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EDILAINY RIZZIERI CALEFFI-MARCHESINI

EFEITO DA ONTOGENIA NA ABSORÇÃO DA LAMOTRIGINA: UMA ABORDAGEM BIOFARMACÊUTICA E FARMACOCINÉTICA

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EDILAINY RIZZIERI CALEFFI-MARCHESINI

Efeito da ontogenia na absorção da lamotrigina: uma abordagem biofarmacêutica e farmacocinética

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Coorientadores: Prof. Dr. Rodrigo Cristofoletti

Profa. Dra. Fernanda B. B. Pangoni

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Este trabalho foi realizado nos Laboratório de Farmacocinética e Biofarmácia (PKBio), bloco K80, sala 24 e Laboratório de Controle de Qualidade Físico-Químico, bloco P02 do Departamento de Farmácia da Universidade Estadual de Maringá.

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sempre me encorajar a sonhar grande,
e ao meu filho Pedro que chegou na minha vida durante este doutorado.

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Verdades e desajustes

Perguntei às minhas pesquisas
Olhando-as com severidade
Por que não me auxiliam?

Pareciam divertir com minhas dúvidas
Harmonizavam e desarmonizavam
Tudo ao mesmo tempo?

As observações me envenenavam
Experimentos beliscavam a alma
Onde estão as comprovações?

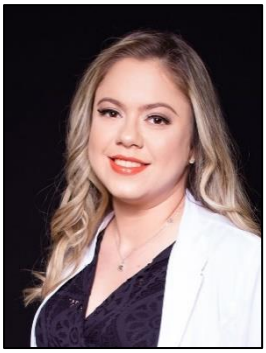
O tempo incomodado trouxe a chama
Conhecimentos ensinaram seus usos
Mas e os devaneios da veracidade?

A Ciência, toda metódica e atenta
com notável conhecimento, respondeu:
Olhe para suas verdades e desajustes!

Andamos juntas e complexas
Nos contínuos procedimentos da vida
Para manutenção da sua espécie.

Pedra Teodora Marchesini &
Paulo Victor dos Santos Marchesini

BIOGRAFIA



Edilainy Rizzieri Caleffi-Marchesini, graduada em farmácia pela Universidade Estadual de Maringá (2011). Mestre em Ciências farmacêuticas (2014) também pela Universidade Estadual de Maringá com dissertação intitulada "Isolamento e caracterização química de polissacarídeos obtidos de raízes de *Pfaffia glomerata* (Spreng.) Pedersen". Atuou como pesquisadora na EMS S/A Indústria Farmacêutica onde trabalhou com perfis de dissolução comparativos, equivalência farmacêutica, validação de metodologias analíticas. Possui experiência em análises por espectrofotometria (UV-VIS), e cromatografia líquida de alta eficiência (CLAE) e operação dos softwares Empower e EZChrom Openlab. Atualmente é professora na União de Faculdades Metropolitanas de Maringá (UNIFAMMA) dos cursos de farmácia e biomedicina ministrando as disciplinas de química analítica, química farmacêutica, farmacotécnica, controle e garantia da qualidade e farmacognosia. Também é socia, analista e consultora na empresa Pharmetrica Consultoria e Treinamento. Possui experiência na área de modelagem e simulação farmacocinética fisiológica (PBPK), modelagem biofarmacêutica fisiológica (PBBM) e relação *in vitro-in vivo* (IVIVR), nos softwares PK-Sim e GastroPlus®, e em análises experimentais de solubilidade em equilíbrio e dissolução, incluindo a aplicação de meios biorrelevantes e a avaliação de condições biopreditivas.

RESUMO

CALEFFI-MARCHESINI, EDILAINY R., 2023. Efeito da ontogenia na absorção da lamotrigina: uma abordagem biofarmacêutica e farmacocinética. Tese de doutorado. Programa de Pós-graduação em Ciências Farmacêuticas. Universidade Estadual de Maringá. 125p.

A lamotrigina (LTG) é um anticonvulsivante frequentemente prescrito para adultos e crianças. LTG é uma base fraca, SCB II, e sua biodisponibilidade é dependente das propriedades biofarmacêuticas. Existem poucos estudos avaliando as características biofarmacêuticas da LTG, principalmente em crianças. Comumente, dados obtidos em adultos são extrapolados para crianças ignorando a ontogenia nessa população. Por esse motivo, o objetivo deste trabalho foi avaliar o impacto da ontogenia na absorção da LTG empregando abordagens biofarmacêuticas e farmacocinéticas. Foi avaliada a capacidade de dissolução da LTG em diferentes meios, tamponados e biorrelevantes. A maior dose adulta (200 mg) e duas doses pediátricas (5 e 15 mg/kg) foram utilizadas para o cálculo da unidade de dose juntamente com o volume de líquido administrado. A LTG demonstrou melhor dissolução em meios biorrelevantes e em pHs mais ácidos. Tanto para adultos quanto para pediátricos, pela classificação oficial, permaneceu como BCS II. No entanto, em crianças menores que 5 anos, quando avaliada a dose de 15 mg/kg, a LTG mostrou-se pouco solúvel em ambientes ácidos. A baixa solubilidade em ambientes gástricos pode impactar os demais processos relacionados a absorção. A partir disso, foram realizadas modelagens e simulações (M&S) farmacocinéticas da LTG utilizando o software GastroPlus®. Foram desenvolvidos modelos farmacocinéticos de base fisiológica (PBPK) e modelos biofarmacêuticos de base fisiológica (PBBM) para adultos e extrapolados para crianças. O modelo PBBM adulto foi empregado para a exploração de um "espaço seguro" de dissolução ou *safe space*, juntamente com a análise de relação *in vitro-in vivo* (RIVIV). O método mecanístico de deconvolução foi o que apresentou menor erro de predição da dissolução *in vivo* e foi adotado para a construção do IVIVR. A melhor relação estabelecida foi empregando o método de dissolução com aparato de células de fluxo e meio FaSSIF, e esta foi validada internamente e externamente. Com a IVIVR foram testados perfis de dissolução teóricos variando 20% ponto a ponto do perfil da formulação referência, o que apresentou perfis plasmáticos preditos bioequivalentes ao referência. Resultado similar foi encontrado utilizando o modelo PBBM por meio do teste de bioequivalência virtual (VBE). Diante dos resultados, sugere-se que o perfil de dissolução de uma formulação de liberação imediata contendo LTG pode variar 20% em quantidade liberada ponto a ponto em relação ao comparador sem prejuízo na bioequivalência. Em sequência, os modelos PBBM adulto e pediátrico foram empregados para a investigação da sensibilidade de parâmetros (PSA). Notou-se que, apesar de a solubilidade e o volume de dose serem parâmetros relevantes, a farmacocinética da LTG não foi significativamente impactada. Assim, é razoável levantar a hipótese de que mesmo que a LTG seja experimentalmente uma BCS II, fisiologicamente pode apresentar o comportamento de BCS I, tanto na população adulta quanto na pediátrica. Tal hipótese é suportada pelos resultados encontrados nos estudos de IVIVR e VBE, que predisseram a manutenção da bioequivalência mesmo alterando o perfil de liberação. Tais informações podem contribuir para mudanças nas estratégias industriais e como guias para o desenvolvimento de formulações por uma abordagem mais científica.

Palavras-chave: Biofarmácia. PBPK. IVIVR. PBBM. VBE

ABSTRACT

CALEFFI-MARCHESINI, EDILAINY R., 2023. Effect of ontogeny on lamotrigine absorption: a biopharmaceutical and pharmacokinetic approach. Ph.D. Thesis. Programa de Pós-graduação em Ciências Farmacêuticas. Universidade Estadual de Maringá. 125p

Lamotrigine (LTG) is an anticonvulsant frequently prescribed for adults and children. It is a weak base, BCS II, and its bioavailability is dependent on biopharmaceutical properties. There are few studies evaluating the biopharmaceutical characteristics of LTG, especially in children. Commonly, data obtained from adults are extrapolated to children, ignoring ontogeny in this population. For this reason, the aim of this work was to evaluate the impact of ontogeny on LTG absorption using biopharmaceutical and pharmacokinetic approaches. The dissolution capacity of LTG in different buffered and biorelevant media was evaluated. The highest adult dose (200 mg) and two pediatric doses (5 and 15 mg/kg) were used for the unit dose calculation together with the dose volume. LTG demonstrated better dissolution in biorelevant media and at more acidic pHs. For both adults and children, according to the official classification, it remained as BCS II. However, in children younger than 5 years, when the dose of 15 mg/kg was evaluated, LTG proved to be poorly soluble in acidic environments. Low solubility in gastric environments can impact other processes related to absorption. From this, LTG pharmacokinetic modeling and simulations (M&S) were performed using the GastroPlus® software. Physiologically based pharmacokinetic models (PBPK) and physiologically based biopharmaceutical models (PBBM) have been developed for adults and extrapolated to children. The adult PBBM model was applied for a dissolution "safe space" exploration, together with the *in vitro-in vivo* relationship analysis (IVIVR). The mechanistic deconvolution method presented the lowest *in vivo* dissolution prediction error and was adopted for IVIVR development. The best relationship established was using the dissolution method with flow cell apparatus and FaSSIF medium, and this was internally and externally validated. From the IVIVR theoretical dissolution profiles varying 20% point by point from the profile of the reference formulation were tested, which presented predicted plasmatic profiles bioequivalent to the reference. A similar result was found using the PBBM model through the virtual bioequivalence test (VBE). In view of the results, it is suggested that the dissolution profile of an immediate release formulation containing LTG may vary in the dissolution profile 20% in amount released point by point in relation to the comparator without prejudice to bioequivalence. Then, the adult and pediatric PBBM models were used to investigate parameter sensitivity (PSA). It was noted that, although solubility and dose volume are relevant parameters, the LTG pharmacokinetics were not significantly impacted. Thus, it is reasonable to raise the hypothesis that even if LTG is experimentally a BCS II, physiologically it may present BCS I behavior, both in the adult and pediatric population. This hypothesis is supported by the results found in the IVIVR and VBE studies, which predicted the maintenance of bioequivalence even changing the release profile. Such information can contribute to changes in industrial strategies and as guides for the development of formulations by a more scientific approach.

Key words: Biopharmaceutics. PBPK. IVIVR. PBBM. VBE.

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ACAT	*Advanced compartmental absorption and transit model*
ADAM	*Advanced dissolution absorption metabolism model*
ADME	Absorção, distribuição, metabolismo e excreção
ANVISA	Agência Nacional de Vigilância Sanitária
ASC	Área sob a curva (no inglês AUC - *Area under curve)*
BCS	*Biopharmaceutical classification system*
BDDCS	*Biopharmeceutics drug disposition classification system*
BE	Bioequivalência
CAT	*Compartmental absorption and transit model*
CIVIV	Correlação *in vitro - in vivo*
CL	Clearance
CL_r	Clearance renal
$C_{máx}$	Concentração plasmática máxima
D/S	Relação dose/solubilidade
DDI	*Drug drug interaction*
EMA	*European Medicines Agency*
F	Biodisponibilidade
Fa	Fração absorvida
FaSSGF	*Fasted state simulated gastric fluid*
FaSSIF	*Fasted state simulated intestinal fluid*
FDA	*Food and Drug Administration*
FeSSGF	*Fed state simulated gastric fluid*
FeSSIF	*Fed state simulated intestinal fluid*
Fg	Fração que resiste ao metabolismo no trato gastrointestinal
Fh	Fração que resiste ao metabolismo hepático de primeira passagem
ICH	*The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use*
IFA	Insumo farmacêutico ativo
IMC	Índice de massa corpórea
IVIVC	*in vitro-in vivo correlation*
IVIVE	*in vitro-in vivo extrapolation*
IVIVR	*vitro-in vivo relationship*
Log D	Logaritmo do coeficiente de distribuição
Log $P_{o:a}$	Logaritmo do coeficiente de partição octanol: água
LTG	Lamotrigina
LTG-2N-glu	LTG-2-N-Glucuronídeo
LTG-5N-glu	LTG-5-N-Glucuronídeo
M-ADAM	*Multi-layer gut wall within* ADAM
OMS	Organização Mundial da Saúde
PBAM	*Physiologically based absorption model*
PBBM	*Physiologically based biopharmaceutics model*
PBPK	*Physiologically based pharmacokinetic model*
pKa	Logaritmo negativo da constante de ionização ácida
RENAME	Relação nacional de medicamentos essenciais
RIVIV	Relação *in vitro - in vivo*
S_0	Solubilidade intrínseca

S_{ap}	Solubilidade aparente
SCB	Sistema de classificação biofarmacêutico
S_{cin}	Solubilidade cinética
S_{eq}	Solubilidade em equilíbrio
SGF	*Simulated gastric fluid*
SIF	*Simulated intestinal fluid*
$t_{1/2}$	Tempo de meia-vida
TGI	Trato gastrointestinal
$T_{máx}$	Tempo para observação da concentração máxima
UGT	UDP-glucuronosiltransferases
VBE	Bioequivalência virtual
Vd	Volume de distribuição aparente

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1. INTRODUÇÃO

Todos os anos, dezenas de novas moléculas ou novas indicações de moléculas já conhecidas são aprovadas pelos órgãos regulatórios. A agência americana *Food and Drug Administration* (FDA) aprovou 50 novas entidades moleculares em 2021 e 37 em 2022 (FDA, 2021a, 2022). O órgão brasileiro, Agência Nacional de Vigilância Sanitária (ANVISA), listou 56 novos medicamentos e novas indicações de tratamento em 2021 e 46 em 2022, destes 20 e 21 eram novos registros respectivamente (BRASIL, 2023). Estes números são o resultado da busca constante por parte das indústrias farmacêuticas, pesquisadores e agências regulatórias pela inovação no desenvolvimento de fármacos e formulações.

Na jornada da pesquisa e desenvolvimento de fármacos, a etapa clínica é uma das mais dispendiosas pelo alto custo envolvido e a alta probabilidade de falhas (PAUL *et al.*, 2010). Na primeira fase desta etapa, o típico critério de inclusão/exclusão de voluntários é limitado a indivíduos saudáveis, caucasianos, adultos de 18 a 65 anos com índice de massa corpórea (IMC) menor que 25 kg/m^2. Já a fase II é realizada geralmente com pacientes. Na fase III, este critério é ampliado e os centros de pesquisa clínica são encorajados a considerar a heterogeneidade da população em geral, por exemplo, aumentando a diversidade étnica, incluindo adultos maiores de 65 anos, pacientes com sobrepeso e realizando estudos com populações especiais (crianças, gestantes, idosos, entre outros) (GRIMSRUD *et al.*, 2015; WINTER *et al.*, 2018). Mas apesar dos incentivos por parte das agências regulatórias para um maior empenho em estudos clínicos em populações especiais, ainda existem muitos hiatos, principalmente, devido à complexidade e às particularidades de cada uma delas (EMA, 2017a; FDA, 2020).

Do nascimento, crescimento até a idade adulta, as crianças passam por uma série de processos físicos, metabólicos e psicológicos, o que as tornam diferentes dentro do mesmo grupo populacional. Este processo de ontogenia ou maturação fisiológica, origina diferenças significativas nos processos de absorção, distribuição, metabolismo e excreção (ADME) de fármacos em comparação com os adultos (ABDEL-RAHMAN *et al.*, 2012). Em razão destas transformações ontogênicas, a organização mundial da saúde (OMS) classifica a população pediátrica em: neonatos pré-termo, neonatos (0 a 28 dias), lactentes (29 dias a 23 meses), crianças (2 a 11 anos) e adolescentes (12 a 18 anos) (EMA, 2013; WHO, 2007). A heterogeneidade dessa população somada às questões éticas relacionadas as condutas dos estudos clínicos desestimulam as pesquisas nesse âmbito. Além disso, levam a extrapolação de dados de ensaios clínicos em adultos para crianças, situação não ideal que pode ocasionar falhas terapêuticas ou mesmo efeitos adversos (GRIMSRUD *et al.*, 2015; KIPPER; KIPPER, 2016; ROCCHI; TOMASI, 2011).

Diante disso, a prescrição de medicamentos para crianças, em muitos casos, segue os mesmos princípios de segurança e dose que é aplicado para adultos. A falta de informações da farmacocinética em pediatria e a rapidez com que as indústrias registram novos medicamento, faz com que a maioria

deles sejam lançados no mercado sem licença para o uso em crianças. Frequentemente, faltam dados em bulas e formas farmacêuticas adequadas, contribuindo para uma baixa adesão ao tratamento devido ao tamanho dos comprimidos e cápsulas e à necessidade de fracionamento (LOUREIRO *et al.*, 2013; MILNE; BRUSS, 2008).

Um fármaco bastante empregado na terapia pediátrica é a lamotrigina (LTG). A LTG é uma das moléculas de escolha para o tratamento da epilepsia tanto em adultos quanto em crianças. Ela é indicada como monoterapia no tratamento de crises focais e generalizadas em pacientes com mais de doze anos de idade com intolerância ou refratariedade aos fármacos de primeira linha e como terapia adjuvante de crises focais e generalizadas em pacientes com mais de dois anos de idade (BRASIL, 2018c). No entanto, existem poucos estudos a respeito da farmacocinética deste fármaco em populações pediátricas, principalmente com enfoque no processo de absorção e nos processos biofarmacêuticos de solubilidade e dissolução.

A fim de contribuir com informações e predições que respondem questionamentos semelhantes a este, a farmacometria, a ciência da farmacologia quantitativa, tem transformado o desenvolvimento de fármacos em um processo menos empírico e mais quantitativo (BONATE, 2011). As ferramentas de modelagem e simulação farmacocinética podem ser aplicadas para a predição de primeira dose em humanos, ajuste de dose, estudos de interação fármaco-fármaco (do inglês *drug-drug interaction* – DDI), estudos do impacto da alimentação no processo de absorção e para estudos de populações especiais, como as crianças por exemplo, além de outras aplicações (GRIMSTEIN *et al.*, 2019). Nesse sentido, a farmacometria pode ser um importante instrumento para a compreensão da farmacocinética da LTG, principalmente na população pediátrica onde existe escassez de dados.

2. REVISÃO BIBLIOGRÁFICA

2.1. Fármacos em pediatria

Durante muitos anos a ciência viu as crianças pela perspectiva do adulto. Muitas ferramentas de extrapolação de dados de adultos para crianças foram desenvolvidas e são empregadas até hoje como, por exemplo, a "árvore de decisão de estudos pediátricos", publicada pelo FDA em 2003. Nesse guia a extrapolação pode ser justificável nos casos em que o curso da doença e o efeito do fármaco são similares em pacientes adultos e pediátricos, com fundamento na farmacodinâmica do fármaco (FDA, 2003).

No entanto, hoje sabe-se que os pacientes pediátricos representam uma população variada e dinâmica em comparação com os adultos, visto que esses indivíduos passam por uma série de processos de maturação, também chamada de ontogênese. A ontogenia é definida como o "processo evolutivo acerca das alterações biológicas sofridas pelo indivíduo, desde o seu nascimento, até seu desenvolvimento final" (MICHAELIS, 2022). Dentre as principais mudanças ontogênicas podemos destacar as proporções corporais, tamanhos de órgãos e composições de tecidos como, proporções de gordura, proteína e teor de água extracelular (SHAWAHNA, 2016; WHO, 2007). A proporção de água, por exemplo, reduz de 80% em recém-nascidos para 60% aos 5 meses de idade (WHO, 2007). Tais mudanças podem resultar em diferenças significativas no que tange a farmacocinética e farmacodinâmica interferindo diretamente na clínica e nas premissas de segurança e eficácia de um fármaco (ABDEL-RAHMAN *et al.*, 2012; BATCHELOR; FOTAKI; KLEIN, 2014; DEL MORAL SANCHEZ *et al.*, 2018; KAYE, 2011).

A maioria dos estudos relacionam o termo ontogenia com o processo de maturação enzimática e metabólica. Sabe-se que a atividade da CYP-3A4, enzima da família do citocromo P450 3A, apresenta-se extremamente baixa no feto e aumenta após o nascimento, atingindo 30 a 40% dos níveis adultos em 3 a 12 meses de idade e 120% após 1 ano de idade (DE WILDT *et al.*, 1999). Para as UDP-glicuronosiltransferases (UGTs) os estudos revelaram uma porcentagem estimada de atividade comparada com a adulta em 1 mês de idade variando de 25% para UGT1A4, 30% para UGT1A6, 60% para UGT2B17 a 94% para UGT1A3. Para essas enzimas a atividade de glicuronidação máxima foi alcançada em diferentes idades, variando de 1 ano para UGT1A1, UGT1A3, UGT1A4, UGT1A9/2B7 até mais de 10 anos para UGT1A6 (BADÉE *et al.*, 2019; MIYAGI; COLLIER, 2007).

Além da maturação enzimática e metabólica outras mudanças fisiológicas são importantes e podem impactar em processos farmacocinéticos como a absorção, por exemplo. A absorção de um fármaco administrado por via oral é dependente da dissolução e permeação ou transporte deste pela mucosa do trato gastrointestinal (TGI). Desse modo, fatores como a acidez gástrica, tempo de esvaziamento gástrico, motilidade intestinal, área de superfície intestinal, além da presença de enzimas e transportadores, secreção de ácidos biliares e lipases pancreáticas (fluidos gástricos e intestinais),

metabolismo de primeira passagem, recirculação entero-hepática e colonização bacteriana do intestino são dependentes do processo ontogênico (Quadro 1) (BATCHELOR, 2014; SHAWAHNA, 2016; WHO, 2007).

Quadro 1 - Fatores fisiológicos dependentes da idade relacionados ao trato gastrointestinal

Parâmetro	**Recém- nascido (0-28 dias)**	**Criança (1 mês – 2 anos)**	**Criança (2-5 anos)**	**Criança (6-11 anos)**	**Adolescente (12-18 anos)**
pH Saliva	7,0		7,1	7,1	7,4
pH Fluido Gástrico	Nascimento: 6-8 24 a 48 h: 1-3,5 Após 20 dias: 6-8	3 meses: 1,4 2 anos de idade: ~Adulto	~Adulto 1,5	~Adulto 1,5	~Adulto 1,5
Tempo de esvaziamento gástrico (min)	54-82	Lento de 6-8 meses Depois de 6 meses: 12-70	~Adulto 12-70	~Adulto 12-70	~Adulto 12-70
Presença de ácido gástrico/pepsina	Relativamente baixo (15-41% ~adulto)	Baseado no peso corporal (41%-100% ~adulto)	~Adulto	~Adulto	~Adulto
Capacidade do estômago (mL)	10-100	90-500	750 -960	750 -960	750 -960
pH intestinal	Reduzido 4,4-7,2	Aumentado 5,9-10,9	~Adulto 5-7,8	~Adulto 5-7,8	~Adulto 5-7,8
Tempo de trânsito intestinal (h)	4,0	4,0	3-7,5	3-7,5	3-7,5
Função pancreática/ biliar	Imaturo	~ Adulto			

Fonte: BATCHELOR; FOTAKI; KLEIN, 2014; KAYE, 2011.
Nota: ~ = Similar

Ao nascer, as crianças apresentam o pH estomacal entre 6-8, devido a presença de fluido amniótico no estômago, e algumas horas depois o pH é reduzido a 1,5-3,0. Após 1 a 10 dias do nascimento o pH aumenta para 6-7 e reduz gradativamente para pH 1-2. Estas variações de pH conforme a idade podem impactar na liberação do fármaco da forma farmacêutica e no processo de solubilização podendo provocar a formação de precipitados (KAYE, 2011).

Sabe-se que a maior janela de absorção de fármacos é na porção do intestino delgado, de forma que o tempo de permanência da substância neste local é um dos fatores que impactam no processo de absorção. Com base nessa premissa, os parâmetros tempo de esvaziamento gástrico e tempo de trânsito intestinal estão diretamente relacionados ao tempo para observação da concentração máxima ($t_{máx}$) acelerando ou atrasando o processo de absorção (BATCHELOR; FOTAKI; KLEIN, 2014; KAYE, 2011). A área de superfície intestinal em crianças é proporcionalmente maior que em adultos o que pode

resultar em maior absorção, em contrapartida, a velocidade do trânsito intestinal é maior o que pode prejudicar a absorção (KAYE, 2011).

Outro parâmetro fisiológico relevante para a absorção é o volume estomacal, que pode ser de 10 a 100 mL em neonatos, chegando a 750-960 mL em crianças de 2 a 5 anos, o que corresponde ao volume em adultos (KAYE, 2011). A capacidade do estômago pode ser determinante para o volume de líquido que é administrado junto com a forma farmacêutica. Em estudos de bioequivalência (BE) em adultos, o volume de líquido administrado concomitante ao medicamento é de 150-250 mL de água (BATCHELOR; FOTAKI; KLEIN, 2014; DRESSMAN *et al.*, 2007; FDA, 2002). Em pediatria não existe um volume padronizado, mas muitos autores reconhecem que é necessário avaliar a população pediátrica em diferentes subgrupos. O volume de líquido presente no TGI permite que a forma farmacêutica libere o ativo e contribui para a solubilidade do fármaco (BATCHELOR; FOTAKI; KLEIN, 2014; CRAWFORD *et al.*, 1990; MARTIR *et al.*, 2020).

Assim como o volume de líquido administrado concomitante ao medicamento, as características dos fluidos gástricos e intestinais também são fundamentais para o processo de solubilização do fármaco e posterior absorção. Diante disso, a concentração de pepsina gástrica após o nascimento (1 a 8 dias) é de aproximadamente 15% da concentração do adulto. Nos neonatos (10-32 dias) e crianças (2-4 meses) essa concentração é de aproximadamente 41% atingindo níveis próximos ao do adulto em crianças de 2 a 5 anos (BATCHELOR; FOTAKI; KLEIN, 2014; MAHARAJ; EDGINTON; FOTAKI, 2016). Já as concentrações de sais biliares demonstraram ser bastante variáveis assim como as concentrações em adultos (FUCHS; DRESSMAN, 2014; MAHARAJ; EDGINTON; FOTAKI, 2016).

As variações decorrentes dos processos de crescimento e amadurecimento são pontos importantes para a farmacocinética e a extrapolação de dados de adultos para crianças pode trazer problemas que não são observados da mesma forma no adulto. Nesse caso, seriam necessários ensaios clínicos em crianças de diferentes idades para demonstrar que um medicamento pediátrico é seguro e eficaz em todos os grupos etários para os quais o medicamento está sendo desenvolvido. Apesar do aumento no número de pesquisas em crianças, esta população representa alguns desafios para a clínica, visto que existem dilemas éticos e dificuldades no recrutamento de voluntários que precisam do consentimento dos responsáveis (EMA, 2017a; FDA, 2020). Dessa forma muitos fármacos são empregados na prática clínica como *off-label* (fora da especificação de registro), quando a prescrição é feita por conta e risco do médico que o prescreve (BRASIL, 2021a).

Segundo Loureiro e colaboradores (2013), em um estudo realizado em ambiente hospitalar, quase a metade dos medicamentos prescritos (45,8%) eram de uso *off-label* em pediatria. Esse número demonstra a realidade de muitos fármacos que ainda são prescritos e administrados em crianças a partir de dados de estudos em adultos (LOUREIRO *et al.*, 2013).

Adicionalmente, a dificuldade de administração de fármacos em crianças pode estar relacionada a problemas farmacêuticos decorrentes do tamanho, formato e volume da forma farmacêutica. Por exemplo, bebês e crianças pequenas apresentam dificuldades em engolir comprimidos de tamanhos

convencionais e neonatos podem aceitar volumes muito pequenos devido a capacidade estomacal. De maneira geral, a aceitabilidade e a preferência entre formas de dosagem pediátrica variam entre as crianças de acordo com a idade, o estado de saúde individual, o comportamento, as deficiências, a formação e a cultura da família (EMA, 2013).

Diante disso, existe um movimento e incentivo, tanto por parte das agências reguladoras quanto pelas indústrias, para a realização de estudos de novos fármacos, ou a adequação dos disponíveis, com foco nas diferentes faixas etárias da população pediátrica. Estes devem considerar as dificuldades dos estudos clínicos com esta população evitando manipulações desnecessárias de voluntários e pacientes, além de contribuir com usos mais apropriados, seguros reduzindo aplicações *off-label* (EMA, 2013, 2017a; FDA, 2020).

2.2. Lamotrigina (LTG)

Considerado um fármaco novo em relação aos anticonvulsivantes mais tradicionais como a fenitoína e a carbamazepina, a LTG, quimicamente 3,5-diamino-6-(2,3-diclorofenil)-1,2,4-triazina, teve seus primeiros relatos em 1984 por Miller e colaboradores e 1985 por Cohen e colaboradores (COHEN *et al.*, 1985; MILLER *et al.*, 1984; PERUCCA, 2013). Esse fármaco anticonvulsivante de segunda geração é comumente de administração por via oral, apresenta longa duração e maior potência quando comparado a outros da mesma classe. No Brasil é amplamente prescrito para o tratamento de crises convulsivas sendo uma das alternativas do Protocolo Clínico e Diretrizes Terapêuticas da Epilepsia publicado pelo Ministério da Saúde e da Relação Nacional de Medicamentos Essenciais (RENAME/2020) (BRASIL, 2020, 2018c; MILOSHESKA *et al.*, 2016).

