib could regulate the proliferation of colon cancer cells and demonstrate that this effect is linked to the PXR agonist property of dabrafenib that is often superior to that of the reference agonist SR12813.

A recommanded dose for dabrafenib (Tafinlar, GSK2118436, MW 519.56) is 150 mg twice daily administered orally giving a plasma concentration of 2160 ng/ml (4 μM) (Puszkiel et al, 2019). At this concentration, dabrafenib is expected to be active on both $BRAF^{V600E}$ and PXR. Since numerous pharmaceuticals like antiandrogens, antiestrogens, anti-epileptic or anti-viral drugs are ligands of hPXR (4, 5, 14 and our unpublished results), our results further emphasize the need to assess the potential agonist activity of drugs that are developed in a more thorough way. Indeed, former preclinical studies evaluating this activity was usually performed in mouse or rat models, but our results clearly demonstrate that in LS74T cells, dabrafenib did not show any significant activity towards the mouse ortholog of PXR. In order to improve the assessment of PXR agonist activity, we therefore

recommend to use adapted cell models to screen for PXR agonist activity, we propose a panel of in vitro techniques enabling such an evaluation for new developed drugs. Those original models could also serve to design new analogs of currently used drugs devoid of PXR binding and activation.

Author contributions

N Creusot, M Gassiot, E Alaterre, B Chiavarina, M Grimaldi, L Toporova, S Gerbal Chaloin, M Daujat Chavanieu and P Balaguer performed experiment. A Boulahouf, A Matheux and R Rahmani established reporter cell lines. P Balaguer and P Pourquier wrote the article. P Balaguer, P Pourquier, A Evrard and C Gongora reviewed and edited the article. P Balaguer supervised the work.

Funding : This work was supported by grants from the Agence Nationale de la Recherche, project SYNERACT (P.B.), the Plan Cancer Inserm project SYNERPXR 2 (P.B.) and the French Agency for Food, Environmental and Occupational Health & Safety project XENOMIX (P.B.) and TOXCHEM (P.B.). The views expressed in the article do not necessarily reflect the views of the funding agencies.

Conflict of interest. The authors declare no conflict of interest.

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