Biomarkers in epilepsy - a modelling perspective

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Review

Biomarkers in epilepsy–A modelling perspective

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ABSTRACT

Biomarkers can be categorised from type 0 (genotype or phenotype), through 6 (clinical scales), each level representing a part of the processes involved in the biological system and drug treatment. This classification facilitates the identification and connection of information required to fully (mathematically) model a disease and its treatment using integrated information from biomarkers. Two recent reviews thoroughly discussed the current status and development of biomarkers for epilepsy, but a path towards the integration of such biomarkers for the personalisation of anti-epileptic drug treatment is lacking. Here we aim to 1) briefly categorise the available epilepsy biomarkers and identify gaps, and 2) provide a modelling perspective on approaches to fill such gaps. There is mainly a lack of biomarker types 2 (target occupancy) and 3 (target activation). Current literature typically focuses on qualitative biomarkers for diagnosis and prediction of treatment response or failure, leaving a need for biomarkers that help to quantitatively understand the overall system to explain and predict differences in disease and treatment outcome. Due to the complexity of epilepsy, filling the biomarker gaps will require collaboration and expertise from the fields of systems biology and systems pharmacology.

1. Introduction

A large unmet medical need exists for the 50 million people with epilepsy worldwide, as up to 30% of them are not satisfactorily treated (Ngugi et al., 2010; Sillanpää and Schmidt, 2006). A major cause of dissatisfactory anti-epileptic drug (AED) treatment outcome is large inter- and intra-individual variability in pharmacokinetics (PK), pharmacodynamics (PD), and pathophysiology (van Dijkman et al., 2016). To better understand and explain such variability, biomarkers are needed. For epilepsy, biomarkers have been defined as "*an objectively measurable characteristic of a biological process that reliably identifies the development, presence, severity, progression, or localization of an epileptogenic abnormality*" which would suggest only a minor focus on the biological features of a treatment effect (Pitkänen and Engel, 2014). Two recent reviews provided an overview of the available epilepsy biomarkers (Pitkänen et al., 2016; Walker et al., 2016). Pitkänen et al. stated that "*Biomarker discovery could be regarded as an advance in the personalisation of medicine, and could allow prevention in the right person at the right time, rather than just symptomatic treatment*". However, biomarker data alone will not automatically lead to personalised medicine. PK, PD and disease models, in conjunction with individual patient data, are required to estimate patient-specific parameters and make subsequent treatment choices (Knibbe and Danhof, 2011; Standing, 2016). Furthermore, the use of biomarkers can result in a

more effective and structured drug development process (Cohen et al., 2015).

A classification system exists that categorises biomarkers with regard to their place in modelling PK, PD and disease processes (Danhof et al., 2005), in terms of cascading types; type 0 (genotype or phenotype), type 1 (drug concentration), type 2 (target occupancy), type 3 (target activation), type 4 (physiological response), type 5 (pathophysiological response), and type 6 (clinical scales). Each type represents part of the interactions between (patho-) physiology and treatment, which, if adequately described, would allow for the prediction of *in vivo* drug effect based on intermediary biomarkers (Fig. 1.). Categorisation of the available epilepsy biomarkers according to this system may aid in the identification of biomarker gaps, and help to determine a way forward in the use of epilepsy biomarkers for personalised medicine and drug development. The aims of this review are therefore to 1) briefly categorise the available epilepsy biomarkers and identify gaps, and 2) provide a modelling perspective on approaches to fill such gaps.

2. Available Biomarkers

2.1. Type 0 – Genotype or Phenotype

Many genes have been discovered that increase the risk of devel-

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Fig. 1. Diagram of a "cascading" PK/PD model for the prediction of *in vivo* drug effects based on intermediary biomarker responses. Reproduced with permission from (Danhof et al., 2005).

oping epileptic seizures or alter the chance of responding to drug therapy (Rossignol et al., 2014; Myers and Mefford, 2015; Pitkänen et al., 2016; Walker et al., 2016). These include the genes that encode for Na + channels, Ca^2 + channels, K + channels, and the nicotine-acetyl-choline- and GABA$_A$ receptors. Some examples of such genetic markers are genetic differences in serotonergic transmission related to the occurrence of temporal lobe epilepsy and epileptogenesis following traumatic brain injury with subsequent changes in GABAergic expression. Epileptic seizures are based on excessive synchronised neuronal excitations, and thus it comes to no surprise that genes related to either increased excitation or decreased inhibition are implicated in seizure susceptibility. Neuro-inflammation is considered an important factor in epileptogenesis after traumatic brain injury or stroke. Genetic markers for CD40, a costimulatory protein found on antigen presenting cells that is required for their activation, interleukins, and oxidative stress seem to support this. Most likely these genes are not single contributors to risk, but part of a large network of interacting genes, some innocuous on their own, and other factors eventually leading to epileptogenesis.

MicroRNA, or miRNA, are small non-coding RNA molecules involved in post-transcriptional gene regulation and gene silencing. Some of these miRNA circulate through the body and thereby provide a bridge between genetic expression, either normal expression of mutated genes, or abnormal expression of otherwise normal genes, and clinical presentation. Two miRNAs, hsa-miR-487a and hsa-miR-9a-3p, have been found to correlate to neuro-inflammation and epileptogenesis, which perhaps one day could allow clinicians to determine if immuno-suppression for the prevention of epileptogenesis is indicated in their patient (Zucchini et al., 2014; Roncon et al., 2015). The miRNA hsa-miR-106b-5p, part of an miRNA family implicated in cell proliferation, was found to diagnose idiopathic generalised epilepsy at a sensitivity and specificity of 80% and 81% respectively (Wang et al., 2015).

