Pharmacological Research 117 (2017) 129–139

Contents lists available at ScienceDirect

Pharmacological Research

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

sec-Butylpropylacetamide (SPD), a new amide derivative of valproic acid for the treatment of neuropathic and inflammatory pain

Dan Kaufmann[a,1], Peter J. West[a], Misty D. Smith[a], Boris Yagen[b,c], Meir Bialer[b,c], Marshall Devor[d], H. Steve White[a,**,1], K.C. Brennan[e,*]

[a] Anticonvulsant Drug Development Program, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT, USA
[b] Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel
[c] David R. Bloom Center for Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel
[d] Department of Cell and Animal Biology, Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel
[e] Department of Neurology, University of Utah, Salt Lake City, UT, USA

ARTICLE INFO

Article history:
Received 27 May 2016
Received in revised form 20 October 2016
Accepted 22 November 2016
Available online 24 November 2016

Keywords:
sec-Butylpropylacetamide (SPD)
Pharmacokinetics
Chung model
Compound action potentials
Formalin test
Carrageenan test
Writhing test

ABSTRACT

Chronic pain is a multifactorial disease comprised of both inflammatory and neuropathic components that affect ~20% of the world's population. *sec*-Butylpropylacetamide (SPD) is a novel amide analogue of valproic acid (VPA) previously shown to possess a broad spectrum of anticonvulsant activity. In this study, we defined the pharmacokinetic parameters of SPD in rat and mouse, and then evaluated its antinociceptive potential in neuropathic and acute inflammatory pain models. In the sciatic nerve ligation (SNL) model of neuropathic pain, SPD was equipotent to gabapentin and more potent than its parent compound VPA. SPD also showed either higher or equal potency to VPA in the formalin, carrageenan, and writhing tests of inflammatory pain. SPD showed no effects on compound action potential properties in a sciatic nerve preparation, suggesting that its mechanism of action is distinct from local anesthetics and membrane stabilizing drugs. SPD's activity in both neuropathic and inflammatory pain warrants its development as a potential broad-spectrum anti-nociceptive drug.

1. Introduction

Chronic pain affects 10–55% [1] of the world's population, causing significant morbidity [2]. Pharmacotherapy for chronic pain is only partially effective and suffers from having a severe side effect profile and high abuse potential [2,3]. Neuropathic pain is caused by traumatic, inflammatory, or dysmetabolic lesions to the central or peripheral nervous system [4]. Traditionally, changes in neuronal or synaptic physiology have been advanced as mechanisms of neuropathic pain [5], however many studies indicate that the traditional focus on these factors provides an incomplete picture [6–9]. Neuropathic pain is typically treated with membrane stabilizing agents (*e.g.* antiepileptic drugs (AEDs) or local anesthetics (*e.g.* lidocaine)), that aim to modulate ion channel activity [10,11]. However, this strategy has met with limited success, suggesting the involvement of other, possibly inflammatory, mediators in chronic pain states [5,7,9]. Inflammatory mechanisms are known to contribute significantly to the development of chronic neuropathic pain [8,10,12]. That said, non-steroidal anti-inflammatory drugs (NSAIDs) have limited efficacy for neuropathic pain conditions [13] and are not included in the pain treatment guidelines [14,15]. Opioids are indicated for both inflammatory and neuropathic pain, although their efficacy in neuropathic pain is still controversial [16]. They also carry the risk of potential tolerance and have a high addiction liability [17]. This supports the need for further development of more tolerable broad spectrum pain medications that target both the inflammatory, and neuropathic components of chronic pain.

Abbreviations: SPD, *sec*-butylpropylacetamide; VPA, valproic acid; CAPs, compound action potentials; AED, antiepileptic drugs; SNL, spinal nerve ligation; PK, pharmacokinetic; PD, pharmacodynamic; VFF, von-Frey filaments; MC, methyl cellulose; TPE, time to peak effect; ACSF, artificial cerebrospinal fluid; PAR, peak-to-peak area ratio; ED_{50}, median effective dose; TD_{50}, median toxic dose; PI, protective index; AUC, area under the SPD plasma concentration *vs.* time curve; CI, confidence interval; MRT, mean residence time; CL/F, oral or extravascular clearance; V/F, apparent volume of distribution; AUMC, area under the first moment (concentration-time product) curve.

* Corresponding author at: Department of Neurology, University of Utah, 383 Colorow Drive, Room 364, Salt Lake City, UT 84108, USA.
** Corresponding author at: Department of Pharmacy, School of Pharmacy, University of Washington, Health Sciences Building H 375X, 1959 NE Pacific Street Box 357630, Seattle, WA 98195 7630, USA.
E-mail addresses: hswhite@uw.edu (H.S. White), K.C.Brennan@hsc.utah.edu (K.C. Brennan).
[1] Previous affiliation where work was done.

http://dx.doi.org/10.1016/j.phrs.2016.11.030
1043-6618/

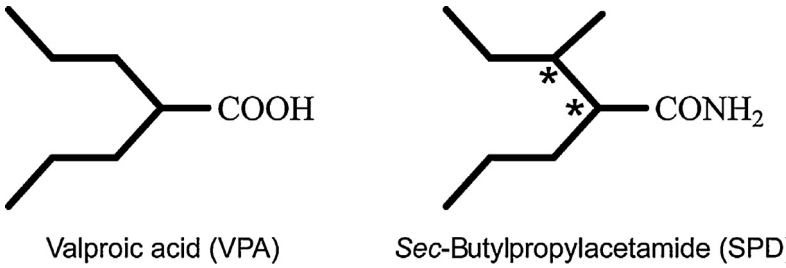

Fig. 1. Chemical structures of valproic acid (VPA) and *sec*-butylpropylacetamide (SPD). Asterisks denote chiral carbons.

