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PERFIL ANTICONVULSIVANTE DAS LACTONAS: UMA REVISÃO SISTEMÁTICA

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DISSERTAÇÃO DE MESTRADO

PORTO ALEGRE
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Dissertação apresentada à Universidade Federal do Rio Grande do Sul, como parte das exigências do Programa de Pós-Graduação em Farmacologia e Terapêutica, para obtenção do título de Mestre em Farmacologia e Terapêutica.

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EPÍGRAFE

"One day, in retrospect, the years of struggle will strike
you as the most beautiful."

(Sigmund Freud)

RESUMO

A epilepsia é uma doença caracterizada por crises recorrentes, sendo uma das doenças neurológicas que mais acomete as pessoas no mundo. Assim, a busca por tratamentos alternativos para pacientes refratários tem sido cada vez mais importante. As lactonas são moléculas conhecidas por suas propriedades terapêuticas, e por isso foram o foco do nosso estudo. Esta dissertação descreve uma revisão sistemática sem meta-análise. A pesquisa utilizou as plataformas PubMed, Web of Science, EMBASE e SciELO (2000–2021) usando as palavras-chave "lactones", "anticonvulsant", "antiseizure" e "antiepileptogenic". Dois investigadores extraíram os dados dos artigos completos de forma independente, quaisquer discordâncias foram resolvidas por um terceiro investigador. Após a aplicação dos critérios de inclusão e exclusão, selecionamos 38 artigos de texto completo para extrair achados sobre a eficácia das lactonas na diminuição de convulsões e padrões epileptiformes. Encontramos 9 moléculas com resultados promissores em estudos clínicos e pré-clínicos. Os resultados da pesquisa sugerem que lactonas como everolimo, rapamicina e gama-decanolactona são muito promissoras, não apenas no tratamento de crises epilépticas, mas na descoberta de novas vias moleculares. A maioria dos estudos tem resultados significativos, e são essencias para compreensão da importância dos ensaios clínicos para o andamento do estudo como evidência.

Palavras-chave: anticonvulsivante, antiepileptogênico, anticonvulsivante, lactonas, epilepsia.

ABSTRACT

Epilepsy is a disease characterized by recurrent seizures. Being one of the neurological diseases that most affects people in the world, the search for alternative treatments for refractory patients has been increasingly important. Lactones are molecules known for their therapeutic properties, and therefore were the focus of our study. This manuscript describes a systematic review without meta-analysis. We searched PubMed, Web of Science, EMBASE, and SciELO (2000–2021) using the keywords "lactones," "anticonvulsant," "antiseizure," and "antiepileptogenic". Two investigators extracted data from the full articles independently, any disagreements were resolved by a third investigator. After applying inclusion and exclusion criteria, we screened 38 full-text articles to extract findings about the effectiveness of lactones in decreasing seizures and epileptiform patterns. We found 9 molecules with promising results in clinical and preclinical studies. Research results suggest that lactones such as everolimus, rapamycin and gamma-decanolactone hold great promise, not just in treating epileptic seizures, but in discovering new molecular pathways. Most studies have significant results, and are essential for understanding the importance of clinical trials to the progress of the study as evidence.

Keywords: anticonvulsant, antiepileptogenic, antiseizure, lactones, epilepsy.

LISTA DE ILUSTRAÇÕES E TABELAS

LISTA DE ABREVIATURAS E SIGLAS

Drogas antiepilépticas

a-etil-a-metil-y-tiobutirolactona

Proteína quinase

Coriana Lactone

Epilepsia do Lobo Temporal

Eletroencefalograma

Everolimo

Administração de Alimentos e Medicamentos dos EUA

Ácido gama-Aminobutírico

Receptor GABA do tipo A
Gama-decanolactona

Liga Internacional Contra Epilepsia

Ácido caínico

Lipopolissacarídeo

Convulsão Máxima de Eletrochoque

Alvo mecanístico da Rapamicina

Alvo mecanístico do Complexo de Rapamicina 1

Alvo mecanístico do Complexo de Rapamicina 2

P-glicoproteína

Pilocarpina

Pentilenotetrazol

Rapamicina

Proteína ribossomal 26 quinase

Status epilepticus

Saikosaponina

Siringaresinol

SUMÁRIO

1. INTRODUÇÃO

1.1. Epilepsia

A epilepsia é uma das doenças neurológicas mais comuns, acometendo cerca de 50 milhões de pessoas no mundo. É caracterizada por crises epilépticas que são classificadas como "uma ocorrência transitória de sinais e/ou sintomas devido a atividade neuronal excessiva ou síncrona anormal no cérebro" (WHO, 2022; FISHER *et al.*, 2017). As alterações podem ocorrer no cérebro inteiro ou apenas em algumas áreas podendo ter diversas etiologias que diferem quanto a idade, metabolismo, genética, presença de infecções e traumatismos cranianos (HAUSER, 2006). Estima-se que 70% dos pacientes podem viver sem crises se devidamente tratados, mas em países em desenvolvimento cerca de 90% destes indivíduos não são adequadamente tratados (WHO, 2022).

A classificação da epilepsia se inicia pelo diagnóstico do tipo de crise, além de achados de eletroencefalograma (EGG) e imagens juntamente com características clínicas que consideram as comorbidades que possam estar presentes (DEVINSKY, 2018). A Liga Internacional Contra Epilepsia (ILAE) elaborou uma classificação revisada com cada tipo de crise epiléptica com intuito de deixar claro a nomenclatura e os tipos de crises, sendo divididas em focais, generalizadas e início desconhecido e subcategorias (Fig. 1) (FISHER *et al.*, 2017). Tais crises podem induzir processos inflamatórios no sistema nervoso central, importantes no momento do insulto. Porém a manutenção de tais processos inflamatórios pode colaborar para a intensificação da crise, desencadear novas crises ou contribuir para o desenvolvimento de outras patologias (KOH, 2018; VARGAS-SÁNCHEZ et al., 2018).

Existem muitos tipos de epilepsia e um paciente pode ser diagnosticado com mais de um tipo. A epilepsia parcial afeta uma parte limitada do cérebro; a generalizada ocorre simultaneamente nos dois hemisférios cerebrais; a do lobo frontal as crises são originadas do lobo frontal e podem evoluir para uma crise parcial ou generalizada; do lobo temporal região que está associada ao processamento da linguagem e audição (SAVAGE, 2014; AABERG, 2017).
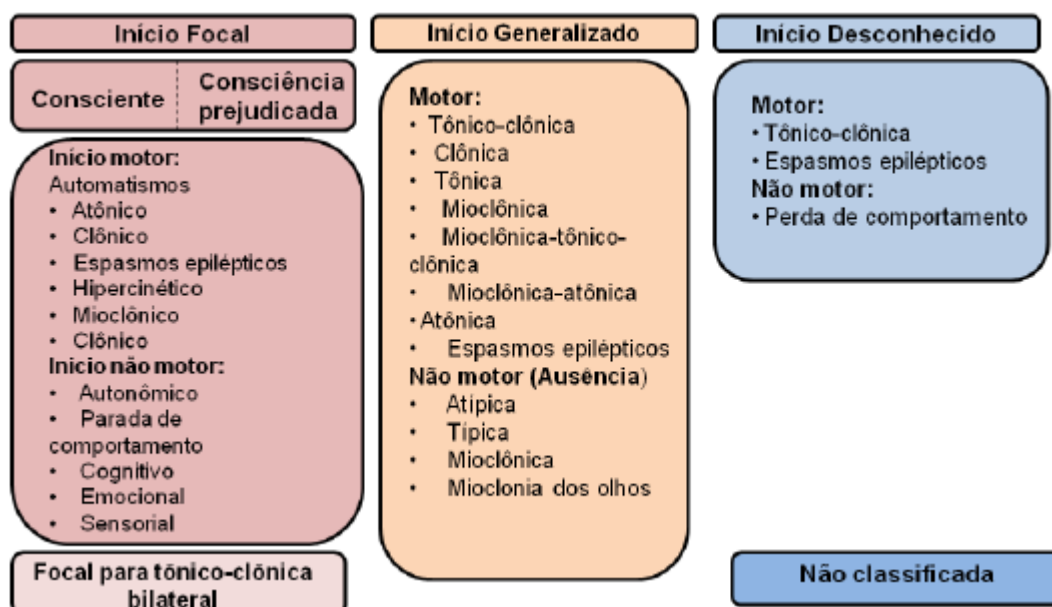
Figura 1: A classificação operacional expandida da ILAE 2017 dos tipos de crises.

A epileptogênese é descrita como um processo crônico que pode ser desencadeado por fatores genéticos ou adquiridos, onde uma rede neuronal normal é alterada com uma maior probabilidade de gerar crises espontâneas (PITKÄNEN *et al.*, 2015). O período da epileptogênese entre o insulto e a manifestação da epilepsia é chamado de período latente. A epileptogênese também se refere à progressão dos sinais e sintomas epilépticos em algumas formas de epilepsia, após a ocorrência das crises iniciais (ENGEL, 2019).

1.2. Fármacos antiepilépticos

O tratamento da epilepsia é tradicionalmente farmacológico, incluindo um amplo espectro de medicamentos antiepilépticos que podem ser usados como monoterapia ou combinação de medicamentos, para melhor eficácia e tolerância do paciente (ABOU-KHALIL, 2019). No entanto, cerca de um terço das pessoas afetadas pela doença não respondem ao tratamento com medicamentos convencionais (LAXER *et al.*, 2014). Diante disto, a pesquisa e o desenvolvimento de novas moléculas com propriedades anticonvulsivantes tem se tornado cada vez mais importante nessa área.

A investigação de tratamentos alternativos mostra-se importante tanto pelo custo-efetividade, pela redução dos efeitos adversos que muitas vezes dificultam a escolha de um tratamento ideal, quanto pelo impacto da melhor efetividade desses medicamentos (TAXAS, 2011).

Os principais fármacos anticonvulsivantes são divididos em basicamente três diferentes mecanismos de ação: 1) aumento da ação do receptotes ácido gama-aminobutírico (GABA); 2) inibição dos canais de sódio; 3) inibição dos canais de cálcio. O objetivo seria inibir a descarga anormal e em seguida corrigir a causa subjacente. Outros fármacos e mecanismos novos já foram desenvolvidos e tem se mostrado eficazes em modelos animais (ROGAWSKI, 2004, RANG; DALE, 2015).

Apesar da disponibilidade de muitos fármacos antiepilépticos, até o presente momento, nenhum deles é capaz de bloquear totalmente ou reverter a epileptogênese. Assim, o entendimento dos mecanismos celulares e moleculares responsáveis pela epilepsia, torna-se extremamente importante para o desenvolvimento de alvos terapêuticos apropriados que possam modificar o curso do processo epiléptico (ZHU *et al.*, 2015).

Considera-se que a epilepsia é uma importante doença neurológica, em que muitos pacientes não atingem um controle satisfatório das crises, embora existam diversos fármacos antiepilépticos disponíveis na terapêutica. Cerca de 20 a 30% dos pacientes não controla as crises, sem haver remissão significativa dos sintomas (LÓPEZ GONZALEZ *et al.*, 2015). Ainda, fármacos utilizados no tratamento da epilepsia desencadeiam muitos efeitos adversos, que tendem a interferir significativamente na qualidade de vida do indivíduo. Recentes estudos clínicos testando diferentes fármacos obtiveram resultados igualmente insatisfatórios, verificando-se um alto risco de evolução para epilepsia resistente aos medicamentos (HERNÁNDEZ-RONQUILLO *et al.*, 2018). Assim, a busca por novas estratégias farmacológicas e não farmacológicas, que tratem efetivamente pacientes epilépticos, torna-se crucial, e neste contexto, a modulação da microbiota intestinal surge como uma nova possibilidade de tratamento.

1.3. Modelos Animais

Pelos últimos 80 anos os modelos animais tornaram-se ferramentas importantes para a descoberta de novas terapias e tratamentos na epilepsia, pois tentam mimetizar os processos fisiopatológicos em humanos e que levam às crises em seres humanos (LÖSCHER, 2017). Tracy Putnam e Houston Merritt, trabalhando na Unidade Neurológica do Boston City Hospital, descobriram as propriedades anticonvulsivantes da fenitoína, usando um modelo de crise por eletrochoque em gatos (GLAZKO, 1986). A maior parte dos mecanismos anticonvulsivantes de fármacos usados clinicamente, foi definida por modelos de crises agudas em roedores usando os testes de eletrochoque máximo e crise induzida por pentilenotetrazol (PTZ) (BECKER, 2018).

