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Dronedarone (a multichannel blocker) enhances the anticonvulsant potency of lamotrigine, but not that of lacosamide, pregabalin and topiramate in the tonic-clonic seizure model in mice

Katarzyna M. Sawicka[a,b], Magdalena Florek-Łuszczki[c], Agnieszka Wawryniuk[a], Jadwiga Daniluk[a], Paula Wróblewska-Łuczka[b], Jarosław Chmielewski[d], Sławomir Karwan[e], Jarogniew J. Łuszczki[b,*]

[a] Department of Internal Medicine in Nursing, Medical University of Lublin, Lublin, Poland
[b] Department of Pathophysiology, Medical University of Lublin, Lublin, Poland
[c] Department of Medical Anthropology, Institute of Rural Health, Lublin, Poland
[d] Institute of Environmental Protection - National Research Institute in Warsaw, Poland
[e] Regional Specialized Children's Hospital, Olsztyn, Poland

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ABSTRACT

Accumulating experimental evidence indicates that some recently licensed antiarrhythmic drugs, including dronedarone (a multichannel blocker) play a crucial role in initiation of seizures in both, in vivo and in vitro studies. Some of these antiarrhythmic drugs elevate the threshold for maximal electroconvulsions and enhance the anticonvulsant potency of classical antiepileptic drugs in preclinical studies.

This study was aimed at determining the influence of dronedarone (an antiarrhythmic drug) on the anticonvulsant potency of four novel antiepileptic drugs (lacosamide, lamotrigine, pregabalin and topiramate) in the maximal electroshock-induced seizure model in mice. To exclude any potential pharmacokinetic contribution of dronedarone to the observed interactions, total brain concentrations of antiepileptic drugs were measured.

Dronedarone (50 mg/kg, i.p.) significantly enhanced the anticonvulsant potency of lamotrigine, by reducing its ED_{50} value from 7.67 mg/kg to 4.19 mg/kg ($P < 0.05$), in the maximal electroshock-induced seizure test in mice. On the contrary, dronedarone (50 mg/kg, i.p.) did not affect the anticonvulsant properties of lacosamide, pregabalin or topiramate in the maximal electroshock-induced seizure test in mice. Measurement of total brain concentrations of lamotrigine revealed that dronedarone did not significantly alter total brain concentrations of lamotrigine in experimental animals. Additionally, the combination of dronedarone with pregabalin significantly impaired motor coordination in animals subjected to the chimney test. In contrast, the combinations of other studied antiepileptic drugs with dronedarone had no negative influence on motor coordination in mice.

It is advisable to combine dronedarone with lamotrigine to enhance the anticonvulsant potency of the latter drug. The combinations of dronedarone with lacosamide, pregabalin and topiramate resulted in neutral interactions in the maximal electroshock-induced seizure test in mice. However, a special caution is advised to patients receiving both, pregabalin and dronedarone due to some possible adverse effects that might occur with respect to motor coordination.

1. Introduction

Accumulating experimental evidence indicates that some antiarrhythmic drugs, owing to their pharmacological properties related with blockade of calcium, sodium and/or potassium channels in neurons, can play an important role during epileptogenesis, especially, with respect to suppression of seizures in preclinical studies on animals (Banach et al., 2016, 2017, 2018; Borowicz-Reutt et al., 2016; Sawicka et al., 2017a, 2017b, 2019).

To the best of our knowledge, epileptogenesis involves numbers of pathophysiological processes that transform a normal and healthy brain tissue into the epileptic one (Clossen and Reddy, 2017). Pathological mechanisms associated with seizure initiation, propagation and amplification involve some irreversible changes in neuronal membrane

* Corresponding author at: Department of Pathophysiology, Medical University, Jaczewskiego 8b, PL 20-090 Lublin, Poland.
E-mail addresses: jluszczki@yahoo.com, jarogniew.luszczki@gmail.com, jarogniew.luszczki@umlub.pl (J.J. Łuszczki).

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potentials (Derera et al., 2017). Deficits in ion channels functioning and improper action of these channels may consequently lead to the appearance of seizures (Navidhamidi et al., 2017). When seizures occur, elimination of pathophysiological processes is not sufficient to restore the normal brain functioning (Curia et al., 2014). Damage evoked by seizures in neuronal network in the brain is permanent (Clossen and Reddy, 2017).

Generally, it is widely accepted that seizures, which occurred in the brain transform and modify a normal neuronal network into pathological circuits and pathways, which can spontaneously trigger seizures by themselves (Avoli et al., 2002). Pathophysiological changes modify the brain structure predisposing neuronal tissue to trigger seizures, even spontaneously (Vismer et al., 2015).

