Epilepsy Research 145 (2018) 116–122

Contents lists available at ScienceDirect

Epilepsy Research

journal homepage: www.elsevier.com/locate/epilepsyres

Combination of phenobarbital with phenytoin and pregabalin produces synergy in the mouse tonic-clonic seizure model: An isobolographic analysis

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ARTICLE INFO

Keywords:
Drug interactions
Isobolographic analysis
Maximal electroshock

ABSTRACT

Aims: Despite many antiepileptic drugs (AEDs) are available to treat epilepsy, there is still about 30% of epilepsy patients inadequately treated with these AEDs. For these patients, polytherapy with two or three AEDs to fully control their seizure attacks is recommended. Unfortunately, polytherapy is always associated with drug interactions, whose nature may be beneficial, neutral or unfavorable. To determine a type of interaction for the combination of three AEDs (i.e., phenobarbital [PB], phenytoin [PHT] and pregabalin [PGB]) at the fixed-ratio of 1:1:1, we used a model of tonic-clonic seizures in male albino Swiss mice.

Materials and method: Tonic-clonic seizures in mice were evoked by a current (sine-wave, 25 mA, 500 V, 0.2 s stimulus duration) delivered via auricular electrodes. The anticonvulsant effects of the three-drug combination (PB, PHT and PGB) in terms of suppression of tonic-clonic seizures in mice were assessed with type I isobolographic analysis. Potential acute side effects for the mixture of PB, PHT and PGB along with total brain concentrations of the AEDs were determined to confirm pharmacodynamic nature of observed interaction.

Results: The three-drug combination of PB, PHT and PGB (at the fixed-ratio of 1:1:1) exerted synergistic interaction (at $P < 0.01$) in the mouse model of tonic-clonic seizures. The combination of PB, PHT and PGB did not produce any side effects in experimental animals, when measuring long-term memory, muscular strength and motor coordination. The measurement of total brain concentrations of PB, PHT and PGB was conducted to confirm that none of the three AEDs significantly influenced total brain concentrations (pharmacokinetic profiles) of the other co-administered AEDs in mice.

Conclusions: The synergistic pharmacodynamic interaction for the combination of PB, PHT and PGB observed in this preclinical study can be translated into clinical settings and this favorable AED combination is worthy of being recommended to some patients with refractory epilepsy.

1. Introduction

Polytherapy with two or three antiepileptic drugs (AEDs) is still a necessary treatment option for ~30% of epilepsy patients, who are inadequately treated with currently available AEDs used in monotherapy (Brodie and Sills, 2011; Kwan and Brodie, 2006). Despite 25 various AEDs are licensed for the treatment of epilepsy, limited information is available on how to combine these AEDs and which drugs can be joined together to reach beneficial effects in patients, resulting in reduction of their seizure activity and/or frequency (Brodie and Sills, 2011; Kwan and Brodie, 2006; Stephen and Brodie, 2002; Stephen et al., 2012). When physicians combine two or three AEDs, they follow a general rule for combining AEDs with diverse molecular mechanisms of anticonvulsant action so as to complementary enhance the anti-seizure effects offered by these AEDs (Perucca, 1995). There exists a hypothesis suggesting that AEDs with diverse molecular mechanisms of action can be combined together so as to enhance their anticonvulsant properties. On the contrary, the AEDs with similar mechanisms of action may competitively affect target/receptors and only additivity can be expected in terms of protection from seizures (Deckers et al., 2000; Perucca, 1995). On the other hand, each AED combination can evoke interactions of pharmacodynamic and/or pharmacokinetic nature

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https://doi.org/10.1016/j.eplepsyres.2018.06.003
Received 3 January 2018; Received in revised form 3 June 2018; Accepted 9 June 2018
Available online 18 June 2018
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(Patsalos and Perucca, 2003a,b; Perucca, 1995).

