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Synthesis and anticonvulsant evaluation of dimethylethanolamine analogues of valproic acid and its tetramethylcyclopropyl analogue

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Subcutaneous metrazol seizure test;
Pharmacokinetic–pharmacodynamic correlation

Summary
Background: Valproic acid (VPA) is a major antiepileptic drug (AED) that is less potent than other AEDs. 2,2,3,3-Tetramethylcyclopropanecarboxylic acid (TMCA) is an inactive cyclopropyl analogue of VPA that serves as a starting material for the synthesis of CNS-active compounds.
Methods: New conjugation products between N,N′-dimethylethanolamine to VPA and TMCA to form N,N-dimethylethanolamine valproate (DEVA) and N,N-dimethylethanolamine 2,2,3,3-tetramethylcyclopropionate were synthesized and their anticonvulsant activity was assessed in the maximal electroshock seizure (MES) and subcutaneous metrazol (scMet) seizure tests and the hippocampal kindling model in mice and/or rats. An amide analogue of DEVA (DEVAMIDE) was also synthesized and evaluated. The pharmacokinetics of DEVA and DEVAMIDE was comparatively evaluated in rats.
Results: In rats DEVA acted as a prodrug of VPA and had ED_{50} values of 73 mg/kg and 158 mg/kg in the MES and the hippocampal kindling models, respectively. At these two anticonvulsant models DEVA was seven-times more potent than VPA. DEVAMIDE was active in the MES test at doses of 100 mg/kg (mice) and its rat-MES-ED_{50} = 38.6 mg/kg however, its protective index ($PI = TD_{50}/ED_{50}$) was twice lower than DEVA's PI. The TMCA analogues were inactive at the mice MES and scMet models. DEVA underwent rapid metabolic hydrolysis to VPA and consequently, in its pharmacokinetic analysis only VPA plasma levels were monitored. In contrast, DEVAMIDE was stable in whole blood.
Conclusion: DEVA acts in rats as a prodrug of VPA yet shows a more potent anticonvulsant activity than VPA. DEVAMIDE acted as the drug on its own and was more potent than DEVA at the rat-MES test.

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Introduction

Epilepsy is a common neurological disorder characterized by recurrent seizures that affect about 1% of the world's population.

Despite all the new antiepileptic drugs (AEDs) introduced in the last two decades about 30% of patients with epilepsy are still not seizure-free (Bialer and White, 2010). In addition all AEDs have side effects. Consequently, there is a significant need to develop new more efficacious AEDs that will be more potent and with less side effects.

Valproic acid (VPA, Fig. 1) one of the leading AEDs is also approved for the treatment of migraine prophylaxis and bipolar disorders. Despite its broad spectrum of antiepileptic activity, VPA is one of the least potent AEDs and therefore clinically used at high doses (Bialer et al., 2004). In addition, VPA's clinical use is restricted by its two rare but potentially life-threatening side effects: teratogenicity and hepatotoxicity (Bialer and Yagen, 2007). 2,2,3,3-Tetramethylcyclopropanecarboxylic acid (TMCA, Fig. 1) is an inactive cyclopropyl analogue of VPA that possesses two quaternary carbons and thus cannot form hepatotoxic metabolites with a terminal double bond and therefore can serve as a starting material for new non-hepatotoxic CNS active compounds (Winkler et al., 2005).

A variety of CNS-active amide derivatives of VPA and TMCA have been designed in our lab and showed activity in animal models for epilepsy, neuropathic pain and bipolar disorder (Isoherranen et al., 2002; Bialer and Yagen, 2007; Bialer and White, 2010; Shaltiel et al., 2004; Shimshoni et al., 2007).

Valnoctamide (VCD) and propylisopropylacetamide (PID) are two CNS-active constitutional isomers of VPA corresponding amide valpromide (VPD) (Bialer and Yagen, 2007; Bialer and White, 2010). Both VCD and PID exhibited more potent anticonvulsant activity than VPA in various animal models and in contrast to VPD acted as drugs on their own and not as prodrugs of their corresponding acids (Isoherranen et al., 2003a,b). VCD recently completed a successful phase IIa clinical trial in patients with bipolar disorder (Bresudsky et al., 2010).

