European Journal of Pharmacology 702 (2013) 20–24

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

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

Neuropharmacology and analgesia

Prolonged analgesic effect of amitriptyline base on thermal hyperalgesia in an animal model of neuropathic pain

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ARTICLE INFO

Article history:
Received 24 April 2012
Received in revised form
14 December 2012
Accepted 19 December 2012
Available online 23 January 2013

Keywords:
Amitriptyline
Depot
Preemptive
Thermal hyperalgesia
Neuropathic pain

ABSTRACT

The best analgesic drugs for neuropathic pain have a long duration of action, can be given via multiple routes, and can be used preemptively. We evaluated the antinociceptive effects and duration of action of subcutaneously injected amitriptyline base (AMT-Base) (in oil). A plantar test in a spinal nerve ligation (SNL) model of neuropathic pain in rats showed that typical amitriptyline HCl (AMT-HCl) (in saline) and AMT-Base had a significant dose-dependent antinociceptive effect: the antinociceptive duration of a single 100 μmol/kg injection of AMT-HCl was 5 h and of AMT-Base was 24 h when given 7 days after a SNL, and of a single 200 μmol/kg injection of AMT-Base was 39 days when given 1 h before and 4 days when given 7 days after a SNL. The post-ligation antinociceptive duration of AMT-Base was 4.8 times that of AMT-HCl, but the duration of preemptive (pre-ligation) AMT-Base treatment was 9.7 times that of AMT-Base. We can conclude that preemptive amitriptyline base provides long-lasting antinociception for neuropathic pain experimentally.

1. Introduction

Neuropathic pain develops as a result of lesions or disease affecting somatosensory nervous system in the periphery or centrally (Baron et al., 2010) and affects one third of the population seen in pain clinics (Crombie et al., 1998). Such nerve injury leads to increased pain sensitivity (hyperalgesia), pain in response to light tactile stimuli (allodynia), and prolonged, persistent pain (Basbaum et al., 2009; Devor, 2001; Sindrup and Jensen, 1999). Although up to now, no clear predictors of therapeutic response have been identified in patients with neuropathic pain, various types of drugs, including antidepressants with norepinephrine and serotonin reuptake inhibition, calcium channel α2-δ ligands, opioid analgesics, and topical lidocaine, have been shown to have consistent efficacy in randomized controlled clinical trials and meta-analyses (Attal et al., 2010; Dworkin et al., 2007, 2010; Finnerup et al., 2005; Jensen et al., 2009; Moulin et al., 2007; O'connor and Dworkin, 2009). Of the tricyclic antidepressants, secondary amine tricyclic antidepressants including nortriptyline and desipramine, are recommended because they provide pain relief that is comparable to amitriptyline and other tertiary amine tricyclic antidepressants while causing several side effects (Baron et al., 2010).

In a rat model of spinal nerve ligation (SNL) (Esser and Sawynok, 1999), amitriptyline alleviated thermal hyperalgesia (systemically, spinally, and locally) but had no effect on mechanical allodynia. Additionally, a rat model of spinal nerve injury (Arsenault and Sawynok, 2009) showed that intraperitoneal (i.p.) amitriptyline 30 min before and immediately after surgery, followed by oral amitriptyline in drinking water for 7 days post-surgery, prevented hypersensitivity to a chemogenic stimulus (αβ-methylene adenosine triphosphate (meATP)). Since amitriptyline base (AMT-Base) (in oil) has a high octanol–buffer partition coefficient ($\log P^0 = 4.95$) and a high lipid solubility (Sudoh et al., 2004), whether it was dissolved in injectable oil and given s.c., its duration of action was longer because of its slow release of from the oily vehicle (Allen et al., 2005; Liu et al., 2006). This indicates that using a free base would be useful for treating neuropathic pain.

Bowsher (1997) reported that preemptive amitriptyline combined with an antiviral drug reduced pain prevalence by more than 50% in elderly patients with acute herpes zoster. The best analgesic drugs for neuropathic pain have a long duration of action, can be given via multiple routes, and can be used

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http://dx.doi.org/10.1016/j.ejphar.2012.12.032

preemptively. Other evidence (Allen et al., 2005; D'Aurizio et al., 2011; Liu et al., 2006) has also shown that depot medication with a prodrug increases the duration of a short-acting drug.

We evaluated the antinociceptive effect and duration of action of amitriptyline base (AMT-Base) (in oil). A SNL model of neuropathic pain (Kim and Chung, 1992) was used to examine the effect of amitriptyline on thermal hyperalgesia. We specifically sought to determine (a) whether subcutaneously (s.c.) administered AMT-Base has a greater antinociceptive effect than amitriptyline HCl (AMT-HCl) (in saline), and (b) whether preemptive treatment with AMT-Base is more effective than post-SNL treatment.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats weighing 120–200 g were purchased from the Taiwan National Laboratory Animal Center. For at least 1 week before the experiments, they were housed in groups of 3 in a climate-controlled room maintained at 21 °C with approximately 50% relative humidity. They were on a 12-h light/dark cycle (lights on at 6:00 a.m.), with food and water available ad libitum except during experiments and tests. All experiments were done in accordance with the recommendations and policies of the International Association for the Study of Pain, and the protocol was approved by the Animal Investigation Committee of the Chi-Mei Medical Center.

