Substrates, Inhibitors and Activators of P-glycoprotein: Candidates for Radiolabeling and Imaging Perspectives

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Abstract: In recent years, several PET tracers for monitoring the activity and expression of P-gp at the BBB have been tested. P-gp substrates such as \([^{11}C]\)verapamil and \([^{11}C]\)loperamide can be employed to visualize P-gp activity, but they display a moderate baseline uptake in the brain and formation of radiolabeled metabolites which hamper the interpretation of PET data. P-gp inhibitors such as \([^{11}C]\)elacridar, \([^{11}C]\)laniquidar and \([^{11}C]\)tariquidar have been tested to investigate P-gp expression and the results need further investigation. Recently, we developed MC18, MC266 and MC80, that have been characterized as an inhibitor, substrate and inducer of P-gp both by in vitro assays and in the everted gut sac method. These compounds have been radiolabelled with \(^{11}C\) and been evaluated in vivo. In the present review, we compare the outcome of biological in vitro assays and the corresponding in vivo PET data for the P-gp inhibitors \([^{11}C]\)MC18 and \([^{11}C]\)elacridar, the P-gp substrates \([^{11}C]\)MC266 and \([^{11}C]\)verapamil, the P-gp inducer \([^{11}C]\)MC80 and the P-gp modulator cyclosporin A. Since a satisfactory overlap was found comparing in vivo results and the corresponding in vitro findings, the proposed biological in vitro assays could be predictive for the in vivo PET data of novel radiotracers. PET tracers could be employed for various purposes: radiolabeled P-gp inhibitors to monitor decreased expression of P-gp at the BBB in neurodegenerative disorders such as Alzheimer’s and Parkinson’s disease; and radiolabeled P-gp substrates with a high baseline uptake to monitor increased expression of P-gp in epileptic foci.

Keywords: P-glycoprotein, PET, inhibitor, substrate, modulator, inducer, function, expression.

INTRODUCTION

P-glycoprotein (P-gp) belongs to the ATP-Binding Cassette (ABC) transporter family and it is expressed in various body tissues, such as liver, kidney, intestine, testes, and brain [1,2]. In the brain, P-gp is localized at the luminal membrane of endothelial cells of blood capillaries where it actively modulates the permeation of xenobiotics [3]. P-gp overexpression has been observed in several tumours causing Multi Drug Resistance (MDR) due to the resistance to Central Nervous System (CNS) drugs, such as antidepressants and antiepileptic or anti-HIV medicine, may also be related to P-gp overexpression [5-7]. Recently, it has been reported that alterations in P-gp expression and function are related to the etiology and pathogenesis of neurologic disorders, such as Alzheimer’s disease (AD) and Parkinson’s disease (PD) [8-10]. Moreover, a recent study reported that P-gp expression and function are strongly decreased during the neuroinflammation process in Multiple Sclerosis (MS) [11]. Several preclinical studies have suggested that changes of P-gp expression in MDR and in diseases of the brain could be imaged by PET and suitable radiotracers binding to the pump. Studies aimed at visualization of P-gp function and expression have been carried out in rats [12] mice [13] and humans [14].

Although compounds that interact with P-gp have various unrelated structures, they have recently been classified into four categories: substrates, inhibitors, modulators, and inducers [15]. In studies performed in recent years, compounds transported by P-gp were considered as substrates, while compounds that compromise the function of the transporter were classified as inhibitors. Moreover, the term inhibitor was often used synonymously with modulator and only few PET studies with P-gp inducers have been reported.

The major PET radiotracers employed in recent PET studies on P-gp function are \([^{11}C]\)verapamil [13], \([^{11}C]\)loperamide [16], \([^{11}C]\)colchicine [17], \([^{11}C]\)carvedilol [18], \([^{64}Cu]\)complexes [19], \([^{68}Ga]\)complexes [20], and \([^{99m}Tc]\)complexes [21], that interact with P-gp as substrates. Only a few P-gp inhibitors such as elacridar, laniquidar and tariquidar (Fig. (1)), have been labeled with \(^{11}C\) for imaging of P-gp expression [22-24].

