Chapter 13

Role of ABC-Transporters in Epileptogenesis and Pharmacoresistant Epilepsy

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Additional information is available at the end of the chapter

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Abstract

According to a recently published article by our group [*The complexity of roles of P-glycoprotein in refractory epilepsy: pharmacoresistance, epileptogenesis, SUDEP and relapsing marker after surgical treatment ADMET & DMPK 3(2) (2015) 110-121*], we have written a chapter related to these concepts.

The manuscript reviews the structure and function of several ABC-transporters, their roles in the transport of different natural compounds, as well as a wide spectrum of drugs. In this regard, it is important to remember that their expression is also related to the highly specialized functions of specific types of cells. In each of these the expression can be transient, permanent or "*de-novo*" induced, also secondary to a wide spectrum of factors. As described initially in cancer, overexpression of ATP-binding cassette (ABC) transporters such as P-glycoprotein (ABCB1, P-gp), multidrug resistance-associated protein (ABCC1, MRP), and breast cancer–resistance protein (ABCG2, BCRP) confers a multidrug-resistant phenotype, by transporting a diverse range of compounds out of the cell against a concentration gradient. This characteristic was also later demonstrated in epilepsy, particularly in cases receiving simultaneously more than 3 antiepileptic drugs (AEDs).

Additional information related to genetic variants such as the Single Nucleotide Polymorphism (SNP) of these transporters, whether alone or associated with a Cytochrome (CYP) system, can modify their functional expression level inducing changes in their pharmacokinetics, their bio-distribution and their brain access to more common AEDs, producing an imbalance in their dose–response equilibrium. Furthermore, the increased production and design of new AEDs, as observed during the last 30 years, has not decreased the high percentage (30–40%) of drug-resistant epileptic cases.

The AEDs design is based on experimental models of seizures induced in "normal/non-epileptic" animals (mice or rats). For this reason, a discussion on the current experimental models of epilepsy will be included, as well as a suggestion that the next generation of AEDs should be developed and assayed via new experimental models where the current AEDs have failed.

One important aspect regarding pharmacoresistant phenotype is that it can be present at the onset of diseases, or it can be acquired progressively. Differences in both conditions can be related with therapeutic error, loss of compliance, or with the specific epileptic syndrome. Of particular interest results is the fact that ABC transporters "P-gp and BCRP" are also the biomarkers of stem cells. In this regard, some epileptic syndromes, secondary to malformations of cortical development or brain tumors, may also serve as a biomarker of risk for seizure relapse after epilepsy surgery.

Finally, we assume that the pathophysiological condition of "hypoxic stress" is produced during each seizure, and this mechanism induces a wide spectrum of biological responses at cellular levels (neurons, astrocytes) in the brain, and on peripheral organs such as the heart. This complex regulatory system can also induce ABC-transporter overexpression in the cells of these different organs. Because P-gp is not expressed in both normal neurons and cardyomiocytes, and P-gp expression can produce membrane depolarization, we can speculate that P-gp could play a role in changing the electric properties of each of these cells. Furthermore, our previous studies suggest that P-gp overexpression in neurons plays a role in epileptogenesis and its expression in cardyomiocytes could be related with Sudden Unexpected Death in Epilepsy (SUDEP).

Keywords: ABC-transporters, Epileptogenesis, Pharmacoresistant Epilepsy, SUDEP, Stem-cell markers

1. Introduction

Epilepsy was described as a clinical entity by Hippocrates in the 5th century BC, however, the oldest inscriptions date from 4000 years BC. Epilepsy, one of the world's oldest recognized disorders, affects currently around 50 million people worldwide. Furthermore, it is the second most common neurological disorder after stroke, with approximately 1-2% of the population being affected by some form of epilepsy. Two features are characteristic of this disease, around 30-40% of epileptic patients are drug refractory and nearly 90% of epilepsy cases are in low-income countries, where both social consequences and different stratagem of treatments affects seriously their gross national product. Several of, if not all, the described properties of ABC-transporters, particularly P-gp, could be involved in the development of AEDs resistance phenotype as well as playing a part in the intimate mechanisms of epileptogenesis. Hence, not only is the control of pharmacoresistnace important, but the prevention of epileptogenesis too, represents challenges to arresting the development of this disease or reach their clinical manifestation full control.

2. ABC transporters and multidrug resistance (MDR) phenotype

P-glycoprotein (P-gp) is member of the ABC (ATP-binding cassette) superfamily of transporters, discovered in pharmacoresistant cancer cells as a 170 kDa plasma membrane protein (**Figure 1**). The ABCB-1 gene, which is also named MDR-1 gene, encodes P-gp, which was the

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first transporter related to the MDR phenotype [1]. P-gp also named as MDR protein 1 (MDR1); in spite that it is commonly reported as the gene product of the ABCB1 gene, can be also encoded by the ABCB4 gene, and this P-gp sister is named MDR-3 protein – mainly expressed in hepatocytes [2].

