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Efavirenz is a substrate and in turn modulates the expression of the efflux transporter ABCG2/BCRP in the gastrointestinal tract of the rat

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ABSTRACT

The oral bioavailability of the antiretroviral efavirenz (EFV) undergoes high inter and intra-individual variability, this fact supporting its therapeutic drug monitoring. Previously, it was demonstrated that the encapsulation of EFV within polymeric micelles increases the oral bioavailability of the drug. The breast cancer resistant protein (BCRP, ABCG2) is known to be inhibited by EFV in vitro. Since ABCG2 is profusely expressed in the gastrointestinal tract, the aim of the present work was to thoroughly investigate whether the intestinal permeability of EFV is modulated by ABCG2. The functional role of ABCG2 in mediating the transport of EFV at the intestinal level was consistent with the following findings: (a) an ABCG2 inhibitor, fumitremorgin C (5–10 μM), significantly potentiated the mucosal-to-serosal permeation of the drug in everted gut sacs; (b) a five-day oral treatment with 20 mg/kg EFV promotes the over-expression of ABCG2 in about 100%, this phenomenon being accompanied by a clear decline in the intestinal permeability of the antiretroviral and (c) the normalization of the ABCG2 expression within 24 h after the last administration of EFV was coincident with the recovery of the ability of the drug to permeate through the small intestine wall. Interestingly, no interactions between EFV and P-glycoprotein (ABCB1) were apparent. Since the intestinal permeability of a drug could be associated with its in vivo absorbability, we suggest that the oral absorption of EFV is affected by modifications in the ABCG2 intestinal expression contributing to the intra-individual bioavailability variations.

1. Introduction

There are 15 million people living with the human immunodeficiency virus (HIV) in low- and middle-income countries [44]. Efavirenz (EFV) is a first-line antiretroviral (ARV) included in the highly active antiretroviral therapy (HAART) that is prescribed to adults and children infected with HIV [1–3]. Its key role as an affordable treatment in developing countries is due to its relatively low cost, manageable pill burden, low viral resistance and well-documented efficacy and safety [4].

EFV is a highly lipophilic non-nucleoside reverse transcriptase inhibitor classified in class II of the Biopharmaceutic Classification System [5]. Its oral bioavailability is about 40–45% and it demands therapeutic drug monitoring (TDM) due to the inter- and intra-individual variability [6,7]. Some studies have highlighted the possible correlation between (a) low EFV plasma exposure and increased risk of therapeutic failure [8–10], and (b) high systemic exposure with toxicity, mainly in the central nervous system [10,11].

At least two factors are known to influence the pharmacokinetics of EFV. First, the very low solubility of EFV in aqueous medium (<4 μg/ml) hinders the absorption and distribution of the drug from the gastrointestinal tract [12,13]. In general, a good correlation has been established between solubility improvement and higher bioavailability for most of the class II drugs [14,15]. In this sense, previous investigations showed that the encapsulation of EFV within polymeric micelles of different poly(ethylene oxide)–poly(propylene oxide) block copolymers [16] significantly improves the oral bioavailability of the drug and reduces the inter-individual variability [17,18].

Second, the hepatic activity of the cytochrome P450 2B6 (CYP2B6) that is the main metabolic route of EFV, can be modified. The inter-individual variability in the CYP2B6 function arises from

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the presence of polymorphic variants as well as from population variables such as age or gender [19–21]. Furthermore, intra-individual differences in CYP2B6 lead to changes in EFV plasma concentrations, as observed by auto-induction [22] and by drug interaction [23,24].

A third factor that could also affect the EFV bioavailability is the activity of efflux transporters belonging to the ATP-binding cassette (ABC) superfamily. These pumps located in the intestinal epithelium actively remove drugs in the basolateral–apical direction, against a concentration gradient. This mechanism leads to sub-therapeutic plasma concentrations and eventually to therapeutic failure. Moreover, drug–drug interactions may take place at the level of these transporters, modulating their activity or expression and influencing the efficacy and toxicity of the substrate drugs. Several studies have investigated the influence of P-glycoprotein (ABCB1, MRP1) polymorphisms on EFV plasma levels. Results were conflicting [25–29], rendering its role unclear. Breast cancer resistance protein (BCRP/MXR) is another important member of the ABC superfamily that effluxes a wide range of endogenous and exogenous substrates across biological membranes and it was identified as ABCG2.

Previous in vitro evidence showed that EFV is able to inhibit ABCG2 in vitro in transfected cell lines [30] and that the chronic treatment with EFV induces the expression of ABCG2 in a leukemia cell line without modifications in the intracellular accumulation of the drug [31].