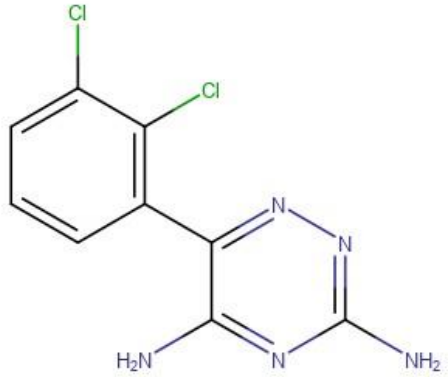

Figura 1. Estrutura química da lamotrigina
Fonte: DRUGBANK, 2021

A LTG é um fármaco que atua por dois mecanismos de ação inibitórios: (1) dos canais de sódio voltagem-dependentes e de canais de cálcio, o que resulta na diminuição dos potenciais elétricos pós-sinápticos, e (2) na redução da liberação de neurotransmissores, principalmente o glutamato. Esse

neurotransmissor é um aminoácido excitatório que desempenha papel-chave no desencadeamento de ataques epilépticos (BRASIL, 2018c; GSK, 2018; LANDMARK, 2007).

A LTG é indicada como monoterapia no tratamento de crises focais e generalizadas em pacientes com mais de doze anos de idade com intolerância ou refratariedade aos fármacos de primeira linha e, como terapia adjuvante de crises focais e generalizadas em pacientes com mais de dois anos de idade (BRASIL, 2018c). Além disso, ela é mais bem tolerada que a carbamazepina em idosos (BRASIL, 2018c; SAETRE *et al.*, 2007).

Os primeiros estudos farmacocinéticos com LTG foram realizados por Cohen e colaboradores (1987). Nestes estudos, foi demonstrada uma relação linear entre a dose de LTG administrada e os parâmetros de concentração plasmática máxima ($C_{máx}$) e exposição dada pela área sob a curva (ASC, em inglês *area under curve* - AUC). Essa relação linear sugere que não ocorre saturação nas etapas de transportes, ocorridas durante a absorção ou eliminação, bem como enzimáticas, durante a eliminação, no intervalo de dose entre 30 e 240 mg (COHEN et al, 1987).

O início do tratamento com a LTG deve ser realizado respeitando um esquema de escalonamento de dose para evitar efeitos colaterais como o exantema ou *rash* cutâneo. Em adultos, o tratamento deve ser iniciado em monoterapia, com dose de 25 mg uma vez ao dia, por duas semanas. Após esse período, a dose é aumentada para 50 mg uma vez ao dia, por outras duas semanas. A partir daí, a dose pode ser aumentada em até 100 mg a cada uma ou duas semanas, de acordo com o paciente e a remissão das crises convulsivas, respeitando a dose máxima de 500 mg administrada fracionada em duas ou três vezes ao dia. A dose usual de manutenção é de 100 a 200 mg/dia administradas uma ou duas vezes ao dia (GSK, 2018; SIDHU *et al.*, 2006; THEIS *et al.*, 2005).

A LTG é amplamente distribuída em todos os órgãos e tecidos com volume de distribuição aparente (V_d) em humanos entre 0,92 e 1,22 L/kg (GSK, 2018; RAMBECK; WOLF, 1993). Apresenta taxa de ligação às proteínas plasmáticas de aproximadamente 55% e, não é afetada pela administração concomitante de outros antiepilépticos altamente ligados a proteínas como o ácido valpróico, fenitoína e fenobarbital, na faixa de concentração terapêutica de 1 a 10 mg/L (RAMBECK; WOLF, 1993).

A via de eliminação predominante da LTG é o metabolismo hepático uma vez que, a excreção da forma inalterada por via renal é inferior a 10% e fecal é inferior a 2% (DOIG; CLARE, 1991; GARNETT, 1997; MILOSHESKA *et al.*, 2016). A inativação metabólica da LTG é catalisada pelas UDP-glucuronosiltransferases (UGT): UGT1A4 e UGT1A3, sendo a UGT1A3 em menor extensão considerando sua baixa expressão no fígado (CONNER; REED; ZHANG, 2019). O principal metabólito inativo formado é o LTG-2-N-glucuronídeo (LTG-2N-glu) (Figura 2), que corresponde a aproximadamente 80% da dose em humanos e é excretado na urina. O metabólito LTG-5-N-glucuronídeo (LTG-5N-glu) também é formado, mas em uma extensão menos significativa (ARGIKAR; REMMEL, 2009; MILOSHESKA *et al.*, 2016). Em adultos saudáveis, o tempo de meia-vida de eliminação ($t_{1/2}$) da LTG é de 24 a 35 h, o clearance (CL) aparente é de 1,50 a 2,60 L/h, e o clearance

renal (CLr) de 0,18 a 0,20 L/h (COHEN et al, 1987; RAMBECK; WOLF, 1993; WOOTTON et al., 1997; GSK, 2018; CONNER; REED; ZHANG, 2019).

No TGI, a LTG é rápida e completamente absorvida apresentando um $t_{máx}$ entre 1 e 3 h. Apresenta biodisponibilidade oral de aproximadamente 98%, indicando nenhum efeito ou efeito insignificante de metabolismo de primeira passagem (GARNETT, 1997; PECK, 1991; RAMBECK; WOLF, 1993). Após alimentação, o $t_{máx}$ da LTG pode ser discretamente retardado, porém, a extensão da absorção não é afetada (GSK, 2018).

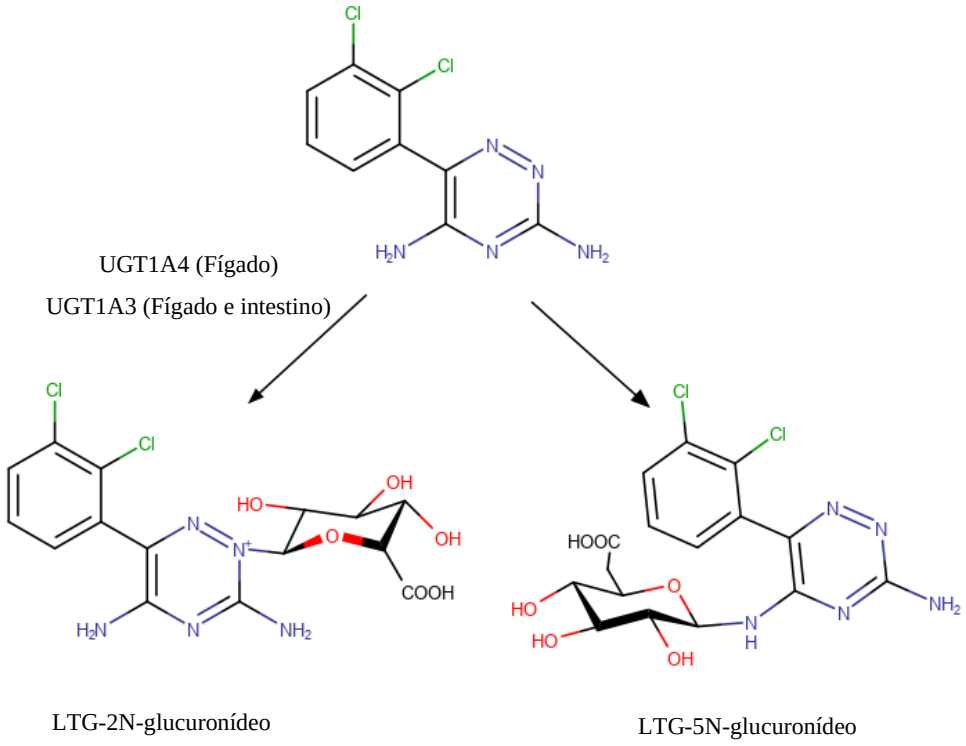

Figura 2. Principais metabólitos inativos da lamotrigina.
Fonte: Adaptado de ARGIKAR E REMMEL, 2009.

O insumo farmacêutico ativo (IFA) de LTG é caracterizado como um pó de coloração branca a creme pálido, apresenta peso molecular de 256,09 g/mol, pKa 5,7 e log P entre 1,19 e 1,93 (CONNER; REED; ZHANG, 2019; DRUGBANK, 2021). Pelo Sistema de Classificação Biofarmacêutica (SCB) (do inglês *Biopharmaceutics Classification System* - BCS) é um fármaco de classe IIb: uma base fraca com baixa solubilidade e alta permeabilidade (AMIDON *et al.*, 1995; BITON, 2006; TSUME *et al.*, 2014; VAITHIANATHAN *et al.*, 2015).

Como base fraca, sua solubilidade em meio aquoso é dependente do pH do meio. Em valores de pH de caráter mais ácidos a LTG tende a exibir alta solubilidade, por apresentar-se na forma ionizada. Em contrapartida, em meios mais neutros a básicos a solubilidade diminui, por apresentar-se majoritariamente na forma molecular (MARTINS; PAIM; STEPPE, 2010; VAITHIANATHAN *et al.*, 2015). Isso significa que apesar de ser considerada altamente solúvel no pH ácido do estômago, esse

fármaco pode precipitar no ambiente intestinal onde o pH é mais neutro (pH 6,0 – 7,5). Esse fenômeno já foi reportado para outros fármacos BCS II, tais como dipiridamol (KOSTEWICZ *et al.*, 2004), cetoconazol (KAMBAYASHI; YASUJI; DRESSMAN, 2016), tamoxifeno e itraconazol (KLEIN; BUCHANAN; BUCHANAN, 2012). Além da precipitação, a mudança repentina do pH ácido para o neutro/básico que ocorre logo após o esvaziamento gástrico, pode levar a mudanças no estado dissolvido como a emulsificação e formação de micro/nanoestruturas, assim como reportado para o fármaco bissulfato de clopidogrel (OLIVEIRA, 2018). Dessa forma, a precipitação ou outra mudança na condição físico-química do fármaco diminui as concentrações dissolvidas desse IFA no ambiente intestinal, onde ocorre a absorção, podendo diminuir a extensão e/ou velocidade da absorção (ICH, 2019; KERNS; DI; CARTER, 2008).

Atualmente, somente formas de administração oral de LTG estão registradas nas agências reguladoras brasileira (BRASIL, 2021b), norte americana (FDA, 2021b) e europeia (*European Medicines Agency* - EMA) (EMA, 2018a), sendo elas comprimidos simples, orodispersíveis, dispersíveis (ou comprimidos para suspensões) e de liberação modificada. Tais formas farmacêuticas acrescentam uma etapa no processo da biodisponibilidade que é a dissolução do fármaco no ambiente gastrointestinal. Em suma, a LTG precisa ser liberada da forma farmacêutica e dissolvida para estar disponível para a absorção (AMIDON *et al.*, 1995).

Á luz dessas características e particularidades biofarmacêuticas da LTG, como a solubilidade, a possibilidade de precipitação em ambiente intestinal, as apresentações farmacêuticas orais e a importância do processo de dissolução, vemos a relevância dos estudos e pesquisas para a compreensão desses processos. Estes estudos podem produzir informações de base e fornecer embasamentos para aplicações desde o âmbito farmacotécnico ao clínico.

2.3. Propriedades biofarmacêuticas

A biofarmácia (do inglês *Biopharmaceutics*) consiste no estudo das relações entre as propriedades físico-químicas dos fármacos, a forma farmacêutica em que são administrados, a via de administração e os efeitos biológicos observados. Fazem parte dessa ciência: a estabilidade, a liberação da forma farmacêutica, a velocidade e taxa de liberação/dissolução no local de absorção e a absorção sistêmica de um fármaco. A base da biofarmácia está na fundamentação científica e nas experimentações *in vitro* e *in vivo* (SHARGEL; WU-PONG; YU, 2004a).

Dentre os estudos e aplicações biofarmacêuticas, um dos mais famosos e útil até os dias atuais é o SCB. Criado por Amidon e colaboradores (1995), esse sistema é baseado na prerrogativa de que a solubilidade de um fármaco e a sua permeabilidade gastrointestinal são parâmetros fundamentais no controle da taxa e da extensão da sua absorção (AMIDON *et al.*, 1995). De acordo com a classificação BCS, os fármacos são categorizados em: classe I – alta solubilidade e alta permeabilidade, classe II – baixa solubilidade e alta permeabilidade, classe III – alta solubilidade e baixa permeabilidade e, classe

IV – baixa solubilidade e baixa permeabilidade. Somada a solubilidade e a permeabilidade, outro fator que influencia a taxa e extensão de absorção é a dissolução de um fármaco ou produto farmacêutico oral. Isso porque, muitas vezes, a velocidade de dissolução é mais lenta que o tempo de esvaziamento gástrico (FDA, 2017).

Baseadas no BCS são levantadas as seguintes suposições: para os fármacos BCS I em formas farmacêuticas de liberação imediata com dissolução muito rápida, a taxa de absorção é dependente da velocidade do esvaziamento gástrico. Para os fármacos de classe II, a taxa de dissolução *in vivo* é responsável por controlar a velocidade de absorção. Os fármacos de classe III diferentemente das outras classes, apresentam a permeabilidade como fator determinante. Por fim, para os fármacos de classe IV ambas as taxas de dissolução e permeabilidade são fatores determinantes para a velocidade e extensão da absorção (AMIDON *et al.*, 1995).

Outro sistema de classificação biofarmacêutico foi proposto em 2005 por Wu e Benet. O sistema de classificação biofarmacêutico com base na disposição do fármaco (BDDCS, do inglês *Biopharmeceutics Drug Disposition Classification System*) assume as mesmas premissas para a solubilidade que o BCS com novas definições para a permeabilidade. Os autores sugerem que se a maior rota de eliminação do fármaco for via metabolismo então este pode ser classificado como alta permeabilidade, ao passo que, fármacos que apresentam a maior rota de eliminação renal ou biliar com eliminação da molécula na forma molecular podem ser classificados como de baixa permeabilidade (BENET *et al.*, 2008; WU; BENET, 2005).

Tanto para o BCS quanto para o BDDCS, a solubilidade é determinada empregando a maior dose única terapêutica em 250 mL ou menos de meio aquoso dentro da faixa de pH de 1,0 a 6,8 a 37º C (BRASIL, 2022; EMA, 2018b). Essa definição é diferente de outros conceitos físico-químicos como: solubilidade em equilíbrio, intrínseca, cinética e aparente. A solubilidade em equilíbrio (S_{eq}), ou termodinâmica, é aquela determinada a partir de uma solução saturada, contendo sólido em excesso, onde a solução e o sólido estão em equilíbrio. A solubilidade intrínseca (S_0) é a S_{eq} de uma substância ionizável, mas que se encontra totalmente na forma não ionizada devido ao pH do meio. Já a solubilidade cinética (S_{cin}) é definida por meio da avaliação da concentração da substância e a velocidade de formação dos primeiros precipitados. Por fim, a solubilidade aparente (S_{ap}) é aquela onde não é garantido o processo de equilíbrio termodinâmico (ELDER; HOLM, 2013; OLIVEIRA, 2018). Definidos os conceitos, a solubilidade empregada para a avaliação do BCS e BDDCS também pode ser determinada por meio da razão entre dose terapêutica e a S_{eq} (D/S), dessa forma D/S menores que 250 mL indicam alta solubilidade do fármaco (OH; CURL; AMIDON, 1993).

A dissolução, por outro lado, é uma prática *in vitro* empregada há muitos anos pela indústria farmacêutica em diversas etapas da pesquisa e desenvolvimento e do controle de qualidade do produto acabado lote a lote. O ensaio de dissolução é um teste físico-químico que avalia a liberação do IFA a partir da forma farmacêutica durante um período de tempo. A resposta é dada pela porcentagem de fármaco dissolvida no meio (BRASIL, 2018a; FDA, 1997a; MARTINS; PAIM; STEPPE, 2010).

Para o desenvolvimento de um bom e discriminativo método de dissolução, alguns tópicos devem ser considerados como a escolha do aparato de dissolução, a velocidade de agitação, tempos de coletas e a seleção do meio de dissolução (composição e volume). Tais escolhas devem ser baseadas nas características físico-químicas da substância ativa, na faixa de dosagem pretendida do medicamento, da formulação a ser testada e também do propósito do teste (EMA, 2017b). O propósito do teste é um fator muito importante e cabe ressaltar que para a dissolução empregada com fins de controle de qualidade lote a lote dá-se preferências a métodos mais simples e rápidos normalmente já descritos nas farmacopeias oficiais e, para a dissolução empregada na pesquisa e desenvolvimento buscam-se métodos mais biodescritivos (PEPIN *et al.*, 2021).

Assim como nos testes de solubilidade, para os testes de dissolução os guias regulatórios sugerem a utilização de meios aquosos com características semelhantes as condições fisiológicas e pHs entre 1,2 a 6,8, seguindo os preparos descritos em farmacopeias oficiais e temperatura de 37° C (BRASIL, 2011, 2018a, 2022; FDA, 1997a; USP, 2020). Nestes compêndios o meio aquoso tamponado de pH 6,8 é também denominado como fluido intestinal simulado (SIF) e o meio aquoso de pH 1,2 sem adição de enzimas como fluido gástrico simulado (SGF) (BRASIL, 2018a; FDA, 1997a).

Apesar dos pHs utilizados nesses meios serem representativos do TGI, sabe-se que os fluidos fisiológicos são mais complexos que os meios farmacopeicos, com capacidade tamponante variada, além de serem constituídos por substâncias como tensoativos e enzimas que podem auxiliar ou prejudicar a dissolução do IFA (CRISTOFOLETTI; DRESSMAN, 2016; HENS *et al.*, 2018; KAUR *et al.*, 2018). À vista disso, nos últimos anos, muitos pesquisadores têm estudado e empregado meios biomiméticos ou biorrelevantes. Dressman e colaboradores introduziram as primeiras propostas de composições destes meios em 1998 e, desde então, muitas atualizações, versões e aplicações foram propostas por diversos autores. O principal objetivo deles foi de mimetizar os fluidos do TGI com o auxílio de reagentes como taurocolato de sódio, lecitina e pepsina e, assim, auxiliar a compreensão de como os medicamentos e formulações se comportam *in vivo* (DRESSMAN *et al.*, 1998; FUCHS; DRESSMAN, 2014; JANTRATID *et al.*, 2008; KAUR *et al.*, 2018; KERNS; DI; CARTER, 2008; MANN *et al.*, 2017; VERTZONI *et al.*, 2004). A fim de refletir todo o TGI, é possível encontrar na literatura meios que representam o estômago no estado em jejum e alimentado, por exemplo: simulado gástrico em jejum *(fasted state simulated gastric fluid* - FaSSGF) e simulado gástrico alimentado (*fed state simulated gastric fluid* - FeSSGF) e, meios que representam as porções intestinais: simulado intestinal em jejum (*fasted state simulated intestinal fluid* - FaSSIF) e simulado intestinal alimentado (*fed state simulated intestinal fluid* - FeSSIF) (FUCHS *et al.*, 2015; JANTRATID *et al.*, 2008; KAUR *et al.*, 2018).

Os meios de dissolução podem ser classificados em diferentes níveis que variam desde os mais simples (Nível 0, que inclui a água) até meios muito complexos que combinam substâncias fisiologicamente relevantes, como enzimas, produtos da digestão de gordura e componentes da bile (Nível 3). O conceito de níveis, para os meios de dissolução, pode ser combinado com os diferentes

tipos de aparatos de dissolução que estão comercialmente disponíveis para gerar uma ampla variedade de possibilidades em potencial. Isso representa um desafio para os pesquisadores e reguladores na decisão de qual teste pode ser ideal para uma determinada combinação de medicamento e forma farmacêutica (MARKOPOULOS *et al.*, 2015; PEPIN *et al.*, 2021). Quaisquer que sejam os métodos *in vitro* escolhidos, é necessária uma abordagem científica para a obtenção de dissoluções biopreditivas.

Nesse contexto, no ano de 2019, algumas terminologias empregadas em técnicas biofarmacêuticas *in vitro* foram definidas durante o workshop "*Current State and Future Expectations of Translational Modeling Strategies to Support Drug Product Development, Manufacturing Changes and Controls*" (MITRA *et al.*, 2021; PARROTT *et al.*, 2021; PEPIN *et al.*, 2021), dentre elas:

> **Método de dissolução biorrelevante:** Conjunto de condições para monitorar a dissolução *in vitro* projetado para simular um fluido biológico relevante e um ambiente fisiológico.
>
> **Método de dissolução biopreditivo:** Conjunto de condições para as quais os perfis de dissolução *in vitro* podem prever perfis farmacocinéticos. Estes são tipicamente baseados na correlação clássica ou mecanística *in vitro-in vivo*.
>
> **Especificações de dissolução clinicamente relevantes:** Conjunto de condições de dissolução *in vitro* e critérios de aceitação, que podem identificar e rejeitar lotes de medicamentos que não devem ser bioequivalentes aos lotes de produtos clínicos de referência.

Vale ressaltar que o objetivo dos métodos de dissolução biopreditivos é apoiar a concepção de estudos clínicos apropriados, identificando diferenças no desempenho farmacocinético entre variantes de medicamentos, informando sobre o risco de bio-inequivalência e apoiando a construção de um "espaço seguro" baseado em estudos *in vitro-in vivo* (PEPIN *et al.*, 2021).

Uma das principais aplicações dos métodos biorrelevantes e biopreditivos é no estudo de fármacos que apresentam baixa solubilidade, como é o caso da LTG, e que podem ter a absorção prejudicada por esta característica (BERBEN *et al.*, 2019; HANSMANN; MIYAJI; DRESSMAN, 2018). Além disso, os métodos de dissolução biorrelevantes e biopreditivos *in vitro* correlacionados com dados farmacocinéticos *in vivo* são ferramentas extremamente úteis para tomadas de decisão dentro do ambiente da pesquisa e desenvolvimento e, para o estudo do impacto das propriedades biofarmacêuticas de um fármaco para a clínica (BERMEJO *et al.*, 2020).

2.4. Farmacometria e abordagens de predição *in vitro-in vivo*

Antes de iniciarmos a discussão sobre a farmacometria e as abordagens de predição *in vitro-in vivo*, é necessário ponderar e diferenciar alguns termos e expressões:

> **Farmacometria:** É a ciência da farmacologia quantitativa que está diretamente associada ao desenvolvimento e aplicação de métodos matemáticos e estatísticos para caracterizar, compreender e prever o comportamento de um fármaco quanto a farmacocinética, farmacodinâmica e biomarcadores (BONATE, 2011; WILLIAMS; ETTE, 2007).
>
> **Correlação *in vitro-in vivo* (CIVIV):** É um modelo matemático preditivo que faz a ligação entre a propriedade *in vitro* de uma forma de dosagem oral e a resposta *in vivo*. Geralmente, a propriedade *in vitro* é a taxa ou extensão da dissolução ou liberação do fármaco, enquanto a resposta *in vivo* é a concentração plasmática ou a quantidade absorvida (FDA, 1997b).

As CIVIV são categorizadas em quatro níveis. O nível A: representa uma correlação ponto a ponto entre os dados *in vitro* e os dados *in vivo*. O nível B: utiliza os princípios da análise de estatística do momento na qual o tempo médio de dissolução *in vitro* é comparado ao tempo médio de residência ou ao tempo médio de dissolução *in vivo*. O nível C: estabelece uma relação de ponto único entre um parâmetro de dissolução e um farmacocinético. E por fim, o nível C múltiplo: relaciona um ou vários parâmetros farmacocinéticos de interesse à quantidade de fármaco dissolvido em diferentes tempos (FDA, 1997b).

> **Relação *in vitro-in vivo* (RIVIV):** Refere-se a outras abordagens semiquantitativas ou de classificação. RIVIV é qualquer tipo de relação entre as propriedades de dissolução *in vitro* e o desempenho *in vivo* que não esteja incluído no conceito clássico de CIVIV. Assim, RIVIV também incluiu casos em que as alterações nas propriedades de dissolução *in vitro* não afetam a farmacocinética *in vivo*, resultando em um "espaço seguro" de dissolução (FDA, 1997c; LOISIOS-KONSTANTINIDIS *et al.*, 2020; NGUYEN *et al.*, 2017).
>
> **Deconvolução:** Técnica empregada para a determinação da porcentagem da fração absorvida ou do perfil de dissolução *in vivo* a partir de dados de concentração plasmática pelo tempo, para o desenvolvimento de uma CIVIV ou RIVIV (MARROUM, 2007).
>
> **Convolução:** É a predição do perfil de concentração plasmática *in vivo*, a partir da CIVIV ou RIVIV estabelecida previamente, empregando dados *in vitro* como perfis de dissolução (MARROUM, 2007).
>
> **Biodisponibilidade (F) (do inglês *bioavailability* - BA):** É a estimação da fração relativa da dose administrada por via oral que é absorvida e se encontra disponível na circulação sistêmica ou sítio de ação utilizando voluntários ou pacientes (FDA, 2002).
>
> **Bioequivalência (BE):** É o estudo da comparação entre as F e demonstração de equivalência terapêutica entre produtos apresentados sob a mesma forma farmacêutica, contendo idêntica composição de princípios ativos (BRASIL, 2021c).
>
> **Bioisenção:** É a isenção de estudos de BE por meio de justificativa formal utilizando dados experimentais *in vitro* (FDA, 2017).

No universo da pesquisa e desenvolvimento de fármacos, como vimos anteriormente, a etapa clínica é a que se destaca pelo tempo gasto e alto custo envolvido, além de ser a etapa com mais chances de falhas (PAUL *et al.*, 2010). Considerando que todos os anos inúmeros estudos clínicos são realizados pelas indústrias, seja para o desenvolvimento de novos fármacos ou para o desenvolvimento de medicamentos genéticos e similares, estudos com resultados não bioequivalentes (ou bioinequivalentes) são experiências indesejadas. Principalmente se considerarmos o custo despendido que varia entre R$ 300 mil a R$ 1 milhão de reais para cada estudo de BE (ICTQ, 2021).

À vista disso, a farmacometria e as técnicas de CIVIV e RIVIV estão sendo empregadas como guia no desenvolvimento de formulações, fornecendo evidências regulatórias em mudanças de pós-registro como alteração de local de fabricação, tamanho de lote, especificações de dissolução que sejam clinicamente relevantes e, principalmente, justificando bioisenções (KOVAĈEVIĆ *et al.*, 2009; NGUYEN *et al.*, 2017; RUIZ PICAZO *et al.*, 2018). Com as bioisenções é possível reduzir o tempo de desenvolvimento, o custo desprendido nas etapas clínicas e a exposição desnecessária de voluntários, além de incentivar a pesquisa e desenvolvimento de fármacos e, ao final do ciclo, beneficiar o paciente com uma maior gama de produtos (ARRUNÁTEGUI *et al.*, 2015; DAVANÇO; CAMPOS; CARVALHO, 2020).

Fundamentado no SCB, o FDA já permite a bioisenção para fármacos de classe I e III em formulações sólidas orais de liberação imediata desde que apresentem excipientes que não interfiram na velocidade e extensão da absorção (FDA, 2017).

O Brasil é membro, desde 2018, do Conselho Internacional para Harmonização de Requisitos Técnicos para Medicamentos de Uso Humano (do inglês *The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use* – ICH). Este conselho reúne autoridades reguladoras e associações de indústrias farmacêuticas para discutir aspectos técnicos e científicos para o registro de medicamentos (BRASIL, 2018b). Assim como o FDA, para o ICH a bioisenção de formulações é permitida desde que a substância ativa satisfaça os critérios de solubilidade e permeabilidade (BCS I e III), e esteja em uma forma farmacêutica oral de liberação imediata com ação sistêmica (ICH, 2019).

No FDA, a abordagem CIVIV foi inicialmente regulamentada em 1997 somente para produtos de liberação modificada, mas seu conceito foi estendido e é hoje também aplicada para formas de dosagem de liberação imediata (EMA, 2014; FDA, 1997b; NGUYEN *et al.*, 2017). Tanto a abordagem CIVIV quanto RIVIV são ferramentas capazes de prever as características de biodisponibilidade esperadas para um produto a partir de dados *in vitro* (FDA, 1997b).

Considerando que o principal experimento *in vitro* empregado nesses modelos é a dissolução, as melhores correlações são esperadas para fármacos classe II do SCB, visto que a absorção destas moléculas é dependente da sua liberação/ dissolução da forma farmacêutica. Os fármacos classe III e IV apresentam limitações por apresentarem a permeabilidade como fator limitante, Já os fármacos classe I podem também apresentar limitações por serem dependentes do tempo de esvaziamento gástrico (DAVANÇO; CAMPOS; CARVALHO, 2020).