Biochemical, molecular, and structural analysis have definitively established that the involvement of P-gp in pharmacoresistance results from its primary function as an ATP- and Ca^{2+}-dependent detoxifying pump, that extrudes potentially toxic compounds out of the cells and can confer resistance levels of 1,000-fold or more to the expressing cells [3]. In spite of the fact that P-gp pumps aqueous soluble drugs, it can also function as a "hydrophobic vacuum cleaner", because many P-gp substrates (largely hydrophobic) bind to P-gp from the lipid bilayer rather than from the aqueous phase.

Currently, 49 different members have been identified in the human genome - these are classified into seven families by the Human Genome Organization (ABC-A to ABC-G). They are encoded in almost all chromosomes [except 5, 8, 15, 18, 20, and Y] [4], and 22 of them have been associated with physiologic or pathological functions. Additional to P-gp, are the

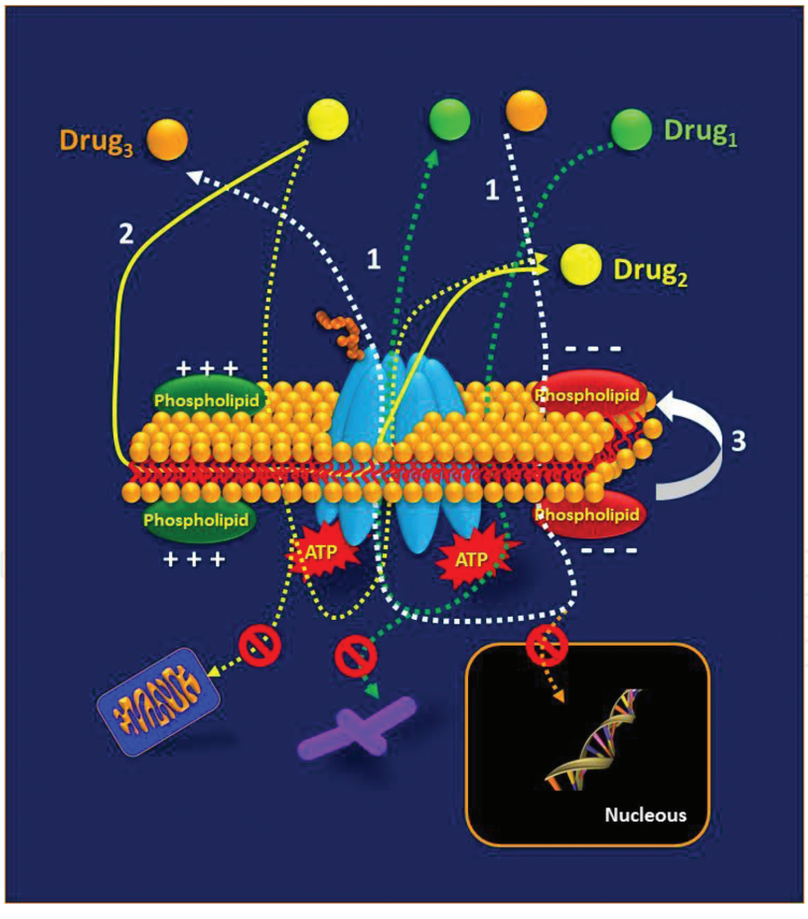

Figure 1. Schematic structure of P-glycoprotein and its typical functional properties. **1**. Efflux system as a pore model. **2**. hydrophobic vacuum cleaner. Both these mechanisms are related to substrate exporting and the pharmacoresistance phenotype in different diseases, including epilepsy. **3**. Phospholipid flippase, related with membrane polarity and suggested as an epileptogenic mechanism.

MDR-associated proteins (MRPs) and breast cancer resistant protein (BCRP) which have also been related to the MDR phenotype. P-gp, MRPs, and BCRP are normally expressed in the luminal surface of most excretory tissues including the capillary endothelial cells in the blood–brain barrier (BBB) or the blood–cerebrospinal fluid (BCSF) barrier (BCSFB), playing together a combined role, i.e., to reduce the brain penetration of many drugs [5].

These three transporters are key in the MDR phenotype of cancer cells and mediate the ATP-dependent unidirectional efflux of different drugs as well as being natural to both endogenous and exogenous compounds.

P-gp and BCRP can export unmodified drugs as well as conjugates, while MRPs can export mainly glutathione and other drug conjugates. Both P-gp and BCRP can transport neutral or cationic compounds, whereas MRPs can transport anionic compounds [6].

A wide spectrum of differentiating agents, hormones, oncogenes, and transcription factors, known to be evolved in apoptosis, stress, inflammation, and hypoxia (e.g., p53, NFkB, NF-IL6, AP-1, HIF-1α, E2F1, and EAPP) can up-regulate the expression of these transporters [7–10], including previously non-expressive cells such as neurons or cardiomyocytes [11, 12]. This property suggests that P-gp and other MDR-like proteins may be also involved in cell survival death–related biological processes [13, 14].

Furthermore, overexpression of microRNAs miR-27a and miR-451 are directly involved in overexpression and activity of P-gp, and treatment of P-gp positive cells with the antagomirs of miR-27a or miR-451 decreased the expression of P-gp and MDR1 mRNA [15].

In the classical pump model, the P-gp alternates between an inward-facing and an outward-facing conformation, and these changes in the transporter induced by either substrate binding or ATP hydrolysis leads to the formation of a hydrophilic channel that permits the release of the substrate from the cytosol to the extracellular space.

