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Anti-inflammatory and anti-hyperalgesic effects of sesquiterpene lactones from Magnolia and Bear's foot

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Abstract

Sesquiterpene lactones possess a variety of biological activities, including anti-inflammatory activity. Two plants native to the southeastern United States, *Magnolia grandiflora* (L.) and *Smallanthus uvedalius* (L.) [syn *Polymnia uvedalius* (L.)], are novel sources of the sesquiterpene lactones parthenolide and enhydrin, respectively. In this study, the anti-inflammatory and anti-hyperalgesic effects of these isolated lactones from these two plant sources were evaluated in the rat carrageenan inflammation model. Rats received ip injections of either vehicle (propylene glycol), indomethacin (5 mg/kg), 11,13-dihydroparthenolide (20 mg/kg), parthenolide (5 or 20 mg/kg) or enhydrin (5 or 20 mg/kg). A 100-μl injection of 2.0% carrageenan was made into the plantar surface of the right hindpaw. Paw withdrawal latencies and paw volumes in both inflamed and non-inflamed paws were recorded at four test intervals: pre-inflammation baseline (0 time point), and 1, 2 and 4 h post-carrageenan injection. Vehicle-treated animals exhibited a significant time-dependent hyperalgesic and edema response that was greatest at the 4-h test interval. Indomethacin significantly blocked the hyperalgesic response and modestly attenuated the edema response. Parthenolide (20 mg/kg) and enhydrin (20 mg/kg) significantly blocked the hyperalgesic response and significantly attenuated the edema response; 11,13-dihydroparthenolide did not affect either inflammation or hyperalgesia. These findings suggest that parthenolide and enhydrin from these plant sources may be useful in the treatment of inflammatory pain.

Keywords: *Magnolia grandiflora*; *Smallanthus uvedalius*; Parthenolide; Enhydrin; Anti-inflammatory; Analgesic

1. Introduction

Acute and chronic inflammatory processes are a major threat to human health and play an important role in the development of various diseases such as rheumatoid arthritis and arteriosclerosis. Natural compounds or compounds derived from natural leads can be used for treatment of inflammatory processes and there are records from traditional medicinal systems for plants being used for such purposes. For example, ethnomedicinal records from Native American Cherokee note the use of Bear's Foot (*Smallanthus uvedalius*) for its analgesic properties (Moerman, 1998) and Southern Magnolia (*Magnolia grandiflora*) for the treatment of fever, diarrhea, rheuma and arthritis (Schühly et al., 2001). It is interesting to note that *M. grandiflora* contains a number of sesquiterpene lactones, a class of lactones that are known to possess anti-inflammatory properties (Hall et al., 1980). The primary sesquiterpene lactone found in *M. grandiflora*, parthenolide, is also found in European feverfew (*Tanacetum parthenium*), a plant which has well-established effects for the treatment of migraine (Heptinstall and Awang, 1998) and inflammation and pain (Jain and Kulkarni, 1999; Schinella et al., 1998).

The mechanism of action of sesquiterpene lactones, such as parthenolide derived from *T. parthenium*, has been found

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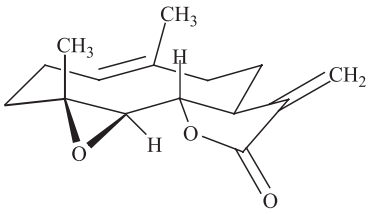
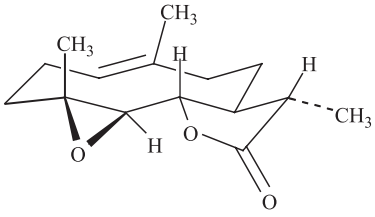
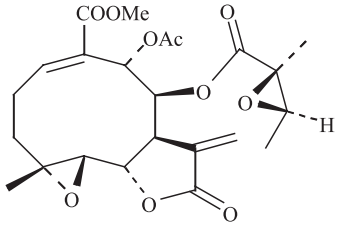


Parthenolide M.W. 248



11,13-Dihydroparthenolide M.W. 250



Enhydrin M.W. 464

Fig. 1. The chemical structure of parthenolide, 11,13-dihydroparthenolide, and enhydrin.

