Electronic Supplementary Material

Physiologically-Based Pharmacokinetic Modeling of Oxcarbazepine and Levetiracetam During Adjunctive Antiepileptic Therapy in Children and Adolescents

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S1. Methods
S1.1. Data

Table S1. List of reported clinical studies in healthy adults that were used for modeling

| Study # | Drug | Route | Dose (mg) | Formulation | Dosing Frequency | Number of Participants (%Female) | Age (year) | WT (kg) or BMI (kg/m^2) | Reference |
|---------|------------------|--------|------------|--------------|-----------------|-------------------------------|-----------|-------------------------|-----------|
| 1       | MHD, OXZ         | IV     | 250, 300   | Solution, Tablet^a | Single, Single | 12 (50%), 12 (100%) | NR, 60-79 | 47.6-80.7, 47.6-87 | Flesch et al. | b 1 |
| 2       | OXZ             | Oral   | 600        | Tablet^a | Single | 12 (100%) | NR, 30-79 | 47.6-80.7, 47.6-87 | van Heiningen et al. | 3 |
| 3       | OXZ             | Oral   | 600        | Tablet^a | Single | 20 (0%) | 21-37 | 53-87 | Flesch et al. | 4 |
| 4       | OXZ             | Oral   | 600        | Tablet^a | Single | 20 (0%) | 21-37 | 53-87 | Flesch et al. | 4 |
| 5       | OXZ             | Oral   | 300        | Tablet^a | Multiple | 8 (0%) | 22-43 | 70-89 | Larkin et al. | 5 |
| 6       | LEV             | IV     | 2000, 4000 | Solution | Single | 6 (50%) | 21-55 | 19-28 | Ramael et al. | 6 |
| 7       | LEV             | IV     | 500        | Solution | Single | 4 (0%) | 31-51 | 69-88 | Benedetti et al. | 7 |
| 8       | LEV             | Oral   | 1000       | Tablet^a | Single | 5 (0%) | 45 ± 9 | 83 ± 5 | Brockmöller et al. | 8 |
| 9       | LEV             | Oral   | 1500       | Tablet^a | Single | 16 (37%) | 22-52 | 18.5-13.3 | Coupez et al. | 9 |
| 10      | LEV             | Oral   | 750        | Tablet^a | Single | 24 (50%) | 33.4 ± 9.78 | 25.2 ± 3.3 | Coupez et al. | 10 |
| 11      | LEV             | Oral   | 500        | Tablet^a | Multiple | 24 (50%) | 18-52 | 19-28 | Rouits et al. | 11 |

OXZ, oxcarbazepine; MHD, mono-hydroxy derivative of OXZ; IV, intravenous; NR, not reported; WT, body weight; BMI, body mass index.

^a Crossover study; b Concentrations of the S and R enantiomers of MHD were reported for both IV and oral doses. However, total MHD concentrations were used for modeling purposes, which were calculated as the summation of S-MHD and R-MHD concentrations. This was a reasonable approximation for modeling purposes since the pharmacokinetic properties of these two enantiomers are not significantly different^1, and also their anticonvulsant efficacy is similar.12
### Table S2. Summary of reported clinical studies in children that were used for modeling

| Study # | Drug | Route | Dose (mg/kg) | Formulation  | Dosing Frequency | Number of Participants (%Female) | Age (year) | WT (kg) or BMI (kg/m²) | Concomitant EIAEDs (% of patients) | Reference |
|---------|------|-------|--------------|--------------|------------------|-------------------------------|------------|------------------------|----------------------------------|-----------|
| 1       | OXZ  | Oral  | 5            | Suspension   | Single           | 6 (NR)                        | 2 - 5      | NR³                   | Carbamazepine (NR), Phenytoin (NR) | Rey et al. ¹³ |
|         |      |       | 15           | Suspension   | Single           | 7 (NR)                        |            |                        |                                   |           |
| 2       | LEV  | IV    | 30           | Solution     | Single           | 14 (NR)                       | 2 – 4      | NR                    | None⁶                             | Weinstock et al. ¹⁴ |
|         |      |       | 30           | Solution     | Single           | 7 (NR)                        | 12 - 16    | NR                    |                                   |           |
| 3       | LEV  | Oral  | 20           | Solution     | Single           | 4 (NR)                        | 2 - 4      | NR                    | Phenobarbital (NR), Phenytoin (NR) | Glauser et al. ¹⁵ |
| 4       | LEV  | Oral  | 20           | NR           | Single           | 24 (37)                       | 6 - 12     | NR                    | Carbamazepine (25%), Primidone (8%) | Pellock et al. ¹⁶ |
| 5       | LEV  | Oral  | 10           | Tablet       | Multiple         | 14 (43)                       | 4 - 12     | 13.5 - 60             | Carbamazepine (42%)                  | Fountain et al. ¹⁷ |