On the other hand, some evidence indicates that P-gp can also decrease the plasma membrane potential of several cell types from normal values (−60 mV) to −10 or 0 mV [16, 17], and under this condition, it can also reduce the convulsive thresholds, and additionally modulates the swelling activated Cl^- currents – both physiologic disturbances observed during brain hypoxia and convulsive stress [18–20].

3. Epilepsy and Refractory Epilepsy

Seizures are defined as "the abnormal excessive or synchronous neuronal activity in the brain" that can be produced secondary to a wide spectrum of injuries. However, "epileptic seizures" are produced spontaneously, so, a seizure is an event and epilepsy is a disorder involving recurrent unprovoked seizures.

So, what is epilepsy? Throughout the last five decades, the definition of epilepsy has been subjected to extensive controversy and debate by different neurological schools. After several years of deliberations on this issue results have been published by the International League

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Against Epilepsy (ILAE) commissioned second task force, to develop a practical (operational) definition of epilepsy, designed for use by doctors and patients and adopted as a position of the ILAE.

The Epilepsy Dictionary, recently published by the ILAE and the World Health Organization (WHO), indicate that epilepsy is defined as a chronic affliction of diverse etiology, characterized by recurring seizures due to excessive neuronal discharge (epileptic seizures), associated with diverse clinical and paraclinical manifestations. However, many variables such as age, risk factors, or genetic mutations, were not included within this definition.

The seizures themselves are the clinical manifestation of an underlying transient abnormality of cortical neuronal activity and the phenotypic expression of each seizure is determined by the point of origin of the hyperexcitability and its degree of spread throughout the brain. From such a minimal expression as loss of awareness to more complex manifestations as tonic–clonic seizures, crisis can last between a few seconds and a few minutes, can be isolated, or can occur in series. All these variations, contribute to the design of the current epilepsy classification. Several causes of sporadic or recurrent seizures include different etiologies such as acquired structural brain damage, altered metabolic states, or inborn brain malformations, and all of them present genetic differences as compared with nonconvulsive individuals. Furthermore, despite the observation that several different illnesses can develop secondary epilepsy, it is clear that only a fraction and not all of the affected patients will develop an epileptic syndrome from the same primary disease. According with this observation, we need to say that epilepsy secondary to other disease cannot be explained only by the primary disease "per se". Perhaps, this difference in susceptibility, could be also based on genetic variants between them, or perhaps all of them share a particular epileptogenic mechanism that is not present in other patients with the same disease but without epilepsy.

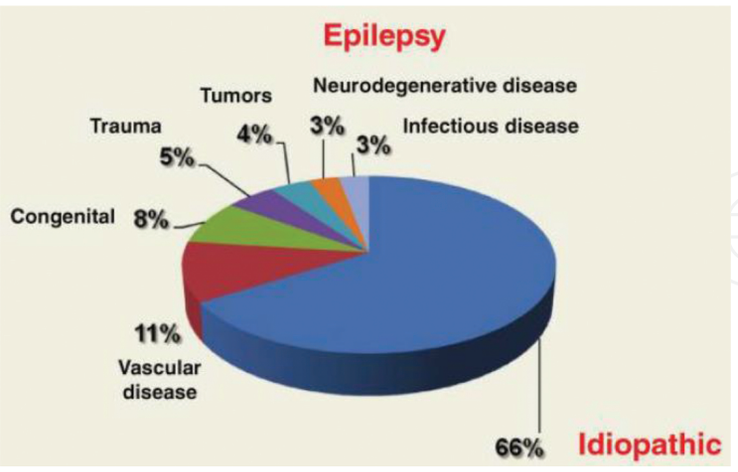

Figure 2. Different causes of epilepsy. In spite the majority of epilepsies are characterized as idiopathic (66%), near 30–40% of epileptic syndromes are secondary to different process that also can induce overexpression of P–gp.

A wide spectrum of syndromes – as diverse as neurodegenerative disorders, mental retardation syndromes, as well as neuronal migration disorders and mitochondrial encephalomyo-

pathies – have been described as capable of developing severe epileptic phenotypes, and to date, more than 200 single-gene disorders are known in which the presence of recurrent seizures are an important part of the phenotype. In 1975, the majority of epilepsies were characterized as 'idiopathic', but today, several of these idiopathic epilepsies comprise autoimmune epilepsies, epilepsies with lesions previously undetected, and the newly defined epilepsies secondary to genetic cause [21]. However, a large group of idiopathic epilepsies have unknown etiologies or epileptogenic intrinsic mechanisms (**Figure 2**).

The treatment of choice for control of epileptic seizures is pharmacologic therapeutics with antiepileptic drugs (AEDs), and the success of this treatment depends on the right selection of AEDs for the specific epileptic syndrome [22].

It is clear the wrong choice of AEDs or lack of therapeutic compliance by the patients may be the cause of treatment failure. However, if a drug resistant phenotype is observed under correct therapeutic stratagem, then patients, who were considered drug resistant, may not remain so because newer AEDs are being developed, targeting newly discovered pathophysiological mechanisms. Furthermore, individuals defined as being drug resistant with their epilepsy considered "drug resistant", perhaps have not yet been prescribed appropriate drugs.