O teste de convulsão máxima por eletrochoque (MES), utilizado em roedores é um dos ensaios mais comuns nos estudos pré-clínicos, sendo bastante eficaz na identificação de fármacos que bloqueiam crises tônico-clônicas generalizadas em humanos (LÖSCHER, 2013). Everett e Richards em 1944 usaram o modelo de PTZ em camundongos para demonstrar o efeito anticonvulsivante de trimetadiona, que posteriormente demonstrou bloquear crises de ausência em humanos. No modelo de *kindling* a administração aguda e repetida do agente convulsivante induz a diminuição do limiar para crises epilépticas e aumenta permanentemente a suscetibilidade do animal para apresentar crises clônicas, mioclônicas e tônico-clônicas (NADER *et al.*, 2018).

Ainda, modelos de crises crônicas e recorrentes em roedores provacadas por ácido caínico (KAI) e a pilocarpina (PIL) são amplamente utilizados, provoca danos em regiões neocorticais (CAVALHEIRO *et al.*, 1991; KNOPP *et al.*, 2008).

Alguns modelos animais permitem investigar as cascatas de sinalização molecular na epileptogênese e tentam elucidar a relação entre moléculas envolvidas na inflamação, crescimento neural e degeneração e plasticidade (BECKER, 2018). A sinalização alvo da rapamicina em mamíferos (mTOR) tem sido extensivamente caracterizada na epilepsia e utilizada como alvo para o tratamento em pacientes com mutações nos genes TSC1/2 portadores do complexo de esclerose tuberosa (TSC), uma doença que se manifesta principalmente por tumores altamente diferenciados ou malformações em muitos órgãos diferentes (GOORDEN *et al.*, 2007; KWIATKOWSKI *et al.*, 2002; CABAN *et al.*, 2017).

1.4. Lactonas

As lactonas estão no contexto para novas estratégias de tratamento de patologias que afetam o cérebro. As lactonas formam uma classe de moléculas com bioatividade comprovada e são muito abundantes na natureza (SARTORI, 2020). Elas são definidas como ésteres cíclicos de ácidos hidroxicarboxílicos contendo uma estrutura 1-oxacicloalcan-2-ona ou análogos com insaturação ou heteroátomos substituindo um ou mais átomos de carbono do anel (IUPAC, 1997).

Várias atividades biológicas já foram associadas às lactonas, como antimicrobiana, antitumoral, inseticida e anti-inflamatória (VIEIRA *et al.*, 2021). De acordo com Tabudravu (2005) há evidências indicando que algumas lactonas como as lactonas KAVA podem mediar seus efeitos ansiolíticos e sedativos através da modulação positiva da função do receptor $GABA_A$ atuando em outros sítios além dos sítios de ligação clássicos dos benzodiazepínicos. Foram estudadas propriedades anticonvulsivantes que aumentam a inibição mediada por GABA pela lactona a-ethyl-a-methyl-y-thiobutyrolactone (a-EMTBL) que teria uma maior ação antiepiléptica e seria mais eficaz no tratamento de epilepsias humanas do que agentes GABAmiméticos ou agentes GABA neutros (HOLLAND, 1992).

Um estudo de Coelho de Souza (1997) demonstrou que a administração sistêmica de gama-decanolactona, estruturalmente relacionada às lactonas presentes no óleo essencial de *A. suaoeolens*, foi capaz de bloquear crises induzidas por PTZ em camundongos. Os resultados deste estudo, demonstraram que gama-decanolactona age no do sistema nervoso, com efeito dose dependente, além de atividade hipnótica e hipotérmica.

Os efeitos neuroprotetores das lactonas observados em modelos de crises epilépticas e epilepsia observados até o momento, permitem delinear o perfil farmacológico dessas substâncias. Considerando que os fármacos antiepilépticos são utilizados, na maioria das situações, de forma contínua, é essencial conhecer seus efeitos neuroprotetores e potencial toxicológico após o tratamento contínuo. Entende-se como de especial importância o conhecimento dos mecanismos de ação e dos efeitos comportamentais das substâncias candidatas ao tratamento das crises epilépticas, a médio/longo prazo no organismo, bem como a sua ação analgésica e moduladora em processos inflamatórios, parâmetros bioquímicos e genotóxicos (PEREIRA *et al.*, 1997; SARTORI, 2020; MUSTO, 2018).

2. OBJETIVOS

O objetivo geral desse estudo foi elaborar uma revisão sobre o perfil anticonvulsivante das lactonas, tanto em estudos pré-clínicos como em estudos clínicos.

Os objetivos específicos são:

a) Avaliar o impacto de lactonas que possuem maior evidência anticonvulsivante;
b) Analisar e investigar se esses estudos podem ser complementares e/ou identificar possíveis limitações.
c) Determinar e descrever os efeitos adversos relacionado as novas moléculas.

3. RESULTADOS

3.1. Artigo Científico

Anticonvulsant profile of lactones: a systematic review

Luciana Martinez Bastian da Costa[1,2], Pricila Fernandes Pflüger[1,2], Marilise Brittes Rott[3], Patrícia Pereira[1,2].

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Anticonvulsant profile of lactones: a systematic review

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Abstract:

Epilepsy is a disease characterized by recurrent seizures. Being one of the neurological diseases that most affects people in the world, the search for alternative treatments for refractory patients has been increasingly important. Lactones are molecules known for their therapeutic properties, and therefore were the focus of our study. This manuscript describes a systematic review without meta-analysis. We searched PubMed, Web of Science, EMBASE, and SciELO (2000–2021) using the keywords "lactones," "anticonvulsant," "antiseizure," and "antiepileptogenic". Two investigators extracted data from the full articles independently, any disagreements were resolved by a third investigator. After applying inclusion and exclusion criteria, we screened 38 full-text articles to extract findings about the effectiveness of lactones in decreasing seizures and epileptiform patterns. We found 9 molecules with promising results in clinical and preclinical studies. Research results suggest that these lactones are very promising, not only in the treatment of epileptic seizures but in the discovery of new molecular pathways. Most studies have significant results, and it is quite clear to understand the importance of clinical trials for the progress of the study as evidence.

Keywords: anticonvulsant, antiepileptogenic, antiseizure, lactones, epilepsy.

Introduction

Epilepsy is one of the major neurological diseases that affects around 50 million people worldwide that may or may not be accompanied by recurrent and spontaneous seizures. (WHO 2022, Musto 2018). The pathology is characterized by a decompensated brain activity where there is an imbalance in the neuronal activity between excitatory and inhibitory neurotransmitters; it can be focal where only a part of the brain is affected or generalized where the neuronal activity is spread throughout the entire brain (Devinsky 2021). The disease is accompanied by cognitive, behavioral, psychological, and social impacts, which can affect people of all ages (Fisher *et al*. 2007).

The treatment of epilepsy is traditionally pharmacological, considering a broad spectrum of anti-epilepsy drugs (AEDs), which can be used as monotherapy or a combination of drugs for

better efficacy and patient tolerance (Abou-Khalil 2019). However, about a third of those affected by the disease do not respond to treatment with conventional drugs (Laxer *et al*. 2014). Given this portion of patients who do not respond to pharmacological treatment, the search and research for new molecules with anticonvulsant properties have become increasingly important in this area.

The investigation of alternative treatments is shown to be important in terms of cost-effectiveness, the reduction of adverse effects that often hinder the choice of ideal treatment, and the impact of the best effectiveness of these drugs. render this search for new treatment opportunities viable (Rates 2011, Manford 2017).

Lactones are in this context for new strategies to treat pathologies that affect the brain and are a class of molecules with proven bioactivity and are very abundant in nature (Sartori 2020). They are defined as cyclic esters of hydroxycarboxylic acids containing a 1-oxacycloalkan-2-one structure or analogs having unsaturation or heteroatoms replacing one or more carbon atoms of the ring (IUPAC 1997). They have several proven applications: antimicrobial, antitumor, insecticide, and anti-inflammatory (Vieira *et al*. 2021). According to Tabudravu (2005) there is evidence indicating that some lactones such as KAVA lactones can mediate their anxiolytic and sedative effects through positive modulation of the $GABA_A$ receptor function acting at sites other than the classical binding sites of benzodiazepines.

Thus, the aim of this study was to summarize the evidence involving the beneficial effects of lactones on epileptic seizures in preclinical and clinical studies, as well as to describe which lactones have the greatest therapeutic potential.

Methods

Search Strategy

This systematic review was based on the literature search using PubMed, Web of Science, EMBASE, and SciELO. The keyword "lactones" was used in combination with other keywords such as "anticonvulsant," "antiseizure," and "antiepileptogenic". The term "AND" was used in each combination. In addition, the reference sections of the studies that met our inclusion criteria

were manually screened for relevant publications (Figure 1). This is a systematic review without meta-analysis.

Inclusion and Exclusion Criteria

Studies had to meet the following criteria: (1) published in English between 2000 and 2021, (2) report original research, (3) preclinical case reports and clinical. Exclusion criteria were: (1) lack of original data (e.g., review articles, editorial material, articles reporting duplicate data); (2) articles addressing only effects of extracts or other product that contain lactones but not isolated ones.

Data Extraction and Outcomes

Two investigators (LC and PFP) extracted data from the full articles independently. Any disagreements were resolved by a third investigator (PP). We summarized the results in a narrative format.

Relevant articles reporting the outcome of interest were identified and standardized tables were used to extract the following variables in preclinical studies and clinical: article type, the molecule used, experiment, convulsion method, species, N, sham group and results/insights.

The first author collected the authors' names, titles, and study design.

Risk of Bias Assessment

Two reviewers (LC and PFP) assessed the risk of bias for each included preclinical study, using SYRCLE's Risk of Bias tool for animal studies (Hooijmans *et al*. 2014). We extracted study characteristics related to constructing and external validity (Henderson *et al*. 2013). We included sex, species, molecule, type of epilepsy model for construct validity.

Results

The last search identified 40 studies. After applying the inclusion and exclusion criteria, we included in the study, articles with different types of designs (33 preclinical/7 clinical) for full-text analysis. We screened the articles according to the primary outcome, the suppression of seizures, and summarized the results separately for preclinical (Table 1) and clinical (Table 2) research. Interestingly, the results obtained from the search in PubMed and Web of Science were the same ending the final search with 38 articles.

Rapamycin

Our search found 17 preclinical studies with Rapamycin (RAP) from zebrafish, mice and rats (Table 1) and two clinical studies. RAP is also known as Sirolimus is a drug already approved by the U.S. Food and Drug Administration (FDA) and it is used as an immunosuppressant and anticancer treatment (Lamming 2016). It is produced by the bacterium *Streptomyces hygroscopicus* found in the soil of Easter Island (Li *et al.* 2015).

This drug is a selective inhibitor of the mechanistic target of Rapamycin (mTOR) which is a protein kinase that controls various cellular processes e forms two complex proteins mTORC1 e mTORC2 (Wong *et al*. 2010). It is known that mTOR can bind to different regulatory subunits and thereby produce complexes with different signaling functions and also RAP sensitivity. Translation, ribosome biogenesis, autophagy, glucose metabolism and others are processes regulated by mTORC1, which phosphorylates ribosomal protein 26 kinase (S6K) and is sensitive to RAP. Unlike the mTORC2 complex, which phosphorylates protein kinase (Akt) and is insensitive to RAP (Ballou *et al*, 2008). So mTOR is a great hope for understanding the cellular targeting mechanism.

Tuberous Sclerosis Complex (TSC) it is an autosomal dominant disease that has variable expression caused by TSC1/TSC2 genes which leads to hyperactivation of mTOR pathway (He *et al*, 2020). The heterogenicity is responsible for forming several tumors in the brain and even in the affected patient's body. The brain, eye, kidney, skin, and heart tumors can lead to seizures and cognitive deficits (Sierra-Paredes 2008). There is no cure, but the treatment has already been

approved so it would be interesting to know what impact it would have seizures. Epilepsy occurs in up to 90% of all individuals with TSC (Samueli 2017).