Candidate tracers for PET analysis should possess two important characteristics: (i) selectivity for the pump and (ii) radiocchemical purity of the signal. Moreover, the magnitude of the signal, defined as the ratio of tissue radioactivity at baseline and after P-gp inhibition, is an important parameter for the substrate [25].

P-gp SUBSTRATES

\([^{11}C]\)verapamil

Initial animal and human PET studies have used racemic \([^{11}C]\)verapamil (Fig. (2)) [26,27]. Both the (R)- and (S)-enantiomers have been applied in \(mdr1ab\) knock-out and wild type mice to measure P-gp activity at the BBB [13]. The brain
uptake of both enantiomers was more than 10-fold higher in P-gp-knock-out mice than in wild-type mice. The (R) and (S)-enantiomers display the same efflux transport activity towards P-gp and are both suitable for P-gp imaging. Since (R)-verapamil is less rapidly metabolized in humans than (S)-verapamil and since it displays lower affinity towards calcium channels, it is the enantiomer of choice for measuring P-gp function \textit{in vivo} [13]. However, verapamil is extensively metabolized by CYP450 enzymes that form radiometabolites, some of which are themselves P-gp substrates [28].

Several PET studies employed $[^{11}\text{C}]$verapamil to assess the kinetic constant $k_1$ and the distribution volume (DV) of the radiotrace within brain [29]. $[^{11}\text{C}]$verapamil has been co-administrated with cyclosporin A (CsA), or tariquidar which is claimed to be a P-gp inhibitor, in humans and rodents [30,31]. After co-administration of CsA in humans, DV and $k_1$ were markedly increased, by about 70% with respect to baseline [30]. In rat PET studies, tariquidar increased the DV and $k_1$ of (R)-$[^{11}\text{C}]$verapamil 12-fold and 8-fold with respect to baseline [31].

In summary, (R)-$[^{11}\text{C}]$verapamil displayed a low brain uptake at baseline, both in humans and in rodents (SUV < 1), and tracer injection led to the formation of radioactive metabolites that are themselves P-gp substrates. To date, $[^{11}\text{C}]$verapamil is the major radiotracer employed in modulator or inhibitor co-administration studies, to quantify P-gp function at the level of the BBB. This technique permits monitoring of decreases of P-gp function in PD and AD, but $[^{11}\text{C}]$verapamil can not be employed to quantify overexpression of P-gp in epilepsy, because of its low brain uptake under baseline conditions.

$[^{11}\text{C}]$loperamide and $[^{11}\text{C}]$N-desmethyl-loperamide

$[^{11}\text{C}]$loperamide (Fig. (3A)) has been utilized as a PET radiotracer to monitor P-gp function both in animals and in humans. A major metabolite of $[^{11}\text{C}]$loperamide is $[^{11}\text{C}]$desmethyl-loperamide (dlop) (Fig. (3B)) which is also a P-gp substrate [16]. In contrast to the parent compound, $[^{11}\text{C}]$dlop presents only a minor concentration of radiometabolites entering the brain [32].

$[^{11}\text{C}]$dlop displays high selectivity for the transporter, as demonstrated by enhanced brain uptake in knock-out mice and in monkeys after co-administration of a P-gp inhibitor [32,33]. The signal purity of $[^{11}\text{C}]$dlop is excellent, since < 10% of total radioactivity in the brain of P-gp knock-out mice represented radiometabolites [34]. However, the magnitude of the
Elacridar is significantly different in rats (low) and in brain slices in vitro. In these analyses, it displayed a 5-fold increase of peak brain activity, which is largely explained by a 5-fold increase of the rate constant describing brain entry \( k_1 \) [22].