Para as modelagens C/RIVIV, os experimentos devem ser planejados de forma a garantir a avaliação da cinética de dissolução *in vitro* por meio de um número suficiente de coletas e análises, que permitam elucidar a forma da curva de porcentagem de dissolução pelo tempo. Nos casos onde os pontos de coleta de dados da dissolução são divergentes dos pontos de concentração plasmática pelo tempo *in vivo*, podem ser aplicados modelos matemáticos não lineares como Weibull, Higuchi, Korsemeyer-peppas entre outros (COSTA; SOUSA LOBO, 2001; DAVANÇO; CAMPOS; CARVALHO, 2020).

Já os dados *in vivo* são provenientes de perfis de concentração plasmática pelo tempo de estudos farmacocinéticos. Estes dados são previamente convertidos em fração da dose absorvida ou fração absorvida (Fa) pelo processo de deconvolução, que estima a entrada do fármaco no sistema biológico com base no balanço de massas. Dentre os métodos de deconvolução mais empregados estão: Wagner-Nelson, Loo-Riegelman, numérica e mecanística (DUTTA *et al.*, 2005; LOO; RIEGELMAN, 1968; MARGOLSKEE *et al.*, 2016; WAGNER; NELSON, 1963).

O método por Wagner-Nelson (WAGNER; NELSON, 1963), que é um dos mais empregados, é baseado na farmacocinética de um compartimento e tem a vantagem de não precisar de dados de perfis intravenosos, somente do perfil plasmático oral. O cálculo pode ser expresso da seguinte forma:

$$F_a(t) = \frac{C + k\int_0^t C_t\ dt}{k\int_0^\infty C_t\ dt} \qquad (1)$$

onde, Fa (t) é a fração absorvível do fármaco no tempo t, C é a concentração do fármaco no compartimento central no tempo t, e k é a constante de eliminação de primeira ordem.

O método de Loo-Riegelman (LOO; RIEGELMAN, 1968) é baseado na farmacocinética de dois compartimentos, e diferentemente do método anterior, requer dados de administração intravenosa do mesmo indivíduo. O método de deconvolução numérica é modelo-independente, ou seja, ele não faz suposições a respeito do número de compartimentos ou da cinética de absorção. O método numérico requer dados de administração de soluções orais ou intravenosa (DAVANÇO; CAMPOS; CARVALHO, 2020).

Outra técnica de deconvolução que vem emergindo com o avanço das abordagens fisiológicas e computacionais é a mecanística. O modelo mecanístico de deconvolução inclui compartimentos de desintegração, dissolução, permeação, trânsito gastrointestinal, metabolismo intestinal e metabolismo de primeira passagem com, aproximadamente, 126 equações diferenciais não lineares (DAVANÇO; CAMPOS; CARVALHO, 2020; YU, Alex *et al.*, 2020).

Em conjunto com o modelo mecanístico adveio a modelagem biofarmacêutica com base fisiológica (do inglês *Physiologically based biopharmaceutics modeling* - PBBM) e a modelagem de absorção de base fisiológica (do inglês *Physiologically based absorption model* – PBAM com o intuito de estabelecer uma ligação entre a dissolução *in vitro* e uma modelagem mecanística de absorção oral utilizando para isso a modelagem farmacocinética fisiológica (*Physiologically based pharmacokinetic* - PBPK) (BERMEJO *et al.*, 2020).

2.5. Modelagens PBPK, PBAM e PBBM

A modelagem farmacocinética com base fisiológica ou PBPK é conceitualmente a aplicação de modelos matemáticos fundamentados na fisiologia do indivíduo, a fim de simular as concentrações de um fármaco ao longo do tempo no(s) tecido(s) e no sangue. Um modelo PBPK considera a taxa de absorção, distribuição nos tecidos, metabolismo e excreção, com base no movimento e disposição do fármaco no organismo (EMA, 2018c; KAUR *et al.*, 2018; SHARGEL; WU-PONG; YU, 2004b).

Atualmente, os principais objetivos dos modelos PBPK em submissões regulatórias são para prever qualitativa e quantitativamente as interações medicamentosas (DDI) e apoiar a seleção da dose em estudos pediátricos e primeira dose em humanos. A modelagem PBPK também pode ser usada como ferramenta para prever o perfil farmacocinético de um composto, criar extrapolação entre espécies, avaliar estados de doença, ajustes de doses em populações especiais como crianças, idosos e grávidas,

auxiliar no desenvolvimento de formulações entre outras (BIESDORF *et al.*, 2019; EMA, 2018c; KAUR *et al.*, 2018).

O modelo PBPK, assim como o modelo tradicional compartimental, é constituído por compartimentos, no entanto, a principal diferença está na forma como eles estão interligados e na entrada de dados. Os modelos compartimentais são tradicionalmente desenvolvidos usando a abordagem "*top-down*", onde toda a informação é proveniente de estudos farmacocinéticos e, além disso, nesses modelos raramente são empregados mais que três compartimentos (UPTON; FOSTER; ABUHELWA, 2016).

No PBPK as informações para a construção do modelo são provenientes de dados de base ou de mecanismos fisiológicos e farmacológicos *a priori*, esta abordagem é identificada como "*bottom-up*". Na modelagem fisiológica também é empregada, e muitas vezes necessária, uma abordagem do tipo "*middle-out*" a qual emprega dados de base, fisiológicos e provenientes de estudos farmacocinéticos. O modelo PBPK é formado por um conjunto de equações diferenciais que descrevem o balanço de massas e o destino de uma substância em cada um dos compartimentos fisiológicos. Esses compartimentos são conectados pelo fluxo do sistema sanguíneo circulante (Figura 3). Os modelos fisiológicos fornecem uma estrutura mecanística quantitativa que permite a extrapolação de parâmetros específicos do fármaco, por meio de técnicas de extrapolação *in vitro-in vivo* (do inglês *in vitro-in vivo extrapolation-IVIVE*). Dessa forma, pode-se prever os perfis de concentração plasmática pelo tempo de fármacos, além da concentração da molécula no(s) tecido(s) alvo (JONES; ROWLAND-YEO, 2013; UPTON; FOSTER; ABUHELWA, 2016).

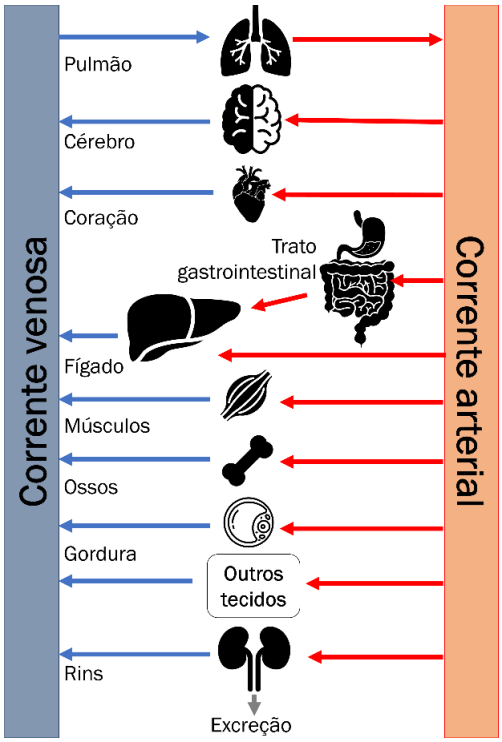

Figura 3. Diagrama esquemático de um modelo farmacocinético fisiológico (do inglês *Physiologically based pharmacokinetic model* – PBPK)
Fonte: Adaptado de Reddy *et al.* 2013 e PIO *et al.* 2003

O uso de modelos multicompartimentais incorporando componentes biológicos e fisiológicos para a simulação de dados farmacocinéticos foi inicialmente proposto por Torsten Teorell em 1937, que hoje é considerado o pai da farmacocinética (PAALZOW, 1995; TORSTEN, 1937). Apesar do conceito de PBPK não ser tão novo, foi só recentemente que as aplicações expandiram devido ao desenvolvimento de sistemas computacionais que pudessem simplificar a complexidade matemática desses modelos. Além disso, esses modelos requerem uma grande quantidade de parâmetros e informações de base, o que envolveu e ainda envolve um importante tempo de pesquisa antes da modelagem PBPK (JONES; ROWLAND-YEO, 2013).

Para o desenvolvimento e construção de um PBPK são necessários dados de base, como já mencionado. Esses dados são compostos por descritores físico-químicos da molécula como: solubilidade em água e em meios biorrelevantes, permeabilidade, logaritmo negativo da constante de ionização ácida (pKa), logaritmo do coeficiente de partição octanol: água (log $P_{o:a}$), logaritmo do coeficiente de distribuição (log D), entre outros. Adicionalmente, são empregados dados farmacocinéticos da molécula obtidos por meio de ensaios *in vitro*, pré clínico e clínico. Outros fatores importantes são intrínsecos e extrínsecos do organismo. Dentre os fatores intrínsecos estão: idade, etnia, doença, gênero, gravidez, lactação entre outros. Já os fatores extrínsecos correspondem ao ambiente, práticas médicas, diretrizes, consumo de álcool, dieta, consumo de cigarros e possibilidade de interação fármaco-fármaco (KUEPFER *et al.*, 2016; ZHAO *et al.*, 2011).

Nos últimos dez anos, ocorreram muitos avanços com as ferramentas para modelagem PBPK. Os modelos podem ser construídos utilizando pacotes de programação como MATLAB®, software R, e também com softwares comercialmente disponíveis como GastroPlus® (Simulations Plus Inc., www.simulations-plus.com), SimCyp (Simcyp, www.simcyp.com) e PK-Sim® (Bayer Technology Services, www.pksim.com). Os softwares comerciais são de interface amigável, o que simplifica o uso técnico dos modelos PBPK, no entanto um bom entendimento dos modelos e das equações ainda é obrigatório, a fim de garantir uma boa interpretação dos resultados (REDDY *et al.*, 2013; SY; WANG; DERENDORF, 2014).

Outro adicional dos softwares comerciais é que eles permitem incorporar parâmetros de absorção ao modelo. O objetivo principal dessas abordagens, que são chamadas de modelagem e simulação de absorção oral, é prever a F de um fármaco, que é o produto da fração absorvida no TGI (Fa), a fração que resiste ao metabolismo do TGI (Fg) e a fração que resiste ao metabolismo hepático de primeira passagem (Fh). O processo de absorção é influenciado por diversos fatores inerentes ao fármaco e a fisiologia. Dessa forma, os modelos mecanísticos concentram-se na predição da absorção oral integrando os processos de trânsito gastrointestinal, dissolução e permeação, e incorporando informações do IFA e dos excipientes que compõem o medicamento, em uma estrutura matemática sistêmica de corpo inteiro (ZHANG *et al.*, 2017; ZHANG; LIONBERGER, 2014).

Os modelos de absorção mecanísticos consideram o TGI dividido em vários compartimentos com suas próprias propriedades. A construção desses modelos inclui parâmetros fisiológicos como o pH do

lúmen gastrointestinal, tempo de esvaziamento gástrico e tempo de trânsito, geometria do órgão, volume, composição dos fluidos e distribuição regional de transportadores e enzimas (JIANG *et al.*, 2011; THELEN *et al.*, 2011; ZHANG; LIONBERGER, 2014).

Um dos primeiros modelos de absorção proposto integrava ao PBPK os processos de dissolução e permeação em um "tanque de mistura" (do inglês, *mixing tank model*), considerando o TGI como um compartimento bastante agitado (do inglês, *well-stirred compartment*). A quantidade de fármaco que entrava no compartimento era considerada instantaneamente misturada no TGI e o movimento era governado pelo tempo de trânsito intestinal (DRESSMAN; FLEISHER, 1986). Apesar deste modelo não considerar o metabolismo intestinal, metabolismo de primeira passagem e as instabilidades químicas do fármaco, ele auxiliou como fundamentação na construção de outros modelos de absorção oral (LIN; WONG, 2017).

Seguindo os princípios do *mixing tank*, Yu e colaboradores criaram o modelo de absorção e trânsito compartimental (CAT – do inglês *Compartmental Absorption and Transit model*) (YU, Lawrence X.; CRISON; AMIDON, 1996). No CAT (Figura 4) o intestino delgado é dividido em sete partes: o primeiro compartimento corresponde ao duodeno, os dois seguintes ao jejuno e os quatro últimos ao íleo. Neste modelo é assumido transporte passivo, dissolução instantânea, cinética de transferência linear entre os compartimentos e, além disso, tempo de trânsito, permeabilidade e diâmetro do órgão constantes para todos os compartimentos (HUANG; LEE; YU, 2009). Mais tarde, foram adicionados ao modelo compartimentos adicionais representando o fármaco dissolvido e não dissolvido (LIN; WONG, 2017; YU, Lawrence X., 1999).

Seguindo os princípios do modelo CAT adveio o modelo de absorção e trânsito compartimental avançado (ACAT – do inglês *Advanced Compartmental Absorption and Transit model*). No ACAT (Figura 5) são considerados os sete compartimentos do modelo CAT adicionados o estômago e o cólon, formando o total de nove compartimentos, acrescidos dos processos de metabolismo intestinal e hepático (LIN; WONG, 2017). No modelo ACAT são consideradas as cinéticas de transferência lineares e não lineares, seis estados do fármaco: não liberado da forma farmacêutica, não dissolvido, dissolvido, degradado, metabolizado e absorvido, e três estados de material excretado: não liberado da forma farmacêutica, não dissolvido e dissolvido. A vantagem do ACAT é a possibilidade de avaliar e investigar os efeitos relacionados a formulação e propriedades biofarmacêuticas. O software comercial GastroPlus® foi desenvolvido com base no modelo ACAT (GOBEAU *et al.*, 2016; HUANG; LEE; YU, 2009; LUKACOVA; DIBELLA, 2022).

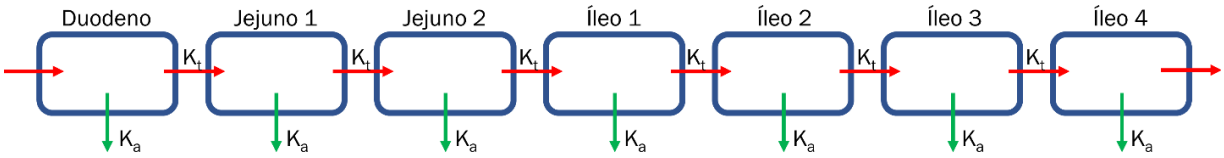

Figura 4. Representação gráfica do modelo de absorção e trânsito compartimental (CAT). Kt: Constante de trânsito, Ka: Constante de absorção.
Fonte: Adaptado de Lin e Wong, 2017

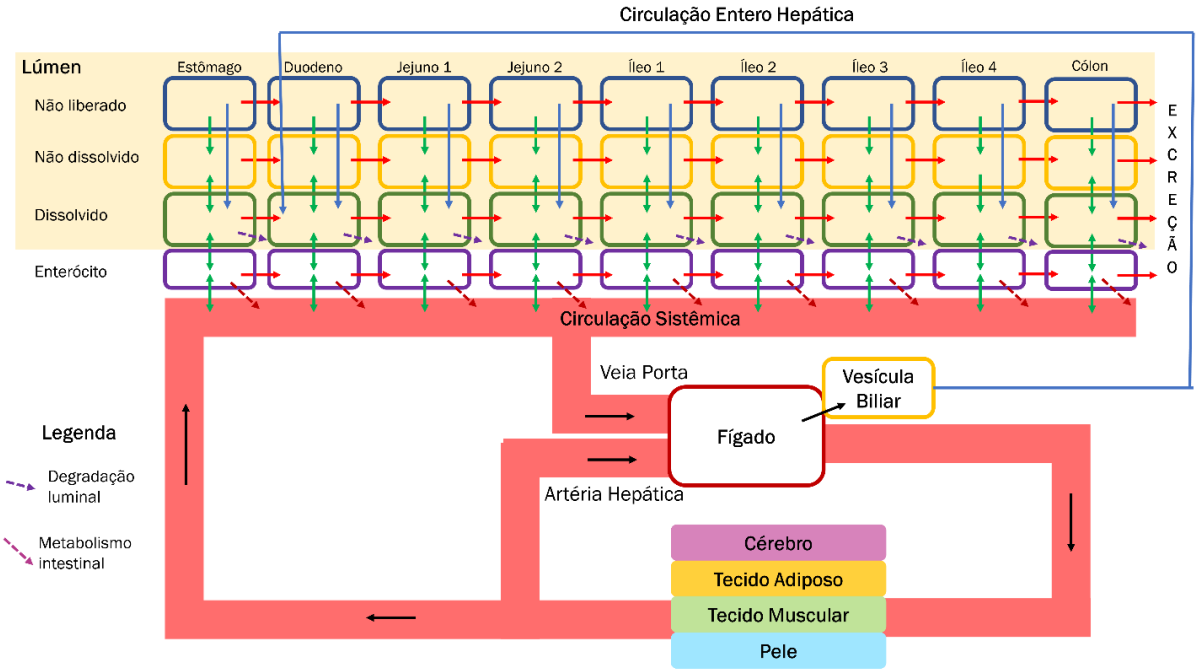

Figura 5. Representação gráfica do modelo de absorção e trânsito compartimental avançado (ACAT)
Fonte: Adaptado de Lukacova e DiBella, 2022.

Similar ao ACAT, o modelo de dissolução, absorção e metabolismo avançado (ADAM, do inglês *Advanced Dissolution Absorption Metabolism model*) está presente no software comercial SimCYP® (LIN; WONG, 2017). Este modelo considera a fisiologia do TGI incluindo tempo de esvaziamento gástrico, tempo de trânsito intestinal além do raio e comprimento do intestino delgado. O modelo ADAM (Figura 6) também divide o TGI em nove compartimentos e considera os processos de dissolução, trânsito de fluidos intestinal, permeação, degradação, metabolismo e transporte (HUANG; LEE; YU, 2009). A partir do ADAM adveio o modelo com parede intestinal de multicamadas (M-ADAM – do inglês *Multi-layer gut wall within* ADAM) com a introdução do conceito de uma membrana basolateral de permeabilidade limitada entre enterócito e fluido intersticial intestinal e, absorção linfática para a circulação sistêmica (DOLTON *et al.*, 2020).

Os modelos mecanísticos descritos, principalmente o ACAT e o ADAM, são atualmente importantes ferramentas para avaliação da cinética de absorção de fármacos. Por meio deles é possível estabelecer um link entre dados de dissolução *in vitro* e dados farmacocinéticos para avaliação da performance de formulações orais (MITRA *et al.*, 2021). À vista disso, várias abordagens e terminologias relacionadas à modelagem PBPK surgiram dando suporte a análise de qualidade de medicamentos e como ferramentas estratégicas de modelagem translacional na pesquisa e desenvolvimento de produtos com base no paciente (PARROTT *et al.*, 2021; PEPIN *et al.*, 2021).

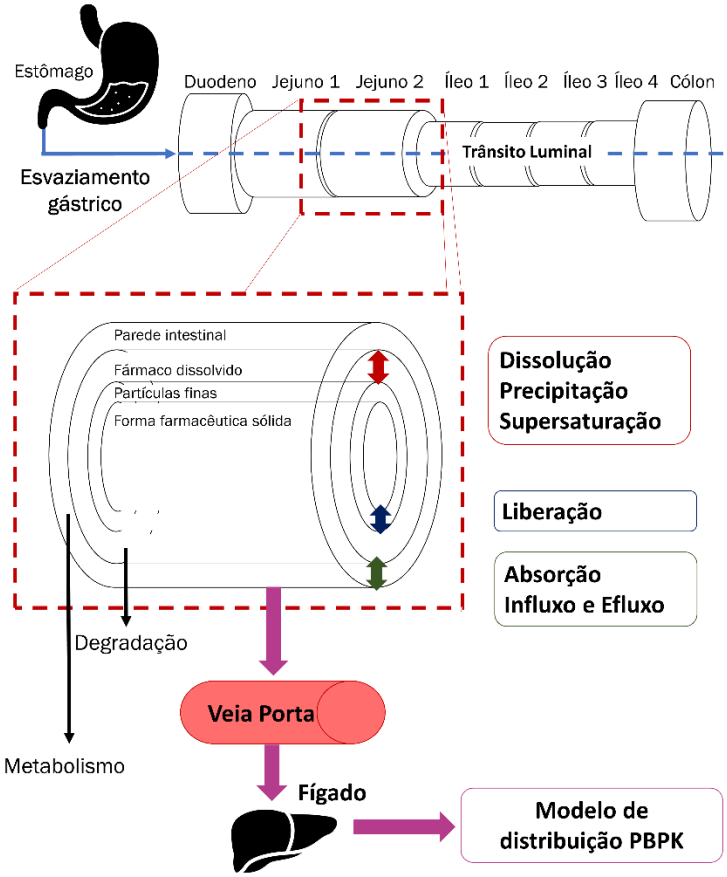

Figura 6. Representação gráfica do modelo de dissolução, absorção e metabolismo avançado (ADAM)
Fonte: Adaptado de Kostewicz *et al.* 2014.

Além do termo PBPK, adveio a modelagem de absorção de base fisiológica (PBAM – do inglês *Physiologically based absorption model*) e a modelagem biofarmacêutica de base fisiológica (PBBM – do inglês *Physiologically based biopharmaceutics modeling*) (BERMEJO *et al.*, 2020). Segundo Mitra e colaboradores (MITRA *et al.*, 2021), os conceitos de PBAM e PBBM são:

> **PBAM:** É por essência um modelo de absorção mecanístico. Nele é possível mimetizar as condições fisiológicas e incorporar a dissolução ou outras informações características de formulação. Ao mesmo tempo, leva em consideração fatores físico-químicos e fisiológicos relevantes e fornece uma previsão de exposição sistêmica em função do tempo (MITRA *et al.*, 2021).
>
> **PBBM:** Concentra-se nas interações formulação-fisiologia para previsões do impacto clínico das variações nos parâmetros e características da formulação. O PBBM é baseado nos mesmos princípios do PBAM, mas tem uma definição mais ampla, abrangendo todas as áreas da biofarmácia. O PBBM pode ser usado para modelar fármacos que são desenvolvidos para não serem absorvidos pelo TGI, ou que são desenvolvidos para exercer uma ação local quando administrados por via parenteral, como para as vias intra-articulares ou intratumorais, por exemplo (MITRA *et al.*, 2021).

Tanto o modelo PBAM quanto o PBBM são potenciais ferramentas de apoio aos processos de desenvolvimento de formulações, para justificativas de bioisenções, alterações pós-registro, desenvolvimento de métodos de dissolução, definição de especificações de qualidade de medicamentos clinicamente relevantes e para os estudos de impacto das propriedades biofarmacêuticas na clínica. O PBBM, embora ainda existam muitas lacunas e muitas perguntas a serem respondidas, tem um imenso

potencial para reduzir testes em animais e humanos, agilizar o desenvolvimento de novos medicamentos e garantir que produtos de qualidade chegue até os pacientes (MITRA *et al.*, 2021; PARROTT *et al.*, 2021).

Uma aplicação do PBBM muito discutida por grupos de pesquisa e agências regulatórias é o conceito de "espaço seguro" (do inglês *safe space*) no desenvolvimento de medicamentos genéricos e similares. *Safe space* é definido como os limites demarcados por especificações *in vitro* (ou seja, dissolução ou, quando aplicável, outros atributos de qualidade do medicamento relevante), dentro dos quais as variantes do medicamento são consideradas bioequivalentes entre si. Dessa forma, a definição de um *safe space* pode ser útil no ciclo de vida de um medicamento, possibilitando o seu uso para suportar alterações pós-registro sem a necessidade de estudos adicionais de bioequivalência (utilizando a bioequivalência virtual – VBE) (MITRA *et al.*, 2021).

Diante disso, considerando a LTG um fármaco BCS II, o qual tem a dissolução como fator limitante do processo de absorção e, por este fármaco estar disponível comercialmente em formas farmacêuticas também dependentes do processo de dissolução, como comprimidos simples e dispersíveis, os estudos biofarmacêuticos e as modelagens farmacocinéticas são recursos extremamente úteis tanto para população adulta quanto para populações especiais como a pediatria. Estas ferramentas podem contribuir fornecendo informações de base que preencham as lacunas e auxiliem em tomadas de decisões tanto na clínica quanto no desenvolvimento de formulações, reduzindo custos, tempo e o número de estudos clínicos necessários para o registro de medicamentos.

3. OBJETIVOS

3.1. Objetivo geral

Avaliar o impacto da ontogenia na absorção da lamotrigina por abordagens biofarmacêuticas e farmacocinéticas

3.2. Objetivos específicos

- Desenvolver e validar método analítico para identificação e quantificação da lamotrigina;
- Avaliar as propriedades biofarmacêuticas do fármaco em estudo, quanto a solubilidade, dissolução e possibilidade da ocorrência de precipitação em meios de dissolução com valores de pH fisiológicos, farmacopeicos e biorrelevantes;
- Desenvolver e validar um modelo farmacocinético baseado em fisiologia (PBPK) em população adulta para medicamentos contendo lamotrigina;
- Desenvolver modelo biofarmacêutico baseado em fisiologia (PBBM) da lamotrigina para população adulta;
- Avaliar a possível relação *in vitro – in vivo* (IVIVR) entre comportamento de dissolução de formulações contendo lamotrigina e a exposição plasmática dos medicamentos;
- Escalonar e validar os modelos PBPK e PBBM para população pediátrica;
- Avaliar os fatores que podem impactar nas propriedades biofarmacêuticas da lamotrigina e no perfil farmacocinético de adultos e crianças.

4. ARTIGO I

Exploring *in vitro* solubility of lamotrigine in physiologically mimetic conditions to prospect the *in vivo* dissolution in pediatric population

Aceito pela revista *Biopharmaceutics & drug disposition*

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Conflict of interest

The authors declare that have no conflicts of interest.

Abstract

Pediatric drugs knowledge still leaves several gaps to be filled, all the while many biopharmaceutic properties applied to adults do not work in pediatrics. The solubility in many cases is extrapolated to the pediatrics, however, sometimes it may not represent the real scenario. In this context, the aim of this study was to assess the possibility of the extrapolation of the solubility from adults to children aged 2 to 12 years using lamotrigine (LTG) as a model. LTG showed its solubility dependent on the pH of the medium, no precipitate formation was seen, and biomimetic media showed greater capacity to solubilize it. Based on dose number (D0) in adults, the LTG was soluble in acidic pH media and poorly soluble in neutral to basic. Similar behavior was found in conditions which mimic children aged 10 to 12 years at a dose of 5 mg/kg, and 15 mg/kg. The D0 for 5-year-old children at a dose of 15 mg/kg showed different behaviors between biorelevant and pharmacopeial buffers media. For children aged 2-3 years, LTG appeared to be poorly soluble in both gastric and intestinal conditions. Solubility was dependent on the volume of fluid calculated for each age group, and this may impact the development of better pharmaceutical formulations for this population, better pharmacokinetic predictions in tools as PBPK and PBBM, greater accuracy in the justifications for biowaiver, and many other possibilities.

Keywords:

Gastrointestinal fluid volume, pH, solubility pH dependent, biorelevant media, dumping test.

1. Introduction

The physical, metabolic, and psychological processes inherent to growth from birth into adulthood reveal that children cannot be regarded as small adults nor they can be regarded as a homogeneous group in themselves (European Medicines Agency, 2013). Among the ontogenic changes in the pediatric population, those that are directly linked to drug absorption process can be highlighted, such as gastric acidity, gastric emptying time, intestinal motility, intestinal surface area, stomach capacity, among others. The main ontogenic variations in the gastrointestinal (GI) tract occur in children younger than two years old (Batchelor, 2014; Kaye, 2011).

In order to facilitate the development and accessibility of drug products for use in the pediatric population, regulatory agencies, pharmaceuticals industries, and academic researchers have intensified their researches in recent years (Food and Drug Administration, 2020). Nevertheless, several tools used in pediatric drug development were initially derived for adults, such as the biopharmaceutical classification system (BCS).

The BCS was proposed by Amidon *et. al* (1995) to identify the limiting step for oral drug absorption. It is widely used in the pharmaceutical community to be an enabling guide for the rational selection of compounds, formulation for clinical advancement, and generic biowaivers (Abdel-Rahman *et al*., 2012; Amidon *et al*., 1995). BCS-based biowaivers eliminate unnecessary human drug exposures, reduce regulatory burden, and has become an important cost-saving tool Food and Drug Administration, 2017; Gandhi *et al.*, 2014). The main update performed on this system was the inclusion of subclasses in classes BCS II and IV that were distributed depending on the acidic (a), basic (b) or neutral (c) characteristics in the physiological pH range (~ pH 7.5) (Tsume *et al*., 2014). The BCS classification became more than a particular property, but something like a surname of the molecule. However, it is necessary to make it clear that this classification was developed in the context of adults.

According to the BCS, drugs are considered highly soluble if the highest single dose is soluble in at least 250 mL of aqueous liquid at a relevant physiological pH range of 1.2 – 6.8, and these aspects concern adult physiology (The International Council for Harmonisation, 2019; Kaye, 2011). So, *in vivo* drug solubility is dependent on the physiological conditions such as the initial gastric volume and characteristics of GI fluids (Kaye, 2011; Martir *et al*., 2020).