As seen in Table 1 the preclinical studies are divided by author, title, type of article, experiment, convulsion method, species and results/insights. The oldest article found was by Zeng *et al*. (2009) and it was the precursor to this type of experiment to study the mTOR pathway for RAP treatment studies. In adult rats, based on a pilot study, the animals received pretreatment with RAP (6mg/kg i.p.) and on the fourth day they were injected with kainate (12mg/kg i.p.) to induce acute SE. This pretreatment had no SE initiation for 6 consecutive days and on latency, severity (stage), or duration of kainate-induced status epilepticus based on another behavioral day in the following weeks until the end point of the analysis. The RAP pretreatment had no effect on the acute kainate-induced seizures (both severity and duration). Also, in anti-epileptogenic models, it did not present effect. Although, it had the ability to decrease or block the effects of kainate seizures on neuronal death, mossy fiber sprouting and neurogenesis showing that mTOR functions managing these cellular processes. When RAP was tested after status epilepticus it blocked the late phase of mTOR activation and reduced mossy fiber sprouting but not neuronal death or neurogenesis. Regardless of the specific mechanisms of action, the partial intervention of RAP treatment suggests that specialty mTOR inhibitors may have antiepileptogenesis effects when administered after the onset of the precipitating initial injury.

The pilocarpine model is used in several studies to induce *status epilepticus* in animals because it is a muscarinic cholinergic agonist (Eyo *et al*. 2016). The authors Hester *et al.* (2016), Buckmaster (2013), Heng (2016) and Tang *et al*. (2012), employed this model in the RAP treatment to inhibit mTOR activity and analyze how that would react on suppressing or reducing seizures. RAP was able to inhibit the increase in mossy fibers, but it was not able to reduce or affect the frequency or severity of induced seizures. Findings reveal that RAP did not affect seizure frequency in pilocarpine-treated mice (Buckmaster 2013).

In a study by Siebel *et al*. (2015) zebrafish was used as an investigation model of the mTORC1 pathway with pretreatment of RAP before PTZ exposure, demonstrating that acute treatment with

RAP has protective effects against seizures in zebrafish, delaying the progression of seizures in different life stages (larvae, juvenile and adult). Showing that the results obtained indicate RAP as an important mechanism in this type of experimental animal model.

A kindling study with Coriana Lactone (CL), an epileptogenic mixture extracted from *Loranthus sp*, was performed by Xiaosa *et al*. (2017) in rats. The objective was to investigate the correlation between the mTOR pathway and P-glycoprotein (P-gp) expression in drug-resistant epilepsy. RAP was administered intraperitoneally at various doses (1, 3 and 6 mg/kg) for six days and then every other day until the end of the study. The mTOR pathway was shown to be activated accompanied by overexpressed P-gp. RAP treatment inhibited the mTOR pathway and down-regulated P-gp expression in non-responders in a dose-dependent manner. Also, RAP attenuated the expression of P-glycoprotein in spontaneous seizures and decreased CL-induced spontaneous seizures in non-responders, showing that the mTOR pathway is conected to pharmacoresistant mechanisms of epilepsy being responsible of regulating the expression of P-gp.

RAP effect was investigated in Temporal Lobe Epilepsy (TLE) model. In the study of van Vilet *et al*. (2012) rats were treated with RAP (6mg/kg/day i.p.) and after they had SE induced via electrical stimulation of the angular bundle. In that study results showed that seizure duration was not different throughout epileptogenesis between groups until 4 weeks after SE and suggests that the effect of RAP on the development of seizures is not due to the control of inflammation but rather the inhibition of mTOR which led to a strong reduction in this development of seizures. Sliwa *et al*. (2012) did the treatment at the same dose of 6mg/kg/day i.p. for two weeks after electrical stimulation of the amygdala. It was demonstrated that the treatment with RAP did not cause epileptogenesis or fiber sprouting and did not decrease the severity of the problem 6 weeks after the insult. It can be that suggested the antiepileptic action of the drug is not a universal phenomenon and may be limited to experimental conditions or models.

In the first study by Drion *et al*. (2016), rats were implanted with intrahippocampal pulse tetanic stimulation electrodes of 50Hz angular trains and then followed up with two types of treatment.

The first so called "stop treatment" experiment where RAP (6 mg/kg/day i.p.) was given after 4h after SE for six days and the pretreatment experiment, it was done for 3 days before SE induction using a lower dose 3 mg/kg/day i.p. of RAP. During the chronic-phase treatment experiment, rats that had developed recurrent seizures were selected for the RAP treatment. After treatment, there was a 14-day washout period to determine whether RAP had an effect on seizures. After that was given an 11-day treatment (150 mg/f/day, orally by gavage of curcumin) as bioavailability is low and cumin at the dosages used is not harmful, a curcumin prediction was then used to maximize the chance of reaching the brain. It was observed that RAP suppressed seizure treatment frequency and effect since continued increase, and during the longer off the drug, seizure frequency increased but first decreased in levels. Seizures had no results from treatment with RAP and did not have duration of treatment with RAP for the duration of treatment. Curcumin, an mTOR inhibitor, was not likely to seizures in the effective phase, probably brain curcumin levels were not sufficient. In the second study of Drion *et al*. (2018) an anti-inflammatory and oxidant comparison of the effects of curcumin and RAP was performed where rats were subjected to tetanic stimulation of the angular bundle (50-Hz pulses every 13s) followed by a treatment of RAP (6mg/kg day) for 7 days or injection via cannula with 2ul of 2Mm curcumin solution. They performed *in vitro* astrocyte cultures where they could conclude that only RAP was able to reduce the level of S6 phosphorylation, thus inhibiting the mTOR pathway. And that the post-treatment with the two drugs did not change SE-induced upregulation of markers of inflammation and oxidative stress, while in vitro, curcumin displayed anti-inflammatory and antioxidant effects.

The first clinical study is a randomized controlled trial by Overwater *et al*. (2016) investigated whether mTORC1 inhibitors could reduce seizure frequency in children with TSC and Sirolimus was titrated to trough levels of 5–10 ng/mL. The study reported Class III evidence that Sirolimus does not significantly reduce seizure frequency in children with TSC and intractable epilepsy. Secondly was a cohort study made by He *et al*. (2020) which 91 children with TSC were enrolled to test the efficacy of controlling seizures in patients where Sirolimus is effective in treating TSC-related epilepsy. For the 45 children whose AEDs were not altered, Sirolimus was the only new additional factor. In this group, the percentage of responders was 75.6 %, and among these,

70.6 % became seizure-free. Also, the antiepileptic effect of Sirolimus is independent of any changes in the number and type of AEDs. The seizure frequency was reduced significantly in both the AEDs-altered group and the AEDs-unaltered group, and there was no statistical difference in the therapeutic effect between the two groups (p = 0.308).

Everolimus

In total, our search found 7 clinical studies on everolimus (EVO) and its association with the treatment of TSC divided by author (year), title, type of study, concentration of EVO, *N*, Sham Group, and the results/insights as seen in Table 2.

With the increasing association of mTOR and TSC, clinical tests have increased to learn more about the relationship of these inhibitors at a molecular level. In the study by Samueli *et al.* (2016) considering that seizures are difficult to cure because children are refractory to conventional treatments with AEDs, the response of this study was good considering that the effect of EVO had different results in different types of seizures, overall percent reduction in seizure frequency of 60 % compared with 80 % in generalized tonic clonic seizures and 87 % in drop attacks.

In the EXIST-1 study by Kotulska *et al*. (2013), the long-term effect of the EVO in children under 3 years of age affected by TSC disease to be treated for astrocytoma was analyzed. All had their seizure diary where 3 were without seizures from the start, one reduced the number of AEDs from 2 to 1 and one child who had very refractory epilepsy had complete cessation of seizures after treatment. Two treatments-resistant children had at least a 50% decrease in the number of seizures in the first six months of treatment. On one child the drug had no impact. All patients had at least one adverse event and a total of 142 were reported, of which 74 were confirmed to be EVO related.

Krueger *et al*. (2013) evaluated EVO in a prospective, open-label,phase I/II clinical trial with patients with refractory epilepsy with TSC and were followed up until the completion of the

study (48 months). This work provides Class IV evidence that prolonged treatment up to 4 years is effective for treating medically refractory epilepsy in patients with TSC. Throughout extension, the median daily dose and corresponding serum trough levels narrowed but did not change significantly (0.47–0.56 mg/kg/d and 7.4–10.8 ng/ml, respectively). It was observed a weak but statistically significant relationship between seizure frequency and EVO dose (r =-0.3394, p = 0.001) and trough level (r = - 0.1606, p =0.045).

Wiegand *et al*. (2013) evaluated the safety and efficacy of seven patients with TSC and intractable epilepsy for nine months. The used dose was increased as necessary to reach a serum concentration of 5-10 ng/ml as tolerated. Seizure reduction was observed in 4/6 patients with a reduction of 25-100%. In addition, the percentage of seizure-free days increased from 3/4 of these patients. In 2/6 patients, no alteration of seizure frequency was noted. One patient discontinued treatment early on because of side effects.

EXIST-3 is a three-arm, prospective, randomized, multicentre, double-blind, placebo-controlled, phase 3 study by French *et al*. (2016) TSC patients taking one to three AEDs concurrently were recruited from 25 countries. Selected from three groups: placebo, low-exposure, or high-exposure. The dose was determined based on patients' age, body-surface area, and concomitant use of cytochrome P450 3A4 (CYP3A4)/P-gp inducers. The response rate was 15·1% with placebo (95% CI 9·2–22·8; 18 patients) compared with 28·2% for low-exposure (95% CI 20·3–37·3; 33 patients; p=0·0077) and 40·0% for high-exposure (95% CI 31·5–49·0; 52 patients; p<0·0001). The median percentage reduction in seizure frequency was 14·9% (95% CI 0·1–21·7) with placebo versus 29·3% with low-exposure (95% CI 18·8–41·9; p= 0·0028) and 39·6% with high-exposure EVO (95% CI 35·0–48·7; p<0·0001). Adjunctive treatment means reduced seizure frequency with a tolerable safety profile compared with placebo in patients with tuberous sclerosis complex and treatment-resistant seizures.

In another EXIST-3 study 3-arm, prospective, randomized, multicenter, double-blind, placebo-controlled, phase 3 study investigating for epilepsy and autism spectrum disorder in TSC by Mizuguchi *et al*. (2019) where 35 patients confirmed with TSC and on treatment for refractory

epilepsy receiving at least 1 to 3 AEDs before randomization were recruited. The study has 3 phases, a baseline phase for 8 weeks and a core phase of 18 weeks, followed by an extension phase of 48 weeks. In the core phase, patients were randomized to receive placebo or titrated to a target trough concentration (Cmin) of 3–7 ng/mL (low-exposure [LE] treatment) or to a target Cmin of 9–15 ng/mL mL (high-exposure [HE] treatment). The response rate was 30.0% and 28.6% versus 0% with the LE and HE versus placebo arm, respectively. Similarly, the median percentage reduction in seizure frequency was 6.88% and 38.06% versus 6.67%. This analysis, along with that of the previous larger study, demonstrates that the adjunctive therapy is a valuable treatment option for the Japanese patients with the treatment-refractory seizures associated with TSC.

In the study done by Kadish *et al.* (2020) was a prospective observational study focused on developmental outcomes in the treatment of TSC. Patients were therefore treated with three different target levels (I: 3–5 µg/l; II: 6–9 µg/l; III: 10–15 µg/l or higher), each for at least nine months. Median follow-up duration was 37 months, ranging from 3 to 70 months. Three patients discontinued treatment with EVO due to insufficient seizure control and additional side effects. Following treatment with EVO, as first results indicated, seizure control was not immediate but improved over time: only one patient (7%) became seizure-free after 9 months, while at their last evaluation, four patients did not experience seizures (27%). While developmental disorders were prominent, we observed an overall progression at a slower pace. Despite a positive effect on seizure occurrence, treatment with EVO did not reverse developmental problems in the observation period of this study.

Ascomycin – calcineurin modulator

Ascomycin (ASC) is a macrolytic lactone with 23-member rings antibiotic that has molecular properties of modulating calcineurin and is also known as phosphatase 2B, FK 506 analog which inhibits calcineurin (Lamming 2016). It was originally isolated in the 60s from the fermentation product of *Streptomyces hygroscopicus var ascomyceticus* (Ballou 2008). It acts by interrupting signaling events mediated by the calcium-dependent serine/threonine protein phosphatase,

calcineurin (CaN, protein phosphatase 2B). The working mechanism involves the formation of a complex with the intracellular FK506-binding protein-12 (FKBP12), thereby acquiring the complex ability to interact with calcineurin and to interfere with the dephosphorylation of substrates (Sierra-Paredes, 2008).

