Kevin Costa Leandro

Relatórios de Estágio e Monografia intitulada "Drug-resistant Epilepsy: An Overview on the relevance of ABC Transporters"

Relatórios de Estágio e Monografia intitulada "Drug-resistant Epilepsy: An Overview on the relevance of ABC Transporters" referentes à Unidade Curricular "Estágio", sob a orientação, respectivamente da Professora Cláudia Furtado, Dr. Pedro Baptista e Professora Doutora Ana Fortuna, apresentados à Faculdade de Farmácia da Universidade de Coimbra para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Setembro de 2018

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Universidade de Coimbra

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UNIVERSIDADE DE COIMBRA

Eu, Kevin Costa Leandro, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o nº 2013145149, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatório de Estágio e Monografia intitulada "Drug-resistant Epilepsy: An overview on the relevance of ABC Transporters Superfamily" apresentados à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade curricular de Estágio Curricular.

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Coimbra, 7 de Setembro de 2018.

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(Kevin Costa Leandro)

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Índice

Resumo

No âmbito da unidade curricular "Estágio" do Mestrado Integrado em Ciências Farmacêuticas da Faculdade de Farmácia da Universidade de Coimbra, o presente documento apresenta, sob a forma de uma análise *SWOT* (Pontos Fortes, Pontos Fracos, Oportunidades e Ameaças), o relatório de estágio na Farmácia Universal, em Coimbra, com início a 16 de Abril e término a 17 de Agosto de 2018, sob orientação do Dr. Pedro Baptista e o relatório de estágio na Direção de Avaliação de Tecnologias de Saúde do INFARMED - Autoridade Nacional do Medicamento e Produtos de Saúde, I.P., em Lisboa, com início a 11 de Janeiro e término a 30 de Março de 2018 , sob orientação da professora Cláudia Furtado. Este documento inclui ainda uma monografia intitulada "Drug-resistant Epilepsy: An overview on the relevance of ABC Transporters Superfamily"

A epilepsia fármaco-resistente afeta aproximadamente um terço dos doentes epiléticos. Das várias teorias subjacentes à epilepsia fármaco-resistente, a teoria dos transportadores de efluxo é uma das mais extensivamente estudadas. Consequentemente, o aumento da expressão de transportadores de efluxo na barreira hemato-encefálica, principalmente da superfamília de transportadores ATP *binding cassette* (ABC), é responsável por dificultar o acesso de fármacos antiepiléticos ao cérebro. Sabe-se que existe expressão exacerbada de glicoproteína P em células endoteliais da barreira hemato-encefálica, astrócitos e neurónios da unidade neurovascular, células predominantemente envolvidas na penetração cerebral de fármacos. A progressão patológica da epilepsia, as crises recorrentes não controladas, a indução de transportadores de efluxo pelos próprios fármacos e os polimorfismos genéticos nestes transportadores estão entre as possíveis causas da expressão acentuada dos transportadores ABC em doentes com epilepsia fármaco-resistente. Para evitar a ação dos transportadores de efluxo, várias abordagens estão sob investigação: a inibição direta destes transportadores, o uso da tecnologia de RNA de interferência ou a inibição do eixo glutamato/receptor NMDA/COX-2.

Palavras-chave: Epilepsia fármaco-resistente; Barreira hemato-encefálica; Antiepilético; Transportador ABC; Glicoproteína P; Multidrug resistance-associated proteins; Breast cancer resistance protein; Sobre-expressão.

Abstract

Within the scope of the course unit "Internship" of the Integrated Master's degree in Pharmaceutical Sciences of the Faculty of Pharmacy of the University of Coimbra, this document presents, in the form of a SWOT analysis (Strengths, Weaknesses, Opportunities and Threats), the internship in Farmácia Universal, in Coimbra, from the 16th of April 16 to the 17th of March 2018, under the guidance of Dr. Pedro Baptista and the report of the internship at the Health Technology Assessment Directorate of INFARMED - National Authority of Medicines and Health Products, I.P, in Lisbon, from the 11th of January to the 30th of March 2018, under the guidance of professor Cláudia Furtado. This document also includes a monograph entitled "Drug-resistant Epilepsy: An overview on the relevance of ABC Transporters Superfamily"

Drug-resistant epilepsy affects approximately one third of epileptic patients. From the various theories underlying drug resistance in epilepsy, the transporter hypothesis is one of the most extensively studied. Accordingly, the overexpression of efflux transporters in the blood-brain barrier, mainly from the ATP binding cassette transporter superfamily, is responsible for hampering the access of antiepileptic drugs into the brain. P-glycoprotein is known to be upregulated in endothelial cells of the blood-brain barrier, astrocytes and neurons of the neuronal unit, cells critically involved in the brain penetration of drugs. Epilepsy pathology progression, uncontrolled recurrent seizures, drug-associated induction and genetic polymorphisms are among the possible causes of the ABC transporters overexpression in drug-resistant epilepsy. In order to evade the action of efflux transporters multiple approaches are being established: direct inhibition, down-regulation through RNA interference or targeting the glutame/NMDA receptor/COX-2 axis.

Keywords: Drug-resistant epilepsy; blood-brain barrier; Antiepileptic drug; ABC transporter; P-glycoprotein; Multidrug resistance-associated proteins; Breast cancer resistance protein; Overexpression.

Parte I - Relatório de Estágio na Direção de Avaliação de Tecnologias de Saúde no INFARMED, I.P

Lista de Abreviaturas

ATS – Avaliação de Tecnologias de Saúde

CATS – Comissão de Avaliação de Tecnologias de Saúde

DATS – Direção de Avaliação de Tecnologias de Saúde

SNS – Serviço Nacional de Saúde

SWOT - strengths, weaknesses, opportunities and threats

1. Introdução

A avaliação de tecnologias de saúde (ATS) tem como objetivo garantir o acesso e o financiamento de medicamentos e dispositivos médicos, quer através da comparticipação quer através do financiamento de tecnologias de saúde nos estabelecimentos e serviços do Serviço Nacional de Saúde (SNS) [1]. A ATS é efetuada desde 1999, como instrumento de suporte à decisão na utilização de tecnologias de saúde, baseando-se não só nos critérios de qualidade, segurança e eficácia, necessários a todos os medicamentos, mas também em critérios de eficiência e efetividade, de modo a permitir o uso racional e ponderado dos recursos públicos [2].

Desde 2015, a ATS é regulada pelo Sistema Nacional de Avaliação de Tecnologias de Saúde, segundo o Decreto Lei n.º 97/2015, de 1 de Julho, através da avaliação técnica, terapêutica e económica das tecnologias de saúde por peritos da Direção de Avaliação de Tecnologias de saúde (DATS) e da Comissão de Avaliação de Tecnologias de Saúde (CATS) [1]. Este permite não só maximizar os ganhos em saúde e garantir a sustentabilidade do SNS, mas também o envolvimento de Portugal na criação de um sistema integrado e europeu de ATS [1].

A possibilidade de estagiar na DATS, surge como uma oportunidade para os estudantes do Mestrado Integrado em Ciências Farmacêuticas (MICF) em contactar com uma nova área da atividade farmacêutica, permitindo a aquisição de conhecimentos e competências em ATS, cada vez mais harmonizada e em linha com as melhores práticas europeias. Tais aprendizagens revelam-se essenciais no exercício da atividade farmacêutica.

O presente estágio decorreu no Infarmed, em Lisboa, no período de 11 de Janeiro a 30 de Março de 2018, sob orientação da professora Cláudia Furtado e colaboração de toda a equipa da DATS.

Este relatório foi elaborado no formato de análise *SWOT* (*strengths, weaknesses, opportunities and threats*), abordando os pontos fortes, fracos, as oportunidades e ameaças identificadas durante a realização do estágio.

2. INFARMED – Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.

O INFARMED, criado em 1993, é um instituto público integrado na administração indireta do Estado, com a principal missão de regular e supervisionar os setores dos medicamentos, dispositivos médicos e produtos cosméticos em Portugal [3]. Com sede no Parque de Saúde de Lisboa, o Infarmed é dotado de autonomia administrativa, financeira e património próprio, com jurisdição em todo o território nacional e sob tutela do membro do Governo responsável pela área da saúde [3]. O Infarmed é constituído por 5 órgãos e 12 unidades orgânicas, das quais 8 são responsáveis por funções de negócio e 4 por funções de suporte, de acordo com o organograma da instituição (Figura 1) [4].

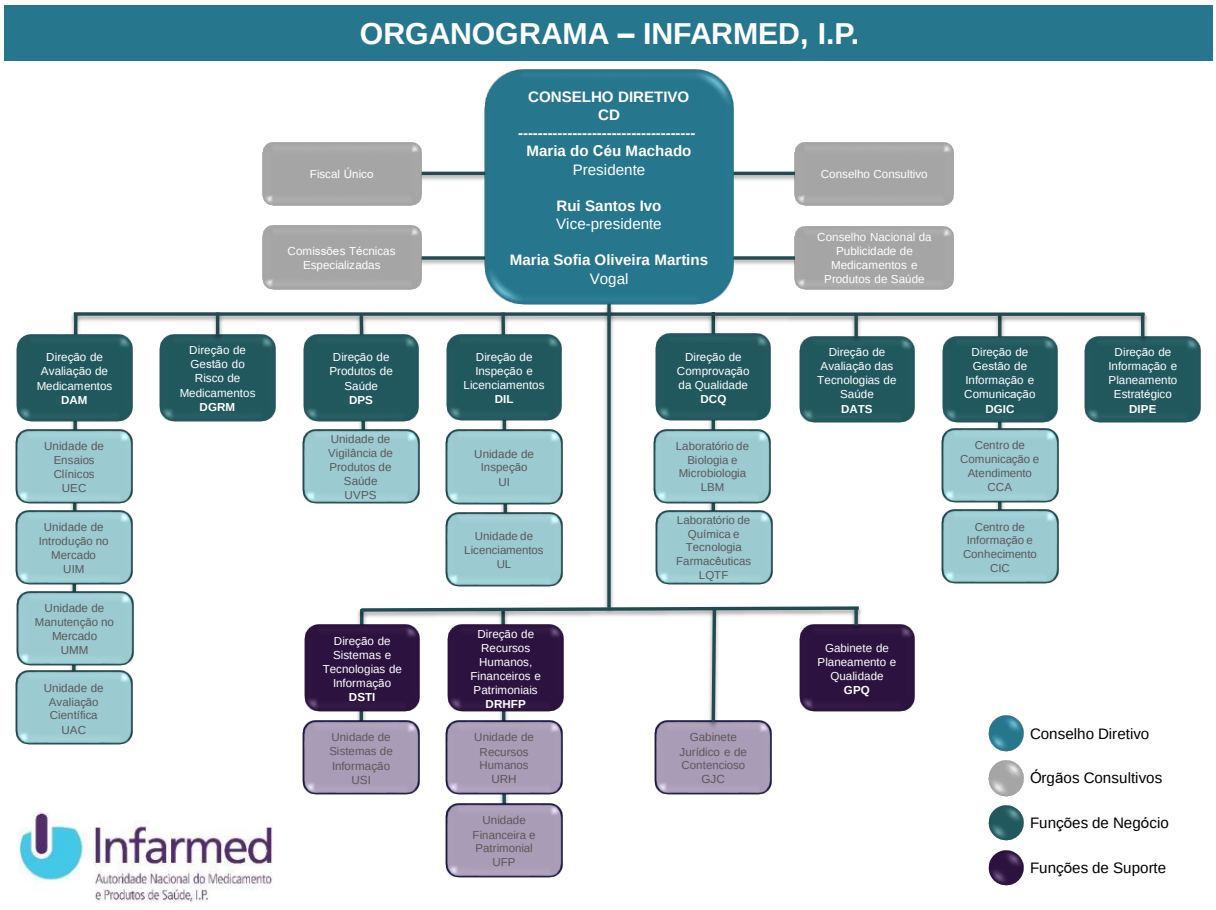

Figura 1: Organograma do INFARMED, I.P.

Disponível na Internet: http://www.infarmed.pt/web/infarmed/institucional/estrutura-e-organizacao. [Consultado a 12 de Agosto de 2018]

3. Análise SWOT

3.1 Pontes Fortes

3.1.1 Equipa da DATS

O pilar fundamental do sucesso deste estágio foi sem dúvida a equipa da DATS. Desde do momento da receção, foi notório o bom ambiente de trabalho que se vivia entre os colaboradores do departamento, que se mostraram sempre disponíveis em ajudar na integração dos estagiários. Ao longo do estágio, o interesse da equipa em atribuir tarefas variadas e proporcionar uma experiência diversificada, contribuíram de forma determinante para os conhecimentos adquiridos a nível de ATS.

De facto, a competência, multidisciplinaridade e profissionalismo demonstrados pelos colaboradores da equipa comprovaram ser valores fulcrais no trabalho de excelência desenvolvido pela DATS.

3.1.2 Plano de Integração

A formação completa e coerente a nível de todas as tarefas e responsabilidades ao encargo da DATS, foi em parte resultado do plano de integração proposto pela professora Cláudia Furtado. Como plano introdutório, os estagiários da DATS foram alvo de formações a nível das várias vertentes do trabalho desenvolvido pela DATS: avaliação farmacoterapêutica e económica de medicamentos genéricos e não genéricos (em ambulatório ou em meio hospitalar), preços (formação, revisão anual e revisão excecional), elaboração e manutenção de contratos e avaliação de dispositivos médicos.

A aprendizagem inicial das tarefas e responsabilidades ao encargo da DATS permitiu compreender a relação e a dinâmica entre os vários grupos de trabalho e a estrutura sequencial de tarefas desde a receção dos pedidos submetidos pelos requerentes até à sua resolução.

3.1.3 Funções desempenhadas

Ao longo do estágio, e sempre sob supervisão dos colaboradores da DATS, desenvolvi diversas tarefas no âmbito da ATS.

De entre as tarefas desenvolvidas, a avaliação de pedidos de comparticipação de medicamentos de uso humano em ambulatório e a avaliação de pedidos de avaliação prévia de medicamentos de uso humano em meio hospitalar foram as que mais frequentemente desempenhadas. Estas caracterizavam-se por verificar a conformidade de uma série de condições descritas numa *checklist*, que após verificação e aprovação farmacêuticas seguia para a avaliação económica à responsabilidade dos economistas da DATS.

A avaliação de processos de transferência de comparticipação de medicamentos de uso humano foi também uma das tarefas que desempenhei com frequência. De um modo similar ao descrito anteriormente, esta avaliação consistia em verificar a conformidade de um conjunto de condições descritas no Artigo 16º da Portaria 195-A/2015, que visa simplificar o processo de comparticipação e avaliação prévia de medicamentos [5].