ABCG2 is highly expressed in the gastrointestinal tract; therefore it is entirely plausible that the poor and variable oral absorption of EFV could, at least in part, be a consequence of its removal from the intestinal mucosa to the lumen by ABCG2. Additionally, the treatment with EFV could lead to a substrate-induced modulation of the transporter. Therefore, the present study aimed to study the involvement of ABCG2 in the permeation of EFV across the intestinal mucosa and to elucidate the relationship between the repeated exposure to EFV and the expression levels of the ABCG2 in the small intestine. Overall data confirm that EFV is pumped into the intestinal lumen by ABCG2. Furthermore, the repeated administration of EFV transiently increases the expression levels of ABCG2 in the intestine, which in turn, reduces the intestinal absorption of EFV.

2. Methods and materials

2.1. Animals

Male Sprague-Dawley rats (280–320 g body weight) were housed under a 12:12-h light:dark cycle, at controlled room temperature with food and water *ad libitum*. Experiments were conducted in accordance to the Guide for the Care and Use of Laboratory Animals of the National Research Council (USA, 1996).

2.2. Preparation of EFV-loaded polymeric micelles

Drug-loaded micelles were prepared according to Chiappetta et al. (2010) [17]. Briefly, 10% (w/v) polymeric micelles made of poloxamer Pluronic® F127 (molecular weight of 12.6 kDa, 70% PEO content) were produced by dissolving the required amount of copolymer in phosphate citrate buffer solution (pH 5.0) at 4 °C and equilibrating the system at 23 °C, at least 24 h before use. EFV (final drug concentration 2%, 20 mg/ml of copolymer solution) was added to the micellar systems (pH 5, 3 ml) in glass vials (10 ml). Samples were shaken (48 h) in a temperature-controlled horizontal shaker at 23 °C Minitherm-Shaker; Adolf Kuhner AG, Switzerland) to enable to complete encapsulation of the drug. Suspensions were filtered (0.45 μm, cellulose nitrate filters) to remove any insoluble residue and stored at 23 °C until use.

2.3. Animal treatment

Either EFV (20 mg/kg) or the vehicle (drug-free F127 micelles, 10%, control) were administered by oral gavage (p.o.) once daily during five days. Between administrations, the animals were housed with food and water *ad libitum*. After the last administration of EFV, the animals were divided into two groups that differed in the wash out period (either 1 h or 24 h) before starting the experiments. These animal groups will referred as 1 h- and 24 h-group, respectively. All groups were analyzed by comparison with the corresponding animals treated with drug-free micelles (control).

2.4. Preparation of brush border vesicles from intestinal mucosa cells (BBM)

The small intestine (from pylorus to ileocecal valve) was removed, carefully rinsed with ice-cold saline and kept in saline at 4 °C. The intestine was divided into four parts of approximately 20 cm each. The most proximal segment, starting from the pylorus, was given the number I, whereas the most distal segment close to the ileocecal valve was given the number IV. Tissues were treated according to Mottino et al. with modifications [32]. Briefly, the intestinal segments were opened lengthwise, the mucus layer was carefully removed, and the mucosa obtained by scraping. Mucosa samples were collected and homogenized in buffer [50 mM mannitol, 2 mM Tris (pH 7.1)], supplemented with protease inhibitors (Complete mini, Roche Applied Sciences, Argentina) and 10 mM PMSF). BBMs were prepared from total homogenates by a divalent cation precipitation method followed by differential centrifugation. The final pellet was re-suspended in a 300 mM mannitol, 10 mM HEPES/Tris (pH 7.5) supplemented with protease inhibitors. Aliquots of BBM preparations were stored at −80 °C immediately after collection until western blot studies were performed.

2.5. Western blot studies

The expression of ABCG2 and ABCB1 in BBM was determined. After thawing, the protein concentration in BBM samples was measured with bovine serum albumin (BSA) as standard [33]. Samples of BBM (three replicates) were heated (95 °C, 5 min) and 30 μg per line were loaded onto 10% SDS-polyacrylamide gels and subjected to electrophoresis. The gels were transferred to nitrocellulose membranes and blocked (room temperature, 1 h) with Tris buffered saline containing 0.1% Tween 20 and 5% non-fat dry milk. Blots were incubated over night at 4 °C with rabbit polyclonal antibodies to either mouse ABCG2 (sc-25822, 1/400), or human ABCB1 (sc-8313, 1/1000) from Santa Cruz Biotechnologies (CA, USA). Rabbit polyclonal anti-actin (A-2066, 1/1000) from Sigma–Aldrich (St. Louis, MI) was used as load control. The immune complex was detected by incubation with the horseradish peroxidase-linked secondary antibody (sc-2004, 1/2000) from Santa Cruz Biotechnologies (CA, USA) for 90 min.