Due to advanced knowledge about epileptogenesis, that increasingly developed in the last two decades, researchers and scientists can, at least in part, modulate and modify this process and control seizures (Clossen and Reddy, 2017). On the other hand, in spite of several currently available antiepileptic drugs (AEDs), there are still some seizure attacks that are refractory to the standard therapy (Zaccara et al., 2019). Considering the facts that seizures result from imbalance in ion channels functioning, as well as that drugs affecting ion channels contribute to seizure suppression, it was of importance to find out whether drugs affecting ion channels that belong to the group of antiarrhythmic drugs exert anticonvulsant action in preclinical studies. Besides, one of the AED – phenytoin (PHT) is also an antiarrhythmic drug that is clinically used to restore normal cardiac rhythm in patients (Ishizue et al., 2016). Moreover, experimental studies indicate that some novel antiarrhythmic drugs (i.e., ivabradine and dronedarone) elevated the threshold for electrically-induced seizures in preclinical studies (Sawicka et al., 2017a, 2017b, 2019). Also some antiarrhythmic drugs (sotalol, propafenone, mexiletine, and amiodarone) exerted the anticonvulsant action in the model of tonic-clonic seizures in mice (Banach et al., 2016, 2017, 2018; Borowicz-Reutt et al., 2016).

Taking into account all mentioned suggestions, it was interesting to investigate whether dronedarone (a novel drug with antiarrhythmic properties that blocks various ion channels) affects the anticonvulsant properties of novel AEDs in mice. Previously, it has been documented that dronedarone reduced the anticonvulsant potency of PHT in the tonic-clonic seizure model (Sawicka et al., 2019). The multichannel blocker had simultaneously no impact on the anticonvulsant potency of phenobarbital (PB), carbamazepine (CBZ) and valproate (VPA) in mice subjected to the maximal electroshock-induced seizure test (Sawicka et al., 2019).

In this study, we selected lacosamide (LCM), lamotrigine (LTG), pregabalin (PGB) and topiramate (TPM) – four novel AEDs which belong to the second- and third-generation AEDs. The choice of AEDs in this study was not serendipitous because all the four AEDs not only suppressed tonic-clonic seizures in preclinical studies, but also the AEDs possess various molecular mechanisms of action and, what is more important, the AEDs suppress tonic-clonic seizures and partial convulsions in epileptic patients (NICE guidelines, 2012). At present, LTG, LCM, PGB and TPM are prescribed as the monotherapy and/or add-on therapy for epileptic patients with generalized tonic-clonic seizures (NICE guidelines, 2012). All these AEDs seem to be efficacious in clinical conditions in patients with tonic-clonic seizures because they suppress these types of seizures offering the patients a substantial reduction in seizure activity.

Up-to-date, a clinical interest of application of antiarrhythmic drugs in epileptic patients increasingly raised because of occurrence of sudden unexpected death in epilepsy (SUDEP) (Massey et al., 2014). The main cause of death of patients with epilepsy is highly likely related to heart problems and arrhythmias that may appear in epileptic patients (Massey et al., 2014). To prevent such problems, doctors prescribe antiarrhythmic drugs, which by affecting ion channels also influence seizure-related phenomena in neuronal tissue of the patients' epileptic brain.

Therefore, the aim of this study was to continue our previous experiments and to determine the influence of dronedarone on the anticonvulsant action of four novel AEDs (i.e., LCM, LTG, PGB and TPM) in the tonic-clonic seizure model in mice. After evaluating the anticonvulsant potencies of novel AEDs in the mouse model of tonic-clonic seizures, acute adverse effects produced by dronedarone in combination with four novel AEDs were assessed in 3 various behavioral tests (including, the chimney, passive avoidance and grip-strength tests). To confirm the nature of interaction between dronedarone and AEDs, pharmacokinetic evaluation of total brain AED concentrations was assessed with high-pressure liquid chromatography (HPLC). Lack of any pharmacokinetic interactions between drugs allows us to indirectly confirm the pharmacodynamic characteristics of interactions.

2. Materials and methods

2.1. Animals

Adult male Albino Swiss mice (8-weeks old, weighing 22–26 g), after 1-week adaptation to laboratory conditions, were randomly assigned to experimental groups, each consisting of 8 mice. All experiments involving animals were approved by the Local Ethics Committee and complied with the ARRIVE guidelines and EU Directive 2010/63/EU for animal experiments. Total number of mice used in this study was 352.