In the study of Vasquez-López *et al.* (2016) it was tested the potential to increase knowledge of the role of calcineurin in microdialyzed picrotoxin-induced epileptic seizures in the hippocampus of rats. Picrotoxin was choosen because it binds to $GABA_A$ receptors results in cellular excitability and can lead to increased dephosphorylation thus calcineurin activity may be associated with some biochemical changes leading to epileptic seizures. ASC alone was not found to significantly prevent seizures but at concentrations of 50μM and 100μM it was shown to be completely suppressing seizures in 41.7% and 75% of the animals studied, respectively. This model shows how phosphorylation and dephosphorylation may be linked to the development of acute epileptic seizures. Making room for yet another strategy to find new anticonvulsant drugs.

Gama-decanolactone

Gama-decanolactone (GD) is a monoterpenic compound with a chemical structure like the lactones present in the essential oil of the plant *Aeollanthus suaveolens*, used by Amazonian caboclos, for its alleged medicinal characteristics (sedative, anticonvulsant, analgesic, hypnotic and anxiolytic) (Elisabetsky 1995).

In the kindling model, considered a fundamental model of epileptogenesis, GD showed a significant protective effect on seizures induced by pentylenetetrazol (PTZ) and demonstrated antigenotoxic activity, as it repaired the DNA damage induced by PTZ (Oliveira 2008). In more recent works, GD reduced acute seizures induced by the chemical agent's isoniazid and 4-aminopyridine and protected against neuronal damage induced by pilocarpine (Pflüger 2018b). The evaluation of the mechanism of action of GD in an epileptic seizure model, using adenosine A1, $A2_A$ receptor antagonists, and GABAA receptor antagonists, suggests that the anticonvulsant effect of GD may be related to the possible modulation of adenosine A1 receptors (Pflüger *et al.*, 2020).

Avermectins

Avermectins are a group of wide spectrum antiparasitic drugs derived from macrolytic lactones and produced through fermentation of actinomycetes of the genus *Streptomyces avermitilis* (de Souza Spinosa *et al*. 2000, Trailovic and Varagic 2007).

The ivermectin, used in human medicine as the drug of choice in the therapy of lymphatic filariasis and onchocerciases, has been shown to protect rats against lidocaine and strychnine-induced seizures at a significantly lower dose than the observed LD50 of ivermectin (18.20 mg/kg) (Trailovic and Varagic 2007). In addition, doramectin shows an anticonvulsant profile in the picrotoxin animal model, since at the times of 24 h ($F_{(3, 39)} = 6,835$, $P < 0.001$), 48 h ($F_{(3, 39)} = 18.964$, $P < 0.001$) and 168 h ($F_{(3, 39)} = 3970$, $P < 0.01$) increased the dose of picrotoxin required to produce seizures in rats (de Souza Spinosa *et al*. 2000).

Dawson *et al*. (2000) tested 25 avermerctin analogues in the PTZ-induced seizure model and found that the anticonvulsant activity produced by some compounds is mediated via the $GABA_A$ receptor.

Dihydrofuran-2(3H)-one (γ-butyrolactone, GBL)

The dihydrofuran-2(3H)-one, (oxolan-2-one, γ butyrolactone, GBL), is a cyclic analogue and precursor of 4 hydroxybutyric acid (GHB).

Countless research shows that lactones in general, as well as GBL and its derivatives, are known to have pharmacological potential in the central nervous system (CNS) including anticonvulsant activity (Hadri *et al*. 2002, Gonzales *et al*. 2004, Wieckowski *et al*. 2012, Pfluger *et al*. 2018, Pfluger *et al*. 2020, Dos Santos *et al*. 2021, Pfluger *et al*. 2021).

Wieckowski *et al*., 2012 synthesized a series of GBL derivatives and tested them for anticonvulsant, neurotoxic and analgesic activity. This study was focused on exploration of the substitution at position 3 by introduction of various alkyl- and arylakyl-substituents linked by nitrogen atom, oxygen atom or aminomethylene group.

A total of ten lactones were effective in the maximal electroshock test (MES) at the highest doses (300 and 100 mg/kg, i.p., mice) 0.5 h after the administration in anticonvulsant screening.

Analgesic activity was also proven in the antinoceptive evaluation of most of the tested compounds.

These results show that possibly the CNS activity of these GBL derivatives depends on the presence of a phenylpiperazine moiety. From eight phenylpiperazine-containing structures, seven compounds were active in the MES test, indicating their penetration into the CNS and inhibitory influence on it.

Saikosaponin

Saikosaponin (SSa), a triterpene saponin, is one of the most important bioactive compounds isolated from *Radix bupleuri*, a root widely used in traditional Chinese medicine for being effective in the treatment of numerous pathologies such as respiratory infections, fever, chronic hepatitis, malaria and premenstrual syndrome. Some studies have shown that SSa isolated exhibited anticonvulsant, antiepileptic, and anti-inflammatory activities (Yu *et al*. 2012; Xie *et al*. 2006).

Ye *et al*. (2015) previously reported that SSa significantly reduced seizure severity and duration while markedly elevated seizure latency in PTZ induced epileptic rats. Also demonstrated that SSa mediates inflammatory cytokines expression and apoptotic such caspase-3. These outcomes are given through the suppression of p-mTOR pathway activation. Corroborating with other studies which point out that the hyperactivation of the mTOR pathway causes neuronal hyperexcitability and is involved in seizures and epileptogenesis (Zeng *et al*. 2009, Lasarge and Danzer 2014).

Syringaresinol (SYR)

(+)-Syringaresinol (SYR; 4,4'-(1S,3aR,4S,6aR)-tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diylbis(2,6-dimethoxyphenol)) is lignan that can be found in the berries of various plants such as *Panax ginseng*, *Prunus mume* and *Magnolia thailandica*. Previous studies show some biological activities of SYR, including the

inhibition of *Helicobacter pylori* bacterium motility and the ability to change the composition of the gut microbiota when it is orally administered to mice (Cho *et al*. 2016, Miyazawa *et al*. 2006).

In addition, SYR stimulates nitric oxide production and SIRT1 gene expression in mammalian endothelial cells and has exhibited neuritogenic activity in neuroblastoma cell lines (Chiba *et al*. 2002, Cho *et al*. 2013, Chung *et al*. 2012, Park *et al*. 2015).

Regarding antiepileptic activity Cho *et al*. (2018) proved in their study that SYR suppressed excitatory synaptic transmission via presynaptic modulation, decreased Ca^{2+} currents and hyperpolarized resting membrane potential. SYR also attenuated picrotoxin-induced epileptiform activity in hippocampal CA1. Considering the results obtained, Cho *et al*. (2018) identifies SYR as a new neuromodulatory agent.

Aminoglutethimide and spironolactone

Aminoglutethimide is a drug commonly used in hormone-dependent breast cancer in women after menopause once it inhibits the enzymatic conversion of cholesterol to pregnenolone, thus reducing the adrenal synthesis of glucocorticoids, estrogens, androgens, and mineralocorticoids. In contrast, spironolactone acts as a nonspecific aldosterone antagonist, being used for patients with primary hyperaldosteronism and drug-resistant edema (Borowicz and Czuczwar 2005).

A previous studies reported that aminoglutethimide, but not spironolactone, increases the antiseizure efficacy in rats of some conventional anticonvulsant drugs such as phenobarbital, clonazepam, and valproate (Borowicz and Czuczwar 2004, Borowicz *et al*. 2004). It is supposed that the antagonists of steroid receptors may interfere with seizure phenomena (Borowicz and Czuczwar 2004).

Borowicz and Czuczwar (2005) evidence that the concomitant treatment of aminoglutethimide (5mg/kg) and carbamazepine (15mg/kg) resulted in antiseizure activity against amygdala-kindled seizures in rats. The similar combination of aminoglutethimide with diphenylhydantoin (2.5 mg/kg) significantly shortened the seizures. These data show that doses of carbamazepine and diphenylhydantoin should be modified in epileptic patients concomitantly treated with aminoglutethimide or spironolactone.

Discussion

In this study, we reviewed preclinical and clinical articles that used lactones for the pharmacological treatment of seizures. The studies we found had a wide range of treatments including kindling, acute or chronic; with the aim of lowering the threshold for the onset of the seizures or even the elimination of seizures. We search for an alternative treatment for those unresponsive to traditional epilepsy treatments getting in depth of the diverse molecular mechanisms since epilepsy has as a characteristic of the hypersynchrony of neuronal activity (Laxer *et al.* 2004).

Lactones are widely used today standing out in recent decades as the use of alternative treatments and phytotherapies (Sartori 2020). The neuroprotective effects of lactones observed in models of epileptic seizures and epilepsy observed so far, as well as the pharmacological profile of this substance were studied. Considering that antiepileptic drugs are used, in most situations, continuously, it is essential to know their neuroprotective effects and toxicological potential after continuous treatment. It is understood as of special importance the knowledge of the mechanisms of action and the behavioral effects of the candidate substances for the treatment of epileptic seizures, in the medium/long term in the body, as well as their analgesic and modulatory action on inflammatory, biochemical, and genotoxic parameters (Pereira *et al.* 1997, Sartori 2020, Musto 2018).

Authors have already shown the inhibitory action of ASC suggesting that this imbalance in neurotransmission may contribute to neuronal excitation and epileptogenesis (Freire-Cobro *et al.* 2014) and it was tested the relationship between CaN and GluN and showed that FK506, inhibited GluN activity and subsequently decreased the frequencies of GluN-dependent excitatory postsynaptic currents (EPSCs) (Lieberman *et al.* 1994) which corroborates the study found by our research by Vázquez *et al.* (2006) neuronal mechanisms which regulate calcineurin dephosphorylation under normal conditions could be highly altered as a result of picrotoxin-induced impairment in GABAergic activity. Calcineurin inhibitors might acutely potentiate GABAergic transmission but, when excessive excitatory activity induces massive Ca^{2+} entry through NMDA or Ca^{2+}-permeable AMPA receptors (Sanchez *et al.* 2005).

Another very well studied lactone is GD which has been proven to have hypnotic, hypothermic and anticonvulsant activity in an animal model (Coelho de Souza *et al.* 1997). The neurochemical evaluation, carried out through a binding assay, showed that GD was able to dose-dependently inhibit the specific binding of L-[3H]-glutamate, an action that may be involved in the anticonvulsant activity of this compound (Pereira *et al.* 1997). An *in vitro* study demonstrated that GD could attenuate the activation of N9 microglial cells in the model of damage induced by lipopolysaccharide (LPS). Furthermore, GD has been shown to decrease the expression of inflammatory markers such as the iNOS enzyme TNF-α and consequently inhibit ROS formation. In addition, GD blocked apoptosis markers such as phosphorylation of p38 and activation of cleaved caspase-9, decreasing DNA damage (Pflüger *et al.* 2016). These data indicate that GD has a neuroprotective therapeutic potential and exerts this effect through the inhibition of neuroinflammation. A study published in 2021 suggests that GD could be a drug with potential utility in Parkinson's Disease (Pflüger *et al.* 2021). Another investigation demonstrated that after 12 days of treatment of CF1 mice, GD was able to protect against DNA damage, reduce COX-2 expression, and increase GluN2B expression (Dos Santos *et al*. 2021).

Results on SYR say they suggest presynaptic glutamatergic suppression by SYR as a new therapeutic strategy to treat not only epilepsy but also other neurological diseases resulting from synaptic hyperexcitation. It still needs an investigation to know how SYR behaves to pass the blood-brain barrier, this makes the need for more studies on this molecule to help the practice of the use of this molecule on *in vivo* studies (Cho *et al.* 2017).

The mechanism of avermectins is that they have effects on GABA receptors and are also able to potentiate neurotransmission by interrupting glutamate-controlled chloride channels (Kane *et al*. 2000 and Fritz *et al*. 1979). Thus, we can observe the results of the studies collected in this article that demonstrate the anticonvulsant profile of avermectins (Dawson *et al*. 2000, de Souza Spinosa *et al*. 2000, Trailovic and Varagic 2007). Cell death initiated by excessive levels of neuronal excitation is linked to several disorders linked to the CNS and a new therapy for this would utilize the silencing of excessive neuronal activity using ivermectin (Crump 2017).

The prominent mechanism in our research was the mTOR target being seen by the RAP and EVO molecules. As a treatment already approved by the FDA for its use as an immunosuppressant, it

facilitates its use in clinical studies with cancer patients. Molecular, energetic and metabolic processes are directly controlled by MTORC1 and cancer cells present fundamental changes in these demands, thus protein synthesis and oncogenic activation of MTORC1 is boosted by promoting a gene expression program involved in the metabolic reprogramming of these cancer cells (Li *et al*. 2014). Knowing how to modulate the activity of MTORC1, which is something very complex that has not been completely unraveled yet, the Rag/ragulator complex recruits mTORC1 to the lysosome when amino acids and glucose are plentiful, whereas the tuberous sclerosis complex 1/2 (TSC1/2) complex, which negatively regulates mTORC1 signaling, departs from the lysosomein response to insulin (Lamming 2016).