The bands were detected by autoradiography using enhanced chemiluminescence (ECL, Amersham Biosciences, MA, USA) and quantified by densitometric analysis using ImageJ software (1.34S National Institutes of Health, USA).

2.6. Transport of EFV across the everted rat intestine

Rats were anesthetized with urethane (1.2 g/kg, i.p. injection). The abdomen was opened and the distal extreme of the small intestine was removed and flushed with 50 ml of ice-cold saline. The isolated intestine was transferred to a poly(methymethacrylate) (Perspex) chamber and perfused with cold Krebs solution

bubbled with 95% O_2/5% CO_2 (in mM: NaCl 118; KCl 4.7; $MgCl_2$ 1.2; NaH_2PO_4 1.0; $CaCl_2$ 2.6; $NaHCO_3$ 25.0; glucose 11.1; sodium ethylenediamine tetraacetic acid (Na_2 EDTA) 0.004; ascorbic acid 0.11; final pH 7.4). The intestine was everted and four sacs (5–7 cm) were prepared. Krebs buffer (1 ml) was introduced into the everted sac (serosal side), and both ends of the sac were ligated tightly. The sac was immersed into 5 ml of Krebs buffer pre-warmed at 37 °C under bubbling with 95% O_2/5% CO_2. After 15 min stabilization, EFV-loaded micelles (final concentration 0.1–1 mM) were added. This time point was considered time 0. In the inhibition studies, either fumitremorgin C (FMT C, final concentration 5–10 μM) or verapamil hydrochloride (VER, final concentration 50–100 μM) were added to the medium 30 min before the addition of EFV samples. The transport of EFV across the intestine from the mucosal to the serosal surface was measured by sampling of the inner medium every 5 min during 40 min. Then, the sacs were gently dried with filter paper and weighed.

2.7. Chromatographic method for EFV analysis

Samples were determined by liquid chromatography (HPLC) using a Phenomenex Luna 5 μm, C18, 150 × 4.60 mm column (Phenomenex, CA, USA) with a UV detector (248 nm, UVIS 204, Linear Instruments, Reno, USA). A previously described technique was used [17]. The mobile phase was a mixture of distilled water, acetonitrile and triethylamine (60:40:0.2, pH 3) pumped at a flow rate of 1.4 ml/min. The analytical method for quantification was validated in the range of 20–5000 ng/ml.

2.8. Drugs

Efavirenz was a gift of Laboratorios LKM, Buenos Aires, Argentina. Poloxamer F127 was a gift from BASF (NJ, USA). The protease inhibitor cocktail Complete mini was purchased in Roche Applied Science (F Hoffman-La Roche Ltd.) Complete mini, Roche. VER, FMT C, phenylmethylsulfonyl fluoride (PMSF), sodium dodecyl sulphate, Tween 20, HEPES, mannitol, Na_2EDTA, glucose, ascorbic acid were purchased in Sigma–Aldrich (St. Louis, MI). NaCl, KCl, $MgCl_2$, NaH_2PO_4, $CaCl_2$, $NaHCO_3$, acetonitrile and triethylamine were purchase in Merck (Merck KGaA, Darmstadt, Germany).

2.9. Statistical analyses

Data are presented as the mean ± SD (n = 4–6) and were analyzed by two-way analysis of variance followed by Bonferroni's post hoc t-test. Exception was made for the statistical analysis of the western blot assay in which one-way analysis of variance followed by Dunnett's multiple comparison tests were performed. In all the cases, a $P < 0.05$ was considered as significant.

3. Results

To characterize the expression profile of ABCG2 transporters in the intestinal tract of Male Sprague-Dawley rats, transporter protein expression was analyzed along the entire small intestine. As becomes apparent from Fig. 1A, ABCG2 is present in all the segments of the small intestine from the segment I comprising the duodenum and proximal part of the jejunum to the segment IV, which extends from the distal jejunum to the ileocecal valve, including the ileum. A significantly higher expression level of ABCG2 was found in the segment IV that includes the distal jejunum and the ileum (Fig. 1B) and therefore, this portion of the gut was selected to assay the possible modulation of the intestinal permeation of EFV by ABCG2.