At present, at least 25 various AEDs are licensed to the treatment of epilepsy patients, therefore, from a theoretical viewpoint, 13,800 possible three-drug combinations exist. In such a situation, it is difficult or impossible to directly examine all these combinations in clinical practice in order to ascertain which of these combinations are the best for epileptic patients. To help physicians in their selective choice of drugs for combinations, preclinical studies on animals provide reliable information on the types of interactions occurring among AEDs and classify these combinations (Loscher, 2015). Accumulating evidence indicates that only few three-drug combinations of AEDs occurred advantageous in epileptic patients (Brodie and Sills, 2011; Stephen and Brodie, 2002, 2012).

On the other hand, it is impossible to theoretically indicate the favorable combinations among AEDs considering only their molecular mechanisms of action. Experiments conducted recently on animals revealed that some three-drug combinations of AEDs are favorable (synergistic) or neutral (additive) in nature and may be recommended to clinical settings. For instance, it was observed that the combination of carbamazepine (CBZ) with phenobarbital (PB) and topiramate (TPM) offered synergistic interaction in the mouse model of tonic-clonic seizures (Luszczki, 2016). The combinations of lacosamide (LCM) with CBZ and lamotrigine (LTG) (Kondrat-Wrobel and Luszczki, 2017); LCM, CBZ and PB (Kondrat-Wrobel and Luszczki, 2016), and LCM, PB and LTG (Kondrat-Wrobel and Luszczki, 2018) exerted additive interactions in the mouse maximal electroshock-induced seizure (MES) model – an experimental model of tonic-clonic seizures (Loscher et al., 1991).

The aim of this study was to continue our experiments and characterize a type of interaction among three AEDs with various molecular mechanisms of anticonvulsant action i.e., PB, phenytoin (PHT) and pregabalin (PGB) in the mouse MES model. The selection of AEDs to this three-drug combination was based on diverse molecular mechanisms of action of PB, PHT and PGB that can mutually complete their anticonvulsant action (Czapinski et al., 2005). For instance, PB enhances γ-aminobutyric acid $(GABA)_A$ receptor mediated neurotransmission in the brain (Czapinski et al., 2005). PHT blocks fast-inactivated state of sodium channels (Mantegazza et al., 2010), and PGB blocks high-voltage activated calcium channels (Czapinski et al., 2005). Undoubtedly, all three AEDs inhibit propagation of pathological discharges in neurons.

When choosing three AEDs (PB, PHT and PGB), we have additionally consulted results obtained from clinical studies (Stephen and Brodie, 2002, 2012), reporting that the drug combinations containing PB, PHT and gabapentin were effective in terms of suppression of seizures in epileptic patients refractory to the standard pharmacological medication. These AEDs in combinations offered the epileptic patients a significant reduction of seizures (Stephen and Brodie, 2002, 2012). Since PGB and gabapentin are quite similar AEDs with respect to their molecular mechanisms of action (Rogawski and Bazil, 2008), we chose PGB to combine it with PB and PHT and to test their ability to interact together in terms of suppression of tonic-clonic seizures in mice.

The assessment of interaction for the three-drug combination of PB, PTH and PGB was performed using type I isobolographic analysis, as described earlier (Kondrat-Wrobel and Luszczki, 2016; Zolkowska et al., 2016). Generally, the isobolographic analysis is thought to be the "gold standard" in examination of interactions (Tallarida, 2012).