OXZ, oxcarbazepine; MHD, mono-hydroxy derivative of OXZ; IV, intravenous; NR, not reported; WT, body weight; BMI, body mass index.

*Children with body weight >2 standard deviations of the weight-for-age profiles were excluded. *⁶ Patients on concomitant EIAEDs were excluded.
S1.2. Model Development

The distribution and elimination processes were described by choosing the appropriate parameterization schemes that are in-built in PK-Sim®.

**Distribution:** Whole-body PBPK modeling considers multi-compartment drug distribution kinetics, where the volume of different compartments represents the physiological volume of the different organs. By default, each (organ) compartment in PK-Sim® is further divided into four sub-compartments (plasma, blood cells, interstitial tissue space, and intracellular tissue space), where drug distribution into the intracellular space can be either perfusion rate-limited or permeability rate-limited. Since there are no data to suggest any transporter involvement in the disposition of LEV, MHD and OXZ, their distributions were assumed to be perfusion rate-limited.

Under the perfusion rate-limited model, the organs are assumed to be well-stirred compartments, where the rate of distribution (that defines the time to achieve steady-state) depends on the organ perfusion \(Q_{\text{org}}\) and organ permeability \(P_{\text{org}}\). PK-Sim® calculates the permeability \(P_{\text{org}}\) for different organs as the products of specific organ permeability \(P_{\text{eff}}\), which is assumed to be constant between organs, and the organ surface area \(SA\) that varies between organs (Equation 1).

\[
P_{\text{org}} \text{ (mL.min}^{-1}\text{)} = P_{\text{eff}} \text{ (cm.min}^{-1}\text{)} \times SA \text{ (cm}^2\text{)}
\]

The specific organ permeability \(P_{\text{eff}}\) is a drug-specific property calculated by PK-Sim® from the lipophilicity \((LogP)\) estimate using an empirical method.\(^{18}\) On the other hand, the extent of distribution (that defines the volume of distribution at steady-state \([V_{ss}]\)) is the summation of the extent of distribution into individual organs, which is dependent on the separate plasma-to-
tissue partition coefficients ($K_p$). In the absence of experimentally determined $K_p$ values, PK-Sim® calculated values by the Rodgers and Rowland algorithm were used.\textsuperscript{19-22} The $K_p$ prediction algorithm uses the user-supplied estimates of certain drug-specific properties, such as plasma free fraction ($f_{u,p}$) and lipophilicity ($LogP$). It also accounts for the differences in tissue composition (of various lipid and non-lipid fractions) between the various organs. As an exception for red blood cells (RBCs), the algorithm uses the blood to plasma ratio ($B/P$) and the hematocrit value ($Hct$) to predict $K_p$.