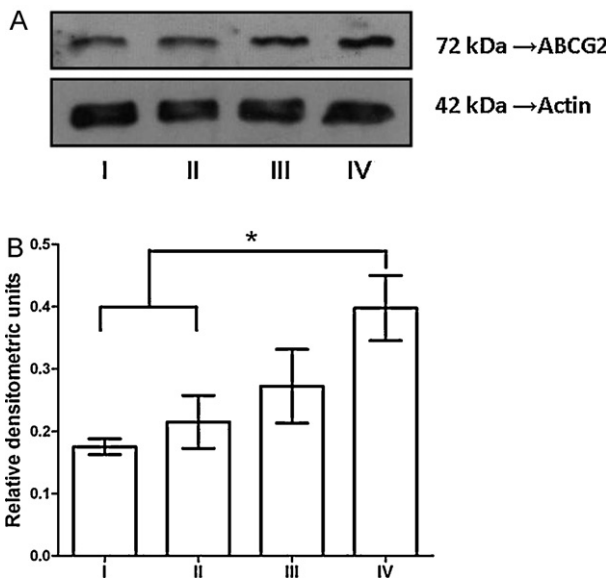

Fig. 1. Expression profile of ABCG2 in the small intestine. (A) Representative immunoblot for ABCG2 (72 kDa) performed in brush border membranes from four 20-cm segments (I–IV) along the entire small intestine of control male rats (see Section 2). Equal amounts of total protein (30 mg) were loaded in all lanes. Actin (42 kDa) was used as loading control. (B) Relative units calculated as ratio between optical density of ABCG2 and actin, data are presented as mean ± SD (n = 4). $^{*}P < 0.01$ compared the segment IV (distal jejunum plus ileum) with respect to the segments I and II.

The addition of increasing EFV concentrations to the mucosal compartment resulted in the progressive recovery of the drug with time in the serosal side, for all concentrations studied (Fig. 2). Moreover, the permeation of EFV across the distal jejunum and ileum was concentration-dependent and at the highest concentration tested (1 mM EFV) the equilibrium of the system was rapidly reached.

To study the involvement of ABCG2 in the passage of EFV from the mucosal to the serosal side, experiments were conducted in the

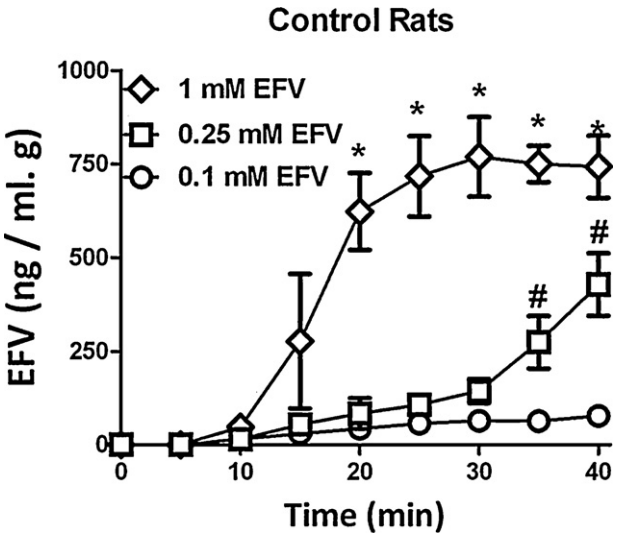

Fig. 2. Permeation of EFV across the everted gut. Mucosal to serosal passage of EFV was assayed in everted sacs obtained from segment IV of the small intestine that comprises distal jejunum and ileum. The tissue was divided into 4 equal parts of approximately 5 cm that were everted and randomly chosen for the experiments. Concentrations of EFV: ○, 0.1 mM; ■, 0.25 mM and ▼, 1 mM were added to the outer side and samples were taken from the inner side of the sac every 5 min up to during 40 min. Data is presented as mean ± SD of the μg of EFV per ml per mg of tissue (n = 4). $^{*}p < 0.001$ comparing 0.1 mM and 0.25 mM EFV; $^{\#}p < 0.0001$ comparing 0.1 mM and 1 mM EFV.