In conclusion, \( [11C]dlop \) displayed a low brain uptake both in humans and in rodents, and little metabolism during the analysis. To date, \( [11C]dlop \) can be employed in modulator or inhibitor co-administration experiments to study P-gp function at the level of the BBB. However, like \( [11C]verapamil \), \( [11C]dlop \) can be employed to monitor decreases of P-gp function in PD and AD, but not to visualize increases of function in epilepsy, because of its low baseline brain uptake [33].

**P-gp INHIBITORS**

Elacridar

Elacridar, a potent third-generation P-gp inhibitor (EC\(_{50} = 0.02 \mu M \)) [35], has been \( ^{11}C \)-radiolabelled (Fig. (4)) and evaluated by in vitro autoradiography and in vivo small-animal PET imaging [22]. In these analyses, it displayed good metabolic stability and high specific binding to rat brain slices in vitro. However, the in vitro brain uptake of \( [11C]elacridar \) is significantly different in rats (low) and in mice (high). Moreover, an immediate increase of brain uptake has been observed in rats after injection of unlabelled elacridar, suggesting that elacridar is a P-gp substrate rather than a P-gp inhibitor. A possible explanation of these unexpected results was that the administration of cold elacridar, at a dose which completely inhibited P-gp activity, displaced \( [11C]elacridar \) from its P-gp binding site, resulting in passive diffusion of unmetabolized \( [11C]elacridar \) from blood into the brain [22]. Specific interaction of \( [11C]elacridar \) with P-gp expressed at the BBB was further demonstrated by PET experiments in wild-type and \( mdr1a/b \) knock-out mice showing comparable blood activity levels but a 2.5-fold higher brain uptake of \( [11C]elacridar \) in knock-out animals. Brain uptake in rats was increased by a factor of 5 following administration of unlabelled elacridar, rather than a factor of 2.5 as in the knock-out mice. This discrepancy could indicate that other transporters such as Breast Cancer Resistance Protein (BCRP, ABCG2) also contribute to limiting the brain uptake of \( [11C]elacridar \). This assumption was supported by the evidence that elacridar inhibited BCRP-mediated transport in vitro at comparable concentrations as it inhibited P-gp. Interestingly, the brain concentration of \( [11C]elacridar \) was about 10-fold higher in wild-type mice than in rats. This difference in brain concentration can most likely be explained by the several-fold higher blood activity levels in mice [22]. In conclusion, \( [11C]elacridar \) is not specific for P-gp and the signal magnitude is low although the radiochemical purity of the signal is good.

![Fig. (3).](image-url) \( ^{11}C \)-loperamide (A) and \( ^{11}C \)-N-desmethyl-loperamide (B).

\( [11C]dlop \) signal in PET is relatively poor: only 4-fold higher in P-gp knock-out mice than in wild-type mice [32].

\( [11C]dlop \) is a promising radiotracer to visualize P-gp function at the BBB. Brain uptake in mice is low at baseline (\%SUV = 2.3) and pharmacological inhibition of P-gp with tariquidar caused about 5-fold increase of peak brain activity, which is largely explained by a 5-fold increase of \( k_1 \), the rate constant describing brain entry [16].

In conclusion, \( [11C]dlop \) displayed a low brain uptake both in humans and in rodents, and little metabolism during the analysis. To date, \( [11C]dlop \) can be employed in modulator or inhibitor co-administration experiments to study P-gp function at the level of the BBB. However, like \( [11C]verapamil \), \( [11C]dlop \) can be employed to monitor decreases of P-gp function in PD and AD, but not to visualize increases of function in epilepsy, because of its low baseline brain uptake [33].

![Fig. (4).](image-url) \( ^{11}C \)-elacridar.

**Laniquidar**

Laniquidar is a P-gp inhibitor displaying submicromolar affinity (IC\(_{50} = 0.51 \mu M \)) and high selectivity towards other ABC transporters [23]. Moreover, in contrast to other third generation P-gp inhibitors, laniquidar does not inhibit the CYP3A4 isoenzyme and is insensitive to liver metabolism. These properties encouraged radiochemists to produce \( [11C]laniquidar \) as a potential P-gp tracer. The compound was evaluated both in control animals and in rats pretreated with P-gp modulators CsA and valspodar [23].