Elimination: OXZ is converted to MHD via a set of carbonyl reductase enzymes that belong to the aldo-keto reductase (AKR) superfamily.\textsuperscript{23} The four enzyme isoforms primarily responsible for the conversion are AKR1C1, AKR1C2, AKR1C3, and AKR1C4, which belong to the AKR1C family.\textsuperscript{24} Among these enzymes, AKR1C4 is mainly concentrated within the liver, whereas the others are widely distributed across hepatic and extra-hepatic tissues, including muscle, lung, and intestine.\textsuperscript{24} The distribution of these isoforms were extracted from the Open Systems Pharmacology gene expression database. In vitro intrinsic clearance ($CL_{int,AKR}$ in $\mu$L/min/pmol of recombinant enzyme) data were available for all four AKR1C isoforms\textsuperscript{24}, which were scaled to organ level intrinsic clearance ($CL_{int,org}$ in mL/min) by multiplying with the respective isoforms’s abundance [AKR] and cellular volume ($V_{org}$ in $L$) of the respective organs (Equation 2). Since the actual enzyme abundance in humans is unknown for the AKR1C isoforms, it was assumed to be 1 (one) $\mu$mol/L. From $CL_{int,org}$, PK-Sim® calculates organ clearance ($CL_{org}$) by accounting for relevant physiological variables such as the organ blood flow ($Q_{org}$). For the liver, $CL_{int,org}$ would represent the hepatic intrinsic clearance ($CL_{int,H}$), and $CL_{org}$ would represent the hepatic clearance ($CL_H$) after accounting for the liver blood flow ($Q_H$). When carbonyl reduction is restricted to the
liver (e.g., in the case of AKR1C4), the total body clearance (CL) of OXZ would be equivalent to $CL_H$. However, for other isoforms, CL would be the summation of $CL_{org}$ of all the respective organs contributing to the reduction process.

$$CL_{int.org} \text{ (mL/min)} = CL_{int,AKR} \times [AKR] \times V_{org}$$

MHD is majorly eliminated by glucuronidation in the liver (45% of the dose) with a minor contribution of renal excretion (28% of the dose).\(^1\) In the absence of in vitro data, the intrinsic clearance for the liver and kidney were back-calculated from the in vivo (plasma) clearance information reported by Flesch et al.\(^1\) The authors estimated the total plasma clearance ($CL$) and the renal clearance ($CL_R$) of MHD following its IV administration in healthy adults. Upon subtraction of $CL_R$ (0.9 L/h) from the total plasma CL (3.7 L/h), hepatic clearance ($CL_H$) was calculated to be 2.8 L/h. Both $CL_H$ and $CL_R$ data were then incorporated into PK-Sim\(^\circledR\) as the input for MHD's hepatic and renal elimination, respectively. From these in vivo clearance data, the respective organ level intrinsic clearance for the liver ($CL_{int,H}$) and kidney ($CL_{int,R}$) were back-calculated using the well-stirred model approximation implemented in PK-Sim\(^\circledR\), which accounts for the relevant physiological variables (e.g., organ volume and perfusion) in a 73 kg adult. These $CL_{int,H}$ and $CL_{int,R}$ estimates (in 1/min) are also referred to as the 'specific clearance' per PK-Sim\(^\circledR\) terminology, and they were inputted as the drug-specific properties of MHD. When performing population simulations, these drug-specific $CL_{int,H}$ and $CL_{int,R}$ estimates are scaled to the respective organ volume and other variables of the virtual individuals, essentially accounting for the inter-individual variation in CL.
LEV is a slowly cleared compound with a reported CL of about 4 L/h in healthy adults. A major portion of a LEV dose (nearly 70%) is filtered unchanged by the kidney, while the rest is metabolized. Since LEV is negligibly bound to plasma proteins ($f_{u,p} = 0.97$), its expected renal clearance ($CL_R$) is comparable to the glomerular filtration rate ($GFR$). However, $CL_R$ of LEV was estimated to be 44 mL/min/1.73 m$^2$ using urinary excretion data, which is substantially lower than $GFR$. This implies that the drug undergoes significant tubular reabsorption. The fraction of $GFR$ that escapes the reabsorption process ($f_{GFR}$) was estimated to be 0.40 per Equation 3, indicating 60% reabsorption by the renal tubules. Therefore, the $CL_R$ of LEV was parameterized as a constant fraction of $GFR$ (Equation 4).