4. Definition of Drug Resistance in Epilepsy

Resistance to drug treatment is a critical problem in the therapy of many brain disorders including epilepsy. When a patient has failed trials of two appropriate AEDs, the probability of achieving seizure freedom with subsequent AED treatments is modest. A useful functional criterion of refractory epilepsy (RE) is the failure to control seizures despite the use of two or more appropriate AEDs, even when maximum tolerated doses are administered (**Figure 3**). Interestingly, it was suggested that patients who were considered drug resistant under a given definition may not remain so as newer AEDs are developed, or designed, to target previously unappreciated underlying pathophysiological mechanisms. Additionally, individuals who we could define as being drug resistant, perhaps his epilepsy is considered "drug resistant" simply because we do not yet have drugs that are appropriate for the treatment of that individual's epilepsy [23].

During the last decade, more than 15 new AEDs have become available, however, the percentage of patients with RE remains near 30–40%, as observed during the early era of treatment with common, older AEDs. In all these cases, the failure of pharmacological therapeutics is observed after altering different combinations of more than 2 or 3 AEDs [24]. This particular phenotype, suggests a common intrinsic mechanism should be evaluated to better design new AEDs able to avoid the pharmacoresistance, and the subsequent development of a new crisis. These observations strongly suggest that all AEDs were wrongly developed using experimental models of seizures induced in healthy animals (without epilepsy), when they should have been developed via models of epilepsy in which all current AEDs have failed. In this regard, developing therapeutics to block P-gp activity, the main factor related with MDR-phenotype, should be addressed [25].

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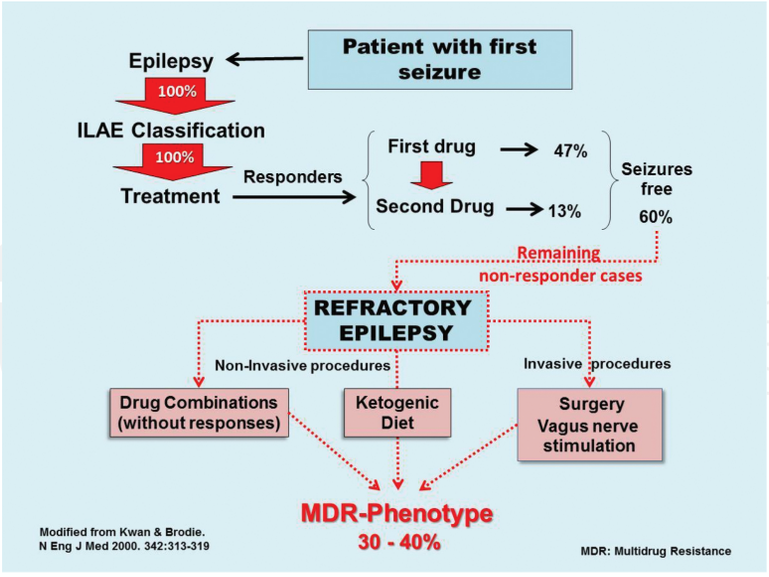

Figure 3. Schematic flow of therapeutic decision: Despite the administration of different therapeutic strategies that include more than two AEDs, almost 30–40% of patients will develop a phenotype of multidrug-resistant (MDR) epilepsy.

5. ABC-transporters and RE

The most important factor regulating the balance between dose and response with a direct impact in the plasmatic levels of drugs is related to the higher expression of the ABC transporters in the transporting epithelia, including the intestine, liver, or kidney, and playing a key role in the absorption, distribution, and removal of AEDs. Consequently, increased functional expression of multidrug transporter proteins, particularly P-gp, which are able to prevent access of AEDs to the brain, and decrease concentration in the sites of action, is an emerging concept of pharmacoresistance in epilepsy, based on extensive clinical and experimental evidence [26–29]. The particular location of these transporters in different excretory organs will induce a unidirectional route for the drugs from the inner to the external body.

The confirmation that P-gp can transport major AEDs (**Table 1**) is in concordance with the potential increased washout of AEDs which could be present in patients with RE [30–32]. The first evidence showing the upregulation of the *mdr1* gene, after experimentally induced seizures, was reported by several different authors showing a highly increased P-gp expression in reactive astrocytes after intracerebroventricular administration of kainite; in BBB and unidentified brain cells after kainate-induced epilepsy [33, 34]; and progressively in neurons after repetitive seizures induced by 3-mercaptopropionic acid [35].

Refractory epilepsy is described in patients receiving recommended AED doses and having adequate therapeutic levels of AEDs in plasma, but who remain without control of seizures. Additionally, it was also demonstrated that therapeutic levels of phenytoin (PHT) in the blood and Cerebrospinal fluid (CSF) can be achieved after 2 hours - enough to reach steady state

concentrations within therapeutic range [36]. Sometimes, persistently low levels in the plasma of at least one of the AEDs administered at recommended doses has been observed during the daily follow-up of patients with different epileptic syndromes' who underwent polytherapy. Interestingly, these cases are currently assumed as non-detectable laboratory errors in the procedures or methods of AED measurement, or a non-complains behavior of the patient with the physicians´ therapeutic indication. However, this particular situation can also be observed in patients with pharmacoresistant epilepsy, for who high expression of P-glycoprotein was also observed in their biopsy specimens from epileptogenic brain areas after surgical treatment [37, 38].