Considering all this information, some authors have discussed the impacts of extrapolating the biopharmaceutical property of a drug in adults to pediatrics (delMoral-Sanchez *et al*., 2019; Gandhi *et al*., 2014; Martir *et al*., 2020). Despite this, in most of these studies, the solubility considered is in water and at 25º C, which does not correspond to the physiological conditions. It is known that GI fluids are more complex and consist of substances such as bile salts, surfactants and enzymes that can often help or hinder the drug solubilization and dissolution (Dressman *et al*., 2007; Vaithianathan *et al*., 2015). Dressman *et al*. introduced the first proposals for compositions of biorelevant media in 1998 and, since then, many updates, versions, and applications have been proposed by several authors (Cristofoletti & Dressman, 2016; Dressman *et al*., 1998; Jantratid *et al*., 2008; Kaur *et al*., 2018; Otsuka, Shono, & Dressman, 2013; Vertzoni *et al*., 2004). In recent years, the biorelevant media has been applied in many studies to improve biopharmaceutics information (Cristofoletti & Dressman, 2017; Kambayashi, Blume, & Dressman, 2013; Van der Vossen *et al*., 2019).

Considering that from two years old and onwards the characteristics of the GI fluids are closer to those of adults (Kaye, 2011; Maharaj & Edginton, 2014), the aim of this study was to assess the possibility of the extrapolation of the solubility from adults to children aged 2 to 12 years. Moreover, to understand and to explore the influence of the pH and the volume of fluids in the segments of the GI tract on the solubility of lamotrigine (LTG), an antiepileptic drug widely administered in adults and children.

2. Material and Methods

2.1 Materials

Pure drug substance of lamotrigine used was a United States Pharmacopeia (USP) grade (Batch R047D0). All chemicals for media preparation and sample analysis were of analytical or HPLC-grade and were purchased commercially: methanol, sodium hydroxide (J.T. Baker, New Jersey, USA), trifluoroacetic acid, hydrochloric acid (Merck KGaA, Darmstadt, Germany), sodium taurocholate, pepsin (Inlab, São Paulo, Brazil), sodium chloride, maleic acid, glacial acetic acid (Sigma-Aldrich, Darmstadt, Germany), potassium phosphate monobasic (Synth, São Paulo, Brazil) and lecithin (Alfa Aesar, Massachusetts, EUA).

2.2 Media composition

Plain buffers: hydrochloric acid buffer pH 1.2, sodium acetate buffer pH 4.5 and monobasic potassium phosphate buffer pH 6.8 and 7.4 were prepared according to USP (United States Pharmacopeial Convention, 2020a).

Biorelevant media (Table 1): fasted state simulated gastric fluid (FaSSGF-V2) and fasted state simulated intestinal fluid (FaSSIF-V2) were prepared according to the literature (Jantratid *et al*., 2008; Otsuka *et al*., 2013; Ottaviani *et al*., 2010; Vertzoni *et al*., 2004; Vertzoni *et al*., 2007). For comparison, a FaSSIF-V2 blank (maleate buffer) was also prepared using the ingredients of FaSSIF-V2 without lecithin and sodium taurocholate (Ottaviani *et al*., 2010).

Table 1 Composition of the fasted state simulated gastric fluid (FaSSGF-V2), fasted state simulated intestinal fluid (FaSSIF-V2) and FaSSIF-V2 blank (maleate buffer) (Jantratid *et al*., 2008; Otsuka *et al*., 2013; Ottaviani *et al*., 2010; Vertzoni *et al*., 2004).

Composition	FaSSGF-V2	FaSSIF-V2	FaSSIF-V2 blank
Lecithin (mM)	0.02	0.20	-
Sodium taurocholate (mM)	0.08	3.00	-
Pepsin (mg/mL)	0.10	-	-
Sodium chloride (mM)	68.00	68.62	68.62
Sodium hydroxide (mM)	-	34.80	34.80
Hydrochloric acid (mM)	qs*	qs*	qs*
Maleic acid (mM)	-	19.12	19.12
pH	1.6	6.5	6.5

Note: * qs = Quantum sufficit (as much as sufficient)

2.3 Instruments and chromatographic conditions

The HPLC analytical method was elaborated and adapted from American Pharmacopoeia Lamotrigine Tablet Monograph (United States Pharmacopeial Convention, 2020c). The HPLC analyses were carried out on Shimadzu LC-20At, (Tokyo, Japan) with a UV-VIS detector, 210 nm wavelength, and Inertsil® ODS-2 150 x 4.6 mm, 5 µm column (GL Science, California, USA). The LTG standard solution was prepared at a concentration of 50.0 µg/mL with ultra-purified water and methanol 40:60 v/v. The analyses were performed using an isocratic elution. The mobile phase was composed of methanol and 0.1% (v/v) trifluoroacetic acid solution in ultra-purified water pH 4.5 (60:40 v/v). The flow rate was 1.00 mL/min at 30° C column temperature. The injection volume of samples was 20 µL.

2.4 Analytical method validation

Specificity, limit of quantification, limit of detection, linearity, range, precision, accuracy, robustness, and matrix effect was determined, and the value of $p < 0.05$ was taken to denote significance (The International Council for Harmonisation, 2005). The following programs were used for data manipulation and statistics analysis: Action Stat Pharma® (Estatcamp, São Carlos, Brazil), Excel (Microsoft, CO), and R 3.5.1 (R Foundation for Statistical Computing, Austria) ggplot2, car, and carData packages.

The specificity of the method was assessed by analysis and comparison of peak retention time of LTG and interfering peaks. LTG standard solutions were prepared at a concentration of 50.0 µg/mL in

the diluent, in pharmacopeial buffers and biorelevant media. Peak purity was determined in Shimadzu Prominence-i LC-2030C HPLC (Tokyo, Japan) with a photodiode array detector (PAD) (Papadoyannis & Gika, 2004; Ramaswamy & Arul Gnana Dhas, 2018; Shimadzu, 2012).

The limit of detection (LOD), as well as quantification (LOQ) were measured based on the signal-to-noise ratio (S/N), S/N of 3:1 and S/N of 10:1 respectively. Determination of the S/N is performed by comparing measured signal samples with a known low concentration of analyte with those of blank samples. After that, linearity was determined by the analysis of three calibration curves with standard solutions of LTG in eight different concentrations each: 0.3, 1.0, 5.0, 10.0, 20.0, 30.0, 50.0, and 60.0 µg/mL. The data for peak area and the LTG concentration analysis were treated by linear regression. The obtained data were subjected to regression analysis using the least-squares method with a weighting factor of 1/x. Data were evaluated for homogeneity by the method Breusch and Pagan (Breusch & Pagan, 1979), normality by the method Anderson-Darling (Anderson & Darling, 1952), and analysis of standardized residuals by visual analysis.

The precision of the method was estimated at two different levels: repeatability (intra-day precision) and intermediate (inter-day precision). It was assessed by injecting nine determinations within the range of linearity, three concentrations and three replicates each, and was expressed as the coefficient of variation (CV%). The acceptance criteria adopted was CV% less than 5.0% taking into account the intrinsic variability of the method and working concentration of the sample (United States Pharmacopeial Convention, 2020d). For accuracy analysis, LTG standards solutions were prepared at three different concentration levels (2.5, 25.0, 55.0 µg/mL) in triplicate. The acceptance criteria adopted was a recovery range of 95.0 to 105.0% in accordance with the requirements adopted for precision.

Robustness was evaluated by measuring the peak area of LTG standard solution by changing temperature (25, 30, and 35° C), flow rate (0.95, 1.00, and 1.05 mL/min), and proportion of mobile phase (35:65, 40:60, and 45:65 v / v). Results were statistically evaluated by the t-Student test by comparing the means of independent samples. The stability of the solutions was also evaluated.

Additionally, matrix effect was determined by comparing the angular coefficients of the curves constructed with the standard in diluent and with the standard in each medium. The curves were prepared with the same 5 concentration levels (3.0, 15.0, 30.0, 45.0, and 60.0 µg/mL) in triplicate. First, the

variance of the slopes of the curves with diluent and matrix (medium) were evaluated and compared by the F-test. Subsequently, the coefficients were evaluated and compared using the t-test and analysis of covariance, at a 5.0% significance level (The International Council for Harmonisation, 2005).

2.5 Solubility Assessment

Amounts of solid LTG were weighed an excess of 30% over the reported solubility in each medium evaluated. Samples were prepared in triplicate and the medium evaluated separately were composed of 5.0 mL of HCl buffer (pH 1.2), acetate buffer (pH 4.5), phosphate buffer (pH 6.8 or 7.4), FaSSGF-V2 pH 1.6, FaSSIF-V2 pH 6.5 or ultrapurified water was added to create saturated solutions. The samples were slightly shaken to remove bubbles and stored on an orbital shaker maintained at 37° C. After 0.5, 1.0, 6.0, 10.0, and 24.0 h, the dispersions were filtered through a 0.45 µm nylon filter (Millex, Millipore, Darmstadt, Germany). At each collection time, the pH was evaluated. Filtered samples were further diluted in diluent to fall within the calibration range (0.3-60.0 µg/mL) (Cristofoletti & Dressman, 2017). The samples were analyzed by the method previously developed and validated (item 2.4). The equilibrium condition was adopted when the last two measurements were similar. For comparison purposes, the 24 h solubility test was also performed for the FaSSIF-V2 blank (de Castro *et al*., 2020; GlaxoSmithKline, 2020).

2.6 Theoretical solubility

The intrinsic solubility (S_0), the solubility of the compound when all the molecules are neutral, was calculated by the Yalkowsky general solubility equation (GSE) (Kerns *et al*., 2008; Yalkowsky & Banerjee, 1992):

$$\log S_0 = 0{,}5 - \log P - 0{,}01(MP - 25) \quad (1)$$

where Log P, the log of the partition coefficient of a compound between octanol and water, assumed was 1.93 and the melting point of the solid (MP), was 181° C (Drugbank, 2021).

From the S_0, theoretical solubilities were calculated considering the ionizability of LTG at the same pH as the experimental media, applying the Henderseon-Hasselbach equation (Henderson, 1908; Kerns *et al*., 2008):

$$S = S_0 (1 + 10^{pKa-pH}) \quad (2)$$

where S is the solubility of LTG at a given pH and pKa = 5.7 (Drugbank, 2021).

2.7 Adult dose number

The dose number (D_0) was calculated assuming the concept of the highest strength single dose, according to Food and Drug Administration BCS guide (2017), (LTG D0 = 200 mg), LTG solubility (mg/mL) for each medium evaluated obtained in this study, and the initial gastric volume available (V_0) using Eq. (01). For adults, V_0 was assumed to be 250 mL which is the volume of liquid taken with oral formulations, derived from typical bioequivalence study protocols (The International Council for Harmonisation, 2019).

$$D_0 = \frac{Dose}{(V_0 \,.\, Solubility)} \quad (3)$$

2.8 Pediatric dose number

Pediatric D_0 was also calculated and interpreted as adult D_0 using Eq. (03), however, it was necessary to adjust the V_0 according to the age group, using Eq. (04):

$$V_0 = \frac{weight\ (kg) \cdot 0.56\ (mL/kg)}{37.1\ (mL)} \,.\, 250\ mL \quad (4)$$

where 0.56 mL/kg and 37.1 mL are estimates of fasted gastric fluids volumes in pediatricians (Crawford *et al*., 1990; Martir *et al*., 2020).

As LTG is indicated for children over 2 years old, for comparison purposes, this study adopted the ages 2, 3, 5, 10, and 12 years old, and their respective average body weights (World Health Organization, 2009).

Considering the dose range from 1 to 15 mg/kg/day, this study adopted as maximum, single doses 5 mg/kg and 15 mg/kg. Fractionation of LTG tablets is not recommend, the most indicated way is rounding up to the nearest whole dose. For example, the calculated dose for 2 years old would be 61 mg and the whole doses available would be 50 or 75 mg. With 50 mg being the closest dose, it was adopted for this

group (GlaxoSmithKline, 2020). Calculated doses greater than the maximum dose in adults were assumed as the adult dose (D_0 = 200 mg).

2.9 Dumping test

A weak base drug, such as LTG, can be easily dissolved in the stomach, and when the drug empties from the stomach into the small intestine, precipitation can occur due to a pH-driven decrease in solubility (Kambayashi, Yasuji, & Dressman, 2016; Psachoulias *et al*., 2011). To assess the possibility of precipitation of some drugs due to a sudden change in pH, some techniques can be used such as two-stage dissolution or the dumping test (Cristofoletti & Dressman, 2017). For this purpose, 200 mg of LTG was solubilized in 50 mL of FaSSGF-V2 medium and stored on an orbital shaker at 37º C for 30 min, simulating the gastric compartment. The resulting solution was "dumped" into 450 mL of the FaSSIF-V2 medium (with concentrations of sodium taurocholate and lecithin 1.11 times more concentrated to attain a final pH of 6.5) in an Erweka DT 800 dissolver (Erweka, Heusenstamm, Germany), USP paddle apparatus, 50 rpm and at 37° C (Cristofoletti & Dressman, 2017). The pH of the solution was evaluated during the experiment. Sampling was performed at 5, 10, 15, 30, and 45 min and samples were filtered through a 0.45 µm nylon filter (Millex, Millipore, Darmstadt, Germany) and analyzed by HPLC. New samples were collected at the bottom of the vessel and evaluated by optical microscopy to detect precipitates. The experiments were carried out in triplicate.

3. Results

Firstly, the method was tested and validated to ensure reliability and safety with solvents and media that would be used for the solubility tests. Further details on method validation are described in the supplementary materials.

The LTG solubility versus time is showed in Figure 1 for plain buffers and biorelevant media. It is possible to see that, right at the beginning of the experiment, in some conditions, the solubility reaches an initial value and, over time, this value presents a fluctuation until reaching the equilibrium state. To confirm the results, the analysis was repeated with collection times of 24, 48, and 72 h and proved the

equilibrium. Based on the different pHs evaluated, LTG equilibrium solubility (S_{eq}) was lower with increasing pH of the medium as can be seen in the results shown in Figure 2.

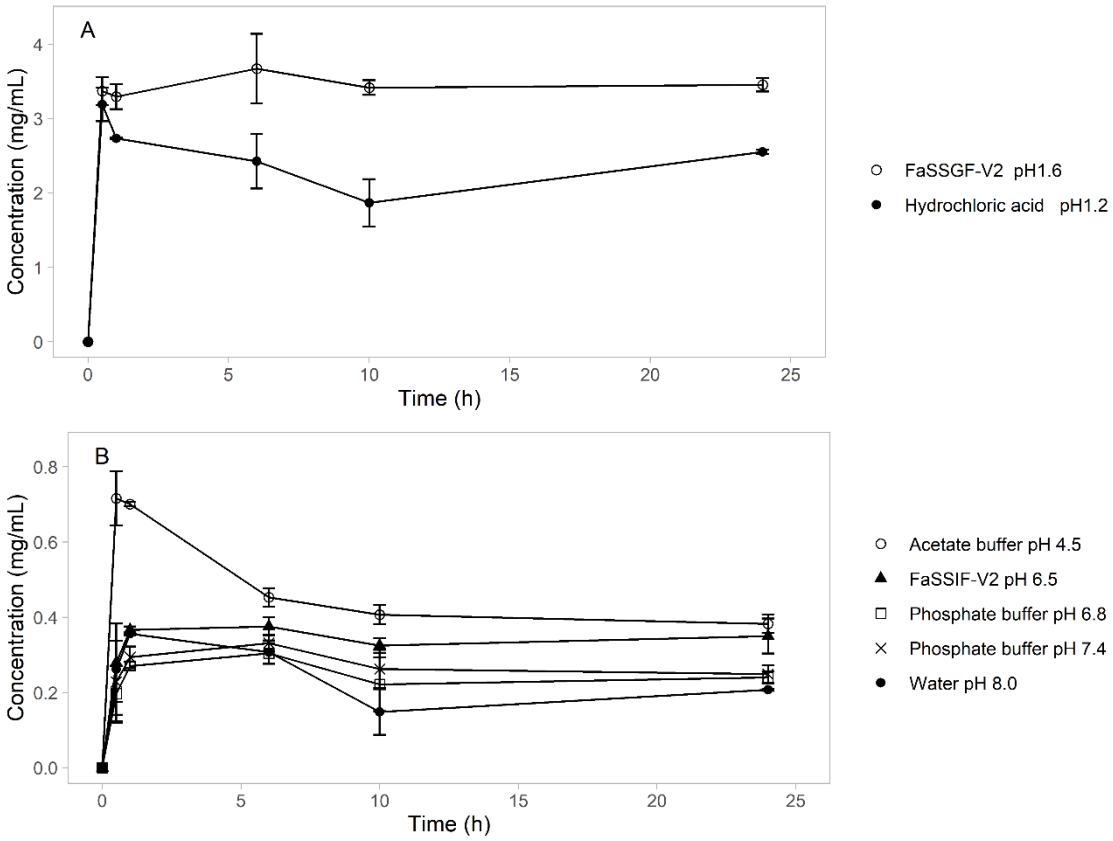

Figure 1 Average solubility of lamotrigine (n = 3) in plain buffers and biorelevant media: (A) gastric conditions and (B) intestinal conditions.

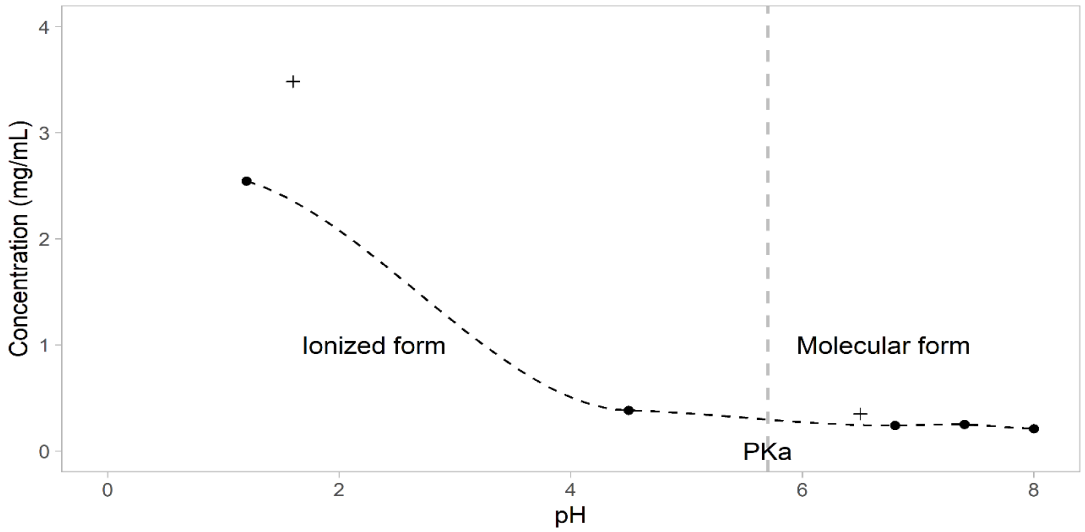

Figure 2 Mean equilibrium solubility profile of lamotrigine according to pH and medium composition (plain buffers and biorelevant media). The gray dashed line represents lamotrigine pKa

The LTG S_{eq} is numerically shown in Table 2 and, we can verify that the biorelevant medium could solubilize higher amount of LTG. When the results in biorelevant media are observed deeply, LTG S_{eq} in FaSSGF-V2 was 36% higher than in Hydrochloric acid buffer pH 1.2, in FaSSIF-V2 was 46% higher than in phosphate buffer pH 6.8, and 67% higher than in blank FaSSIF-V2. The LTG solubility in blank FaSSIF-V2 medium was 0.21 ± 0.02 mg/mL. Media pH did not change throughout the experiments.

Table 2 Lamotrigine equilibrium solubility in plain buffers and bio-predictive media

Media	Solubility (mg/mL)
Hydrochloric acid buffer pH 1.2	2.54 ± 0.03
Acetate buffer pH 4.5	0.38 ± 0.02
Phosphate buffer pH 6.8	0.24 ± 0.02
Phosphate buffer pH 7.4	0.25 ± 0.02
Ultrapure water pH 8.0	0.21 ± 0.01
FaSSGF-V2 pH 1.6	3.45 ± 0.08
FaSSIF-V2 pH 6.5	0.35 ± 0.05
FaSSIF-V2 blank	0.21 ± 0.02

Theoretical solubility is demonstrated in Table 3. It is possible to see that the solubility of LTG, when the medium is pH 8.0, is equal to the intrinsic solubility. Furthermore, the calculated solubilities when the media presents a pH between 6.8 and 8.0 are close to those found experimentally.

Table 3 Lamotrigine theoretical solubility

Condition	Solubility (mg/mL)
Intrinsic	0.26
pH 1.2	8287.17
pH 4.5	4.42
pH 6.8	0.28
pH 7.4	0.27
pH 8.0	0.26

In the dumping test, the final solution in FaSSIF-V2 medium resulted in a clear and colorless one. There were no significant changes in the pH of the medium and the concentration of LTG in the times of 5 to 45 minutes was constant (Figure 3). In optical microscopy it was not possible to identify the presence of precipitates.

D_0 was also calculated for adults, at a dose of 200 mg, and children, taking the doses: 5 mg/kg (Table 4) and 15 mg/kg (Table 5) rounding up to the nearest whole dose. It was possible to notice that for the

dose of 5 mg/kg, D_0 for gastric conditions were less than one that suggest the LTG is soluble, on the other hand, for intestinal conditions were greater than one indicating poor solubility for all ages (2-12 years), similar for adult condition. For the LTG dose of 15 mg/kg, the ages of 10 and 12 years also showed adult-like solubility behaviors, however, for younger children (2-3 years) LTG was poorly soluble in gastric conditions, with a D_0 greater than one. For 5 years old, LTG in gastric conditions seems to be soluble in biorelevant media and poorly soluble in plain buffer media.

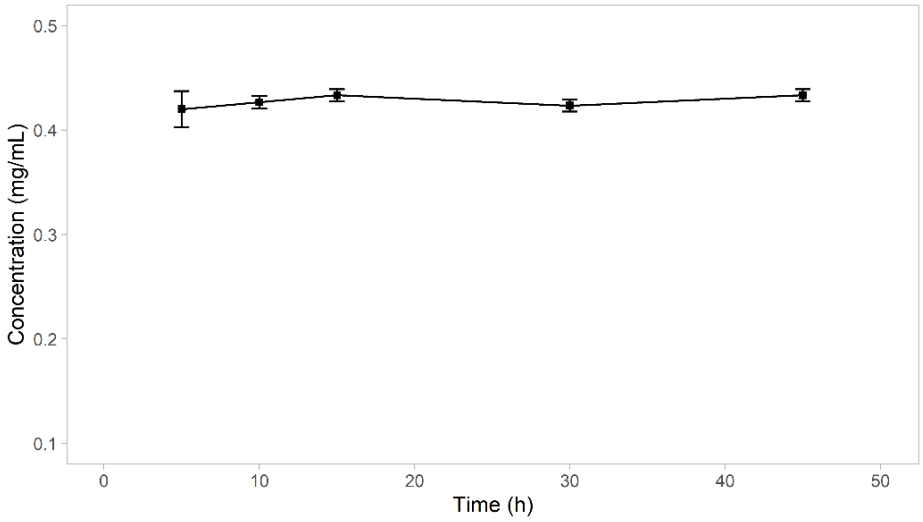

Figure 3 Dumping test of a lamotrigine suspension in FaSSGF-V2 medium to a FaSSIF-V2 medium 1.11 times concentrated using a USP paddle apparatus at 50 rpm and 37º C. Arithmetic mean of three replicates and standard deviation.

Table 4 Table 4 Dose unit (D_0) in different age groups assuming a dose of 5 mg/kg for pediatric and 200 mg for adult

Media	**Age group (Body weight*)**					
	Adult	**2 years (12.2 kg)**	**3 years (14.3 kg)**	**5 years (18.3 kg)**	**10 years (31.2 kg)**	**12 years (41.5 kg)**
Hydrochloric acid buffer pH 1.2	0.31	0.43	0.36	0.57	0.67	0.50
Acetate buffer pH 4.5	2.11	2.86	2.44	3.81	4.47	3.36
Phosphate buffer pH 6.8	3.33	4.53	3.86	6.03	7.08	5.32
Phosphate buffer pH 7.4	3.20	4.34	3.71	5.79	6.79	5.11
Ultrapure water pH 8.0	3.81	5.17	4.41	6.90	8.09	6.08
FaSSGF-V2 pH 1.6	0.23	0.31	0.27	0.42	0.49	0.37
FaSSIF-V2 pH 6.5	2.29	3.10	2.65	4.14	4.85	3.65
FaSSIF-V2 blank	3.81	5.17	4.41	6.90	8.09	6.08
Dose (mg)	200.00	50.00	50.00	100.00	200.00	200.00
V_0 (mL)	250.00	46.04	53.96	69.06	117.74	156.60

Note: Average body weights according to World Health Organization,2009.

Table 5 Dose unit (D_0) in different age groups assuming a dose of 15 mg/kg

Media	Age group (Body weight*)				
	2 years (12.2 kg)	**3 years (14.3 kg)**	**5 years (18.3 kg)**	**10 years (31.2 kg)**	**12 years (41.5 kg)**
Hydrochloric acid buffer pH 1.2	1.71	1.46	1.14	0.67	0.50
Acetate buffer pH 4.5	11.43	9.75	7.62	4.47	3.36
Phosphate buffer pH 6.8	18.10	15.44	12.07	7.08	5.32
Phosphate buffer pH 7.4	17.38	14.83	11.58	6.79	5.11
Ultrapure water pH 8.0	20.69	17.65	13.79	8.09	6.08
FaSSGF-V2 pH 1.6	1.26	1.07	0.84	0.49	0.37
FaSSIF-V2 pH 6.5	12.41	10.59	8.27	4.85	3.65
FaSSIF-V2 blank	20.69	17.65	13.79	8.09	6.08
Dose (mg)	200.00	200.00	200.00	200.00	200.00
V_0 (mL)	46.04	53.96	69.06	117.74	156.60

Note: Average body weights according to World Health Organization, 2009.

4. Discussion

4.1 Adults

The experimental S_{eq} of LTG at pH 6.8 was similar to that found by Vaithianathan *et al*. (2015), 0.21 mg/mL. In water, it was close to what was reported by Shayanfar, Acree, & Jouyban (2009), 0.17 mg/mL, and Rahman *et al*. (2012), 0.23 mg/mL. LTG showed slightly soluble at pH 1.2 and 1.6, and very slightly soluble at pH between 4.5 to 8.0 (USP, 2020a) which was demonstrated as well by Martins, Paim, & Steppe (2010).

Both experimental and theoretical Seq showed similar results under pH conditions between 6.8 and 8.0. Furthermore, the calculated theoretical S_0 was similar to Seq in pH 8.0 medium, indicating that at this pH the LTG was almost entirely in the non-ionized form. In contrast, the theoretical S_{eq} overestimated the solubilization capacity of LTG at pH 4.5 and 1.2. By the theoretical calculation, at pH 4.5 LTG would be slightly soluble and at pH 1.2 very soluble, which was not confirmed experimentally. Considering that many studies use predicted data, including those used in pharmacometrics, for LTG in some conditions the theoretical data does not represent the real one. This may be a warning case for other weak base drugs.

As far as we know, this is the first time that LTG solubility has been reported in biorelevant media, FaSSGF-V2 pH 1.6 and FaSSIF-V2 pH 6.5. Biomimetic media showed greater capacity to solubilize

LTG than media without sodium taurocholate and lecithin, in about 50%. It can be explained by the capacity of these reagents to decrease the interfacial energy between the drug and the medium, increase the effective surface area, and increase wetting and/or micellar solubilization (Andrieux *et al*., 2004; Elder & Holm, 2013; Mithani *et al*., 1996; Ottaviani *et al*., 2010). According to Mithani *et al*. (1996), the driving force for bile salt solubilization of a drug is determined by the hydrophobicity rather than their affinity for bile salt micelles. LTG presents a log P of 1.93 which means that it has a greater affinity for organic than aqueous media, and therefore has considerable hydrophobicity. This hydrophobicity of LTG may also explain the improvement in its solubility in media with biorelevant reagents.

However, despite the improvement in LTG solubility capacity, it was not enough to change its equilibrium solubility rating for active pharmaceutical ingredient according to United States Pharmacopeial Convention (2020b). LTG was slightly soluble at pH 1.6 and very slightly soluble at pH 6.5, as in similar pH plain buffers. The correlation between sodium taurocholate concentration and log P of some drugs were tested by Mithani *et al*. (1996). They observed that only molecules with high lipophilicity show a considerable increase in their solubility in the presence of taurocholate, and it was necessary a log P greater than 2.5 to double the amount solubilized. Therefore, LTG seems not to have enough lipophilicity to significantly alter its solubility in this situation.

