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New model of pharmacoresistant seizures induced by 3-mercaptopropionic acid in mice

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ABSTRACT

About 30% of the patients with epilepsy do not respond to clinically established anticonvulsants, despite having effective concentrations of the antiepileptic drug in plasma. Therefore, new preclinical models of epilepsy are needed to identify more efficacious treatments. We describe here a new drug-resistant seizure model in mice to be used at the early stages of pre-clinical trials. This model consists in inducing daily generalized seizures for 23 consecutive days by administration of 3-mercaptopropionic acid (MP). As a result, 100% of animals become resistant to phenytoin and 80% to phenobarbital. Such resistance is strongly associated with the overexpression of P-glycoprotein (Pgp), observed in cerebral cortex, hippocampus and striatum while resistance to Pgp nonsubstrate drugs such as carbamazepine, diazepam and levetiracetam is not observed.

This model could be useful for screening novel anticonvulsant drugs with a potential effect on pharmacoresistant seizures treatment.

1. Introduction

Epilepsy is a chronic neurological disorder characterized by recurrent and spontaneous seizures (Meldrum, 1984). About 30% of the patients with epilepsy do not respond to clinically established anticonvulsants despite effective antiepileptic drug (AED) plasma concentrations, defining a multidrug resistance (MDR) phenotype (Kwan and Brodie, 2000). Therefore, there is a genuine need to incorporate new models of refractory epilepsy (RE) at the preclinical stage of drug development, in order to identify new AEDs that overcome the problem of drug resistance (White, 2003).

The inability of the AEDs to reach their molecular targets and the high frequency and severity of seizures have been proposed as explanations to drug resistant epilepsy (Lazarowski et al., 2007; Potschka, 2010; Rogawski, 2013). Drug concentrations in several tissues (including the brain) are regulated by drug efflux transporters of the ATP-binding cassette (ABC) superfamily, such as P-glycoprotein (Pgp), the multidrug resistance associated proteins (MDRPs), and Breast Cancer Resistance Protein (BCRP) (Kuteykin-Teplyakov et al., 2009). Back in 1995, Tishler observed increased expression of Pgp in drug-resistant patients, suggesting that the loss of response may be caused by limited brain bioavailability of the AEDs (Tishler et al., 1995). Thereafter, several reports indicated high levels of Pgp expression in epileptogenic brain tissue from patients with RE (Sisodiya et al., 2002; Lazarowski et al., 1997; Aronica et al., 2004; Löscher and Potschka, 2005a, b).

A diversity of experimental models of pharmacoresistant epilepsy in rats has demonstrated the inducible Pgp overexpression at the blood brain barrier (BBB) which is associated to the MDR phenotype (Seegers et al., 2002; Rizzi et al., 2002; Jing et al., 2010; Volk and Löscher, 2005; Bankstahl and Löscher, 2008). In these models, Pgp levels in vessel-related cells and neurons correlated with the loss of protective effects of phenytoin (PHT), a proven Pgp substrate (Zhang et al., 2012). These models require several weeks of treatment and only about 40% of the surviving animals become resistant to drugs (Löscher et al., 1993; Löscher, 2011; Potschka, 2012). These

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characteristics make the use of these models difficult at the first stages of the preclinicalal assays of new AEDs due to the high number of animals needed to test a considerable number of candidate drugs.

Brain Pgp expression pattern has previously been studied in a rat model of seizure induced by 3-mercaptopropionic acid (MP) (Lazarowski et al., 2004). MP is a classic seizure-inductor as a consequence of the inhibition of gamma aminobutyric acid (GABA) synthesis. (De Sarro et al., 2003; Giraldez et al., 1999; Giraldez and Girardi, 2000; Girardi et al., 1989, 2004; Sprince et al., 1969). The repetitive seizure activity induced by daily administration of MP in rats during 7 days induces an increase in Pgp expression in capillary endothelial cells of the blood brain barrier, neurons, and glial cells from cortex, striatum, and hippocampus (Lazarowski et al., 2004). This situation is associated with astrocyte hypertrophy, characterized by an increase in number and thickness of branching and in soma size (Girardi et al., 2004) and lower brain penetration of PHT (Hocht et al., 2007).

Although the repetitive administration of MP in rat represents a good experimental model to reproduce pharmacoresistant seizures, an important disadvantage is that experiments in rats require high amount of the experimental drugs.Concerning this issue, mouse represents an attractive cost-efficient model.

The aim of this study was the development of a new model of pharmacoresistant seizures linked to Pgp overexpression induced by repetitive administration of MP in mice. This model could be used to screen for drugs capable of controlling RE in short time-periods.

The model was validated by evaluating the protective effects of standard AEDs: PHT and Phenobarbital (PB), (proven Pgp substrate AEDs (Zhang et al., 2012)) and Carbamazepine (CBZ), Levetiracetam (LEV) and Diazepam (DZP) (Pgp nonsubstrates). Based on the hypothesis that there is an overexpression of Pgp in animals treated with MP, we expect to observe drug resistance to Pgp-substrate AEDs, and a protective response to those AEDs that are not Pgp substrate. The effect of co-administration of nimodipine (NIMO) (a Pgp blocker) with PHT and PB was also studied.