Another molecule that we have also seen that involves the regulation of mTOR is Saikosaponine, where in studies with cancer cells, a decrease in MTOR phosphorylation can be detected when they added SSa in hepatoma cells, increasing the apoptosis of these cells (Wang *et al*. 2020). Thus, with the mediation of Ssa in the expression of inflammatory cytokines through the suppression of activation of the P-MTOR/P70S6K pathway, the results demonstrate that Ssa may hold promise in the treatment of epilepsy and neurological disorders (Ye *et al*. 2016).

A lot of evidence has suggested that the MTORC1 pathway may be involved in several neurological disorders, not only as shown in the article as TSC, but also in many neurological diseases involving cognitive dysfunction and autism as we could see in the article by Mizuguchi *et al*. (2013) and Kadish *et al*. (2020) So, inhibition of this pathway is a therapeutic option that we can see in evidence.

What we can corroborate with the research of several authors of clinical research is that the adverse effects of the treatment of these molecules trigger adverse effects that can end up in the abandonment of the treatment, such as stomatitis, upper respiratory tract infections, skin rash and flushing (Kotulska *et al*. 2013, Krueger *et al*. 2016, Wiegand *et al*. 2013, French *et al*. 2016).

Finally, this investigation is important to keep us up to date with new and promising studies that can give us direction and open our eyes to where the best investment of research and resources is.

Conclusion

Considering the information obtained in this study we can conclude that lactones are becoming a considerable and viable option for more comprehensive studies and new perspectives for the future.

The two molecules that stood out the most were RAP and EVO, one with many preclinical studies and the other with advanced clinical studies, respectively. But even so, in all studies adverse effects were reported which could be an impediment to treatment, so it should be investigated how this can be improved.

The other molecules with fewer studies found still proved to be very interesting and with a lot of potential and that should still be studied to know how all the mechanisms work molecularly and how this could be used to either decrease the frequency, intensity or even cease the seizures. Considering the lower price and fewer adverse effects than traditional treatments. Although there are several trials published in this field, further evidence is needed to understand the potential role of using lactones treat epilepsy.

Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

All authors participated in the study design and wrote the manuscript. PP and PFP participated in the study coordination and helped write the manuscript. All authors read and approved the final manuscript.

References

Abou-Khalil BW. Update on Antiepileptic Drugs 2019. Continuum (Minneap Minn). 2019 Apr;25(2):508-536. doi: 10.1212/CON.0000000000000715. PMID: 30921021.

Ballou, L. M. and Lin, R. Z. (2008) 'Rapamycin and mTOR kinase inhibitors', Journal of Chemical Biology, 1(1–4), pp. 27–36. doi: 10.1007/s12154-008-0003-5.

Borowicz KK, Czuczwar SJ. Influence of aminoglutethimide and spironolactone on the efficacy of carbamazepine and diphenylhydantoin against amygdala-kindled seizures in rats. Eur J Pharmacol. 2005;516(3):212-218. doi:10.1016/j.ejphar.2005.01.040.

Borowicz, K. K. and Czuczwar, S. J. (2005) 'Aminoglutethimide but not spironolactone enhances the anticonvulsant effect of some antiepileptics against amygdala-kindled seizures in rats.', Journal of neural transmission (Vienna, Austria: 1996), 112(7), pp. 891–903. doi: 10.1007/s00702-004-0238-4.

Borowicz, K.K., Czuczwar, S.J., 2004. Effect of aminoglutethimide and spironolactone on the efficacy of valproate, phenobarbital and clonazepam against amygdala-kindled seizures in rats. J. Neural Transm. 238, 1 – 13

Borowicz, K.K.,xuszczki, J., Swiader, M., Kleinrok, Z., Czuczwar, S.J., 2004. Influence of sexual hormone antagonists on the anticonvulsant action of conventional antiepileptic drugs against electrically- and pentylenetetrazol-induced seizures in mice. Eur. Neuropsychopharmacol. 13, 257 – 265.

Buckmaster, P. S. and Lew, F. H. (2011) 'Rapamycin suppresses mossy fiber sprouting but not seizure frequency in a mouse model of temporal lobe epilepsy.', The Journal of neuroscience: the official journal of the Society for Neuroscience, 31(6), pp. 2337–2347. doi: 10.1523/JNEUROSCI.4852-10.2011.

Cawello W. Clinical pharmacokinetic and pharmacodynamic profile of lacosamide. Clin Pharmacokinet. 2015;54(9):901-914.

Chachua, T. et al. (2012) 'Rapamycin has age-, treatment paradigm-, and model-specific anticonvulsant effects and modulates neuropeptide y expression in rats', Epilepsia, 53(11), pp. 2015–2025. doi: 10.1111/j .1528-1167.2012.03674.x.

Chi, X. et al. (2017) 'Inhibition of mTOR Pathway by Rapamycin Decreases P-glycoprotein Expression and Spontaneous Seizures in Pharmacoresistant Epilepsy', Journal of Molecular Neuroscience, 61(4), pp. 553–562. doi: 10.1007/s12031-017-0897-x.

Chiba K, Yamazaki M, Umegaki E, et al. Neuritogenesis of herbal (+)- and (-)-syringaresinols separated by chiral HPLC in PC12h and Neuro2a cells. Biol Pharm Bull. 2002;25(6):791-793.

Cho SY, Cho M, Seo DB, Lee SJ, Suh Y. Identification of a small molecule activator of SIRT1 gene expression. Aging (Albany NY). 2013;5(3):174-182.

Cho SY, Kim J, Lee JH, et al. Modulation of gut microbiota and delayed immunosenescence as a result of syringaresinol consumption in middle-aged mice [published correction appears in Sci Rep. 2017 Jan 31;7:41667]. Sci Rep. 2016;6:39026.

Cho YS, Song WS, Yoon SH, Park KY, Kim MH. Syringaresinol suppresses excitatory synaptic transmission and picrotoxin-induced epileptic activity in the hippocampus through presynaptic mechanisms. Neuropharmacology. 2018;131:68-82.

Cho, Y. S. et al. (2018) 'Syringaresinol suppresses excitatory synaptic transmission and picrotoxin-induced epileptic activity in the hippocampus through presynaptic mechanisms', Neuropharmacology, 131, pp. 68–82. doi: 10.1016/j.neuropharm.2017.12.014.

Choi, D., Stables, J.P., Kohn, H., 1996. Synthesis and anti-convulsant activities of N-benzyl 2-acetamidopropionamide derivatives. J. Med. Chem. 39, 1907—1916.

Chung BH, Kim S, Kim JD, et al. Syringaresinol causes vasorelaxation by elevating nitric oxide production through the phosphorylation and dimerization of endothelial nitric oxide synthase. Exp Mol Med. 2012;44(3):191-201.

Dawson, G. R. et al. (2000) 'Anticonvulsant and adverse effects of avermectin analogs in mice are mediated through the gamma-aminobutyric acid(A) receptor.', The Journal of pharmacology and experimental therapeutics, 295(3), pp. 1051–1060.

De Oliveira, P. A. et al. (2008) 'Effects of gamma-decanolactone on seizures induced by PTZ-kindling in mice', Experimental Brain Research, 187(1), pp. 161–166. doi: 10.1007/s00221-008-1295-y.

de Souza Spinosa, H., Gerenutti, M. and Bernardi, M. M. (2000) 'Anxiolytic and anticonvulsant properties of doramectin in rats: behavioral and neurochemistric evaluations.', Comparative biochemistry and physiology. Toxicology & pharmacology : CBP, 127(3), pp. 359–366. doi: 10.1016/s0742-8413(00)00165-1.

Dos Santos FM, Pflüger PF, Lazzarotto L, et al. Gamma-Decanolactone Alters the Expression of GluN2B, A1 Receptors, and COX-2 and Reduces DNA Damage in the PTZ-Induced Seizure Model After Subchronic Treatment in Mice. Neurochem Res. 2021;46(8):2066-2078.

Doty P, Rudd GD, Stoehr T, Thomas D. Lacosamide. Neurotherapeutics. 2007;4(1):145-148.

Drion, C. M. et al. (2016) 'Effects of Rapamycin and curcumin treatment on the development of epilepsy after electrically induced status epilepticus in rats.', Epilepsia, 57(5), pp. 688–697. doi: 10.1111/epi.13345.

Drion, C. M. et al. (2018) 'Effects of Rapamycin and curcumin on inflammation and oxidative stress in vitro and in vivo - in search of potential anti-epileptogenic strategies for temporal lobe epilepsy.', Journal of neuroinflammation, 15(1), p. 212. doi: 10.1186/s12974-018-1247-9.

El Hadri A, Abouabdellah A, Thomet U, et al. N-Substituted 4-amino-3,3-dipropyl-2(3H)-furanones: new positive allosteric modulators of the GABA(A) receptor sharing electrophysiological properties with the anticonvulsant loreclezole. J Med Chem. 2002;45(13):2824-2831.

French, J. A. et al. (2016) 'Adjunctive EVO therapy for treatment-resistant focal-onset seizures associated with tuberous sclerosis (EXIST-3): a phase 3, randomised, double-blind, placebo-controlled study.', Lancet (London, England), 388(10056), pp. 2153–2163. doi: 10.1016/S0140-6736(16)31419-2.

Gonzales EB, Bell-Horner CL, de la Cruz MA, Ferrendelli JA, Covey DF, Dillon GH. Enantioselectivity of alpha-benzyl-alpha-methyl-gamma-butyrolactone-mediated modulation of anticonvulsant activity and GABA(A) receptor function. J Pharmacol Exp Ther. 2004;309(2):677-683.

Guo, D. et al. (2013) 'Rapamycin attenuates the development of posttraumatic epilepsy in a mouse model of traumatic brain injury.', PloS one, 8(5), p. e64078. doi: 10.1371/journal.pone.0064078.

Hartman, A. L. et al. (2012) 'The mTOR inhibitor Rapamycin has limited acute anticonvulsant effects in mice.', PloS one, 7(9), p. e45156. doi: 10.1371/journal.pone.0045156.

He, W. et al. (2020) 'Sirolimus improves seizure control in pediatric patients with tuberous sclerosis: A prospective cohort study.', Seizure, 79, pp. 20–26. doi: 10.1016/j.seizure.2020.03.018.

Heng, K., Haney, M. M. and Buckmaster, P. S. (2013) 'High-dose Rapamycin blocks mossy fiber sprouting but not seizures in a mouse model of temporal lobe epilepsy.', Epilepsia, 54(9), pp. 1535–1541. doi: 10.1111/epi.12246.

Hester, M. S. et al. (2016) 'Impact of Rapamycin on status epilepticus induced hippocampal pathology and weight gain.', Experimental neurology, 280, pp. 1–12. doi: 10.1016/j.expneurol.2016.03.015.

Huang, X. et al. (2012) 'Rapamycin down-regulates KCC2 expression and increases seizure susceptibility to convulsants in immature rats.', Neuroscience, 219, pp. 33–47. doi: 10.1016/j.neuroscience.2012.05.003.

IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). Online version (2019-) created by S. J. Chalk. ISBN 0-9678550-9-8. https://doi.org/10.1351/goldbook.

Kadish, N. E. et al. (2020) 'Developmental outcomes in children/adolescents and one adult with tuberous sclerosis complex (TSC) and refractory epilepsy treated with EVO.', Epilepsy & behavior: E&B, 111, p. 107182. doi: 10.1016/j.yebeh.2020.107182.

Kotulska, K. et al. (2013) 'Long-term effect of EVO on epilepsy and growth in children under 3 years of age treated for subependymal giant cell astrocytoma associated with tuberous sclerosis complex.', European journal of paediatric neurology: EJPN: official journal of the European Paediatric Neurology Society, 17(5), pp. 479–485. doi: 10.1016/j.ejpn.2013.03.002.

Krueger, D. A. et al. (2016) 'Long-term treatment of epilepsy with EVO in tuberous sclerosis.', Neurology, 87(23), pp. 2408–2415. doi: 10.1212/WNL.0000000000003400.

