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Imaging of P-glycoprotein function and expression to elucidate mechanisms of pharmacoresistance in epilepsy

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Abstract

The issue of pharmacoresistance in epilepsy has received considerable attention in recent years, and a number of plausible hypotheses have been proposed. Of these, the so-called transporter hypothesis is the most extensively researched and documented. This hypothesis assumes that refractory epilepsy is associated with a localised over-expression of drug transporter proteins such as P-glycoprotein (Pgp) in the region of the epileptic focus, which actively extrudes antiepileptic drugs (AEDs) from their intended site of action. However, although this hypothesis has biological plausibility, there is no clinical evidence to support the assertion that AEDs are sufficiently strong substrates for transporter-mediated extrusion from the brain. The use of modern brain imaging techniques to determine Pgp function in patients with refractory epilepsy has started only recently, and may ultimately determine whether increased expression and function of Pgp or other efflux transporters are involved in AED resistance.

Introduction

Drug efflux transporters that are constitutively expressed at the blood-brain barrier (BBB) limit the ability of many drugs to enter the brain [1-3]. The prototypic drug efflux transporter P-glycoprotein (Pgp) was initially discovered in the 1970s as a transporter involved in multidrug resistance of cancer cells, but was also the first drug efflux transporter that was detected in endothelial cells of the human BBB [4,5]. Subsequently, Pgp was located in brain capillary endothelial cells of several species, including humans and other primates, rats, mice, cattle, and pigs, suggesting that Pgp may serve as a general defense mechanism in the mammalian BBB, protecting the brain from intoxication by potentially harmful lipophilic compounds from natural sources and other lipophilic xenobiotics that otherwise could penetrate the BBB by simple diffusion without any limitation [6,7].

The multidrug transporter hypothesis of pharmacoresistant epilepsy

Epilepsy, which is characterized by spontaneous recurrent seizures, is one of the most common neurological disorders. Despite advances in antiepileptic drug (AED) therapy, about one-third of epileptic patients are resistant to drug treatment [8]. The consequences of

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uncontrolled epilepsy can be severe and include shortened lifespan, bodily injury, neuropsychological and psychiatric impairment, and social disability. Most patients with refractory epilepsy are resistant to several, if not all, AEDs, even though these drugs act by different mechanisms [8]. This multidrug type of resistance argues against epilepsy-induced alterations in specific drug targets as the main cause of pharmacoresistant epilepsy, but rather points to nonspecific and possibly adaptive mechanisms [6,7].

Epilepsy was the first CNS disorder for which drug resistance was associated with enhanced expression of Pgp in the brain [9]. Pathologically elevated expression of Pgp has been found in resected brain tissue of patients with refractory temporal lobe epilepsy (TLE) undergoing epilepsy surgery as well as in limbic brain regions of mouse and rat models of TLE [6,7]. In such animal models, increased expression of Pgp was associated with decreased brain concentration of AEDs such as phenytoin. These observations have led to the multidrug transporter hypothesis, which proposes that refractory epilepsy may be the consequence of a localized overexpression of transport proteins that prevents AEDs from penetrating the BBB in sufficient concentration - and would explain why patients are resistant to multiple AEDs with distinct mechanisms of action [6-8,10-12].

The transporter hypothesis has gained enormous interest in recent years, because, if true, it would allow overcoming pharmacoresistance by either inhibiting or bypassing Pgp or other involved efflux transporters [13]. Increased brain expression of efflux transporters, such as Pgp, could be either a result of prolonged or frequent seizures, as demonstrated in rodent models of epilepsy, or of genetic factors, such as polymorphisms in the *ABCB1* gene or, theoretically, of both [6,7]. Similar to the famous Koch's postulates, which were originally used to establish the role of bacteria in infectious disease, Sisodiya [12] suggested that at least four criteria must be satisfied for a proposed drug-resistance mechanism of epilepsy to be accepted; the mechanism must: (1) be detectable in epileptogenic brain tissue; (2) have appropriate functionality; (3) be active in drug resistance; and (4) drug resistance should be affected when the mechanism is overcome. All these criteria recently have been satisfied for the transporter hypothesis, however as yet, only in rodent models of epilepsy [14]. Accordingly, Pgp expression is: 1) increased in epileptogenic brain tissue of rodents; 2) associated with lower brain levels of AEDs; 3) higher in AED-resistant rats than in responsive animals; and most importantly, 4) co-administration of the highly selective Pgp inhibitor, tariquidar, reverses AED resistance. Thus, it is reasonable to conclude that Pgp plays a significant role in mediating resistance to AEDs in rodent models of TLE and that inhibition of Pgp can circumvent this mechanism - however, whether this phenomenon extends to at least some human refractory epilepsy remains unclear [14].