A realização de relatórios de avaliação de medicamentos de uso humano não genéricos, em ambulatório ou em meio hospitalar, após emissão do parecer da CATS e decisão final da Secretaria de Estado da Saúde, foi também uma tarefa atribuída aos estagiários da DATS. A elaboração destes relatórios, publicados na plataforma online do Infarmed, visa informar a comunidade e os profissionais de saúde, de uma forma simples e prática, das razões pelas quais o pedido de avaliação de uma determinada tecnologia de saúde foi alvo de deferimento/indeferimento.

Para além disto, tive a oportunidade de colaborar com a Direção de Informação e Planeamento Estratégico, na recolha e organização dos dados de registo mínimo de medicamentos biológicos fornecidos pelos hospitais abrangidos pelo SNS, obrigatório à luz da Portaria nº48/2016, de 22 de Março [6].

A elucidação das condições de dimensionamento das embalagens de medicamentos para efeitos de comparticipação foi também uma das tarefas atribuídas aos estagiários.

O diverso leque de atividades que realizei permitiu por um lado compreender o enquadramento legal da ATS, como também constatar e participar ativamente nas várias vertentes do trabalho ao encargo da DATS.

3.1.4 Reuniões Farmacêuticas

As reuniões farmacêuticas, realizadas semanalmente, foram um dos momentos de maior aprendizagem durante o estágio no Infarmed. Estas reuniões apresentavam 2 objetivos principais: em primeiro lugar harmonizar os processos de avaliação farmacêuticas, de forma a uniformizar o sentido das decisões ao encargo da DATS; em segundo lugar debater

eventuais processos de maior complexidade e manter os vários membros da equipa informados acerca dos mesmos.

Pela relevância e diversidade dos assuntos aqui tratados, que muitas vezes despertavam a atenção para determinados pormenores nos processos de avaliação farmacoterapêutica, as reuniões farmacêuticas foram uma mais valia para o desenvolvimento das competências adquiridas na DATS.

3.1.5 Caráter singular das atividades desenvolvidas

Dada natureza do trabalho sob responsabilidade da DATS, e a competência dos colaboradores altamente especializados na ATS, o estágio no Infarmed foi uma grande oportunidade de contactar com a área de acesso ao mercado na perspetiva da Autoridade Nacional do Medicamento e Produtos de Saúde, o Infarmed, I.P. Em Portugal, a aquisição de competências e experiência prática em ATS é apenas concretizável na DATS.

Esta oportunidade prendeu-se com a aquisição de conhecimentos e competências únicas em ATS, especialmente tendo em consideração a futura prática profissional na área da indústria farmacêutica.

3.2 Pontos Fracos

3.2.1 Duração do Estágio

Um dos pontos de maior fragilidade do estágio, tendo em conta a ampla diversidade de tarefas ao encargo da DATS, foi a duração do mesmo.

Considerando a evolução rápida e constante da ATS, tanto em Portugal como a nível europeu, e em que cada decisão tem um impacto significativo no funcionamento e na sustentabilidade do SNS, um estágio de 3 meses é insuficiente para uma compreensão plena das numerosas vertentes do trabalho da DATS. Não só é fundamental compreender o contexto da ATS, como é necessário perceber o seu enquadramento com as restantes áreas regulamentares do medicamento.

Apesar da breve duração do estágio, este foi importante na obtenção de uma visão geral do trabalho desenvolvido pela DATS, sendo uma mais valia na minha futura prática profissional.

3.2.2 Autonomia

A natureza das responsabilidades ao encargo da DATS e as tarefas diariamente desempenhadas pelos seus colaborados caracterizam-se pelo elevado impacto que têm na sustentabilidade do SNS. O sentido das decisões no financiamento de medicamentos e dispositivos médicos requer um elevado nível de responsabilidade por parte da equipa da DATS.

Na qualidade de estagiário, foi evidente o quanto o impacto de cada tarefa sob responsabilidade da DATS impossibilitava que os estagiários desempenhassem as tarefas delegadas de forma mais autónoma. Deste modo, era necessária a supervisão constante por parte dos colaboradores de todo o trabalho desenvolvido pelos estagiários.

3.2.3 Desconhecimento da área

A atividade da DATS engloba um vasto conjunto de tarefas na área de ATS, atividade em que os farmacêuticos desempenham um papel fundamental. Neste sentido, ao iniciar o estágio senti alguma falta de preparação nesta área, que requer um conhecimento aprofundado da legislação subjacente à ATS, conteúdos não abordados no plano de estudos do MICF.

O desconhecimento da legislação subjacente a esta área e das atividades sob responsabilidade da DATS exigiram um maior esforço da minha parte na preparação inicial, para poder posteriormente desempenhar algumas tarefas mais práticas no contexto real da ATS. Apesar de grande parte da atividade deste departamento não constar do plano de estudos do MICF, unidades curriculares como Comunicação e Marketing Farmacêutico e Farmácia Hospitalar contribuíram para a minha formação em ATS.

Este estágio provou ser uma grande oportunidade para descobrir uma área totalmente nova, na qual toda a equipa da DATS e o plano de integração inicial foram fundamentais para o sucesso das minhas aprendizagens.

3.3 Oportunidades

3.3.1 Formação Contínua

Considerando a constante evolução da ATS tanto a nível nacional como a nível europeu, a aquisição constante de novos conhecimentos é essencial para um bom desempenho no exercício desta área.

No decorrer deste estágio tive a oportunidade de adquirir novos conhecimentos em diversas áreas, como em farmacoeconomia, ensaios clínicos de medicamentos biológicos (amplamente debatidos nas reuniões da CATS), VIH/SIDA, e dispositivos médicos. A inovação não só se reflete na constante introdução de novos medicamentos e dispositivos médicos, mas também na constante atualização dos procedimentos e técnicas de avaliação dos mesmos.

Por estas razões, a inovação terapêutica e a evolução da ATS constituem um desafio constante para a equipa de trabalho da DATS e uma oportunidade para os estagiários em enriquecer a sua formação.

3.3.2 Reuniões da CATS

A CATS segundo o Decreto-Lei n.º 97/2015, de 1 de Julho, é responsável pela emissão de pareceres e recomendações, apreciação de estudos de avaliação económica e proposta de medidas que vão ao encontro dos interesses da saúde pública e do SNS, relativamente às tecnologias de saúde [1].

A presença nas reuniões semanais entre a CATS e os colaboradores da DATS foi uma oportunidade singular de perceber mais de perto os métodos da avaliação farmacoterapêutica de medicamentos não genéricos. Esta comissão relativamente nova e em permanente evolução, tem uma importância primordial no acesso à inovação farmacêutica e na sustentabilidade do SNS, dado o seu papel na avaliação de medicamentos e terapias inovadoras, que frequentemente envolvem custos avultados ao SNS.

Por estas razões, a compreensão do funcionamento deste organismo foi uma oportunidade ímpar de reconhecer as variáveis e implicações do financiamento de medicamentos inovadores na sustentabilidade do SNS.

3.4 Ameaças

3.4.1 Cumprimento de metas e objetivos

Dada a responsabilidade da DATS na avaliação e na decisão de financiamento de medicamentos e dispositivos médicos, esta é também alvo de pressão externa por parte dos *stakeholders* do setor farmacêutico, pelo cumprimento rigoroso de prazos e metas estabelecidos pelos mesmos.

Desde da determinação de objetivos e metas temporais por parte do Ministério da Saúde, ao interesse da Indústria Farmacêutica na resolução rápida e eficiente dos processos, até à relevância na celeridade de análise de pedidos de Autorização de Utilização Excecional de medicamentos, a DATS está sujeita a pressão constante nas diversas funções ao seu encargo.

Deste modo, a necessidade de cumprimentos de prazos e a pressão externa por parte dos *stakeholders*, apresentam-se como um desafio constante para os colaboradores da DATS.

4. Conclusão

A possibilidade de estagiar na DATS surgiu como uma oportunidade de conhecer de perto a área regulamentar do medicamento no contexto português, a sua matriz de funcionamento e importância na sustentabilidade do SNS. Destacou-se pela relevância que manifesta na atividade farmacêutica, cada vez mais competitiva e dispendiosa para os sistemas de saúde nacional e internacionais.

Desde o plano de integração, às inúmeras funções desempenhadas, a equipa da DATS mostrou uma inestimável disponibilidade e apoio que asseguraram o sucesso da realização deste estágio. A diversa variedade de tarefas executadas permitiu não só compreender a estrutura e funcionamento da ATS em Portugal, mas também apreender o funcionamento dos diversos órgãos do Infarmed, entre si e com os diversos *stakeholders,* demonstrando a importância destes no desenvolvimento e na sustentabilidade do SNS.

O estágio no Infarmed revelou-se como uma excelente experiência no meu percurso académico, não só pela obtenção de conhecimentos e competências técnicas na área, mas também pela aquisição de valores a nível de trabalho em equipa e de integridade profissional, que decerto serão uma importante mais valia ao longo do meu percurso.

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Parte II - Relatório de Estágio na Farmácia Universal

Lista de Abreviaturas

DCI – Denominação Comum Internacional

MICF - Mestrado Integrado em Ciências Farmacêuticas

SWOT - *strengths, weaknesses, opportunities and threats*

1. Introdução

O farmacêutico enquanto especialista do medicamento e agente de saúde pública desempenha um papel vital na salvaguarda da saúde e na promoção do bem-estar da comunidade [1]. Por representar o profissional de saúde mais facilmente acessível pelas populações, tem o dever de promover o uso seguro, eficaz e racional do medicamento.

Dada a evolução rápida e constante da área do medicamento, quer pela introdução de novos medicamentos e produtos de saúde, quer pela atualização dos já existentes a nível de segurança e eficácia, o farmacêutico de oficina encontra-se em formação contínua ao longo do desempenho da sua atividade.

O estágio em Farmácia Comunitária permite aos estudantes do Mestrado Integrado em Ciências Farmacêuticas (MICF) consolidar os conhecimentos adquiridos ao longo do seu percurso académico e adquirir as competências necessárias à prática da atividade farmacêutica.

O presente estágio decorreu na Farmácia Universal, em Coimbra, no período de 16 de Abril a 17 de Agosto de 2018, sob orientação do Dr. Pedro Baptista e colaboração de toda a equipa da Farmácia Universal.

Este relatório foi elaborado no formato de análise *SWOT* (*strengths, weaknesses, opportunities and threats*), abordando os pontos fortes, fracos, as oportunidades e ameaças identificadas durante a realização do estágio.

2. Análise SWOT

2.1 Pontes Fortes

2.1.1 Equipa da Farmácia Universal

A equipa da Farmácia Universal, constituída exclusivamente por farmacêuticos, foi sem dúvida a chave do sucesso deste estágio. Desde cedo foi notória a capacidade da equipa em integrar-me na farmácia e em atribuir tarefas diversificadas. O bom ambiente que se vivia na farmácia também contribuiu para o desenvolvimento de competências de trabalho, por permitir que esclarecesse todas as minhas dúvidas mais livremente.

A competência e dedicação dos profissionais da Farmácia Universal permitiram também denotar a confiança e reconhecimento que os utentes depositavam no aconselhamento dado pelos mesmos. Este reconhecimento também se expressou pela minha motivação constante em realizar eficazmente todas as tarefas que me eram atribuídas.

De facto, a relação de trabalho e o bom ambiente entre farmacêuticos em contexto profissional revelou-se como um valor fundamental na prestação de um serviço de excelência à sociedade.

2.1.2 Diversidade de Tarefas

A diversidade de tarefas desempenhadas foi também um dos pontos fortes deste estágio. O facto de ter desempenhado as várias tarefas necessárias ao bom funcionamento da farmácia permitiu-me encarar as várias vertentes do trabalho do farmacêutico de oficina e perceber o quão importantes são as tarefas de *backoffice*.

Para além do atendimento ao público, desempenhei diversas tarefas relacionadas com a gestão da farmácia. De entre elas destaca-se a gestão e receção de encomendas e a organização do espaço e produtos na farmácia. Pela confiança em mim depositada na realização destas tarefas, considero que estas foram essenciais à minha evolução ao longo do estágio.

2.1.2.1 Atendimento ao público

O atendimento ao público foi sem dúvida umas das tarefas em que mais progredi durante o estágio, e também uma das mais desafiantes que encarei. Para além de ser uma atividade de extrema responsabilidade, pela importância que representa na saúde e bem-estar da população, requer um elevado nível de confiança e compromisso para com o utente, de modo a transmitir a informação correta e pertinente à sua situação.

Desde cedo fui incentivado pelos farmacêuticos a iniciar a atividade no atendimento ao público, e a aperfeiçoar a prática do uso do Sifarma2000. Ao longo do estágio, os profissionais da Farmácia Universal sempre tiveram a preocupação em explicar-me o que aconselhar em determinadas situações usualmente questionadas pelos utentes, o que me facultou mais confiança no atendimento. Foi também notório o impacto que o aconselhamento farmacêutico tem na educação e na atitude dos utentes no uso consciente dos medicamentos.

Esta componente do estágio permitiu-me também aplicar e consolidar os conhecimentos adquiridos ao longo da minha formação académica. Neste aspeto relevo a minha evolução no aconselhamento da posologia indicada a determinada situação, e na recomendação de produtos de dermocosmética. Um elemento extremamente importante na transmissão de informação e educação para a saúde é adaptar a linguagem utilizada ao entendimento do utente. Este ponto é particularmente importante para que o utente entenda a mensagem e fique totalmente esclarecido.

Assim, de entre as atividades desempenhadas na farmácia, o atendimento ao público foi não só uma das mais desafiantes, mas também a mais gratificante, pelo impacto que tem na satisfação dos utentes.

2.1.2.2 Gestão de Encomendas

Uma componente de elevada importância para o bom funcionamento da farmácia de oficina é a gestão de encomendas. A este nível, fiquei muitas vezes encarregado de rececionar todo o fluxo de encomendas diárias.

As encomendas diárias são efetuadas com base na definição de stocks mínimos e máximos, de modo a que os medicamentos e produtos de maior rotatividade estejam sempre disponíveis. A receção deste tipo de encomendas permitiu-me ter noção quais os medicamentos com maior rotatividade e conhecer os produtos que melhor se ajustavam ao público-alvo da Farmácia Universal.

Por outro lado, as encomendas instantâneas revelaram-se de grande importância por permitirem a aquisição rápida de produtos sem stock, solicitados pelos utentes. Este tipo de encomendas era importante para satisfazer num curto espaço de tempo as necessidades dos mesmos.

De notar que a eficaz gestão de encomendas significa a disponibilidade permanente de medicamentos e produtos de saúde solicitados pelos utentes, que promove credibilidade junto dos mesmos, levando também à sua fidelização.

A gestão de encomendas permitiu-me adquirir uma visão mais integrada das empresas de distribuição farmacêutica a atuar em território nacional e obter uma visão geral das margens de lucro praticadas para cada tipo de produto.