2.2. Drugs

Dronedarone (Multaq®, Sanofi-Aventis, Paris, France), LCM (Vimpat®, UCB Pharma, Brussels, Belgium), PGB (Lyrica®, Pfizer Ltd., Sandwich, Kent, UK), LTG (Lamictal®, Glaxo Wellcome, Greenford, Middlesex, UK) and TPM (Topamax®, Cilag AG, Schaffhausen, Switzerland) were suspended in a 1% aqueous solution of Tween 80 (Sigma-Aldrich). All drugs were administered intraperitoneally (i.p.) as follows: PGB and dronedarone – 120 min, LTG and TPM – 60 min, LCM – 30 min before electroconvulsions and behavioral tests. The pretreatment times and route of i.p. administration were based upon information from experimental studies published elsewhere (Sawicka et al., 2019, 2017b). To avoid pharmaceutical interaction that would occur when combining the drugs in one syringe, dronedarone and one of the AEDs were administered separately, as two independent injections.

2.3. Maximal electroshock seizure test

Maximal electroshock (seizure activity) was evoked in experimental animals by a current (sine-wave, 25 mA, 50 Hz, 500 V, stimulus duration 0.2 s) delivered via ear-clip electrodes from a rodent shocker generator (Hugo Sachs Elektronik, Freiburg, Germany). The protective activity of the studied AEDs against maximal electroshock-induced seizures was determined by means of log-probit method (Litchfield and Wilcoxon, 1949), allowing for calculating median effective dose (ED_{50}) values for the AEDs administered either singly or in combination with dronedarone, as published elsewhere (Kondrat-Wróbel and Łuszczki, 2016; Zolkowska et al., 2016). Dronedarone was applied at a maximal dose of 50 mg/kg that by itself did not significantly affect the threshold for maximal electroconvulsions in mice (Sawicka et al., 2019). Total number of animals used in this procedure was 256.

2.4. Measurement of total brain AED concentrations

Total brain concentrations of lamotrigine were measured in mice receiving the combination of dronedarone (50 mg/kg) with lamotrigine at a dose corresponding to the ED_{50} value of the AED from the maximal electroshock-induced seizure test. Total brain concentrations of lamotrigine were analyzed by HPLC, as described in details earlier (Luszczki et al., 2013), and were expressed in μg/ml of brain supernatants as

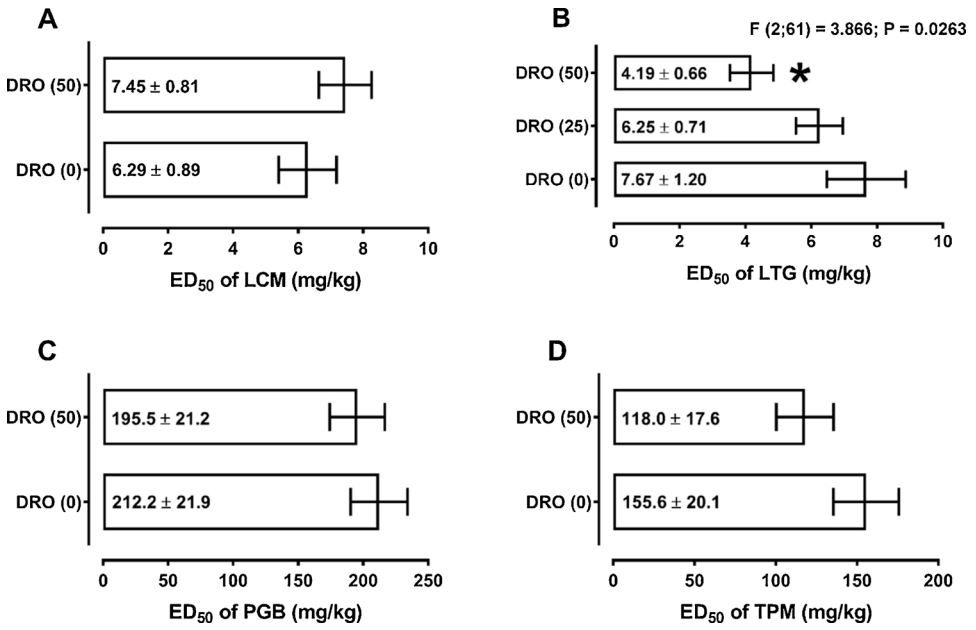

Fig. 1. A–D. Effect of dronedarone (DRO) on the protective activity of various novel antiepileptic drugs against MES-induced seizures in mice.
Columns represent median effective doses (ED_{50} in mg/kg ± S.E.M.) of antiepileptic drugs, protecting 50% of animals tested against MES-induced seizures in mice. All antiepileptic drugs were administered i.p.: pregabalin (PGB) – 120 min., lamotrigine (LTG) and topiramate (TPM) – 60 min., and lacosamide (LCM) – 30 min. prior to the MES-induced seizure test. Dronedarone was administered i.p. at 120 min. before the MES test. Statistical analysis of data was performed with log-probit method and one-way ANOVA followed by the post-hoc Tukey-Kramer test for multiple comparisons. n – total number of animals used at those doses whose anticonvulsant effects ranged between 4th and 6th probit. *$P < 0.05$ vs. control (antiepileptic drug + vehicle-treated [DRO (0)]) animals.

means ± S.E.M. of 8 separate brain preparations. Total number of animals used in this procedure was 16.