Surprisingly, \( [11C]laniquidar \) displayed even lower cerebral uptake than that seen for the P-gp substrate \( [11C]verapamil \). Several mechanisms could affect cerebral uptake of a tracer such as its metabolism, its lipophilicity, and differences in functionality between MDR1 in humans and \( mdr1a \) in rodents.

As shown in Fig. (5), laniquidar was \( ^{11}C \)-labeled at the ester moiety, which may be unstable in vivo [36,37]. However, a metabolic study indicated that the rate of metabolism of \( [11C]laniquidar \) in rats was quite low because thirty minutes after injection, 68% of radioactivity in plasma still represented parent radiotracer. Thus, the low brain uptake is not caused by rapid metabolism of \( [11C]laniquidar \).

Moreover, a good correlation between P-gp interactions at the human and rodent BBB was found, suggesting that rodent and human P-gp have similar characteristics [37]. Finally, since the lipophilicity of laniquidar is rather high (Clog P =
6.89) [38], it could bind to plasma proteins resulting in a low free fraction of $[^{11}C]$laniquidar in plasma and consequently in a low cerebral uptake. In rats pretreated with CsA, a significantly increased brain uptake of $[^{11}C]$laniquidar was observed. By contrast, in the same condition valsapodar was found unable to increase brain uptake of the radiotracer [23]. These findings led to consider laniquidar at low concentration as a P-gp substrate rather than an inhibitor although further kinetic studies are needed to investigate the mechanism of interaction of laniquidar with P-gp. In conclusion, $[^{11}C]$laniquidar can not be considered a prominent tool for imaging P-gp expression and function by PET.

\[ \text{Fig. (5).} \; ^{11}C\text{-laniquidar.} \]

**Tariquidar**

Tariquidar, an anthranilic derivative and a potent P-gp inhibitor (IC\textsubscript{50} = 0.04-0.4 \mu M) [39], has been evaluated in clinical trials for restoring the efficacy of antineoplastic drugs in chemoresistant tumours. The results were quite unsatisfactory because of pharmacokinetic and pharmacodynamic limitations, such as inhibition of the detoxification enzyme CYP3A4 and a poor selectivity against other ABC transporters which are not involved in MDR. Recently, tariquidar has been evaluated in rats using (R)-$[^{11}C]$verapamil as a P-gp substrate to image P-gp function at the BBB [31]. The results indicated that (R)-$[^{11}C]$verapamil with tariquidar co-administration permits the evaluation of P-gp function in the brain. In particular, this study demonstrated that tariquidar is a potent and fast-acting inhibitor of cerebral P-gp and that, at the highest dose, it increased the brain uptake of (R)-$[^{11}C]$verapamil 12-fold with respect to baseline. Moreover, the increased uptake is due to an 8-fold increased influx rate constant $k_1$ in the brain.

In order to visualize P-gp expression at BBB, tariquidar has been $^{11}C$-radiolabelled (Fig. (6)) and administrated to rats both in the absence and in the presence of cold compound [24]. Surprisingly, brain uptake of the radiotracer was rather low and comparable to $[^{11}C]$verapamil uptake. Moreover, in the presence of unlabeled ligand, an increased influx of radioactivity into the brain has been observed. These findings have been explained by assuming that non-carrier-added $[^{11}C]$tariquidar is fully captured by P-gp at the BBB, but a saturating dose of unlabeled tariquidar results in a breakthrough of activity into brain parenchyma and blocks the specific interaction of $[^{11}C]$tariquidar with P-gp.