2.1.2.3 Organização do Espaço e Produtos

Uma das primeiras tarefas realizadas durante o estágio prendeu-se com a organização do espaço e produtos de saúde. A arrumação de medicamentos nas respetivas gavetas, organizadas por forma farmacêutica e ordem alfabética, permitiu-me não só conhecer a vasta variedade de medicamentos disponíveis no mercado e o seu fluxo de vendas, mas também tornou o atendimento ao público mais rápido e eficaz, por conhecer exatamente onde se encontrava o produto que procurava.

Tendo em conta o público-alvo da Farmácia Universal, a disposição dos produtos de saúde e medicamentos não sujeito a receita médica no espaço da farmácia, provou ser uma componente de extrema importância de forma a cativar o público à compra dos mesmos. Esta componente é de grande importância para a sustentabilidade e bom funcionamento de uma farmácia, por representar os produtos cuja margem pode ser gerida pela mesma.

2.1.3 Localização da Farmácia

A Farmácia Universal localiza-se na Praça 8 de Maio, em Coimbra. Possui uma entrada através desta Praça, e outra em frente à Câmara Municipal, junto à Rua da Sofia. A sua localização privilegiada na Baixa de Coimbra, numa área densamente habitada e com grande circulação de pessoas, permite que um elevado número de utentes pontuais se desloque à Farmácia Universal. A sua posição estratégica, com duas frentes expostas para zonas diferentes, permite grande visibilidade do exterior, aumentando também a área para divulgação de informação.

Deste modo, a localização estratégica da Farmácia Universal, garante a afluência constante de utentes muito heterógenos, de diferentes faixas etárias e estratos socioeconómicos, possibilitando o contacto com uma grande variedade de situações e a constante adaptação às distintas realidades.

2.1.4 Serviços Farmacêuticos

A Farmácia Universal oferece um vasto número de serviços farmacêuticos, permitindo o fácil acesso dos utentes e que reforçando a importância do farmacêutico junto das populações. Dos serviços farmacêuticos prestados posso destacar a determinação da altura e peso corporal, do índice de massa corporal, a medicação da pressão arterial, a determinação de parâmetros bioquímicos, como a glicémia e colesterol total e consultas semanais de nutrição.

Estes revelaram ser um dos pontos fortes deste estágio por permitirem que consolidasse alguns conhecimentos, relembrando também as técnicas de manuseamento e medicação dos parâmetros bioquímicos. Esta componente permitiu também incutir alguns hábitos de saúde aos utentes e promover a adesão à terapêutica. Neste sentido, é importante reforçar sistematicamente a importância dos hábitos saudáveis e incutir medidas não farmacológicas, que muitas vezes são as suficientes para manter alguns dos elementos acima descritos dentro dos valores desejáveis. Noutras situações, era também importante promover a adesão à terapêutica, quando o utente já era medicado, situação que muitas vezes denotei ser a causa da não eficácia dos tratamentos.

Deste modo, os serviços farmacêuticos para além de permitirem a aproximação do farmacêutico ao utente, possibilitam também uma participação mais ativa na sociedade.

2.1.5 CashGuard

O uso do CashGuard, sistema de gestão de numerário automático, na Farmácia Universal demonstrou ser muito vantajoso principalmente em dois aspetos. Em primeiro lugar, este sistema oferece uma gestão eficiente do dinheiro resultantes das vendas, diminuindo eventuais erros relacionados com a devolução de trocos e reforçando a segurança nas transações. Por outro lado, ao permitir um menor dispêndio de tempo no ato do pagamento e impossibilitando falhas a este nível, permite que durante o atendimento o foco total sejam as necessidades do utente.

Assim, para além de permitir uma melhor gestão e controlo de caixa no final do dia, representa um instrumento de confiança para a farmácia, por se tornar menos suscetível a roubos.

2.2 Pontos Fracos

2.2.1 Medicamentos manipulados

Atualmente, face à constante evolução tecnológica na área farmacêutica, e com o aumento de produtos de saúde disponíveis no mercado, a preparação de medicamentos manipulados nas farmácias tem vindo a decrescer progressivamente. No entanto, este tipo de medicamentos revela-se ainda importante na salvaguarda da preparação de medicamentos ainda não disponíveis no mercado, ou cujo um ajuste de dose seja necessário, garantindo também a preparação de produtos para situações patológicas específicas.

Apesar da Farmácia Universal possuir um pequeno laboratório para preparação de medicamentos manipulados, este deixou de ser utilizado pela escassa solicitação de preparação dos mesmos. Deste modo, considero que este aspeto representou um ponto fraco no meu estágio por não ter tido oportunidade de preparar qualquer medicamento manipulado, não podendo também colocar em prática conhecimentos adquiridos na minha formação nas unidades curriculares de Farmácia Galénica e Tecnologia Farmacêutica.

2.2.2 Espaço Pequeno

Um dos pontos fracos deste estágio foi o reduzido espaço de trabalho na Farmácia Universal. A equipa técnica da farmácia é constituída por 5 farmacêuticos, indispensáveis dado o fluxo de utentes ao longo do dia. No entanto, a existência de apenas 4 balcões de atendimento não permite que a velocidade de atendimento seja a desejada em determinadas alturas do dia com maior afluência de utentes. Este fator tornou-se limitante em determinados momentos, impedindo que efetuasse um maior número de atendimentos, ato que constitui a maior fonte de aprendizagem em farmácia comunitária.

Outro aspeto importante prende-se com o espaço físico para disposição de produtos de saúde e medicamentos não sujeitos a receita médica. Um espaço mais amplo em redor da zona de atendimento para colocação de expositores e disposição de uma maior variedade de produtos de saúde seria uma mais valia para a sustentabilidade da farmácia, por permitir uma

maior variedade de escolha ao utente. Este aspeto não só é importante financeiramente para a farmácia, mas também permite a eventual fidelização de utentes.

2.2.3 Variedade de Produtos no Mercado

A vasta variedade de produtos no mercado, especialmente a nível de dermofarmácia e cosméticas e de suplementos alimentares refletiu-se na dificuldade no aconselhamento destes produtos no ato do atendimento. Apesar dos notáveis conhecimentos e competência adquiridos ao longo da minha formação académica, reconheço algumas fragilidades na minha formação nestas áreas, considerando a enorme diversidade de produtos disponíveis no mercado.

A diversidade de linhas de cosmética, cada uma com uma vasta gama de produtos específicos para determinados tipos de pele e afeções dermatológicas, refletiu-se na minha falta de confiança e autonomia no aconselhamento destes produtos. Neste aspeto, a equipa da Farmácia Universal foi crucial na aprendizagem e preparação para o ato do atendimento, especialmente a nível da gama de solares, amplamente solicitada no período em que realizei estágio.

A constante introdução de suplementos alimentares no mercado é também responsável pela formação contínua a que o farmacêutico de oficina está sujeito. Nesta área, apesar das fortes bases concedidas pelo MICF, senti também alguma relutância da minha parte no que respeita ao seu aconselhamento.

Todos estes aspetos puderam ser colmatados pela constante disponibilidade e preocupação da equipa técnica da Farmácia Universal em esclarecer todas as minhas dúvidas e incentivar a minha contínua aprendizagem.

2.3 Oportunidades

2.3.1 Formação Contínua

Os sucessivos avanços tecnológicos e científicos na área da saúde exigem a formação contínua e progressiva dos farmacêuticos de oficina, de modo a prestarem aconselhamento de acordo com a evidência científica mais atual. Neste sentido tive a oportunidade de participar em formações na área de dermofarmácia e cosmética, que ocorrem na farmácia sob orientação dos delegados de informação médica dos laboratórios em questão.

Estas formações foram de grande importância para adquirir conhecimentos em dermofarmácia e cosmética no contexto da realidade profissional, que também se traduziram numa melhoria de desempenho no atendimento ao público. Apesar de não ter participado em formações noutras áreas, foram notórios o impacto e a relevância destas formações no bom exercício da prática farmacêutica em farmácia comunitária.

A formação contínua na área da saúde apresenta-se com uma valiosa oportunidade tanto para os estagiários como para os profissionais da Farmácia Universal para atualizar os seus conhecimentos, permitindo assim a prestação de um serviço de qualidade à população.

2.3.2 Receita Eletrónica

O sistema de prescrição eletrónica foi introduzido com o intuído de racionalizar o acesso ao medicamento no âmbito do Serviço Nacional de Saúde, simplificando o ato da prescrição e desmaterializando as receitas [2]. A prescrição eletrónica permite que o número da receita, o código de acesso e o código de direito de opção sejam facultados aos utentes via email, através do telemóvel ou a mais frequentemente utilizada, em formato de papel.

Este novo sistema de receita eletrónica surgiu como forma de simplificar a dispensa de medicamentos nas farmácias, proporcionando uma maior rapidez, eficácia e segurança no ato da dispensa. As principais vantagens que posso salientar são por um lado a diminuição de ocorrência de erros, tanto ao nível da verificação do prazo de validade como da troca de medicamentos, mas também por permitirem ao utente aviar apenas os medicamentos prescritos de acordo com as suas necessidades.

Desta forma, o uso da receita eletrónica permitiu-me efetuar os atendimentos de forma mais rápida e eficiente, perdendo menos tempo a verificar a conformidade da receita e a introduzir os dados necessários no Sifarma2000, como se verifica para as receitas manuais, e focando-me mais no aconselhamento ao utente.

2.3.3 Prescrição por DCI

A prescrição por denominação comum internacional (DCI) representa a possibilidade por parte do utente em escolher entre o medicamento de referência e o medicamento genérico de acordo com a sua preferência, permitindo também ao utente intervir diretamente nos seus encargos com medicamentos e assumir um papel ativo na gestão do seu tratamento.

Para além das vantagens para o utente acima nomeadas, a prescrição por DCI permitiu-me sobretudo participar ativamente na educação da população através da introdução de alguns conceitos importantes no entendimento do conceito de medicamento genérico. Por outro lado, permitiu que esclarecesse e assegurasse os utentes para o facto de medicamentos com apresentações diferentes poderem efetivamente ter igual composição e ser usados para a mesma indicação, e explicasse a razão pela qual os preços entre o medicamento de referência e o medicamento genérico serem muitas vezes tão discrepantes.

Assim, a prescrição por DCI manifestou-se como uma oportunidade de educar a população para o conceito de medicamento genérico, conceito ainda pouco claro por um número significativo de pessoas.

2.4 Ameaças

2.4.1 Concorrência entre farmácias

A Baixa de Coimbra caracteriza-se pela elevada densidade populacional e circulação constante de pessoas em redor da Rua da Sofia, Praça 8 de Maio, Rua Visconde da Luz até ao Largo da Portagem, tornando-se assim num local privilegiado para o funcionamento de uma farmácia. A Farmácia Universal, tal como muitas outras da mesma zona, iniciou atividade antes da implementação da distância mínima obrigatória de 350 metros entre farmácias [4]. Assim, num raio de 350 metros, existem aproximadamente 5 outras farmácias, que inevitavelmente fazem concorrência entre si.

A elevada densidade de farmácias nesta zona de Coimbra acaba por dispersar os utentes pelas várias farmácias, o que pode justificar a menor afluência de utentes à Farmácia Universal em determinados períodos do dia. Por outro lado, a competitividade entre farmácias pode também funcionar como fator impulsionador para a dinamização de campanhas, implementação de serviços e fidelização de utentes.

2.4.2 Conceito de medicamento genérico

Medicamento genérico, à luz do Decreto-Lei 176/2006, de 30 de agosto, define-se como "medicamento com a mesma composição qualitativa e quantitativa em substâncias ativas, a mesma forma farmacêutica e cuja bioequivalência com o medicamento de referência haja sido demonstrada por estudos de biodisponibilidade apropriados" [5].

Tal como descrito anteriormente, na secção 2.3.3, a prescrição por DCI apresenta algumas vantagens para o utente na escolha do medicamento de acordo com a sua preferência. Deste modo, e sendo dever do profissional de farmácia questionar sempre o utente sobre a sua preferência, em muitas situações expliquei aos utentes no que consiste um medicamente genérico. O que verifiquei em muitos casos foi o desconhecimento deste conceito, e uma desconfiança permanente de alguns utentes quanto à qualidade e segurança destes medicamentos. Considero que neste sentido o uso da terminologia medicamento "de marca" ou medicamento "original" muitas vezes é erradamente associado a outros produtos fora da área da saúde em que o entendimento geral é de que o produto que não é "de marca" é de qualidade inferior. Assim, a adoção da terminologia medicamento "de referência", por ser mais correta e a implementada pela Autoridade Nacional do Medicamento e Produtos de Saúde, o INFARMED, I.P., permite uma melhor compreensão e menor hesitação de alguns utentes quanto à qualidade, segurança e eficácia destes medicamentos.

Deste modo, é fundamental a dedicação dos profissionais de saúde na educação dos utentes no que respeita aos medicamentos genéricos.

2.4.3 Roturas de Stock

Uma das maiores ameaças que presenciei no estágio na Farmácia Universal foi a constante rotura de stock de medicamentos muitas vezes necessários para tratamentos prolongados de doenças e afeções de caracter crónico. Destes posso destacar a rotura de stock do sensor de medição da glicémia FreeStyle Libre®, e dos medicamentos Cardura® 4mg, Pritor® 80mg e Doce Alívio® durante o período em que efetuei o estágio.

As roturas de stock são de facto preocupantes por comprometerem a continuidade do tratamento dos doentes, que muitas vezes são inflexíveis quanto à troca para um medicamento equivalente, e principalmente quando o medicamento em causa é o único com determinada indicação terapêutica. Estas constituem também uma ameaça para a relação entre os profissionais da farmácia e os utentes.

Assim, as roturas de stock para além de comprometerem a continuidade das terapêuticas, são também uma causa de inquietação para os farmacêuticos pela insatisfação que fomentam junto dos utentes.

3. Caso Clínico

Uma senhora de idade deslocou-se à farmácia e explicou que se encontrava obstipada há mais de uma semana, situação que se verificava com muita frequência, pelo que pede aconselhamento sobre o laxante mais indicado. Questionei a senhora sobre a medicação que tomava regularmente, para despistar se esta situação se encontrava relacionada com efeitos secundários de outros medicamentos e para perceber a situação clínica da senhora. Apenas tomava medicação para a hipertensão arterial e hipercolesterolémia. Assim, tentei perceber o tipo de alimentação da utente, incutindo a importância das medidas não farmacológicas, como a ingestão de frutas e legumes variados, devido à constituição rica em fibra, beber muita água e a adoção de um horário definido para defecar. A senhora aceitou tentar implementar algumas destas medidas, mas mesmo assim considerou necessário mais alguma coisa dado se encontrar obstipada frequentemente.