2.5. Grip-strength test

Skeletal muscular strength of forelegs in mice receiving dronedarone alone, AEDs alone and their combinations (in doses reflecting their ED_{50} values from the MES-induced seizure test) was determined with the grip-strength test, as described elsewhere (Meyer et al., 1979; Zadrozniak et al., 2009). Skeletal muscular strength in experimental animals was expressed in newton (N) as means ± S.E.M. of 8 determinations. Total number of mice used in this procedure was 80.

2.6. Chimney test

Motor coordination in mice receiving dronedarone alone, AEDs alone and their combinations (in doses reflecting their ED_{50} values from the MES-induced seizure test) was determined with the chimney test, as described elsewhere (Boissier et al., 1960). Motor coordination in experimental animals was expressed as a number of animals with impairment of motor coordination per total number of animals in experimental group (n = 8). Total number of mice used in this procedure was 80.

2.7. Step-through passive avoidance task

Long-term memory (acquisition, learning and remembering) in mice receiving dronedarone alone, AEDs alone and their combinations (in doses reflecting their ED_{50} values from the MES-induced seizure test) was determined with the passive avoidance task, as described in details elsewhere (Luszczki et al., 2005; Venault et al., 1986). Long-term memory in mice was expressed as median retention times (in seconds with 25th and 75th percentiles) of 8 determinations indicated. Total number of mice used in this experimental procedure was 80.

2.8. Statistical analysis

The experimentally-derived ED_{50} values (± S.E.M.) for AEDs administered alone and in combination with dronedarone were calculated from log-probit equations, determined according to the method described by Litchfield and Wilcoxon (Litchfield and Wilcoxon, 1949). Single comparisons between two ED_{50} values (± S.E.M.) were statistically analyzed with log-probit method (Litchfield and Wilcoxon, 1949). Multiple comparisons among three ED_{50} values (± S.E.M.) were performed with one-way analysis of variance (ANOVA) followed by the *post-hoc* Tukey-Kramer test. Total brain concentrations of lamotrigine were statistically verified with unpaired Student's *t*-test. Mean muscular strength (± S.E.M.) from the grip-strength test was statistically analyzed with one-way ANOVA. Median retention times (with 25th and 75th percentiles) from the passive avoidance task were statistically verified with non-parametric Kruskal-Wallis test. Number of animals with impaired motor coordination from the chimney test was statistically analyzed with the χ^2 (Chi-squared) test. Statistical significance was established at $P < 0.05$ and GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego, CA, USA) was used as a statistical software.

3. Results

3.1. Effects of dronedarone on the anticonvulsant potency of lacosamide, lamotrigine, pregabalin and topiramate in the MES-induced seizure test in mice

Dronedarone administered i.p., at a maximally tested dose of 50 mg/kg (which was considered a subthreshold dose), significantly enhanced the anticonvulsant action of lamotrigine by reducing its ED_{50} value in the mouse MES test from 7.67 mg/kg to 4.19 mg/kg (by 45%; $P < 0.05$; Fig. 1B). Dronedarone (25 mg/kg) also potentiated the antiseizure effects of lamotrigine in the mouse MES test by decreasing the ED_{50} value of lamotrigine from 7.67 mg/kg to 6.25 mg/kg (by 18%; Fig. 1B), however, the observed change did not reach statistical significance (Fig. 1B). In contrast, dronedarone in a subthreshold dose of 50 mg/kg (i.p.) had no significant impact on the anticonvulsant potency of lacosamide, pregabalin or topiramate in the MES-induced seizure test in mice (Fig. 1A–D).

3.2. Effect of dronedarone on total brain antiepileptic drug concentrations

With HPLC, total brain concentrations in animals receiving lamotrigine alone did not significantly differ from those assessed for lamotrigine combined with dronedarone (50 mg/kg) (Fig. 2). The Student's *t*-test revealed no significance between the analyzed brain content of lamotrigine in experimental animals (Fig. 2).

