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Pharmacological characterization of a novel gastrodin derivative as a potential anti-migraine agent

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ABSTRACT

Migraine is a highly prevalent neurovascular disorder in the brain. An optimal therapy for migraine has not yet been developed. Gastrodin (Gas), the main effective constitute from *Gastrodiae Rhizoma* (Tianma in Chinese), has been indicated for migraine treatment and prophylaxis more than 30 years, with demonstrated safety. However, Gas is a phenolic glycoside, with relatively low concentrations and weak efficacy in the central nervous system. To develop more effective anti-migraine agents, we synthesized a novel Gas derivative (Gas-D). In the present study, comparative pharmacodynamic evaluations of Gas and Gas-D were performed in a model of nitroglycerin (NTG)-induced migraine in rats and the hot-plate test in mice. Following behavioral testing in this migraine model, external jugular vein blood and the trigeminal nucleus caudalis (TNC) were collected to analyze plasma nitric oxide (NO) and calcitonin gene-related peptide (CGRP) concentrations and c-Fos expression in the TNC. The acute oral toxicity of Gas and Gas-D was also examined. We found that Gas-D had potent anti-migraine effects, likely attributable to inhibition of both trigeminal nerve activation at central sites and the peripheral release of CGRP following NO scavenging. Additionally, Gas-D exerted significant anti-nociceptive effect in response to thermal pain compared with Gas. Furthermore, a single dose of 2.048 g/kg Gas or Gas-D presented no acute oral toxicity in mice. Altogether, the potent anti-migraine and anti-hyperalgesic effects of Gas-D suggest that it might be a potentially novel drug candidate for migraine treatment or prophylaxis.

1. Introduction

Migraine is one of the most important primary headaches (*i.e.*, independent disorders that are not caused by other diseases) that require effective treatment or prophylaxis. Epidemiological studies have shown that migraine ranks seventh on the World Health Organization's list of all diseases that cause disability, thereby greatly decreasing quality of life in patients and having severe socioeconomic consequences [1,2]. Although several important pathophysiological events, such as the transmission of nociceptive impulses, vascular reactions, and neurogenic inflammation, are well known to be implicated in migraine attacks, the precise pathogenesis of migraine is still not well understood, which has greatly hampered drug treatment for migraine. The current therapeutic approach for migraine mainly consists of acute and prophylactic treatments. Acute treatment drugs that are used for symptomatic control mainly include 5-hydroxytryptamine-1B/1D receptor agonists (*e.g.*, triptans) and nonsteroidal antiinflammatory drugs (NSAIDs). Unfortunately, triptan-associated chest symptoms and NSAID-induced gastrointestinal and renal toxicity restrict their clinical use [3,4]. Almost all prophylactic drugs with variable mechanisms of action, such as the antihypertensive drug propranolol, antiepileptic drug valproate, and calcium antagonist flunarizine, were initially developed for other indications, followed by their application for migraine treatment [5–7]. These preventive agents generally have relatively low efficacy with unclear mechanisms of anti-migraine action. Calcitonin gene-related peptide (CGRP) receptor antagonists and monoclonal antibodies (mAbs) against CGRP or its receptor may be promising anti-migraine treatment [8]. However, the hepatotoxicity that is produced by CGRP receptor antagonists affects their clinical application [9] and mAbs are not available yet. Collectively, the optimal therapy for all migraine sufferers has not yet been developed. Therefore, there is a huge unmet need for more effective and safer therapies for migraine.

One of the leading hypotheses for the pathomechanism of migraine involves trigeminovascular system (TGVS) activation at peripheral and central sites and the subsequent release of endogenous vasodilating and algogenic mediators [10]. Nitric oxide (NO) and CGRP concentrations were significantly higher interictally in peripheral blood in migraine patients [11,12]. Previous studies have shown that NO and

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CGRP are crucial mediators of the generation of migraine [13]. And nitric oxide synthase (NOS) inhibition with the nonselective NOS inhibitor L-NMMA significantly improved migraine attacks, and CGRP receptor antagonists exerted anti-migraine effects through inhibition of CGRP receptor and spinal trigeminal activity [14,15]. Therefore, targeting NO and CGRP has been regarded as the potential strategies for the development of new anti-migraine drugs [8,16]. Nitroglycerin (NTG), a donor of NO, has been known for more than a century to induce primary headaches [17]. Recent studies showed that the systemic administration of NTG may trigger endogenous NO production, thereby inducing the release of CGRP in the trigeminal pain pathway [18,19]. Therefore, NTG administration in animal models is often used to screen anti-migraine drugs and perform different levels of analysis, including migraine-like behavioral responses, trigeminal nucleus caudalis (TNC) activation in the brainstem, and circulating levels of NO and CGRP [20,21].