to inhibit both cyclooxygenase (COX-2) and pro-inflammatory cytokines in macrophages (Hwang et al., 1996). These processes represent an early stage of the inflammatory cascade. The activity of parthenolide is related to the α-methylene-γ-lactone moiety and to the presence of an epoxide group of the molecule. These functional groups may cause alkylations of sulfhydryl-groups of proteins (Hwang et al., 1996). The absence of these structural components in the inactive lactone 11,13-dihydroparthenolide, derived from *Ambrosia artemisiifolia*, confirms this view. Furthermore, enhydrin, derived from *S. uvedalius*, shows very similar activity to parthenolide in this in vitro system (see Fig. 1). To our knowledge, parthenolide, 11,13-dihydroparthenolide and enhydrin derived from these plant sources (*M. grandiflora*, *A. artemisiifolia*, *S. uvedalius*, respectively) have not been evaluated in an in vivo model of inflammatory nociception. Thus, the present study sought to explore the anti-inflammatory and anti-hyperalgesic effects of these lactones from these plants using the rat carrageenan model of inflammatory nociception.

2. Materials and methods

2.1. Plant material

M. grandiflora (Magnoliaceae) leaves were collected on the Campus of University of Mississippi in the Fall of 2001. A voucher specimen (voucher number WS-4) is deposited at the Pullen Herbarium at the University of Mississippi.

2.2. Extraction and isolation

The crude plant material of *M. grandiflora* was dried at room temperature, ground and exhaustively extracted with dichloromethane (DCM). Parthenolide was isolated from this DCM crude extract by a previously described method (Castaneda-Acosta et al., 1993); its spectra (NMR, MS) were in full accord with our previously reported data (Castaneda-Acosta et al., 1993). Enhydrin and 11,13-dihydroparthenolide were obtained from the natural products repository of one of the authors (N.H.F.). Enhydrin had been previously isolated from the aerial parts of *S. uvedalius* (Asteraceae) (Tak et al., 1994) and 11,13-dihydroparthenolide was obtained from aerial parts of common ragweed, *A. artemisiifolia* L. (Asteraceae) (Parodi et al., 1989). The spectral data of enhydrin (Tak et al., 1994) and 11,13-dihydroparthenolide (Parodi et al., 1989) used for the present study were in full agreement with our previously reported physical data.

2.3. Subjects

Male Holtzman rats (225–250 g, Harlan, Indianapolis, IN, USA) were obtained and individually housed in suspended stainless steel cages (18×25×18 cm) with food (Purina 5001 Laboratory Rodent Chow, St. Louis, MO, USA) and water available ad libitum. Room temperature was maintained at 22±1 °C, and overhead fluorescent illumination was maintained on a 12-h light–dark cycle. Animals received 3–4 days of handling and 2 days of apparatus habituation prior to treatment.

2.4. Procedure

Groups in this study formed a two-way repeated-measures factorial design that combined seven drug treatments with four tests intervals. The drug treatments were vehicle, indomethacin (5 mg/kg), 11,13-dihydroparthenolide (20 mg/kg), parthenolide (5 or 20 mg/kg) or enhydrin (5 or 20 mg/kg). The four test intervals were a 0-time point pre-inflamed baseline and 1, 2 and 4 h post-carrageenan injection test intervals. The vehicle was propylene glycol and sample sizes were 9–10 per cell.

Immediately following pre-inflammation baseline measures, rats received ip injections of either vehicle or drug probe. These injections were immediately followed by administration of 100 μl of 2.0% carrageenan into the plantar surface of the right hindpaw. Animals were returned to their home cage until subsequent testing. A Plantar Analgesiometer (Model 390, IITC/Life Sciences Instruments, Woodland Hills, CA, USA) was used to index thermal hyperalgesia (Hargreaves et al., 1988). Mean paw withdrawal latency from a focused beam of light on two

consecutive tests served as the measure of thermal nociception. A 20-s cutoff was employed for animals that did not respond. A Ugo Basile Plethysmometer (Model 7140, Ugo Basile, Italy) was used to quantify paw volume via fluid displacement. Nociceptive and paw volume measures were taken on both the right (inflamed) and the left (non-inflamed) feet. Nociceptive and inflammation scores (as presented in Figs. 2 and 3) were derived by subtracting left from right foot measures. These procedures were approved by the University of Mississippi IACUC (Protocol # 02-023) and conducted in accordance with the ethical guidelines specified by the Animal Welfare Act and the National Institutes of Health guide for the care and use of laboratory animals (Publication no. 85-23, revised 1985).