2.2. Type 1 – Drug Concentration

For infectious diseases, therapeutic drug monitoring (TDM) of antibiotic plasma concentrations is commonly used to assure sufficient drug exposure; plasma concentrations are considered representative for the antibiotic target site. In AED therapy, plasma AED concentrations are theoretically simple biomarkers, yet much controversy exists regarding its use in clinical practice. Objections to plasma PK optimisation of AED therapy include the lack of a clear correlation between plasma concentrations and AED therapy outcome in the individual patient in the clinic, the burden on the patient, and the cost of sampling. While target site exposure is undeniably the main driving force behind efficacy, pharmacokinetic variability obscures the correlation between dose, plasma concentration and target site concentration, and thereby efficacy. Yet, plasma exposure has been shown to correlate to efficacy for a number of AEDs (Nakashima et al., 2015; Ogusu et al., 2014; Delattre et al., 2012; Van Den Broek et al., 2012; Girgis et al., 2010), suggesting that clinical titration to a certain efficacious level of systemic exposure may still be optimised by TDM (van Dijkman et al., 2017b), especially in the face of polytherapy (van Dijkman et al., 2017a).

Few studies have investigated the correlation between plasma and cerebrospinal fluid (CSF) or target site AED concentrations in humans. In 22 pharmacoresistant patients undergoing epileptic focus resection, large differences in AED concentrations were found between plasma, medial temporal gyrus dialysate, and excised tissue (Rambeck et al., 2006). However, plasma and CSF concentrations of topiramate were

highly correlated in a group of 14 regular, non-pharmacoresistant patients (Christensen et al., 2001). Finally, one study showed parallel profiles of valproic acid concentrations in plasma, CSF, and extracellular fluid (ECF), albeit not at the same concentration level (Lindberger et al., 2001). These three studies suggest that regular patients' AED concentrations in CSF, and possibly at the target site, can be seen as a simple ratio of plasma concentrations, whereas CSF concentrations in non-responding patients may not show the same correlation, requiring more elaborate methods for determining target site exposure. For the moment, evidence is too limited for it to be used in clinical decision making, or in drug development to determine the source of inadequate response.

As an alternative to CSF sampling, total or local brain AED concentrations could be measured by positron emission tomography (PET) (Kim et al., 2010; Syvänen et al., 2013). Such an intensive PK analysis and optimisation is not indicated in the patient that responds well to initial drug titration, but may still be indicated for refractory or non-responding patients to differentiate between failure to reach adequate target site exposure and failure to respond pharmacodynamically. Inadequate target site exposure could be adjusted for by dose adjustments or blood-brain barrier (BBB) manipulation, for example by Pgp inhibition (Clinckers et al., 2005), whereas pharmacodynamic failure would require switching to another AED or add-on therapy. Although an intensive PK workup (*i.e.* determining an individual's systemic, and possibly cerebral concentrations) incurs extra costs and burden, it should be offset against the costs of hospital admissions, missed work days, and other adverse results from seizures or severe side effects in non-responding patients. In well-responding patients, it is still highly recommended to measure drug plasma concentrations, to be able to differentiate between inadequate exposure due to changes in PK, the development of pharmacoresistance, or non-compliance in patients that becomes treatment resistant at a later time (Patsalos et al., 2008). Some of the burden of plasma sampling may be preventable by substituting it with the newer, lesser invasive techniques like dried blood spot and saliva sampling, which have seen some increased interest in recent years (Milosheska et al., 2015; Patsalos and Berry, 2013), but would add another source of variability to the analysis.

2.3. Type 2 – Target Occupancy & Type 3 – Target Activation

Target occupancy is a major determinant in drug efficacy, but few suitable biomarkers are available. *In vitro* binding kinetics, investigated for many AEDs, are not directly translatable to *in vivo*. PET occupancy studies can be performed to investigate target occupancy *in vivo*, but it requires at least 12 research subjects, and two PET scans per individual (Zhang and Fox, 2012), making it impractical for AED therapy personalisation in the clinic and very costly for drug development. Further complicating binding kinetics, it has been proposed that epilepsy may structurally or functionally modify target sites, for which no biomarker is currently available (Beck, 2007).

Once a target has been occupied, it should translate into an activation of the cellular processes that are involved in the treatment effect. Important to some of these processes is the second messenger cAMP, which is central to the phosphorylation of ion channels (Misonou et al., 2004), but is also implicated in epileptogenesis (Zhu et al., 2012). As these processes take place inside the cell, measuring them is highly invasive in *in vivo* situations. Alternatively, products that are a direct result of activation and readily cross the BBB, such as

miRNA's, could be measured peripherally. For example, miRNA has-miR-301a-3p was found to correlate to AED resistance in a study of drug-resistant patients, drug-responsive patients, and healthy controls, which may imply its down-stream role after target activation (Wang et al., 2015). As their patients were already drug-resistant, it cannot be determined if this miRNA was part of the cause or effect of drug-resistance. Some evidence exists for the so-called use-dependent blocking effects of AEDs, resulting in a higher AED effect at higher levels of neuronal activity (Beck, 2007), which may be quantified by an electro-encephalogram (EEG), hot glucose PET, or functional magnetic resonance imaging (fMRI).

2.4. Type 4 – Physiological Response & Type 5 – Pathophysiological Response

Neuro-inflammation plays a role in epileptogenesis and is a result of seizure activity, making biomarkers that can detect it at an early stage highly valuable in both prevention and treatment. PET can visualise neuro-inflammation by targeting the peripheral benzodiazepine receptor (PBR), otherwise known as translocator protein 18 kDa (TSPO), expressed in many parts of the body, amongst which microglia. In the post-status epilepticus model TSPO peaks around 2 weeks after the initial status epilepticus (Galovic and Koepp, 2016; Bogdanović et al., 2014). TSPO binding is also increased in foci of pharmaco-resistant patients with focal-onset epilepsy (Gershen et al., 2015). So far, no quantitative data is available to support setting threshold levels of neuro-inflammation above which anti-inflammatory treatment is indicated. Some miRNAs may be involved in neuro-inflammation by regulating interleukin 1β, cell adhesion molecules, and neuronal growth (Henshall et al., 2016). How well these miRNAs monitor response in terms of neuro-inflammation (and subsequent neuronal damage) in the clinical setting is as of yet unclear and needs to be further investigated.