Bile salts are surface-active compounds and their amphipathic molecular structures are important to the digestion and absorption of lipophilic drugs (Gass *et al*., 2007). It is known that in the postprandial phase there are changes in pH, motility, gastric emptying rate and increased concentration of bile salts (Food and Drug Administration, 2002). Considering that, according to Garnett (1997) and GlaxoSmithKline (2020), the pharmacokinetics of LTG does not change in the presence of food, this fact agrees with the results of this work, since the presence of bile salts in the medium did not significantly increase the solubility of LTG.

Additionally, based on the equation proposed by Mithani *et al*. (1996), which predicts solubility in the presence of sodium taurocholate as a function of water solubility, molecular weight and salt concentration, LTG would present similar solubilities in FaSSGF-V2 and FaSSIF-V2, which contain 0.08 mM and 3 mM sodium taurocholate respectively. Thus, the concentration of bile salts does not seem to be as significant for the solubility of LTG. On the other hand, the LTG experimental Seq

demonstrated greater solubilization capabilities in acidic pH media and these data were in agreement with the experiments of Vaithianathan *et al*. (2015) and Martins, Paim, & Steppe (2010).

LTG is a weak base and the pH-solubility profile is characterized by two regions, driven by the ionizability and principles of the Henderson-Hasselbalch equation (eq. 02) (Henderson, 1908; Kerns *et al*., 2008). The first region, where the pH of the medium is lower than the pKa of the molecule, is in this situation, the predominant form is the ionized. As for the second region, where the pH of the medium is greater than the pKa of the molecule, the predominant form is the non-ionized or molecular one.

Therefore, although the highest dose of LTG is soluble in acidic pH conditions such as the stomach, it is poorly soluble in neutral to basic pHs such as the intestine. In this way, supersaturation and precipitation of the drug could occur precisely at the site of greatest absorption, to evaluate these processes the dumping test was carried out. In acid condition at a dose of 200 mg with 50 mL of HCl medium the theoretical concentration of LTG would be 4 mg/mL, considering the Seq in this condition of 2.54±0.03 mg/mL the solution would be supersaturated. After dumping, increasing the medium to 500 mL of FaSSIF-V2 the theoretical of LTG would be 0.4 mg/mL, considering the Seq in this condition of 0.35±0.05 mg/mL the solution would be close to saturation. However, there was no formation of a precipitate which can be explained by the final concentration being slightly below the saturated condition. Thus, the highest dose was not enough to provoke the precipitation process in the physiological conditions mimicked for adults.

4.2 Pediatrics

It is known that pediatric physiological conditions are different from those of adults. Such conditions can alter the interpretations of some biopharmaceutical properties of a given drug (Elder, Holm, & Kuentz, 2017; Maharaj, Edginton, & Fotaki, 2016; Purohit, 2012). Some authors have warned about problems related to extrapolation of biopharmaceutical properties from adults to children. Martir *et al*. (2020) worked with the classic concept of BCS using water as the medium for solubility and warned that the use of BCS-based biowaivers for pediatric products needs to be undertaken with caution due to differences in the drug D0 between adults and pediatrics.

In this work we look for more physiologically relevant data that can inform possible dissolution processes under conditions different from those used in the BCS for pediatrics. LTG, in the smallest dose (5 mg/kg), had the same behavior for all ages (2-12 years). It seems to be soluble in gastric conditions and poorly soluble in intestinal conditions, similar to that calculated for adults.

At the same time, for the dose of 15 mg/kg, different behaviors according to age could be observed. At ages 10 and 12 years, LTG seems to be soluble in gastric conditions and poorly soluble in intestinal conditions, as demonstrated for adults and for the lower pediatric dose. For 5 years old, LTG in gastric conditions seems to be soluble in biorelevant media and poorly soluble in plain buffer. In this case, the increased solubilization capacity of LTG, in the presence of bile salts, seems to be an important factor since the solubilized drug is more easily absorbed (Amidon *et al*., 1995). In contrast, for younger children (2-3 years) LTG appears to be poorly soluble in all conditions tested. In this case, the amount of drug and the volume of fluid administered concomitantly seem to be more impactful than the pH of the fluids. So, although BCS was not changed, according to the guidelines (The International Council for Harmonisation, 2019), the solubilization capacity of LTG was impacted by the dose and volume of liquid in children. Therefore, extrapolation from adult data to children can be misleading. Taking into account that the solubility and dissolution of a drug is the first step for the absorption and bioavailability (Elder & Holm, 2013), the poor solubility of LTG in gastric conditions in young children can result in impaired pharmacological effect. Variations in solubilization capacity of LTG can also explain cases of non-bioequivalence between different formulations in different age groups. Like so, a good strategy would be to apply eq. (3) and (4) together, shown in eq. (5), individually to predict the D0 and the solubilization capacity.

$$D_0 = \frac{Dose}{(3{,}77\,.\,Weight\,.\,Solubility)} \tag{5}$$

Knowing that LTG, at high doses, may have low solubilization capacity in the gastric environment for children under 3 years old, can help in guiding pharmaceutical companies and researchers towards the development of more suitable formulations for this population. Additionally, basic biopharmaceutics information, such as solubility, has been extremely important as input into physiologically-based

pharmacokinetic modelling studies (PBPK) and physiologically based biopharmaceutics modelling (PBBM). Hence, the data generated in this study can be used in new pharmacokinetic modeling studies.

5. Conclusion

The extrapolation of biopharmaceutical properties from adult to children needs to be undertaken with caution, due to differences in physiology between adults and pediatrics. Considering the BCS official classification form LTG seems to be a BCS II for both adults and pediatric patients. As LTG is quite soluble in the gastric medium, it will arrive in the first intestinal portions in this way, which may favor absorption in this region the absorption window for adults. On the other hand, for children, solubility was dependent on the volume of fluid calculated for each age group, and this may impact the development of formulations for these populations. The improvement of knowledge about the classification of medicines used in pediatrics can help in the development of better pharmaceutical formulations for this population, better pharmacokinetic predictions in tools as PBPK and PBBM, greater accuracy in the justifications for biowaiver, and many other possibilities.

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Supplementary material

Validation results

Initially, to define the best wavelength for the analytical condition, a scanned spectrum was performed in the range of 200–400 nm. The wavelength that showed the LTG highest absorption intensity and the best chromatogram peak was 210 nm. This wavelength was then adopted for the validation analysis.

A key factor in the development of analytical methods is the equipment chosen. The equipment condition is critical to ensure reliable results, and, for this, a system suitability testing was performed. Thereby, the system suitability parameters were as expected for LTG peak: CV% was less than 2.0% (0.7 ± 0.2%), peak tailing was less than 2.0 (1.5 ± 0.1) and capacity factor was greater than 1.0 (1.4 ± 0.2). In these conditions the run time adopted was 5 minutes and the LTG retention time determined was approximately 3 minutes.

Once developed, the analytical method was tested and validated to ensure reliability and safety. The first parameter analyzed was the specificity that is the ability to assess the compound unequivocally in the presence of other components. Method specificity could be proven by the presence of a well-defined LTG peak with reproducible retention time (3.04 min ± 0.19) (Figure S1). Comparing the chromatograms of the mobile phase, diluent, LTG standard, and media, it suggests that there were no interfering peaks at the same retention time as the LTG peak. LTG peak purity was observed in the purity graphic with the similarity curve above the threshold curve. The peak purity index was 1.0 and no impurity was observed. Thus, peak purity analysis demonstrates that the method is highly specific and no other components were co-eluting with LTG (Figure S2).

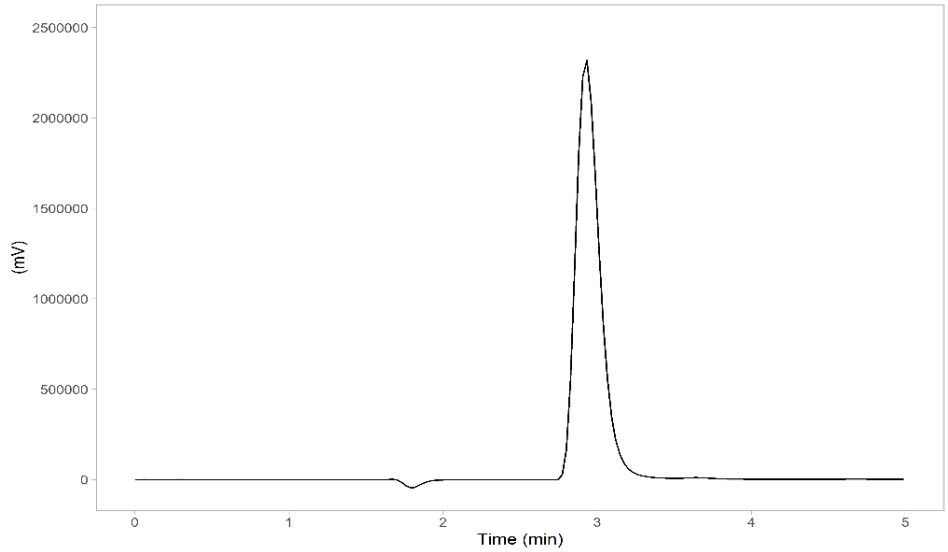

Figure S1 Lamotrigine standard solution chromatogram profile in HPLC with UV-VIS detector at 210 nm

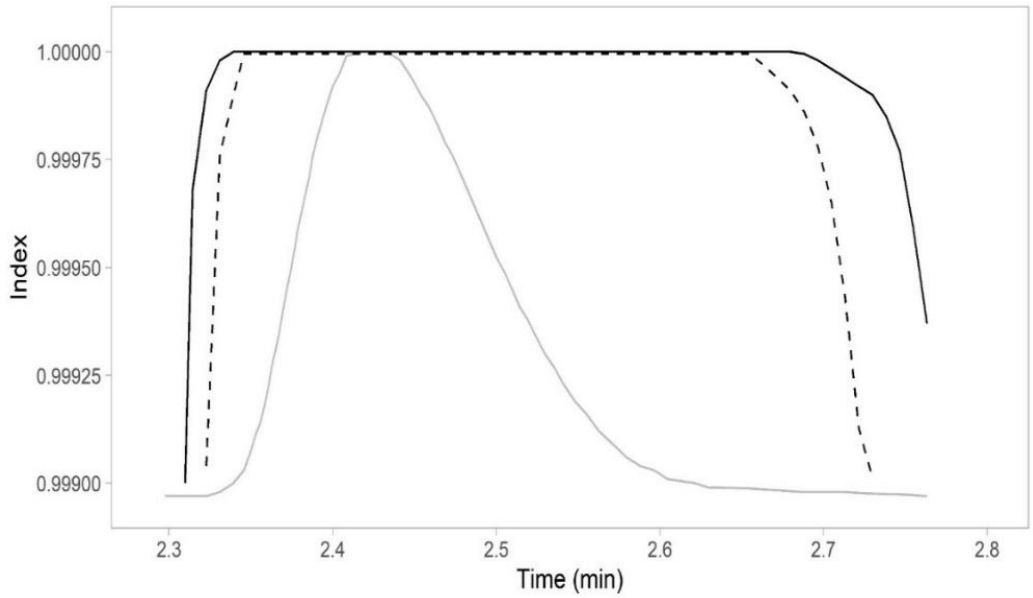

Figure S2 Peak purity graph of the lamotrigine standard solution performed on HPLC with PAD detector. The solid black line is the similarity curve, the dashed black line is the threshold curve and the solid gray line is the lamotrigine chromatogram

To evaluate the lower limits of the method, LOQ and LOD tests were performed. Therefore, according to the determined signal-to-noise ratio (S/N), LTG presented LOD of 0.05 µg/mL (S/N = 2.4 ± 0.1) and LOQ of 0.30 µg/mL (S/N = 11.9 ± 0.1). From the LOQ data, the linearity of the method was evaluated.

Linearity is the ability to find responses directly proportional to the concentration of an analyte within a given range. A linear correlation was found between the peak areas and the concentrations of LTG in the assayed range with the regression equation y = 249607x – 68753 (Figure S3). The correlation coefficient (R) obtained was higher than 0.99 which attests to the linearity of the method and means that there is a good relationship between the two variables (concentration and area). To evaluate the significance of the model was used the ANOVA F test. As the p-value was less than 0.01, the null hypothesis (significance of the linear model) was not rejected. And, to evaluate the intercept (linear coefficient) was used the t-student statistic test. The zero-intercept hypothesis was not rejected with a p-value of 0.14. The residual distribution was evaluated visually and statistically, by applying the Anderson-Darling test for normality and Breusch-Pagan test for homogeneity. In the visual analysis, no extreme values were observed (Figure S4). The hypothesis of residual normality, with the Anderson-Darling test, was not rejected with a p-value of 0.29. The hypothesis of equality of variance, with the Breusch Pagan-test, was not rejected too with a p-value of 0.15, so these tests showed a homoscedastic model.

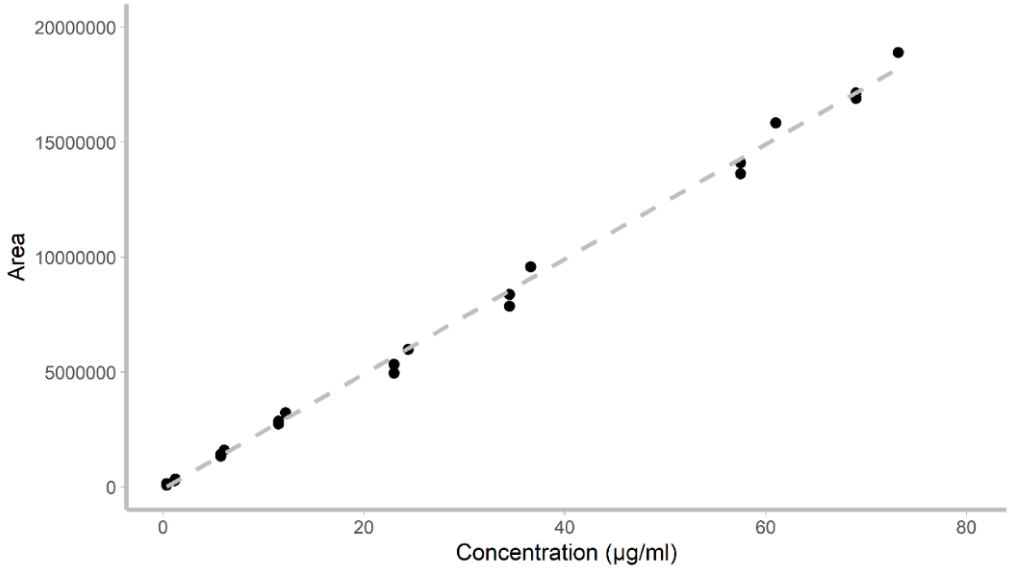

Figure S3 Linearity profile of lamotrigine obtained by HPLC with UV-VIS detector at 210 nm

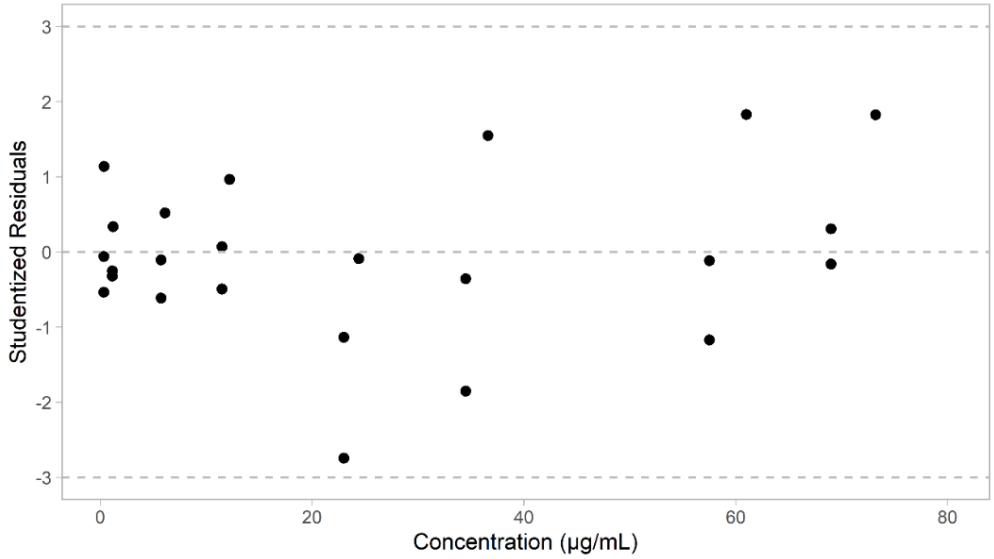

Figure S4 Studentized residuals dispersion analysis from the lamotrigine linearity test

Sequentially, two other critical parameters were evaluated: precision and accuracy. The precision assesses the variations between the series of analyses obtained from the same homogeneous sample on the same day and on different days. In intra-day and inter-day precision were analyzed three concentrations (2.5, 25.0, and 55.0 µg/mL). For the lowest concentration, the CV% was 1.9% intra-day and 1.4% inter-day. The CV% calculated for intermediate concentration was 3.0% in intra-day and 2.7% in inter-day. And, for the highest concentration, the CV% of intra-day was 4.9% and 3.3% for inter-day. The results were in accordance with the acceptance criteria adopted, CV% less than 5.0%, and demonstrated a good precision of the method. Accuracy of an analytical procedure expresses the variations between the true value and the value found experimentally. The accuracy was analyzed in three different concentrations (2.5, 25.0, and 55.0 µg/mL) and data was expressed in the percentage of the recovery. The mean value obtained was 104.8 ± 2.0%, 97.0 ± 2.9%, and 98.1 ± 4.8% for low,

intermediate, and high concentration respectively. According to acceptance criteria, recovery range between 95.0 to 105.0%, the results found were in agreement with the limits established and demonstrated the accuracy of the method.

The robustness of an analytical procedure is the ability to remain unaffected by small variations in some parameters and provides reliability during use. For robustness analysis, changes in column temperature and mobile phase ratio did not show statistically significant differences between the LTG areas obtained. On the other hand, changes in flow rate presented results with significant differences in LTG areas, so the flow rate of 1.0 mL/min is an important factor for the analysis and should not be changed. The standard solution presented a good stability period, it was stable up to 8 days when stored in a refrigerator at 4º C.

Finally, matrix interference was analyzed. The results in Table S1 indicate that all curves showed a good relationship between the concentration and area variables according to its R coefficient (higher than 0.99). At a significance level of 0.05, the angular and linear coefficients did not show significant differences with the changes of diluent/matrix, which means that the components of the medium did not interfere in the LTG analysis.

Table S1 Evaluation of matrix interference in angular and linear coefficients in lamotrigine analysis in HPLC .

Matrix	**Angular coefficient**	**Linear coefficient**	**R**
Diluent	224771.08[a]	277732.29[b]	0.9953
Hydrochloric acid buffer pH 1.2	233885.23[a]	-8824.60[b]	0.9988
Acetate buffer pH 4.5	234055.08[a]	47942.43[b]	0.9991
Phosphate buffer pH 6.8	230007.93[a]	-110776.31[b]	0.9984
Phosphate buffer pH 7.4	219170.10[a]	182312.51[b]	0.9972
FaSSGF-V2 pH 1.6	227522.64[a]	65653.01[b]	0.9989
FaSSIF-V2 pH 6.5	229004.85[a]	-139843.15[b]	0.9982

[a-b] Statistically equal at a 5.0% significance level.

5. ARTIGO II

Adult and pediatric physiologically based biopharmaceutics model to explain lamotrigine immediate release absorption process

Manuscrito será submetido à revista *CPT: Pharmacometrics & Systems Pharmacology*

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Abstract

Physiologically based biopharmaceutics modeling (PBBM) has an immense potential benefit in speeding up the development of new drugs and formulations, ensuring quality, reducing unnecessary human testing, and exploring the biopharmaceutical risk of a drug, particularly in children where data is scarce due to ethical reasons. Taking this in consideration, the main goal of this study was to explore biopharmaceutic properties of lamotrigine (LTG) and its effects in absorption process in adults and children by applying PBBM. An oral physiologically based pharmacokinetic model (PBPK) and PBBM was developed using GastroPlus™ software. A pediatric model was also developed and verified. Exploring biopharmaceutical parameters using sensitivity analysis, solubility had the greatest impact on LTG PK, more expressively in t_{max}. The PK of LTG is dependent on the solubility in the gastrointestinal tract. It is important to understand the quality of the data input in the PBBM/PBPK model since there are different ways of evaluating the solubility. Fluid volumes did not significantly interfere with LTG PK. The dose volume discreetly affected the t_{max} in pediatrics. Despite having shown a relationship between the volume available for drug solubilization (dose volume) and the PK parameters, it was not

significant. Hence, the underline hypothesis raised is even if LTG, experimentally classified as a BCS II, with low solubility, physiologically it may present a behavior of BCS I, when solubility is not a limiting factor. Further studies are needed to prove this, such as *in vitro-in vivo* relationship (IVIVR) and virtual bioequivalence (VBE).

Keywords: biopharmaceutics, modeling & simulation, solubility, dissolution, permeability

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The biopharmaceutical properties of a drug are important parameters for drug development. The traditional way of evaluating these properties is, in most cases, through the empirical interpretation of *in vitro* experiments.

WHAT QUESTION DID THIS STUDY ADDRESS?

The present work aimed, using physiology-based biopharmaceutical modeling, to understand whether the biopharmaceutical properties impact on pharmacokinetics of lamotrigine in adults and pediatrics.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Using physiologically based biopharmaceutical models, it is possible to evaluate the properties of drugs and formulations in a more mechanistic way, contributing to decision-making in the development of new products.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

This work can contribute to the development of formulations and change industrial strategies since the models developed and verified can be applied to virtual bioequivalence analysis and to define a dissolution safe space.

INTRODUCTION

The increase of pharmacokinetic modeling and simulation (M&S) applications has been notorious in recent years[1-2]. El-Khateeb *et al.* (2021) estimated a rate of growth for physiologically-based pharmacokinetic (PBPK) modeling (>40 fold/20 years) much steeper than the general pharmacokinetic modeling (< 3-fold/20 years). The M&S tools have been used for the most varied applications, in the last 10 years, among the publications using PBPK, the main goals were for study design (28%), formulation development (22%), drug-drug interaction studies (17%), and special populations (18%)[1]. Special populations such as children, elderly, pregnant women, and patients are challenging for the therapeutic sphere due to the difficulty of including these individuals in clinical studies[3].

From birth, growth to adulthood, children go through a series of physical, metabolic, and physiological processes, which make them different within the same population group. This process of ontogeny or physiological maturation originates significant differences in the pharmacokinetics (PK) processes: such as absorption, distribution, metabolism, and excretion (ADME) of drugs compared to adults[4,5]. Given this scenario, PBPK brings great advantages by considering the physiological characteristics and the variability needed to evaluate a population as heterogeneous as the pediatric one[2].

Another important application offered by PBPK is the possibility to evaluate and develop mechanistic absorption models. Through them, it is possible to explore and establish a link between *in vitro* data and pharmacokinetic data[6]. Among the mechanistic models, physiology-based biopharmaceutical modeling (PBBM) has a wide application and can cover several areas of biopharmaceutics. The PBBM is a support tool for formulation development processes, for justifying biowaivers, post-registration changes, developing dissolution methods, defining quality specifications for clinically relevant drugs and for studying the impact of biopharmaceutical properties in the clinic. PBBM, while there are still many gaps and many questions to be answered, has immense potential benefit in reducing unnecessary animal and human testing[6,7].

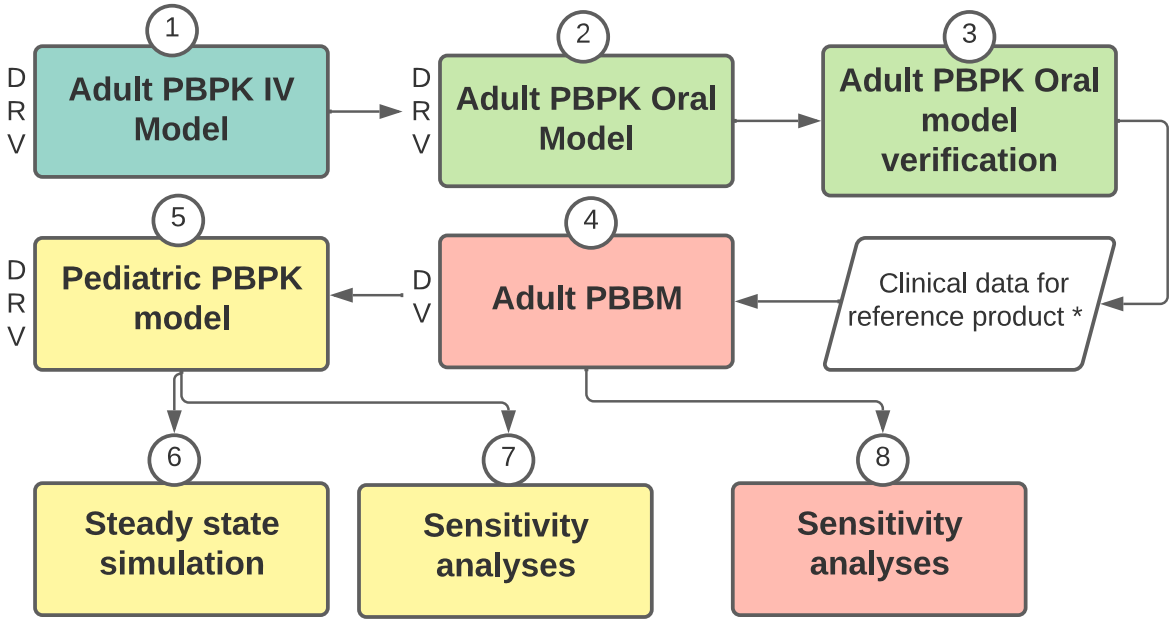
Currently, one of the main applications of PBBM is in studies of drugs with low solubility that may have their absorption impaired by biopharmaceutical properties[8,9]. Lamotrigine (LTG) is an antiepileptic drug widely used by adults and children that is categorized in the biopharmaceutical classification system (BCS) as II, having low solubility and high permeability. So, the mail goal of this study was to assess the biopharmaceutic risk on the properties of LTG and the effect on its absorption in adults and children applying PBBM.

METHODS

Workflow

The Workflow of the Adult PBPK, Adult PBBM, and Pediatric PBPK model development and performance evaluation for LTG is demonstrated in Fig 1.

Fig 1 Workflow of the Adult PBPK, Adult PBBM, and Pediatric PBPK studies for lamotrigine. The circled numbers are the sequential steps, D, R, and V means Development, Refinement and Verification, respectively for each model. *Clinical data for reference product.



Software

For the development of the PBPK and PBBM model GastroPlus® software (version 9.8, Simulations Plus Inc., CA, USA) and the modules ADMETPredictor®, PBPKPlus®, Metabolism & Transporter, and Optimization were used.

PK data

Clinical observed data was collected from literature for model development and evaluation, and are summarized in table S1 for adults and table S2 for pediatrics. The clinical studies were conducted in healthy volunteers [10-22] and in pediatric patients with epilepsy[23-25] for doses ranging from 25 to 200 mg for adults via intravenous and oral administration, and from 25 to 100 mg via oral administration for pediatrics following single or multiple doses. The data were extracted digitalized using WebPlotDigitizer® (Version 4.4 Released, PLOTCON; Oakland, USA).

Adult PBPK Model for Intravenous Administration

First, a whole PBPK model was developed for LTG for intravenous (IV) administration to accurately describe LTG distribution and elimination.

The metabolic parameters K_m and V_{max} for the enzymes UGT1A4 and UGT1A3 were fitted by parameter optimization. The tissue: plasma partition coefficient (K_p) perfusion-limited model was calculated using the Lukacova (Rodger-single) method. The volume of distribution (V_d) was also predicted according to Lukacova method[26]. The volume of distribution was adjusted by the refinement of the partition coefficient parameter (log P) using *in vivo* IV data[10-11]. Equilibrium solubility of LTG

determined in different pH conditions[27] was used to predicted pKa and the solubility factor with Henderson-Hasselbalch equation available in GastroPlus®. Solubility factor is the ratio that relates the solubility of a completely ionized state of a compound to the solubility of its un-ionized state[28].

The physicochemical, pharmacokinetic, and physiological parameters descriptors[29-33] of the LTG PBPK model are presented in Table S3.

Adult PBPK Model for Oral Administration

After a good agreement of distribution and eliminations phase, the parameters were fixed and a PBPK model was developed for oral administration including the inputs for *in vitro* solubility in biorelevant media, effective permeability (P_{eff}), formulation properties and the physiological factors for first-pass metabolism, gastric emptying, intestinal transit time, and transport. At first, the software default immediate-release tablet and the Johnson dissolution model was assumed.