Kwiatkowski, David J. et al. A mouse model of TSC1 reveals sex-dependent lethality from liver hemangiomas, and up-regulation of p70S6 kinase activity in Tsc1 null cells. Human molecular genetics, v. 11, n. 5, p. 525-534, 2002.

L.C. Fritz, C.C. Wang, A. Gorio, Avermectin B1a irreversibly blocks postsynaptic potentials at the lobster neuromuscular junction by reducing muscle membrane resistance, Proc Natl Acad Sc.

Lamming, D. W. (2016) 'Inhibition of the mechanistic target of Rapamycin (mTOR)–Rapamycin and beyond', Cold Spring Harbor Perspectives in Medicine, 6(5), pp. 1–14. doi: 10.1101/cshperspect.a025924.

Lasarge CL, Danzer SC. Mechanisms regulating neuronal excitability and seizure development following mTOR pathway hyperactivation. Front Mol Neurosci. 2014;7:18.

Laxer, K. D. et al. (2014) 'The consequences of refractory epilepsy and its treatment', Epilepsy and Behavior, 37, pp. 59–70. doi: 10.1016/j.yebeh.2014.05.031.

Mandro, M. et al. (2020) 'Ivermectin as an adjuvant to anti-epileptic treatment in persons with onchocerciasis-associated epilepsy: A randomized proof-of-concept clinical trial.', PLoS neglected tropical diseases, 14(1), p. e0007966. doi: 10.1371/journal.pntd.0007966.

Miyazawa M, Utsunomiya H, Inada K, et al. Inhibition of Helicobacter pylori motility by (+)-Syringaresinol from unripe Japanese apricot. Biol Pharm Bull. 2006;29(1):172-173.

Mizuguchi, M. et al. (2019) 'EVO for epilepsy and autism spectrum disorder in tuberous sclerosis complex: EXIST-3 substudy in Japan.', Brain & development, 41(1), pp. 1–10. doi: 10.1016/j.braindev.2018.07.003.

Musto, A. R. and A. E. (2018) 'The role of inflammation in the development of epilepsy', Journal of Neuroinflammation, 15, p. 144. doi: 10.3109/02713688808997245.

N.S. Kane, B. Hirschberg, S. Qian, D. Hunt, B. Thomas, R. Brochu, S.W. Ludmerer, Y. Zheng, M. Smith, J.P. Arena, C.J. Cohen, D. Schmatz, J. Warmke, D.F. Cully, Drug-resistant Drosophila indicate glutamate-gated chloride channels are targets for the antiparasitics nodulisporic acid and

ivermectin, Proc Natl Acad Sci U S A 97 (25) (2000) 13949–13954, https://doi.org/10.1073/pnas.240464697.

Nicholas Rensing, Lirong Han, and M. W. (2010) 'Intermittent dosing of Rapamycin maintains antiepileptogenic effects in a mouse model of tuberous sclerosis complex', Neurology, 34(2), pp. 291–299. doi: 10.1111/epi.13031.Intermittent.

Overwater, I. E. et al. (2016) 'Sirolimus for epilepsy in children with tuberous sclerosis complex: A randomized controlled trial.', Neurology, 87(10), pp. 1011–1018. doi: 10.1212/WNL.0000000000003077.

Park HW, Cho SY, Kim HH, et al. Enantioselective induction of SIRT1 gene by syringaresinol from Panax ginseng berry and Acanthopanax senticosus Harms stem. Bioorg Med Chem Lett. 2015;25(2):307-309.

Pereira, P. et al. Effect of γ-decanolactone on glutamate binding in the rat cerebral cortex. Neurochemical research, v. 22, n. 12, p. 1507-1510, 1997.

Pfluger P, Coelho VR, Regner GG, et al. Neuropharmacological Profile of Gamma-Decanolactone on Chemically-induced Seizure in Mice. Cent Nerv Syst Agents Med Chem. 2018;18(3):222-227.

Pflüger P, Pereira P, Loza MI, et al. Gamma-decanolactone: Preliminary evaluation as potential antiparkinsonian drug. Eur J Pharmacol. 2021;906:174276.

Pflüger P, Regner GG, Luft JG, et al. Gamma-decanolactone attenuates acute and chronic seizures in mice: a possible role of adenosine A1 receptors. Behav Pharmacol. 2020;31(6):544-552.

Pflüger, P. et al. (2018) 'Gamma-Decanolactone Improves Biochemical Parameters Associated with Pilocarpine-Induced Seizures in Male Mice.', Current molecular pharmacology, 11(2), pp. 162–169. doi: 10.2174/1874467210666171002114954.

Pflüger, P. et al. (2020) 'Gamma-decanolactone attenuates acute and chronic seizures in mice: A possible role of adenosine A1receptors', Behavioural Pharmacology, 31(6), pp. 544–552. doi: 10.1097/FBP.0000000000000554.

Pflüger, Vanessa Rodrigues Coelho, Gabriela Gregory Regner, Lucas Lima da Silva, Karina Martinez, Alan Fonseca, Cassiana Macagnan Viau, P. P. (2018) 'Neuropharmacological Profile of Gamma-Decanolactone on Chemically-induced Seizure in Mice', Central Nervous System Agents in Medicinal Chemistry, 18(3).

Samueli, S. et al. (2016) 'Efficacy and safety of EVO in children with TSC - associated epilepsy - Pilot data from an open single-center prospective study.', Orphanet journal of rare diseases, 11(1), p. 145. doi: 10.1186/s13023-016-0530-z.

Sartori, S. K., Diaz, M. A. N. and Diaz-Muñoz, G. (2021) 'Lactones: Classification, synthesis, biological activities, and industrial applications', Tetrahedron, 84. doi: 10.1016/j.tet.2021.132001.

Siebel, A. M. et al. (2015) 'Rapamycin suppresses PTZ-induced seizures at different developmental stages of zebrafish.', Pharmacology, biochemistry, and behavior, 139 Pt B, pp. 163–168. doi: 10.1016/j.pbb.2015.05.022.

Sierra-Paredes, G. and Sierra-Marcuño, G. (2008) 'Ascomycin and FK506: pharmacology and therapeutic potential as anticonvulsants and neuroprotectants.', CNS neuroscience & therapeutics, 14(1), pp. 36–46. doi: 10.1111/j.1527-3458.2008.00036.x.

Sliwa, A. et al. (2012) 'Post-treatment with Rapamycin does not prevent epileptogenesis in the amygdala stimulation model of temporal lobe epilepsy.', Neuroscience letters, 509(2), pp. 105–109. doi: 10.1016/j.neulet.2011.12.051.

Stöhr T, Kupferberg HJ, Stables JP, et al. Lacosamide, a novel anti-convulsant drug, shows efficacy with a wide safety margin in rodent models for epilepsy. Epilepsy Res. 2007;74(2-3):147-154.

Stöhr, T. et al. (2007) 'Lacosamide, a novel anti-convulsant drug, shows efficacy with a wide safety margin in rodent models for epilepsy.', Epilepsy research, 74(2–3), pp. 147–154. doi: 10.1016/j.eplepsyres.2007.03.004.

Tabudravu, Jioji & Jaspars, Marcel. (2005). Anticancer activities of constituents of kava (Piper methysticum). The South Pacific Journal of Natural Science. 23. 10.1071/SP05005.

Tabudravu, Jioji N.; Jaspars, Marcel. Anticancer activities of constituents of kava (Piper methysticum). The South Pacific Journal of Natural and Applied Sciences, v. 23, n. 1, p. 26-29, 2005.

Tang, H. et al. (2012) 'Rapamycin suppresses the recurrent excitatory circuits of dentate gyrus in a mouse model of temporal lobe epilepsy.', Biochemical and biophysical research communications, 420(1), pp. 199–204. doi: 10.1016/j.bbrc.2012.02.143.

Trailović, S. M. and Varagić, V. M. (2007) 'The effect of ivermectin on convulsions in rats produced by lidocaine and strychnine', Veterinary Research Communications, 31(7), pp. 863–872. doi: 10.1007/s11259-007-0050-3.

van Vliet, E. A. et al. (2012) 'Inhibition of mammalian target of Rapamycin reduces epileptogenesis and blood-brain barrier leakage but not microglia activation.', Epilepsia, 53(7), pp. 1254–1263. doi: 10.1111/j.1528-1167.2012.03513. x.

Vázquez-López, A., Sierra-Paredes, G. and Sierra-Marcuño, G. (2006) 'Anticonvulsant effect of the calcineurin inhibitor ascomycin on seizures induced by picrotoxin microperfusion in the rat hippocampus.', Pharmacology, biochemistry, and behavior, 84(3), pp. 511–516. doi: 10.1016/j.pbb.2006.06.015.

VIEIRA, Perla R. N. et al. Antimicrobial activity of sesquiterpene lactones from yacon (Smallanthus sonchifolius, Asteraceae) leaves. Fitos Magazine, Rio de Janeiro, v. 15, no. 1, p. 108-114, 2021.

Więckowski K, Sałat K, Bytnar J, et al. Search for anticonvulsant and analgesic active derivatives of dihydrofuran-2(3H)-one. Bioorg Med Chem. 2012;20(21):6533-6544.

Wieckowski, K. et al. (2012) 'Search for anticonvulsant and analgesic active derivatives of dihydrofuran-2(3H)-one', BIOORGANIC \& MEDICINAL CHEMISTRY, 20(21), pp. 6533–6544. doi: 10.1016/j.bmc.2012.08.037.

Wiegand, G. et al. (2013) 'EVO in tuberous sclerosis patients with intractable epilepsy: A treatment option?', European Journal of Paediatric Neurology, 17(6), pp. 631–638. doi: 10.1016/j.ejpn.2013.06.002.

World Health Organization (2021) Epilepsy. https://www.who.int/health-topics/epilepsy. Accessed 05 May 2021

Xie Wei, Bao Yong. Dynamic observation of the effect of saikosaponin a on mice with experimental seizure. J. Guangdong Coll. Pharm. 1 (2006) 025.

Y.-H. Yu, W. Xie, Y. Bao, H.-M. Li, S.-J. Hu, and J.-L. Xing, "Saikosaponin a mediates the anticonvulsant properties in the HNC models of AE and SE by inhibiting NMDA receptor current and persistent sodium current," PLoS ONE, vol. 7, no. 11, Article ID 50694, 2012.

Ye M, Bi YF, Ding L, Zhu WW, Gao W. Saikosaponin a functions as anti-epileptic effect in pentylenetetrazol induced rats through inhibiting mTOR signaling pathway. Biomed Pharmacother. 2016;81:281-287.

Ye, M. et al. (2016) 'Saikosaponin a functions as anti-epileptic effect in pentylenetetrazol induced rats through inhibiting mTOR signaling pathway', Biomedicine and Pharmacotherapy, 81, pp. 281–287. doi: 10.1016/j.biopha.2016.04.012.

Yu YH, Xie W, Bao Y, Li HM, Hu SJ, Xing JL. Saikosaponin a mediates the anticonvulsant properties in the HNC models of AE and SE by inhibiting NMDA receptor current and persistent sodium current. PLoS One. 2012;7(11):e50694.

Zeng LH, Rensing NR, Wong M. The mammalian target of Rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. J Neurosci. 2009;29(21):6964-6972.

Zeng, L.-H., Rensing, N. R. and Wong, M. (2009) 'The mammalian target of Rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy.', The Journal of neuroscience : the official journal of the Society for Neuroscience, 29(21), pp. 6964–6972. doi: 10.1523/JNEUROSCI.0066-09.2009.

4. CONCLUSÃO

De acordo com os resultados encontrados neste estudo é possível afirmar que a busca por novas moléculas para o tratamento de crises epilépticas é relevante levando em conta que uma considerável parcela de pacientes é refratária. Para que alguma substância seja a escolha terapêutica adequada uma série de parâmetros devem ser considerados na sua avaliação, para que além de servir seu objetivo na amenização ou cessação total das crises também se almeja manter o bem-estar dos pacientes. As moléculas encontradas se mostram definitivamente promissoras e obtiveram efeitos positivos quando experimentadas junto com uma diversa gama de testes que induzem a epileptogênese em diferentes modelos animais.

Apesar de nenhuma lactona ter sido aprovado ainda para tratamento da epilepsia, em certos tipos de crises algumas delas mostram-se eficazes, além de promissoras em tratamentos experimentais em pesquisas pré-clínicas e em clínicas. Desta forma, conclui-se que a classe das lactonas poderão se tornar estratégias farmacológicas importantes no tratamento da epilepsia, em associação com outros fármacos ou até mesmo em monoterapia. Portanto, a continuidade dos estudos dos mecanismos de ação das lactonas, como por exemplo gama-decanolactona, torna-se crucial para o desenvolvimento de novas alternativas farmacológicas.