Cerebral micro damage due to seizure activity may contribute to disease progression, either due to kindling (*i.e.* seizures initiate a pathological process of inflammation and dysregulated neuronal growth leading to sustained seizures) or worsening of existing patho-physiology. fMRI characterises brain region activity, active seizure foci and/or inflammation (Olszewska and Costello, 2014). It can also be coupled to EEG to increase its accuracy of locating epileptic foci and determining the involvement of different brain structures (Gotman and Pittau, 2011). Diffusion tensor imaging (DTI) traces nerve fibres, thereby illustrating structural connectivity and hierarchical structure, which may be affected both as a cause and as a result of epilepsy (Gong et al., 2009; Iturria-Medina et al., 2008). BBB disruption occurs during and after seizures, which has a significant impact on cerebral AED concentrations. Dynamic contrast enhanced MRI is able to quantify this BBB disruption, it can track epileptogenesis, and may one day help the physician to determine dose adjustments when a change in cerebral AED exposure is due to changes in BBB permeability or transport.

Brainwaves on an EEG are a direct result of micro processes that are otherwise difficult to measure, allowing insight in the underlying physiology. Standard EEG is non-invasive, does not present a large burden to the patient, and is inexpensive in terms of equipment or application. Interpretation of EEG signals, however, can be time consuming and requires a trained professional with expert knowledge, although advances in machine-based EEG analysis and interpretation are slowly eliminating these issues. A limitation of the use of EEG in the clinic is the reliance on EEG characteristics that were discovered when EEGs could only be analysed with the naked eye, such as high-frequency oscillations, focal slowing, spindles, *etc.*, while these characteristics are not very sensitive to drug effect. Slow adoption of pharmaco-EEG (PEEG) has been attributed to its unreliable translational accuracy, a lack of standardised operating procedures (SOPs), and large inter-individual variability (Jobert et al., 2013). Methodologies more sensitive to microphysiology and treatment effect are being

developed, such as beta wave spectral power correlations with GABA-ergic inhibition in AED use (Lopes da Silva, 2002).

2.5. Type 6 – Clinical Response

The definition of response to AED treatment is ill-defined and may differ between patient, physician, and clinical researcher. Typically, clinical response is a reduction in seizures, but patient and physician may disagree on what would qualify as a satisfactory reduction, and the impact of side effects may be underestimated, possibly leading to poor adherence (Smithson et al., 2012). In clinical trials, treatment success is defined as a reduction of at least 50% in seizure frequency between baseline and end of the study. This binarisation and the reduction of data leads to a large information loss, and unnecessarily complicates the characterisation of correlations between AED dose, exposure, and response, especially given the large variability between and within individuals (van Dijkman et al., 2016).

3. Modelling Perspective

There is a simple adage that states: *to measure is to know*. Quantification of the intricate mechanisms involved in the disease and its therapy is required to allow optimal prevention, prognosis and treatment. Hence, single biomarkers will not suffice to make informed decisions in the clinic, nor in drug development. Instead, models that integrate biomarkers across the entire range of types (Fig. 1) are needed. In systems biology and systems pharmacology, problems are considered as part of a network of interactions and complex mechanisms. Systems biology of epilepsy entails the quantification of physiological and pathological processes, roughly corresponding to biomarkers types 0, 4 and 5. Systems pharmacology allows the quantification of physiology-based (PB/)PK/PD, roughly corresponding to biomarker types 1, 2, 3, and 6 (Danhof, 2016). Fig. 2, adapted from Sheiner (Sheiner, 1997), depicts how a physician would determine the individual patient's optimal treatment based on the dimensions of a treatment regimen, its benefits, and the patient's prognostic factors. Whereas this figure may adequately represent diseases with relatively few dimensions, the number of dimensions in epilepsy treatment grows to levels that can no longer be handled without the aid of computer algorithms. Personalised medicine in epilepsy will ultimately require the combination of models from systems biology and systems pharmacology, leading to systems medicine. Epilepsy biomarker literature generally focuses on *qualitative* biomarkers that categorise patients for diagnosis and prognosis, while systems medicine needs *quantitative* biomarkers that aid in understanding the systems involved in disease and treatment mechanisms. Using the biomarker classification system, gaps in biomarkers were identified with regard to target occupancy and target activation (Table 1, Fig. 3). Such biomarkers characterise how AED
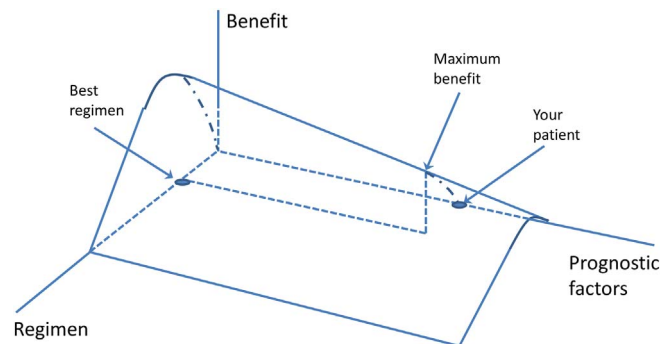

Fig. 2. Example of a simple therapeutic response surface, relating patient prognostic factors and dose regimen to benefit, as net utility of efficacy and toxicity. The best regimen for your patient corresponds to the maximum of the surface on the benefit axis. Adapted with permission from (Sheiner, 1991).

Table 1
Currently available biomarkers in epilepsy research and development, categorised per type.