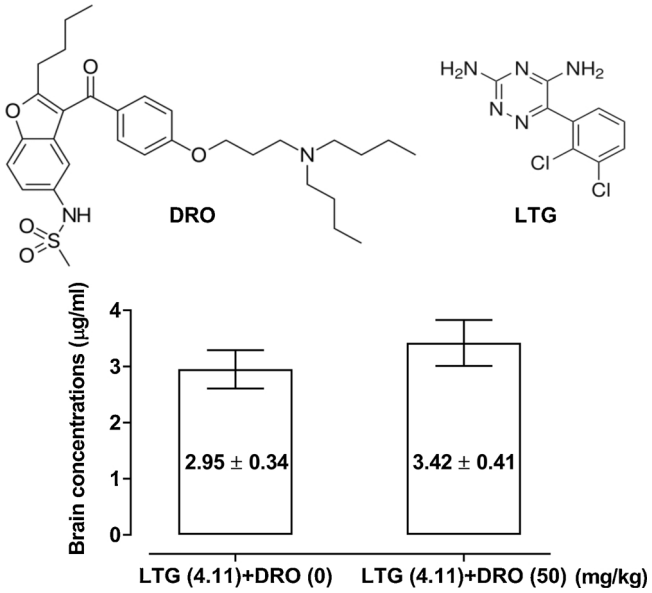

Fig. 2. Influence of dronedarone (DRO) on total brain concentrations of lamotrigine (LTG) in the tonic-clonic seizure test in mice.
Columns illustrate total brain concentrations (means ± S.E.M. of 8 separate determinations) of lamotrigine. The unpaired Student's *t*-test was used to statistically analyze data.

3.3. Effects of dronedarone alone, the studied antiepileptic drugs alone and their combination on muscular strength, motor coordination and long-term memory in mice

When administered singly, the four studied AEDs (LCM, LTG, PGB and TPM) and dronedarone neither significantly affected skeletal muscular strength, nor long-term memory in experimental animals (Table 1). Similarly, the combinations of dronedarone (50 mg/kg) with the studied AEDs (at doses corresponding to their ED_{50} values from the MES-induced seizure test) did not significantly impair skeletal muscular strength and long-term memory in mice (Table 1). In case of the chimney test and assessment of motor coordination in experimental animals, it was found that pregabalin (195.5 mg/kg) in combination with dronedarone (50 mg/kg) considerably disturbed motor coordination in mice ($P < 0.05$), as compared to the control group (vehicle-treated animals; Table 1). Pregabalin (195.5 mg/kg) administered alone also slightly impaired motor coordination in the mice (Table 1). However, in this case, statistical analysis of data with chi-squared test revealed no statistical significance. The other AEDs (LCM, LTG and TPM) tested either alone or in combination with dronedarone did not affect motor coordination in mice subjected to the chimney test (Table 1).

4. Discussion

Results presented in this study indicate that dronedarone (administered at a subthreshold dose of 50 mg/kg) significantly potentiated the anticonvulsant action of LTG, but not that of LCM, PGB and TPM in the mouse model of tonic-clonic seizures. Considering molecular mechanisms of action of the studied second- and third-generation AEDs, it is very likely that dronedarone through the action on various channel types in neurons, contributes to the potentiation of the antiseizure activity of LTG. So, dronedarone potentiates the effects of LTG by interacting probably with sodium and/or calcium channels. On the other hand, dronedarone neither potentiated the antiseizure action of PGB with its main influence on alpha2delta subunit of calcium channels (Calandre et al., 2016; Rogawski and Bazil, 2008), nor that of TPM with its action on sodium and calcium channels, $GABA_A$, NMDA and AMPA receptors (Angehagen et al., 2004; Braga et al., 2009; Gibbs et al., 2000; Gryder and Rogawski, 2003; Herrero et al., 2002; Motaghinejad et al., 2017). Additionally, it was found that dronedarone partially alleviated the anticonvulsant activity of LCM with its action on low-potential sodium channels (Abdelsayed and Sokolov, 2013; Biton et al., 2015; Errington et al., 2006, 2008). As reported earlier, the multichannel blocker dronedarone significantly reduced the anticonvulsant action of PHT in mice challenged with the tonic-clonic seizures (Sawicka et al., 2019). More specifically, it was observed that

Table 1
Influence of dronedarone (DRO) alone and its combination with AEDs on skeletal muscular strength, long-term memory and motor coordination in mice.