In conclusion, $[^{11}C]$tariquidar seems to be a useful radiotracer for visualizing P-gp expression at BBB, although further investigations are needed.

\[ \text{Fig. (6).} \; ^{11}C\text{-tariquidar.} \]

**P-gp MODULATOR**

**Cyclosporin A**

CsA (Fig. (7)) is an immunosuppressive agent and a potent MDR modulator but unfortunately, it also interferes with CYP3A4 metabolizing activity.

A modulator differs from a substrate because it interacts with P-gp binding sites and reduces substrate binding through negative allosteric interactions. In radioligand binding studies, it has been demonstrated that a modulator modifies substrate binding in a noncompetitive manner so that the maximal receptor density ($B_{\text{max}}$) for substrate binding is changed, but the equilibrium constant of dissociation ($K_d$) is unchanged. These results demonstrate that the modulator interacts with P-gp at a site distinct from that of the substrate. Moreover, it suggests allosteric communication between the substrate and modulator binding sites [40,41].

In many papers, co-administration of CsA with a radiolabelled P-gp substrate such as $[^{11}C]$verapamil [30] has been reported to visualize P-gp function because CsA increases brain uptake of the radiotracer with respect to radiotracer administered alone. However, CsA treatment enhances uptake of the radioligand in all regions of the brain including targeted and nontargeted tissues [42].

The modulation of P-gp by CsA is a rapid process because P-gp is quickly blocked when CsA is administered, but examination of the pharmacokinetics of $[^{11}C]$verapamil in brain has shown that this inhibition is also quickly reverted [43].

Some experiments have indicated that the increased uptake of $[^{11}C]$verapamil in brain is due to negative modulation of brain efflux rather than by increased transport into the brain. Moreover, not the concentration of CsA reached in the blood is directly responsible for the P-gp inhibition, but rather the concentration reached in an effect compartment, probably the cytoplasm of endothelial cells at the BBB [43,44].

**BIOLOGICAL IN VITRO EVALUATION OF RADIOTRACER CANDIDATES**

Recently, we developed three compounds $[^{11}C]$MC18 [45], $[^{11}C]$MC266 [45] and $[^{11}C]$MC80 [46] a P-gp inhibitor, sub-
strate and substrate-inducer, respectively, and evaluated these in microPET studies. Moreover, the unlabeled compounds, elacridar, verapamil, and CsA have been evaluated in vitro following the steps illustrated in Fig. (8).

Compounds MC18 and elacridar (inhibitors), MC266 and verapamil (substrates), CsA (substrate-modulator), MC80 (substrate-inducer) have been compared to verify if their in vitro results could predict the outcome of in vivo PET studies of the corresponding radiolabelled analogs Fig. (9).

Firstly, for each compound the apparent permeability ($P_{app}$) in Caco-2 cells has been determined [47,48]. This cell line, deriving from human colonic carcinoma, displays high levels of P-gp expression although other ABC transporters are expressed both at the apical and at the basolateral cell membrane [49]. In this model the basolateral-apical (BA) and apical-basolateral (AB) fluxes for each test compound have been evaluated. The first flux represents passive diffusion, while the second represents P-gp-mediated active transport. The BA/AB ratio discriminates between compounds which are transported and not transported by P-gp. In particular, compounds displaying $BA/AB > 2$ are transported while ligands having $BA/AB < 2$ are not transported by P-gp [50]. In the same cell model, compounds not transported by P-gp did not deplete ATP while compounds transported by P-gp depleted ATP in a dose- and time-dependent manner. By combination
of these assays it is possible to discriminate between a P-gp inhibitor and a P-gp substrate [50].

The potency (EC50) of each P-gp modulating agent is determined in Madin-Darby Canine Kidney cells, stably transfected for P-gp overexpression (MDCK-MDR1) [51]. The evaluation of potency in these cells was carried out using a prodrug such as acetoxymethyl ester of calcine (calcine-AM) that is a known P-gp substrate. In the presence of a P-gp modulator, calcine-AM diffuses into the cytosol where it is hydrolyzed to the fluorescent dye calcine which can no longer be transported by P-gp [51].