Gaps exist in all types, but especially types 2 and 3 are underrepresented. Development of current biomarkers is aimed at those biomarkers that translate directly into a factor of clinical response (*i.e.* reduction in seizures, prediction of non-responders) while disregarding the intermediate steps with regard to pharmacology. PK/PD modelling methodologies that help improve or supplant biomarkers in that area are shown in brackets.

Type 0 genotype or phenotype	Type 1 drug concentration	Type 2 target occupancy	Type 3 target activation	Type 4 physio-logical response	Type 5 patho-physio-logical response	Type 6 clinical response
$Na+$, Ca^2+, $K+$, nicotine-acetyl-choline, $GABA_A$, serotonin receptors	Blood sampling CSF sampling	[Translational modelling of binding kinetics]	cAMP miRNA	fMRI DWI DTI	fMRI DWI DTI PET	Seizure count, seizures *vs* no seizures
CD40 interleukins oxidative stress	[(PB)PK modelling]	PET	PEEG	PEEG	PEEG	Responder *vs* non-responder
miRNA	PET					Side effect measures

$Na+$ – Sodium channel. Ca^2+ – Calcium channel. $K+$ – Potassium channel. GABA – gamma-aminobutyric acid channel. CD40 – cluster of differentiation 40. miRNA – microRNA. PEEG – pharmaco-electro-encephalogram. CSF – cerebrospinal fluid. PET – positron emission tomography. cAMP – cyclic adenosine monophosphate. fMRI – functional magnetic resonance imaging. DWI – diffusion weighted imaging. DTI – diffusion tensor imaging. *vs* – versus.
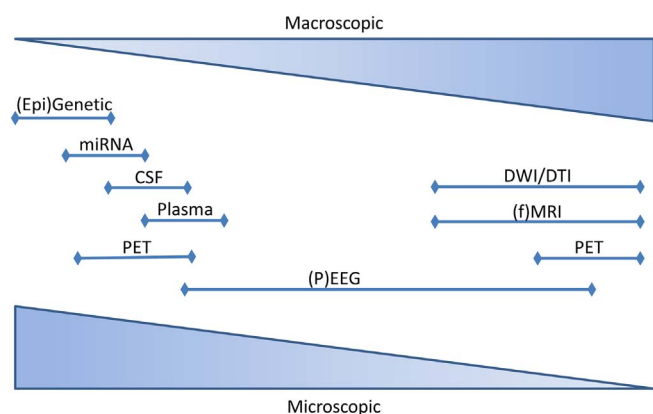

Fig. 3. A diagram of the scale span of the main discussed biomarkers. A large gap exists between the microscopic and macroscopic scale, where only pharmaco-EEG and modelling methodologies may provide a bridge.

exposure at or inside the neuron translates to changes in (patho-) physiology, and thus are essential to determine the cause of pharmacoresistance. Although biomarkers were found that relate to epileptogenesis and prognosis, these did not provide quantitative data allowing the modelling of disease predisposition, generation, and progression. In the following sections we present modelling approaches that may augment the use of existing biomarkers, or reduce the need for biomarkers.

3.1. From Dose to Target Site Concentrations

Covariates such as weight, age, sex, comedication, drug binding and cytochrome P450 polymorphisms strongly influence dose to plasma PK and can be pre-emptively adjusted for using PK models, resulting in a more personalised medicine compared to current weight-based dosing algorithms (Knibbe and Danhof, 2011). A greater challenge is to relate concentrations in plasma to those available for interaction at the target site. Much progress has been made on the PBPK modelling of concentrations in plasma and local brain areas (Westerhout et al., 2012; de Lange and Hammarlund-Udenaes, 2015; Yamamoto et al., 2016), based on the Mastermind Approach (Fig. 4) (de Lange, 2013). These models, developed on preclinical data, have been shown, as proof-of-concept, to translate well to humans. Where information was available on human drug concentrations in particular brain compartments (such as lateral ventricle CSF, lumbar spine region, or brain extracellular fluid) this could be adequately predicted by the generic PBPK model after translation of the rat physiological parameter values to the human values. This could replace the need for large invasive

studies in humans, although at this stage, it may still require lumbar punctures to measure drug concentrations in CSF for increased accuracy for the individual patient.

3.2. From Target Site Concentrations to Target Occupancy & Activation

PET occupancy studies in animals advance the understanding of binding kinetics, which can be translated to humans, assuming that the involved receptor is similar. Such translational approaches led to the discovery of brivaracetam, based on knowledge of SV2A binding kinetics of levetiracetam (Kaminski et al., 2012). Performing a PET in each patient is infeasible, even if it is restricted to only refractory or pharmacoresistant patients. Instead, transgenic animals with human (mutated) transporter genes could be used to explore how *in vitro* binding kinetics translate to those *in vivo*. With sufficient studies, models could be constructed that describe these binding kinetics based on receptor theory (Ploeger et al., 2009). A promising alternative is the use of PET studies to investigate receptor density and affinity directly (Syvänen et al., 2011). Binding kinetics either from translational studies or from human studies may then be used in the clinic to predict target occupancy (with subsequent target activation and efficacy) based on the individual's genetic profile, combined with PET to ensure adequate total brain AED exposure. *In vitro* or *in vivo* data on receptor density and affinity can be used to inform models on the relation between *in vivo* binding kinetics, target occupancy and effect profiles (de Witte et al., 2015).

3.3. Epileptogenesis, Ictogenesis & Clinical Outcome

Advances such as next-generation sequencing, epigenetic profiling and proteomics are leading to improved understanding of epileptogenic and ictogenic factors (Rossignol et al., 2014). Systems biology models based on such knowledge may one day aid in the personalisation of AED therapy by predicting which AED will best interact with or prevent pathological mechanisms (Loeb, 2011). On a larger scale, modelling of the inhibitory and excitatory processes in the cortex show how their balance breaks down during seizures (Dehghani et al., 2016). Some neural mass and EEG models are able to describe AED side effects (Vlooswijk et al., 2011), inter-ictal activity (Caballero-Gaudes et al., 2013), and seizure activity (Helling et al., 2015). Due to the increase in understanding of physiology and EEG, PEEG-based personalised medicine may become a reality in the near future (Swatzyna et al., 2015), and may also prevent AED-related neurotoxicity (Salinsky et al., 2002).

