Epilepsy Research 134 (2017) 9–15

Contents lists available at ScienceDirect

Epilepsy Research

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

Efficacy of anticonvulsant substances in the 6 Hz seizure test: Comparison of two rodent species

Elise Esneault*, Guillaume Peyon, Vincent Castagné

Porsolt S.A.S., Z.A. de Glatigné, 53940 Le Genest Saint Isle, France

ARTICLE INFO

Keywords:
6 Hz
Epilepsy
Lamotrigine
Levetiracetam
Phenytoin
Rat
Valproate

ABSTRACT

Usually performed in the mouse, the 6 Hz seizure test is used for screening potential new anticonvulsant substances against complex partial seizures. Nevertheless, advanced models of temporal lobe epilepsy (TLE) are more often performed in rats, so that possible species-related differences may complicate the development of anticonvulsant substances. The aim of the present study was to evaluate the feasibility of adapting the 6 Hz test in the rat.

We first compared the effects of increasing current intensities for inducing seizures in the mouse and in the rat. This step was followed by the evaluation of the activity of anticonvulsant substances. Animals received an electrical stimulation with a constant current via corneal electrodes. The seizure was characterized by the presence of forelimb clonus immediately after stimulation. Spontaneous locomotion was evaluated following the 6 Hz test.

In the rat, the forelimb seizure score was intensity-dependently increased and seizures were observed in all animals tested at 44 mA. In the mouse, the seizures were of lower magnitude and they were not observed in all mice stimulated at 44 mA. In both species, levetiracetam (LEV) clearly decreased the forelimb seizure score over the dose-range 100–300 mg/kg without affecting locomotion. Valproate (VPA) displayed anticonvulsant activity at 200 mg/kg and fully protected both species at 300 mg/kg, a dose producing sedative effects in the mouse. Phenytoin (PHT) showed slight to moderate anticonvulsant activity at 100 mg/kg in the mouse and at 60 and 100 mg/kg in the rat without modifying locomotor activity. Lamotrigine (LTG) partially antagonized forelimb seizure at 60 mg/kg in the mouse and at 30–60 mg/kg in the rat, but it induced clear motor impairments at high dose in both species.

Our data suggest that in the 6 Hz test, the magnitude and the nature of seizures differed between the mouse and the rat for a given current intensity. Nevertheless, the pharmacological profile of anticonvulsant substances was similar in both species for the 4 substances tested. Dose-dependent efficacy of LEV and VPA was observed and LTG and PHT also showed anticonvulsant activity, even though the magnitude of the effects remained moderate for these two last substances.

The 6 Hz test in the rat therefore appears as a useful model which may be performed prior to follow-up models of partial seizures performed in the same species.

1. Introduction

In Humans, complex partial seizure is characterized by psychiatric manifestations such as hallucinations associated with the occurrence of complex motor seizure. The 6 Hz test was originally described as a model of complex partial seizure in the mouse by Toman (1951). Its use in drug development remained relatively rare due to its lack of sensitivity to phenytoin (PHT) which was considered to be inconsistent with the clinical efficacy of PHT in the treatment of complex partial seizures (Brown et al., 1953). Several years later, the model was reconsidered due to its sensitivity to levetiractam (LEV) (Barton et al., 2001) whereas this substance was devoid of activity in the traditional acute tests (Maximal Electroconvulsive Shock (MES) and Pentylenetetrazole (PTZ) tests).

The 6 Hz test has now been included in the Anticonvulsant Drug Development (ADD) program recommended by National Institutes of

Abbreviations: ADD, anticonvulsant drug development; AED, antiepileptic drug; g, grams; Hz, hertz; i.p., intraperitoneal; LEV, levetiracetam; LTG, lamotrigine; MES, maximal electroconvulsive shock; NMRI, national marine research institute; PD, pharmacodynamics; PHT, phenytoin; PK, pharmacokinetic; PTZ, pentylenetetrazole; s, second; TLE, temporal lobe epilepsy; VPA, valproate

* Corresponding author.
E-mail address: eesneault@porsolt.com (E. Esneault).

http://dx.doi.org/10.1016/j.eplepsyres.2017.05.002
Received 10 January 2017; Received in revised form 31 March 2017; Accepted 3 May 2017
Available online 10 May 2017
0920-1211/

Health (NIH) in United States. It is considered as a screening tool to evaluate new antiepileptic drugs (AED) with potential activity against therapy-resistant epilepsy (Löscher, 2011). The compounds found inactive in the MES and PTZ tests are evaluated in the 6 Hz test in advance of strategic decisions concerning their development.

The 6 Hz test is described as an acute model using a low-frequency and a long-duration stimulation paradigm to induce partial seizures in the mouse (Barton et al., 2001). At low intensity, the test is non-discriminating and can be used as a screening test to identify new AEDs. At high intensity, the 6 Hz test becomes less sensitive to some AEDs and can be used to identify new drugs efficacious against difficult-to-treat partial epilepsy (Löscher, 2016). For example, non-toxic doses of PHT and lamotrigine (LTG) are efficacious in the 6 Hz test at the current intensity of 22 mA but they lose their efficacy at 32 and 44 mA in CF-1 mice whereas LEV remains active even at high stimulation intensities (Barton et al., 2001).