One major problem in this respect is that species differences in substrate recognition by Pgp have been reported, and there is as yet only limited evidence that human Pgp transports any AED [15-18]. Furthermore, the question of whether overexpression of Pgp is exclusive to patients with pharmacoresistant seizure disorders remains to be resolved. Even if differences in Pgp expression between AED-resistant and responsive patients exist, overexpression of Pgp in pharmacoresistant epilepsy patients may be an epiphenomenon related to the frequency of seizures or underlying pathology without any functional consequences for brain levels and efficacy of AEDs. Noninvasive brain imaging of Pgp function in AED resistant and responsive epilepsy patients is the only strategy to evaluate whether overexpression of Pgp has any functional consequences that may be relevant for the mechanisms underlying pharmacoresistance in epilepsy.

Brain imaging of ABC transporter function and expression

Modern brain imaging methods now provide unprecedented opportunities for the *in vivo* study of CNS function. There are a number of methods for imaging compounds in the brain, including positron emission tomography (PET), single photon emission computed tomography (SPECT) and magnetic resonance spectroscopy (MRS). PET and SPECT are imaging techniques in which a radionuclide is synthetically introduced into a molecule of potential biological relevance and administered to a patient or experimental animal [19-21]. PET or SPECT cameras monitor the distribution of these radiolabelled molecules ("radiotracers") in the living organism over time. The concentration-time curves of radiotracers are analyzed by biomathematical modelling to derive measures of *in vivo* neurochemistry (e.g. blood flow, metabolic rate of glucose, density of receptor proteins). The great advantage of PET over SPECT is a higher sensitivity, better temporal and spatial resolution and the ability to provide a quantitative measure of radioactivity in tissue. These methods are particularly attractive to clinical researchers, because they can be applied to the living human brain. PET typically makes use of the radioisotopes carbon-11 (^{11}C), nitrogen-13 (^{13}N), oxygen-15 (^{15}O) or fluorine-18 (^{18}F). These decay by emitting a positron, with radioactive half-lives of 20.4, 9.96, 2.04 and 109.7 min, respectively. The emitted positron annihilates with an electron and emits two colinear annihilation photons (gamma rays) that can be detected outside the body. Typically, the radioisotopes are made in a cyclotron and because of their short half-lives need to be rapidly incorporated into the molecule of interest immediately prior to administration. In SPECT, isotopes that decay by electron capture and/or γ emission are used, and include both iodine-123 (^{123}I , half-life: 13.1 h) and technetium-99m ($^{99\text{m}}\text{Tc}$, half-life: 6.01 h).

Both PET and SPET, together with radiolabelled substrates and inhibitors of Pgp, have been used for quantitative assessment of Pgp function in the BBB [2,21]. For PET studies on Pgp function, radiotracers based on known Pgp substrates have been developed and characterized *in-vitro* and *in-vivo* in laboratory animals, including [^{11}C]daunorubicin, [^{11}C]colchicine, [^{18}F]paclitaxel, [^{11}C]carvedilol, [^{11}C]loperamide, [^{11}C]-*N*-desmethyl-loperamide, and [^{11}C]verapamil [2,19,21]. Only three of these radiotracers have so far advanced to studies in human subjects ([^{11}C]loperamide, [^{11}C]-*N*-desmethyl-loperamide, and [^{11}C]verapamil). Of these, [^{11}C]verapamil is the best validated PET tracer to date [2, 21, 22]. In Pgp-knockout mice, a 10-fold increased brain uptake of [^{11}C]verapamil was found as compared to wild-type mice [23]. A quantitatively similar effect has been described in normal mice by administration of the Pgp inhibitor cyclosporin A (CsA). In humans, a 70% increase in brain concentrations of [^{11}C]verapamil was determined following CsA infusion as compared to baseline scans, which confirmed that [^{11}C]verapamil is transported by Pgp at the human BBB [24]. The (*R*)-enantiomer of [^{11}C]verapamil has been suggested to be superior for PET imaging than racemic radiotracer, due to differences in metabolism and plasma protein binding between the two enantiomers, with the (*R*)-enantiomer being metabolically more stable than the (*S*)-enantiomer [25,26].