Perante este caso recomendei Laevolac® 666,7mg/mL xarope (uma a duas colheres de sopa por dia) por ser indicado para a obstipação crónica e ter como ingrediente ativo a lactulose, laxante osmótico que promove o aumento da quantidade de água no conteúdo do colon, estimulando os movimentos intestinais e normalizando a consistência das fezes [6].

4. Conclusão

O estágio em Farmácia Comunitária revelou ser uma das experiências mais enriquecedoras do meu percurso académico. Foi não só uma oportunidade de consolidar os conhecimentos adquiridos ao longo destes cinco anos do meu percurso académico, mas também a oportunidade de os aplicar no contexto da realidade profissional e em benefício da saúde pública.

A minha experiência na Farmácia Universal caracterizou-se pela aquisição de competências essenciais ao desempenho da atividade farmacêutica. Desde as atividades de *backoffice*, mais focadas na gestão e organização da farmácia, até ao atendimento ao público, a grande fonte de aprendizagem nesta área, permitiram percecionar a importância do farmacêutico na sociedade, na promoção da saúde e do bem-estar da sociedade. A formação contínua e a implementação do conceito de acompanhamento farmacoterapêutico, cada vez mais especializado e individualizado, decerto serão os caminhos a seguir para continuar a conquistar a confiança dos utentes e implementar valores em saúde que se reflitam nos comportamentos da população.

O sucesso desta etapa teve como base fundamental toda a equipa da Farmácia Universal, que foi incansável na sua disponibilidade e determinação em proporcionar uma experiência o mais gratificante e completa possível.

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Parte III – Monografia Intitulada "Drug-resistant Epilepsy: An overview on the relevance of ABC Transporters Superfamily"

List of Abbreviations

ABC - ATP binding cassette

AED - Antiepileptic drug

BBB - Blood-brain barrier

BCRP - Breast cancer resistance protein

BCSFB - Blood cerebral spinal fluid barrier

CETA - Concentration equilibrium transport assay

CNS - Central Nervous System

COX-2 - Cyclooxygenase 2

CYP – Cytochrome P450

DRE - Drug-resistant epilepsy

DS - Dravet syndrome

EC - Endothelial cell

EP1 - Prostaglandin E2 receptor 1

FCD - Focal cortical dysplasia

GLUT1 – Glucose transporter 1

HPLC - High-performance liquid chromatography

HS - Hippocampal sclerosis

LLC - Lewis-lung cancer

LLC-PK1 - Lewis-lung cancer porcine kidney 1

MCD - Malformations of cortical development

MDCK - Madin-Darby Canine Kidney

MDCKII - Madin-Darby Canine Kidney type II

MDR1 - Multidrug resistance protein 1

MPPF - 2'- methoxyphenyl-(N-2'-pyridinyl)-p-fluoro-benzamido-ethyipiperazine

miRNA – MicroRNA

mRNA – Messenger ribonucleic acid

MRP - Multidrug resistance-associated protein

NBD - Nucleotide-binding domain

NMDA - N-methyl-D-aspartate

PET - Positron emission tomography

PET/MR - Positron emission tomography and magnetic resonance imaging

P-gp - P-glycoprotein

RT-PCR - Reverse transcription polymerase chain reaction

SE – Status epilepticus

shRNA - Short hairpin RNA

siRNA - Small interfering RNA

SLC - Solute carrier

SNP - Single nucleotide polymorphism

TLE - Temporal lobe epilepsy

TMD - Transmembrane domain

TQD – Tariquidar

UV – Ultraviolet

WHO – World Health Organization

3-MP - 3-mercaptopropionic acid

1. Introduction

According to International League Against Epilepsy, epilepsy is generally defined as having at least two unprovoked (or reflex) seizures apart from each other for not less than 24 hours [1]. Epilepsy affects around 50 million people worldwide, with a higher prevalence in resource-poor countries, making it one of the most common neurological diseases, according to the World Health Organization (WHO) [2]. Despite the antiepileptic drugs (AEDs) available in clinical practice, approximately one third of patients with epilepsy remain refractory to treatment [3]. This clinical situation is usually referred to as "drug-resistant", "pharmacoresistant", "refractory" or "intractable" epilepsy, and it is generally defined as the failure of two rationally chosen AED posologic schedules, in monotherapy or in combination with other AEDs, to achieve seizures control or freedom [4]. These patients fail to achieve a seizure free lifestyle, which highly increases morbidity and mortality [5], rushing the need of discovering the mechanisms underlying drug-resistant epilepsy (DRE) and find effective treatments to avoid seizures.

In this regard, there are numerous theories that attempt to explain DRE [6]. Amongst them emerges the pharmacokinetics hypothesis, which states that efflux transporters located in peripheral organs reduce the systemic bioavailability of AEDs and consequently the quantity available to cross the blood-brain barrier (BBB) and attain the biophase [6]. Complementarily, the transporter hypothesis emphasises that those efflux transporters are overexpressed in endothelial cells of the BBB and in epileptogenic cells of epileptic patients, hampering the AEDs access into the brain and decreasing the concentration of the AEDs in the epileptogenic focus [6]. In parallel, the neuronal network hypothesis is also gaining force, claiming that seizure activity is responsible for the remodelling and degeneration of the neuronal structure and therefore restrains AEDs from their site of action as well as the intrinsic severity hypothesis, which proposes that drug resistance is due to the intrinsic physiopathological evolution of epilepsy [6]. More recently, the gene variant hypothesis started to postulate that drug resistance results from genetic variations that occur in the pharmacodynamic targets of AEDs (voltage-gated Na^+ channels) and in the biomolecules that determine AEDs pharmacokinetics [cytochrome P450 (CYP) 2C9] [6]. Similarly, the target hypothesis also states that modifications in AED target receptors causes unresponsiveness to their action. DRE has certainly a multifactorial explanation, considering that each theory individually cannot fully explain refractoriness to treatment [6].

This review will focus on the transporter and pharmacokinetics hypothesis, giving particular emphasis to the ATP binding cassette (ABC) transporter superfamily. Firstly, the

causes and consequences of BBB disruption in epilepsy will be discussed, followed by reviewing the scientific evidence regarding if AEDs are effective substrates of ABC transporters as well as the mechanisms underlying DRE, pointing out the currently available clinical and non-clinical models that aim at explaining the overexpression of such proteins. After providing proof of ABC efflux transporters upregulation, various strategies to evade their range of action will be also herein commented.

2. The blood-brain barrier in epilepsy

The BBB is a vital physiological apparatus responsible for protecting the Central Nervous System (CNS) and regulating its narrow homeostatic state. The BBB controls the exchange of molecules, ions and cells between the blood and the CNS [7]. Its restrictive action is partially explained by the continuous fenestrated capillaries that compose the microvasculature of the CNS [8]. The neurovascular unit of the BBB is composed of different cells: endothelial cells (ECs), connected to each other by tight junctions, pericytes, astrocytes, which form the astrocytic endfoot processes, and the free microglia that plays a central role in the innate and adaptive immune responses of the CNS [9].

In epilepsy, and as a result of consecutive and successive seizures, the physiological function of the BBB is disrupted, with upregulation of adhesion molecules in the microvascular ECs that stimulate immune cells trafficking, causing inflammation and BBB leakage [10]. Recent findings also point out that neurovascular events go beyond leakage of the BBB, concerning also the abnormal interstitial fluid in epileptogenic areas that favour the accumulation of serum proteins (albumin, immunoglobulin G) in the perivascular space of the brain [11].

This reminds a vicious cycle, since the disruption of the BBB and inflammation are thought to be caused by epilepsy, but other contributing factors are also known to be their cause, such as peripheral infection, autoimmunity, brain trauma and stroke [12]. Other triggers to neuro-inflammation and BBB disruption can be high blood pressure, low blood pH, hypoxia and certain chemotherapy regimens [13,14]. Brain inflammation caused by these triggering events results in neuronal hyperexcitability that leads to the development of epilepsy and the generation of seizures [12]. Angiogenesis has also been reported as a relevant consequence of BBB damage [15].

ECs from the neurovascular unit express two main types of transporters, responsible for moving compounds into and out of the CNS: the nutrient transporters and the efflux transporters [8].

The nutrient transporters comprise a vast group of proteins mainly responsible for the influx of nutrients, ions and metabolites that are required for the appropriate function of the CNS. Many of these transporters belong to the solute carrier (SLC) family, a group of membrane transport proteins with over 400 members, including slc2a1 [glucose transporter 1 (GLUT1)] [16]. Interestingly, the GLUT1 deficiency is a genetic syndrome responsible for juvenile onset of epilepsy, causing great comorbidity in epileptic patients [17].

The majority of efflux transporters expressed in the BBB belong to the ABC superfamily, and are essentially responsible for keeping xenobiotics and toxins out of the CNS, protecting the sensitive balance of the neural network [18]. However, when regarding central-acting drugs, ABC transporters often limit their access to the target site [19]. Efflux transporters present a major obstacle to drug penetration into de CNS, being a fundamental key point throughout drug discovery and development programs of new drugs.

From the different types of transporters expressed in the BBB cells, including ions channels, solute transporters, aquaporins and the ABC transporters, the latest are currently ascribed as the most clinically relevant and responsible for drug efflux and drug resistance, particularly when concerning DRE [20]. For this reason, this superfamily will be in deeply described in the following section.

3. The ABC transporters and the efflux of AEDs

The ABC superfamily includes several transport proteins mainly responsible for exporting substrates using the catalytic energy of adenosine triphosphate hydrolysis. In humans, as in all eukaryotic systems, this superfamily only works as active efflux pump, extruding toxins and drugs out of the cells [21]. They have a broad variety of substrates, not only small substances such as ions and molecules but also larger highly organised structures like peptides, lipids, polysaccharides [22].

In humans, there are 48 ABC transporters classified as 7 different subfamilies, from A to G, regarding their genetic and amino acid structure [20]. The most widely studied subfamilies and significant for their drug efflux potential, are the ABCB, ABCC and ABCG subfamilies. Among them, the transporter ABCB1, encoded by *ABCB1* gene and also named P-glycoprotein (P-gp), has been the efflux transporter most studied, followed by the multidrug resistance-associated proteins (MRPs) and the ABCG2, also named breast cancer resistance protein (BCRP) [23].

Constitutively, ABC efflux transporters have a variable number of nucleotide-binding domains (NBDs) and transmembrane domains (TMDs), generally being two of each in order

to be functional [24]. The NBDs contain singular motifs (Walker A and Walker B), common in all ABC proteins, and the TMDs contain between 6 and 12 membrane spanning α-helixes, responsible for substrate specificity [25]. However, there are some exceptions, namely the ABCG subfamiliy, which includes BCRP, as they comprise only one TMD and one NBD, requiring homo or heterodimerization to attain adequate functionality [24].

ABC proteins are codified by a very conserved and well-known genome dispersed in a vast number of genes, which can be studied to evaluate function or associated disease phenotypes [20,26]. Mutations in the genes that codify ABC transporters are responsible for several genetic disorders, namely cystic fibrosis (*ABCC7*), Tangier disease (*ABCA1*), gout (*ABCG2*) and Dubin-Johnson syndrome (*ABCC2*) [25]. Nevertheless, certain ABC genetic polymorphisms have demonstrated to be involved in the abnormal regulation of efflux proteins, and therefore prompt increased efflux of AEDs in the BBB, contributing to the development of the DRE [27] as it will be in deeply discussed in section 4.3.

Apart from the impact of genetic polymorphisms associated to the ABC transporters, their overexpression has been demonstrated to be a result of epilepsy progression. Indeed, seizures and neural stress seem to induce the expression and activity of efflux proteins as a defensive pathway to counteract the increased CNS permeability observed during seizures and epilepsy progression [6]. Some studies also corroborate the concept that the chronic use of AED may also promote the excessive expression of ABC transporters [28].

These physiopathological dysfunctions are considerably important for the development of resistance against AEDs because most of them seem to be substrates of ABC transporters. Nevertheless, even though multiple studies have evidenced such correlation throughout the past 20 years, doubts still existing concerning whether AEDs are effectively ABC transporter substrates and if this has, or not, clinical real impact. At this point, P-gp, MRPs, BCRP and their AED substrates will be focused on the following sections as they are strongly important when regarding epilepsy and DRE.

3.1 P-glycoprotein

Composed by 1280 amino-acids and encoded by the *ABCB1* gene on the 7q21.1 human chromosomal region [23], P-gp or ABCB1 is the most extensively studied transporter of its superfamily. It is acknowledged by its poly-specificity that results from the large and variable binding pocket of P-gp structure, transporting a remarkable number of substrates [29].

P-gp is mainly expressed in the apical membrane of cells from organs responsible for absorption, distribution, metabolism and excretion [30]. Its main sites of expression include the

small intestine, liver, kidney, and blood tissue barriers such as the BBB and the blood cerebral spinal fluid barrier (BCSFB) [30]. In the small intestine, colon, and in the liver, it works as first-pass elimination process for drugs orally administered, critically affecting their bioavailability [31]. On the other hand, its induction/inhibition seems to be a major player in drug-drug interactions [30]. In the kidneys, it works as an excretion mechanism because of its location in the apical membrane of the epithelial cells of proximal tubes [32].

One of the main clinical issues of P-gp, together with its impact on intestinal absorption, concerns its localization in the BBB. Indeed, P-gp has been identified in the luminal side of the ECs and in the abluminal side of the astrocyte endfoot processes of the CNS microvasculature [33–35]. Moreover, P-gp is currently known to be upregulated in neurons, astrocytes and neuroglia of the epileptogenic brain [36–39]. The aforementioned localization of P-gp will actively expel drugs from the brain, even high BBB permeability compounds that passively diffuse through the membrane. In addition, P-gp expression in the epithelial cells of the choroid plexus in the BCSFB may also prevent the entry of drugs into the brain [33].

The broad number of P-gp substrates includes several classes of pharmaceutical compounds such as anticancer drugs, immunosuppressive agents, corticoids, analgesics, antiviral drugs, calcium channel blockers, antidepressants, antibiotics and AEDs [23]. In order to access whether common AEDs used in clinical practise are in fact substrates of P-gp, evidence from *in vitro* and *in vivo* studies were compiled in Table 1. It should be noted that the results presented in Table 1 and 2 are based on the studies herein mentioned and it was assumed that there was a positive substrate-transporter association if the majority of studies pointed that way, and vice-versa. We assumed conflicting evidence when equal number of studies had opposite results. Accordingly, phenytoin, phenobarbital and lamotrigine are definitely substrates of P-gp, considering the number of experiments that confirm this fact, both *in vitro* and *in vivo* models. Some studies suggest that levetiracetam, carbamazepine, topiramate, tiagabine, gabapentin and felbamate might also be P-gp substrates, even though other studies suggest the opposite. Regarding carbamazepine, most of the studies did not find any proof that carbamazepine was in fact a P-gp substrate. Nonetheless, the interpretation of those results should be done carefully, due to the fact that many methodological difficulties and confounding factor might influence the results. Taking a closer look into these studies, a few considerations must be presented.

