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Anticonvulsant effects of acetaminophen in mice: Comparison with the effects of nonsteroidal anti-inflammatory drugs

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ABSTRACT

Objective: The appropriate use of analgesic drugs based on their degree of analgesia and adverse effects is important for pain management. Although it has been reported that AM404, a metabolite of acetaminophen, has anticonvulsant effects in several animal seizure models, little is known about the relation between acetaminophen and seizures. We therefore investigated the effects of acetaminophen on seizure susceptibility in several mouse seizure and epilepsy models and compared the effects with those of nonsteroidal anti-inflammatory drugs (NSAIDs).

Methods: Anticonvulsant activity was evaluated in ICR mice using maximum electroshock-induced seizure tests and acute pentylenetetrazol-induced seizure tests. Electrical kindling via corneal stimulation and pentylenetetrazol administration were used to establish animal kindling epilepsy models. Proconvulsive activity was examined using an electroconvulsive shock test with low-stimulus currents.

Results: Acetaminophen showed slight, but not statistically significant, anticonvulsant activity in both the maximum electroshock-induced seizure test (300–600 mg/kg i.p.) and acute pentylenetetrazol-induced seizure test (100–600 mg/kg i.p.). In contrast, acetaminophen exhibited significant anticonvulsant effects in corneal electroshock-kindled and pentylenetetrazol-kindled mice (ED_{50} values: 251 and 310 mg/kg i.p., respectively). When the proconvulsive effects of NSAIDs were examined in the low-current electroconvulsive shock-induced seizure model, the nonselective cyclooxygenase (COX)-1 and COX-2 inhibitors indomethacin, diclofenac, and loxoprofen induced dose-dependent proconvulsant activity. Celecoxib, a COX-2 selective inhibitor, had no proconvulsant activity.

Conclusion: These findings suggest that acetaminophen has a significant anticonvulsant effect and that its profile is completely different from that of NSAIDs.

1. Introduction

The World Health Organization's analgesia ladder for the treatment of cancer-related pain provides a three-step sequential approach based on pain severity: Nonopioids are recommended for mild pain, with the addition of mild opioids for moderate pain and strong opioids for severe pain. The appropriate use of analgesic drugs based on their degree of analgesia and their adverse effects is important for cancer pain management. For example, high doses of short-acting opioids, such as morphine or fentanyl, have been reported to cause generalized seizures. Accordingly, treatment with opioids should be avoided in patients with uncontrolled seizures (Perks et al., 2012).

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase (COX)-1 or COX-2 isozymes, or both, and have analgesic, antipyretic, and anti-inflammatory effects. NSAIDs are associated with a number of adverse effects, however, including gastrointestinal bleeding and renal impairment (Ong et al., 2007). Seizures have rarely been documented for overdoses of NSAIDs (Smolinske et al., 1990). The combination of new quinolone antibacterial agents with some nonselective COX inhibitors induces functional blockade of γ-aminobutyric acid (GABA) receptors and induces convulsions (Yakushiji et al., 1992). In contrast, celecoxib, a COX-2 selective inhibitor, reportedly does not

Abbreviations: AM404, N-(4-hydroxyphenyl)-5Z,8Z,11Z,14Z-eicosatetraenamide); COX, cyclooxygenase; GABA, γ-aminobutyric acid; PG, prostaglandin; TRPV1, transient receptor potential vanilloid-1

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induce convulsions in the presence of the new quinolones in mice (Yoshino et al., 2005).

Acetaminophen, a centrally acting analgesic and antipyretic agent, has few adverse effects when used in therapeutic doses, but an excessive dose may cause hepatotoxicity. The pharmacological mechanisms underlying the analgesic effects of acetaminophen are complex and have not been fully elucidated (Bertolini et al., 2006). It has reported that AM404, a metabolite of acetaminophen, inhibits the uptake of endocannabinoid anandamide (Högestätt et al., 2005) and that the endocannabinoid system is involved in the anti-nociceptive actions of acetaminophen (Ottani et al., 2006; Dani et al., 2007). It is well known that cannabinoids are potent anticonvulsants (Wallace et al., 2002), and the anandamide transport blocker AM404 has been reported to have had anticonvulsant effects in several animal seizure models (Solbrig et al., 2005; Shubina et al., 2017). Acetaminophen is reported to inhibit status epilepticus-like activity through cannabinoid type 1 receptors in cultured hippocampal neurons (Deshpande and DeLorenzo, 2011). In acute seizure models, acetaminophen delays the onset of pentylenetetrazol-induced convulsions in rats (Wallenstein, 1985) but reportedly has no effect against maximum electroshock-induced seizures in mice (Kaminski et al., 1998). Thus, the anticonvulsant property of acetaminophen is not yet clearly understood. In this study, we investigated the effects of acetaminophen on seizure susceptibility in mouse seizure and epilepsy models and compared the effects of acetaminophen with those induced by NSAIDs.

2. Materials and methods

2.1. Animals

All animal care and experimental procedures were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals adopted by the Japanese Pharmacological Society and were approved by the Ethics Committee for Animal Experimentation of Shujitsu University (approval code 025-002).