However, currently available radiotracers are not ideal for assessing differences in Pgp function in different brain regions (e.g. epileptic *versus* non-epileptic brain regions). [^{11}C]loperamide, [^{11}C]-*N*-desmethyl-loperamide and (*R*)-[^{11}C]verapamil are all high-affinity substrates of Pgp. They are therefore very effectively transported by Pgp at the BBB which results in low brain uptake of these tracers. Brain entry of these tracers may be maximally blocked by Pgp-mediated efflux and therefore be independent of regional differences in cerebral Pgp function and expression. [^{11}C]loperamide [27] and (*R*)-[^{11}C]verapamil [26] undergo extensive cytochrome P450-mediated metabolism. In the case of (*R*)-[^{11}C]verapamil it has been shown that a polar radiometabolite is generated which is taken up into brain tissue independent of Pgp function [26]. The situation is further

complicated by the fact that (*R*)-[¹¹C]verapamil metabolism differs between healthy volunteers and epilepsy patients, possibly due to the enzyme-inducing properties of AEDs [28]. [¹¹C]-*N*-desmethyl-loperamide is metabolised to a lesser extent than (*R*)-[¹¹C]verapamil and does not appear to give radiometabolites which are taken up in brain independent of Pgp function [29]. However, it is a high-affinity Pgp substrate that shows virtually no brain uptake, which makes it unsuitable to map regional differences in Pgp function [21,30].

A possible strategy to overcome the limitation of low brain uptake of currently available Pgp substrate radiotracers is to perform PET scans after partial blockade of Pgp by Pgp modulating drugs, such as CsA or tariquidar. For this strategy, it is important that Pgp blockade is incomplete as brain uptake of the radiolabelled Pgp substrate would otherwise be driven by passive diffusion only and fail to delineate regional differences in Pgp function and expression [31]. The third-generation Pgp inhibitor tariquidar [32] is safer than CsA for use in human subjects and was shown to lack interactions with metabolism and plasma protein binding of (*R*)-[¹¹C]verapamil [33,34]. A pilot study in 5 healthy volunteers showed that the brain distribution volume of (*R*)-[¹¹C]verapamil was increased by a mean of 25±14% following administration of tariquidar at a dose of 2 mg/kg body weight [34].

A complementary approach to assessing the Pgp system with PET is the use of radiotracers which bind with nanomolar affinity to Pgp without being transported by it, in analogy to PET tracers which are commonly used for mapping the distribution of other transporter proteins in brain, such as [¹¹C]-labelled 2beta-carbomethoxy-3beta-(4-fluorophenyl)-tropane ([¹¹C]CFT) for the dopamine transporter [35] or [¹¹C]3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile ([¹¹C]DASB) for the serotonin transporter [36]. Such a radiotracer would give a signal increase rather than a decrease (as will a Pgp substrate tracer) in brain regions that overexpress Pgp. A combined use of PET tracers based on Pgp inhibitors with radiolabelled transporter substrates, such as (*R*)-[¹¹C]verapamil, might be particularly informative, as inhibitors would allow for assessing transporter distribution, whilst substrates assess transporter function. The principle of this new study approach is illustrated in Figure 1.

Pgp is a target protein which is ubiquitously and abundantly expressed in brain tissue. Kamiie and colleagues [37] reported a density ($B_{\max}$) of Mdr1a Pgp of 15 fmol/μg protein in mouse brain capillaries as measured with liquid chromatography tandem mass spectrometry (LC/MS/MS) analysis. When assuming a protein content of brain capillaries of approximately 10%, the $B_{\max}$ value determined by Kamiie et al. [37] translates to a concentration value of 1500 nM, which is by far greater than for instance the concentration of the highly abundant dopamine D₂ receptor in the basal ganglia (approximately 30 nM) [38]. Four PET radiotracers based on third-generation Pgp inhibitors have been reported to date: [¹¹C]laniquidar, [¹¹C]MC-18 (6,7-dimethoxy-2-{3-[4-methoxy-3,4-dihydro-2*H*-naphthalen-(1*E*)-ylidene]-propyl}-1,2,3,4-tetrahydro-isoquinoline), [¹¹C]elacridar and [¹¹C]tariquidar [39-42]. The ratio of the $B_{\max}$ of a given target protein over the equilibrium dissociation constant (K_d) of a prospective radioligand is commonly used as a predictor of the target-to-nontarget ratio to be expected in *in vivo* imaging [43]. Martin and co-workers [44] reported a K_d of 5.1 nM for the binding of tritium-labelled tariquidar to membranes of Chinese hamster ovary resistant cells (CH^TB30). Although, to the best of our knowledge, no K_d values have so far been reported for elacridar, most *in vivo* data indicate that elacridar is equipotent or even more potent than tariquidar in inhibiting Pgp-mediated transport of Pgp substrates which points to comparable or even lower K_d values for elacridar [31]. Laniquidar, on the other hand, appears to be at least 10-fold less potent as compared to elacridar and tariquidar in inhibition of Pgp transport [41]. For tariquidar and elacridar, the $B_{\max}/K_d$ is therefore approximately 300, which should allow for obtaining a sufficiently