To avoid the binarisation of treatment effect, seizure count models connect an underlying parameter to efficacy (Plan, 2014). Recently, the use of stochastic differential equations was proposed to model intra-individual variability in this parameter, allowing further exploration of
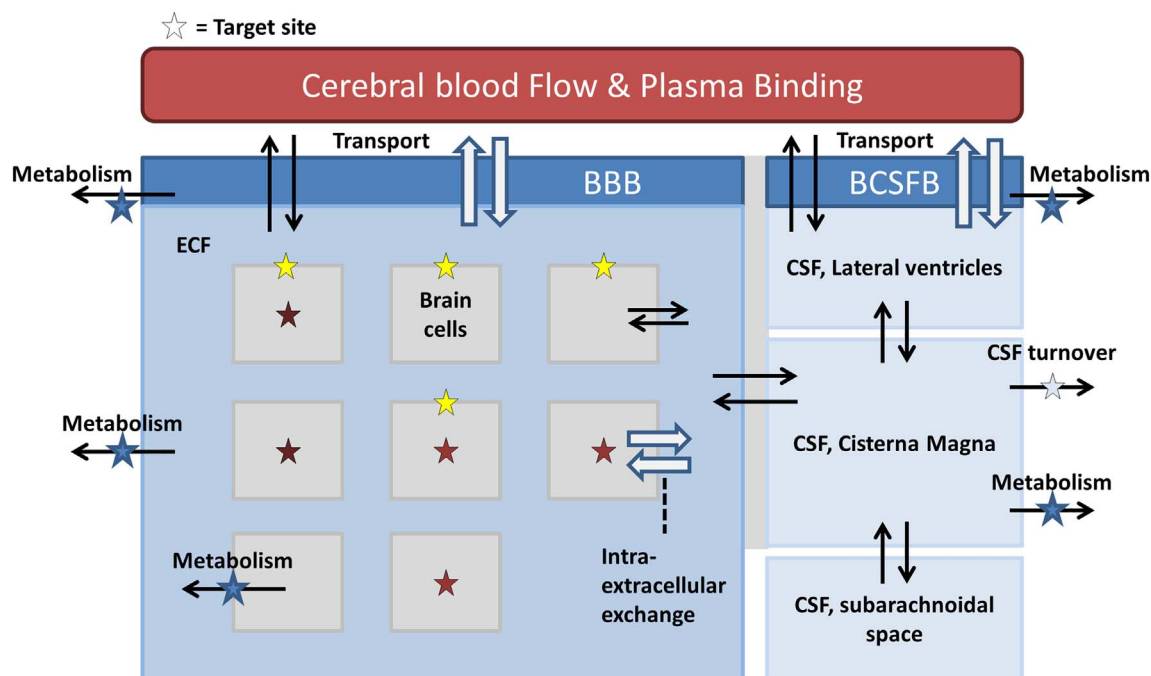
Fig. 4. Schematic presentation of the major compartments of the mammalian brain and routes for drug exchange; extracellular fluid (ECF), brain cells, lateral ventricular CSF, cisterna magna CSF and lumbar CSF, passive transport (black arrows) and active transport (white arrows), as well as metabolism and CSF turnover. Drug targets may be present at different sites within the brain (stars). The blood brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB) limit direct diffusion of molecules from the blood into the brain. Reproduced with permission from (de Lange, 2013).

trends in disease progression (Deng et al., 2016). The lack in the modelling of AED side effects needs to be addressed, as they have a large impact on treatment cessation and non-adherence. Ideally, models will become available that correlate exposure to both response and side effects, which -as part of a benefit-risk balance- allow patient and physician to make the most informed decision (Bellanti et al., 2015).

4. Conclusions

We categorised the available epilepsy biomarkers using a biomarker classification system (Danhof et al., 2005), and identified gaps (Table 1). Biomarkers are lacking which provide more overlap between each other in scale (Fig. 3). Subsequently, we provided examples of advances in systems biology and systems pharmacology that can make better use of -, or even supplant biomarkers. Some of the most pertinent issues in the field of epilepsy pharmacotherapy are: being able to target specific mutations or receptors, to predict disease progression, and to achieve synergy by combining specific AEDs. For this to be achieved, more quantitative biomarker models are needed.

Acknowledgements

References

Beck, H., 2007. Plasticity of antiepileptic drug targets. Epilepsia 48 (Suppl. 1), 14–18.
Bellanti, F., van Wijk, R.C., Danhof, M., Della Pasqua, O., 2015. Integration of PKPD relationships into benefit-risk analysis. Br. J. Clin. Pharmacol. 80 (5), 979–991.
Bogdanović, R.M., Syvänen, S., Michler, C., Russmann, V., Eriksson, J., Windhorst, A.D., Lammertsma, A.A., De Lange, E.C., Voskuyl, R.A., Potschka, H., 2014. (R)-[11C] PK11195 brain uptake as a biomarker of inflammation and antiepileptic drug resistance: evaluation in a rat epilepsy model. Neuropharmacology 85, 104–112.
Caballero-Gaudes, C., Van de Ville, D., Grouiller, F., Thornton, R., Lemieux, L., Seeck, M., Lazeyras, F., Vulliemoz, S., 2013. Mapping interictal epileptic discharges using mutual information between concurrent EEG and fMRI. NeuroImage 68, 248–262.
Christensen, J., Højskov, C.S., Dam, M., Poulsen, J.H., 2001. Plasma concentration of topiramate correlates with cerebrospinal fluid concentration. Ther. Drug Monit. 23