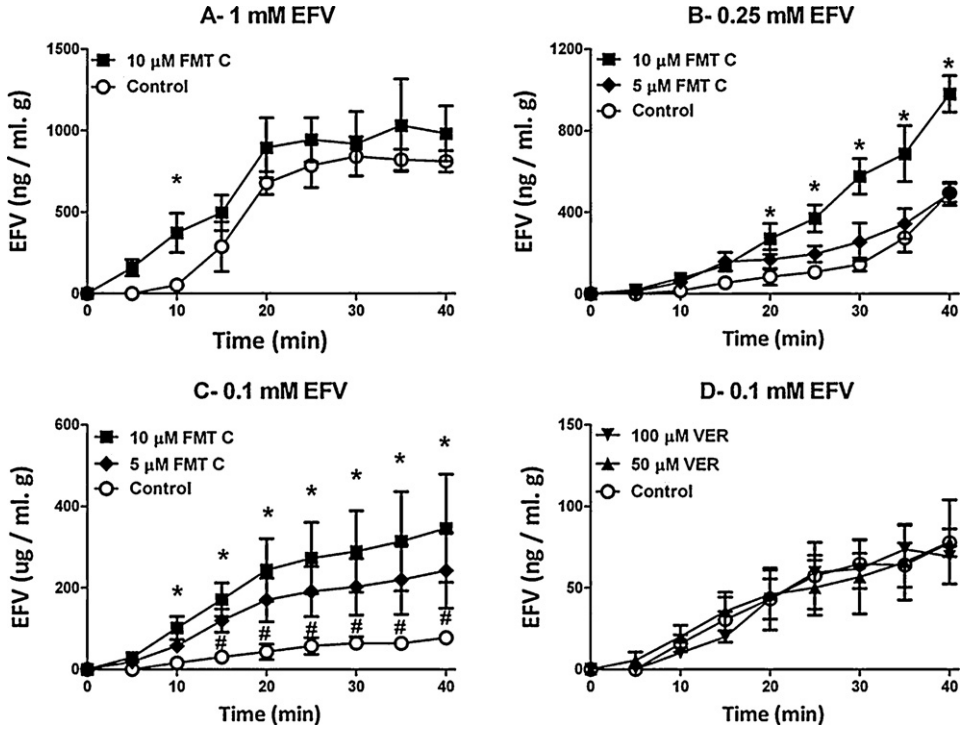

Fig. 3. Effect of ABC-transporters inhibition on the permeation of EFV across the everted gut. Passage of EFV was assayed in everted sacs obtained from the segment IV (distal jejunum plus ileum). The tissue was divided into 4 pieces of approximately 5 cm to prepare the sacs that were randomly chosen for the experiments. Concentrations of EFV of 1 mM in (A), 0.25 mM in (B) and 0.1 mM in (C) and (D) were added to the serosal side and samples were taken from the inner side every 5 min during 40 min. When required, the ABCG2 inhibitor fumitremorgin C [final concentrations: 5 μM (◆) or 10 μM (■)] or the MDR1 inhibitor verapamil [final concentrations 50 μM (▲) or 100 μM (▼)] were added to the incubation medium 30 min before starting experiments. Data is presented as mean ± SD ($n = 4$). $^{*}p < 0.01$ when compare 10 μM fumitremorgin C and $^{\#}p < 0.01$ when compare 5 μM fumitremorgin C to the corresponding controls.

presence of the highly active and selective ABCG2 inhibitor FMT C. Fig. 3 shows that the highest concentration of FMT C (10 μM) potentiated the permeation of EFV in the linear portion of the curves for all the tested EFV concentrations. Moreover, the passage of 0.1 mM EFV was significantly potentiated also with a lower concentration of FMT C and therefore, this pair (0.1 mM EFV + 5 μM FMT C) was selected to perform the subsequent assays.

Moreover, ABCB1 also modulates drug absorption of a broad spectrum of drugs, the effect of the VER (50–100 μM), a quite selective ABCB1 inhibitor, was analyzed. However, we found no changes in the passage of EFV through the distal portion of the small intestine in the presence of VER (Fig. 3D).

As shown in Fig. 4, the repeated daily oral administration of 20 mg/kg EFV over 5 days significantly reduced the permeation of EFV from the mucosal to the serosal side of the intestinal wall in the experiments conducted only 1 h after the administration of the last dose of the antiretroviral (Fig. 4A). This difference was not observed in the presence of the ABCG2 inhibitor 5 μM FMT C (Fig. 4B) but persisted when the tissues were incubated with VER (Fig. 4C). On the other hand, when experiments were performed 24 h after the last oral administration of EFV, the intestinal permeation of the drug almost recovered to the levels obtained in rats treated with drug-free micelles and no significant differences were found (Fig. 4D).

It is interesting to note that the repeated administration of oral EFV significantly increased the expression levels of ABCG2 with respect to the control rats (Fig. 5). However, 24 h after the last exposition to EFV, the protein abundance of ABCG2 was significantly lower than that only 1 h after the last administration (Fig. 5B). Moreover, the abundance of ABCB1 in every segment of the small intestine between control and the EFV-treated rats 1 h after the last oral administration remained unchanged (Fig. 6).