high Pgp-specific *in vivo* signal. Interestingly, elacridar and tariquidar appear to bind to distinct sites on Pgp, which seem to interact allosterically [45].

In vivo, [^{11}C]elacridar and [^{11}C]tariquidar possess low brain activity uptake (peak standardized uptake values *SUV* in rat brain, <1), which might be explained by the fact that they bind at tracer doses with high affinity to Pgp at the BBB and are thereby hindered from entering brain parenchyma [40]. In line with this assumption, uptake of [^{11}C]elacridar and [^{11}C]tariquidar in rat brain was severalfold increased following pretreatment with unlabelled tariquidar or elacridar [39,40]. Due to the limited spatial resolution of small-animal PET (about 1 mm), brain capillaries cannot be resolved from surrounding brain tissue in rodents. The presumably high signal of [^{11}C]elacridar and [^{11}C]tariquidar binding to Pgp located on brain capillaries, which account for only approximately 5% of total brain tissue volume, might thus be diluted by a background of low radiotracer uptake in surrounding brain tissue thus giving an overall low Pgp signal. Although the apparently small Pgp-specific PET signal obtained with [^{11}C]elacridar and [^{11}C]tariquidar might not be optimal for *in vivo* imaging, the advantage might be that non-specific binding is quite low or absent. This is of particular importance given the high lipophilicity of these molecules. In addition, [^{11}C]elacridar and [^{11}C]tariquidar appear to display excellent metabolic stability which avoids the problem of contamination of the Pgp-specific signal by radiometabolites. It has yet to be determined in how far brain distribution of these radiolabelled Pgp inhibitors correlates with regional Pgp expression levels. Pilot data acquired for [^{11}C]elacridar and [^{11}C]tariquidar in rats¹ indicate that this is indeed the case (see Figure 1).

[^{11}C]MC-18 possesses considerably higher brain uptake as compared to [^{11}C]elacridar, [^{11}C]tariquidar and [^{11}C]laniquidar (peak brain uptake, *SUV*, in rat brain approximately 1.5) [42]. In addition, *in vivo* brain activity uptake was reduced by approximately 30% after pretreatment with unlabelled MC-18 (15 mg/kg). This is in contrast to the behavior of [^{11}C]elacridar and [^{11}C]tariquidar, which both showed increased rather than decreased brain activity uptake following pretreatment with unlabelled inhibitor [39,40]. However, proof is still lacking that the brain PET signal obtained with [^{11}C]MC-18 is indeed specific to Pgp [42].

Currently available SPECT tracers are not ideal for imaging Pgp function. The most widely used radiotracer utilized as a transport substrate for Pgp in SPECT studies is [$^{99\text{m}}\text{Tc}$]sestamibi, a cationic radiotracer originally developed for myocardial scintigraphy. Because of its unique role as a Pgp-specific substrate, this compound can be used to examine Pgp functional activity *in vitro* and *in vivo* under pathologic conditions. [$^{99\text{m}}\text{Tc}$]sestamibi is widely used to detect *in vivo* Pgp activity in tumors, and may be a useful technique for monitoring the development of Pgp-mediated multidrug resistance [19,21]. In rats, inflammation-mediated downregulation of brain *mdr1a* mRNA, the gene encoding Pgp in brain capillary endothelial cells in rodents, is associated with higher accumulation of [$^{99\text{m}}\text{Tc}$]sestamibi in the brain, further demonstrating the potential utility of [$^{99\text{m}}\text{Tc}$]sestamibi for noninvasively assessing Pgp function *in vivo* [46]. However, to a lesser extent, [$^{99\text{m}}\text{Tc}$]sestamibi is also transported by multidrug resistance-associated protein 1 (MRP1), which may form a bias for results obtained with [$^{99\text{m}}\text{Tc}$]sestamibi [47]. Alternative Pgp substrates for SPECT analysis are [$^{99\text{m}}\text{Tc}$]tetrafosmin and a $^{99\text{m}}\text{Tc}$ (I)-tricarbonyl complex of sestamibi [19,21]. However, because all these compounds are cationic radiotracers, cellular functions other than Pgp and MRP1 function, most likely cationic ion transporters, may affect their distribution. Furthermore, in contrast to PET, SPECT does not