(5), 529–535.
Clinckers, R., Smolders, I., Meurs, A., Ebinger, G., Michotte, Y., 2005. Quantitative in vivo microdialysis study on the influence of multidrug transporters on the blood-brain barrier passage of oxcarbazepine: concomitant use of hippocampal monoamines as pharmacodynamic markers for the anticonvulsant activity. J. Pharmacol. Exp. Ther. 314 (2), 725–731.
Cohen, A.F., Burggraaf, J., Van Gerven, J.M.A., Moerland, M., Groeneveld, G.J., 2015. The use of biomarkers in human pharmacology (phase I) studies. Annu. Rev. Pharmacol. Toxicol. 55 (September), 55–74.
Danhof, M., 2016. Systems pharmacology–towards the modeling of network interactions. Eur. J. Pharm. Sci. 94, 4–14.
Danhof, M., Alvan, G., Dahl, S.G., Kuhlmann, J., Paintaud, G., 2005. Mechanism-based pharmacokinetic-pharmacodynamic modeling-a new classification of biomarkers. Pharm. Res. 22 (9), 1432–1437.
Dehghani, N., Peyrache, A., Telenczuk, B., Van, Quyen, Le, M., Halgren, E., Cash, S.S., Hatsopoulos, N.G., Destexhe, A., 2016. Dynamic balance of excitation and inhibition in human and monkey neocortex. Sci. Report. 6 (March 16).
Delattre, M., Savic, R.M., Miller, R., Karlsson, M.O., Lavielle, M., 2012. Analysis of exposure-response of CI-945 in patients with epilepsy: application of novel mixed hidden Markov modeling methodology. J. Pharmacokinet. Pharmacodyn. 39 (3), 263–271.
Deng, C., Plan, E.L., Karlsson, M.O., 2016. Approaches for modeling within subject variability in pharmacometric count data analysis: dynamic inter-occasion variability and stochastic differential equations. J. Pharmacokinet. Pharmacodyn. 43 (3), 305–314.
van Dijkman, S.C., Alvarez-Jimenez, R., Danhof, M., Della Pasqua, O., 2016. Pharmacotherapy in pediatric epilepsy: from trial and error to rational drug and dose selection–a long way to go. Expert Opin. Drug Metab. Toxicol. 12 (10), 1143–1156.
van Dijkman, S.C., Rauwé, W.M., Danhof, M., Della Pasqua, O., 2017a. Pharmacokinetic interactions and dosing rationale for antiepileptic drugs in adults and children. Br. J. Clin. Pharmacol (In press).
van Dijkman, S.C., Wicha, S.G., Danhof, M., Della Pasqua, O., 2017b. Individualised dosing algorithms and therapeutic monitoring for antiepileptic drugs. Clin. Pharmacol. Ther (In press).
Galovic, M., Koepp, M., 2016. Advances of molecular imaging in epilepsy. Curr. Neurol. Neurosci. Rep. 16 (6).
Gershen, L.P., Zanotti-Fregonara, P., Dustin, I.H., Liow, J.S., Hirvonen, J., Kreisl, W.C., Jenko, K.J., Inati, S.K., Fujita, M., Morse, C.L., Brouwer, C., Hong, J.S., Pike, V.W., Zoghbi, S.S., Innis, R.B., Theodore, W.H., 2015. Neuroinflammation in temporal lobe epilepsy measured using positron emission tomographic imaging of translocator protein. JAMA Neurol. 72 (8), 882–888.
Girgis, I.G., Nandy, P., Nye, J.S., Ford, L., Mohanty, S., Wang, S., Ochalski, S., Eerdekens, M., Cox, E.E., 2010. Pharmacokinetic-pharmacodynamic assessment of topiramate dosing regimens for children with epilepsy 2 to 10 years of age. Epilepsia 51 (10), 1954–1962.
Gong, G., He, Y., Concha, L., Lebel, C., Gross, D.W., Evans, A.C., Beaulieu, C., 2009. Mapping anatomical connectivity patterns of human cerebral cortex using in vivo diffusion tensor imaging tractography. Cereb. Cortex 19 (3), 524–536.

Gotman, J., Pittau, F., 2011. Combining EEG and fMRI in the study of epileptic discharges. Epilepsia 52 (Suppl. 4), 38–42.

Helling, R.M., Koppert, M.M.J., Visser, G.H., Kalitzin, S.N., 2015. Gap junctions as common cause of high-frequency oscillations and epileptic seizures in a computational cascade of neuronal mass and compartmental modeling. Int. J. Neural Syst. 25 (6), 1550021.

Henshall, D.C., Hamer, H.M., Pasterkamp, R.J., Goldstein, D.B., Kjems, J., Prehn, J.H.M., Schorge, S., Lamottke, K., Rosenow, F., 2016. MicroRNAs in epilepsy: pathophysiology and clinical utility. Lancet Neurol. 15 (13), 1368–1376.

Iturria-Medina, Y., Sotero, R.C., Canales-Rodríguez, E.J., Alemán-Gómez, Y., Melie-García, L., 2008. Studying the human brain anatomical network via diffusion-weighted MRI and graph theory. NeuroImage 40 (3), 1064–1076.

Jobert, M., Wilson, F.J., Roth, T., Ruigt, G.S.F., Anderer, P., Drinkenburg, W.H.I.M., 2013. Guidelines for the recording and evaluation of pharmaco-sleep studies in man: the international pharmaco-EEG society (IPEG). Neuropsychobiology 67 (3), 127–167.

Kaminski, R.M., Gillard, M., Klitgaard, H., 2012. Targeting SV2A for discovery of antiepileptic drugs. In: Noebels, J.L., Avoli, M., Rogawski, M.A., Olsen, R.W., Delgado-Escueta, A.V. (Eds.), Jasper's Basic Mechanisms of the Epilepsies. National Center for Biotechnology Information.