AEDs as ABC-transporters (ABC-t) substrates	
Antiepileptic drug	**ABC-t substrate**
Phenobarbital	P-gp
Phenytoin	P-gp/MRP
Carbamazepine	P-gp/MRP
Valproate	P-gp/MRP
Benzodiazepines	P-gp
Ethosuximide	?
Vigabatrin	P-gp
Lamotrigine	P-gp
Gabapentin	P-gp
Felbamate	P-gp
Topiramate	P-gp
Tiagabine	?
Oxcarbazepine	?
Levetiracetam	MRP
Pregabalin	?

P-gp: P-glycoprotein; *MRP:* multidrug resistant-associated proteins

Table 1. Several AEDs share their status as substrates of P-gp, and some of them are also substrates of MRP (multidrug resistant related protein).

All these data, suggests that several, if not all AEDs, could be substrates of P-gp - brain overexpression being related to RE phenotype and a simultaneous systemic P-gp overexpression, something which can induces persistent subtherapeutic levels in plasma, of at least one AED administered.

Over the last 20 years, subsequent to the firsts three clinical reports [37, 39, 40], more than 300 clinical and experimental publications, related to P-gp and/or MDR-1 gene and refractory

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epilepsy, were registered (**Figure 4**). One of the hypotheses of RE proposes that P-gp as well as others ABCt, could play a significant role in pharmacoresistance in epilepsy by extruding AEDs from their intended site of action to brain outside. Both *in-vitro* and *in-vivo* experiments have demonstrated that several AEDs such as phenytoin, phenobarbital, lamotrigine, and levetiracetam, are substrates of human P-gp and MRPs [41–43].

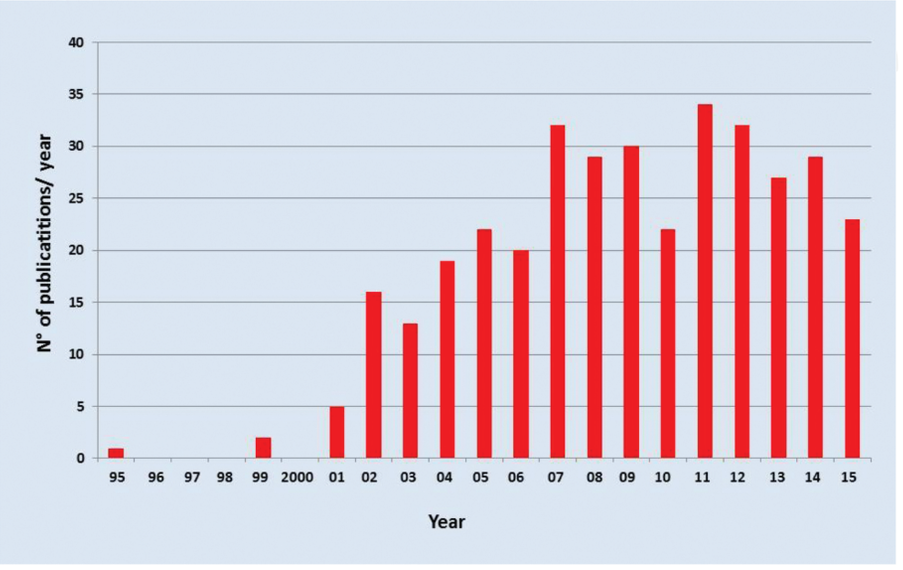

Figure 4. Number of publications each year focusing on ABC-transporters and epilepsy.

The initial interpretation of these investigations was that ABC-transporters such as P-gp, MRPs, and BCRP, whether individually or combined, could be responsible for the phasmacoresistant phenotype, RE. These transporters can induce the efflux of AEDs from the brain as well as increasing bodily excretion and/or inhibiting absorption, via the alteration of the pharmacokinetics of these agents [30–32].

Additionally, experimental evidence has demonstrated that seizures can induce the overexpression of these transporters, particularly P-gp, not only at the BBB level, but also in neurons and astroglial cells. Furthermore, these seizure-induced expressions can be progressively increased, according to the number and/or severity of the crisis. Consequently, we can assume that seizures without control can increase the risk of developing pharmacoresistant epilepsy because seizures can also induce a progressively increased brain expression of P-gp [33–35].

Interestingly, epilepsy is the second most common neurological disorder after cerebrovascular accident (CVA) (stroke). This very important position in the international statistics of brain diseases is concordant with the wide spectrum of very different factors causing epilepsy [44]. Furthermore, these particular and multifactorial processes can advance in clinically silent ways, and later can lead to a first spontaneous crisis, something which is recognized as latency phase or "epileptogenesis". Under this context, persistent neuronal excitability, secondary to an also wide spectrum of chronic mechanisms, in response to complex and progressive

processes, leads to a prolonged and increased depolarization and reduces the convulsive thresholds which precede seizures.