In vitro bidirectional transport assays should be examined cautiously. In fact, the high permeability of AEDs, together with the fact that AEDs appear to be weak ABC transporter substrates (specially of P-gp) [48], renders this type of assays inadequate, given the fact that

negative results do not necessarily mean that a specific AED is not a substrate of a determined efflux transporter. In bidirectional transport assays, the passive transport of AEDs can lead to false negatives and contribute to the conflicting evidence [48]. For this reason, Luna-Tortós et al established the concentration equilibrium transport assay (CETA), in which passive permeability is discarded by applying equal concentration of drug in the apical and basolateral chambers, allowing a better evaluation of active transport [48].

Another important issue in drug-transporter association studies is the drug concentrations employed in each experiment, a crucial variable to take into consideration. Zhang et al conducted a bidirectional transport assay and a CETA, using animal cell lines transfected with human multidrug resistance protein 1 (MDR1) cDNA. They demonstrated that concentration of phenytoin and phenobarbital affected their P-gp transport, probably because saturation may occur when testing higher concentrations [49]. Thus, the ideal drug concentration used when assessing P-gp-substrates should be within the therapeutic window, especially in the CETAs. Loscher et al also suggested that very low concentrations should not be used, since highly lipophilic drugs like phenobarbital may bind to cell membrane macromolecules, and only become available for transport after binding saturation [62].

Table 1: Commonly prescribed AEDs that are P-gp substrates: evidence from non-clinical studies

? = conflicting evidence; - = no evidence found

Antiepileptic drugs	P-glycoprotein					
	In Vitro			*In Vivo*		
	Cell culture		Human brain tissue/ Humanized BBB model	Brain microdialysis in rats	Animal models of P-gp overexpression	mdr1 knockout mice
	Non-human P-gp	Human P-gp				
Carbamazepine	No	No	-	Yes	?	?
Felbamate	No	-	-	Yes	-	-
Gabapentin	Yes	-	-	-	-	-
Lamotrigine	Yes	Yes	-	Yes	-	-
Levetiracetam	Yes	?	-	No	-	-
Phenobarbital	Yes	Yes	-	Yes	Yes	-
Phenytoin	No	Yes	Yes	-	Yes	Yes
Tiagabine	-	-	-	-	-	Yes
Topiramate	No	Yes	-	-	-	Yes
Valproate	No	No	-	No	-	-
References	40–45	41,46–50	51,52	42,53–55	56–59	47,59–61

In vitro and *in vivo* models which express efflux transporters in physiological concentrations fail to simulate the pathologic conditions observed in DRE. This was reported in a study using endothelial cells of normal epileptic brain tissue (which expressed physiological levels of P-gp), where P-gp inhibitor tariquidar (TQD) had no effect on the permeability of phenytoin [51]. On the contrary, phenytoin permeability in drug-resistant epileptic brain tissue was partially abolished by TQD [51]. This is also corroborated in *in vitro* models where P-gp is only overexpressed in *MDR1*-transfected cells compared to wildtype [48]. Luna-Tortós et al reported that phenytoin directional transport only seems to occur in *MDR1*-transfected Lewis-lung cancer (LLC) cells (which overexpress P-gp) compared to wildtype cells (which express physiological levels of P-gp) [48]. In point of fact, several ABC efflux transporters are overexpressed in specific brain regions, such as ECs of the BBB, astrocytes and neurons of the neurovascular unit [36,37,39,63]. This variation in transport expression, combined with the fact that most AEDs do not seem to be high affinity substrates, may dramatically influence experimental results [64,65]. Marchi et al corroborated this concept when they proved an inverse correlation exists between oxcarbazepine metabolite concentration and MRP1 expression in epileptic tissue [66].

Inter-species differences observed in efflux transporters structure and activity between humans and animal models (namely the rodents, mouse or rat, that are the most usually used) are undeniably an important cause of discrepancy between several experiments [67]. In order to investigate whether carbamazepine, levetiracetam and phenytoin are substrates of efflux transporters, Baltes et al performed bidirectional transport studies through monolayers of polarized Madin-Darby Canine Kidney type II (MDCKII) cells and Lewis-lung cancer porcine kidney 1 (LLC-PK1) cells transfected with either human *ABCB1* or *MRP2* or mouse *mdr1a* or *mdr1b*[41]. Accordingly, both phenytoin and levetiracetam were transported by mouse but not by human P-gp. In opposition, carbamazepine was not transported by either model. The evidence from this study points out differences in substrate recognition and transporter efficacy between species and makes questionable the real impact of ABC transporters on AEDs efflux [41].

Therefore, it is obvious that clinical studies would be the most reliable model type, however for ethical reasons, difficulties in studies design and results interpretation, they are not easily performed or informative. Rambeck et al conducted an intraoperative microdialysis to measure the concentration of multiple AEDs in human neocortical tissue [68]. Although the extracellular space concentration of AEDs in the neocortical tissue was lower than in the cerebrospinal fluid, the lack of comparable data from healthy subjects restrains us

from having meaningful conclusions. The study also suggested that intra and inter-individual variations are considerable in DRE.

For these reasons, negative results gathered in the previous tables should be acknowledged carefully, as a negative result does not necessarily mean these drugs are not ABC transporters substrates. The studies herein collected, especially for MRPs and BCRP (that mainly had negative results as we will see further on), do not allow us to take conclusions about their involvement in AEDs efflux in DRE. On the other hand, cumulative positive results, such as observed for phenytoin, phenobarbital and lamotrigine concerning P-gp, can be taken as proof that multiple AEDs are in fact object of efflux transport.

3.2 Multidrug resistance-associated proteins

MRPs belong to the ABCC subfamily, a group composed of 13 members. Among them, 9 are associated to multidrug resistance (MRP1-MRP9) [69]. Their chromosomal location is heterogeneous, with each protein associated to a specific location usually in different chromosomes [20]. The most studied transporters are MRP1-MRP6, which are importantly expressed in very different levels in healthy and epileptogenic brain tissues [70–73], as we will see in section 4.

Their tissue distribution is varied and depends on each subtype: while MRP1 and MRP5 have an ubiquitous distribution, MRP2, MRP3 and MRP6 are mainly distributed in the liver, small intestine, kidneys and in some secretory organs [69]. In the CNS, MRP1, MRP2 and MRP3 are located in the choroid plexus of the BCSFB, with differential distribution [23,33]. MRP1, MRP2, MRP4 and MRP5 were also identified in the endothelial cells of the BBB, with MRP1 in the abluminal side, and MRP4 and MRP5 in the luminal side of rat and murine cerebral tissues [33,34]. Their ample location in the BBB proves their potential role in central-drug resistance.

There is still lack of definitive evidence that common AEDs are MRPs substrates. Nonetheless, Table 2 summarizes the studies that aimed at answering this issue, despite the fact that very few studies were able to identify the substrate-transporter relation between AEDs and MRPs. Accordingly, it is suggested that the frequently used AEDs are not substrates of MRPs, even though the evidence is conflicting and insufficient. Indeed, the experimental obstacles mentioned in the previous section for P-gp also apply for MRPs and BCRP studies. Some additional setbacks will be clarified.

In vitro models like Madin-Darby Canine Kidney (MDCK), MDCKII and LLC-PK1 may express endogenous drug transporters (canine and porcine, respectively) that cloud the true

contribution of transfected drug transporters [79], thus leading to false positive results. In order to understand this question, Goh et al evaluated the expression of endogenous transporters in MDCK, MDCKII and LLC-PK1 cell lines by reverse transcription polymerase chain reaction (RT-PCR) and by using specific transporter inhibitors [79]. They found MDR1 and MRP1 messenger ribonucleic acid (mRNA) in all cell lines and MRP2 mRNA in MDCKII and LLC-PK1 cell lines. MDCKII cells proved to have high levels of functional P-gp compared with the other cell lines [79].

Some MRP non-selective inhibitors, like probenecid and indomethacin, might inhibit both influx and efflux transporters at the BCSFB and the BBB [42]. When accessing valproate uptake in bovine brain microvessel endothelial cells by MRPs, Gibbs et al noticed that low inhibitor (probenecid and indomethacin) concentration increased valproate influx, and high concentration of these inhibitors had the opposite effect [74]. Consequentially, this can hamper studies meant to evaluate the real impact of MRP in AEDs efflux. Another issue when studying MRPs is the lack of inhibitors that specifically inhibit one member of the MRP family, hindering the results validation [65].

Table 2: Commonly prescribed AEDs that are MRPs or BCRP substrates: evidence from non-clinical studies

? = conflicting evidence; - = no evidence found

Antiepileptic drugs	MRPs				BCRP		
	In Vitro		*In Vivo*		*In Vitro*		*In Vivo*
	Cell culture		Brain microdialysis in rats	MRPs deficient mice	Cell culture		BCRP deficient mice
	Non-human MRPs	Human MRPs			Non-human BCRP	Human BCRP	
Carbamazepine	-	No	?	Yes	No	No	-
Felbamate	-	-	No	No	-	-	-
Gabapentin	-	-	-	-	-	-	Yes
Lamotrigine	-	No	No	No	?	Yes	-
Levetiracetam	-	No	No	-	No	No	Yes
Phenobarbital	-	?	-	-	No	No	Yes
Phenytoin	-	No	-	-	No	No	-
Tiagabine	-	-	-	-	-	-	Yes
Topiramate	-	No	-	-	No	No	-
Valproate	?	No	?	-	No	No	-
References	42,74	41,42,48,50,72	42,53,55,75,76	75	77,78	77	60

3.3 Breast Cancer Resistance Protein

BCRP, a 655 amino acid ABC transporter, is codified by the gene *ABCG2* located on chromosome 4q22 [20]. It has an unusual structure compared to its family members previously discussed, since BCRP is a half-transporter, requiring the formation of homo or heterodimers to actively function [80]. BCRP is expressed in a variety of tissues, having its peak of expression in the placental tissue, brain (putamen, substantia nigra, thalamus), liver and also in the kidneys, small intestine and colon [81].

Similarly to the other subfamilies, BCRP has a wide range of substrates, most of them in parallel with P-gp, including negative to positively charged molecules, organic anions and sulfate conjugates [80].

BCRP is the most studied transporter from its family particularly because of its well-known relevance underlying multidrug resistance phenomena in breast cancer [81,82]. Indeed, BCRP has currently a recognized role in drug resistance, but particularly regarding anticancer compounds with little knowledge concerning AEDs. From the few studies founded, most of them could not prove that common AEDs are BCRP substrates (Table 2). Some notes should be made on the results reported in Table 2, in order to consider some weaknesses of these experiments.

Nakanishi et al evaluated the contribution of P-gp and BCRP on the brain distribution of 12 AEDs. To access this hypothesis they used knockout mouse models, Mdr1a/1b(-/-) and Mdr1a/1b(-/-)/Bcrp(-/-) mice [60]. The blood and brain concentrations were measured 60 minutes after simultaneous intravenous administration of 6 AEDs, to determine the brain-to-plasma concentration ratio of each one. When interpreting the brain-to-plasma concentration ratio of each AED, the authors concluded that phenobarbital, levetiracetam, tiagabine and gabapentin are substrates of BCRP, even though these results might be due to the cooperative effect of P-gp and BCRP, since the comparison was made between Mdr1a/1b(-/-) and Mdr1a/1b(-/-)/Bcrp(-/-) mice [60]. Thus, the positive results reported in Table 2 for BCRP deficient mice might be due to the cooperative effect of P-gp and BCRP.

In another study, using CETA in MDCKII cells, overexpressing the murine transduced Bcrp1 or the human transduced BCRP, Romermann et al only found BCRP-mediated transport of lamotrigine, out of seven AEDs that were investigated [77]. The authors reported that the lack of drug-transporter association between BCRP and the other AEDs studied can be due to the low expression of BCRP in MCDKII cells, so that weak BCRP substrates might be missed [77]. Apart from this, species differences are highly relevant in BCRP expression. In

humans, it is the most abundant transporters with 30% higher expression than P-gp, whereas in mice its expression is 70% lower than P-gp [83].

4. Upregulation of ABC efflux transporters in epilepsy

Although the overexpression of ABC transporters in epileptic brain is currently scientifically supported [84], the challenge is to know whether this upregulation is caused by the epilepsy pathology itself, the uncontrolled recurrent seizures, the drug-associated induction, genetic polymorphisms or a combination of all these factors [6]. Another question is whether this upregulation is significant enough to have a clinical impact and cause multidrug resistance in epilepsy and other CNS disorders.

The first clinical evidence of the transporter theory was postulated by Tishler et al who found increased levels of ABCB1 mRNA and P-gp in brain sample from patients with intractable epilepsy [85]. Out of the 19 brain samples, RT-PCR investigations revealed that 11 samples presented levels of ABCB1 mRNA at least 10 times higher than those observed in samples from normal brains; 14 samples showed an increased staining of P-gp expressed in capillary endothelium from epileptic tissue comparing to normal samples [85]. Expression of P-gp was also identified in astrocytes of epileptic brain specimens [85]. In another study, with surgically resected brain tissue and post-mortem brain tissue from drug-sensitive and drug-resistant epileptic patients, P-gp was overexpressed in the epileptogenic hippocampus of drug-resistant epileptic tissues. This overexpression was only associated to P-gp with no significant variations found regarding BCRP or MRP1 [86].

A pilot study performed by Bauer et al with seven patients diagnosed with drug-resistant temporal lobe epilepsy (TLE) showed that patients who underwent surgery and became seizure free were associated with higher levels of P-gp preoperatively [87]. They noted normalization of P-gp activity in patients with long-lasting seizure-freedom after surgery, demonstrating the change in P-gp behaviour. However, the study could not address if P-gp upregulation was the cause of drug-resistant TLE although it was suggested that it probably contributed to the development of DRE [87].

In a meta-analysis of 9 studies recently conducted, a strong relationship between MRP1 overexpression and drug-resistant epilepsy was revealed [88]. Interestingly, this upregulation was found mostly on astrocytes and neurons rather than endothelial cells of lesioned brain tissue [88].

The possible causes that explain the upregulation of ABC transporters in DRE will be focussed in the following sections.

4.1 Pathophysiological upregulation of ABC transporters

The cornerstone of the transporter hypothesis in DRE is the evidence of efflux transporters upregulation in epilepsy and epileptogenic malformations. This can either be caused by the natural evolution of the pathology and/or the effect of recurrent seizures [86,89] as it will be presented in this section together with the current state of art .