5. REFERÊNCIAS BIBLIOGRÁFICAS

AABERG, K. M. et al. (2017) 'Seizures, syndromes, and etiologies in childhood epilepsy: The International League Against Epilepsy 1981, 1989, and 2017 classifications used in a population-based cohort', Epilepsia, 58(11), pp. 1880–1891. doi: 10.1111/epi.13913.

ABOU-KHALIL BW. Update on Antiepileptic Drugs 2019. Continuum (Minneap Minn). 2019 Apr;25(2):508-536. doi: 10.1212/CON.0000000000000715. PMID: 30921021.

ASLA Pitkanen, Katarzyna Lukasiuk, F. Edward Dudek, and K. J. S. (2014) 'Epileptogenesis', Paper Knowledge . Toward a Media History of Documents, pp. 1–17.

BECKER, A. J. Animal models of acquired epilepsy: insights into mechanisms of human epileptogenesis. Neuropathology and applied neurobiology, v. 44, n. 1, p. 112-129, 2018.

CABAN C, Khan N, Hasbani DM, Crino PB. Genetics of tuberous sclerosis complex: implications for clinical practice. Appl Clin Genet. 2016 Dec 21;10:1-8. doi: 10.2147/TACG.S90262. PMID: 28053551; PMCID: PMC5189696.

DE OLIVEIRA, P. A. et al. (2008) 'Effects of gamma-decanolactone on seizures induced by PTZ-kindling in mice', Experimental Brain Research, 187(1), pp. 161–166. doi: 10.1007/s00221-008-1295-y.

DEVINSKY O, Vezzani A, O'Brien TJ, et al (2018) Epilepsy. Nat Rev Dis Prim 4:. https://doi.org/10.1038/nrdp.2018.24

DEVINSKY, Orrin et al. Epilepsy. Nature Reviews Disease Primers, v. 3, p.1, 2018.

DOS SANTOS FM, Pflüger PF, Lazzarotto L, et al. Gamma-Decanolactone Alters the Expression of GluN2B, A1 Receptors, and COX-2 and Reduces DNA Damage in the PTZ-Induced Seizure Model After Subchronic Treatment in Mice. Neurochem Res. 2021;46(8):2066-2078.

ELISABETSKY, E. Effects of linalool on glutamatergic system in the rat cerebral cortex. Neurochemical research, v. 20, n. 4, p. 461-465, 1995.

ENGEL, J., Jr (2019), Evolution of concepts in epilepsy surgery*. Epileptic Disorders, 21: 391-409. https://doi.org/10.1684/epd.2019.1091

FALCO-WALTER, J. (2020) 'Epilepsy-Definition, Classification, Pathophysiology, and Epidemiology', Seminars in Neurology, 40(6), pp. 617–623. doi: 10.1055/s-0040-1718719.

FISHER, R. S. et al. (2017) 'Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology', Epilepsia, 58(4), pp. 522–530. doi: 10.1111/epi.13670.

GLAZKO AJ. Discovery of phenytoin. Ther Drug Monit. 1986;8(4):490-7. doi: 10.1097/00007691-198612000-00021. PMID: 3547783.

GONZÁLEZ, FJ López et al. Drug-resistant epilepsy: definition and treatment alternatives. Neurología (English Edition), v. 30, n. 7, p. 439-446, 2015.

GOORDEN SM, van Woerden GM, van der Weerd L, Cheadle JP, Elgersma Y. Cognitive deficits in Tsc1+/- mice in the absence of cerebral lesions and seizures. Ann Neurol. 2007 Dec;62(6):648-55. doi: 10.1002/ana.21317. PMID: 18067135.

HERNÁNDEZ-RONQUILLO, Lizbeth et al. Epilepsy in an elderly population: classification, etiology and drug resistance. Epilepsy research, v. 140, p. 90-94, 2018.

HOLLAND KD, McKeon AC, Canney DJ, et al (1992) Relative Anticonvulsant Effects of GABAmimetic and GABA Modulatory Agents. Epilepsia 33:981–986. https://doi.org/10.1111/j.1528-1157.1992.tb01747.x

IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). Online version (2019-) created by S. J. Chalk. ISBN 0-9678550-9-8. https://doi.org/10.1351/goldbook.

JUNIOR, E. (2016) 'Epileptogenesis, Traumatic Brain Injury, and Biomarkers', Physiology & behavior, 176(1), pp. 139–148. doi: 10.1016/j.nbd.2018.04.002.Epileptogenesis.Devinsky, O. et al. (2018) 'Epilepsy', Nature Reviews Disease Primers, 4(May). doi: 10.1038/nrdp.2018.24.

KOH S. Role of Neuroinflammation in Evolution of Childhood Epilepsy. J Child Neurol. 2018 Jan;33(1):64-72. doi: 10.1177/0883073817739528. PMID: 29246095.

LAXER, K. D. et al. (2014) 'The consequences of refractory epilepsy and its treatment', Epilepsy and Behavior, 37, pp. 59–70. doi: 10.1016/j.yebeh.2014.05.031.

LÖSCHER, W. (2017) 'Animal Models of Seizures and Epilepsy: Past, Present, and Future Role for the Discovery of Antiseizure Drugs', Neurochemical Research, 42(7), pp. 1873–1888. doi: 10.1007/s11064-017-2222-z.

LÖSCHER, W. et al. (2020) 'Drug resistance in epilepsy: Clinical impact, potential mechanisms, and new innovative treatment options', Pharmacological Reviews, 72(3), pp. 606–638. doi: 10.1124/pr.120.019539.

MUSTO, A. R. and A. E. (2018) 'The role of inflammation in the development of epilepsy', Journal of Neuroinflammation, 15, p. 144. doi: 10.3109/02713688808997245.

NADER, M. A. et al. Sitagliptin enhances the neuroprotective effect of pregabalin against pentylenetetrazole-induced acute epileptogenesis in mice: implication of oxidative, inflammatory, apoptotic and autophagy pathways. Neurochemistry international, v. 115, p. 11-23, 2018.

PALARETI, G. et al. (2016) 'Comparison between different D-Dimer cutoff values to assess the individual risk of recurrent venous thromboembolism: Analysis of results obtained in the DULCIS study', International Journal of Laboratory Hematology, 38(1), pp. 42–49. doi: 10.1111/ijlh.12426.Jr.,

PEREIRA, P. et al. Effect of γ-decanolactone on glutamate binding in the rat cerebral cortex. Neurochemical research, v. 22, n. 12, p. 1507-1510, 1997.

PFLUGER P, Coelho VR, Regner GG, et al. Neuropharmacological Profile of Gamma-Decanolactone on Chemically-induced Seizure in Mice. Cent Nerv Syst Agents Med Chem. 2018;18(3):222-227.

PFLÜGER P, Pereira P, Loza MI, et al. Gamma-decanolactone: Preliminary evaluation as potential antiparkinsonian drug. Eur J Pharmacol. 2021;906:174276.

PFLÜGER P, Regner GG, Luft JG, et al. Gamma-decanolactone attenuates acute and chronic seizures in mice: a possible role of adenosine A1 receptors. Behav Pharmacol. 2020;31(6):544-552.

PFLÜGER, P. et al. (2018) 'Gamma-Decanolactone Improves Biochemical Parameters Associated with Pilocarpine-Induced Seizures in Male Mice.', Current molecular pharmacology, 11(2), pp. 162–169. doi: 10.2174/1874467210666171002114954.

PFLÜGER, P. et al. (2020) 'Gamma-decanolactone attenuates acute and chronic seizures in mice: A possible role of adenosine A1receptors', Behavioural Pharmacology, 31(6), pp. 544–552. doi: 10.1097/FBP.0000000000000554.

RANG, Rang et al. Rang & Dale Farmacologia. Elsevier Brasil, cap. 45, 2015.

ROGAWSKI, M. A.; LÖSCHER, W. The neurobiology of antiepileptic drugs. Nature Reviews Neuroscience, v. 5, n. 7, p. 553-564, 2004.

SARTORI, S. K., Diaz, M. A. N. and Diaz-Muñoz, G. (2021) 'Lactones: Classification, synthesis, biological activities, and industrial applications', Tetrahedron, 84. doi: 10.1016/j.tet.2021.132001.

SAVAGE, Neil. The complexities of epilepsy. Nature, v. 511, n. 7508, p. 50–51, 2014.

SHETTY, A. K.; UPADHYA, D. GABA-ergic cell therapy for epilepsy: Advances, limitations and challenges. Neuroscience & Biobehavioral Reviews, v. 62, p. 35-47, 2016.

TABUDRAVU, Jioji & Jaspars, Marcel. (2005). Anticancer activities of constituents of kava (Piper methysticum). The South Pacific Journal of Natural Science. 23. 10.1071/SP05005.

VARGAS-SÁNCHEZ, Karina et al. Astroglial role in the pathophysiology of status epilepticus: an overview. Oncotarget, v. 9, n. 42, p. 26954, 2018.

VIEIRA, Perla R. N. et al. Antimicrobial activity of sesquiterpene lactones from yacon (Smallanthus sonchifolius, Asteraceae) leaves. Fitos Magazine, Rio de Janeiro, v. 15, no. 1, p. 108-114, 2021.

WORLD HEALTH ORGANIZATION (2022) Epilepsy. https://www.who.int/health-topics/epilepsy. Accessed 05 January 2022

6. ANEXOS

Figure 1: Flowchart of the query on lactones and their work suppressing seizure activities in the main databases.

| 1 – LACTONES |
| 2 – ANTICONVULSANT |
| 3 – ANTISEIZURE |
| 4 - ANTIEPILEPTOGENIC |

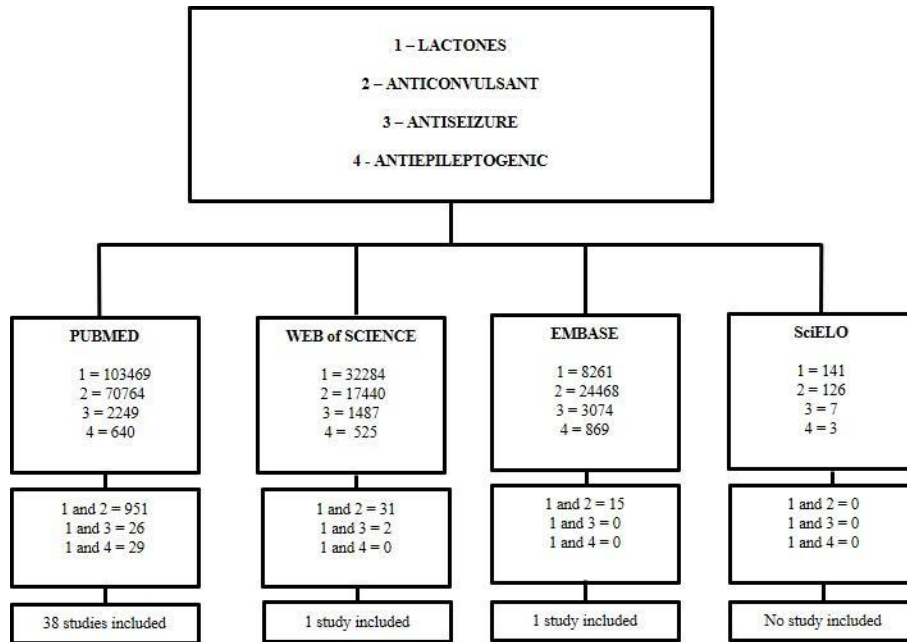| PUBMED	WEB of SCIENCE	EMBASE	SciELO
1 = 103469 2 = 70764 3 = 2249 4 = 640	1 = 32284 2 = 17440 3 = 1487 4 = 525	1 = 8261 2 = 24468 3 = 3074 4 = 869	1 = 141 2 = 126 3 = 7 4 = 3
1 and 2 = 951 1 and 3 = 26 1 and 4 = 29	1 and 2 = 31 1 and 3 = 2 1 and 4 = 0	1 and 2 = 15 1 and 3 = 0 1 and 4 = 0	1 and 2 = 0 1 and 3 = 0 1 and 4 = 0
38 studies included	1 study included	1 study included	No study included

Table 1 – Summary of pre-clinical studies with RAP.