¹Kuntner, C.; Bankstahl, J. P.; Bankstahl, M.; Stanek, J.; Wanek, T.; Dörner, B.; Bauer, F.; Mairinger, S.; Erker, T.; Müller, M.; Löscher, W.; and Langer, O. A new PET study paradigm to assess cerebral P-glycoprotein expression and function. *Nuklearmedizin* **2009**, *48*, A152 (abstract).

enable quantitative measurements of uptake and efflux kinetics of Pgp substrates and thus has only limited use in studying Pgp function in the BBB.

Imaging of P-glycoprotein function and expression to elucidate mechanisms of pharmacoresistance in epilepsy

The use of PET or SPECT to determine Pgp function in patients with refractory epilepsy has started only recently. In seven patients with drug-resistant unilateral TLE, the Pgp substrate (*R*)-[¹¹C]verapamil and PET were used to test for differences in Pgp activity between epileptogenic and nonepileptogenic brain regions [48]. Unfortunately, the most interesting epileptogenic brain region, i.e., the hippocampus, could not be analyzed due to spill-in of radioactivity from the adjacent choroid plexus. However, in five of the seven patients, increased efflux of radioactivity was determined in ipsilateral parahippocampal regions, indicating increased Pgp expression in the epileptogenic hemisphere [48]. These pilot data in a small sample size of patients may indicate that regionally enhanced Pgp activity contributes to drug resistance in some patients with TLE.

Differences in Pgp expression in patients with epilepsy may be a consequence of polymorphisms in drug transporter genes. One of these, a single nucleotide polymorphism (SNP) in exon 26 (C3435T) of the *Multidrug Resistance 1* (*MDR1*) gene is associated with altered expression, functionality and substrate specificity of the *MDR1* product Pgp [49]. Because the efflux drug transporter Pgp is highly expressed at the BBB, genetic variation in its expression or functionality could directly affect brain uptake and extrusion of AEDs. Indeed, several recent genetic association studies have indicated an association of the 3435CC genotype, which is associated with increased Pgp expression, with AED resistant epilepsy [49]. However, other studies did not support such an association. In healthy volunteers differing in *MDR1* haplotypes, no difference in the brain distribution of [¹¹C]verapamil could be detected by PET analyses [50,51]. On the other hand, in a recent SPECT study in patients with epilepsy (which is as yet only available as abstract²), the 3435CC genotype was associated with reduced brain uptake of [^{99m}Tc]sestamibi, which was correlated with AED resistance. Furthermore, cerebrospinal fluid concentrations of the AED phenobarbital were significantly lower in epilepsy patients with the 3435CC genotype [52].

Of course, the ideal PET or SPECT radiotracer for studies in epilepsy patients would be a radiolabelled AED. About 30 years ago, [¹¹C]phenobarbital and [¹¹C]phenytoin were synthesized and the latter radioligand has been used to study in vivo distribution of phenytoin in monkeys and humans [53-55]. In a study in eight patients with medically resistant partial epilepsy and two patients without epilepsy, [¹¹C]phenytoin was used to evaluate the brain kinetics of this AED [55]. At steady-state, brain:blood ¹¹C-activity concentration ratios were determined for the visual cortex, resulting in an average value of 1.32 (range 1.05-1.66) in epilepsy patients compared to 1.61 (1.34-1.87) in nonepileptic patients [55]. Whether phenytoin steady-state concentration is lower within the epileptic focus was not addressed in this study. Preliminary data indicated that [¹¹C]phenytoin kinetics within the estimated epileptogenic area were not significantly different when compared to the contralateral homologous brain region in the same epilepsy patients [55], but additional data with high-resolution PET are needed for such an analysis. Furthermore, as indicated by the study on (*R*)-[¹¹C]verapamil in epilepsy patients, the uptake and efflux kinetics rather than steady-state levels of Pgp substrates should be examined in epileptogenic brain regions [48].