4.1.1. Epileptogenic malformations

The aetiology of epilepsy is known to be a dominant aspect in the development of DRE. In this section we will give particular focus on structural abnormalities that are associated with DRE. Malformations of cortical development (MCD), hippocampal sclerosis (HS) and brain tumours are more often associated to drug resistance than the idiopathic forms of epilepsy [84]. Studies that evaluated the influence of epileptogenic malformations in the upregulation of ABC transporters are summarized in Annex 1.

Several studies were conducted on HS samples, a pathology strongly associated with drug-resistant TLE [63,90–94]. A case-control study evaluated the activity of P-gp on patients with TLE caused by unilateral HS [94], using positron emission tomography (PET). Drug-resistant patients and healthy controls received an infusion of TQD for 30min, followed by (R)-^{11}C-verapamil PET scan for 60 min to access the plasma-to-brain transport rate constant of (R)-^{11}C-verapamil [94]. A higher activity of P-gp was suggested in drug-resistant patients compared to seizure-free patients and healthy controls, because of the lower plasma-to-brain transport rate of (R)-^{11}C-verapamil observed in the experimental group. Furthermore, there was an inverse correlation between (R)-^{11}C-verapamil brain uptake in the hippocampus and the average monthly seizure frequency [94], suggesting an association between P-gp activity and seizure frequency.

P-gp was found overexpressed in reactive astrocytes and ECs in epileptogenic tissue from HS samples [63,90] and MRP1 in reactive astrocytes, but not in ECs [63,93]. Aronica et al detected upregulation of P-gp, MRP1 and MRP2 in the epileptogenic tissue from samples of TLE patients with HS, but no change of BCRP was detected compared with control hippocampus samples [91,92]. P-gp and BCRP localization was confirmed in the microvascular endothelium [93].

As aforementioned, other common causes of DRE include MCD and certain brain tumours. Sisodiya et al performed one of the first studies on DRE related to MCD [95]. Accordingly, a constitutive P-gp overexpression was identified when analysing brain samples

of MCD. Although P-gp and MRP1 were not detectable in neuroglia and neurons under physiological conditions [39,63], those transporters were both detected in astrocytes, dysplastic neurons and also ECs of epileptogenic tissue from focal cortical dysplasia (FCD) patients [36,37,39,63]. Furthermore, they were also identified in reactive astrocytes and dysplastic neurons of brain neoplastic epileptogenic tissue samples (dysembryoplastic neuroepithelial tumours and gangliogliomas) [37,63]. In other samples of FCD, BCRP was identified in the brain microvascular endothelium, and was overexpressed in glial and neuronal cells [92,93].

On the other hand, tuberous sclerosis, a genetic disease that causes benign tumours growth in the brain and other vital organs, is also underlying DRE [5,37]. Resected tissue analysis showed increased levels of P-gp, with intralesional cell distribution [96,97]. P-gp and MRP1 were also upregulated in dysplastic neurons, astrocytes and microglial cells [38].

Recently, Banerjee et al studied human samples of mesial TLE and FCD, in which BCRP mRNA was found upregulated [98], suggesting that BCRP can also be involved in DRE in spite of the scarce information regarding this point.

Marchi et al conducted a study in a rat model of human developmental brain malformations, treated with methylazoxymethanol acetate [99]. Pilocarpine was administered to induce recurrent generalized seizures, and 18 hours afterwards, ondansetron (P-gp substrate) was intravenously administered. Plasma and centrifuged brain tissue samples were processed and analysed by high-performance liquid chromatography (HPLC) with ultraviolet detection (UV) to measure ondansetron concentrations. In comparison to normal rats, those treated with methylazoxymethanol presented ondansetron brain levels increased by 4-fold and 2-fold in the hippocampus and frontal cortex, respectively [99]. After seizures induction, ondansetron brain levels decreased 50% and 34% in the same brain regions, event that was found fully reversed after TQD administration [99]. Finally, upregulation of P-gp was also observed in blood vessels and astrocytes in malformed tissues [99]. Thus, this model proved the effect of intrinsic brain pathology on brain permeability, and the impact of seizure activity on ondansetron brain penetration [99]. The authors suggested that brain injury in this model was associated with the expression of P-gp in ECs.

Worthy to note is the new imaging techniques such as PET, positron emission tomography and magnetic resonance imaging (PET/MR), single-photon emission computerized tomography that are currently propelling the non-invasive quantitative analysis of live DRE patients, instead of the classical histopathological analysis of resected or post-mortem epileptogenic brain tissue. Their potential regard the identification of the epileptic focus and the anatomical changes that result from seizure activity, helping in DRE diagnosis and its underlying aetiology [100,101]. Additionally, given a radiolabelled P-gp substrate we can

study the activity of P-gp inhibitors in vivo, and also substantiate if a radiolabelled-drug is a target of P-gp mediated-efflux [102,103].

In a pilot PET study, Langer et al used a radiolabelled substrate of P-gp, R-[^{11}C]-verapamil, to access the differences in P-gp activity between epileptogenic and normal brain regions [104]. Even though the differences were not statistically significant, there was an increased efflux of the radiolabelled compound in epileptogenic ipsilateral parahippocampal regions, suggesting an increased P-gp expression in TLE patients [104]. Shin et al simultaneously used PET/MR imaging with R-[^{11}C]-verapamil and cyclosporine A, a well-known P-gp inhibitor, in patients with DRE [105]. Their results showed that all patients with DRE had significantly different asymmetry patterns compared to the healthy controls [105]. This means that P-gp activity is significantly different between the epileptogenic focus and the normal areas of the brain. The drug-sensitive epileptic patients had a similar asymmetry profile compared with healthy controls, corroborating the usefulness of this methodology and its potential to localize the epileptic focus [105].

The previous reports undoubtedly evidenced that multidrug resistance ABC proteins are upregulated in common causes of refractory epilepsy, and their expression is probably a consequence of intrinsic pathological mechanisms, ultimately working as defensive systems against xenobiotics. Nevertheless, studies should be performed on MRPs and BCRP to attest if these efflux transporters are also altered in pathologic conditions. Further studies should be performed to authenticate these findings.

4.1.2. Seizure-mediated upregulation

A distinct explanation for the upregulation of several ABC efflux transporters in DRE, besides the pathologic process itself, is the induction of ABC proteins by the recurrent seizure activity in the epileptic focus and surrounding regions. A considerable number of studies, mostly experimental animal studies, take into examination this hypothesis. Over and above, it has been demonstrated that the frequency of seizures in early stages of epilepsy is a dominant prognostic factor for the development of DRE [89]. Studies that evaluated the influence of seizure activity in the upregulation of ABC transporters are summarized in Annex 2.

Generally, animal experiments aimed at studying the regulation of P-gp over the other ABC transporters. Acute seizure-mediated upregulation of P-gp has been proven with a single audiogenic induced seizure, with mdr1a overexpression in the cortex and midbrain region after 24 hours and in the cortical regions 7 days after stimulation [106]. This hypothesis

has also been exploited and proven in other rodent models, namely the kainite-induced seizures model and amygdala kindled seizures model [107]. Overexpression of P-gp has been identified both in endothelial cells and in neurons of the hippocampus in a rat model of status epilepticus (SE) induced by pilocarpine and kainate [108]. Other experiments not only proved the correlation with acute exposure, but also with chronic seizures, showing increased levels of mdr mRNA and P-gp in the epileptogenic regions [59,109]. Van Vliet et al noted that upregulated P-gp was located in glia-like cells in close apposition to blood vessels and also in blood vessels, event that was correlated to the occurrence of SE [109].

Some non-clinical studies went beyond the identification of P-gp, and also related P-gp expression to AEDs response [110,111]. P-gp upregulation was found associated with rats non-responsive to phenobarbital and phenytoin therapy. These non-responsive rats exhibited an overexpressed level of P-gp confined to the brain ECs of the BBB [111]. In opposition, other investigations performed in 2 rat SE models showed that P-gp was not significantly involved in DRE [112].

Bartmann et al carried out a microPET evaluation of the effects of TQD on the brain kinetics of P-gp substrate [^{18}F]-[2'- methoxyphenyl-(N-2'-pyridinyl)-p-fluoro-benzamido-ethyipiperazine] [[^{18}F]-MPPF] in a rat model of spontaneous recurrent seizures [113]. They had previously demonstrated that phenobarbital non-responder animals had increased expression of P-gp compared to phenobarbital responders. After the microPET evaluation with TQD, they concluded that phenobarbital non-responders had an increase of 142% [^{18}F]-MPPF unidirectional blood-brain clearance, compared to the 92% increase observed in the responder group [113].

To the best of our known, the only clinical study regarding this topic was performed by Kwan et al, who examined temporal lobe tissue from patients who underwent lobectomy for drug-resistant TLE [114]. They noticed increased capillary staining of P-gp from those who relapsed to seizures compared to the ones who did not have seizures after surgery [114].

At this point it is noteworthy that the majority of studies is performed in rodents and that P-gp was practically the only transporter investigated. However, MRP2 also showed to be overexpressed in the hippocampus and cortex of a pentylenetetrazole-kindled rat model [115]. The same study also proved that co-administration of probenecid (MRP2 inhibitor) increased phenytoin levels on both normal and kindled rats [115].Overall, it can be recognised that both acute and chronic seizures have the potential to increase efflux transporter levels on the epileptic focus and this may play significant part in the development of drug resistance in some patients. Even though, more studies are required for other efflux transporters and not only for P-gp.

4.2 Drug-mediated upregulation

Another hypothesised cause of cerebral upregulation of ABC efflux proteins is the intrinsic effect of AEDs, which results from the chronic administration often observed in epilepsy. This theory is still controversial by virtue of a considerable number of studies that show contradictory results. *In vivo* experiments in rodent models have shown that therapeutic doses of phenytoin and carbamazepine have no effect on the expression of P-gp in the brain [59,116], albeit other results showed that therapeutic doses of levetiracetam, sodium valproate, topiramate, phenobarbital, phenytoin and carbamazepine positively modulate brain levels of P-gp, these last 3 specifically in capillary endothelial vessels [117–119]. *In vitro* studies are similarly controversial, with phenytoin, phenobarbital and carbamazepine demonstrating increased expression of P-gp in astrocytes and endothelial cells in some studies [28,120] while Ambroziak et al evidenced that these AEDs did not provoke any significant induction in P-gp expression or function [121].

Grewal et al investigated the effect of phenytoin, carbamazepine, valproate, lamotrigine, topiramate and levetiracetam on the expression and function of P-gp, MRP1, MRP2 and BCRP in *in vitro* cell models applying Caco2 and HepG2 cell lines [122]. They concluded that carbamazepine caused a significant induction of P-gp and BCRP in both cell lines, and valproate caused a similar effect on P-gp in HepG2 cell lines, whereas the other AEDs studied did not show significant modulation of these efflux transporters [122].

Indeed, the few studies available in literature are *in vitro* or non-clinical *in vivo* studies, which may justify the lack of coherence aforementioned. Moreover, the differences regarding the studies design may also contribute to contradictory results. More studies are required before taking conclusions, even though DRE seems more likely to be related to the pathology itself and the effect of seizure activity.

4.3 Genetic polymorphisms

Genetic factors are increasingly recognized as responsible for drug pharmacokinetic inter-individual variability in multiple diseases; and epilepsy is not an exception. It is widely proven that specific genetic polymorphisms are responsible for hepatic metabolism variability as for instance CYP3A4 and CYP2C9 [123,124]. In particular, CYP2C9 is a major metabolic pathway of phenytoin and determines the wide inter-individual variability observed in its plasma concentrations [124].

When considering the ABC efflux transporters, scientific evidence is still unclear, although several studies hypothesise that genetic polymorphisms also occur in efflux transporters of the BBB. In fact, this theory has been vastly explored in the last few years and the most studied polymorphisms are the ones related to the P-gp encoding gene, *ABCB1*, specifically the C3435T, C1236T, T129C and G2677T/A polymorphisms [27,125]. A meta-analysis carried out by Bournissen et al with 3371 patients, examining 3435CC ABCB1 polymorphism, in a European and Asian cohort, found no association between this ABCB1 genotype and patient response to AEDs [126]. Similarly, another meta-analysis taking 6755 patients (3231 drug-resistant patients and 3524 drug-responsive oo healthy controls) also failed to prove an allelic association between ABCB1 C3435T polymorphism and risk of drug resistance (under the fixed-effects model and the random-effects model) [127]. On the other hand, a meta-analysis conducted by Lv et al in 8331 patients analysing the same ABCB1 polymorphism, found significant differences between the drug-resistant group and the control group, both in allele model (C vs T: OR=1,13; 95% CI: 1,02-1,25) and genotype model (CC vs CT+TT: OR=1,27; 95% CI: 1,08-1,50), between C3435T ABCB1 polymorphism and DRE in the Caucasian population [128]. Another meta-analysis, performed with 8604 patients, reported similar results, in both allele model (C vs T: OR=1,09; 95% CI: 1,00-1,18 P=0,05) and genotype model (CC vs CT+TT: OR=1,20; 95% CI: 1,04-1,40 P=0,01), between the drug-resistant group and the control group in Caucasian populations [129]. These meta-analyses performed a global and ethnic based-analysis in Asian and Caucasian populations. Even between studies which find an association between C3435T ABCB1 polymorphism and drug-resistant patients, they failed when associating the risk of developing DRE to the same genotype, i.e., resistance to AEDs in epilepsy is associated with different genotypes of the same polymorphism [130,131]. Some clinical assays associate the risk of developing drug resistance against AEDs with the TT genotype (recessive) and others with the CC genotype (dominant) [130,131].

Besides P-gp, other genotyping studies were carried out in order to investigate the association ABCC2, ABCC5 and ABCG2 polymorphisms and refractory epilepsy [132,133]. A meta-analysis of ABCC2 G1249A polymorphisms involving 2213 patients suggested there is decreased risk of AED resistance associated with this polymorphism [134]. Wang et al did a similar meta-analysis and suggested the recessive model of G1249A ACBC2 polymorphism was linked to decreased risk of DRE in Asian populations [135]. However, some limitations should be duly noted, since these meta-analyses used a small number of published studies, which might not have enough statistical power [134,135].

In fact, in spite of the several studies that succeeded to associate certain polymorphisms to drug resistance in epilepsy, multiple factors can contribute to discrepancies between genetic association studies and compromise their clinical use as a biomarker. Among those factors, the following ones must be always taken into account:

1) The same functional differences in P-gp may be caused by multiple single nucleotide polymorphisms (SNPs), rather than just by a single genetic SNP. In fact, variations in efflux transporters expression and function might be caused by a combination between different alleles in multiple *loci* [136,137]. As aforementioned, several meta-analysis studies demonstrated no involvement of the C1236T, G2677T/A or C3435T P-gp polymorphisms on the response to AEDs in 7067 patients [125,138]. Even though, in the subgroup analyses, an association between C3435T polymorphism and susceptibility to AEDs was found in the Caucasian population [138]. This clearly emphasizes the importance of other intrinsic characteristics and the superiority of combining genetic and non-genetic factors as composite phenotypes and biomarkers.

