European Journal of Pharmacology 752 (2015) 40–48

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Immunopharmacology and inflammation

Anti-inflammatory effect of natural and semi-synthetic phthalides

Mayela Del-Ángel, Antonio Nieto, Teresa Ramírez-Apan, Guillermo Delgado *

Instituto de Química de la Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán, 04510 México, D.F, México

ARTICLE INFO

Article history:
Received 22 September 2014
Received in revised form
13 January 2015
Accepted 15 January 2015
Available online 23 January 2015

Chemical compounds studied in this article:
Z-ligustilide (PubChem CID: 529865)
diligustilide (PubChem CID: 70698035)
tokinolide B (PubChem CID: 70681959)

Keywords:
Inflammation
Semi-synthetic phthalides
12-*O*-tetradecanoylphorbol-13-acetate
Carrageenan
Myeloperoxidase
Oxide nitric synthase

ABSTRACT

This study evaluated the potential anti-inflammatory effects of natural phthalides, isolated from *Ligusticum porteri*, and of semi-synthetic phthalides. Anti-inflammatory activity was investigated in two mouse models; one with ear edema, induced with 12-*O*-tetradecanoylphorbol-13-acetate, and the other with paw edema, induced with carrageenan. The effect on the RAW 264.7 stimulated with lipopolysaccharide cells was evaluated and after application of 12-*O*-tetradecanoylphorbol-13-acetate, the activity of myeloperoxidase was assessed to serve as an index of leukocytes infiltration together with the histological evaluations. We also assessed the inhibition of cyclooxygenases 1 and 2 *in vitro*. Our results demonstrated that administration of semi-synthetic phthalides significantly inhibited the ear edema induced by 12-*O*-tetradecanoylphorbol-13-acetate, and reduced the paw edema caused by carrageenan. The anti-inflammatory activity of phthalides could, in part, be explained by the reduction in myeloperoxidase activity and the infiltration of leukocytes. The semi-synthetic phthalides also inhibited the production of oxide nitric in RAW cells.

1. Introduction

Inflammation is a response that link innate and adaptive immunity to soluble pro-inflammatory mediators as: leukotrienes, histamine, bradykinin, platelet activator factor, interleukins, and reactive oxygen species (Hawiger, 2001; Lo Faro et al., 2014). This response protects the body against infection and injury, but it can also produce deleterious consequences to the host (Gorzalczany et al., 2011; Hawiger, 2001). Neutrophils are infiltrating cells which represent the primary carriers of myeloperoxidase (MPO); thus, MPO is a biochemical marker of tissue neutrophil content (De Young et al., 1989; Laight et al., 1994; Lo et al., 1982). Other enzymes involved in inflammatory reactions include the cyclooxygenases (COXs), which produce prostaglandins (White, 1999), and the oxide nitric synthase (NOS). COX-1 is constitutively expressed; COX-2 is inducible by pro-inflammatory stimuli (Vane and Botting, 1998). The large amounts of oxide nitric produced by the NOS, mainly the inducible isoform, have shown to be harmful in the inflammatory process (Lo Faro et al., 2014). The inflammation is involved in various diseases, such as rheumatoid arthritis, inflammatory bowel disease, among others (Souren et al., 2012). For that reason, the development of new anti-inflammatory agents is of interest. Secondary metabolites from plants used in folk medicine constitute an important source for the search of new anti-inflammatory agents (Butler, 2005; Gorzalczany et al., 2011; Newman and Cragg, 2012; Payá et al., 1996; Souren et al., 2012).