$$f_{GFR} = \frac{\text{Observed } CL_R}{\text{Expected } CL_R} = \frac{\text{Observed } CL_R}{f_{u,p} \times GFR} = \frac{44}{0.97 \times 110} = 0.40 \quad (3)$$

$$CL_R = f_{GFR} \times f_{u,p} \times GFR \quad (4)$$

The major metabolic pathway of LEV (i.e., nearly 30% of the dose) has been identified to be deamination to its acid metabolite L057, which is mediated by Type B esterase enzymes. Generally, esterase enzymes are mostly uncharacterized and are known to be ubiquitously expressed in the body. However, it has been postulated that the esterase-mediated metabolism of LEV exclusively occurs within the RBCs. Therefore, in vitro data generated from a whole blood assay were used to characterize LEV's metabolism. Since the enzyme isoforms were not characterized, a hypothetical enzyme was created in PK-Sim®, which was assumed to be exclusively localized within the RBCs at a concentration of 1 µmol/L. The whole blood assay reported a metabolic capacity ($V_{max}$) = 129 pmol/min/mL of whole blood and an affinity ($K_m$) = 439 µmol/L whole blood, which is equivalent to a $V_{max} = 287$ pmol/min/mL of RBC and
a $K_m = 439 \, \mu\text{mol/L}$ of RBC, assuming hematocrit ($Hct$) = 0.45 and $B/P = 1$ of a 73 kg adult individual. These $V_{\text{max}}$ and $K_m$ values normalized to per unit volume of RBC were inputted in PK-Sim as drug-specific properties. When performing population simulations, PK-Sim® uses this information to predict the individual clearance ($CL_{RBC}$) using Equations 5 and 6 where $V_{\text{blood}}$ and $Hct$ values are varying in the population.

\[
CL_{\text{int,RBC}} \, (1/\text{min}) = \frac{V_{\text{max}}}{K_m} \tag{5}
\]

\[
CL_{RBC} \, (L/\text{min}) = CL_{\text{int,RBC}} \times V_{\text{blood}} \times Hct \tag{6}
\]

Absorption: The absorption processes for OXZ are depicted in Figure S1. The absorption modeling (along with parameters) of OXZ and LEV are described in detail in the main text.

Figure S1 Schematic representation of the processes involved following oral administration of oxcarbazepine (OXZ), including its (1) dissolution, (2) conversion to its mono-hydroxy derivative (MHD) during and post-absorption, (3) permeation of MHD, and (4) distribution of OXZ and MHD. Solid-colored arrows represent processes 1 to 3, and process 4 is represented by the dashed arrow.
S1.3. Simulations of Steady-State Exposure in Children

Doses of OXZ and LEV used for steady state simulations are summarized in Table S3.

Table S3. Maintenance doses of oxcarbazepine (OXZ) and levetiracetam (LEV) used for the simulation of steady-state exposure of MHD and LEV using physiologically-based pharmacokinetic modeling with and without considering the effect of EIAEDs

| Age Group (years) | Drug          | Weight Range (kg) | Maximum Recommended Dose (MRD) $^{a,b}$ | Exploratory Doses $^b$ |
|------------------|---------------|-------------------|----------------------------------------|------------------------|
| 2 to <4          | Oxcarbazepine | None              | 60 mg/kg/day                            | 45 mg/kg/day           |
|                  | Levetiracetam | None              | 50 mg/kg/day                            | -                      |
|                  |               | 20 - 29           | 900 mg/day                              | -                      |
|                  | Oxcarbazepine | 29.1 - 39         | 1200 mg/day                             | -                      |
|                  |               | >39               | 1800 mg/day                             | -                      |
| 4 to 6           | None          | 60 mg/kg/day      | -                                      |                        |
| Levetiracetam    | <50           | -                 | 50 mg/kg/day                            |                        |
|                  | >50           | -                 | 1250 mg/day                             |                        |