2) As ethnic variability is a source of heterogeneity that promotes discrepant results, analysis of ethnicity subgroups should always be performed [139]. As it was emphasized from the studies mentioned in the previous topic, C3435T ABCB1 polymorphisms and DRE seem to be more associated with the Caucasian population.

3) Although difficult, inclusion criteria should be identical between the several studies. On one hand, the definition of DRE tends to diverge among studies, due to the complexity of epilepsy, and therefore different pathological conditions may be a cause of different results. On the other hand, as exposed in section 3 of the present review, the knowledge regarding which AEDs are ABC efflux transporters substrates is not totally clear and there are some studies that do not analyse substrates of P-gp. Thus, if there is indeed a relation between genetic factors and DRE, it can never be found if the target drugs are not even substrates of active efflux transporters. Other criteria, such as aetiology, seizure types and definition of treatment outcomes also contribute to inclusion criteria variability [125];

4) Chance can lead to publication bias in the initial studies that try to find genetic associations [139]. When the first evidence appears with positive results, there is a considerable probability that chance alone can lead to false positive conclusions, considering that the first studies tend to evaluate a vast number of *loci* and polymorphisms, in a small number of subjects [139].

Overall, lack of definitive proof and heterogeneity in study design still limit the current knowledge regarding the main question: do the aforementioned genetic variations have a dominant impact on the clinical outcomes in DRE?

5. Evasion strategies for enhanced therapeutic outcomes

5.1 Inhibition of ABC efflux transporters

In the scope of developing new therapeutic strategies against DRE, one of the most extensively exploited is the inhibition of ABC efflux transporters expressed in the BBB, particularly P-gp.

Multiple experiments resort to new imaging techniques, such as PET, to study the impact of P-gp inhibition in (R)-^{11}C-verapamil brain penetration. Using the P-gp substrate (R)-^{11}C-verapamil, Bauer et al analysed its distribution volume in 42 brain regions from healthy subjects before and after TQD intravenous administration [140]. After TQD administration, the authors detected a moderately higher distribution volume of (R)-^{11}C-verapamil in cerebellum, parahippocampal gyrus, olfactory gyrus and middle temporal lobe and cortex, which may be due to decreased P-gp function. Even though P-gp differential expression was only moderated, the authors suggested that this could be explained by the lack of P-gp overexpression in healthy human brain tissues [140 141]. Also applying PET in healthy volunteers, Muzi et al found a significant increase in (R)-^{11}C-verapamil transport across the BBB after cyclosporine A (P-gp inhibitor) injection, proving the impact of P-gp in (R)-^{11}C-verapamil brain penetration [142]. In a PET study performed in Wistar rats, the brain distribution volume and plasma-to-brain influx rate of (R)-^{11}C-verapamil increased 12-fold and 8-fold, respectively, after P-gp inhibition by TQD [143]. The authors ruled out the influence of TQD on (R)-^{11}C-verapamil kinetics, by demonstrating that TQD had no considerable impact on its plasma binding or metabolism [143]. On the contrary, Langer et al failed to detect statistical significant differences regarding (R)-^{11}C-verapamil transport between epileptogenic and non-epileptogenic brain regions of drug-resistant unilateral TLE patients [104].

Due to the differences on BBB and brain tissues from healthy humans and epileptic patients, administration of P-gp inhibitors started to be vastly employed to access the brain penetration of AEDs in drug-resistant human patients and animal models. In order to explore this question, the influence of nimodipine (P-gp inhibitor) on the brain uptake of phenytoin was investigated in rats with epilepsy inducted by 3-mercaptopropionic acid (3-MP) [144]. Two experimental groups were evaluated – the control and the 3-MP pre-treated group – and both of them were divided into two branches, one injected with vehicle and the other with nimodipine. Among the rats injected with vehicle, phenytoin hippocampal concentrations were lower in the rats pre-treated with 3-MP, compared to control [144]. This could theoretically be explained by increased P-gp expression and function in 3-MP induced

epileptic rats. It was also concluded that, amongst the nimodipine pre-treated rats, those who had induced epilepsy showed higher brain phenytoin levels than the control group, showing an active inhibition of P-gp by nimodipine [144]. As reported by other authors [145], the lack of effect of nimodipine pre-treatment on phenytoin brain levels in the control group might be due to the fact that P-gp is not upregulated in non-epileptic rats [144]. Other P-gp inhibitors, like valspodar, verapamil and sodium cyanide, were also found to increased brain extracellular fluid concentrations of phenytoin [146] and carbamazepine [53], using *in vivo* microdialysis in rats. In rat epilepsy models, P-gp inhibition by TQD showed to increase phenytoin concentrations in specific brain regions that overexpressed P-gp [58] and significantly improve control of spontaneous recurrent seizures in combination with phenytoin [147]. Additionally, TQD showed to restore the anticonvulsant activity of phenobarbital in the drug-resistant subgroup of a rat model of TLE [141].

Clinical studies were also conducted. In 19 patients with drug-resistant TLE and different medication regimens, Asadi-Pooya et al studied the significance of P-gp inhibition by verapamil on seizure frequency [148]. 7 of the 19 patients achieved more than 50% reduction in seizure frequency, and 2 out of the 7 became seizure free [148].

Several case-control studies also show some positive results using a similar approach. For example, Summers et al described a complex case of a 24-year-old woman with high number of hospitalizations, who underwent temporal lobe lobectomy and placement of vagus nerve stimulation device [149]. AEDs were used at maximum tolerated doses and the patient was adherent to a ketogenic diet, but still classified as medically intractable. With few treatment options, verapamil was added to her therapy, which greatly improved seizures control and overall quality of life [149]. In addition, Schmitt et al also described a clinical case report of a patient with frontal lobe epilepsy; co-administration of verapamil with here current therapy significantly reduced seizure frequency [150]. Verapamil has also improved the condition of two young girls who suffered from Dravet syndrome (DS), a common cause of DRE [151]. Verapamil allowed one of the patients to control SE and seizures for 13 months while the other girl still had her condition controlled at the time of this report, 20 months after verapamil administration [151]. The same author reported the improvement of a 11-year-old boy with drug-resistant SE after verapamil administration [152]. In these cases a doubt emerges, questioning whether the final outcome results of inhibition of P-gp by verapamil, or because of the calcium channel blocker action of this drug, which modulates calcium influxes and preserves resting membrane potentials, increasing the threshold of excitability [151]. Another possible mechanism of calcium channel blockers is by inhibiting AED metabolism [149], through the inhibition of liver metabolizing enzymes, specifically CYP3A4, CYP2D6 and

CYP2C9 [153], thus resulting in higher AEDs bioavailability. More recently, Nicita et al assessed the effect of verapamil as add-on therapy to relieve the symptomatology of 7 children with DRE. Among them, 3 had a reduction between 50 and 99% in seizures (DS with positive mutation on *SCN1A* gene), 1 presented an initial improvement with following worsening of condition (DS without genetic mutation) and the other 3 showed no recovery (two with structural epilepsy and the other one with Lennox-Gastaut Syndrome) [154]. These results indicate that P-gp inhibition might be beneficial to certain subgroups of DRE patients [154].

Although these reports have low statistical power concerning the low number of subjects, one cannot exclude the significant condition improvement in some DRE patients.

5.2 Down-regulation of ABC transporters

With the discovery of microRNAs (miRNAs) and their role in gene expression, increasing interest has been given to further explore their function and potential clinical implications as biomarkers and therapeutic targets in multiple diseases [155,156]. These small endogenous molecules, with around 23 nucleotides, have a crucial regulatory action in post-transcriptional gene expression, directly repressing protein translation by targeting and destabilizing specific mRNAs [157,158]. Efforts have been made on developing new strategies to effectively modulate cellular levels of miRNAs, by selectively targeting them with "antagomirs", engineered oligonucleotides that silence miRNAs [159].

In the past few years, variations in miRNA levels have been implicated in the pathogenesis of epilepsy [160,161]. Studies suggest that they affect a wide range of biological processes, namely inflammation, neural morphology, function and death, gliosis, synaptic structure and neurotransmission, ion channels and transporters [162,163]. Although a relationship was found between miRNA alterations and epilepsy, there is still uncertainty about the impact they have on the pathophysiology of epilepsy and which biological processes are involved [160].

Particularly focusing on the role of miRNAs on ABC transporters expression and modulation, there is increasing evidence that miRNAs are indeed involved in their regulation [164]. Even though an association is undeniable, very few information is known concerning DRE. A recent study accessed the association between miR-466b-1-3p and P-gp expression in rat cerebral microvascular endothelial cells [165]. The authors demonstrated that cells overexpressing P-gp had decreased levels of miR-466b-1-3p compared to normal cells [165]. This finding was corroborated later, when using a recombinant miR-466b-1-3p (mimic) and the correspondent artificial antisense RNA of miR-466b-1-3p: P-gp expression was down-

regulated when the mimic was employed, whereas the use of the antisense RNA of miR-466b-1-3p upregulated P-gp [165].

Down-regulation of ABC transporters, mainly P-gp, resorting to RNA interference had some preliminary positive results. Two experiments using rat astrocyte models overexpressing P-gp, one targeting *mdr1b* coding regions [166] and the the other targeting mdr1b mRNA [167], through RNA interference, attested decreased levels of mdr1b mRNA and P-gp expression, and reduced rhodamine 123 efflux compared to control groups. Chen et al verified these results during the first 7 days after infection, with the maximum down-regulation observed 48 hours after [166]. Both experiments used short hairpin RNA (shRNA) targeting P-gp expression, although through different vectors.

Tian et al also demonstrated reduced mdr1b mRNA levels and P-gp expression and increased intracellular rhodamine 123 fluorescence intensity, using a rat brain microvascular endothelial cell model overexpressing P-gp [168]. The group used adenoviral delivery of small interfering RNA (siRNA) to efficiently target mdr1b [168]. In another study, using multidrug-resistant cancer cells and adenoviral delivery of shRNA against mdr1, it was observed down-regulation of P-gp mRNA and its expression and reversal of the multidrug resistance phenotype [169]. Likewise, intravenous injection of siRNA against P-gp in mouse brain capillary endothelial cells decreased P-gp labelled area in the hippocampal hilus and parietal cortex [170].

The increasing knowledge about the relationship between miRNAs and ABC transporters makes RNA interference a potential method to effectively overcome multidrug resistance. Further studies are required to understand the full impact of RNA interference in cell biology and metabolism, and the clinical relevance of this technique in DRE.

5.3 The role of neuro-inflammation

Neuro-inflammation is known to play a crucial role in epilepsy, causing neurodegeneration, cell death and neurotoxicity during the onset of epilepsy and after intense seizure activity. It is also thought to be a key factor in disease severity and aggravation, and even related to the pathogenesis of seizures [171]. More interestingly, increasing evidence suggests it also regulates efflux transporters expression, specially P-gp, through the glutamate/N-methyl-D-aspartate (NMDA) signalling pathway, following seizure-mediated glutamate release, and possibly as a defence mechanism after brain injury [172]. In fact, this is due to the excessive glutamate release following seizures [173], which is thought to increase P-gp expression though a signalling cascade beginning in the NMDA receptor. This evidence makes the glutamate/NMDA/ cyclooxygenase 2 (COX-2) pathway a viable target to

efficiently manage efflux transporters expression and regulation in the BBB and the epileptogenic focus [174,175] (Figure 1).

Several studies showed that glutamate is responsible for P-gp brain upregulation and that inhibition of its signalling pathway reverses this effect. In a rat pilocarpine model, in which P-gp was upregulated in the capillary endothelial cells of the hippocampus, the administration of MK-801 (NMDA receptor antagonist) prevented P-gp overexpression in comparison to control groups [176]. Similar results were observed in isolated rat or mouse capillaries, in which MK-801 and celecoxib (COX-2 inhibitor) counteracted P-gp upregulation that followed glutamate exposure [177]. The same research group showed that

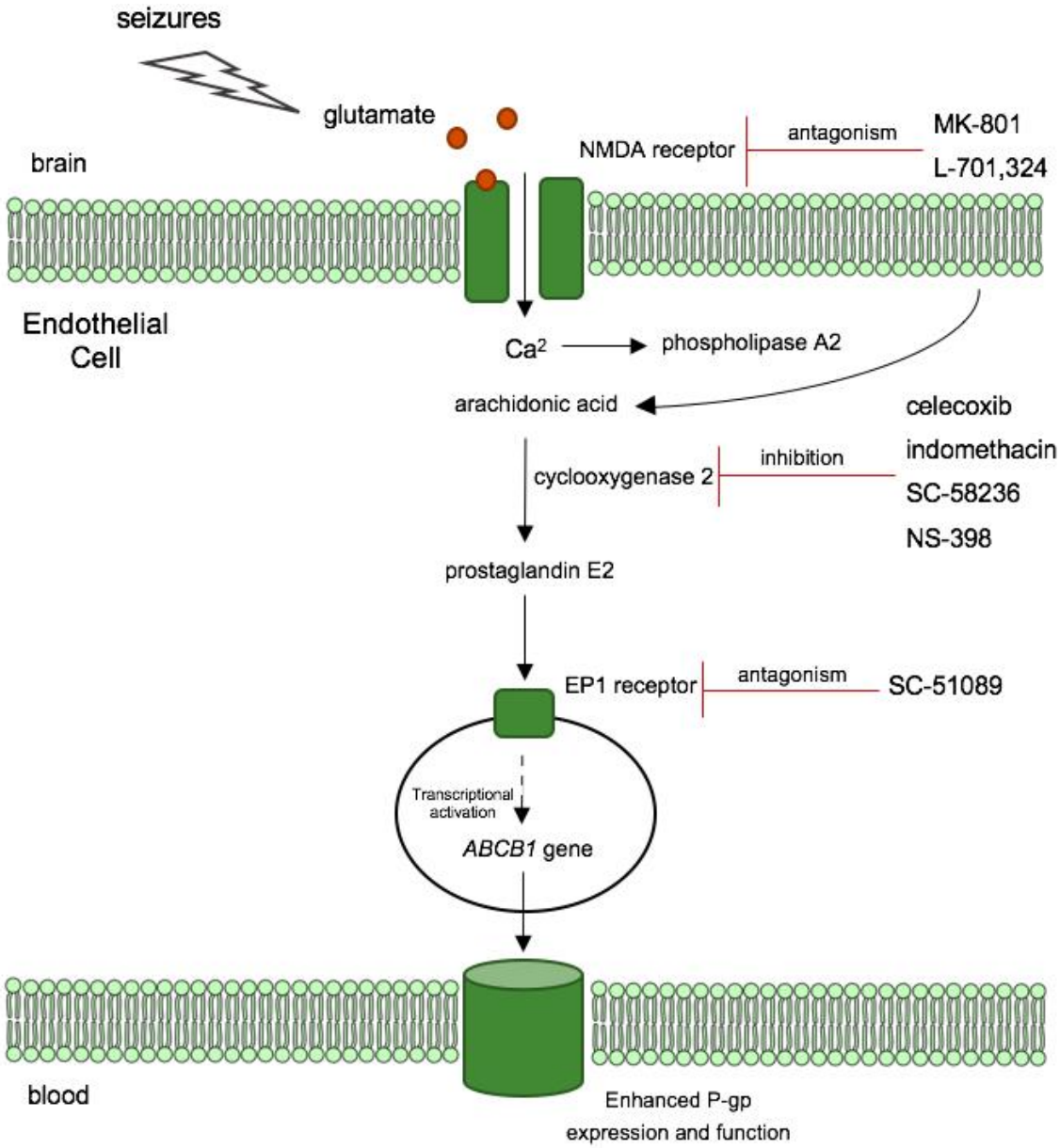

Figure 1: Neuro-inflammation hypothesis. Glutamate release following seizures signals through the NMDA receptor/COX-2/EP1 receptor axis to increase the transcriptional activation of *ABCB1* gene, enhancing P-gp expression and function.

pre-treatment with COX inhibitor indomethacin prevented P-gp upregulation in a pilocarpine SE rat model. Another interesting fact was that in capillaries from COX-2 knockout mice, P-gp remained unaltered following glutamate administration, which in opposition was increased in normal rats [177]. In contrast to COX-2 inhibition, COX-1 inhibitors do not seem to be involved in P-gp regulation [177]. Surprisingly, COX-2 inhibitors only seem to affect P-gp levels when it is upregulated, taking it to basal levels rather than just causing its down-regulation [178]. Zibell et al proved this theory both *in vivo* and *in vitro*, since different COX-2 inhibitors (celecoxib, NS-398 and indomethacin heptyl ester) blocked P-gp upregulation, restoring it to control levels. They also showed that induction of P-gp is due to high COX-2 substrate levels (arachidonic acid) rather than enhanced COX-2 enzyme [178].