In this regards, as previously mentioned, an alternative mechanism to the classic pumping function of P-gp was described in cells expressing the MDR-1 gene, exhibiting significantly low membrane potential ($\Delta\Psi 0$= –10 to –20 mV) compared to physiological potential ($\Delta\psi 0$ of –60 mV) [16, 17].

The main function of neurons is electrical conductivity which depends on the action potential of the membrane and its polarity. Neurones, therefore, are key to communication, with interneuronal connections being dependent on both chemical and electrical synaptic transmission [45]. Near the rest potential, low glutamic acid concentration induces a "weak" stimulus and only activates the AMPA/Kainate receptors with the NMDA receptor remaining closed. Interestingly, neurons from epileptogenic brain areas overexpressing P-gp could exhibit a pre-depolarized membrane potential, and lead to a persistent reduced threshold to stimulate these cells. So, they could become more sensitive to new seizures under normal or lightly elevated concentrations of glutamic acid. So, under these conditions, the same normal "weak" stimulus could open KA/AMPA and NMDA channels producing total activation of neurons and inducing a new seizure. Furthermore, recently it was demonstrated that chronically elevated extracellular glutamate is a common pathological feature among epilepsies with different etiology [46].

All these observations suggest that P-gp dependent membrane potential alterations ($\Delta\psi 0$), not only could contribute to the development of the refractory phenotype, but also to the intrinsic mechanisms of the epileptogenicity. In agreement with these concepts, a preliminary collaborative study showed the first evidence that repetitive seizures induce high neuronal P-gp overexpression associated with refractoriness and a concomitant progressive enrollment of hippocampal cells with a depolarized membrane. Both refractoriness and depolarization were reversed after administration of nimodipine, a calcium channel blocker that also inhibits P-gp activity [47].

P-gp overexpression in neurons can be induced by many silent non-convulsive processes such as inflammation, hypoxia, and toxic agents, and can also constitutively be expressed in immature brain cells. All these conditions can contribute to a progressive lowering of membrane potential, particularly in neurons.

Consequently, how much time P-gp can be expressed in brain cells after an initial inducer insult, waiting a new stimulus producing a persistent chronically P-gp expression, and ending in spontaneous seizures commonly named EPILEPSY?.

So, irrespective of the well-known drug transport property, there could be an additional mechanism that increases the risk that new seizures play a role in epileptogenesis. Because seizures also induce a greater expression of P-gp, all these mechanisms could explain popular comments like: "seizures induce seizures" and "seizures without control induce refractoriness".

Patients with RE carry an increased mortality risk than patients with well-controlled seizures. This clinical phenomenon named Sudden Unexpected Death in Epilepsy (SUDEP), needs a

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mechanistic or molecular explanation because post-mortem examination does not reveal a toxicological or anatomical cause of death [48]. Several clinical behaviors of these particular RE cases are in concordance with the MDR phenotype, and different studies have suggested seizure activity as an inducer of cardiovascular alterations. Again, potential membrane alterations, secondary to a high expression of P-gp, but now in cardiomyocytes, could also explain sudden death in these patients. Interestingly, P-gp overexpression in this type of cell, was demonstrated in both chronic and acute heart hypoxic models, as well as in induced fatal status epilepticus after repetitive, induced seizures in rats [49–51].

It was reported that the potential pathomechanisms of SUDEP comprise cardiac arrhyth-mia, due to electrolyte disturbances, arrhythmogenic drugs, or transmission of epileptic activity via the autonomic nervous system to the heart, central or obstructive apnea, and myocardial ischemia [52].

A variety of seizure-related cardiac dysrhythmias such as lengthening of the QT interval, ST depression and T-wave inversion, ventricular fibrillation and asystole, bradyarrhythmias, as well as atrial fibrillation and sinus and supraventricular tachycardias were documented. Interestingly, atrial and ventricular premature depolarizations were also documented under the same conditions [53–54]. So, we could suggest that a similar mechanism of progressive P-gp overexpression, inducing an also progressive depolarization in the brain, is related with epileptogenesis, and that a progressive P-gp overexpression in cardiomycoites may induce an also progressive heart depolarization increasing heart dysfunction.

It was mentioned above that RE is observed in approximately one-third of patients with epilepsy. In the same way, it was described that refractory status epilepticus (RSE), defined as status epilepticus (SE) that fails to respond to acute administration of two antiepileptic medications, also occurs in approximately one- third of patients with SE, and is associated with an increase in the length of time patience stay in hospital, functional disability, as well as mortality [55].

Taking these data together, we can speculate that after a long period of RE, an accumulated high brain and heart P-gp expression, increases the risk of SE development and, under severe stress, can also increase the risk of sudden and fatal heart failure.

