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Characterization of the anticonvulsant profile of isonicotinic acid benzylamide in various experimental seizure models in mice

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Abstract

This study focused on the evaluation of anticonvulsant properties of isonicotinic acid benzylamide (iso-Nic-BZA) in numerous experimental seizure models (maximal electroshock [MES]-, bicuculline [BIC]-, pentylenetetrazole [PTZ]-, pilocarpine [PILO]-, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA]-, kainic acid [KA]- and *N*-methyl-D-aspartic acid [NMDA]-induced seizures). Moreover, acute adverse-effect profile of the agent with respect to impairment of motor coordination was assessed in animals subjected to the chimney test. The evaluation of time–course and dose–response relationships for iso-Nic-BZA provided evidence that the compound produced the peak to maximum antielectroshock action and acute adverse effects at 5 min after its systemic (i.p.) administration. Iso-Nic-BZA exerted a clear-cut anticonvulsant action against maximal electroshock-induced seizures in mice and its ED_{50} value was 70.6 (56.4–88.4) mg/kg. The assessment of acute adverse effects in the chimney test revealed that the agent produced acute neurotoxic effects and its TD_{50} value was 135.6 (108.8–169.0) mg/kg. Additionally, iso-Nic-BZA showed the anticonvulsant activity in numerous chemically-induced seizures (AMPA-, BIC-, KA-, and PTZ-evoked clonic convulsions), remaining virtually ineffective (at doses up to 200 mg/kg) in PILO- and NMDA-induced seizures in mice. Based on this study, one can conclude that iso-Nic-BZA due to the short time to peak of its maximum anticonvulsant effects (5 min after its i.p. administration), deserves more attention as a potential antiepileptic drug for patients in status epilepticus.

Keywords: Isonicotinic acid benzylamide; Maximal electroshock; Chimney test; Protective index; Bicuculline; Pentylenetetrazole; AMPA; Kainic acid; NMDA; Pilocarpine

Overwhelming evidence indicates that imbalance between excitatory and inhibitory neurotransmission in the brain is a main cause contributing to seizure development in both, experimental and clinical conditions [4,5,8,13,14,20]. Numerous experimental studies have documented that *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainic acid (KA) receptor antagonists exert anticonvulsant effects in various experimental models of epilepsy [2,3,6,17].

Quite recently, it has been reported that some benzylamide (BZA) derivatives, containing a cyclic amino acid structure (such as nicotinic acid BZA and picolinic acid BZA), showed anticonvulsant effects against maximal electroshock (MES)- and various chemically-induced seizures (i.e., bicuculline (BIC)-, pentylenetetrazole (PTZ)-, pilocarpine (PILO)-, NMDA-, AMPA-, and KA-induced seizures) in rodents, suggesting that these BZA derivatives have AMPA/KA receptor antagonist properties [15,16]. Moreover, the evaluation of acute neurotoxicity profile for these BZA derivatives allowed for the calculation of their protective indices (PIs), as ratios of the respective TD_{50} and ED_{50} values [10]. Considering the PI values of nicotinic acid BZA and picolinic acid BZA, it has been documented that both BZA derivatives had a broad-spectrum of

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anticonvulsant activity and low potential to produce side effects in preclinical studies [15,16]. Unfortunately, these agents underwent rapid metabolic transformation in rodents and that fact limited their potential clinical use [15,16].

In the present study, we evaluated time–course and dose–response relationships for both antielectroshock action and acute neurotoxic activity of isonicotinic acid BZA (iso-Nic-BZA)—a new BZA derivative. Generally, in the MES test, one can determine the antiseizure effects of agents or drugs suppressing tonic-clonic seizures and, to a certain extent, partial convulsions in humans [9]. The acute neurotoxic profile of iso-Nic-BZA was determined in the chimney test, which allowed for the assessment of acute neurotoxic (adverse) effects produced by drugs with respect to motor coordination impairment in animals subjected to this test [1,9]. Additionally, the anticonvulsant properties of iso-Nic-BZA were evaluated in BIC-, PTZ-, PILO-, NMDA-, AMPA-, and KA-induced seizures in mice in order to assess pharmacological characteristics of this BZA derivative in preclinical study.

The experiments were carried out on male Swiss mice weighing 20–25 g. The animals were housed in colony cages with free access to food (chow pellets) and tap water. The experimental temperature was $21 \pm 1\,^{\circ}C$ and mice were on a natural light–dark cycle. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups (consisting of eight animals). Each mouse was used only once. Local Ethical Committee at the Medical University of Lublin approved all experimental procedures described in this study.