4. Discussion

EFV is a first-choice antiretroviral in the pharmacotherapy of patients infected with HIV. Current clinical experience has revealed important differences in patient response to the treatment, which to a large extent, can be attributed to high inter and intra-individual variability. This supports the TDM to optimize viral load suppression and to minimize central nervous system toxicity [6]. The present work showed that the BCRP/ABCG2 efflux pump is able to efflux EFV into the intestinal lumen negatively modulating the permeability of the drug in the rat small intestine. Moreover, this phenomenon is more pronounced after the repeated oral administration of the antiretroviral as a consequence of a transient increase in the protein levels of ABCG2 in the intestinal epithelium.

BCG2 is a member of the ABC family that is expressed in the epithelium of the small intestine in human beings [34]. The selection of the last portion of the small intestine to assay the role of ABCG2 on the intestinal permeability of EFV relied on the observation that ABCG2 is expressed throughout the small intestine of control rats though with a significantly higher level in the segment that comprises the distal portion of jejunum and the ileum. These findings were in full agreement with a previous work by [35]. Moreover, the improvement of the EFV aqueous solubility (from 4 μg/ml to 20 mg/ml) by means of its encapsulation within nano-sized polymeric micelles made of 10% (w/v) poloxamer Pluronic F127 [17] was capitalized herein to achieve EFV aqueous solutions concentrated enough to enable the assessment of the intestinal passage of the drug in a concentration range that could be quantified by HPLC.

The concentration-dependent passage of EFV across the mucosal membrane into the serosal side of the intestinal wall, that could be considered representative of the oral absorption of