N-Methyl-2,2,3,3-tetramethylcyclopropanecarboxamide (M-TMCD), 2,2,3,3 tetramethylcyclopropancarbonylurea (TMCU) and α-fluoro-TMCD (F-TMCD) are CNS-active amide derivatives of TMCA that are more potent than VPA in various anticonvulsant animal models (Isoherranen et al., 2002; Sobol et al., 2006; Pessah et al., 2009).

VPA has a partial brain penetration; a fact that contributes to its less potent anticonvulsant activity. In rat the VPA brain-to-plasma AUC ratio (BPR) is only 0.16 and is significantly lower than that of VCD (1.0), M-TMCD (1.0) or TMCU (3.3) (Blotnik et al., 1996, 1998; Sobol et al., 2006).

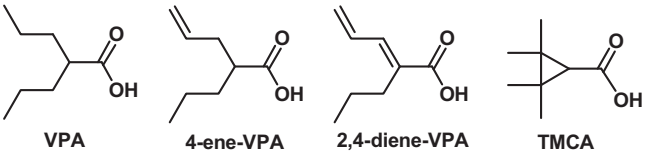

Figure 1 Chemical structures of VPA, 4-ene-VPA, 2,4-diene-VPA and TMCA.

N,N-Dimethylethanolamine and choline are important constituents of phospholipids.

In fact, choline has been shown in several animal studies to penetrate the blood brain barrier (BBB) by assisted diffusion (Cornford et al., 1978; Klein et al., 1991) and to enter cholinergic neurons by low-affinity and high affinity pumps (Klein et al., 1992). Thus, attaching this molecule to a CNS drug might improve the drug's potency.

In order to enhance VPA brain permeability, in the current study we synthesized and evaluated the anticonvulsant activity of new conjugation products between VPA and TMCA to N-N-dimethylethanolamine and choline as well as amide analogues of these esteric adducts.

Materials and methods

Chemicals

Chemicals were purchased from Sigma—Aldrich. N-(2-methoxyethyl) methylamine was purchased from Tokyo chemical industry, Tokyo, Japan. Methanol, diethylether and ethylacetate were purchased from Frutarom Israel. Dry dichloromethane was obtained by reflux over CaH_2 for 2 h and fresh distillation prior to use.

VPA and TMCA were converted by $SOCl_2$ to the corresponding acyl chlorides by a method described in the literature (Pessah et al., 2009).

Animals

Adult male CF No. 1 albino mice (18—25 g) and adult male Sprague-Dawley albino rats (100—150 g) were used for the anticonvulsant and neurotoxicity evaluation. Animals were maintained in the animal facilities of the University of Utah. Animals were allowed to acclimatize for 24—48 h before testing. A 12-h light, 12-h dark cycle was maintained, and the animals were allowed free access to food and water, with the exception of the testing times. The animals were maintained and handled according to the recommendations of the U.S. Department of Health, Education and Welfare publication (NIH) No. 8623, Guide for the Care and Use of Laboratory Animals. All the animal experiments were approved by the Institutional Animal Care and Use Committee of the University of Utah.

Male Sprague-Dawley rats weighing 250 ± 24 g were used in the pharmacokinetic studies. The animals were maintained at 22 °C with a 12-h light:12-h dark cycle. Prior to each experiment, the animals were allowed to acclimatize for at least 2 days in communal cages with standard rat chow and tap water provided *ad libitum*. For urine collection animals were kept in metabolic cages. The animal use in the PK studies was approved by the Institutional Animal Care and Use Committee of The Hebrew University of Jerusalem.

Anticonvulsant activity and neurotoxicity

The evaluation of the anticonvulsant activity in the maximal electroshock (MES), subcutaneous pentylenetetrazol or metrazol (scMet) and hippocampal kindling seizure tests were performed at the NIH Epilepsy Branch as a part of Anticonvulsant Drug Development Program according to the protocols described by White et al., 2002. Metrazol was administered at its convulsive dose (CD_{97} — 85 mg/kg in mice and 56.4 mg/kg in rats). In addition to the efficacy studies, the neurotoxicity of the compounds was established using the rotorod ataxia test (mice) and positional sense test (rats). In the rotorod test a mouse is placed on a rotor that rotated at a speed of 6 rpm. Inability of the mouse to maintain its equilibrium in three trials during 1 min on this rotating rod is used as an indication