Chinese medicines have a long history for the treatment of headache. *Gastrodiae Rhizoma*, called Tianma in Chinese, is the dried rhizome of *Gastrodia elata* Bl of the Orchidaceae family. Tianma was recorded in *Sheng Nong's Herbal Classic*, a famous compendium of Chinese medicines in the Han Dynasty (25 AD–220 AD), and has been widely used for the treatment of headaches for thousands of years. Modern phytochemical and pharmacological studies have shown that gastrodin (Gas; 4-[β-D-glucopyranosyloxy] benzyl alcohol; Fig. 1) is the most active constituent of Tianma [22]. It has been applied clinically in the treatment of migraine and other neurovascular headaches for more than 30 years. To date, however, the mechanism of anti-migraine action of Gas is unclear. Gas is a phenolic glycoside that has low blood–brain barrier (BBB) permeability; therefore, relatively low concentrations and weak activity of Gas are observed in the central nervous system (CNS) [23]. To enhance the efficacy of Gas for migraine treatment, we synthesized a series of Gas derivatives, one of which was a novel Gas ester derivative (Gas-D; Fig. 1), which contains a ferulate moiety and thereby confers on it a higher CNS profile. Ferulate is another common Chinese medicine for the treatment of cardiovascular and cerebrovascular diseases and prevention of thrombosis [24]. Moreover, ferulate is reported to be effective for migraine treatment in clinic [25], which is probably associated with the antioxidant activity of ferulate through direct clearance of oxygen free radicals [26]. We hypothesized that Gas-D might possess more potent anti-migraine actions by inhibiting trigeminal nerve activation at central sites and inhibiting the peripheral release of CGRP, followed by NO scavenging by ferulate. The aim of the present study was to compare the efficacy and safety of Gas and Gas-D for migraine treatment in animal models. Pharmacodynamic comparative studies of two compounds were first performed in a model of NTG-induced migraine in rats to evaluate hyperalgesia, c-Fos expression in the TNC, and plasma concentrations of NO and CGRP. The anti-nociceptive effects of Gas and Gas-D on acute thermal pain were also tested in mice, in addition to the evaluation of acute oral toxicity. The present preliminary pharmacodynamic and safety results suggest that Gas-D may be a drug candidate for migraine treatment or prophylaxis.

2. Materials and methods

2.1. Drug preparation

Gastrodin (Gas) was purchased from Chengdu, Must Bio-Technology Co., Ltd. (Chengdu, China). Gas-D was prepared by Dr. Chu Chen (Sichuan Academy of Chinese Medicine Sciences, Chengdu, China). Both drugs had >98% purity, determined by high-performance liquid chromatography (Fig. 1). Gas and Gas-D were suspended in 0.5% sodium carboxymethyl cellulose (CMC-Na) at the indicated

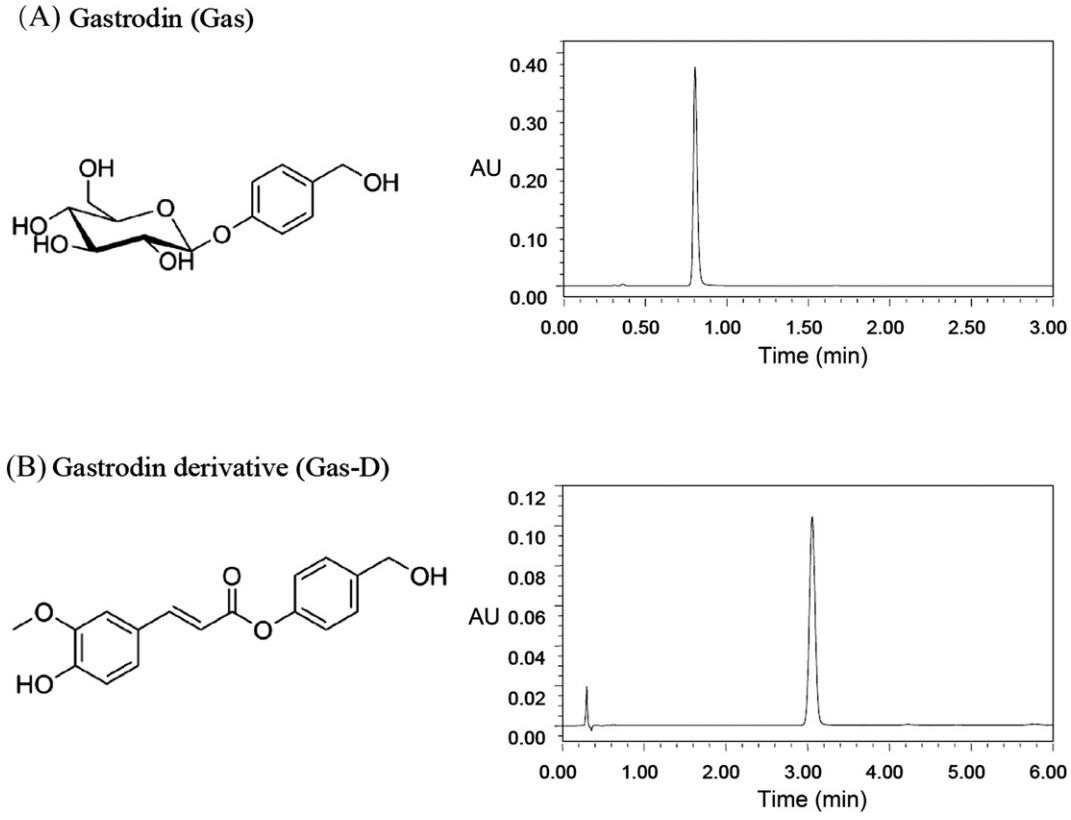

Fig. 1. Chemical structures and UPLC chromatograms of gastrodin (Gas) and its derivative (Gas-D). The separations were performed on a Waters BEH C18 (50 × 2.1 mm i.d., 1.7 μm) column. The mobile phase consisted of acetonitrile (A) and 0.1% aqueous phosphoric acid (v/v, B) using isocratic programs of 3% A for Gas and 25% A for Gas-D, respectively. The flow rate was 0.4 ml/min, and the column temperature was maintained at 30 °C. The detective wavelengths for Gas and Gas-D were set at 220 nm and 330 nm, respectively. As a result, the respective purities of Gas and Gas-D were 99.1% and 98.5% based on peak area normalization.