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Research article

Resveratrol lacks protective activity against acute seizures in mouse models

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HIGHLIGHTS

- The natural product resveratrol has anti-inflammatory and neuroprotective properties.
- Anticonvulsant effects of resveratrol were evaluated in standard seizure models.
- The reference compound phenobarbital displayed dose-dependent protection against seizures.
- Acute resveratrol treatment did not provide any significant protection in the models.
- A therapeutic interest of resveratrol in epilepsy is questionable.

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ABSTRACT

Resveratrol (3,4',5-stilbenetriol) is a natural product having diverse anti-inflammatory and antioxidant properties. The compound has a wide spectrum of pharmacological and metabolic activity, including cardioprotective, neuroprotective, anticarcinogenic and anti-aging effects reported in numerous studies. Some reports also suggest potential anticonvulsant properties of resveratrol. In the present study, we used in mice three different seizure models which are routinely applied in preclinical drug discovery. The protective effects of resveratrol were evaluated in the pentylenetetrazole (PTZ), maximal electroshock (MES) and 6-Hz electrical seizure models. Resveratrol (up to 300 mg/kg) administered ip (5–60 min pre-treatment time) remained without any protective activity against seizures induced in these models. There was only a trend towards a delay in seizure latency, which reached statistical significance after treatment with resveratrol (100 mg/kg; 15 min) in case of tonic convulsions induced by PTZ. Phenobarbital (PHB, ip, 45 min), used as a reference compound, displayed a clear-cut and dose-dependent protection against seizures in all the models. The ED_{50} values obtained with PHB were as follows: 7.3 mg/kg (PTZ model), 13.3 mg/kg (MES model) and 29.7 mg/kg (6-Hz model). The present data demonstrate that an acute treatment with resveratrol does not provide any significant protection in three seizure models which collectively are able to detect anticonvulsants with diverse mechanisms of action. However, it cannot be excluded that chronic treatment with resveratrol may offer some protection in these or other seizure models.

1. Introduction

Resveratrol (3,4',5-stilbenetriol) is a polyphenolic phytoalexin present in high concentration in grapes, cranberries, peanuts and red wine. These sources contain both *cis*- and *trans*-isomers of resveratrol, but the latter is the preferred and relatively more stable steric form.

Resveratrol affects a number of molecular mechanisms involved in inflammation, oxidative stress and carcinogenesis [1–3]. In fact, resveratrol is a naturally occurring inhibitor of both forms of cyclooxygenase thereby having anti-inflammatory properties [4]. Resveratrol has also been shown to increase plasma antioxidant capacity and to decrease lipid peroxidation [5,6]. It also enhances the expression of both endothelial and inducible nitric oxide synthase [7]. An important action of resveratrol includes the activation

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of the silent information regulator 2 (Sir2) family of proteins, or sirtuins, and the compound acts as a substrate-specific activator of yeast Sir2 and human SIRT1 [8,10]. Activation of SIRT1 by resveratrol provides strong protective effects in an in vitro β-amyloid model of Alzheimer's disease [9].

Such diverse properties of resveratrol triggered numerous studies exploring its utility in different therapeutic areas [1–3]. The last 10–15 years brought a plethora of evidence indicating a wide spectrum of beneficial effects attributed to resveratrol treatment. For example, the compound has been shown to have anti-cancer properties, to delay progression of cardiovascular disease and to prolong the lifespan of various organisms from yeast to vertebrates [1].

Neuroprotective effects of resveratrol have also been reported [11–15]. In vivo experiments indicate that chronic treatment with resveratrol weakens kainic acid-induced neuronal injury [13–15]. Furthermore, resveratrol also protects against traumatic brain injury in rats [11,12]. Traumatic brain injury is one of the leading causes of epilepsy and kainic acid is widely used as a model of epilepsy in rodents [16]. Therefore, neuroprotective properties of resveratrol may be of particular importance for possible treatment of epilepsy. Several of the above-described actions of resveratrol, i.e. antioxidant or anti-inflammatory activity, may have beneficial impact on epilepsy pathophysiology [17,18]. However, there is only very limited data concerning anticonvulsant activity of resveratrol [19–21]. Consequently, the aim of this study was to evaluate the anticonvulsant effects of resveratrol in standard models used for antiepileptic drug discovery, i.e. pentylenetetrazole (PTZ)-, maximal electroshock (MES)- and 6-Hz-induced seizures in mice. Phenobarbital (PHB) was used as a comparator because it displays protective activity in all these three models.