The human oral absorption was predicted using the advanced compartmental absorption and transit model (ACAT®) under fasting conditions based on intestinal permeability, dissolution, and absorption scale factors (ASF). ASF was estimated using the software and the Opt logD Model SA/V 6.1. The used clinical observed data for the development of the oral model was obtained from a published PK study where the individuals received oral (solution) administration in a single dose of 100 mg (Table S1, dataset 2)[12].

Model evaluation

The model was evaluated calculating the observed *versus* predicted for area under the concentration-time curve (AUC_{0-t}) and maximum concentration (C_{max}). Additionally, a visual inspection of the predicted plasma profile overlapped with the clinical observed data collected from literature[34] was performed. Different pharmaceutical forms (capsule, tablet, dispersible and orodispersible tablets) were also simulated for doses of 25, 100, and 200 mg. Dispersible and orodispersible tablets[16,17,21] were assumed as "controlled release (CR): dispersed" dosage forms, tablets[14,18-20,22] as "IR: tablet", and capsule[13,15] as "IR: capsule" (Table S1).

To support the model evaluation, data of a 2 x 2 crossover clinical study for bioequivalence (BE) evaluation were kindly provided by Prati-Donaduzzi Pharmaceutical Company (Toledo, Paraná, Brazil) (an unpublished study identified with the code P10/12). The PK profile of the reference product (Lamictal®) (n = 34) was also used for model evaluation.

The model was deemed acceptable if the percentage of prediction error (%PE) of AUC_{0-t} and C_{max} was less than 25%, criteria like those used for bioequivalence studies[36,36], which is more stringent than the 2-fold range that is typically applied for evaluating the predictive performance of PBPK models[37,38].

Dissolution data

The *in vitro* dissolution data was kindly provided by Prati-Donaduzzi Pharmaceutical Company (Toledo, Paraná, Brazil). The samples were from the same batch of reference (Lamictal®) and test product used in the BE clinical trial (biobatch). Dissolution data were obtained from flow-through cell apparatus in open mode with biorelevant medium FaSSIF-V1. For adult dosage form (100 mg), the flow rate was 6 mL/min.

A pediatric condition was simulated adopting a flow rate of 4 mL/min[39] for the biorelevant medium FaSSIF-V1 with 25 mg orodispersible tablets.

Adult PBBM Model

The PBBM model was developed assuming the *in vitro* dissolution as input function for oral administration in adults. The trial simulation was performed for the reference product. The dissolution data for reference and test formulations were inserted in GastroPlus® as a table of percentual dissolved per time and fitted as z-factor[40].

Pediatric PBBM Model

The evaluated PBPK model developed for healthy adult volunteers was scaled for pediatrics of different age groups: 3.8 to 11.8 years and 6 months to 4.5 years respectively to the studies of Chen *et al.* (1999) and Vauzelle-Kervroëdan *et al.* (1996). GastroPlus™ PEAR Physiology module was used to build the pediatric PBPK model (table S2). The blood to plasma ratio (B/P) was scaled from adults to children accounting for age-dependent changes in hematocrit. For age and weight dependency, gut size, intestinal and stomach transit time, intestinal and stomach pH, regional lengths, and radii were kept as default pediatric gut physiology in the ACAT™ model. The drug-dependent parameters were evaluated the same as in the adult PBPK[41]. Pediatric PBBM was obtained by entering the dissolution data. The model evaluation followed the same criteria as mentioned before.

Simulations for multiple dose regimen were also considered for model evaluation. The study of Otoul *et al*. (2007) was reproduced for the dose of 7.7 mg/kg once a day (QD) in children aged 3.3 to 17.2 years[25], also, data from a therapeutic drug monitoring (TDM) study, conducted at the *Hospital das Clínicas* in Ribeirão Preto - Brazil, where the patients received ranging from 50 to 100 mg once (QD), twice (BID) and three times (TID) a day in children aged 4 to 11 years old (Table S2). The multiple dose model performance was evaluated by visual inspection of the predicted plasma profile overlapped with the clinical observed data and by the predicted over observed ratio rough plasma concentration (C_{tr}).

Parameter sensitivity analyses

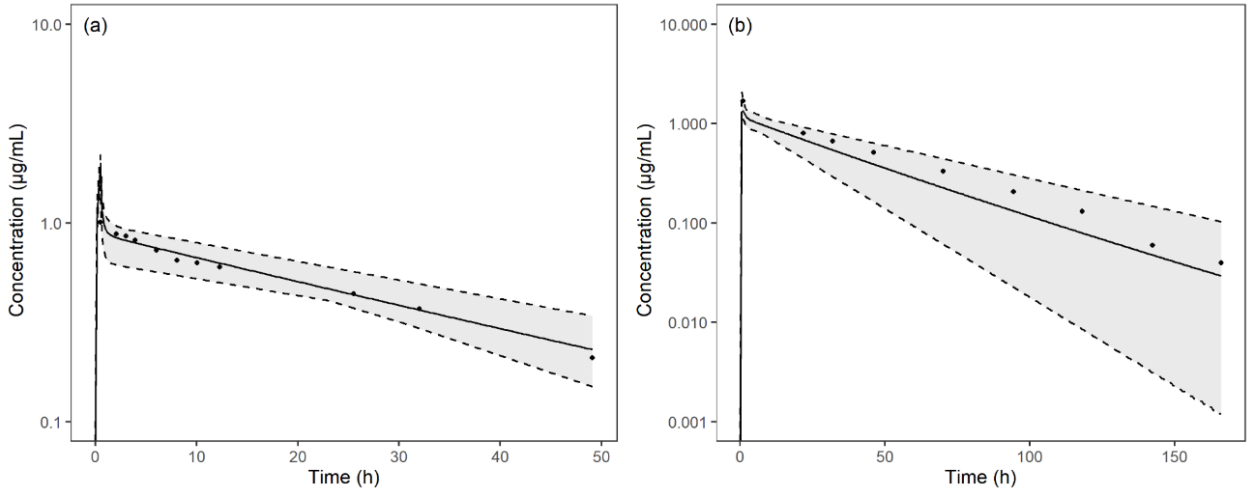
Parameter sensitivity analyses (PSA) were also performed to assess the importance of the biopharmaceuticals properties, permeability (Peff), solubility, and dissolution (z-factor)[42], to predict the percentage of drug absorbed and to mechanistically explore the comparison between adults and pediatrics. In addition, the influence of the gastric and intestinal fluids volumes was also investigated.

RESULTS

Intravenous adult PBPK model

The intravenous model accurately predicted the distribution and elimination of LTG (Fig 2). The calculated mean V_d was 1.13±0.09 L/kg, which agreed with reported in literature (0.9 to 1.5 L/kg)[30,31,43]. Total clearance (including metabolic clearance and renal excretion) was estimated as 2.08±0.39 L/h comparable with reported values in literature, ranging from 1.3 to 2.51 L/h[11,4].

Fig 2 Predicted and observed mean plasma concentration-time profiles of lamotrigine as single dose intravenous infusion[10] (a) and oral solution[12] (b). The solid line represents the predicted mean. The dashed lines and the gray area represent the 5th and 95th percentile of the predicted values for a virtual population. Filled circles are the mean observed data digitized from the literature



Oral PBBK model

The absolute oral bioavailability estimated was 98.04±0.54%, which agrees with previous reported data by Garnett (1997) and Conner *et al.* (2019)[30,31]. Simulated LTG oral cp-time profiles showed %PE less than 25% (-23.48 – 20.43%) comparing to the clinical observed mean data. Observed and predicted PK parameters are summarized in Tab 1.

Tab 1 Verification model results. Pharmacokinetic parameters predicted (Pred) and observed (Obs) for oral administration of a single dose of lamotrigine.

Dose (mg)[Ref]	Dosage form	AUC 0-t (µg/mL. h)			Cmax (µg/mL)			tmax (h)		
		Pred	Obs	%PE	Pred	Obs	%PE	Pred	Obs	%PE
25 [13]	Capsule	8.56	7.82	9.46	0.31	0.26	19.23	0.95	2.38	-60.08
25 [14]	Tablet	10.95	14.31	-23.48	0.31	0.35	-11.43	0.89	1.95	-54.36
100 [15]	Capsule	32.72	31.75	3.06	1.45	1.31	10.69	0.77	2.99	-74.25
100 [16]	Dispersible	41.89	54.02	-22.45	1.59	1.66	-4.22	0.90	1.00	-10.00
100 [17]	Orodispersible	49.44	50.39	-1.89	1.42	1.19	19.33	0.82	0.96	-14.58
100 [18]	Tablet	42.96	51.73	-16.95	1.42	1.73	-17.92	0.94	0.58	62.07
100 [19]	Tablet	51.20	66.40	-22.89	1.72	1.70	1.18	0.87	0.72	-20.83
100 *	Tablet	41.13	38.43	7.03	1.42	1.32	7.58	0.93	1.92	-51.56
100 [20]	Tablet	32.14	33.10	-2.90	1.37	1.28	7.03	0.86	1.99	-57.78
200 [21]	Dispersible	92.42	107.46	-14.00	2.84	2.82	0.71	1.02	2.00	-49.00
200 [22]	Tablet	99.36	91.37	8.74	2.83	2.35	20.43	1.11	3.53	-68.56

Note: Ref. reference; AUC_{0-t}, area under the concentration-time curve from time zero to time t; C_{max}, maximum concentration; Obs, observed; %PE, percentage predicted error; Pred, predicted; t_{max}, time for observation of maximum concentration. * Data from a bioequivalence study unpublished kindly provided by Prati-Donaduzzi Pharmaceutical Company.

PBBM model

For the z-factor calculation, an artificial volume of 1,000,000 mL was assumed for the medium to ensure unsaturation since the dissolution in flow-through cell apparatus was performed in open-loop mode with constant replacement of medium and complete sink condition. The z-factor calculated was 8.87 x 10^{-4} mL/mg/s and 8.94x10^{-4} mL/mg/s for reference and test formulation respectively. The adult PBPK model using z-factor improved the PK prediction and reduced the t_{max} %PE from -51.56 to -12.50% for reference formulation and from -54,32 to -25,48% for test formulation (Tab 2). Based on this founding, the PBBM model showed to have enough predictive capacity for applications in biopharmaceutical studies.

Tab 2 Pharmacokinetic parameters predicted and observed for oral administration of a single dose of lamotrigine in adults applying the dissolution models: z-factor and Johnson

Formulation	Dissolution mode	AUC 0-t (µg/mL. h)			Cmax (µg/mL)			tmax (h)		
		Pred	Obs	%PE	Pred	Obs	%PE	Pred	Obs	%PE
Reference	Johnson	41.13	38.43	7.11	1.45	1.32	9.85	0.93	1.92	-51,56
	Z-factor	42.74	38.43	11.22	1.22	1.32	-7.58	1.68	1.92	-12.50
Test	Johnson	43.61	39.47	10.49	1.44	1.31	9.92	0.95	2.08	-54,33
	Z-factor	39.54	39.47	0.18	1.19	1.31	9.16	1.55	2.08	-25.48

Note: AUC_{0-t}, area under the concentration-time curve from time zero to time t; C_{max}, maximum concentration; Obs, observed; %PE, percentage predicted error; Pred, predicted; t_{max}, time for observation of maximum concentration.

Pediatric PBPK model

The V_d for pediatrics population was adjusted by optimization of log P to 1.87[29]. The fed state was assumed given that is difficult to achieve the fasted state in children. Employing pediatric dissolution condition, by z-factor calculated of 1.86 x 10^{-4} mL/mg/s, the %PE for AUC, C_{max} and t_{max} were between -27.07 and 12.68% in single doses simulations (Tab 3).

Tab 3 Pharmacokinetic parameters predicted (Pred) and observed (Obs) for oral administration of lamotrigine in children.

Dose[Ref]	Protocol	AUC $_{0-t}$ (µg/mL.h)			C_{max} (µg/mL)			t_{max} (h)		
		Pred	Obs	%PE	Pred	Obs	%PE	Pred	Obs	%PE
2.0 mg/kg[23]	SD	37.67	37.60	0.18	1.30	1.48	-12.16	2.99	4.10	-27.07
2.0 mg/kg[24]	SD	28.62	25.40	12.68	1.16	1.11	4.50	4.75	4.93	-3.65
		AUC $_{0-t}$ (µg/mL.h)			C_{tr} (µg/mL)					
		Pred			Pred			Obs		%PE
7.7 mg/kg[25]	QD	16860			6.10			6.33		-3.63
50 mg*	BID	754			3.01			3.56		-15.44
100 mg*	BID	1648			6.63			8.86		-25.17
100 mg*	TID	1714			7.59			6.02		26.08

Note: AUC_{0-t}, area under the concentration-time curve from time zero to time t; C_{max}, maximum concentration; C_{tr}: trough plasma concentration; %PE, percentage predicted error; t_{max}, time for observation of maximum concentration, SD: single dose, QD: once a day, BID: twice a day, TID: three times a day. * Experimental data from a therapeutic drug monitoring in Brazilian pediatric patients unpublished.

Additionally, multidose plasma profiles were also simulated with once-a-day, twice-a-day, and three times-a-day protocols. The parameter used for this analysis was C_{tr} and the %PE were between -25.17 and 26.08. All pediatric PBPK simulations are present in Fig 3.

Impact of biopharmaceutical parameters in adult and pediatric absorption models

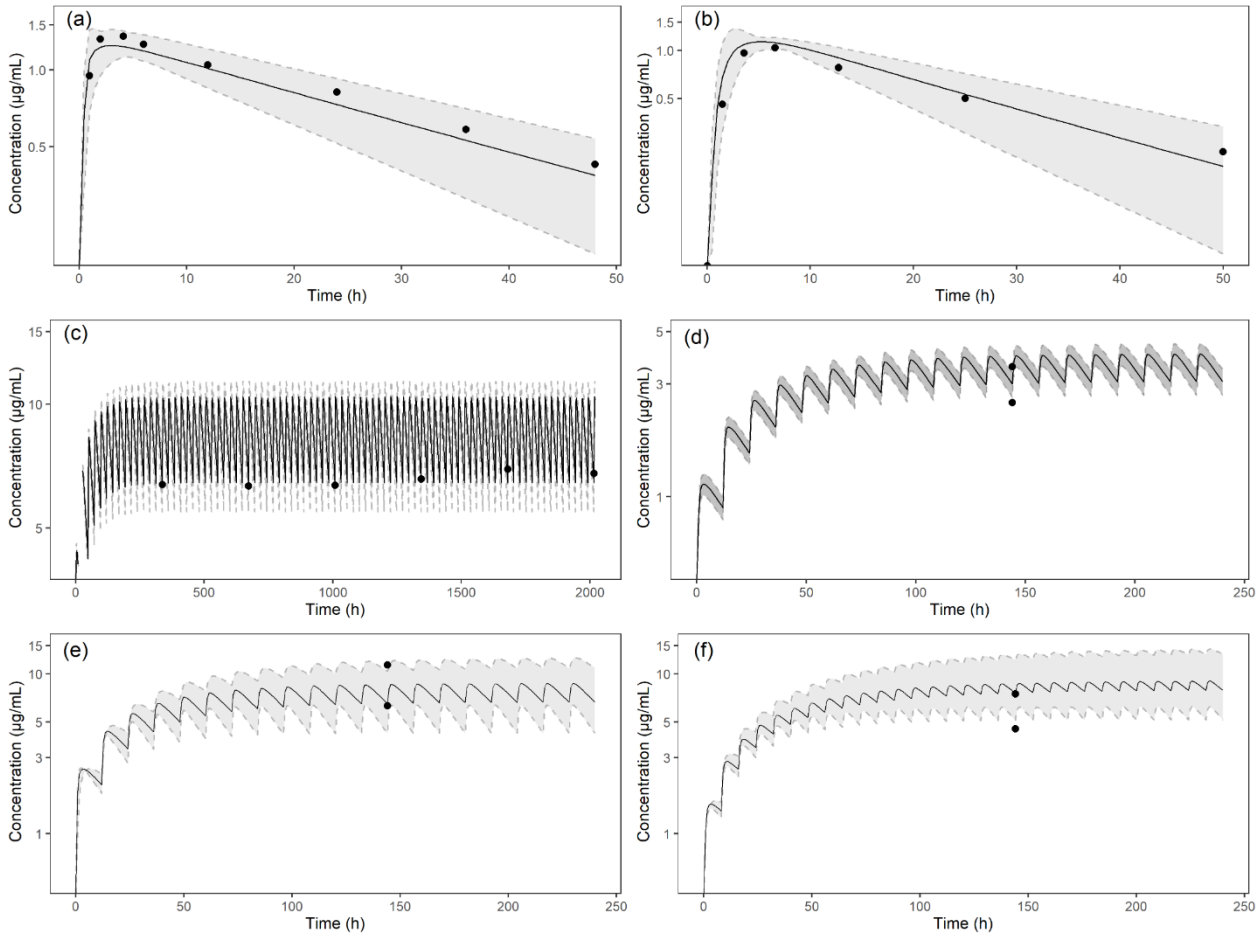
For the PSA evaluation, high doses were simulated for adults and pediatrics (assuming a worst case-scenario). For adults, simulations were performed for 200 mg and for children under 3 years old, dose of 15 mg/kg (200 mg)[27].

PSA (Fig 4) demonstrated that solubility is the most important property analyzed impacting PK parameters in both adult and pediatric populations. Reducing solubility from 0.21 to 0.021 mg/mL AUC and C_{max} was reduced by half and the t_{max} increased sixteen times. Peff does not seem to have an impact on the PK parameters evaluated.

On the other hand, dissolution can influence the LTG PK in adults and children with different intensities. For adults, low z-factors (less than 5.86 x 10^{-4} mL/mg/s) seem to significantly impact t_{max} in an inversely proportional manner by up to five times. For pediatrics, this proportion was smaller compared to adults.

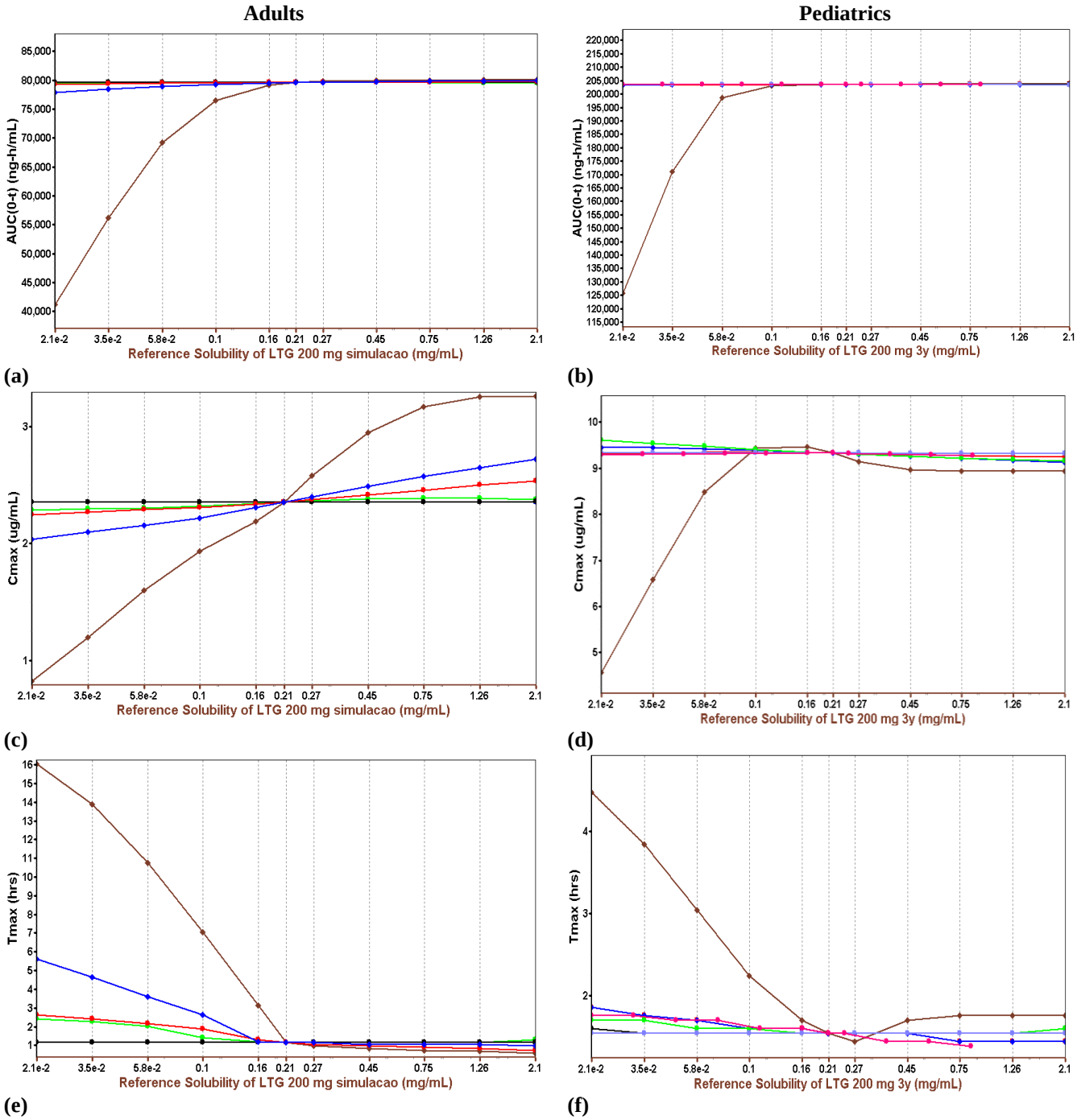
Variations in stomach, small intestine and colon fluid volumes did not show major impacts on the PK parameters evaluated in both the adult and pediatric models. The variation in the volume of liquid administered with the pharmaceutical form (dose volume) also appeared to influence the t_{max}. With the volume reduced from 250 to 25 mL in the pediatric model, the t_{max} increased by 13%.

Fig 3 Predicted and observed mean plasma concentration-time profiles of orally administered pediatric tablets of lamotrigine. The solid line represents the predicted mean. The dashed lines and the gray area represent the 5th and 95th percentile of the predicted values for a virtual population. Circles are the mean observed data for (a) a single dose of 2 mg/kg in children aged 3.8 to 11.3 years old[23], (b) a single dose of 2 mg/kg in children aged 0.5 to 4 years old[24], (c) 7,7 mg/kg QD in children aged 3.3 to 17.2 years old[25], (d) 50 mg BID in children aged 3 to 11 years old*, (e) 100 mg BID in children aged 6 and 7 years old*, (f) 100 mg TID a day in children aged 10 and 11 years old*.



Note: QD: Once a day, BID: Twice a day, TID: Three times a day. * Experimental data from a therapeutic drug monitoring study conducted in Brazil with pediatric patients diagnosed with epilepsy (unpublished data).

Fig 4 Sensitivity analysis of lamotrigine PBPK model. Parameters: permeability (Peff) in red, solubility in brown, dissolution (z-factor) in blue, stomach volume in black, small intestinal volume in green, colon volume in light blue, and dose volume in pink. (a), (c), (e) adults and (b), (d), (f) pediatrics.



DISCUSSION

PBPK have been used for the most varied purposes, including exploring biopharmaceutical scenarios and the PK on special populations[1]. Pediatrics is one of the special populations which represents a heterogeneous stage of physiological maturation. This differences in maturity are given by the ontogeny and it plays an important role in comparison of PK processes between adults and pediatrics or even

between pediatric ages[4,5]. Given this scenario, PBPK brings great advantages by considering the physiological characteristics and the variability required to evaluate a population as heterogeneous as the pediatric[2].

A commonly prescribed drug for children is LTG, an antiepileptic drug. LTG is classified as BCS II, which presents solubility and dissolution as limiting factors of the absorption process[32]. It is known that these biopharmaceutical properties can be impaired due to the immaturity of the gastrointestinal tract, but as far as we know, there are no studies evaluating the impact of biopharmaceutical properties on the pharmacokinetics of LTG in children.

Keeping with this in mind, the first stage of this work was to use the PBPK/PBBM to develop a LTG model for adults including dissolution data for a more mechanistic model. The adult PBBM model showed an improvement of the t_{max} parameter. Although this parameter is not traditionally used in pharmacokinetic studies of bioavailability and bioequivalence. According to the Food and Drug Administration guide[36], t_{max} should be used when the assessment of AUC and C_{max} are not sufficient, thus improving the prediction of t_{max} represents greater reliability on the developed model.

For the construction of the pediatric LTG model, it was necessary to adjust the V_d. The calculated mean V_d was 35.54 L, Chen *et al.* (1999) reported V_d value of 37.70 L for children aged between 3.8 to 11.3 years old[23]. Comparing by weight, the mean adult V_d is 1.16 L/kg[43] while the pediatric is 1.50 L/kg[23]. For this adjustment, the log P of the model was within the range found in the literature (1.19 to 1.93)[30,45,46], and it was assumed that this difference in input data between adult and pediatric models will not have a major impact on the purpose of this work. Thus, the pediatric model was considered able to predict the LTG PK behavior. With the adult and pediatric PBBM models developed and verified, it was possible to explore some biopharmaceutical parameters.

According to Amidon *et al.* (1995) three parameters are fundamental for the biopharmaceutical evaluation of a drug that control the rate and extent of absorption: permeability, solubility and dissolution[42]. The sensitivity analysis indicated that permeability did not influence LTG PK. This was described by Vaithianathan *et al.* (2015), where they reported that since LTG is classified as BCS II (high permeability) therefore does not interfere in the drug absorption[32].

On the other hand, both in the adult and in the pediatric models, the PK parameters were sensitive to variations in dissolution with greater intensity for the adult. It is important to remember that the pharmaceutical form used for adults is the immediate-release tablet and for children it is the orodispersible tablet[47]. Although the two formulations present a very fast dissolution type, which releases 85% of the active within 15 minutes[48], the orodispersible formulation dissolves almost instantly and, therefore, the data found in the PSA may be related to these differences.

Solubility was the parameter that had the most impact on AUC, t_{max} and C_{max} PK parameters in both adults and pediatrics, with a greater affect in t_{max} than in C_{max} and AUC. It is known that solubility is a property of the drug and can be influenced by the amount, the pH of the medium, the ionization of the molecule (pka), the volume, among other factors. LTG, as previously mentioned, is a BCS II which

classifies it as a molecule with low solubility, moreover, LTG is also a weak base and the pH of the analyzed medium dictates its solubility[32,49]. Therefore, as demonstrated by PSA, the PK of LTG depends on the solubility of this drug in the gastrointestinal tract.

Thus, it is important to understand the quality of the data used in the PBPK model, since there are different ways of evaluating the solubility of a molecule. The most used in pharmacokinetic models are equilibrium solubility (S_{eq}), or thermodynamics, which is determined from a saturated solution containing excess solids, where the solution and solid are in equilibrium, and intrinsic solubility (S_0) which is the S_{eq} of an ionizable substance, but which is entirely in non-ionized form due to the pH of the medium[50].

Intimately linked to the solubility of the drug, variations in the volumes of fluid in the stomach (30.28-121.12 mL), small intestine (20-80 mL) and colon (5-20 mL), and dose volume (water) (25-1000 mL) were also analyzed. Fluid volumes did not significantly interfere with LTG PK in both adults and children. Although several authors indicate the possible impact of the gastrointestinal tract ontogeny[51-52], in the condition tested (3-year-old child at a dose of 200 mg) this was not proven by fluids volume applying the PBBM model.

The dose volume discreetly affected the t_{max} in the pediatric model. According to Caleffi-Marchesini *et al.* (2023) the volume of liquid available is responsible for the total or partial solubilization of LTG. This volume is even more important in children younger than 3 years and in higher doses (15 mg/kg), since the dose/volume ratio is higher in children than adults[27]. So, using the PBBM model, it was possible to confirm this information, in which case the impact of volume was less than 15% on t_{max}.

It can then be seen that, despite having shown a relationship between the volume available for drug solubilization and the PK parameters, LTG does not seem to have exposure (AUC), C_{max} and t_{max} significantly influenced by the tested parameters. Under these conditions, the hypothesis can be raised that even if the drug is experimentally classified as a BCS II, physiologically it may show a BCS I behavior, when solubility is not a limiting factor. Such information can contribute to the development of new formulations and change industrial strategies since with BCS I drugs the regulatory guides that allows waiver of *in vivo* bioavailability and bioequivalence studies for the registration of generic and similar drugs can be applied[48].

However, further studies are needed to prove the possibility of waiver of *in vivo* bioavailability and bioequivalence studies, such as *in vitro-in vivo* relationship studies, virtual bioequivalence analysis and definition of a dissolution safe space. These strategies can be employed by industries and academy in discussions and justifications together with health authorities.