Author (year)	Title	Type of article	Experiment	Convulsion Method	Species	Sham group	Results/Insights
Zeng et al. (2009)	The Mammalian Target of RAP Signaling Pathway Mediates Epileptogenesis in a Model of Temporal Lobe Epilepsy	Original article	Treatment with RAP (6 mg/kg 1 d 1, i.p.) or vehicle for 3 consecutive days before receiving kainate	KAI	Adult Male Sprague-Dawley Rats	Yes	Findings indicate that mTOR signaling mediates mechanisms of epileptogenesis in the kainate rat model and that mTOR inhibitors have potential antiepileptogenic effects in this mode
Buckmaster et al. (2011)	RAP Suppresses Mossy Fiber Sprouting But Not Seizure Frequency in a Mouse Model of Temporal Lobe Epilepsy	Original article	Mice were treated daily (intraperitoneally) with 1.5 mg/kg RAP, 3 mg/kg RAP, or vehicle alone.	PIL	Male and Female Mice FBV strain	Yes	These findings suggest RAP does not affect seizure frequency or severity in pilocarpine-treated mice.
Tang et al. (2012)	RAP suppresses the recurrent excitatory circuits of dentate gyrus in a mouse model of temporal lobe epilepsy	Original article	RAP (6 mg/kg d, i.p.) for RAP 24 h after the onset of PILO induced status epilepticus for 6 consecutive days	PIL	Male Mice C57BL/6	Yes	Findings suggested an anti-epileptogenic role of RAP by suppressing the recurrent excitatory circuits of dentate gyrus. RAP suppressed recurrent excitatory circuits in the dentate gyrus, reduced epileptiform activity, and inhibited both spontaneous and evoked EPSCs.
Chachua et al. (2012)	RAP has age-, treatment paradigm-, and model-specific anticonvulsant effects and modulates neuropeptide Y expression in rats	Original article	RAP was injected intraperitoneally (i.p.) in a dose of 3 mg/kg in PN15 rats and 3 or 6 mg/kg in the adult rats.	Flurothyl, PTZ, NMDA, KA	Male Rats (PN15 and adult)	Yes	These findings suggest that RAP is a poor anticonvulsant and may have beneficial effects only against epileptogenesis.
Sliwa et al. (2012)	Post-treatment with RAP does not prevent epileptogenesis in the amygdala stimulation model of temporal lobe epilepsy	Original article	Animals were treated with RAP (6 mg/kg) or vehicle daily for 2 wks, beginning 24 h after stimulation	Electrical stimulation of the amygdala	Adult Male Sprague-Dawley Rats	Yes	Data indicate that post-treatment with RAP for 2 wks following amygdala stimulation-induced SE does not prevent epileptogenesis nor mossy fiber sprouting and does not decrease the disease severity in the 6 wks period after SE.
Huang et al. (2012)	RAP down-regulates KCC2 expression and increases seizure susceptibility to convulsants in immature rats.	Original article	Rats were pretreated with RAP at 5 mg/kg/day i.p. for one to three consecutive days prior to the induction of seizures	PTZ, KAI, PIL	Adult Male Sprague-Dawley Rats	Yes	Rapamycin also reduces the minimal dose of pentylenetetrazol (PTZ) necessary to induce clonic seizures. However, in mature rats, Rapamycin does not significantly change the seizure sensitivity to pilocarpine and PTZ
Vliet et al. (2012)	Inhibition of mammalian target of Rapamycin reduces epileptogenesis and blood–brain barrier leakage but notmicroglia activation	Original article	RAP was given intraperitoneally (6 mg/kg/day under isoflurane anesthesia) starting 4 h after status epilepticus (SE), once daily for 7 days	Electrical stimulation	Adult Male Sprague-Dawley Rats	Yes	Rapamycin treatment did not alter SE severity and duration compared to vehicle treatment rats. RAP treatment leads to a suppression of seizure development in the rat TLE model.
Hartman et al. (2012)	The mTOR Inhibitor Rapamycin Has Limited AcuteAnticonvulsant Effects in Mice	Original article	Mice were injected intraperitoneally with Rapamycin 4.5 mg/kg or 6 mg/kg body weight	MES-T test, 6 Hz test, KAI and PTZ	Mice (NIH - Swiss)	Yes	The only seizure test where short-term Rapamycin treatment protected mice was against tonic hindlimb extension in the MES threshold test, though this protection waned with longer RAP treatment.
Heng et al. (2013)	High-dose RAP blocks mossy fiber sprouting but not seizures in a mouse model of temporal lobe epilepsy	Original article	Beginning 24 h after status epilepticus, daily treatment began with 10 mg/kg RAP	PIL	Male and Female GIN Mice	Yes	These findings suggest mossy fiber sprouting is not necessary for epileptogenesis in the mouse pilocarpine model. They also reveal that RAP does not have anti-seizure or anti-epileptogenic effects in this model.
Guo et al. (2013)	RAP Attenuates the Development of Posttraumatic Epilepsy in a Mouse Model of Traumatic Brain Injury	Original article	RAP (6 mg/ kg/d, i.p) or vehicle solution was injected 1 hour after TBI	Controlled cortical impact (CCI) injury model	Mice CD-1	Yes	These results suggest that RAP may represent a rational treatment for preventing posttraumatic epilepsy in patients with TBI.

			and continued once daily for up to 4 weeks.			
Rensing *et al.* (2015)	Intermittent dosing of RAP maintains antiepileptogenic effects in a mouse model of tuberous sclerosis complex	Original article	Mice were injected with RAP for 4 days at doses ranging between 0.1 to 10 mg/kg/d i.p. and harvested 24 hours after the last injection for western blot analysis	None	Male and Female (Tsc1GFAPCKO) mice with conditional inactivation of the Tsc1(GFAP)	Yes	Intermittent RAP, with drug holidays of up to 24 days, almost completed prevented epilepsy in mice. mTOR activity is inhibited by RAP in a dose- dependent fashion and recovers to baseline by 10 days after
Siebel *et al.* (2015)	RAP suppresses PTZ-induced seizures at different developmental stages of zebrafish	Original article	Zebrafish larvae were individually placed in 96 well plates and submitted to treatment with 0.1% DMSO (control group), RAP at 0.12, 0.25, 0.5, 1, and 2.5 μM or VPA at 3 mM for 30 min	PTZ	Zebra-fish	Yes	Data support that RAP has immediate antiseizure effects and could be a potential alternative therapy for seizure control in epilepsy
Hester *et al.* (2016)	Impact of RAP on status epilepticus induced hippocampal pathology and weight gain	Original article	RAP was administered daily (6mg/kg, i.p; or vehicle control) for six weeks	Tamoxifen, PIL	Mice	Yes	. RAP had modest effects on mossy fiber sprouting, and was ineffective in preventing changes among the other parameters, strongly suggesting that the beneficial effects of the drug in epilepsy are not mediated by changes in any of these processes.
Drion *et al.* (2016)	Effects of RAP and curcumin treatment on the development of epilepsy after electrically induced status epilepticus in rats	Original article	RAP (6 mg/kg/day, i.p.) was started 4 h after SE and continued once daily for 6 days. Hereafter, RAP was given every other day for 2 weeks.	Intracranial electrodes for stimulation	Adult Male Sprague-Dawley Rats	Yes	RAP (3 and 6 mg/kg) shows seizure-suppressing effects in the post-SE rat model for TLE Effects of RAP are transient; RAP was not able to prevent or reverse epileptogenesis in this TLE model
Xiaosa *et al.* (2017)	Inhibition of mTOR Pathway by RAP Decreases P-glycoprotein Expression and Spontaneous Seizures in Pharmacoresistant Epilepsy	Original article	Injected with various doses of RAP (1, 3, and 6 mg/kg) or the same volume of vehicle for six consecutive days, and then every other day until the end point of the study.	Kindling Coriaria Lactone	Human Tissue/Male Sprague-Dawley Rats	Yes	RAP significantly inhibited spontaneous seizures in pharmacoresistant epileptic rats, demonstrating that RAP may be a potential therapy for pharmacoresistant epilepsy
Drion *et al.* (2018)	Effects of RAP and curcumin on inflammation and oxidative stress in vitro and in vivo — in search of potential anti- epileptogenic strategies for temporal lobe epilepsy	Original article	Rats (n = 5) were injected intraperitoneally with 6 mg/kg RAP solution, starting 4 h after SE and once daily for 7 days thereafter.	Tetanic stimulation of the angular bundle in the form of 50 Hz pulse trains	Adult Male Sprague-Dawley Rats	Yes	1-week post-SE treatment with RAP or curcumin did not alter SE-induced upregulation of markers of inflammation and oxidative stress, while in vitro, curcumin displayed anti-inflammatory and antioxidant effects

Table 2 – Summary of clinical studies with EVO.

Author (year)	Title	Type of article	Concentration	N	Sham-group	Results/Insights
Kotulska et al. (2013)	Long-term effect of EVO on epilepsy andgrowth in children under 3 years of age treated forsubependymal giant cell astrocytoma associatedwith tuberous sclerosis complex	EXIST-1 study	Concentration was 4.5 mg per square meter of body surface area and was subsequently adjusted to attain a whole-blood through EVO concentration of 5e15 ng/mL	8 children	Yes	At their last examination, four patients were seizure-free (27%), and four experienced a reduction of seizures N50% (27%). With treatment, (slight) increase was seen in absolute values of developmental age (DA) regarding both development and adaptive functioning.
Wiegand et al. (2013)	EVO in tuberous sclerosis patients with intractable epilepsy: A treatment option?	Prospective observational study	Concentration started with 1 mg per square meter of body surface area. Increased until a trough serum concentration of 5e10 ng/ml was obtained.	7 children	No	Reduction of seizures in 4/6 patients In addition, the percentage of seizure-free days increased in 3/4 of these patients. In 2/6 patients, no alteration of seizure frequency was noted.
Krueger et al. (2016)	Long-term treatment of epilepsy with EVO in tuberous sclerosis	Prospective, open-label, phase I/II clinical trial design	Concentration was initiated at 5 mg/m2 /d, rounded to the nearest 2.5 milligram, and dosed once daily. Adjusted for a target range between 5 and 15 ng/mL.	20 patients	No	Clinical focal-onset seizures were the most common (69% of seizures overall). The largest reduction was observed with focal-onset seizures (83% reduction, 13 of 15 participants).
French et al. (2016)	Adjunctive EVO therapy for treatment-resistant focal-onset seizures associated with tuberous sclerosis (EXIST-3): a phase 3, randomised, double-blind, placebo-controlled study	(EXIST-3): a phase 3, randomised, double-blind, placebo-controlled study	Concentration (Cmin) of 3–7 ng/mL (low-exposure EVO), or everlimus titrated to a target Cmin of 9–15 ng/mL (high-exposure EVO), in addition to a stable regimen of one to three antiepileptic drugs.	366 patients	Yes	Adjunctive EVO treatment significantly reduced seizure frequency with a tolerable safety profile compared with placebo in patients with tuberous sclerosis complex and treatment-resistant seizures.
Samueli et al. (2016)	Efficacy and safety of EVO in children with TSC - associated epilepsy – Pilot data from an open single-center prospective study	Open single-center prospective study	Concentration starting with 4.5 mg/m2 and titrated to achieve blood trough concentrations (measured with the LC-MS/MS method) between 5 and 15 ng/ml.	15 patients	No	At last observation, 80 % (12/15) of the patients were responders, 58 % of them (7/12) were seizure free. The overall reduction in seizure frequency was 60 % in focal seizures, 80 % in generalized tonic clonic seizures and 87 % in drop attacks.
Mizuguchi et al. (2018)	EVO for epilepsy and autism spectrum disorder in tuberous sclerosis complex: EXIST-3 substudy in Japan	EXIST-3 substudy in Japan	Concentration (Cmin) of 3–7 ng/mL (EVO low-exposure [LE] treatment) or to a target Cmin of 9–15 ng/mL (everoli- mus high-exposure [HE] treatment) in addition to a stable regimen of 1–3 AEDs	35 patients	Yes	The response rate was 30.0% and 28.6% versus 0% with the EVO LE and HE versus placebo arm, respectively. Similarly, the median percentage reduction in seizure frequency was 6.88% and 38.06% versus 6.67%.
Kadish et al. (2020)	Developmental outcomes in children/adolescents and one adult with tuberous sclerosis complex (TSC) and refractory epilepsy treated with EVO	Prospective observational study	Patients were treated with three different target levels (I: 3–5 µg/l; II: 6–9 µg/l; III: 10–15 µg/l or higher)	14 children/adolescents	No	At their last examination, four patients were seizure-free (27%), and four experienced a reduction of seizures N50% (27%).