6. ABC-t genetic polymorphisms, Refractory Epilepsy and Epileptogenesis

One intriguing and unresolved question is whether the ABC-transporter's polymorphisms could play a role in pharmacoresistance to AED treatment, as well as in epileptogenesis. Remembering that epilepsy constitutes a heterogeneous group of disorders that is characterized by recurrent unprovoked seizures due to widely different etiologies, discrepant observation in genetic studies related with refractoriness could be attributed to variety of factors such as variable definitions of AED-resistance, variable epilepsy phenotypes, and ethnicities among studies. In this regard, a significant number of studies have been developed to establish whether different haplotypes, resulting from the combination of polymorphisms of ABC-t and

enzyme systems of drug metabolism, are associated with the development of drug-resistant phenotype in epilepsy.

To date, all scientific literature indicates controversial results, where several studies suggest a positive relationship and several others indicate the opposite. A trend showing a negative correlation appears to be observed in caucasian cases [60–62], and a positive correlation in Mexican or Asiatic patients [56–59].

According to these contradictory observations, a more recent study of 738 ethnically matched Malayalam speaking subjects were enrolled into a genetic study of the ABC-transporter. All of them were residents of Kerala, south India, for more than three generations, of which 259 were RE (AED resistant), 201 were AED responsive, and the remaining 275 were non-epilepsy control subjects. Interestingly, this study concluded that variants in the ABCB1 and ABCG2 do not confer a significant risk to AED-resistance in the south Indian population of Kerala, but instead demonstrate an increased vulnerability to epilepsy and associated phenotypes [63].

Perhaps, irrespective of genetic polymorphisms, the final result of a high histological brain expression of these transporters could be the prognostic hallmark for the clinical evolution of the disease. Neuropathological alterations secondary to repetitive seizures may be adaptive and reversible, while other alterations may be permanent. Furthermore, in others cases, similar brain alterations can be present as constitutive lesions, playing a role in the epileptogenesis, as proposed in epilepsies secondary to mesial temporal sclerosis [64], brain malformations [65], or tumors [66].

Epilepsy surgery has been established as an effective treatment option in pharmacoresistant epilepsies [67]. However, in one study of long-term outcomes in 325 people having anterior temporal resection, the rate of seizure freedom was 41% after 10 years [68]. More recently, the long-term outcome of surgery for epilepsy in 615 adults, indicated that although most patients showed a substantial reduction in seizures, only 40% entered long-term remission by virtue of having no seizures from the time of surgery, and only 28% of those who were seizure-free at last follow-up had discontinued antiepileptic drugs and could therefore be regarded as being cured [69]. Furthermore, ABC-transporters, such as P-gp and BCRP, could be interpreted as stem-cell markers present in several brain cortical malformations, as previously described in epileptogenic subependimal giant astrocytoma (SEGA) [70], being constitutive components of immature not fully differentiated cells, as observed in dysplastic neurons and ballooned cells or brain tumor cells. Interestingly, all these abnormal cells play a role in epileptogenesis, have high expression of ABC-transporters, and are also refractory to AEDs.

Malformations of cortical development as well as brain tumors arise from abnormal progenitor cells where ABC-transporters, together with others stem cell markers, could help to improve the identification of these abnormal progenitor cells and serve as biomarkers for seizure relapse risk after epilepsy surgery [71].

In this regard, the functional activity of P-gp measured at the BBB level was evaluated in patients with temporal lobe epilepsy by a positron emission tomography (PET) study using [^{11}C]-verapamil, before and after temporal lobe surgery, to assess whether postoperative changes in seizure frequency and antiepileptic drug load are associated with changes in P-gp

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function. In this study, only 7 cases were enrolled and followed up for a median of 6 years after surgery. P-gp immunoreactivity in surgically resected hippocampal specimens was also determined. Patients with optimal surgery outcomes, defined as seizure freedom and withdrawal of AEDs, had global PET scan parameter increases as compared with presurgery PET scans, suggesting a reduced P-gp function at the BBB of different evaluated brain areas. Consequently, an optimal surgical outcome, defined as seizure freedom and withdrawal of AEDs, was associated with higher temporal lobe P-gp function before surgery, higher P-gp-positive staining in surgically resected hippocampal specimens, and reduction in global P-gp function postoperatively, compared with nonoptimal surgery outcomes. This pilot study suggests that Pgp overactivity in epilepsy is dynamic, and complete seizure control and elimination of antiepileptic medication is associated with reversal of overactivity [72].

These particular observations indicate that presurgery overexpression and overactivity of P-gp can be a reactive process secondary to chronic stimulation that can disappear when convulsive stress is also eliminated by surgical treatment, with a minimal risk of seizure relapse. In contrast, abnormal stem cells with aberrant location have a constitutive P-gp (or BCRP) overexpression which can induce a persistent membrane depolarization associated refractoriness and epileptogenesis. So, ABC-transporters and other stem cell markers, if they are presents in those mentioned abnormal cells, could contribute to build a risk score or prognostic profile for long-time seizure relapse [71].

In spite of the high success rate of many surgical procedures for pharmacoresistant epilepsy, a substantial number of patients do not become seizure-free. Alternative strategies using brain electrical modulation by deep brain/vagal nerve/transcraneal magnetic stimulations, have gained considerable interest in the last decade as potential therapies in medically refractory epilepsy. Under these conditions, it was suggested that electrical modulation of the brain may reduce the overexpression of P-gp, and combined with pharmacotherapy, may represent an innovative approach to avoid epileptogenesis, reduce seizure activity, induce beneficial effects during the postictal state, diminish the amount of antiepileptic drugs, and improve alertness, memory, and mood in pharmacoresistant epilepsy [73].