²Jensen, S.; DiPaolo, A.; Lastella, M.; Erba, P.; Baldini, M.; Perini, D.; Pecori, C.; Danesi, R.; Iudice, A.; Mariani, G.; Del Tacca, M.; and Murri, L. Pharmacogenetics of ABCB1 and brain kinetics of 99m-Tc-MIBI in epilepsy patients. *Epilepsia* **2006**, *47* (Suppl. 3), 88-89 (abstract).

Future Goals

In the past few years, methods for measuring the role of Pgp expression in cerebral kinetics of drugs in humans have become more accessible. By using imaging techniques such as PET or SPECT, a future goal will be the comparison of brain kinetics of Pgp substrates in AED-resistant and -responsive patients with epilepsy. Furthermore, if such studies support a role of Pgp in AED resistant epilepsy, PET or SPECT may be used to identify AED-resistant patients with regionally enhanced Pgp activity, which would most likely benefit from coadministration of Pgp inhibitors. Recent studies from our laboratory using AED resistant epileptic rats with increased Pgp expression in epileptogenic brain regions have demonstrated that AED resistance could be counteracted by coadministration of the selective Pgp inhibitor, tariquidar, in such animals [56]. Thus, animal models may be used (i) to develop diagnostic techniques for identification of Pgp overexpression and (ii) to evaluate novel treatment strategies for drug-resistant subjects.

Recent advances in small-animal imaging have provided new ways to perform biologic research. These non-invasive, in vivo imaging techniques permit longitudinal studies of the same animals over an extended period of time. Both SPECT and small-animal PET ("microPET") have been extensively used to study brain kinetics of Pgp substrates in mice or rats. We plan to use a high-resolution small-animal PET system for studying the potential role of Pgp in AED resistance by two strategies in rats. In the first strategy, a pilocarpine-induced status epilepticus (SE) is used. We have shown recently that such SE is followed by a regionally restricted, significant overexpression of Pgp in brain capillary endothelial cells forming the BBB with 48 h [57]. The overexpression of Pgp in the hippocampus is associated with a significantly reduced brain:plasma ratio of phenytoin [57]. Thus, such rats are an ideal model to study whether the regionally restricted increase in Pgp expression and function can be detected by PET using radiolabelled Pgp substrates such as [^{11}C]verapamil or [^{11}C]phenytoin.

The second approach is much more time- and labor-extensive. In this approach, AED resistant and responsive epileptic rats are selected by prolonged treatment with phenobarbital. Epilepsy is induced by prolonged electrical stimulation of the basolateral amygdala (BLA), resulting in spontaneous recurrent seizures after about 4-6 weeks following the SE. About 30-40% of the epileptic rats are resistant to both phenobarbital and phenytoin, whereas the remaining rats respond to these AEDs [58]. We have shown recently that AED nonresponders exhibit a significant overexpression of Pgp in brain capillary endothelial cells of the hippocampus, dentate gyrus and parahippocampal areas [59]. Inhibition of Pgp by tariquidar counteracts the AED resistance [56]. We now plan to use small-animal PET to study the differences in brain kinetics of Pgp substrates between AED responders and nonresponders. Because our rat model has strong parallels with human TLE, we hope that the results from these studies will help to develop strategies for evaluating the multidrug transporter hypothesis in patients with refractory TLE.

In conclusion, the recent development of radiolabelled Pgp substrates for human use now allows to directly evaluating the role, if any, of Pgp in medically refractory epilepsy. Furthermore, PET and SPECT are useful to explore the relationship between polymorphisms of *MDR1* and the pharmacokinetics of Pgp substrates within the brain of patients with epilepsy. Information about the transport function of Pgp and the effect of modulating this function may improve the therapeutic treatment of patients with pharmacoresistant epilepsy.

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[¹¹C]Inhibitor scan **[¹¹C]Substrate scan** **Figure 1.**

Assessing the cerebral Pgp system with a novel double PET scan paradigm in a rat model of Pgp overexpression. A scan with an [¹¹C]-labeled Pgp inhibitor is followed by a second scan with the Pgp substrate (*R*)-[¹¹C]verapamil, recorded after administration of 3 mg/kg tariquidar (see study timeline). Shown are horizontal PET summation images as well as dose-normalized time-activity curves for both scans. Note that despite low brain uptake of [¹¹C]inhibitor the rank order of brain activity uptake is inverse for the [¹¹C]inhibitor and [¹¹C]substrate scans. Brain regions that show high uptake of [¹¹C]inhibitor, suggesting binding of [¹¹C]inhibitor to Pgp, show low uptake of (*R*)-[¹¹C]verapamil, suggesting Pgp-mediated efflux.