CONFLICT OF INTEREST SECTION

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Supplementary material

Tab S1 Summary of studies of lamotrigine from the literature used for adult model development and evaluation

Dataset	Population	Age (years)	Dose (mg)	n	Route/ regimen	Reference
Datasets for model development and refinement						
1	HV	21-48	67.82	8	IV/S	[10,11]
2	HV	35-59	100	12	Oral/S	[12]
Datasets for model evaluation						
3	HV	21-29	25	10	Oral/S	[13]
4	HV	21-48	25	32	Oral/S	[14]
5	HV	19-54	100	17	Oral/S	[15]
6	HV	22-47	100	12	Oral/S	[16]
7	HV	25-39	100	12	Oral/S	[17]
8	HV	22-27	100	12	Oral/S	[18]
9	HV*	19-24	100	24	Oral/S	[19]
10	HV	18-50	100	33	Oral/S	*
11	HV	20-52	100	24	Oral/S	[20]
12	HV	20-28	200	14	Oral/S	[21]
13	HV	35-57	200	12	Oral/S	[22]

Note: HV: Healthy adult volunteers; HV*: Thai healthy adult volunteers. IV: Intravenous administration; S: Single administration. *Data from a bioequivalence study unpublished kindly provided by Prati-Donaduzzi Pharmaceutical Company.

Tab S2 Summary of studies of lamotrigine from the literature used for pediatrics oral model extrapolation

Dataset	Population	Age (years)	Body weight (kg)	Dose	n	Regimen	Reference
Datasets for model extrapolation							
1	PP	3.8-11.3	12.8-51.3	2.0 mg/kg	12	SD	[23]
2	PP	0.5-4.5	7.0-20.5	2.0 mg/kg	10	SD	[24]
Datasets for model evaluation							
3	PP	3.3-17.2	16.0-80.0	7.7 mg/kg	20	QD	[25]
4	PP	4.0-11.0	21.4-22.0	50 mg	2	QD	*
5	PP	6.0-7.0	20.2-22.8	100 mg	2	BID	*
6	PP	10.0-11.0	25.7-39.0	100 mg	2	TID	*

Note: PP: pediatric patients; SD: single dose, QD: once a day, BID: twice a day, TID: three times a day. * Experimental data from a therapeutic drug monitoring study conducted in Brazil with pediatric patients diagnosed with epilepsy (unpublished data).

Tab S3 Physicochemical, *in vitro*, and physiological data used in the lamotrigine PBPK model

Parameters	Value	Reference / Comments
Physicochemical & Blood Binding		
MW (g/mol)	256.09	[29]
log P	1.70	Fitted assuming observed V_d data
pK_a (Base)	4.41	Predicted with Henderson-Hasselbalch equation using GastroPlus®
Solubility factor	12.09	Predicted using GastroPlus®
Solubility (mg/mL)	2.54 pH 1.2	Experimental[27]
	0.38 pH 4.5	
	0.24 pH 6.8	
	0.25 pH 7.4	
	0.21 pH 8.0	

B:P	1.00	[30]
Fu	0.45	[30,31]
Distribution		
Model	Full PBPK	Predicted with Lukacova equation [26] uring GastroPlus®
K_p Lung	0.59	
K_p Adipose	1.35	
K_p Muscle	0.96	
K_p Liver	1.35	
K_p Spleen	1.00	
K_p Heart	0.80	
K_p Brain	1.92	
K_p Kidney	1.00	
K_p Skin	1.17	
K_p ReproOrg	1.01	
K_p Red Marrow	1.95	
K_p Yellow Marrow	1.35	
K_p Rest of Body	1.01	
V_d (L/kg)	1.16	
Metabolism		
UGT1A3		Fitted by optimization module
V_{max} (mg/s/mg UGT1A3) *	6.81×10^{-5}	
K_m (mg/L) *	18.83	
UGT1A4		Fitted by optimization module
V_{max} (mg/s/mg UGT1A4) *	4.66×10^{-3}	
K_m (mg/L) *	147.95	
Excretion		
C_L renal (L/h)	0.20	[30,31]
Absorption		
Dosage form	IR	
P_{eff} (cm/s)	7.76×10^{4}	[31,32]
Diffusion coefficient (cm^2/sec)	0.84×10^{-5}	Predicted using GastroPlus®
Particle size distribution	Log-normal	Default GastroPlus®
Particle radius (µm)	25.00	Default GastroPlus®
Particle density (g/mL)	1.20	Default GastroPlus®
Dose volume (mL)	250	Default GastroPlus®
Precipitation model	First order	Default GastroPlus®
Precipitation time (sec)	900	Default GastroPlus®
Solubility FaSSGF (mg/mL)	3.48	Measurement *in vitro*
Solubility FaSSIF (mg/mL)	0.35	Measurement *in vitro*

Note: MW, molecular weight; log P, logarithm of octanol: water partition coefficient; pK_a, $-\log_{10}$ Ka, where Ka is the acid dissociation constant; B:P, blood to plasma concentration ratio; *fu*, fraction unbound in plasma; K_p, tissue: plasma partition coefficient; V_d, volume of distribution; K_m, Michaelis-Menten constant; V_{max}, maximum rate of drug metabolism; $C_{L\ renal}$, fraction of drug cleared unchanged renally; IR, immediate release formulation; P_{eff}, effective permeability; FaSSGF, fasted state simulated gastric fluid; FaSSIF, fasted state simulated intestinal fluid. * It was fitted by optimization module from the data of Argikar et al.[33], UGT1A3: K_m=70 µm and V_{max}=17 pmol/min/mg; UGT1A4: K_m= 50 µm and V_{max}=153 pmol/min/mg.

6. ARTIGO III

Exploration of lamotrigine IR tablets dissolution safe space using virtual bioequivalence and *in vitro-in vivo* relationship

Manuscrito que será submetido à *European Journal of Pharmaceutics and Biopharmaceutics*

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Abstract

Generic drug development involves significant investments in terms of time and resources from the drug development until the approval and registration of a new generic formulation. A crucial part of this process is the clinical study of bioequivalence (BE) between the test and reference products. However, modeling & simulation tools such as *in vitro-in vivo* relationship (IVIVR) and physiologically based biopharmaceutical modeling (PBBM) have emerged as alternatives to expedite the generic drug development and approval by health agencies. In this study, the IVIVR between dissolution tests and pharmacokinetic exposure of lamotrigine (LTG) was explored. Different dissolutions apparatus and media were utilized, and both the traditional Wagner-Nelson and the mechanistic deconvolution forms were tested. Additionally, a PBBM model was developed to explore and propose a safe space for immediate release (IR) tablets of LTG. Results showed that the IVIVR was best when using the flow-through cell apparatus and FaSSIF as dissolution medium and, this method was deemed biopredictive. The Wagner-Nelson and mechanistic deconvolution methods showed similar IVIVR in terms of apparatus and dissolution media, but the mean absolute percent prediction error was lower for the mechanistic deconvolution method. Using the LTG IVIVR and PBBM to simulate a clinical safe space,

the convoluted Cp-time and virtual BE suggested that variations of up to 20% in the dissolution profile may not impair the BE. Therefore, this study provides guidance for the development of new LTG formulations and contributes to biowaiver requests based on IVIVR and virtual BE.

Keywords: PBPK. BCS II. Biorelevant media. Biopharmaceutics. Apparatus 4

1. Introduction

Pharmaceutical companies and surveillance agencies are actively seeking alternatives to reduce time and cost of drug development phase for generic formulations [1]. One key aspect of new generic medicine approval is the bioequivalence test (BE), which compares the systemic exposure of a test and a reference drug product. The bioequivalence is achieved if the products exhibit the same rate and extent of absorption [2].

Clinical studies are a time-consuming and costly part of the generic drug approval, making *in vitro* techniques, which are shorter and less costly, a golden pot in the rainbow for manufacturers. I*n vitro-in vivo* relationship (IVIVR) can also be a key factor for post-registration of generic drugs, including changes in site producers, batch sizes and dissolution specifications. If dissolution tests are clinically relevant, they can justify waivers from bioavailability and BE studies (biowaiver) under certain conditions [3–5].

In vitro analyses used in IVIVR typically measure biopharmaceutical properties such as solubility and dissolution. Biopharmaceutics tools enable to link these critical quality attributes of the drug product to measured human exposure, and are therefore critical to understanding product performance, and to evaluate any limitations to drug dissolution and absorption [6]. For a good and discriminative dissolution method, some main aspects must be considered such as the choice of the dissolution apparatus, the agitation hydrodynamics, agitation speed, collection times and the selection of the dissolution medium (composition and volume). Such choices must be based on the physicochemical characteristics of the active substance, the intended dosage range of the drug, the formulation to be tested and the purpose of the test. For the development of IVIVR more biopredictive dissolution methods are ideal [6,7].

In addition to dissolution, it is also necessary to consider the dynamics of gastrointestinal variables that control transit form and time, disintegration, absorption, and metabolism throughout the human gastrointestinal (GI) tract [8]. From this perspective, the physiologically based biopharmaceutical modeling (PBBM) is an attractive tool for establishing a link between *in vitro* dissolution and a mechanistic modeling of oral absorption using for this the physiological based pharmacokinetic modeling (PBPK) [8].

Currently, IVIVR and PBBM are particularly useful for drugs with low solubility that may have their absorption impaired by biopharmaceutical properties [9,10]. A drug that shows low solubility is Lamotrigine (LTG), an antiepileptic drug categorized in the biopharmaceutical classification system (BCS) as II. Therefore, the aim of this work was to evaluate and establish the best LTG IVIVR by testing

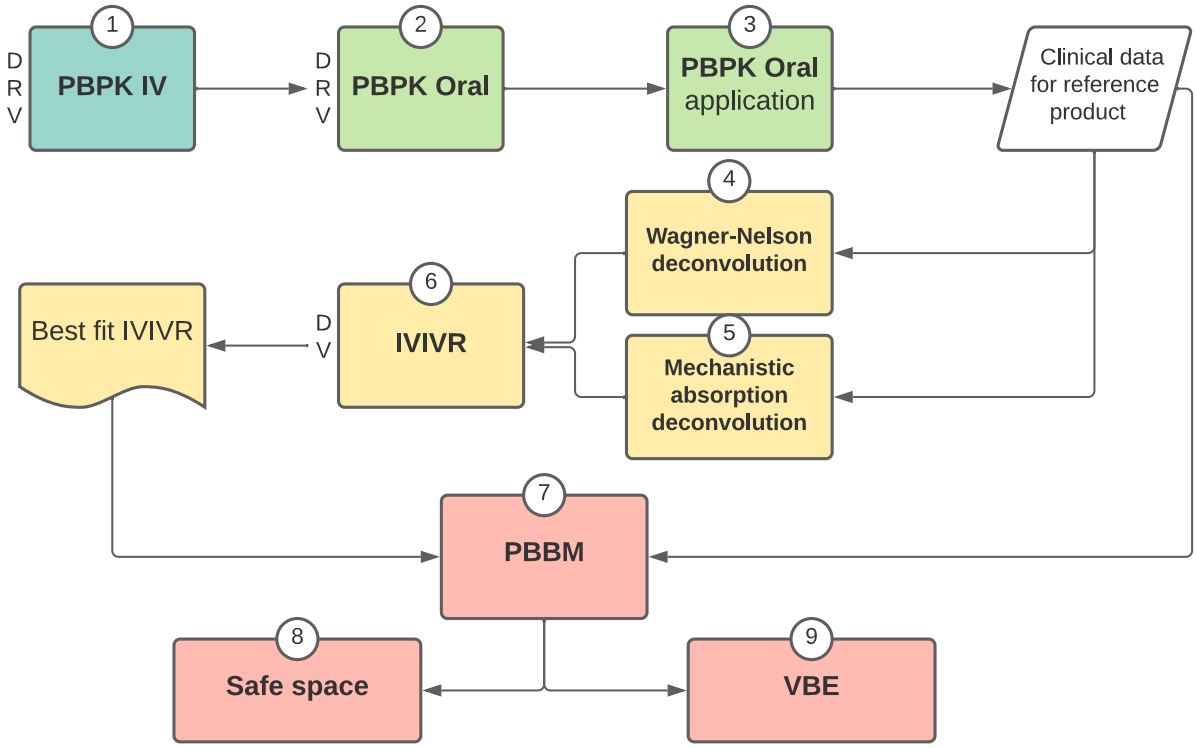
different dissolution apparatus and media, and comparing the traditional Wagner-Nelson and mechanistic deconvolution forms. Additionally, explore a LTG immediate release (IR) safe space through PBBM-IVIVR.

2. Materials and methods

2.1. Workflow

The Workflow of the PBBM, IVIVR, Safe space and Virtual bioequivalence (VBE) studies for LTG is demonstrated in Figure 01. Items 1,2,3 and 7 are described in Caleffi-Marchesini *et al.*, (2023) unpublished data (item 6).

Figure 01 Workflow of the PBBM, Safe space and virtual bioequivalence (VBE) studies for lamotrigine. The numbers are the sequential steps, D R V means Development, Refine and Verification, respectively for each model.



2.2. Software

The GastroPlus™ software (version 9.8, Simulations Plus Inc., CA, USA) with its modules: PBPKPlus™, PKPlus™, IVIVCPlus™ and Advanced Compartmental Absorption Transit (ACAT™) were used.

2.3. PBPK and PBBM

The oral and intravenous PBPK and PBBM models have been developed and verified and are described in Caleffi-Marchesini *et al.*, (2023) unpublished data (item 6).

2.4. Model evaluation

The models were evaluated calculating the errors for observed *versus* predicted for area under the concentration-time curve (AUC_{0-t}) and maximum concentration (C_{max}). Additionally, a visual inspection of the predicted plasma profile overlapped with the clinical observed data collected from literature [11]. Simulations were also carried out for different pharmaceutical forms and doses as showed by Caleffi-Marchesini *et al.*, (2023) unpublished data (item 6). Dispersible and orodispersible tablets [12-14] were assumed as "controlled release (CR): dispersed" dosage forms, tablets [15-19] as "IR: tablet", and capsule [20,21] as "IR: capsule".

To support the model evaluation, data of a 2 x 2 crossover clinical study for BE evaluation were kindly provided by Prati-Donaduzzi Pharmaceutical Company (Toledo, Paraná, Brazil) (an unpublished study identified by P10/12). The PK profile of the reference product (Lamictal®) (n = 34) was also used for model verification.

The model was deemed acceptable if the percentage of prediction error (%PE) of AUC_{0-t} and C_{max} was less than 25%, criteria like those used for bioequivalence studies [22-23] which is more stringent than the 2-fold range that is typically applied for evaluating the predictive performance of PBPK models [24-25].

2.5. Dissolution Input

The dissolution data of the same batch of reference (Lamictal) and test product used to BE was provided by Prati-Donaduzzi Pharmaceutical Company (Toledo, Paraná, Brazil).

Dissolution data for the reference and test formulation were obtained from paddle apparatus and flow-through cell apparatus with plain buffer and biorelevant medium. For paddle dissolution apparatus was used volume of 900 mL, 50 rpm, and dissolution media (a) hydrochloric acid 0.1 M pH 1.2, and (b) fasted stated intestinal fluid (FaSSIF-V1). For flow-through cell apparatus was used open mode, flow rate of 6 mL/min and dissolution media (c) hydrochloric acid 0.1 M pH 1.2 and (d) FaSSIF-V1.

2.6. IVIVR by Wagner-Nelson and mechanistic absorption modelling

The observed plasma concentration data of the reference formulation in Brazil (Lamictal®) was used for two approaches of deconvolution techniques: the traditional Wagner-Nelson [26] method and mechanistic method.

The Wagner Nelson deconvolution assumes the equation 1.

$$\frac{A_t}{A_\infty} = \frac{C_t + k_e \cdot \int_{t=0}^{t=t} C dt}{k_e \cdot \int_{t=0}^{t=\infty} C dt} \quad (1)$$

where A_t is the amount of drug absorbed at time t, A_∞ is the amount of drug absorbed at time infinite, k_e is the elimination rate, $\int_{t=0}^{t=t} Cdt$ is the area under the curve of plasma concentration versus time profile for the period between 0 and t and, $\int_{t=0}^{t=\infty} Cdt$ is is the area under the curve of plasma concentration versus time profile for the period between 0 and ∞ [26].

For mechanistic deconvolution, the platform accommodates any combination of nonlinearities and complexities in the behavior of a drug, generating a final Weibull parameter which represents the best function to describe the *in vivo* plasma exposition [27].

All deconvolved data obtained was confronted with the *in vitro* dissolution profiles in different media and apparatus (section 2.4.). The level of IVIVR were stablish by the slope of the regression line, the determination coefficient (R_{sq}), and visually on the fraction by time graph [28]

The model internal validation was performed applying the convolution tool. The predicted PK parameters (AUC_{0-t} and C_{max}) of the reference formulation were compared to observed ones. Additionally, the external validation of the model was performed with the same tool. The dissolution profile of a generic formulation was tested and the predicted and observed PK profiles were compared. Variations between predicted and observed values of a maximum of 15% were accepted in internal and external validation [29].

2.7. Exploratory analysis of the dissolution safe space

For the safe space exploration, the final PBBM model was assumed as a reference profile, then the theorical dissolution profiles were simulated by decreasing and increasing of the percentage of release (5, 10, 15 and 20%) at every reported time point.

Each theoretical dissolution profile was used to predict the systemic exposition by the convolution tool in the IVIVCPlus™. The predicted AUC and C_{max} were compared and variations of a maximum of 20% were accepted as similar to the reference profile and considered as a safe space for dissolution specifications for the BE.

2.8. Virtual bioequivalence (VBE)

All theorical dissolution profiles generate as describe in the safe space analysis session (item 2.6) were assumed as distinct input data of the hypothetical test formulations. These data were entered in GastroPlus™ as a table time (.dsd files) and fitted using the calculated z-factor.

In the PEAR™ module a of healthy American subjects ages between 18-50 years and male: female ratio 1:1 was selected. Simulations were performed for 150 subjects (10 trials x 15 subjects) to better reflect inter-trial and inter-individual variability, for each hypothetical formulation.

Predicted AUC_{0-t} and C_{max} parameters for each theorical formulation performed were compared to the observed reference parameters. The formulations were considered bioequivalent if 90% confidence

intervals (CI) of the mean test/reference for C_{max} and AUC ratios fall within the limits of 0.80-1.20 [2,28].

3. Results

3.1. In vitro-in vivo relationship (IVIVR)

Applying the Wagner-Nelson method for deconvolution, the *in vivo* systemic fraction showed 100% LTG available in 1.5 h, however, using the mechanistic absorption model (GastroPlus™) approach, the result showed that only 71.63% LTG was dissolved in the same time.

In the figure 02 can be observed the overlapping of the deconvoluted *in vivo* systemic fraction, the *in vitro* dissolution profiles, and the IVIVR for Wagner Nelson and figure 03 show the same results for the mechanistic absorption deconvolution.

For both approaches the *in vitro* method which better described the *in vivo* dissolution was the flow-through cell apparatus and FaSSIF-V1 as medium. This dissolution system showed 80.24±3.77% of LTG dissolved *in vitro* in 1.5 h for reference formulation. For the other dissolution conditions, there was a mismatch between the *in vitro* and *in vivo* times to dissolved 80% of LTG, showing the *in vitro* dissolution was faster than *in vivo*.

Therefore, the IVIVR for both deconvolution methods were verified internally and externally. The verifications were carried out using dissolution data referring to the *in vitro* methodology that demonstrated the best relationship. As can be seen in table 01, all %PE of internal and external verifications were below 15%. However, when comparing the absolute means of %PE, the mechanistic absorption deconvolution method presents smaller errors than the deconvolution method by Wagner Nelson.

Figure 02 Time-dependent profiles and *in vitro-in vivo* relationship of deconvolved *in vivo* dissolution, using Wagner-Nelson method. In the left column are represented the *in vitro* (squares) and *in vivo* deconvolved (circles) profiles. The right column presents the *in vitro-in vivo* relationships where the solid lines and dashed lines represent identity line and regression line, respectively. For paddle apparatus, (a) and (b) - the *in vitro* dissolution with HCl 0.1 M; (c) and (d) - FaSSIF-V1. For flow-through cell apparatus, (e) and (f)- FaSSIF-V1; (g) and (h) - gradient condition of 15-minute of 0.1 M HCl medium followed by FaSSIF-V1.

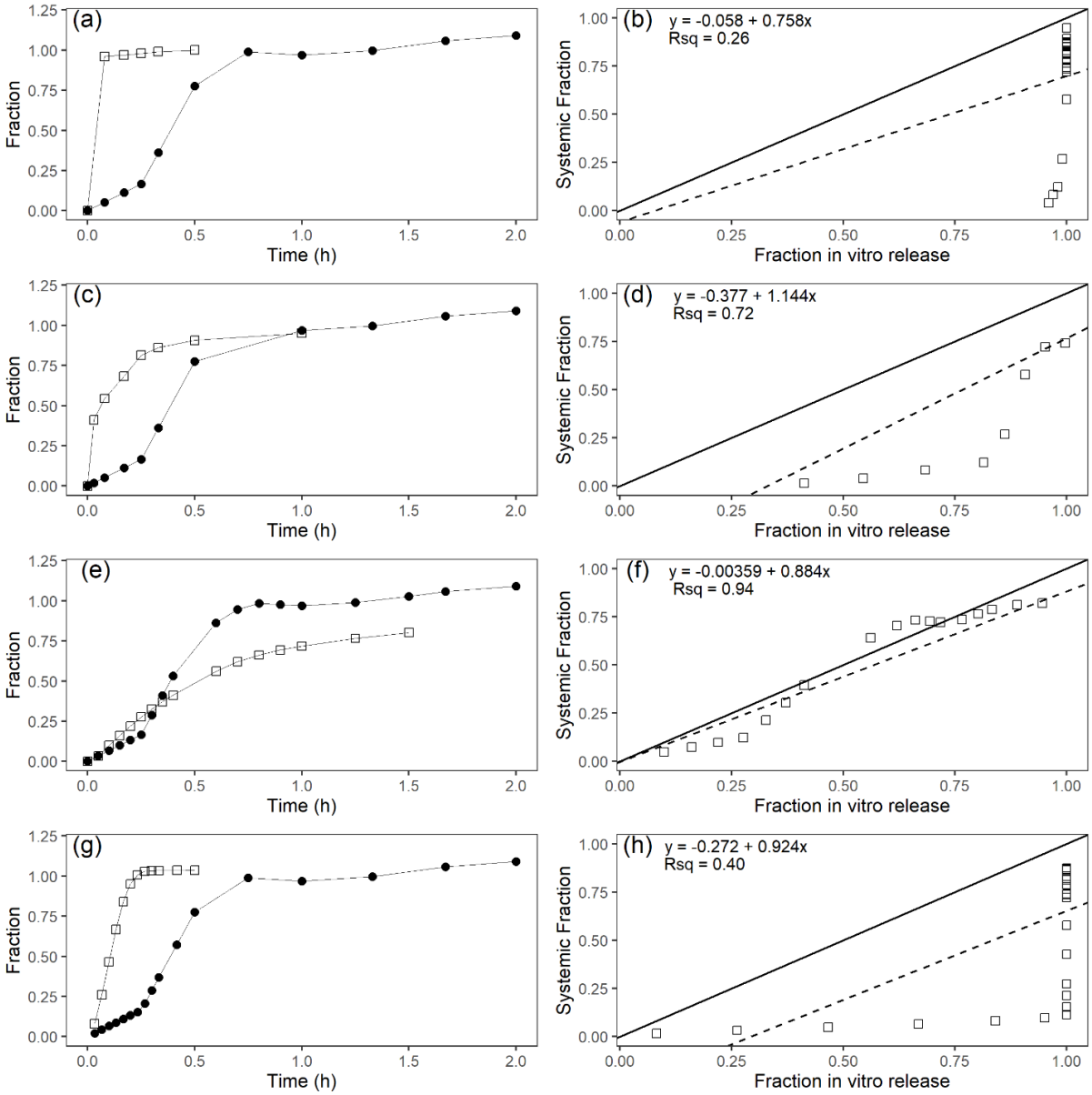


Figure 03 Time-dependent profiles and *in vitro-in vivo* relationship of deconvolved *in vivo* dissolution, using the mechanistic absorption model (GastroPlus™). In the left column are represented the *in vitro* (squares) and *in vivo* deconvolved (circles) profiles. The right column presents the *in vitro-in vivo* relationships where the solid lines and dashed lines represent identity line and regression line, respectively. For paddle apparatus, (a) and (b) - the *in vitro* dissolution with HCl 0.1 M; (c) and (d) - FaSSIF-V1. For flow-through cell apparatus, (e) and (f)- FaSSIF-V1; (g) and (h) - gradient condition of 15-minute of 0.1 M HCl medium followed by FaSSIF-V1.

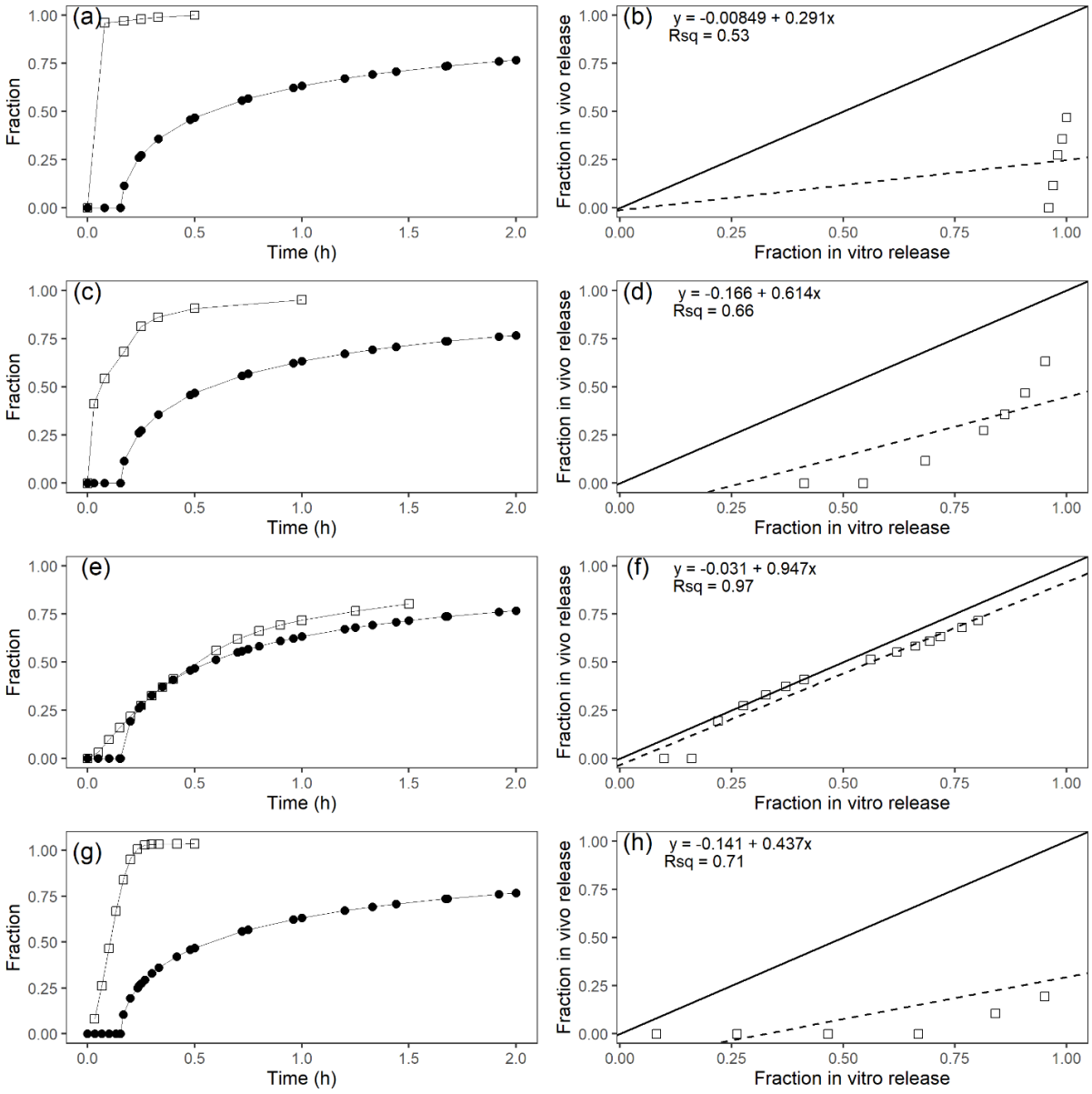


Table 01 Internal and external verification of IVIVR methods using deconvolution techniques: Wagner-Nelson deconvolved method and Mechanistic absorption model (GastroPlus™)

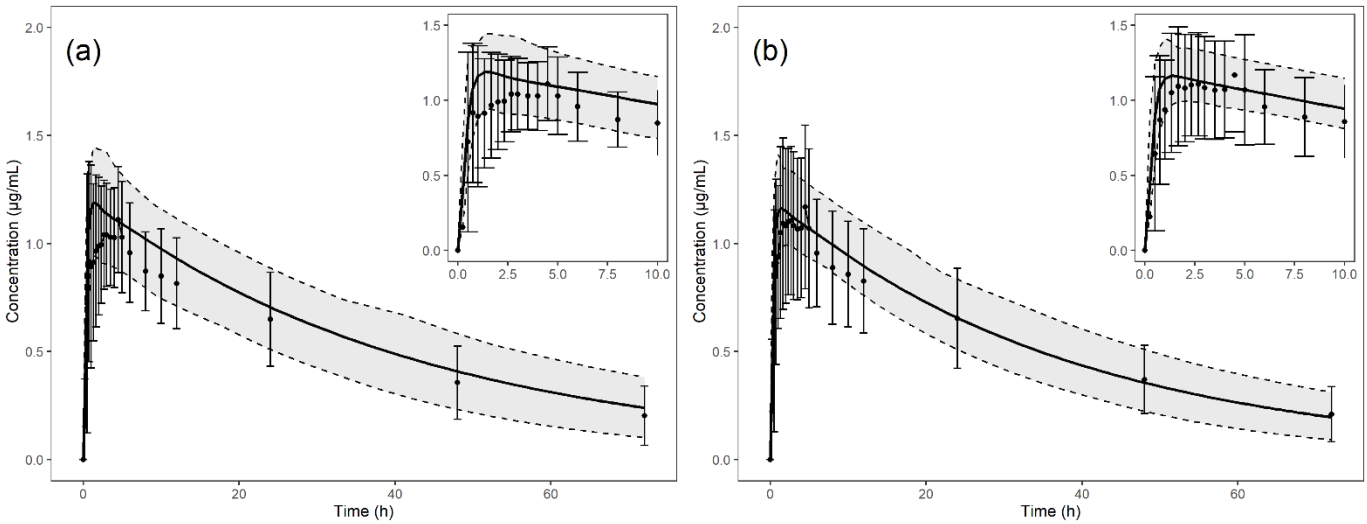
Wagner Nelson deconvolved method						
Formulation	AUC_{0-t} (µg/mL. h)			C_{max} (µg/mL)		
	Predicted	Observed	%PE	Predicted	Observed	%PE
Reference	42.41	37.83	-12.11	1.06	1.11	4.78
Test	42.10	38.73	-8.70	1.02	1.17	12.57
Mean absolute percent prediction error			10.41			8.67
Mechanistic absorption (GastroPlus™) deconvolved method						
Formulation	AUC_{0-t} (µg/mL. h)			C_{max} (µg/mL)		
	Predicted	Observed	%PE	Predicted	Observed	%PE
Reference	41.62	37.83	-10.01	1.20	1.11	-8.29
Test	41.40	38.73	-6.91	1.21	1.17	-3.40
Mean absolute percent prediction error			8.46			5.85

From the IVIVR evaluation, the dissolution profile from flow-through cell apparatus using FaSSIF-V1 were selected as the best data for the sequential steps. These data were used as an input function in the PBPK model to evaluate the influence of *in vitro* drug dissolution rate on LTG plasma concentration profiles.