$^a$ Recommended by U.S. Food and Drug Administration, $^b$ Maintenance doses are administered in two divided doses at 12 h intervals, MHD monohydroxy derivative of oxcarbazepine
Figure S2 Visual inspection of the final model predictions for oxcarbazepine (OXZ) and levetiracetam (LEV) using steady-state data (solid circles) from clinical studies in adults. The arithmetic mean (solid lines) and the 90% prediction interval (shaded areas) of the predicted plasma concentrations following oral (PO) administration of OXZ (a) and LEV (b) are shown. Data Source: Larkin et al. 5, Rouits et al. 11
Figure S3 Observed vs. predicted area under the plasma concentration-time curve (AUC) of oxcarbazepine (OXZ), its mono-hydroxy derivative (MHD) metabolite (a), and levetiracetam (LEV) (b) up to the last observed concentration for several clinical studies in adults. The solid line represents the line of unity, and the dashed lines represent ±25% prediction error. The studies marked with an asterisk (*) in sub-plot (a) imply that the corresponding data points represent the AUC of MHD, while the others represent the AUC of OXZ. Data Source: Flesch et al. 1,4, Lloyd et al. 2, van Heiningen et al. 3, Ramael et al. 6, Benedetti et al. 7, Brockmöller et al. 8, Coupez et al. 9,10
Figure S4 Simulated values of individual clearance (CL) of the monohydroxy derivative of oxcarbazepine (MHD) and levetiracetam (LEV) in children, adolescents, and young adults (n=1000) ranging from 2 to 20 years of age using the respective final models (a, b), and the effect size of enzyme-inducing antiepileptic drugs (EIAEDs) on the CL of respective drugs (c, d). The individual effect size was calculated as the percentage increase in the CL due to the presence of concomitant EIAEDs compared to the CL in the absence of EIAEDs. The solid lines represent the smoothing lines (LOESS).
Figure S5. Influence of age on physiological variables that are relevant for the clearance of the monohydroxy derivative of oxcarbazepine (MHD) and levetiracetam (LEV).
Table S4. Comparison of observed vs. simulated pharmacokinetics from the scaled model in children with and without considering EIAEDs as shown in Figure 3 and Figure 4 in the main text.

| Drug                  | Age Group | Dose (mg/kg) | Observed AUC (µM.h) and %\(f_{u,dose}\) | Simulated AUC and %\(f_{u,dose}\) |
|-----------------------|-----------|--------------|---------------------------------------|----------------------------------|
|                       |           |              | Without EIAEDs (Bias)\(^b\) | With EIAEDs (Bias)\(^b\) |
| Oxcarbazepine \(^a\) | 2-5 years | 5            | AUC = 183.5                          | AUC = 264.2 (44%)               | AUC = 208.6 (14%)               |
|                       |           | 15           | AUC = 587.2                          | AUC = 801.1 (36%)               | AUC = 632.0 (8%)                |
|                       | 6-12 years| 5            | AUC = 247.3                          | AUC = 317.5 (28%)               | AUC = 253.7 (3%)                |
|                       |           | 15           | AUC = 827.1                          | AUC = 952.4 (15%)               | AUC = 760.9 (−8%)               |
| Levetiracetam         | 2-4 years | 20           | AUC = 1360.3                         | AUC = 2023.1 (49%)              | AUC = 1659.7 (22%)              |
|                       | 6-12 years| 20           | AUC = 1424.2                         | AUC = 2151.2 (51%)              | AUC = 1696.4 (19%)              |
|                       |           |              | %\(f_{u,dose}\) = 51.9              | %\(f_{u,dose}\) = 65.2 (27%)    | %\(f_{u,dose}\) = 55.5 (27%)    |
|                       | 4-12 years| 10 (Twice Daily) | AUC = 839.7                         | AUC = 1068.0 (27%)              | AUC = 839.6 (−0.01%)            |
|                       |           |              | %\(f_{u,dose}\) = 60.7              | %\(f_{u,dose}\) = 73.3 (21%)    | %\(f_{u,dose}\) = 59 (−2.8%)    |

AUC Area under plasma concentration-time curve from time zero to infinity (for single doses) or steady state AUC from time zero to 12 h for twice daily dosing (only levetiracetam), %\(f_{u,dose}\) percentage of dose excreted unchanged in urine (data were available for levetiracetam only), EIAEDs Enzyme-inducing antiepileptic drugs, \(^a\)AUC of 10-monohydroxy derivative (MHD) of oxcarbazepine are shown. \(^b\) Bias was calculated as [(simulated – observed)\*100/observed]
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