To better characterize a P-gp interacting agent, the everted gut sac (isolated from rat ileum) method permits to study P-gp-mediated intestinal absorption and interactions with CYP450 enzymes [52,53]. Since CYP450 enzymes are present in intestinal epithelial cells, it is possible to examine both the effect of transport and the effect of CYP450 metabolizing activity in this model. This combined study is needed because inhibitors and substrates may display overlapping activities towards CYP450 enzymes and the P-gp pump [54].

In this method, the transport of a known P-gp radiolabelled or fluorescent substrate in the absence and presence of a P-gp interacting agent, has been evaluated. The flux of a P-gp substrate from serosal to mucosal compartment and vice versa, is represented by the efflux (k2) and influx rate constants (k1), respectively (Fig. (10A)).

Then, the same evaluations have been carried out in the presence of a P-gp interacting agent to determine k'/2 and k'/1, the efflux and influx constants of the test substrate after P-gp interaction (Fig. (10B, 10C)). For each tested compound, the k%/k'2 and k'/k'1 ratios are representative for the alteration of the efflux, reported as k2, and the influx, reported as k1, of the P-gp substrate. The ratios k%/k'2 and k'/k'1, rather than k%/2 and k'/1, sic et simpliciter, leaving out the experimental conditions, are representative for the efflux and influx of the tested substrate in the presence of a P-gp interacting agent. In the studies carried out in the everted gut sac, the experimentally determined ratio k%/k1 for a compound in the presence of a P-gp interacting agent, has been employed as another test to characterize it as an inhibitor, substrate, substrate-inducer or substrate-modulator (Fig. (8)).

**COMPARISON IN VITRO AND IN VIVO OF P-gp INHIBITORS MC18 AND ELACRIDAR**

At a concentration of 20 μM, MC18 increased the intracellular accumulation of doxorubicin in the MCF7/adr cell line 5.7-fold and potentiated the antiproliferative effect of 5 μM doxorubicin from 5% to 95%. The ratio of drug transport through Caco-2 monolayers in the basolateral-apical and apical-basolateral directions (F BA/AB = BA/AB) was 1.6 and the compound did not activate ATPase enzyme within the monolayer. On the basis of these data, MC18 was classified as a potent P-gp inhibitor (EC50 = 1.6 μM) [47].

Elacridar was defined as a P-gp inhibitor because it displayed BA/AB < 2, was unable to deplete endocellular ATP and inhibited P-gp transport showing an EC50 value in the nanomolar range [50].

Both P-gp inhibitors at 50 μM have been tested in the everted gut sac employing rhodamine-123 as a fluorescent P-gp substrate. As depicted in Fig. (11), MC18 poorly decreased the efflux (Fig. (11A)) and the influx (Fig. (11B)) of rhodamine-123. From each assay k2 was 1.4 and k1 was 1.5, and these values are listed in Table 1. Elacridar markedly modified the transport (Fig. (11C) and (11D)) of the fluorescent substrate (k2 = 4.1 and k1 = 5.1, Table 1). However, k2/k1 values for MC18 and elacridar were comparable (0.93 vs 0.80).

MC18 and elacridar displayed superimposed results in all in vitro biological assays, although their potency in inhibiting P-gp activity was different. However, in PET studies several differences were found [22,45]. The first aspect involves brain uptake: [11C]MC18 displayed higher uptake than [11C]elacridar and the uptake of the last was lower than verapamil, an avid P-gp substrate. Moreover, [11C]MC18 targeted peripheral organs such as intestine, lung and kidney where the presence and the role of P-gp is largely demonstrated. By contrast, [11C]elacridar poorly targeted the peripheral organs above mentioned. In the presence of cold MC18 and elacridar, two different effects were observed: pretreatment with unlabeled MC18 decreased binding of the corresponding radiotracer to P-gp in various brain areas and in all target organs (from 35% to 70%) while elacridar showed an increased uptake of radiotracer into the brain. These findings allow us to consider MC18 as a true P-gp inhibitor while elacridar seems to be a dose-dependent inhibitor: at low concentration it is a P-gp
Fig. (10). Rhodamine-123 transport in everted gut sac in the absence (A) and presence (B) of an inhibitor (a), substrate (b), substrate-modulator (c) and substrate-inducer (C) of P-gp.
substrate while at high concentration it shows P-gp inhibitory activity [22]. Moreover, a significant activity of elacridar towards BCRP could be another aspect that distinguishes MC18 from elacridar during in vivo experiments. In conclusion, MC18 and elacridar are both characterized as P-gp inhibitors in vitro, but only MC18 displayed the expected results in PET studies. Additional investigations are needed to establish the interaction of elacridar with P-gp and BCRP transporters.