Rodents are widely used in epilepsy models, with the mouse and the rat representing standard animal species for AED preclinical development (Rogawski, 2006; Löscher, 2011). Although the majority of animal models of seizures can be performed in both mice and rats, the 6 Hz test, used as a screening model at an early stage of drug development, is generally performed in mice. This complicates the drug development process when data obtained in the mouse 6 Hz test have to be confirmed in rat models of temporal lobe epilepsy (TLE). Rowley and White (2010) showed that LEV, valproate (VPA) and LTG were effective in the 6 Hz test at 32 mA in the mice and in the hippocampal kindling test in the rat although the effective doses (ED_{50}) were different based on the model used and the species tested. They also showed that PHT was toxic at doses that were not fully efficacious in both models, suggesting the utility of the 6 Hz test to predict drug efficacy against TLE models. Mice and rats can display species-related differences in pharmacokinetic/pharmacodynamic (PK/PD) properties of drugs, including AED (Kagan, 2014). Extending the use of the 6 Hz test to the rat would thus present many advantages for programs of AED preclinical research.

The aim of the present study was to compare data obtained in mice or in rats evaluated in the 6 Hz test. We first evaluated the effects of different stimulations intensities to induce complex partial seizures either in the mouse or in the rat. Then, the pharmacological profile of different AEDs (VPA, LEV, PHT and LTG) was evaluated and compared between both species. In order to evaluate the therapeutic index of the different AEDs, their possible effects on spontaneous locomotion were also evaluated. We measured locomotion as this is a very sensitive behavioral endpoint, often able to detect side effects of drugs at lower doses than the rotarod or the chimney tests (Lynch et al., 2011).

2. Material and methods

2.1. Animals

Experiments were carried out on male NMRI mice and Wistar rats (Janvier-Labs, Le Genest Saint Isle, France). The animals were housed in groups of 5–8 on wood litter with nesting material and free access to food and water up to administration of drugs. The body weight range on the testing days was comprised between 22 and 33 g for mice and 197 and 258 g for rats. The animal house was maintained under artificial lighting (12 h with lights on at 7:00) with constant temperature and humidity. Experiments were performed during the light phase, at the same moment of the day.

The procedures used in the present study have been approved by the ethical committee of Porsolt S.A.S. The experiments were performed in accordance with French legislation and European Directive No 2010/63/UE concerning the protection of laboratory animals and in accordance with a currently valid license for experiments on vertebrate animals, issued by the French Ministry for Agriculture, Agro-food and Forestry. Experiments were conducted in a manner attempting to maintain an optimal level of animal welfare (Lidster et al., 2016). No death was observed before or after administration of the anticonvulsant substances and no death was observed after the 6 Hz test.

2.2. Treatments

LEV (Sequoia Research Ltd, United Kingdom), VPA and PHT (Sigma-Aldrich Inc., France) were dissolved in physiological saline which served as vehicle. LTG (Sigma-Aldrich Inc., France) was dispersed in 0.2% hydroxypropylmethyl-cellulose in physiological saline. Treatments were administered intraperitoneally (i.p.) as a single injection in a volume of 10 ml/kg body weight for the mice and 5 ml/kg for the rats. The solutions and suspensions were prepared freshly on each testing day. The doses of the different AEDs were selected on the basis of internal data obtained in epilepsy studies to evaluate anticonvulsant activity and on the literature in the mouse (Barton et al., 2001; Leclercq and Kaminski, 2015). The maximal concentration was detected in the brain at 120 min postinjection with PHT (Leclercq and Kaminski, 2015). In our experiments, PHT was administered 30 min before the 6 Hz test in the rat and 120 min before the test in the mouse, based on previous data indicating that the half-life of PHT was longer in the mouse as compared with the rat and differences on peak efficacy between both species (Gerber et al., 1969 and 1971; Löscher et al., 1991). The same doses of VPA, LEV and LTG at the same pre-treatment time were evaluated in the mice and in the rats, assuming that the pharmacokinetic data were relatively close in both species (Bialer et al., 2004). VPA was administered 30 min before the 6 Hz test, a pre-treatment time considered as sufficient to ensure an adequate plasma or brain exposure in the mouse and in the rat (Löscher et al., 1991; Parveen et al., 2015; Gynther et al., 2016). LEV was administered 60 min before the test, thereby allowing good brain penetration in mice and in rats (Leclercq and Kaminski, 2015; Nicolas et al., 2016). The pre-treatment time of LTG was 60 min and was considered as adequate to ensure brain penetration as already reported (Castel-Branco et al., 2005). The tests were performed blind for all studies with AEDs.

2.3. 6 Hz seizure test

Mice and rats (15 per experimental group) were manually restrained and were administered a rectangular current (rectangular pulse: 0.2 ms pulse width, 3 s duration, 6 Hz) via corneal electrodes connected to a constant current shock generator (Ugo Basile: type 7801).

Several behavioral symptoms such as stunned attitude, forelimb clonus, twitching of the vibrissae, rearing and Straub tail are usually reported for evaluating seizure in the mouse (Brown et al., 1953; Barton et al., 2001; Florek-Luszczki et al., 2014; Tutka et al., 2017).

In our experiments, forelimb clonus was homogeneously observed in both species and was thus considered as the most reliable parameter. It was evaluated using a score comprised between 0 and 2 after electrical stimulation.

Following electrical stimulation, the animals were placed in a Plexiglas cage and behaviour was observed during 30 s. Seizure was reflected by the presence of forelimb clonus immediately after current administration. Forelimb clonus was scored as absent (0), mild (1 = clonus with one forelimb) and strong (2 = clonus with two forelimbs). In a first series of experiments, mice and rats were evaluated at different intensities (24, 28, 32, 36, 40 and 44 mA) and the magnitude of forelimb clonus was evaluated for each species. Following these experiments, the effects of four AEDs were evaluated in a second series of experiments using the current intensity of 44 mA in both species.