Kim, S., Salamon, N., Jackson, H.A., Blüml, S., Panigrahy, A., 2010. PET imaging in pediatric neuroradiology: current and future applications. Pediatr. Radiol. 40 (1), 82–96.

Knibbe, C.A.J., Danhof, M., 2011. Individualized dosing regimens in children based on population PKPD modelling: are we ready for it? Int. J. Pharm. 415 (1–2), 9–14.

de Lange, E.C.M., 2013. The mastermind approach to CNS drug therapy: translational prediction of human brain distribution, target site kinetics, and therapeutic effects. Fluids Barriers CNS 10 (1).

de Lange, E.C.M., Hammarlund-Udenaes, M., 2015. Translational aspects of blood-brain barrier transport and central nervous system effects of drugs: from discovery to patients. Clin. Pharmacol. Ther. 97 (4), 380–394.

Lindberger, M., Tomson, T., Wallstedt, L., Ståhle, L., 2001. Distribution of valproate to subdural cerebrospinal fluid, subcutaneous extracellular fluid, and plasma in humans: a microdialysis study. Epilepsia 42 (2), 256–261.

Loeb, J.A., 2011. Identifying targets for preventing epilepsy using systems biology. Neurosci. Lett. 497 (3), 205–212.

Lopes da Silva, F.H., 2002. Pharmaco-EEG: from cellular to the network level. EEG beta spectral power as a biomarker for GABAergic inhibition and its role in the assessment of AEDs. Methods Find. Exp. Clin. Pharmacol. 24 (p. Supplement D3).

Milosheska, D., Grabnar, I., Vovk, T., 2015. Dried blood spots for monitoring and individualization of antiepileptic drug treatment. Eur. J. Pharm. Sci. 75, 25–39.

Misonou, H., Mohapatra, D.P., Park, E.W., Leung, V., Zhen, D., Misonou, K., Anderson, A.E., Trimmer, J.S., 2004. Regulation of ion channel localization and phosphorylation by neuronal activity. Nat. Neurosci. 7 (7), 711–718.

Myers, C.T., Mefford, H.C., 2015. Advancing epilepsy genetics in the genomic era. Genome Med. 7 (1), 91.

Nakashima, H., Oniki, K., Nishimura, M., Ogusu, N., Shimomasuda, M., Ono, T., Matsuda, K., Yasui-Furukori, N., Nakagawa, K., Ishitsu, T., Saruwatari, J., 2015. Determination of the optimal concentration of valproic acid in patients with epilepsy: a population pharmacokinetic-pharmacodynamic analysis. PLoS One 10 (10), e0141266.

Ngugi, A.K., Bottomley, C., Kleinschmidt, I., Sander, J.W., Newton, C.R., 2010. Estimation of the burden of active and life-time epilepsy: a meta-analytic approach. Epilepsia 51 (5), 883–890.

Ogusu, N., Saruwatari, J., Nakashima, H., Noai, M., Nishimura, M., Deguchi, M., Oniki, K., Yasui-Furukori, N., Kaneko, S., Ishitsu, T., Nakagaswa, K., 2014. Impact of the superoxide dismutase 2 Val16Ala polymorphism on the relationship between valproic acid exposure and elevation of gamma-glutamyltransferase in patients with epilepsy: a population pharmacokinetic-pharmacodynamic analysis. PLoS One 9 (11), e111066.

Olszewska, D.A., Costello, D.J., 2014. Assessment of the usefulness of magnetic resonance brain imaging in patients presenting with acute seizures. Ir. J. Med. Sci. 183 (4), 621–624.

Patsalos, P.N., Berry, D.J., 2013. Therapeutic drug monitoring of antiepileptic drugs by use of saliva. Ther. Drug Monit. 35 (1), 5–29.

Patsalos, P.N., Berry, D.J., Bourgeois, B.F.D., Cloyd, J.C., Glauser, T.a., Johannessen, S.I., Leppik, I.E., Tomson, T., Perucca, E., 2008. Antiepileptic drugs – best practice guidelines for therapeutic drug monitoring: a position paper by the subcommission on therapeutic drug monitoring, ILAE Commission on Therapeutic Strategies. Epilepsia 49 (7), 1239–1276.

Pitkänen, A., Engel, J., 2014. Past and present definitions of epileptogenesis and its biomarkers. Neurotherapeutics 11 (2), 231–241.

Pitkänen, A., Löscher, W., Vezzani, A., Becker, A.J., Simonato, M., Lukasiuk, K., Gröhn, O., Bankstahl, J.P., Friedman, A., Aronica, E., Gorter, J.A., Ravizza, T., Sisodiya, S.M., Kokaia, M., Beck, H., 2016. Advances in the development of biomarkers for epilepsy. Lancet Neurol. 15 (8), 843–856.

Plan, E.L., 2014. Modeling and simulation of count data. CPT: Pharmacometrics Syst. Pharmacol. 3 (August 13), e129.

Ploeger, B.A., van der Graaf, P.H., Danhof, M., 2009. Incorporating receptor theory in mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) modeling. Drug Metab. Pharmacokinet. 24 (1), 3–15.

Rambeck, B., Jürgens, U.H., May, T.W., Wolfgang Pannek, H., Behne, F., Ebner, A., Gorji, A., Straub, H., Speckmann, E.J., Pohlmann-Eden, B., Löscher, W., 2006. Comparison of brain extracellular fluid, brain tissue, cerebrospinal fluid, and serum concentrations of antiepileptic drugs measured intraoperatively in patients with intractable epilepsy. Epilepsia 47 (4), 681–694.

Roncon, P., Soukupovà, M., Binaschi, A., Falcicchia, C., Zucchini, S., Ferracin, M., Langley, S.R., Petretto, E., Johnson, M.R., Marucci, G., Michelucci, R., Rubboli, G., Simonato, M., 2015. MicroRNA profiles in hippocampal granule cells and plasma of rats with pilocarpine-induced epilepsy–comparison with human epileptic samples. Sci. Report. 5 (April 2015), 14143.