Ligusticum porteri (Apiaceae) is a plant that grows in the northern Mexico and southern USA (Linares and Bye, 1987). This plant has been used traditionally by Rarámuri and Zuni communities for the treatment of bronchitis, stomachaches, and other ailments (León et al., 2011); root infusions have been applied topically to alleviate body aches (Linares and Bye, 1987). Several genera of the Apiaceae family produce phthalides (Beck and Chou, 2007). Natural phthalides isolated from the rhizome of *L. porteri* include *Z*-ligustilide (**1**), diligustilide (**2**), tokinolide B (**3**) and riligustilide (**4**) (Fig. 1). Several *in vitro* and *in vivo* studies have evaluated the anti-inflammatory effects of *Z*- ligustilide (**1**) (Chung et al., 2012; Liu et al., 2005; Wang et al., 2010; Zhaoji and Lunhao, 2012), diligustilide (**2**), and tokinolide B (**3**) (Huang et al., 2013). Other studies have investigated the chemical reactivities of some natural phthalides (Beck and Stermitz, 1995; Quiroz et al., 2003, 2004; Radcliff et al., 2008; Ríos et al., 1998a; 1998b). We previously reported the preparation of some semi-synthetic phthalides, including: ketoacid of tokinolide B (**5**), cyclotokinolide B (**6**), ketoester of tokinolide B (**7**), demethylwallichilide (**8**), diketodiacid of diligustilide (**9**) and an intramolecular condensation product (**10**) (Quiroz et al., 2003; Ríos et al. 1998a).

The present study was aimed to investigate the anti-inflammatory effects of natural and semi-synthetic phthalides (Fig. 1) in two mouse

* Corresponding author. Tel.: +52 55 56 22 44 45; fax: +52 55 56 16 22 17.
E-mail addresses: mayela.delangelm@comunidad.unam.mx (M. Del-Ángel), delgado@unam.mx (G. Delgado).

http://dx.doi.org/10.1016/j.ejphar.2015.01.026
0014-2999/

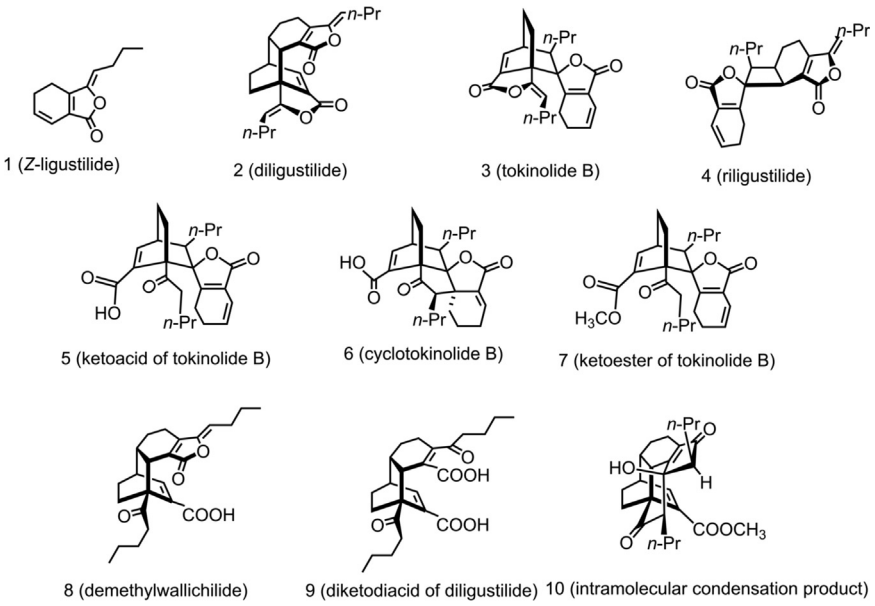

Fig. 1. Chemical structures of natural (**1**–**4**) and semi-synthetic phthalides (**5**–**10**).

models of inflammation. In one inflammation was induced by applying 12-*O*-tetradecanoylphorbol-13-acetate (TPA) to the ear. In the other model, edema was induced by injecting carrageenan into the hind paw. Furthermore, we evaluated the activities of MPO, COX-1, COX-2, and NOS.