In order to further investigate the mechanisms that explain the influence of glutamate on P-gp regulation, the impact of prostaglandin E_2 receptor 1 (EP1) antagonism was exploited through the sub-chronic administration of SC-51089 and the consequent effect on P-gp expression [179]. EP1 inhibition in a rat pilocarpine model, where it has previously been proved that P-gp was overexpressed, retained P-gp at control levels, proving that prostaglandin E2 and EP1 also take part in P-gp regulation. It was also shown that, in the SC-51089 pre-treated group, a reduced dosage of phenobarbital had anticonvulsant effects, compared to the lack of action in the control group [179].

Some studies resort to the blockage of the glutamate/NMDA receptor/COX-2 axis to access if this pathway can effectively increase brain penetration of AEDs. In a rat chronic model of drug-resistant TLE, in which P-gp was overexpressed, a 6-day treatment with celecoxib restored the anticonvulsant effect of phenobarbital in the non-responder group [180]. In another study by Van Vliet et al, using SC-58236 (COX-2 inhibitor) in a 2-week regimen, P-gp expression was kept at basal levels and therefore enhanced brain delivery of phenytoin in chronic epileptic rats was observed [181].

Although these results are positive and might pave the way for a new strategy to increase drug permeability and reduce disease severity, some considerations should be made. As neuro-inflammation is involved in the pathogenesis of epilepsy and its aggravation [171], anti-inflammatory drugs such as COX-2 inhibitors might exert neuroprotective effect and contribute for the reduction of disease severity observed in some studies [180]. Thus, some positive results might not be only caused by increased penetration of AEDs, but also because of the neuroprotective role of some anti-inflammatory drugs.

When considering COX-2 inhibition it is important to note their adverse effects, namely the risk of renal and cardiovascular events. Besides, some studies point out that the

use of COX-2 inhibitors and NMDA antagonists in epilepsy patients can lead to increased disease severity [175,182].

6. Conclusion

The transporter hypothesis is the theory that most thoroughly explains drug resistance in epilepsy patients, although a long road is still ahead to transpose it into clinical practice. As it was herein reviewed, multiple ABC efflux transporters are upregulated in brain areas responsible for epileptogenic activity and seizures, not only on the ECs of the BBB, but also in astrocytes, neurons, microglia and brain parenchymal cells. This overexpression of efflux transporters occurs not only as a result of diverse pathologies that explain the onset of epilepsy, as MCD and even brain tumours, but also as a result of frequent and successive seizures. This means that the more uncontrolled seizures occur, the more prone the patient is to develop resistance to AEDs therapy. Recent studies point out a direct mechanistic link between the high glutamate concentrations released during seizures and the increase expression and function of P-gp in brain epileptogenic areas. Genetic predisposition is also a vastly debated problem, although confounding factors in study design and experimental difficulties still cloud its true impact.

Currently, one of the weakest pillars in the transporter hypothesis concerns the lack of proof that the marketed AEDs are indeed substrates of ABC transporters and that efflux transport is clinically relevant. There is confirmation that a vast number of AEDs are ABC transporters substrates, with phenytoin, phenobarbital and lamotrigine showing some strong evidence. However, as herein emphasized, the lack of evidence does not necessarily mean that AEDs are not substrates of those efflux transporters, considering the methodological difficulties aforementioned and the fact that AEDs might be weak substrates of these transporters. On this context, new imaging techniques like PET will aim to clarify the brain location where transporters are upregulated and which AEDs are effective substrates in live patients.

Different approaches have been employed in order to overcome drug resistance in refractory patients. Among direct transporter inhibition, their regulation using miRNAs or inhibitors of the glutamate/NMDA/COX-2 pathway, all of them are making strong progresses and showing positive results. Direct transporter inhibition showed limited efficacy in cancer clinical trials, although its role in DRE is still under scrutiny. Down-regulation of efflux transporters via the glutamate/NMDA/COX-2 axis seems optimistic since COX-2 inhibitors seem to restore pharmacosensivity and lower P-gp back to its basal

levels, without impairing its normal function. Regulating efflux transporters through RNA interference has still a long road ahead until clinical application since this technology is considerably new.

All in all, experimental evidence is still required to clarify the mechanisms underlying DRE, although incredible steps have been made in the past few years. Moreover, new approaches to evade the action of efflux transporters are making their way to success, even though none have yet reached clinical trials.

7. Annexes

7.1 Annex 1: Evidence of epileptogenic malformations influence on ABC transporters expression

Study/Author	Aetiology	Brain Area sampled	Variable	Sample Size	Method	Findings	
						Overexpressed ABC Transporter	Location
Aronica et al [91]	Hippocampal sclerosis; ganglioglioma	Hippocampus	P-gp, MRP1, MRP2 expression	16 TLE patients; 8 healthy controls	Immunocytochemistry	P-gp	Astrocytes and blood vessels
						MRP1	Glial endfoot processes, blood vessels, neurons
						MRP2	Blood vessels
Dombrowsky et al [90]	Mesial temporal sclerosis; vascular malformation; left hippocampal atrophy	Endothelial cells from temporal lobe blood vessels	P-gp expression	5 TLE patients; 4 controls	cDNA array; Immunocytochemistry; Western Blot	P-gp	ECs from temporal lobe blood vessels
Sisodiya et al [63]	Dysembrioplastic neuroepithelial tumours; focal cortical dysplasia; hippocampal sclerosis	Lesional tissue	P-gp and MRP1 expression	30 refractory epilepsy patients;	Immunocytochemistry	P-gp	Reactive astrocytes and dysplastic neurons
						MRP1	Reactive astrocytes
Aronica et al [92]	Dysembrioplastic neuroepithelial tumours; focal cortical dysplasia; hippocampal sclerosis; oligodendroglioma; astrocytoma; glioblastoma multiforme	Lesional tissue	BCRP expres sion	55 refractory epilepsy patients; 9 healthy control tissues;	Western Blot; Immunocytochemistry	BCRP	ECs of tumour blood vessels
Sisodiya et al [93]	Hippocampal sclerosis; focal cortical dysplasia type IIb	Microvascular endothelium from epileptogenic brain tissue	P-gp, MRP1 and BCRP expression	3 refractory epilepsy patients; 2 healthy controls	Double labelling immunofluorescence	P-gp and BCRP	Microvascular endothelium

Aronica et al [37]	Focal cortical dysplasia; ganglioglioma	Lesional, perilesional and normal brain regions	P-gp and MRP1 expression	30 refractory epilepsy; 8 healthy controls; 10 perilesional disease-control samples; 3 refractory complex partial epilepsy controls	Immunocytochemistry	P-gp and MRP1	Dysplastic neurons and neuronal component
Lazarowski et al [96]	Tuberous sclerosis	Lesional tissue	P-gp expression	1 Tuberous sclerosis	Immunocytochemistry	P-gp 170	Lesional tissue
Dixit et al [98]	MTLE; FCD type I and II	Lesional tissue	MRP1 and BCRP mRNA	16 mesial TLE; 12 FCD; 6 tumour periphery controls; 4 healthy controls	PCR	BCRP	Lesional tissue
Ak et al [39]	FCD	Lesional tissue	MDR1 and MRP1 expression	28 FCD; 10 healthy controls	Immunocytochemistry	P-gp and MRP1	Neurons, reactive astrocytes and ECs
Boer et al [97]	Tuberous sclerosis complex	Lesional tissue	P-gp distribution	1 tuberous sclerosis complex;	Immunocytochemistry	P-gp	Intralesional cell-specific distribution
Lazarowski et al [38]	Tuberous sclerosis	Cortical tuber brain tissue	MDR1 and MRP1 expression	3 refractory epilepsy	Immunocytochemistry	MDR1 and MRP1	Ballon cells, dysplastic neurons, astrocytes, microglia and blood vessels
Lazarowski et al [144]			BCRP expression			BCRP	Vascular ECs
Shin et al [105]	-	-	(R)-[11C]verapamil kinetics	5 drug-resistant epilepsy; 5 drug sensitive epilepsy; 8 healthy controls	PET Scan	P-gp	Significantly different asymmetry indices between drug-resistant and drug-sensitive patients
Feldmann et al [94]	Unilateral hippocampal sclerosis	-	(R)-[11C]verapamil kinetics after tariquidar inhibition	14 drug-resistant; 8 seizure free patients; 13 healthy controls	PET scan	P-gp	Ipsilateral amygdala; bilateral parahippocampus; fusiform gyrus; inferior temporal gyrus; middle temporal gyrus

7.2 Annex 2: Evidence of seizure-mediated upregulation of ABC transporters

Study/Author	Study model	Variable	Method	Findings		
				Overall Result	Location	Comment
Loscher et al [89]	Rat model of spontaneous recurrent seizures after SE induction by prolonged electrical stimulation	Response to phenobarbital treatment	Electroencephalography	Phenobarbital non-responders with higher seizure frequency than responders	-	Seizure frequency is a dominant risk factor for refractoriness
Bartmann et al [113]	Rat model of spontaneous recurrent seizures after SE induction by prolonged electrical stimulation	[18F]-MPPF kinetics after tariquidar inhibition	PET scan	P-gp overexpression	-	Increased [18F]-MPPF brain distribution after tariquidar treatment in phenobarbital non-responders
Van Vliet et al [109]	Rat model of spontaneous recurrent seizures after SE induction by prolonged electrical stimulation	mdr1a and mdr1b expression; P-gp expression	RT PCR; P-gp immunostaining	mdr1a mRNA upregulation	-	Increased 1 week after SE (dentate gyrus)
				mdr1b mRNA upregulation	-	Increased 1 week after SE (dentate gyrus) and chronically (parahippocampal cortex)
				P-gp overexpression	Glia like cells in close apposition to blood vessels; blood vessels	Chronic and SE-dependent
Potschka et al [110]	Rat amygdala-kindling model by electrical stimulation	P-gp expression (between phenytoin responsive and non-responsive rats)	Immunohistochemistry	P-gp overexpression	Capillary endothelial cells of the amygdala	Overexpression associated to phenytoin non-responders
Volk and Loscher [111]	Rat model of spontaneous recurrent seizures after SE induction by prolonged electrical stimulation	P-gp expression (between phenobarbital responsive and non-responsive rats)	Immunohistochemistry	P-gp overexpression	Brain capillary endothelial cells of the hippocampus, dentate gyrus and piriform cortex	Overexpression associated to phenobarbital non-responders

Yao et al [115]	Pentylenetetrazole-kindled rats	MRP2 expression, bromosulfophthalein (MRP2 substrate) and phenytoin distribution (with and without probenecid)	Western Blot	MRP2 overexpression	Hippocampus and cerebral cortex	-
			Liquid chromatography – mass spectrometry	bromosulfophthalein decreased distribution	Hippocampus and cerebral cortex	Decreased brain distribution in kindled rats, reversed by probenecid
			HPLC-UV	Phenytoin decreased distribution	Hippocampus and cerebral cortex	Decreased brain distribution in kindled rats, reversed by probenecid
Volk et al [108]	Rat model of SE induced by pilocarpine or kainate	mdr1a, mdr1b and P-gp expression	Immunohistochemistry	P-gp overexpression	ECs and neurons of the hippocampus and dentate gyrus	24h after SE
			RT-PCR	mdr1a and mdr1b upregulation	Hippocampus neurons	-
Van Vliet et al [183]	Rat model (after electrically induced SE)	MRP1, MRP2 and BCRP expression	Immunocytochemistry; Western Blot	MRP1, MRP2 and BCRP overexpression	Astrocytes in close apposition to blood vessels and blood vessels (limbic structures)	-
			HPLC	phenytoin decreased distribution	-	Decreased distribution in chronic epileptic rats, reversed by probenecid
Rizzi et al [59]	Rat model of kainic acid-induced limbic seizures	Mdr mRNA, phenytoin and carbamazepine distribution	RT-PCR	mdr mRNA upregulation	Hippocampus	3-24h after kainic acid induction
			HPLC	phenytoin and carbamazepine decreased distribution	-	Decreased brain distribution associated with seizure activity
Volk et al [107]	Rat amygdala-kindling model by electrical stimulation	P-gp expression	Immunohistochemistry	P-gp overexpression	Dentate gyrus, hippocampus and cortex	-
Marchi et al [99]	Methylazoxymethanol acetate-treated rats	P-gp expression	Immunohistochemistry; Double immunostaining; Western blot	P-gp overexpression	Vessels and perivascular/parenchymal astrocytes (hippocampus)	-
Kwan et al [106]	Genetically epilepsy-prone rat brain after a single audiogenic seizure	mdr1a and mdr1b expression	-	mdr1a overexpression	Cortex and midbrain	24h-7days after a single audiogenic seizure

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