2. Materials and methods

2.1. Animals and drug administration

This study was carried out on adult male albino Swiss outbred mice (weighing 20–26 g), purchased from a licensed breeder (Dr. J. Kolacz, Warszawa, Poland). All experimental procedures, described below, comply with the ARRIVE guidelines and were approved by the Second Local Ethics Committee at the University of Life Sciences in Lublin, Poland (License no.: 45/2014). Three AEDs: PB (Polfa, Krakow, Poland), PHT (Sigma-Aldrich, Poznan, Poland) and PGB (Lyrica®, Pfizer Ltd., Sandwich, Kent, UK), were suspended in an aqueous (1%) solution of Tween 80 (Sigma-Aldrich, Poznan, Poland) and administered intraperitoneally (i.p.) in a volume of 5 ml/kg body weight. PB was administered 60 min.; PHT and PGB – 120 min.; before the MES test, evaluation of potential acute adverse effects and collection of brain samples for the measurement of PB, PHT and PGB concentrations. These treatment times indicated the times to peak of maximal anticonvulsant effects produced by the AEDs (Luszczki, 2009a,b; Luszczki et al., 2013). The time periods between i.p. administration of the AEDs and the MES test were identical as for the brain sampling for the measurement of total brain concentrations of AEDs and evaluation of potential acute adverse effects. Total number of mice used in this study was 184 (i.e., 15 groups per 8 mice in the tonic-clonic seizure model, when evaluating ED_{50} values for the studied AEDs and $ED_{50\ exp}$ for the mixture [120 mice]; 2 groups per 8 mice in the passive avoidance, chimney and grip-strength tests, when assessing acute adverse effects [16 mice], and 6 groups per 8 mice during the measurement of brain AEDs concentrations [48 mice]. Totally, it was 23 groups per 8 mice). According to the ARRIVE guidelines, all experimental procedures involving animals were performed in this study in a blind manner by researchers who were blind to the respective treatment.

2.2. Maximal electroshock-induced seizure (MES) test

Mice were subjected to maximal electroshock-induced seizures (MES), produced by a current (50 Hz, 25 mA, 500 V, 0.2 s stimulus duration), generated by a rodent shocker (RS Type 221; Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany), delivered via auricular electrodes. The occurrence of seizure activity, which manifested as tonic hind limb extension in mice, was considered as the endpoint. In our study, the animals received increasing doses of the AEDs in order to obtain a variable percentage of animals protected from MES-induced seizures, which allowed us to construct dose-response effects for the studied AEDs when administered singly, as described earlier (Litchfield and Wilcoxon, 1949). The anticonvulsant activities of three AEDs administered alone (i.e., PB, PHT and PGB) were expressed as their median effective doses (ED_{50} values ± S.E.M.) that protected 50% of the mice tested from MES-induced seizures, as described earlier (Kondrat-Wrobel and Luszczki, 2016; Luszczki, 2016; Zolkowska et al., 2016). To determine the ED_{50} values of the studied AEDs, the drugs were administered i.p. at the following doses: PB – 20, 25, 30 mg/kg; PHT – 8, 10, 12, 14 mg/kg; and PGB – 50, 75, 100, 150 mg/kg. The anticonvulsant activity of the mixture of PB, PHT and PGB at the fixed drug-dose ratio combination of 1:1:1 was expressed as its experimental median effective dose ($ED_{50\ exp}$ value ± S.E.M.) against MES-induced seizures, as described earlier (Kondrat-Wrobel and Luszczki, 2016; Luszczki, 2016; Zolkowska et al., 2016). After documenting the response of animals to the electrically-induced seizures, the animals underwent euthanasia with carbon dioxide, as recommended elsewhere (AVMA, 2013).

2.3. Isobolographic analysis

The type of interaction among PB, PHT and PGB in combination at the fixed-ratio of 1:1:1 was assessed by the use of type I isobolographic analysis, as described earlier (Kondrat-Wrobel and Luszczki, 2016; Luszczki, 2016; Zolkowska et al., 2016). Percentages of the mice showing protection from MES-induced seizures per doses of each AED administered singly or doses of the mixture of three AEDs (PB, PHT and PGB) were analyzed with log-probit linear regression in the mouse MES model (Litchfield and Wilcoxon, 1949). Test for parallelism of three AEDs when used alone was performed in strict accordance with the log-probit method described by Litchfield and Wilcoxon (Litchfield and Wilcoxon, 1949). In this test, we compared slope ratios derived from

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