2. Materials and methods

2.1. Isolation of compounds

All natural compounds were isolated from the dried rhizomes of *L. porteri* according to procedures described previously. The acetone extract was separated by column chromatography (SiO_2, gradient, *n*-hexane/EtOAc) to afford *Z*-ligustilide (**1**), diligustilide (**2**), tokinolide B (**3**) and riligustilide (**4**). These structures were confirmed by spectroscopic analyses and by direct comparison with authentic samples isolated previously in our laboratory.

2.1.1. Preparation of ketoacid of tokinolide B (*5*), cyclotokinolide B (*6*) and ketoester of tokinolide B (*7*)

Preparation of compounds **5**–**7** (Fig. 1) was carried out in accordance with the methodology described by Quiroz et al. (2003).

2.1.2. Preparation of demethylwallichilide (*8*), diketodiacid of diligustilide (*9*) and the intramolecular condensation product (*10*).

Preparation of compounds **8**–**10** (Fig. 1) was carried out in accordance with the methodology described by Ríos et al. (1998a).

2.2. Experimental animals

Experiments were conducted with adult male CD-1 mice (25–30 g) purchased from the Universidad Autónoma Metropolitana-Xochimilco, México, D. F. They were housed at 22 ± 2 °C under a 12 h light/12 h dark cycle, with access to food and water *ad libitum*. The mice were acclimatized for at least three days before testing and were used only once throughout the experiments. Experiments reported in this study were performed in accordance with the Mexican Official Regulations NOM-062-ZOO-1999.

2.2.1. 12-O-tetradecanoylphorbol acetate induced mouse ear edema

The natural and semi-synthetic phthalides were evaluated for their anti-inflammatory effects on dermal inflammation. Mice were anesthetized with an intraperitoneal (i.p.) injection of sodium pentobarbital (31.5 mg/kg, PiSA). Each mouse received a topical application on the right ear of 10 µl ethanolic TPA (0.25 mg/ml; Sigma-Aldrich) solution to induce edema. 10 min later, the same ear received 1 µmol of a natural or semi-synthetic phthalide in 20 µl of methanol (vehicle). Mice were divided into 11 treatment groups of 4 mice each, including: 1) TPA alone (positive control), 2) *Z*-ligustilide (**1**)+TPA, 3) diligustilide (**2**)+TPA, 4) tokinolide B (**3**)+TPA, 5) riligustilide (**4**)+TPA, 6) ketoacid of tokinolide B (**5**)+TPA, 7) cyclotokinolide B (**6**)+TPA, 8) ketoester of tokinolide B (**7**)+TPA, 9) demethylwallichilide (**8**)+TPA, 10) diketodiacid of diligustilide (**9**)+TPA, 11) intramolecular condensation product (**10**)+TPA. The left ears (negative control) received only 10 µl of ethanol and 20 µl of methanol. After 4 h, animals were euthanized in a CO_2 chamber; then, disks of ear tissue (7 mm in diameter) were removed from each ear and weighed. The edema, which represented inflammation, was defined as the difference in weight between the disks from right (treated) and left (negative control) ears. The percentage of edema inhibition was calculated with the formula:

$$\%\ \text{of inhibition} = [(C-E)/C] \times 100,$$

where

C=was the edema measured in the positive control group (TPA alone);

and

E=edema measured in the phthalide treatment group (Payá et al., 1996; Rao et al., 1993).

Phthalides that showed more than 50% inhibition of ear edema were selected for testing at different doses. The dose range was 0.031, 0.1, 0.31, 0.56, and 1 µmol in 20 µl of methanol (vehicle). Indomethacin (Sigma-Aldrich) was used as a reference anti-inflammatory drug. The treatment groups included 5–8 mice, and the same test procedure was repeated. A dose–response plot was analyzed with linear regression to determine the dose that provided 50% of inhibition (ID_{50}).

2.2.2. Carrageenan-induced hind paw edema in mice

The natural and semi-synthetic phthalides were evaluated for their anti-inflammatory effects on carrageenan-induced paw edema. Paw edema was induced with a subplantar injection of 0.05 ml 1% sterile lambda carrageenan in saline solution (0.9%) into the right hind paw.