3.2. PBBM model

The z-factor calculated was 8.87 x 10^{-4} mL/mg/s and 8.94 x1 0^{-4} mL/mg/s for reference and test formulation respectively. The PBBM model (figure 04) improve the absorption prediction. Then, the PBBM model was assumed with enough predictive capacity for applications in Biopharmaceutical studies. More details of the PBBM model were discussed by Caleffi-Marchesini *et al.*, (2023) unpublished data (item 6).

Figure 04 Predicted and observed mean plasma concentration-time profiles of orally administered reference formulation of lamotrigine as single doses of 100 mg as tablet using dissolution models: (a) Johnson and (b) z-factor for the reference formulation. The solid and dashed lines represent the predicted mean, 5th and 95th percentile of the predicted values for a virtual population. Circles represent the mean observed data.



3.3. Safe space analysis

Applying IVIVR, predicted plasma profiles were obtained by varying the dissolution profiles theoretically. The predicted plasma profiles obtained are shown in figure 05. Pharmacokinetic parameters C_{max} and AUC_{0-t} predicted and observed, and %PE are shown in table 02.

Figure 05 Convoluted IVIVR plasma concentration-time profiles of lamotrigine employing theoretical dissolution profiles with slow and fast release, decreasing, and increasing 5, 10, 15 and 20% at each reported time point of the reference formulation.

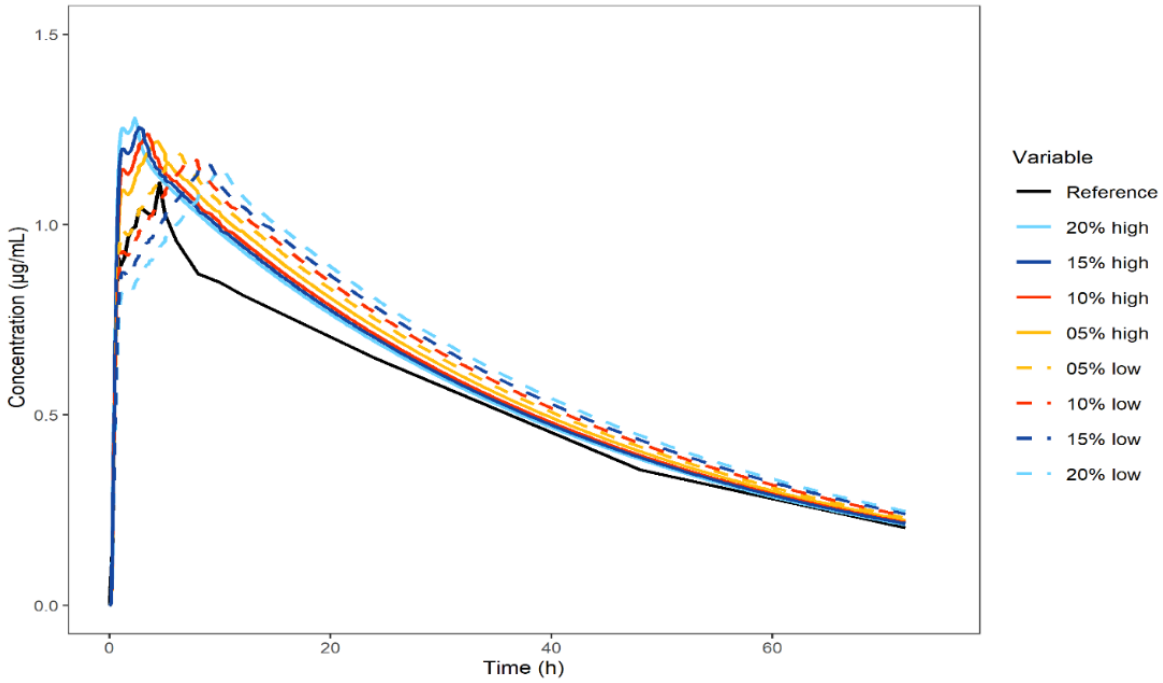


Table 02 Pharmacokinetic parameters predicted and observed for oral administration of a single dose of lamotrigine 100 mg applying theoretical dissolutions by traditional IVIVR

Theoretical dissolution	C_{max} (µg/mL)			AUC 0-t (µg/mL. h)		
	Predicted	Observed	%PE	Predicted	Observed	%PE
20% low	1.15	1.11	-3.27	41.24	37.83	-9.03
15% low	1.16	1.11	-4.50	41.38	37.83	-9.39
10% low	1.17	1.11	-5.73	41.48	37.83	-9.66
5% low	1.19	1.11	-7.03	41.56	37.83	-9.86
5% high	1.22	1.11	-9.81	41.66	37.83	-10.13
10% high	1.24	1.11	-11.48	41.69	37.83	-10.22
15% high	1.26	1.11	-13.14	41.72	37.83	-10.29
20% high	1.28	1.11	-15.35	41.74	37.83	-10.35

The predicted profiles showed %PE for C_{max} between -3.27 and -15.35, and for AUC_{0-t} between -9.03 and -10.35. Considering that two formulations are bioequivalent when the differences between C_{max} and AUC of the test and the reference remain within a 20% variation [2], all theoretical profiles tested would be bioequivalent to the reference.

3.4. Virtual bioequivalence (VBE)

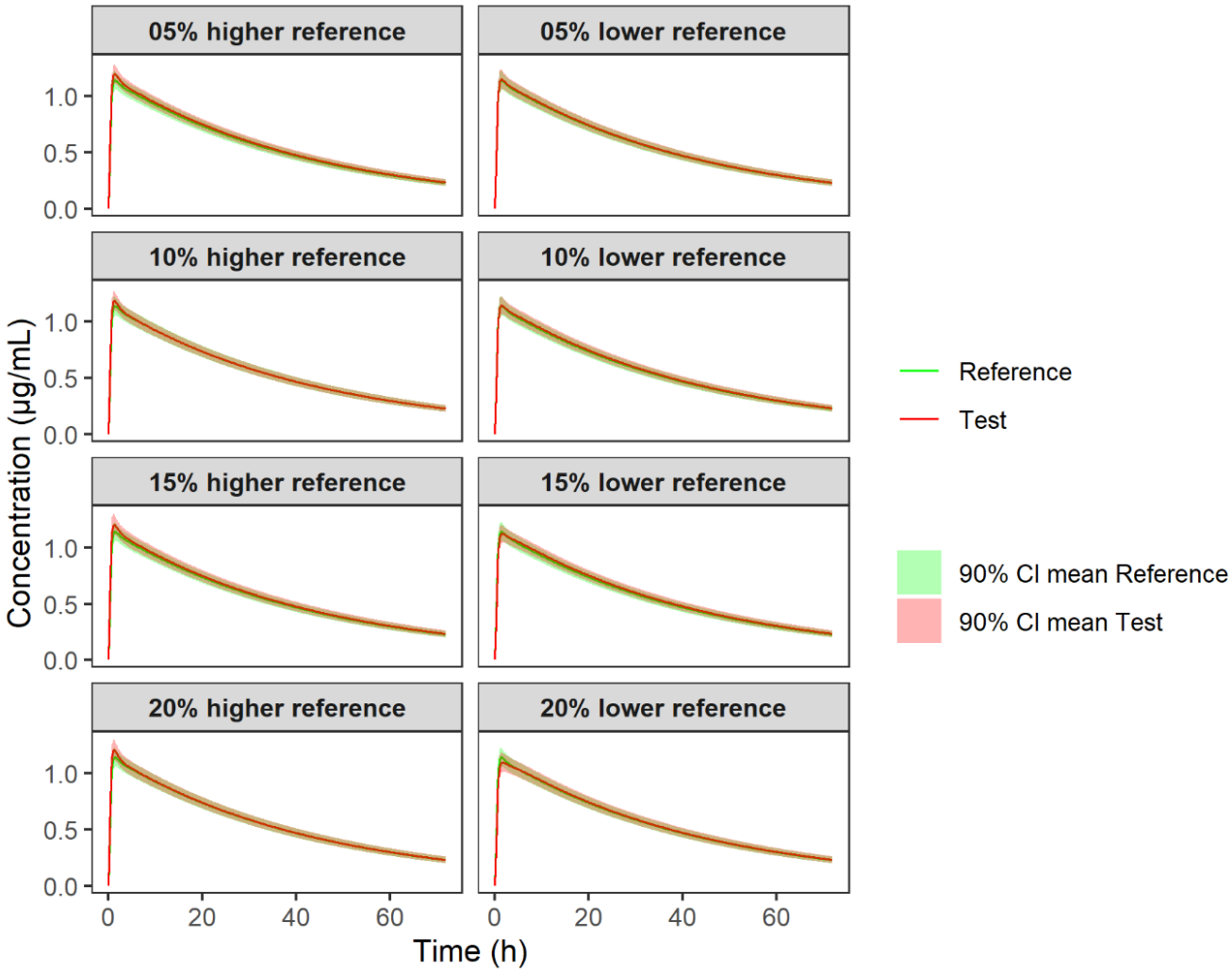
The theoretical dissolution profiles were also used to assess VBE using the PBBM model (Table 03). The figure 06 shows the simulated PK profiles for theoretical tests and reference.

Table 03 Pharmacokinetic parameters predicted for oral administration of a single dose of lamotrigine 100 mg applying theoretical dissolutions as test formulation by virtual bioequivalence

Theoretical dissolution	C_{max} (µg/mL)				AUC 0-t (µg/mL. h)			
	Test	Reference	Ratio	90% CI	Test	Reference	Ratio	90% CI
20% low	1.17	1.24	0.94	0.87 - 1.02	41.15	41.69	0.99	0.89 - 1.07
15% low	1.18	1.24	0.95	0.88 - 1.04	41.35	41.69	0.99	0.90 - 1.08
10% low	1.20	1.24	0.97	0.89 - 1.05	41.40	41.69	0.99	0.90 - 1.08
5% low	1.22	1.24	0.98	0.90 - 1.05	41.42	41.69	0.99	0.89 - 1.09
5% high	1.27	1.24	1.02	0.94 - 1.10	41.47	41.69	0.99	0.90 - 1.08
10% high	1.28	1.24	1.10	0.95 - 1.10	41.52	41.69	1.00	0.90 - 1.08
15% high	1.29	1.24	1.04	0.96 - 1.12	41.66	41.69	1.00	0.89 - 1.09
20% high	1.29	1.24	1.04	0.96 - 1.14	41.47	41.69	0.99	0.89 - 1.09

The predicted profiles, called as test formulation, showed mean test/ reference ratio for C_{max} between 0.94 to 1.10 and 90% confidence interval (CI) within the range of 0.87 to 1.14. For AUC_{0-t}, mean test/reference ratio was between 0.99 to 1.00 and 90% CI within the range of 0.89 to 1.09

Figure 06 Virtual bioequivalence simulation for the reference formulation and test formulations employing theoretical dissolution profiles with slow and fast release.



As in the predicted data obtained by IVIVR, all plasma profiles predicted by the PBBM model passed the VBE, presenting test/reference ratio between 0.80 and 1.25, that is the traditional BE limit for non-narrow therapeutic range drugs according to the Food and Drugs Administration (FDA) [2]

4. Discussion

In the routine of new drug development, the *in vivo* prediction tools such as IVIVR, PBPK and PBBM are extremely useful to reduce testing time and cost as well as reduce the amount of testing in humans [1]. Such tools have been extremely useful for drugs with low solubility, where the limiting factor for the absorption and bioavailability process is drug dissolution rate. In this sense, LTG was chosen in this study as a model drug, a weak base BCS II (low solubility and high permeability). Despite being classified as low solubility, the LTG solubility is dependent on the pH of the medium [35], which suggests that the dissolution and solubilization process are important for absorption.

In visual analysis, the most favorable relationship, in both deconvolution methods, was observed when it was applied the flow-through cell apparatus and FaSSIF as dissolution medium. This method displays the highest biopredictivity in this given situation. Biopredictive dissolution methods are more clinically relevant and are a key element for successful modeling and simulation [6], for applications with different goals.

Medina *et al*. (2017) conducted a study comparing the USP apparatus 2 and 4 for *in vitro* performance of ibuprofen. They observed that in the flow-through cell apparatus the drug release was slightly slower than the paddle apparatus, as well as for LTG. They explained that by the hydrodynamic and agitation conditions of the USP apparatus 4 and the fact that the dosage form and the drug particles are continuously exposed to uniform laminar flow, like the natural environment of the gastrointestinal tract [36,37], permitting a continuous dissolution of the drug simulating absorption into the systemic circulation [37], which also appears to be an important factor in the rate of dissolution of LTG. The flow-through cell apparatus has been applied specially to demonstrate the *in vitro* dissolution performance of poorly soluble drugs [38]. As is already known, LTG has better solubility in gastric environments [35], thus, the best relationship with FaSSIF medium agrees that intestinal conditions are critical for LTG dissolution and absorption.

In IVIVR approaches, the choice and application of a suitable deconvolution method is crucial as it determines the absorption kinetics after an oral administration [39]. The mechanistic deconvolution method showed a lower mean error compared to the Wagner-Nelson method. The deconvolution method proposed by Wagner-Nelson is composed of a hybrid function with several kinetic processes involved, such as dissolution, permeability, and intestinal transit [39,40]. On the other hand, the PBPK mechanistic deconvolution method is used to estimate the *in vivo* dissolution instead of the systemic entry rate, separately accounting for permeation, gastrointestinal transit, and first-pass elimination [41,42]. Although the Wagner-Nelson method is more traditional, the mechanistic method was able to predict *in vivo* dissolution data in a more accurate and pure way. Therefore, the deconvolution method chosen for the other analyses.

Most IVIVR methods are designed for controlled release formulations. For LTG, Shah *et al*. (2009) developed an *in vitro-in vivo* correlation (IVIVC) level A for modified release formulations with dissolution profiles in two-stage paddle apparatus (0.1 N HCl and pH 6.8 buffer) [43]. However, as far as is known, there are no IVIVR for IR dosage forms of LTG.

LTG IVIVR was tested using the dissolution profiles of two formulations, a reference and a generic one. From the reference formulation, the IVIVR was developed and validated internally. The best relationship was obtained with the combination of flow-through cell, FaSSIF medium, and mechanistic deconvolution method. The LTG PK parameters predicted by IVIVR model adequately described the observed ones and the model was considered internally and externally validated. The IVIVR was able to explain and predict the plasma LTG data for both formulations, which suggests that it can be applied to other formulations, and can be useful in routine formulation development.

Additionally, in order to confirm the chosen dissolution method for the IVIVR, the dissolution profile of the reference and test formulation were used to simulate a plasma profile by PBBM modeling. The introduction of the dissolution profile in the PBBM model improved the t_{max} prediction for both formulations. This fact supports that the absorption of LTG is dependent on the dissolution process and confirms the importance and applicability of an IVIVR for LTG. Hence, the LTG IVIVR can be a useful tool to guide new formulations development and to predict the clinical impact by using dissolution data as the input into the model.

Adopting the LTG IVIVR and PBBM, a clinical safe space was explored. A clinical safe space indicates how the dissolution of a new formulation can vary without impairing the BE with the reference formulation [44]. From that, the convoluted IVIVR Cp-time of LTG suggested that a 20% variation from the reference formulation dissolution profile would possibly not impair the BE since the theoretical formulations presented %PE is less than 20%. Although, variations in dissolution rate seem to affect t_{max}.

At the same time, in VBE simulations using PBBM, the variations in dissolution profile of 20% higher and lower were bioequivalent to the reference, and they did not show large variations in t_{max}. It should be emphasized that in studies of VBE, inter-occasion variability is considered, which is important to fill gaps in the accuracy of *in vivo* variability [28].

According to the FDA, the PK parameters that must be evaluated in a BE study are AUC and C_{max}. In both simulations, the variations in the *in vitro* dissolution profiles did not bring significant changes to BE with the reference formulation. Therefore, the biowaiver of IR formulations containing LTG seems to be justifiable based on the IVIVR and PBBM, when the *in vitro* dissolution profile of the test formulation is within the 20% variation range of the reference formulation.

The models presented in this work can support drug research and development of generic formulations reducing time, expenses, and unnecessary exposure of healthy volunteers to antiepileptic drugs for BE studies. The verified models for LTG can be explored for special populations extrapolation that present the dissolution and absorption process as limiting factors.

In conclusion, it is important to highlight that in this present work the amount released was considered, but not the dissolution speed. Future studies are needed to evaluate the impact from dissolution speed. Considering that dissolution safe space is, by definition, “the boundaries of *in vitro* dissolution, and relevant quality attributes, within which drug product variants are expected to be bioequivalent to each other” [45], this was not possible to be assessed, since there were no data available from non-BE studies.

5. Conclusion

Biopharmaceutical assessment tools such as PBBM, IVIVR and VBE are extremely useful in the routine of pharmaceutical companies to guide the development of new formulations, *in vivo* studies and to assess possibilities for biowaiver. The models suggested that the dissolution profile of a LTG test

formulation can vary by up to 20% point-to-point compared to the reference formulation and remain bioequivalent to each other. Also, the present LTG models may be applied and tested for other formulations and extrapolated to special populations that have the dissolution and absorption process as limiting factors.

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Conflict of interest section

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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7. DISCUSSÃO GERAL

A LTG é um fármaco cuja biodisponibilidade e absorção no TGI são dependentes dos processos biofarmacêuticos de solubilidade, precipitação e dissolução (VAITHIANATHAN *et al.*, 2015). Para estudar estas propriedades foi desenvolvido um método analítico para identificação e quantificação da LTG em meios de dissolução tamponados de pHs fisiológicos e meios biorrelevantes. O método mostrou-se linear, exato, preciso e robusto, e foi considerado validado.

A análise de solubilidade em equilíbrio evidenciou a dependência do pH do meio para a dissolução da LTG. Houve um aumento da solubilidade em meios biorrelevantes e, portanto, pode existir o efeito dos sais biliares e formação de micelas neste processo. No entanto, esta melhora não foi suficiente para mudar a classificação e a LTG continuou sendo pouco solúvel em pHs ácidos e muito pouco solúvel em pHs neutros a básicos independente da presença de reagentes biorrelevantes.

Em adultos, assim como esperado, a maior dose de LTG demonstrou característica de classe II do SCB, sendo totalmente solúvel em ambiente ácido e pouco solúvel em ambientes neutros a básicos. Em crianças de 10 a 12 anos, as doses de 5 mg/kg e 15 mg/kg apresentaram comportamentos semelhantes ao do adulto. Já em crianças menores que 5 anos na maior dose, a LTG apresentou-se pouco solúvel em todos os meios avaliados. Assim, em pediatria, apesar de não haver mudança na classificação biofarmacêutica da LTG, visto que nesta classificação é sempre adotado o pior caso no TGI, a dose e o volume de líquido administrado concomitante demonstraram ser fatores importantes. Estes fatores podem alterar a solubilidade no ambiente gástrico e impactar os demais processos envolvidos na absorção da LTG.

Paralelamente, foi desenvolvido e verificado um modelo PBPK completo de administração oral que foi capaz de prever de forma satisfatória os dados plasmáticos de 10 estudos farmacocinéticos publicados e 1 estudo de bioequivalência. Após o desenvolvimento do modelo PBPK da LTG, foram testadas as RIVIV entre dados de perfis de dissolução *in vitro* e perfis plasmáticos *in vivo*. Para a deconvolução dos dados *in vivo*, o modelo mecanístico, que emprega o PBPK nos seus cálculos, foi o que apresentou menor erro de predição e assim foi o escolhido para esta análise.

Dentre os métodos de dissolução testados o que apresentou melhor relação com os dados *in vivo* foi o que empregou o dissolutor com células de fluxo (aparato 4) e FaSSIF como meio de dissolução. Este método apresentou correlação linear com $R^2 = 0,97$ e angulação da reta de 0,95. O aparato de células de fluxo apresenta um comportamento similar ao do TGI por meio de um fluxo contínuo de meio e, dessa forma, mostrou ser mais biodescritivo para a LTG em formulações de liberação imediata.

A RIVIV desenvolvida foi validada internamente a partir de dados do medicamento referência, que foi empregada para a construção do modelo, e externamente a partir de dados do medicamento teste (candidato à genérico). A partir da RIVIV foram testados perfis de dissolução teóricos variando até 20%

ponto a ponto do perfil da formulação referência. Nessa análise os perfis plasmáticos previstos foram bioequivalentes ao medicamento referência.

Um modelo PBBM também foi desenvolvido com a introdução de dados de perfil de dissolução no modelo PBPK. Os mesmos perfis de dissolução teóricos empregados na análise por IVIVR foram testados e, originaram perfis plasmáticos preditos bioequivalentes a formulação referência.

Dessa forma, os modelos PBBM e RIVIV para a LTG podem ser ferramentas úteis para aplicação em outras formulações e uso em extrapolações para populações especiais que tenham o processo de dissolução como fator limitante para a absorção.

Uma dessas populações especiais é a pediátrica. As crianças compõem uma população complexa devido aos processos ontogênicos de cada faixa etária, de forma que, as respostas farmacocinéticas podem ser diferentes de acordo com a maturação do indivíduo (BATCHELOR; FOTAKI; KLEIN, 2014). Diante disso, neste trabalho, a partir do PBBM adulto foi extrapolado e desenvolvido um modelo adotando perfil de dissolução em condições pediátricas. Este modelo foi verificado utilizando dados de literatura e obtidos por um estudo de monitorização terapêutica com administração única e múltiplas doses.

A partir disso, foi avaliada a sensibilidade das características biofarmacêuticas na farmacocinética da LTG. Como esperado, por ser uma molécula classe II do SCB, a permeabilidade não é o fator limitante para a absorção da LTG (VAITHIANATHAN *et al.*, 2015). Já a dissolução demonstrou sensibilidade principalmente quanto a formulação empregada. Em adultos foi testado um comprimido de liberação imediata e em crianças um comprimido orodispersível, ambos com dissolução muito rápida, liberando 85% ou mais do IFA em 15 minutos(FDA, 2002). O comprimido de liberação imediata apresentou maior efeito nas respostas farmacocinéticas da LTG.

A solubilidade, por sua vez, corroborando com as análises *in vitro*, apresentou efeito significativo para a absorção da LTG tanto no adulto quanto no pediátrico principalmente no t_{max}. Considerando a LTG uma base fraca, existe uma variação no processo de dissolução do IFA no TGI que depende do pH do meio e do volume de líquido disponível.

Seguindo essa linha, foram testadas variações nos volumes de fluidos do estômago, intestino delgado e grosso, e volume da dose (ou volume administrado concomitantemente ao fármaco).

Os volumes de fluidos não interferiram significativamente na farmacocinética da LTG em adultos e crianças. Embora vários autores indiquem o possível impacto da ontogenia do TGI (BATCHELOR; FOTAKI; KLEIN, 2014; KAYE, 2011), na condição testada isso não foi comprovado para o volume de fluidos aplicando o modelo PBBM.

O volume da dose afetou discretamente o t_{max} no modelo pediátrico. Este resultado corroborou com o determinado nos experimentos *in vitro*, nos quais o volume de líquido disponível foi responsável pela solubilização total ou parcial do LTG. Esse volume é ainda mais importante em crianças menores de 3 anos e em doses maiores, pois a relação dose/volume é maior. Entretanto, é preciso observar que a variação determinada em t_{max} para o volume da dose foi menor que 15%. Percebe-se então que, apesar

de existir relação, a exposição (ASC), C_{max} e t_{max} da LTG não foram significativamente influenciados pelos parâmetros biofarmacêuticos testados.

Perante o exposto, pode-se levantar a hipótese de que mesmo que a LTG seja experimentalmente classificada como classe II, fisiologicamente pode apresentar o comportamento de classe I. Tal hipótese é suportada pelos resultados encontrados nos estudos de RIVIV e VBE, que predisseram a manutenção da bioequivalência mesmo alterando a quantidade do fármaco liberado em até ±20%.

Tais informações podem contribuir para mudanças nas estratégias industriais, pois para os medicamentos classe I pode-se pleitar a dispensa de estudos de bioequivalência para registro de medicamentos genéricos e similares (BRASIL, 2022; FDA, 2017). Além disso, os modelos poderão ser empregados como guias para o desenvolvimento de formulações de forma mais assertiva aplicando conceitos da estratégia *quality by design,* por uma abordagem fundamentada no conhecimento científico e no gerenciamento de risco.

8. CONCLUSÃO

Neste trabalho a LTG apresentou solubilidade dependente do pH do meio. Em meios biorrelevantes a capacidade de solubilização foi maior que em meios tamponados farmacopeicos. No entanto, este aumento não foi suficiente para mudar a classificação deste fármaco, permanecendo pouco solúvel em ambientes ácidos e muito pouco solúvel em ambientes neutros a básicos, independente da concentração de reagentes biorrelevantes. De acordo com os critérios oficiais de classificação biofarmacêutica, a LTG apresentou característica de classe para adultos (200 mg) e crianças de 2 a 12 anos (5 e 15 mg/kg). No entanto, para crianças menores de 5 anos houve redução significativa da capacidade de dissolução em ambiente gástrico, dependendo da dose e do volume de líquido administrado concomitantemente.

Para o desenvolvimento da RIVIV, a técnica de deconvolução mecanística mostrou-se mais adequada por apresentar menor erro de predição. Já dentre os métodos de dissolução avaliados, a combinação entre dissolutor com células de fluxo e meio FaSSIF foi a mais biodescritiva. Aplicando a RIVIV, variações teóricas ponto a ponto do perfil de dissolução referência, em até 20%, originaram perfis plasmáticos preditos bioequivalentes a formulação referência. Resultado semelhante foi observado aplicando os mesmos perfis teóricos pelo método de VBE.

Por fim, por meio de ferramentas de modelagem biofarmacêuticas levantou-se a hipótese de que mesmo que a LTG seja experimentalmente classificada como classe II, fisiologicamente pode apresentar o comportamento de classe I. Tal hipótese é suportada pelos resultados encontrados nos estudos de RIVIV e VBE.

Em síntese, os modelos apresentados poderão auxiliar nas estratégias industriais de desenvolvimento e registro de medicamentos visto que, para os fármacos classe I pode-se aplicar a dispensa de estudos de biodisponibilidade e bioequivalência *in vivo*. Além disso, poderão guiar o desenvolvimento de formulações de forma mais assertiva e aplicando conceitos da estratégia *quality by design*.

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