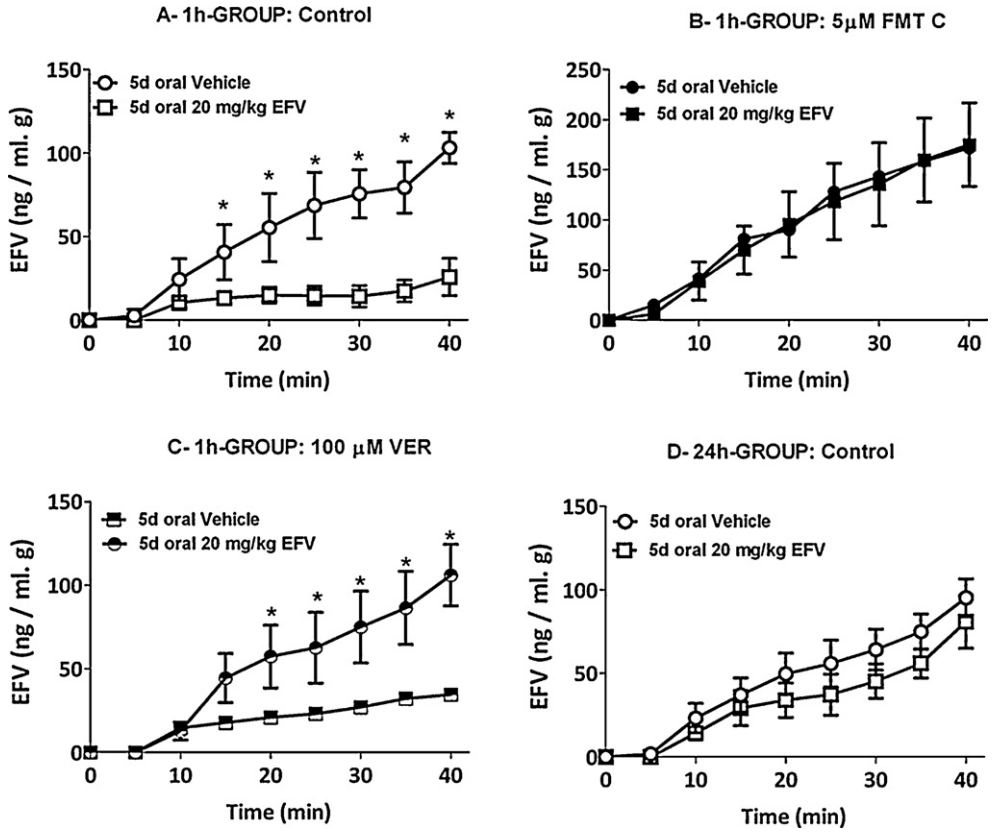

Fig. 4. Effect of repeated oral administration of EFV on its permeation across the everted gut. Mucosal to serosal passage of EFV was assayed in everted sacs of segment IV (distal jejunum plus ileum) obtained from rats daily administered with 20 mg/kg EFV (squares) or the corresponding vehicle (circles). Animals were sacrificed 1 h (A-C) or 24 h (D) after last oral administration. EFV (0.1 mM) was added to the outer side and samples were taken from the inner side of the sac every 5 min up to during 40 min. The experiments were performed in control conditions (white symbols) or in the presence of the selective BCRP inhibitor Fumitremorgin C (black symbols) or the MDR1 inhibitor verapamil (black–white symbols). The sacs were randomly chosen for the experiments. Data is presented as mean ± SD ($n = 4$). $^{*}p < 0.01$ when compare 20 mg/kg EFV-treated rats and vehicle-treated rats.

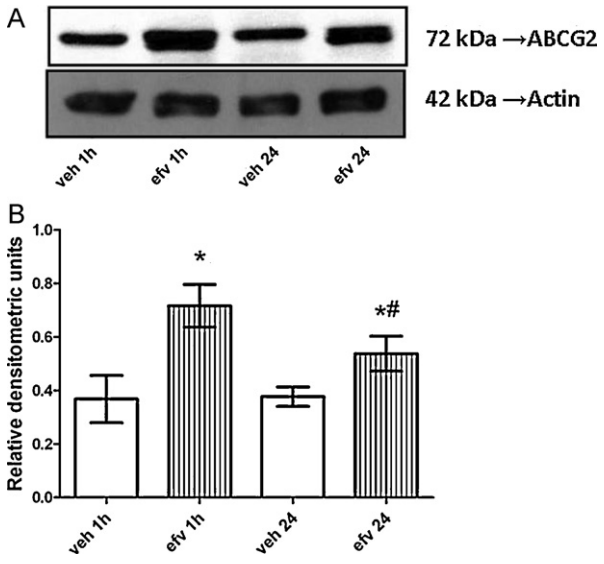

Fig. 5. Effect of repeated oral administration of EFV on the expression levels of ABCG2 in the small intestine. (A) Representative immunoblot for ABCG2 (72 kDa) performed in brush border membranes from segment IV (distal jejunum and ileum) obtained 1 h or 24 h last oral administration of 20 mg/kg EFV or the vehicle. Equal amounts of total protein (30 mg) were loaded in all lanes. Actin (42 kDa) was used as loading control. (B) Relative densitometric units calculated as ratio between optical density of ABCG2 and actin, data are presented as mean ± SD ($n = 4$). $^{*}P < 0.01$ compared EFV-treated rats and the corresponding vehicle. $^{\#}P < 0.01$ compared 1 h-group with 24 h-group.

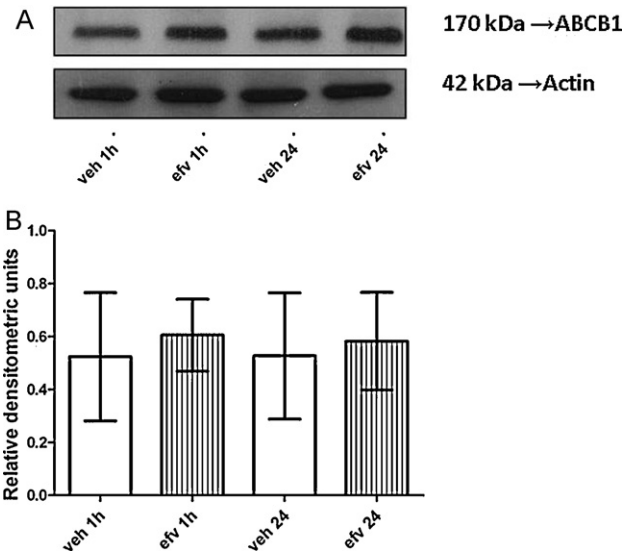

Fig. 6. Effect of repeated oral administration of EFV on the expression levels of ABCB1 in the small intestine. (A) Representative immunoblot for ABCB1 (170 kDa) performed in brush border membranes from segment IV (distal jejunum and ileum) obtained 1 h or 24 h last oral administration of 20 mg/kg EFV or the vehicle. Equal amounts of total protein (30 mg) were loaded in all lanes. Actin (42 kDa) was used as loading control. (B) Relative densitometric units calculated as ratio between optical density of ABCB1 and actin, data are presented as mean ± SD ($n = 4$).