2.2. Drugs (preparation of long-acting amitriptyline)

Amitriptyline HCl was purchased from Sigma-Aldrich Chemical Co. (Saint Louis, MO). Amitriptyline base was obtained using a method of precipitation. After a saturated solution of 5% $NaHCO_3$ (Riedel-de Haën, Seelze, Germany) added drop by drop into an AMT-HCl solution, AMT-Base was precipitated. The precipitate was extracted using ethyl acetate and evaporated to dryness. After drying the residue, the long-acting formulation of amitriptyline depot was therefore obtained by dissolving AMT-Base into injectable sesame oil.

2.3. Drug administration

Amitriptyline HCl (200 μl) (in saline) or amitriptyline base (200 μl) (in sesame oil) was injected subcutaneously using a 27-G needle into the midline of the back, 2 cm below the neck of each rat.

2.4. Spinal nerve ligation (SNL)

We used the peripheral neuropathy model described by Kim and Chung (1992) for the SNL procedure. The rats were first anesthetized with pentobarbital (60 mg/kg intraperitoneally). They were surgically clipped and given adequate fluids. The surgical area was aseptically scrubbed with alcohol and iodine, and then a 3-cm midline dorsal incision was made using the ischium as the midline. The L5 and L6 spinal nerves were exposed using blunt dissection and partial removal of the articular facet and the fourth lumbar spinal nerve (L4) transverse process. The left L5/L6 spinal nerves were then tightly ligated with sterilized 6-0 silk. After ensuring hemostasis, the wound was closed in layers using subcutaneous and cutaneous 3.0 monofilament polybutester sutures. Rats that underwent the same surgical procedures but without nerve ligation were assigned to the sham-operation group (SHAM). The rats were then placed in a heated area and monitored as they recovered from the surgery.

2.5. Thermal hyperalgesia

The antinociceptive effects were measured using the plantar test (7371; Ugo Basil, Italy). In the test, an infrared source located under the glass floor of the cage was positioned by the operator directly beneath the hind paw of the tested rat. Latency from the beginning of the infrared stimulus to paw withdrawal was classified as response latency. To prevent tissue damage, a 20-s cut-off time was set. The sensitivity of the test was 0.1 s. The onset time was defined as the first significantly different time point between the groups treated with AMT and the group treated with vehicle. The duration of the effect was defined as the interval between onset and the last time point of the significant effect.

In experiment 1, the antinociceptive effects of AMT-HCl (25, 50, 75, and 100 μmol/kg) 7 days after nerve injury were evaluated; in experiment 2, the effects of AMT-Base (25, 100, and 200 μmol/kg) 7 days after nerve injury; and in experiment 3, the effects of AMT-HCl or AMT-Base (200 μmol/kg) 1 h before nerve injury.

2.6. Data analysis

The latency of foot withdrawal to noxious heat stimuli was measured using a method described (Bennett and Xie, 1988; Hargreaves et al., 1988). The rat was placed on a glass plate under which a light box was located. A radiant heat stimulus was applied to the heal of each hind paw through the glass plate. The light beam was turned off automatically by a photocell when the rat lifted the foot, allowing the measurement of time between start of light beam and the foot lift. The time was defined as the foot withdrawal latency. Five minutes were allowed between stimulations and 5 measurements were averaged for each side.

Data are reported as means ± S.E.M. To describe the time course of thermal hyperalgesia before and after AMT treatment, mean paw withdrawal latencies with standard errors were plotted and then compared using the Mann–Whitney *U* test between two groups. To compare more than two treatment groups, the Kruskal–Wallis *H* test was initially done for overall equality testing; if the significance level was met, Dunn's test was used to detect differences between the AMT-treated and SHAM or Control groups. The familywise type I error of overall testing was set at 0.05, and the correction of significance levels of post hoc comparisons were divided by the number of comparisons. All data analyses were using SPSS for Windows 17.0 (SPSS Inc. Chicago, IL).

3. Results

3.1. Decreased thermal withdrawal latencies 7 days after SNL

There was a significant increase in rat hind-paw withdrawal latency in response to a thermal stimulus 7 days after SNL compared with controls (Figs. 1 and 2). In experiment 1, AMT-HCl was injected (s.c.) 7 days after SNL, and in experiment 2, AMT-Base was injected (s.c.) 7 days after SNL.

3.2. AMT-HCl injected 7 days after SNL caused antinociception

A single injection of AMT-HCl (50, 75, or 100 μmol/kg) 7 days after SNL produced a significant dose-dependent antinociceptive effect with rapid onset times of 2 h, 2 h, and 1 h, and durations of