Male ICR mice were purchased from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan). They were maintained in an air-conditioned room with controlled temperature (22 ± 2 °C) under a 12-h light/dark cycle with lights on at 0800. They were housed in standard-size plastic cages (32 × 18 × 24 cm) with sawdust (4–6 mice per cage). The mice were allowed free access to food and water except during the experiments.

2.2. Drugs

Acetaminophen (Sigma-Aldrich, St. Louis, MO, USA) and celecoxib (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) were dissolved in a polyoxyethylene castor oil (Cremophor®) vehicle (18:1:1, saline/Cremophor/ethanol). Pentylenetetrazol (Sigma-Aldrich), indomethacin (Sigma-Aldrich), diclofenac (Tokyo Chemical Industry Co., Ltd.), loxoprofen (Wako, Osaka, Japan), sodium valproate (Wako), pilocarpine hydrochloride (Wako), and methyl-scopolamine (Sigma-Aldrich) were dissolved in saline. All drugs were administered intraperitoneally (i.p.) or subcutaneously in a volume of 0.1–0.2 mL/10 g body weight. Acetaminophen, valproate, and NSAIDs were administered intraperitoneally 30 min before the test. Control groups were administered only the vehicle but in the same manner.

2.3. Maximal electroshock-induced seizure

Mice were stimulated with corneal electrodes from a stimulator (ECT Unit 57800-001; Ugo Basile, Comerio, Italy) using a suprathreshold current (60 Hz = 50 mA; shock duration 0.2 s; frequency 100 pulses/s; pulse width 0.5 ms). The electrodes were placed in saline (0.9% sodium chloride solution) before application. The animals were observed for 1 min after the stimulation. Tonic hind-limb extension was used as the criterion for convulsion. The ED_{50} value, which is the dose at which tonic hind-limb seizures are prevented in 50% of the animals, and the 95% confidence interval (CI) were calculated.

2.4. Acute pentylenetetrazol-induced seizure

Pentylenetetrazol 80 mg/kg i.p. was injected 30 min after administration of acetaminophen or valproate. The animals were observed for 20 min after the injection, and clonic and tonic seizures were monitored. The ED_{50} values and 95% CIs for drugs to prevent clonic seizures were calculated.

2.5. Corneal electroshock kindling

Kindling was induced by twice-daily stimulation on 12 consecutive days with a current intensity of 5.5 mA for 3 s delivered via corneal electrodes connected to a stimulator (SEN-3301 and SS-403J; Nihon Kohden, Tokyo, Japan). A drop of 0.5% tetracaine hydrochloride was placed on each eye before stimulation. The animals were observed for 1 min after the stimulation. The seizure intensity score was determined using the Racine scale (stages 0–5) as follows: 0, no response; 1, ear and facial twitching; 2, myoclonic body jerks; 3, forelimb clonus, rearing; 4, clonic seizures, turning onto the side; 5, generalized clonic seizures, turning onto the back (Racine, 1972). “Fully kindled” was defined as the occurrence of three consecutive stage 4 or 5 seizures. The kindled mice were pretreated with saline in the morning and then stimulated and observed for convulsive behavior. The animals were treated similarly in the afternoon after pretreatment with the vehicle, valproate, or acetaminophen. The ED_{50} values and 95% CI for drugs against clonic seizures (stage 4 or 5) were calculated.

2.6. Pentylenetetrazol kindling

Pentylenetetrazol kindling was induced by daily administration of pentylenetetrazol 40 mg/kg i.p. 5 days per week for 2 weeks. The animals were observed for 20 min after injection. The seizure intensity was scored using the same scale as that for corneal electroshock kindling. “Fully kindled” was defined as the occurrence of three consecutive stage 4 or 5 seizures. The kindled mice were pretreated with saline, injected with pentylenetetrazol, and observed for convulsive behavior. The same procedure was repeated the following day after pretreatment with vehicle, acetaminophen, or valproate. The ED_{50} values and 95% CI for drugs to prevent clonic seizures (stage 4 or 5) were calculated.

2.7. Pilocarpine-induced seizure

Mice were injected intraperitoneally with the vehicle, acetaminophen, or valproate. Five minutes later, scopolamine methyl bromide (1 mg/kg) was injected subcutaneously to prevent peripheral cholinergic activation. Ten minutes later, seizures were induced by injection of pilocarpine (300 mg/kg i.p.). Animals were observed for 1 h after the pilocarpine injection. The seizure intensity was scored using a modified Racine’s scale (stages 1–5) (Racine, 1972), as follows: 0, no response; 1, ear and facial twitching; 2, myoclonic body jerks; 3, clonus seizure; 4, tonic-clonic seizure; 5, generalized clonic seizure or status epilepticus.

2.8. Proconvulsive test

Proconvulsive activity was evaluated using an electroconvulsive shock test with low-stimulus currents according to Tanaka et al. (2014). The mouse cornea was stimulated with a current of 5.5 mA (60 Hz) for 3 s. A drop of saline was placed on the eye before stimulation. The animals were observed for 1 min after the stimulation. The seizure intensity was scored according to the Racine scale (stages 1–5), and the incidence of generalized seizure (seizure stage 4 or 5) was calculated.