EFV in the gastrointestinal tract, was dependent of ABCG2; a significant enhancement of the intestinal drug penetration in the linear portion of the curves was observed in the presence of FMT C that is a selective, potent and competitive inhibitor of ABCG2 that completely inhibits and reverses ABCG2 activity at low micromolar concentrations [36]. Previous evidence about inhibition of ABCG2 by EFV with the consequent increase of the accumulation of the ABCG2-substrate pheoforbide A in a canine kidney cell line [30] altogether with the present results indicate that EFV is a substrate of the ABCG2. Therefore, EFV may compete with other ABCG2-substrates once in the intestine. According to this concept, the affinity of each substrate for ABCG2 defines whether this competition will be reflected in an increased accumulation either of EFV or the other substrate, as has been observed for FTM C (current results) and for pheoforbide A [30], respectively.

Since it has been reported that the chronic treatment with EFV modulates the expression of several drug transporters, including ABCG2, in a leukemia cell line critically modulating the kinetics of other drugs [31], we decided to analyze the effects of the repeated oral dosing with EFV on the intestinal protein expression of ABCG2. For the in vivo experiments, EFV-loaded micelles were used again to increase the bioavailability and reduce the inter-individual variability [17,18]. The greater levels of efflux transporter protein in animals treated for 5 days with a daily dose of 20 mg/kg EFV reinforces the assumption that ABCG2 plays an important role in intestinal absorption of this anti-retroviral, especially because unlike what happens in the peripheral blood mononuclear cells [31] the over-expression of this transporter was accompanied by a marked decrease in intestinal permeability of the drug. This decrease was overcame when the transporter was selectively inhibited, reaching values similar to those obtained under the same conditions in control rats.

The ABCB1 is the most extensively characterized ABC-transporter and it is also expressed in the last portion of the small intestine [35]. In addition, many of its substrates are also substrates of ABCG2. However, since the permeability of the EFV in the everted gut sac was not affected in the presence of the inhibitor VER in a range of concentrations which previously was shown to inhibit intestinal ABCB1 in rat [37], the intestinal absorption of EFV appeared not to be dependent on the presence of this transporter. Moreover, the involvement of ABCB1 in the decrease of the intestinal permeability of EFV in animals chronically treated with the drug was also precluded given that (i) the modifications in this parameter were observed in the presence of VER and (ii) no alterations in the ABCB1 expression compared to control were found. In this regard, the literature yields that in several tissues no evidence for EFV–ABCB1 interactions were found. With respect to the intestine, the ABCB1 function was not modified after 6 days of oral treatment with EFV in everted gut sac experiments [38], while in Caco-2 cells the EFV transport is not affected by VER and the induction of ABCB1 attributable to EFV is quantitatively negligible [39]. Moreover, neither modifications in the accumulation ratio of EFV nor induction of ABCB1 activity or expression were observed after chronic treatment with EFV in peripheral blood mononuclear cells from healthy volunteers [40] or at blood–brain barrier [41].

Turning to the interaction between EFV and ABCG2, when this phenomenon was evaluated at the time when a new drug administration should take place (i.e. at 24 h), we found that expression levels of ABCG2 protein decreased significantly as the drug disappeared from plasma, given that the elimination half-life is approximately 5 h in rats [17,18]. However, the expression of ABCG2 protein was still significantly greater than the value found in control animals after 24 h. This residual over-expression was not accompanied by significant changes in intestinal permeability of EFV. Uncoupling events that lead to the loss of the ability of ATP hydrolysis by ABCG2 [42] as well as changes in the cholesterol content in the cell membrane [43] generate quantitative loss in the functional transporter capacity without changing its localization or expression levels. The occurrence of any of these phenomena in our experimental conditions could explain the dichotomy between the increased expression levels and the absence of changes in intestinal permeability that occurs within 24 h after the administration of EFV that could also be observed at 1 h. However, evaluating the ATPase activity and the cholesterol content in the brush border membrane was beyond the scope of the present work, but gaining a deeper insight into these phenomena is the aim of our further investigations.

In conclusion, since the intestinal permeability of a drug could be associated with its in vivo absorbability, we suggest that oral absorption of EFV is affected by modifications in the ABCG2 intestinal expression contributing to the intraindividual bioavailability variations.

Finally, since ABCG2 is expressed in tissues that are crucial for the pharmacodynamics of EFV, as peripheral blood mononuclear cells and central nervous system, a preclinical analysis of the relevance of the efflux transporter ABCG2 on the distribution of EFV to those tissues is the aim of our ongoing investigation. While these changes may not result in a modification of the therapeutic effectiveness or even cause no elevated toxicity to the central nervous system, they account for the significant potential to induce substantial drug interactions observed for EFV [31].

Acknowledgments

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Further reading

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