Treatment (mg/kg)	Muscular strength (N)	Retention time (s)	Motor coordination deficits
[a]Vehicle + vehicle	0.979 ± 0.046	180 (180; 180)	0/8
[b]DRO (50) + vehicle	0.982 ± 0.038	180 (172.5; 180)	0/8
LCM (7.45) + vehicle	0.978 ± 0.052	180 (177.3; 180)	0/8
DRO (50) + LCM (7.45)	0.948 ± 0.060	180 (160; 180)	0/8
Statistics	F (3;28) = 0.103; P = 0.958	KW = 1.750; P = 0.626	–
[a]Vehicle + vehicle	0.979 ± 0.046	180 (180; 180)	0/8
[b]DRO (50) + vehicle	0.982 ± 0.038	180 (172.5; 180)	0/8
LTG (4.19) + vehicle	0.946 ± 0.054	180 (180; 180)	0/8
DRO (50) + LTG (4.19)	0.968 ± 0.041	180 (176.3; 180)	0/8
Statistics	F (3;28) = 0.131; P = 0.941	KW = 1.008; P = 0.800	–
[a]Vehicle + vehicle	0.979 ± 0.046	180 (180; 180)	0/8
[b]DRO (50) + vehicle	0.982 ± 0.038	180 (172.5; 180)	0/8
PGB (195.5) + vehicle	0.934 ± 0.056	180 (152.5; 180)	3/8
DRO (50) + PGB (195.5)	0.946 ± 0.061	180 (151.8; 180)	4/8 *
Statistics	F (3;28) = 0.220; P = 0.882	KW = 2.149; P = 0.542	χ^2 = 9.326; df = 3; P = 0.025
[a]Vehicle + vehicle	0.979 ± 0.046	180 (180; 180)	0/8
[b]DRO (50) + vehicle	0.982 ± 0.038	180 (172.5; 180)	0/8
TPM (118.0) + vehicle	0.961 ± 0.064	180 (180; 180)	0/8
DRO (50) + TPM (118.0)	0.948 ± 0.044	177.5 (147.2; 180)	1/8
Statistics	F (3;28) = 0.106; P = 0.956	KW = 4.676; P = 0.197	χ^2 = 3.097; df = 3; P = 0.377

Column represents doses of the studied drugs corresponding to the ED_{50} values of novel AEDs from the tonic-clonic seizure model. Results are presented as: mean muscular strengths (in newtons [N] ± S.E.M.) in mice from the grip-strength test; median retention times (with 25th and 75th percentiles in parentheses) of the mice from the passive avoidance task; and number of animals with impairment of motor coordination / per total number of animals in each experimental group, from the chimney test, respectively. *P < 0.05 vs. the control (vehicle + vehicle-treated) group.

[a] Results from the control are identical because one control group of animals was tested in this study.

[b] Results from the DRO (50) + vehicle are identical because only one group of animals receiving dronedarone (50 mg/kg) + vehicle was tested in this study.

dronedarone considerably elevated the ED_{50} value of PHT in the mouse maximal electroshock-induced seizures, but dronedarone did not significantly alter the anticonvulsant action of CBZ, whose molecular mechanisms of action are also associated with blockade of voltage-gated sodium channels in neurons (Sawicka et al., 2019). Thus, it can be suggested that dronedarone-mediated effects through the blockade of sodium and calcium channels interfere with effects evoked by LTG, PHT and partly, by LCM. It can be hypothesized that dronedarone disturbs conductance and/or ion balance in neurons, making PHT and LCM incapable of exerting their anticonvulsant effects. However, a competition of dronedarone and PHT or LCM to bind to sodium channels should be also taken into consideration while explaining the observed effects in animals. Simultaneously, dronedarone can enhance neuronal conductance thereby sodium and/or calcium channels that finally contribute to the potentiation of the anticonvulsant action of LTG in mice challenged with the maximal electroshock-induced seizure test. Moreover, as reported earlier, dronedarone did not enhance the anticonvulsant action of CBZ, PB and VPA (Sawicka et al., 2019). At present, it is not clear why dronedarone selectively potentiated the antielectroshock effects of LTG, significantly reduced that of PHT, partially diminished that of LCM and had no impact on the antiseizure effects of CBZ, PGB, PB, VPA and TPM in the mouse tonic-clonic seizure model. More advanced neurochemical and biochemical studies are required to fully explain this phenomenon in the context of molecular mechanisms of action of these AEDs and their effects on sodium and calcium channels.