Rossignol, E., Kobow, K., Simonato, M., Loeb, J.A., Grisar, T., Gilby, K.L., Vinet, J., Kadam, S.D., Becker, A.J., 2014. WONOEP appraisal: new genetic approaches to study epilepsy. Epilepsia 55 (8), 1170–1186.

Salinsky, M.C., Binder, L.M., Oken, B.S., Storzbach, D., Aron, C.R., Dodrill, C.B., 2002. Effects of gabapentin and carbamazepine on the EEG and cognition in healthy volunteers. Epilepsia 43 (5), 482–490.

Sheiner, L.B., 1991. The intellectual health of clinical drug evaluation. Clin. Pharmacol. Ther. 50 (1), 4–9.

Sheiner, L.B., 1997. Learning versus confirming in clinical drug development. Clin. Pharmacol. Ther. 61 (3), 275–291.

Sillanpää, M., Schmidt, D., 2006. Natural history of treated childhood-onset epilepsy: prospective, long-term population-based study. Brain 129 (3), 617–624.

Smithson, W.H., Hukins, D., Colwell, B., Mathers, N., 2012. Developing a method to identify medicines non-adherence in a community sample of adults with epilepsy. Epilepsy Behav. 24 (1), 49–53.

Standing, J.F., 2016. Understanding and applying pharmacometric modelling and simulation in clinical practice and research. Br. J. Clin. Pharmacol. 83 (2), 247–254.

Swatzyna, R.J., Kozlowski, G.P., Tarnow, J.D., 2015. Pharmaco-EEG: a study of individualized medicine in clinical practice. Clin. EEG Neurosci. 46 (3), 192–196.

Syvänen, S., de Lange, E.C., Tagawa, Y., Schenke, M., Molthoff, C.F.M., Windhorst, A.D., Lammertsma, A.A., Voskuyl, R.A., 2011. Simultaneous in vivo measurements of receptor density and affinity using [11C]flumazenil and positron emission tomography: comparison of full saturation and steady state methods. NeuroImage 57 (3), 928–937.

Syvänen, S., Russmann, V., Verbeek, J., Eriksson, J., Labots, M., Zellinger, C., Seeger, N., Schuit, R., Rongen, M., van Kooij, R., Windhorst, A.D., Lammertsma, A.A., de Lange, E.C., Voskuyl, R.A., Koepp, M., Potschka, H., 2013. [11C]quinidine and [11C] laniquidar PET imaging in a chronic rodent epilepsy model: impact of epilepsy and drug-responsiveness. Nucl. Med. Biol. 40 (6), 764–775.

Van Den Broek, M.P.H., Groenendaal, F., Toet, M.C., van Straaten, H.L.M., Van Hasselt, J.G.C., Huitema, A.D.R., de Vries, L.S., Egberts, T.C.G., Rademaker, C.M.A., 2012. Pharmacokinetics and clinical efficacy of phénobarbital in asphyxiated newborns treated with hypothermia. Clin. Pharmacokinet. 51 (10), 671–679.

Vlooswijk, M.C.G., Vaessen, M.J., Jansen, J.F.A., de Krom, M.C.F.T.M., Majoie, H.J.M., Hofman, P.A.M., Aldenkamp, A.P., Backes, W.H., 2011. Loss of network efficiency associated with cognitive decline in chronic epilepsy. Neurology 77 (10), 938–944.

Walker, L.E., Janigro, D., Heinemann, U., Riikonen, R., Bernard, C., Patel, M., 2016. WONOEP appraisal: molecular and cellular biomarkers for epilepsy. Epilepsia 1354–1362.

Wang, J., Yu, J.-T., Tan, L., Tian, Y., Ma, J., Tan, C.-C., Wang, H.-F., Liu, Y., Tan, M.-S., Jiang, T., Tan, L., 2015. Genome-wide circulating microRNA expression profiling indicates biomarkers for epilepsy. Sci. Report. 5, 9522.

Westerhout, J., Ploeger, B., Smeets, J., Danhof, M., Lange, E.C.M., 2012. Physiologically based pharmacokinetic modeling to investigate regional brain distribution kinetics in rats. AAPS J. 14 (3), 543–553.

de Witte, W.E.A., Wong, Y.C., Nederpelt, I., Heitman, L.H., Danhof, M., van der Graaf, P.H., Gilissen, R.A.H.J., de Lange, E.C.M., 2015. Mechanistic models enable the rational use of in vitro drug-target binding kinetics for better drug effects in patients. Expert Opin. Drug Discovery 11 (1), 45–63.

Yamamoto, Y., Välitalo, P.A., van den Berg, D.-J., Hartman, R., van den Brink, W., Wong, Y.C., Huntjens, D.R., Proost, J.H., Vermeulen, A., Krauwinkel, W., Bakshi, S., Aranzana-Climent, V., Marchand, S., Dahyot-Fizelier, C., Couet, W., Danhof, M., van Hasselt, J.G.C., de Lange, E.C.M., 2016. A generic multi-compartmental CNS distribution model structure for 9 drugs allows prediction of human brain target site concentrations. Pharm. Res. 34 (2), 333–351.

Zhang, Y., Fox, G.B., 2012. PET imaging for receptor occupancy: meditations on calculation and simplification. J. Biomed. Res. 26 (2), 69–76.

Zhu, X., Han, X., Blendy, J.A., Porter, B.E., 2012. Decreased CREB levels suppress epilepsy. Neurobiol. Dis. 45 (1), 253–263.

Zucchini, S., Marucci, G., Paradiso, B., Lanza, G., Roncon, P., Cifelli, P., Ferracin, M., Giulioni, M., Michelucci, R., Rubboli, G., Simonato, M., 2014. Identification of miRNAs differentially expressed in human epilepsy with or without granule cell pathology. PLoS One 9 (8), 1–8.