2. Materials and methods

2.1. Animals and drugs

We employed Swiss albino male mice (25–35 g) provided by the Faculty of Veterinary, National University of La Plata. The animals were housed in colonies of ten, under a regime of 12 h light/dark with water and food ad libitum. Every effort was made to minimize animal stress. The animal care for this experimental protocol was conducted in accordance with the NIH guidelines for the Care and Use of Laboratory Animals and it was approved by the Ethical Committe of Exact Sciences Faculty of University of La Plata.

MP was acquired from Merck (Hohenbrunn, Germany). LEV was provided by Glaxosmithline. CBZ and NIMO were a generous gift from Bagó Laboratories. DZP was acquired from Roche, PB from Serva (Heidelberg, Germany) and PHT from Sigma-Aldrich.

PHT was dissolved in saline and the pH = 11.2 was adjusted with NaOH. Carboxymethylcellulose 1% (CMC) was used as vehicle for CBZ, NIMO and PB. Finally, DZP and LEV were dissolved in saline. All AEDs were evaluated at their time of maximum response. All drugs except MP were administered at 10 ml/kg.

The study was not performed blinded to treatment groups. Animals were randomized to treatment.

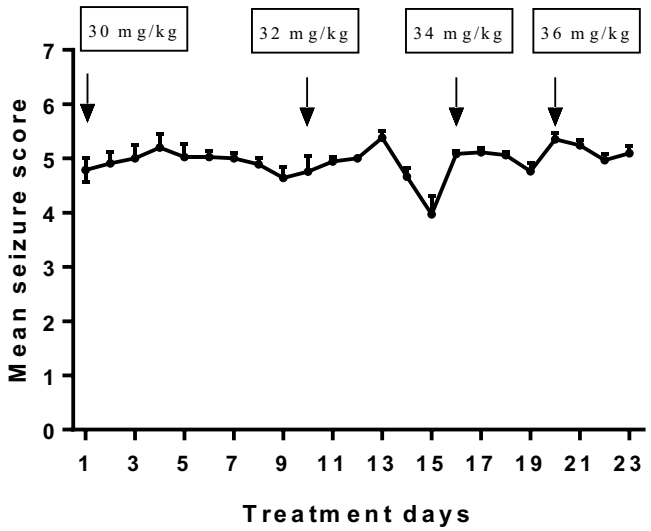

Fig. 1. *MP treatment.* Swiss albino mice were administered once a day with a variable dose of MP from 30 to 36 mg/kg. The dose was increasing according to animal behavior in order to maintain the generalized seizure response. Mean seizure severity score ± SEM vs MP treatment days (n = 47).

2.2. 3-Mercaptopropionic acid (MP) treatment

Initially, mice received a daily administration of saline (0.1 ml intraperitoneal [i.p.]) during 7 days. This procedure allows the animals' habituation to handling, avoiding down regulation of GABA receptors induced by acute handling, which could affect the susceptibility to convulsions (Skilbeck et al., 2010). Thereafter they were daily administered with i.p. MP for 23 consecutive days. MP dose was increased through the treatment, starting from 30 mg/kg on day 1 to 36 mg/kg on day 23 in order to obtain generalized seizures throughout the 23 days (Fig. 1). The MP starting dose (30 mg/kg) is the necessary dose to induce generalized seizures without causing the death of the animals and it was chosen from preliminary dose-response experiments. 23 MP treatment days was the optimal time to avoid PHT anticonvulsant effect. (See Section 2.2.1).

MP was daily prepared in saline (5 ml/kg) and neutralized with trizma base immediately before its administration. After each MP injection, the mice were placed individually in transparent Plexiglass cages and they were observed for 30 min. Seizure activity was classified according to a behavioral scale adapted to the MP induced seizures (Racine, 1972):

0- no response.
1- ear and facial twitching, nodding, piloerection, chewing.
2- myoclonic jerks.
3- forelimb clonus.
4- kangaroo position with bilateral clonic jerks of the forelimbs.
5- generalized clonic seizures with falling.
6- sudden running fits and generalized clonic seizures.
7- generalized tonic-clonic seizures.

2.2.1. Determination of the initial dose of MP and treatment time

To determine initial MP dose, 3 different mice groups (n = 4) were administered with 27, 30 and 33 mg/kg during 4 days. The percentages of animals with generalized seizures and mortality were recorded each day.

MP convulsant dose was administered i.p. once a day for 0, 7, 10, 13, 19 or 23 consecutive days to determine the necessary treatment time to develop PHT resistance. MP19 and MP23 groups were injected from Monday to Friday; the other groups were administered from Monday to Sunday. 24 h after the last administration of MP, PHT 18 mg/kg, i.p., and 2 h later, MP convulsant dose were