Amiodarone, a multi-channel blocker, enhances anticonvulsive effect of carbamazepine in the mouse maximal electroshock model

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ABSTRACT

Cardiac arrhythmia may occur in the course of epilepsy. Simultaneous therapy of the two diseases might be complicated by drug interactions since antiarrhythmic and antiepileptic agents share some molecular targets. The aim of this study was to evaluate the influence of amiodarone, an antiarrhythmic drug working as a multi-channel blocker, on the protective activity of four classical antiepileptic drugs in the maximal electroshock test in mice. Amiodarone at doses up to 75 mg/kg did not affect the electroconvulsive threshold in mice. Acute amiodarone at the dose of 75 mg/kg significantly potentiated the anticonvulsive effect of carbamazepine, but not that of valproate, phenytoin or phenobarbital in the maximal electroshock-induced seizures in mice. The anti-arrhythmic agent and its combinations with antiepileptic drugs did not impair motor performance or long-term memory in mice, except for the combination of amiodarone and phenobarbital. Brain concentrations of anti-epileptic drugs were not changed. Despite favourable impact of amiodarone on the anticonvulsive action of carbamazepine in the maximal electroshock, co-administration of the two drugs should be carefully monitored in clinical conditions. Further studies are necessary to evaluate effects of chronic treatment with amiodarone on seizure activity and the action of antiepileptic drugs.

1. Introduction

Amiodarone, primarily used as an antianginal agent, is a well described class III (according to Vaughan Williams classification) antiarrhythmic drug. Both, European Resuscitation Council and American Heart Association guidelines for resuscitation recommend amiodarone for the treatment of ventricular fibrillation (VF) or ventricular tachycardia (VT) (Laina et al., 2016). Additionally, amiodarone is also used in the treatment of supraventricular arrhythmias, especially atrial fibrillation, even in patients with severe heart failure (Connolly, 1999).

Cardiac arrhythmia may affect up to 42% of epileptic patients and is believed to be the most frequent mechanism of sudden unexpected death in epilepsy (SUDEP) (Borowicz and Banach, 2014). Synchronization of cardiac sympathetic and less common vagal discharges with both ictal and interictal spikes on the concurrent electroencephalogram (EEG) was demonstrated in a cat model of seizures induced by pentylenetetrazole (PTZ) (Lathers et al., 1987). Furthermore, cardiac rhythm disturbances were observed during seizure activity in a model of focal epilepsy induced by chemical stimulation in hemispherectomized rats and in amygdala-kindled rats (Goodman et al., 1990; Mameli et al., 1988). These results support theory that seizures activates neurocardiac axis and disrupts autonomic balance, which may be followed by fatal arrhythmia (Davis and Natelson, 1993). In humans, simultaneous recordings of EEG and ECG during episodes of seizures revealed typical onset of tachycardia just before seizures with both atrial and ventricular ectopy. Significant prolongation of QTc interval was reported in patients who died of SUDEP in comparison with other epileptic individuals (Earnest et al., 1990). Prolonged QTc is a well-known risk factor for sudden death and may indicate electrical instability resulting from central nervous system stimulation or dysfunction (Davis and Natelson, 1993). Biet et al. (2015) suggested that alterations in cardiac sodium channels in epileptic patients may be at least partially responsible for conduction anomalies and QT prolongation. Interestingly, SUDEP is relatively uncommon in childhood, while it is more frequent in adults. The most probable reason is shorter epilepsy duration and reduced seizure severity in children. Children and adolescents may be also more resistant to SUDEP. Finally, some other factors are taken into consideration, the most frequently better treatment adherence, closer supervision by parents and better health status and adaptability. In fact, pediatric SUDEP cases are observed mostly in children with developmental delay, genetic disorders and early-onset of severe epilepsy (Donner et al., 2017; Morse and Kothare, 2016).

Numerous previous studies showed interesting interactions between representatives of four classes of antiarrhythmic agents and

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antiepileptic drugs (AEDs) in animal models of seizures. Propafenone (class Ic) potentiated the action of valproate (VPA), carbamazepine (CBZ), phenytoin (PHT) and phenobarbital (PB) in maximal electroshock (MES) test in mice (Banach et al., 2016). Isobolographic analysis of interactions between mexiletine (class Ib) and classic AEDs indicated additivity between mexiletine and CBZ, PHT and PB, whereas in the case of VPA antagonistic interactions were observed (Borowicz-Reutt et al., 2016). Beta-adrenolytics (class II) and calcium channel blockers (class IV) were most thoroughly investigated in various seizure models in rodents (Borowicz and Banach, 2014), albeit the results were inconsistent. Interestingly, sotalol − representative of class III, which acts as a betablocker and potassium channel inhibitor, enhanced the antielectroshock action of VPA and PHT, remaining indifferent to the action of CBZ and PB (Banach et al., 2017).

Pharmacological properties of AMD are characteristic of all four classes of antiarrhythmic drugs. AMD blocks the inward sodium current, noncompetitively inhibits sympathetic stimulation, suppress outward potassium currents: iKr, iKs, iKAch, iKNa, iKATP and calcium influx through voltage-dependent calcium channels (Doggrell, 2001). And consequently, AMD slows conduction and increases refractoriness of the atrioventricular node, increases atrial and ventricular refractoriness, prolongs QTc interval (Connolly, 1999; Campbell and Williams, 2001). Moreover, as a structural analogue of thyroid hormone, AMD may interact with nuclear thyroid hormone receptors (Doggrell, 2001). Chronic AMD was shown to modulate potassium-channel gene expression in rat hearts (Kodama et al., 1999).