sec-Butylpropylacetamide (SPD) (Fig. 1) is a second generation amide derivative of valproic acid (VPA, Fig. 1), previously found to be active in numerous animal seizure models [18,19]. Epilepsy and neuropathic pain are thought to share some aspects of their underlying pathophysiology [11], and some AEDs are used for the treatment of neuropathic pain (*e.g.* gabapentin) [20,21]. Owing to its broad-spectrum and promising effect as an anticonvulsant compound, we evaluated SPD's potential in both neuropathic and acute inflammatory pain models. In this work, we show SPD to be active in the spinal nerve ligation (SNL) model for neuropathic pain and three models of acute inflammatory pain. Compared to its parent compound VPA, SPD had higher potency in the SNL model and a higher or equivalent potency in the inflammatory pain models. Its effects suggest a different pharmacological profile than either conventional membrane stabilizers or NSAIDs. We also evaluated SPD's pharmacokinetic profile to demonstrate its favorable PK profile and to explore its pharmacokinetic (PK) – pharmacodynamic (PD) correlation. Our results support further evaluation of SPD for the treatment of refractory pain.

2. Materials and methods

All experiments, analysis, and reporting were performed according to the ARRIVE criteria [22].

2.1. Animals

All acute inflammatory pain experiments were conducted using male CF-1 mice (20–30 g, Charles River Laboratories, Raleigh, NC). Neuropathic pain models were performed on male Sprague-Dawley rats (200–250 g, Harlan Laboratories, Jerusalem). Electrophysiology recordings were done *in vitro* on acutely dissected sciatic nerves from male Sprague Dawley rats (300–450 g). Animals were housed in clear acrylic glass cages in an Institutional Animal Care and Use Committee (IACUC) approved animal facility and were allowed free access to food (product 8640, Harlan Teklad, WI, USA) and water. All animal cages were maintained in a temperature, humidity, and 12:12 h light:dark cycle controlled environment. Every attempt was made to minimize the pain experienced by experimental animals. All experiments were approved by the University of Jerusalem and University of Utah's IACUC.

2.2. Materials used

sec-Butylpropylacetamide (SPD), *N*-methyl tetramethylcyclopropyl acetamide (MTMCD) used as a positive control in the SNL model, and N-methyl valnoctamide (N-methyl VCD) used as internal standard in the PK studies were synthesized according to a previously published procedure [19].

Formalin solution, 37% v/v in water (10%–15% in methanol), glacial acetic acid, methyl cellulose (MC, 4000 Centipoise), λ–carrageenan, Ibuprofen sodium salt, sodium valproate and sodium pentobarbital were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Lidocaine hydrochloride was purchased from Spectrum Chemicals Mfg. Corp. (Gardena, CA, USA). Both SPD and VPA were suspended in 0.5% MC before they were injected i.p. to mice, at doses lower than their TD_{50} values previously evaluated (88 mg/kg and 391 mg/kg i.p. for SPD and VPA respectively [18]).

2.3. Spinal nerve ligation (SNL) model for neuropathic pain

Rats were evaluated twice for their tactile allodynia threshold, 1 and 2 days prior to surgery. All rats included in the experiment had pain threshold >15 g in both hind paws before surgery. The surgical procedure used to produce allodynia was previously described by Kim and Chung [23]. Briefly, rats were anesthetized following i.p. administration of 85 mg/kg ketamine and 15 mg/kg xylazine. With the rats in prone position, the paraspinal muscles on the left were carefully separated from the L4 to the S2 transverse processes, followed by removal of the L6 transverse process in order to visualize the L5-L6 spinal nerves. These were tightly ligated with a 5-0 silk thread and cut distal to the ligature. The paraspinal muscles were closed with sutures, and the skin was closed with Michel clips. A bacteriostatic powder was then applied topically, followed by intramuscular administration of ampicillin. Recovery after surgery lasted 5 days before commencement of the behavioral tests. The rat's foot withdrawal in response to tactile stimulus was used to detect tactile allodynia using a set of nine nylon von-Frey filaments (VFF). VFF produced an initial bending force of (in mN): 5.8, 13.7, 19.6, 39.2, 58.7, 78.3, 97.9, 146.9, and 254.5, equivalent to a mass of (in grams): 0.6, 1.4, 2, 4, 6, 8, 10, 15, and 26. The same set was calibrated and used in all experiments. SPD was administered intraperitoneally (i.p.) at 40, 60, and 80 mg/kg *vs.* negative control (MC) at 7, 14, and 21 days post-surgery using a Latin square design protocol where the experimenter who performed the behavioral tests was not aware of the dose nor substance given to the animals tested. MTMCD was used as a positive control comparator. The VFF were applied briefly, just before and 30, 60, 120, 180, and 240 min after injection at 1–2 s interval to the mid plantar skin of the hind paw. Stimulation began with the 0.6 g VFF, using a perpendicular force to the skin that was just sufficient to bend the monofilament. If the animal failed to respond with a brief paw withdrawal to at least 3 out of 5 stimuli, the next monofilament was tested using an ascending staircase protocol. The response threshold was set as the average of the minimal force required to obtain a criterion response on the two repeats. Rats were considered "protected" from allodynia if they failed to respond to the 15 g VFF (considered 100% protection).

2.4. Formalin test

Formalin test was performed according to a previously published paradigm [24,25]. A total of ten groups, each having eight mice per group, were used in the study; a control group (MC) was paired with each of the five dose groups. Mice were placed in cylindrical clear acrylic glass chambers (6″ tall × 4″ diameter)