Iso-Nic-BZA was suspended in a 1% aqueous solution of Tween 80 (Sigma, St. Louis, MO, USA) and administered intraperitoneally (i.p.), in a volume of 0.01 ml/g body weight, at 5, 15, 30 and 60 min prior to electroconvulsions and chimney test. In all six chemically-induced seizures (BIC, AMPA, KA, NMDA, PILO, and PTZ), iso-Nic-BZA was administered i.p., at 5 min before the tests in order to ensure the maximum anticonvulsant effect of iso-Nic-BZA, corresponding to the onset of seizures from the MES test. BIC (Sigma, St. Louis, MO, USA) was brought into solution with a drop of glacial acetic acid and made up with sterile saline to a volume of 5 ml. The final pH was adjusted to 5.0 with 0.2 N NaOH. PTZ and *N*-methyl-scopolamine (*N*-SCO) (both from Sigma, St. Louis, MO, USA) were dissolved in distilled water in a volume of 5 ml. BIC, PTZ and *N*-SCO were administered subcutaneously (s.c.), in a volume of 0.005 ml/g body weight, into a loose fold of skin in the midline of the neck. PILO (Sigma, St. Louis, MO, USA) was dissolved in distilled water and given i.p., in a volume of 0.01 ml/g body weight, to animals pretreated with *N*-SCO 30 min earlier. All solutions were prepared freshly before the experiments. NMDA, AMPA and KA (all three drugs from ICN Biomedicals Inc., Costa Mesa, CA, USA) were administered intracerebroventricullarly (i.c.v.), in a volume of 5 μl. The pH of all three excitatory amino acid solutions was adjusted to 7.2 with 0.2 N NaOH.

Electroconvulsions were produced by means of an alternating current (50 Hz, fixed current intensity of 25 mA, maximum stimulation voltage of 500 V, stimulus duration of 0.2 s) delivered via ear-clip electrodes by a generator (Rodent Shocker, Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic hindlimb extension. The protective activity of iso-Nic-BZA was determined as its ED_{50} values (in mg/kg), protecting 50% of the animals tested against MES-induced seizures. The animals were administered iso-Nic-BZA at increasing doses ranging between 50 and 300 mg/kg, so as to obtain a variable percentage of protection against MES, allowing the construction of a dose–response relationship curve for iso-Nic-BZA, according to log-probit method [7]. This experimental procedure has been described in detail in our earlier studies [11].

The effects of iso-Nic-BZA on motor performance impairment were quantified by the chimney test of Boissier et al. [1]. In this test, animals had to climb backwards up a transparent plastic tube (3 cm inner diameter, 25 cm length) and motor impairment was indicated by the inability of the animals to climb backward up the tube within 60 s. The adverse effects of iso-Nic-BZA were expressed as its TD_{50} values, representing the doses at which iso-Nic-BZA impaired motor coordination in 50% of the animals tested. To evaluate each TD_{50} value, at least four groups of animals (each group consisted of eight mice) injected iso-Nic-BZA, at increasing doses ranging between 100 and 400 mg/kg were challenged with the chimney test. A dose–response relationship curve was calculated on the basis of the percentage of mice showing motor deficits by means of the log-probit method according to Litchfield and Wilcoxon [7]. This experimental procedure has been described in detail in our earlier studies [12].

Protective index (PI) for iso-Nic-BZA (administered i.p. at various pretreatment times), was calculated by dividing a given TD_{50} value, evaluated in the chimney test, by the respective ED_{50} value determined in the MES test. The PI is considered an index of the margin of safety and tolerability between anticonvulsant doses and doses of iso-Nic-BZA exerting acute adverse effects (e.g. sedation, ataxia, impairment of motor coordination or other neurotoxic manifestations) in preclinical studies [10]. This experimental procedure has been described in detail in our earlier studies [11,16].

BIC and PTZ were administered s.c. in doses of 3 mg/kg and 95 mg/kg, respectively, being their CD_{97} values (i.e., the doses of BIC and PTZ that evoked clonic seizures in 97% of animals tested). NMDA, AMPA and KA were administered i.c.v., in a volume of 5 μl, at doses of 1.0, 1.2, and 1.4 nmol, respectively. These doses correspond to their CD_{97} values (i.e., the doses of NMDA, AMPA and KA that evoked clonic seizures in 97% of animals tested). Following the injection of BIC, PTZ, NMDA, AMPA, and KA mice were placed separately into transparent Plexiglas cages (25 cm × 15 cm × 10 cm) and observed for 30 min for the occurrence of clonic seizures. The clonic seizure activity was defined as clonus of whole body lasting over 3 s, with an accompanying loss of righting reflex. The number of animals convulsing out of the total number of mice tested was noted for each treatment condition. The observations were double-blind and the anticonvulsant potencies of iso-Nic-BZA were evaluated as its ED_{50} values (in mg/kg), necessary to protect 50% of the animals against clonic seizures. This experimental procedure has been described in more detail elsewhere [3,19].