2. Material and methods

2.1. Animals

Male NMRI mice (IFFA Credo, Belgium) weighing 20–30 g were used in all experiments. The animals were kept on a 12/12-h light/dark cycle with lights on at 0600 h and were housed at a temperature maintained at 20–21 °C and at humidity of about 40%. The mice were housed in groups of 10 per cage (38 × 26 × 14 cm). All animals had free access to standard pellet food and water before random assignment to experimental groups consisting of 10 mice each. All animal experiments were conducted in accordance with the guidelines of the European Community Council directive 2010/63/EU. A local ethical committee approved the experimental protocol.

2.2. Drugs

Pentylenetetrazole (PTZ), *trans*-resveratrol and phenobarbital sodium (PHB) were purchased from Sigma (Bornem, Belgium). PTZ and PHB were dissolved in sterile saline, while resveratrol required a suspension containing 0.1% Tween 80. PHB and resveratrol were administered intraperitoneally (ip), while PTZ was administered subcutaneously (sc) (all compounds in a 10 ml/kg injection volume). Resveratrol was administered at different pretreatment times (5–60 min) to detect its peak pharmacological activity, whereas PHB was injected 45 min before seizure tests.

2.3. PTZ seizure model

PTZ was used at the previously established CD_{97} dose of 89 mg/kg; a convulsive dose inducing clonic convulsions of all four extremities in 97% of mice [22]. Immediately following PTZ injection, the mice were placed individually in Perspex cages and observed for the presence of clonic convulsions in all four extremities and tonic hindlimb extension during a 60-min period. Latency time to the onset of these endpoints was also noted.

2.4. MES model

MES was produced by a stimulator (WITT IndustrieElektronik, Berlin, Germany) using a current of 50 mA delivered with a pulse frequency of 50 Hz for 0.2 s through corneal electrodes [22]. A drop of 0.4% oxybuprocaine hydrochloride (Unicaine, Thea, France) was placed on the eyes before the electrical stimulation to ensure good conductivity and local anesthesia. The mice were observed for 10 s following the stimulation and the incidence of tonic hindlimb extension was noted.

2.5. 6-Hz model

The 6-Hz model was carried out according to a previously described protocol [23]. Briefly, corneal stimulation (44 mA, 0.2 ms-duration monopolar rectangular pulses at 6 Hz for 3 s) was delivered by a constant-current device (ECT Unit 57800; Ugo Basile, Comerio, Italy). A drop of 0.4% oxybuprocaine hydrochloride (Unicaine, Thea, France) was placed on the eyes before electrical stimulation. During the stimulation, mice were manually restrained and gently released into an observation cage (38 × 26 × 14 cm) immediately after the current application. The seizures were often preceded by a brief period (~2–3 s) of intense psychomotor agitation (wild running and jumping). The animals then exhibited a "stunned" posture associated with rearing, forelimb automatic movements and clonus, twitching of the vibrissae, and sometimes Straub tail. At the end of the seizure, animals resumed their normal exploratory behavior. The experimental endpoint was protection against the seizure. An animal was considered to be protected if it resumed its normal exploratory behavior within 7 s from the stimulation. The duration of seizures was also noted.

2.6. Data analysis

The ED_{50} values (dose protecting 50% of animals) and their 95% confidence intervals were determined by a nonlinear curve fitting of the percentage protection data at different doses constrained between 0 and 100% protection. The latency to the onset of PTZ-induced seizures was statistically compared with Student's *t*-test, while the proportion of seizing mice were compared versus vehicle group with Fisher's exact probability test. The duration of 6-Hz seizures at different pretreatment times was compared with one-way ANOVA. All statistical evaluations were done with GraphPad Prism 4 (GraphPad Software, San Diego, CA, USA) and differences were considered statistically significant when p-value was <0.05.

3. Results

3.1. Effects of resveratrol and PHB on PTZ-induced seizures

Resveratrol administered at the doses of 40 and 80 mg/kg, 30 min before PTZ injection, did not protect the animals against clonic and tonic convulsions, but the latencies to the occurrence of these seizures were prolonged (Table 1). This effect, nonetheless, did not reach statistical significance. At a higher dose (100 mg/kg), resveratrol injected 15 min before PTZ also remained without any significant effect against clonic seizures (Table 1). However, all animals treated at this dose were protected against tonic seizures and the latency to these seizures was significantly prolonged (Table 1).