Relatively recently, some cardiac antiarrhythmic drugs, used clinically in patients with heart diseases, were investigated in terms of their influence on the anticonvulsant properties of AEDs in experimental models of epilepsy to find out the most promising cardiac drugs that could potentiate the anticonvulsant effects of the AEDs in the mouse tonic-clonic seizure model. Of note, dronedarone can be used as an alternative drug to amiodarone because both drugs belong to the same antiarrhythmic group of drugs (Camm and Savelieva, 2008; Deneer and van Hemel, 2011; Manakshe and Rao, 2012; Saklani and Skanes, 2012). This is the reason that dronedarone can be preferentially used in patients with heart problems, who could not be treated with amiodarone because of its severe adverse effects that could negatively influence the patients' quality of living. On the other hand, experimental evidence indicates that amiodarone potentiated the anticonvulsant action of CBZ, but not that of PB, PHT or VPA (Banach et al., 2018). In contrast, dronedarone alleviated the anti-electroshock potency of PHT, but not that of CBZ, PB or VPA. Comparing the results exerted by these two antiarrhythmic drugs on AEDs, the preclinical profile of both antiarrhythmic drugs (amiodarone and dronedarone) considerably differs, when combined with AEDs in the mouse model of tonic-clonic seizures. Unfortunately, experimental data reporting interactions between amiodarone and novel AEDs (including, LTG, LCM, PGB and TPM) in the mouse maximal electroshock-induced seizure model, do not exist, as yet. Therefore, we cannot compare our results with those obtained for amiodarone. Since the influence of dronedarone and amiodarone considerably differed, when comparing their effects on classical AEDs (CBZ, PHT, PB and VPA), a similar difference can be expected for interactions of both antiarrhythmic drugs with novel AEDs in the mouse maximal electroshock-induced seizure model.

Noticeably, the pretreatment times of AEDs and dronedarone had no impact on the observed anticonvulsant effects of AEDs in this study. Both, PHT and dronedarone were administered at 120 min before the maximal electroshock-induced seizure test (Sawicka et al., 2019). LTG was administered at 60 min, whereas CBZ and LCM at 30 min before maximal electroconvulsions (Sawicka et al., 2019). So, it was impossible that dronedarone, when administered 60 min. before LTG, significantly potentiated the anticonvulsant effect of the latter drug and simultaneously, when administered 90 min. before CBZ and LCM, it had no effect or partially reduced the anticonvulsant potency of CBZ and LCM, respectively. Evidently, PGB was administered at 120 min. before the maximal electroshock-induced seizure test and dronedarone had no impact on the antiseizure action of the later AED. In contrast, dronedarone significantly reduced the anticonvulsant action of PHT that was administered i.p. at 120 min prior to the MES test. Simultaneously, dronedarone considerably potentiated the antiseizure action of LTG (administered 60 min. before the maximal electroshock-induced seizure test), but not that of PB and TPM, two AEDs administered i.p. at the same pretreatment time (60 min.) before the maximal electroshock-induced seizure test. Thus, it seems that the pretreatment times of AEDs and dronedarone had no correlation with the anticonvulsant action of the classical and novel AEDs. Taking into account all mentioned facts, it was impossible to correlate the pretreatment times with effects exerted by dronedarone on AEDs in experimental animals. Of note, all pretreatment times described in this study were considered to be the times required for AEDs that could reach maximal anticonvulsant effects in animals. These times were experimentally confirmed in earlier studies and were considered as times to peak anticonvulsant effects in animals.

In this study, a pharmacokinetic profile of LTG when combined with dronedarone was also evaluated. It was reported that dronedarone (administered i.p., 120 min. before the brain tissue sampling for the estimation of LTG content in experimental animals), did not affect total brain concentration of LTG (administered i.p., at 60 min. prior to the collection of animals' brains). Thus, it can be stated that the interaction between dronedarone and LTG was pharmacodynamic. Of note, in our pharmacokinetic study we evaluated only total brain concentrations of LTG because dronedarone significantly potentiated only the anticonvulsant action of LTG in experimental animals. Since dronedarone did not significantly affect the anticonvulsant potency of LCM, TPM or PGB, their total brain concentrations were not estimated in this study. We are aware of the fact that if the final effects produced by a drug combination are additive, they could result from pharmacodynamic, pharmacokinetic or both types of interactions. Lack of pharmacokinetic interactions between drugs indicates pharmacodynamic nature of interaction. However, some pharmacokinetic changes (increase or decrease) in AED content may counteract pharmacodynamic (antagonistic or synergistic) interaction resulting finally in additivity. In such a situation, the final effect (even though is entirely or partially masked by pharmacokinetic interactions occurring between drugs), is still additive. In this study pharmacokinetic estimation of total brain AEDs concentrations was performed in strict accordance with ARRIVE guidelines related with the rule 3R (reduction, refinement and replacement). Following this rule, we were obliged to reduce the number of animals used to the smallest one, but allowing us to obtain the most scientifically reliable data. This was the main reason why we did not estimate total brain concentrations of the other AEDs in this study.