Data were analyzed using two-way repeated measures analysis of variance (ANOVA) and one-way simple effects ANOVA. Post hoc analyses were performed using Fisher's LSD. For reasons of clarity, the 11,13-dihydroparthenolide and parthenolide data (A) and enhydrin data (B) will be presented in separate panels within each figure. For comparative purposes, vehicle and indomethacin data are presented in both panels.

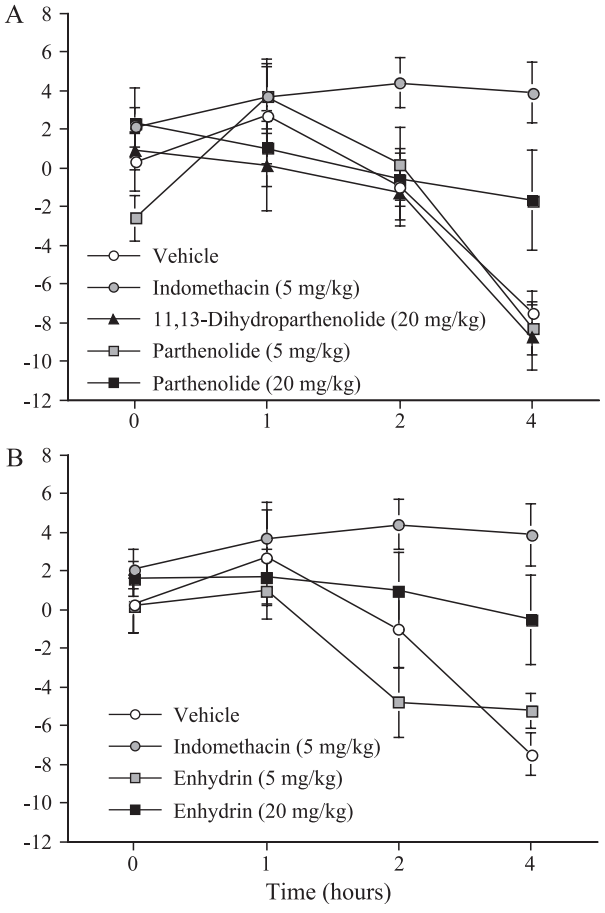


Fig. 2. The effects of vehicle, indomethacin, 11,13-dihydroparthenolide, parthenolide and enhydrin on carrageenan-induced thermal hyperalgesia. Data are presented as the mean (±S.E.M.) difference between inflamed minus non-inflamed paw withdrawal latencies (sec). Negative scores indicate carrageenan-induced hyperalgesia.

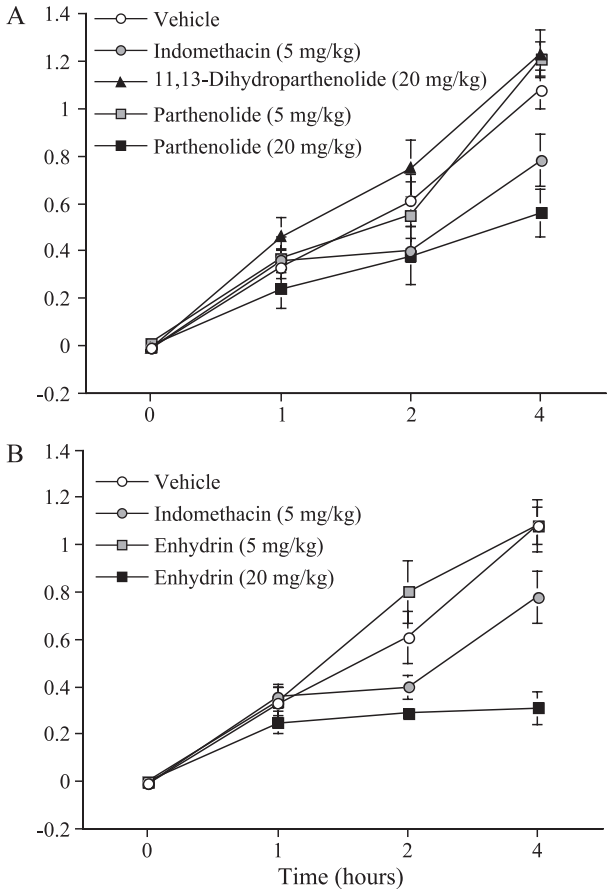


Fig. 3. The effects of vehicle, indomethacin, 11,13-dihydroparthenolide, parthenolide and enhydrin on carrageenan-induced edema. Data are presented as the mean (±S.E.M.) difference between inflamed minus non-inflamed paw volumes (ml). Positive scores reflect carrageenan-induced edema.