COMPARISON IN VITRO AND IN VIVO OF P-gp SUBSTRATES MC266 AND VERAPAMIL

Compound MC266 at a concentration of 20 μM increased the intracellular accumulation of doxorubicin in MCF7/adr cells 4.8-fold and potentiated the antiproliferative effect of 5 μM doxorubicin from 5% to 85%. BA/AB ratio of this compound was 18, and it strongly activated ATPase within Caco-2 cells. These combined results led to define MC266 as a P-gp substrate [47]. Verapamil (EC50 = 20 μM) [47] is the most widely employed and best known P-gp substrate although it differed from MC266 because the BA/AB ratio for verapamil was only 1.2 so that these results led to define verapamil as atypical P-gp substrate classified as not transported substrate [47].

Both P-gp substrates at 50 μM have been tested in the everted gut sac as depicted in Fig. (12). MC266 and verapamil caused a decrease of rhodamine-123 efflux as depicted in Fig (12A) and Fig. (12C) (k2 = 2.2 and 24.7, respectively). In contrast, MC266 caused a weak increase of rhodamine-123 influx (Fig. (12B)) whereas verapamil increased (Fig. (12D)) influx (k1 = 1.3 and 5.8, respectively). Consequently, k2/k1 was 1.7 for MC266 and 4.3 for verapamil.

In summary, MC266 and verapamil displayed different potencies (EC50 = 6.35 μM and EC50 = 20 μM, respectively) in modulating P-gp activity and different BA/AB ratios (18 vs 1.2, respectively). These compounds have been employed in PET studies as [11C]verapamil and [11C]MC266 demonstrating some similarities and several interesting differences. At baseline, [11C]MC266 displayed a 10-fold higher brain uptake than [11C]verapamil. However, after CsA pretreatment a higher and superimposed brain uptake was observed for both radiotracers [45].

These findings confirmed that MC266 and verapamil showed different k2/k1 ratios and different values of brain uptake, although both are claimed to be P-gp substrates. In contrast to [11C]verapamil, [11C]MC266 could be employed in PET studies to detect upregulation of P-gp function in neurological disorders such as epilepsy.

COMPARISON IN VITRO AND IN VIVO OF P-gp MODULATORS MC80 AND CYCLOSPORIN A

MC80, a potent P-gp interacting agent (EC50 = 0.30 μM) displayed a BA/AB ratio of 3.6, and it did not activate ATPase within Caco-2 cells [48].
In the same in vitro assays CsA (EC<sub>50</sub> = 80 μM) [47] was less potent than MC80, it displayed a BA/AB ratio of 9.6 and did not activate ATPase. Combining the results of these assays, MC80 and CsA were classified as transported substrates [48].