The transporter theory of pharmacoresistance in epilepsy could also be completed with additional properties of P-gp such as:

1. P-gp expression inducible by a wide spectrum of factors such as hypoxia, convulsions, inflammation, trauma, cancer, toxics, metabolic imbalance, infection, etc.
2. P-gp inducing membrane depolarization and possibly being related with epileptogenesis when P-gp is expressed in neurons.
3. Seizures also inducing P-gp overexpression at the BBB, neurons, and in the heart.
4. Further expression of P-gp related with further pharmacoresistance, more severity of seizures, and increased risk of develop of SE and/or SUDEP.

Perhaps, pharmacological modulation of expression and function of P-gp could avoid invasive surgical treatment of refractory epilepsy, the relapse of seizures after surgery, and SE and/or the SUDEP.

7. Conclusion and remarks

The drug transporter properties of P-gp producing pharmacokinetic changes and pharmacoresistance phenotype should be distinguished from those related to plasmatic membrane depolarization directly related with epileptogenesis. ABC-transporters, such as P-gp and BCRP, could also be markers of the presence of stem cells or immature, not fully differentiated, brain cells, as observed in dysplastic neurons and ballooned cells in several brain cortical malformations, or brain tumor cells. In all these cases, high expression of ABC-transporters, were documented and they are also refractory to AEDs. The condition of ABC-transporters as stem cell markers, if they are present in those mentioned abnormal cells, could contribute to the creation of a risk score or predictive profile for long-time seizure relapse after surgical treatment.

Finally, repetitive seizures and/or apneas can induce simultaneous P-gp overexpression in both the brain and heart, and it could represent a high-risk factor for developing an acute heart failure under severe stress triggered by SE resulting in death (SUDEP) (**Figure 5**).

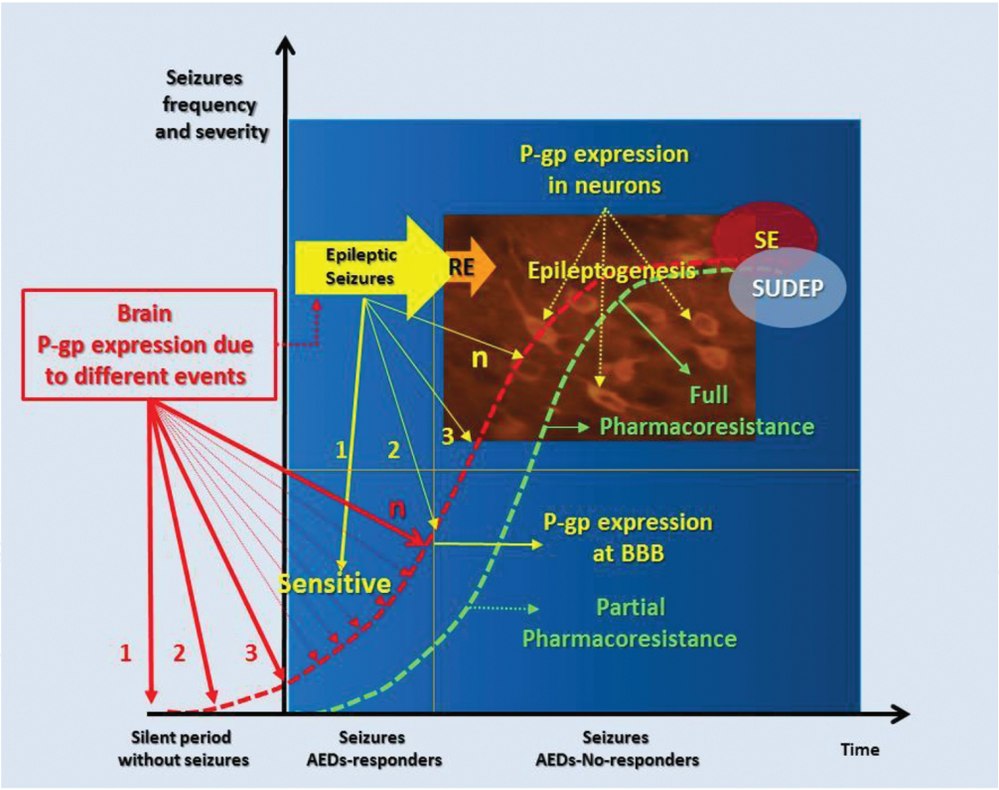

Figure 5. Progressive brain overexpression of P-gp. Initially, a wide spectrum of different stimuli can affect the brain without seizures, however, with a light induction of P-gp expression, it is most noticeable at the BBB level. This up-regulation can be reversible, except if new stimuli are added and spontaneous epileptic seizures are also started. The early pharmacological control of seizures is the key to avoiding the installation of a secondary epileptic syndrome. If not, the progressive increased expression of P-gp will develop epilepsy with drug resistant phenotype, and later expression of P-gp, at neurons, will have a direct participation in epileptogenesis. Under these conditions, SE and/or SUDEP can be the final scenario.

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