Unfortunately, the use of AMD may be complicated by its unusual pharmacokinetic properties and serious undesired effects. In humans, oral administration of AMD is followed by variable bioavailability (20–80%). AMD is metabolized by cytochrome P450 oxidase (CYP3A4)-dependent oxidative de-ethylation to desethylamiodarone (DEA), an active metabolite (Doggrell, 2001). Both AMD and DEA accumulate in fat tissue, nonetheless brain distribution is poor (Latini et al., 1984; Najjar, 2001; Plomp et al., 1987; Plomp et al., 1989). Half-time of elimination is calculated to be 35–40 days, but may exceed 100 days. Therefore, it may take quite a long time (several weeks to months) to achieve tissue saturation and steady-state concentration. Commonly recommended maintenance dose of AMD is 200 mg/day, whereas loading dose varies between 600 and 2000 mg/day (Connolly, 1999). Therapeutic drug monitoring has limited application for AMD, since it is based on the concentration of the parent drug without its metabolite (DEA) and the correlation between antiarrhythmic effect or toxicity and drug concentration is scant. Putative therapeutic range is 0,5–2,5 μg/ml (Campbell and Williams, 2001).

Acute treatment with AMD may be complicated by sinus bradycardia, atrioventricular nodal blockade, hypotension and phlebitis. However, the hemodynamic effects at least partially may be ascribed to widely used solvent polysorbate 80 (Lindquist et al., 2015). Furthermore, long-term treatment with AMD may result in side effects involving several organs like lungs, thyroid gland, skin, central nervous system and eyes (Goldschlager et al., 2007; Connolly, 1999).

Since AMD is a lipid soluble agent that can cross blood-brain barrier and acts on multiple molecular targets we were tempted to assess its action on seizure phenomena in mice. The aim of the study was to determine the influence of amiodarone on anticonvulsive activity of four classical AEDs: VPA, CBZ, PB and PHT in the maximal electroshock (MES) model of tonic-clonic seizures. To the best of our knowledge this is the first evaluation of amiodarone in electrically-induced seizures in mice.

2. Materials and methods

2.1. Animals

All experiments were carried out on adult female Swiss mice weighing 20–25 g. The animals were housed in colony cages with free access to food (chow pellets) and tap water, under standardized laboratory conditions (temperature 21 ± 1 °C, a natural light-dark cycle). After 7-day acclimatization, the tested groups, consisting of 8–10 mice, were randomly assigned. All procedures were performed between 9.00 a.m. and 3.00 p.m. Each mouse was used only once. The Local Ethical Committee of Lublin Medical University approved all experimental procedures of this study (license No 30/2013). All animal experiments complied EU Directive 2010/63/EU for animal experiments.

2.2. Drugs

Amiodarone (AMD, Opacorden, Polpharma, Starogard Gdański, Poland) and three AEDs: carbamazepine (CBZ, Sigma-Aldrich, St. Louis, MO, USA), phenytoin (PHT, Sigma-Aldrich, St. Louis, MO, USA), phenobarbital (PB, UNIA Pharmaceutical Department, Warszawa, Poland) were suspended in a 1% solution of Tween 80 (Sigma-Aldrich, St. Louis, MO, USA) in distilled water. Valproate (VPA, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in distilled water. Fresh solutions were prepared each day.

All drugs were administered intraperitoneally (*i.p.*) in a volume of 10 ml/kg body weight. Control mice were injected with vehicle. AMD, CBZ and VPA were administered 30 min before experiments, whereas PB and PHT 60 min and 120 min before test, respectively. Doses of AEDs were based on our experimental experiences and calculations of median effective doses made in our previous research (Banach et al., 2016, 2017; Borowicz et al., 2013). In the case of AMD, we translated pharmacological human doses to mice, according to the body surface area-related formula described by Reagan-Shaw et al. (2008). This formula is mainly recommended for appropriate conversion of drug doses from animal to human studies, because it correlates well with several metabolic and pharmacokinetic parameters of both compared species (Reagan-Shaw et al., 2008).

2.3. Electroconvulsive threshold and maximal electroshock seizure test

The electroconvulsive seizure threshold and MES tests are well known models of tonic-clonic seizures. Electrically-induced seizures in rodents are used in the preclinical evaluation of possible anticonvulsant medications (Castel-Branco et al., 2009). Both tests were extensively described by Borowicz et al. (2013).

Tonic hindlimb extension (i.e., the hindlimbs of animals outstretched 180° to the plane of the body axis) was considered as the endpoint. CS_{50} is a current strength (expressed in mA) necessary to induce tonic convulsions in 50% of animals. To estimate the electroconvulsive threshold, at least three groups of mice were challenged with currents of various intensities. An intensity-response curve was calculated on the basis of the percentage of convulsing animals (convulsions in less than 50%, around 50% and more than 50% of mice). In the electroconvulsive threshold test AMD was applied in a dose range of 25–75 mg/kg. The median effective dose (ED_{50}) represents the ability of the drug to protect 50% of animals against maximal electroshock-induced tonic hindlimb extension. To evaluate the ED_{50} (mg/kg) at least three groups of mice received progressive doses of AEDs or their combinations with AMD (dose range 25–75 mg/kg) and were challenged with the MES test. According to Litchfield and Wilcoxon (1949), a dose-response curve was constructed based on the percentage of mice protected (protection in less than 50%, around 50% and more than 50% of mice).

2.4. Chimney test

The effects of acute treatment with conventional AEDs at their ED_{50s}, AMD and its combinations with AEDs on motor performance in mice were determined in the chimney test (Boissier et al., 1960). The procedure was conducted according to the description provided by