MC80 has been radiolabelled with <sup>11</sup>C and tested in mice. This radiotracer displayed good uptake in mouse brain followed by an efficient wash-out [46]. Brain uptake was always greater than tracer concentrations in blood. <sup>11</sup>C-MC80 is cleared from plasma via the hepatobiliary system as shown by the high liver uptake and the increase in radioactivity uptake over time in small and large intestine. <sup>11</sup>C-MC80 also demonstrated a high initial uptake in kidney and a subsequent urinary clearance (data not shown). Pretreatment of animals with CsA caused a significant increase of radioactivity uptake in mice brain compared to FVB mice treated with physiological saline. Brain uptake was raised 1.3 - 2 fold compared to mice without CsA pretreatment. Except at 1 min p.i., CsA also increased the uptake of <sup>11</sup>C-MC80-derived radioactivity in several peripheral organs including testes, pancreas, spleen, kidneys, and liver. Compared to mice treated with saline, radioactivity uptake in blood, heart and lungs was already higher at 1 min p.i. and remained higher at each time point. In contrast, intestinal uptake of <sup>11</sup>C-MC80 was significantly lower after CsA pretreatment. A possible reason is the reduced binding to P-gp caused by CsA modula-

### Table 1. Biological in vitro Assays and Classification of the Mechanism of Interaction of the Tested Compounds with P-gp

<table>
<thead>
<tr>
<th>Compound</th>
<th>P&lt;sub&gt;app&lt;/sub&gt; (BA/AB)</th>
<th>ATPase Activation</th>
<th>Potency EC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
<th>Everted gut sac</th>
<th>Classification</th>
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<tr>
<td>MC18</td>
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<td>No</td>
<td>1.6</td>
<td>1.4</td>
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<tr>
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<td>Yes</td>
<td>6.35</td>
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<tr>
<td>MC80</td>
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<td>No</td>
<td>0.30</td>
<td>0.80</td>
<td>Substrate-Inducer</td>
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<tr>
<td>elacridar</td>
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<td>0.02&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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<td>80</td>
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<sup>a</sup>see references 47 and 50

Fig. (12). Rhodamine-123 efflux (A or C) and influx (B or D) in the absence (solid line) and presence (dotted line) of MC266 or verapamil.
tion. Taken together, CsA administration has an effect on the tissue uptake of $[^{11}C]MC80$ suggesting that P-gp plays a role in the pharmacokinetics of $[^{11}C]MC80$.

Moreover, cold MC80 has been co-administered with $[^{11}C]dlop$ causing a reduction of brain uptake of the radiotracer (unpublished data) suggesting MC80 is a P-gp inducer [46].

Considering these results, CsA and MC80 allosterically modulate P-gp substrate sites: CsA causing a decrease of P-gp efflux activity whereas MC80 increases the efflux efficiency of P-gp. These suggestions were consistent with everted gut sac results, as depicted in Fig. (13). MC80 increased rhodamine-123 efflux (Fig. (13A)) displaying $k_2 = 0.80$ while CsA (Fig (13C)) decreased substrate efflux ($k_2 = 3.5$). Similarly, MC80 and CsA (Fig. (13B) and (13D)) strongly decreased the influx of rhodamine-123 ($k_1 = 0.084$ and 0.06, respectively). MC80 showed $k_2/k_1 = 10$ while for CsA $k_2/k_1 = 58$. In detail, MC80 and CsA differently affected rhodamine-123 efflux but displayed the same effect on rhodamine-123 influx.

CONCLUSION

The present report illustrates tentatively how a P-gp ligand could be characterized as a radiotracer candidate for PET. The tests that have been carried out to identify the mechanism of interaction of each studied compound with P-gp and its ability to target the transporter are highlighted in Fig. (8). Measured pharmacokinetic parameters are presented in Table 1.

The present findings aimed to assess specific and sensitive biological in vitro and ex vivo assays to screen potential PET radiotracers for P-gp imaging. The objective is to minimize the discrepancies that could be observed between in vivo PET studies and the corresponding in vitro results.

This review, considering all in vitro assay and everted gut sac results, is a contribution to predict if the in vitro tests could match the corresponding in vivo PET data. Thus, the present paper is an attempt to define an in vitro test procedure for potential PET radiotracers, with a high predictive value for the in vivo results.

REFERENCES


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Received: December 17, 2010   Accepted: February 15, 2010