3. Results

The effects of vehicle, indomethacin, 11,13-dihydroparthenolide, parthenolide and enhydrin on thermal hyperalgesia are summarized in Fig. 2A and B. Vehicle-treated animals exhibited a time-dependent thermal hyperalgesic response that was blocked in animals treated with indomethacin. Parthenolide (20 mg/kg) and enhydrin (20 mg/kg) similarly blocked this thermal hyperalgesic response whereas 11,13-dihydroparthenolide did not. A two-way ANOVA revealed significant main effects for drug [F (6, 183)=4.509, p<0.001] and time [F (3, 183)=19.810, p<0.0001], and a significant drug×time interaction [F (18, 183)=2.433, p<0.005]. A simple effects ANOVA for the vehicle-treated group across test intervals revealed a significant main effect for time [F (3, 24)=7.525, p=0.001] and post hoc analyses demonstrated a significant hyperalgesic effect at the 4-h test interval, p<0.01. These findings

prompted a second simple effects ANOVA across drug groups at the 4-h test interval and revealed a significant main effect [F (6, 61)=9.842, p<0.0001]. Post hoc analyses revealed that indomethacin, 20 mg/kg parthenolide and 20 mg/kg enhydrin significantly attenuated hyperalgesic responses, p<0.0001, 0.05, and 0.01, respectively.

The effects of vehicle, indomethacin, 11,13-dihydroparthenolide, parthenolide and enhydrin on paw volume difference scores are summarized in Fig. 3A and B. Vehicle-treated animals exhibited a time-dependent paw edema response that was modestly attenuated in animals treated with indomethacin. Parthenolide (20 mg/kg) and enhydrin (20 mg/kg) produced a significant attenuation of edema scores whereas 11,13-dihydroparthenolide did not. A two-way ANOVA revealed significant main effects for drug [F (6, 183)=5.125, p<0.0005] and time [F (3, 183)=230.384, p<0.0001] and a significant drug×time interaction [F (18, 183)=6.336, p<0.0001]. These results prompted our use of a simple effects ANOVA for the vehicle-treated group across test intervals and revealed a significant main effect for time [F (3, 24)=46.683, p<0.0001]. Post hoc analyses demonstrated significant edema responses at the 1-, 2- and 4-h test intervals, p^s<0.005, 0.0001 and 0.0001, respectively. For consistency with the thermal hyperalgesia analyses, a single simple effect ANOVA across drug groups at the 4-h test interval was conducted which revealed a significant main effect [F (6, 61)=29.732, p<0.0001]. Post hoc analyses demonstrated that 20 mg/kg parthenolide and 20 mg/kg enhydrin significantly attenuated edema responses, p<0.005 and 0.0001, respectively.

4. Discussion

The carrageenan model is a well-established paradigm for studying inflammatory nociception (Winters et al., 1962; Joris et al., 1990). Consistent with other reports, carrageenan-induced hyperalgesia and edema peaked at 4 h post-injection (Vincent et al., 1978; Zhang et al., 2002). Treatment with indomethacin significantly blocked the hyperalgesic response and modestly attenuated the edema response. These findings are consistent with earlier reports that NSAIDs affect inflammatory nociception (Insel, 1990). Parthenolide and enhydrin demonstrated anti-hyperalgesic and anti-inflammatory properties, with enhydrin producing greater anti-inflammatory effects than parthenolide. These observations are consistent with earlier reports (Hwang et al., 1996) that sesquiterpine lactones are highly active in an in vitro COX-2 assay. As expected, 11,13-dihydroparthenolide was ineffective at reversing carrageenan-induced inflammatory nociception and this finding is consistent with the observation that the absence of an α-methylene-γ-lactone moiety and an epoxide group renders this sesquiterpine lactone inactive in the COX-2 assay. The ability of these sesquiterpine lactones to modulate edema and hyperalgesia in the carrageenan model is most likely due to their ability to inhibit COX-2 as well as the pro-inflammatory cytokines in macrophages. These findings suggest that parthenolide and enhydrin derived from *M. grandiflora* and *S. uvedalius*, respectively, may be useful in the treatment of inflammation and pain.

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