Evaluation of acute adverse effect profile in experimental animals revealed that dronedarone had no significant impact on skeletal muscular strength in the animals receiving combinations of dronedarone and AEDs. Similarly, dronedarone did not significantly disturb long-term memory in mice subjected to the passive avoidance task after receiving LCM, LTG, PGB and TPM. In the chimney test, it was reported that only PGB (when administered alone and in combination with dronedarone) significantly impaired motor performance in mice. For the combination of PGB and dronedarone, 4 out of 8 mice were unable to climb backward up a plastic tube within 1 min. PGB administered alone produced also a slight, but non-significant impairment in motor coordination (3 out of 8 mice displayed motor deficits). Considering the facts that motor impairment in mice was observed only in the animals that received PGB alone and PGB with dronedarone, it can be ascertained that the observed side effects in the chimney test were associated with application of PGB, but not with dronedarone. Moreover, dronedarone (in the same dose of 50 mg/kg) co-administered with other AEDs had no impact on motor coordination in mice receiving LCM, LTG and TPM. So, it can be unequivocally ascertained that the observed deficits in locomotor function in experimental animals were exclusively ascribed to PGB application.

Another fact needs a short explanation because in this study we used the chi-squared test instead of the Fisher's exact probability test to analyze data from the chimney test. Previously, statistical analysis of data from the chimney test was performed with independent comparisons of the results to the control value. However, in this study, we also compared the results from the dronedarone- and AED + dronedarone–treated animals to the control value as well as we tend to determine whether the observed effects in the chimney test resulted from some specific conditions related with the application of AEDs. This is why, we statistically compared the results from 4 groups, namely, the control (vehicle-treated), dronedarone-alone, AED-alone and AED + dronedarone-treated animals. By analyzing the data with the contingency table (4 × 2), we obtained significant difference for PGB + dronedarone-treated animals, as compared to both, the control and dronedarone-alone-treated animals. Moreover, statistical comparison with chi-squared test revealed that the significant impairment in motor coordination resulted from the PGB application. Of note, with the Fisher's exact probability test, one could assess differences only between two groups, i.e., the control group and/or each of the tested groups of animals separately.

It is important to note that only motor coordination deficits were observed in mice receiving PGB with dronedarone. No side effects were observed in the passive avoidance task or grip-strength test in mice, receiving the same combination of PGB with dronedarone. It should be mentioned that PGB at a dose of 190 mg/kg, administered i.p. alone or in combination with dronedarone did not impair long-term memory in mice subjected to the passive avoidance task. As reported earlier, PGB in doses ranged between 50–200 mg/kg elevated the threshold for the first pain reaction in animals subjected to the hot-plate test (Luszczki, 2010). Since PGB possesses the antinociceptive properties, it was of importance to change experimental conditions in the passive avoidance task in order to adequately evoke the first pain reaction (aversive stimulus) in animals. Generally, the passive avoidance task is based on the observation that animals remembered situation, which is closely associated with aversive and painful condition. In the passive avoidance task, the animals remember not to entry the dark box of the apparatus on the next day (24 h later) of experimentation, because they received an aversive (painful) stimulus after crossing the door between the dark and light compartments of the apparatus during the first day of testing session (Venault et al., 1986). The electrical stimulus delivered via grid floor during the first session allows the mice to immediately acquire information and remember the painful condition in the dark box. In other words, after receiving painful (aversive) stimulus in the passive avoidance task, the animals remember (even 24 h later) not to enter the dark box. In the case of drugs that produce antinociceptive effects, including tiagabine, vigabatrin and PGB, we were obliged to increase the current strength (up to 6 mA) to properly generate and evoke aversive stimulus in the mice. As reported earlier, PGB, tiagabine and vigabatrin increased the threshold for the first pain reaction in mice (Luszczki, 2010; Luszczki et al., 2003, 2005). Owing to antinociceptive properties of PGB, it was reasonable to elevate the current strength delivered via grid floor in the passive avoidance task. Otherwise, the current strength would be weak enough and the animals could not feel this aversive stimulus in the passive avoidance task and could not remember to avoid the entrance into the dark box in this task on the next day. Obviously, the animals would not remember to avoid the entrance into the dark box if they did not perceive the painful action on the animals' behavior. When translating the results from this passive avoidance task to clinical conditions, a quite similar lack of acute adverse effects is expected in humans receiving the combination of AEDs with dronedarone.

Summing up, dronedarone can be used by patients suffering from tonic-clonic seizures and treated with LTG, as well as, receiving the antiarrhythmic medication. This favorable combination is worthy of recommendation to further clinical practice. In case of the combinations of dronedarone with other AEDs belonging to the second- and third-generations, the combined application of dronedarone with LCM, PGB or TPM should be neutral from a clinical viewpoint. A special caution is advised for patients receiving PGB with dronedarone because of some side effects related with impairment of motor coordination, which might appear during the combined treatment with these two drugs.

Disclosure of conflictsof interest

Prof. JJ Luszczki has been involved in the design and development of new AEDs and CNS drugs. The author have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. The remaining authors have no conflict of interest to disclose.

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