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Anti-inflammatory effects of the essential oils of ginger (*Zingiber officinale* Roscoe) in experimental rheumatoid arthritis

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ABSTRACT

Ginger and its extracts have been used traditionally as anti-inflammatory remedies, with a particular focus on the medicinal properties of its phenolic secondary metabolites, the gingerols. Consistent with these uses, potent anti-arthritic effects of gingerol-containing extracts were previously demonstrated by our laboratory using an experimental model of rheumatoid arthritis, streptococcal cell wall (SCW)-induced arthritis. In this study, anti-inflammatory effects of ginger's other secondary metabolites, the essential oils (GEO), which contain terpenes with reported phytoestrogenic activity, were assessed in female Lewis rats with SCW-induced arthritis. GEO (28 mg/kg/d ip) prevented chronic joint inflammation, but altered neither the initial acute phase of joint swelling nor granuloma formation at sites of SCW deposition in liver. Pharmacologic doses of 17-β estradiol (200 or 600 μg/kg/d sc) elicited the same pattern of anti-inflammatory activity, suggesting that GEO could be acting as a phytoestrogen. However, contrary to this hypothesis, GEO had no *in vivo* effect on classic estrogen target organs, such as uterus or bone. En toto, these results suggest that ginger's anti-inflammatory properties are not limited to the frequently studied phenolics, but may be attributable to the combined effects of both secondary metabolites, the pungent-tasting gingerols and as well as its aromatic essential oils.

1. Introduction

Ginger (*Zingiber officinale* Roscoe, Zingiberaceae), a commonly used botanical in the United States [1], is primarily known for its anti-emetic properties [2]. However, it has also been used medicinally since antiquity as an anti-inflammatory [3–5]. In modern usage, particular attention has focused on the cyclooxygenase (COX)-inhibiting effects of the gingerols, phenolic compounds that are responsible for ginger's pungent taste, and their potential use in treating inflammatory disorders such as arthritis [4,5]. Previously, we demonstrated potent anti-arthritic effects of gingerol-containing extracts of ginger in an experimental model of rheumatoid arthritis (RA) [6]. However, crude extracts containing both of ginger's secondary metabolites, the gingerols and the essential oils, were even more potent in inhibiting joint swelling than gingerols alone [6]. Having previously demonstrated anti-arthritic effects of both the phenolic and essential oil fractions of turmeric (*Curcuma longa* L., Zingiberaceae), a plant that is botanically and chemically related to ginger [7–10], we postulated that the essential oils of ginger could similarly be bioactive with respect to inhibition of joint inflammation and thus contribute to ginger's potential anti-arthritic effects.

To test this postulate, studies were undertaken to examine the joint protective effects of the isolated essential oils of ginger (GEO), secondary metabolites that are responsible for ginger's characteristic aroma [11,12]. For these studies, the streptococcal cell wall (SCW)-induced arthritis model of RA previously employed by our laboratory to test other ginger (and turmeric) extracts was employed to facilitate comparisons with chemically-related extracts [6–10]. In this model, the inflammatory reaction in response to streptococcal cell wall (SCW) deposition within joints recapitulates the histopathology of RA; female Lewis rats develop an initial, transient phase of joint swelling that is characterized by an influx of neutrophils and other inflammatory cells (acute phase,

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days 0–5), followed by a recrudescence of joint swelling that is associated with synovial hyperplasia and progressive destruction of periarticular bone by the invading synovium (chronic phase, days 10–28) [6–10,13]. Additionally, classic granulomas form within the liver at sites of hepatic SCW deposition [6,7,10,14], an inflammatory response that can be protective in certain settings, such as pulmonary tuberculosis where invading bacilli are walled off within granulomas, thus helping to quell the spread of infection [15,16].

In our previous studies, SCW-induced arthritis and granulomatous inflammation were each more effectively blocked by a crude ginger extract containing GEO and gingerols as compared to a gingerol-only fraction [6]. The crude extract almost completely prevented both phases of joint swelling (93% and 97% inhibition of acute and chronic arthritis, respectively), while the gingerol-only fraction was less effective (78% and 62% inhibition, respectively) [6]. The crude extract also blocked granulomatous inflammation by 76%, while the gingerol-only fraction was without effect [6]. Therefore, effects of isolated GEO on joint inflammation and the granulomatous hepatic response were tested here using the SCW model. In addition, because estrogenic effects have been reported *in vitro* for certain monoterpenes present in GEO [17,18], *in vivo* treatment effects of GEO in the SCW model were compared to those of 17-β estradiol (E_2). While joint protective effects of estrogen have previously been reported in pre-clinical RA models and have been postulated for RA itself due to the clinical observation of improved disease activity during pregnancy [19], effects of estrogen in the SCW-model in female rats have, to our knowledge, not previously been reported.

2. Material and methods

2.1. Preparation of GEO

A crude ginger extract was prepared as previously described by extracting ground ginger rhizome (2500 g) with CH_2Cl_2 (dichloromethane) at 25 °C for 36 h (6.4% yield) [6,12]. After filtration, washing and work up, 40 g of the resultant extract ("crude ginger extract") were applied to a silica gel column and sequentially eluted with solvents of increasing polarity to yield fractions 1 through 11, which were chemically characterized by HPLC (Fig. 1C) and/or GC–MS and screened *in vitro* for their ability to inhibit PGE_2 production from an LPS-stimulated human macrophage cell line, as previously described [6,12]. For the studies reported here, fraction 1, a lipophilic, sesquiterpene-containing gingerol-free fraction was used (23% yield, 'GEO'; Fig. 1A) [12]. In previous SCW experiments, essential oil-free fractions (fractions 4–9) containing gingerols and their derivatives, as identified by HPLC and GC–MS, were combined to constitute a single "gingerol fraction" (Fig. 1B) (approximately 50% yield) that was used for *in vivo* testing [6,12].

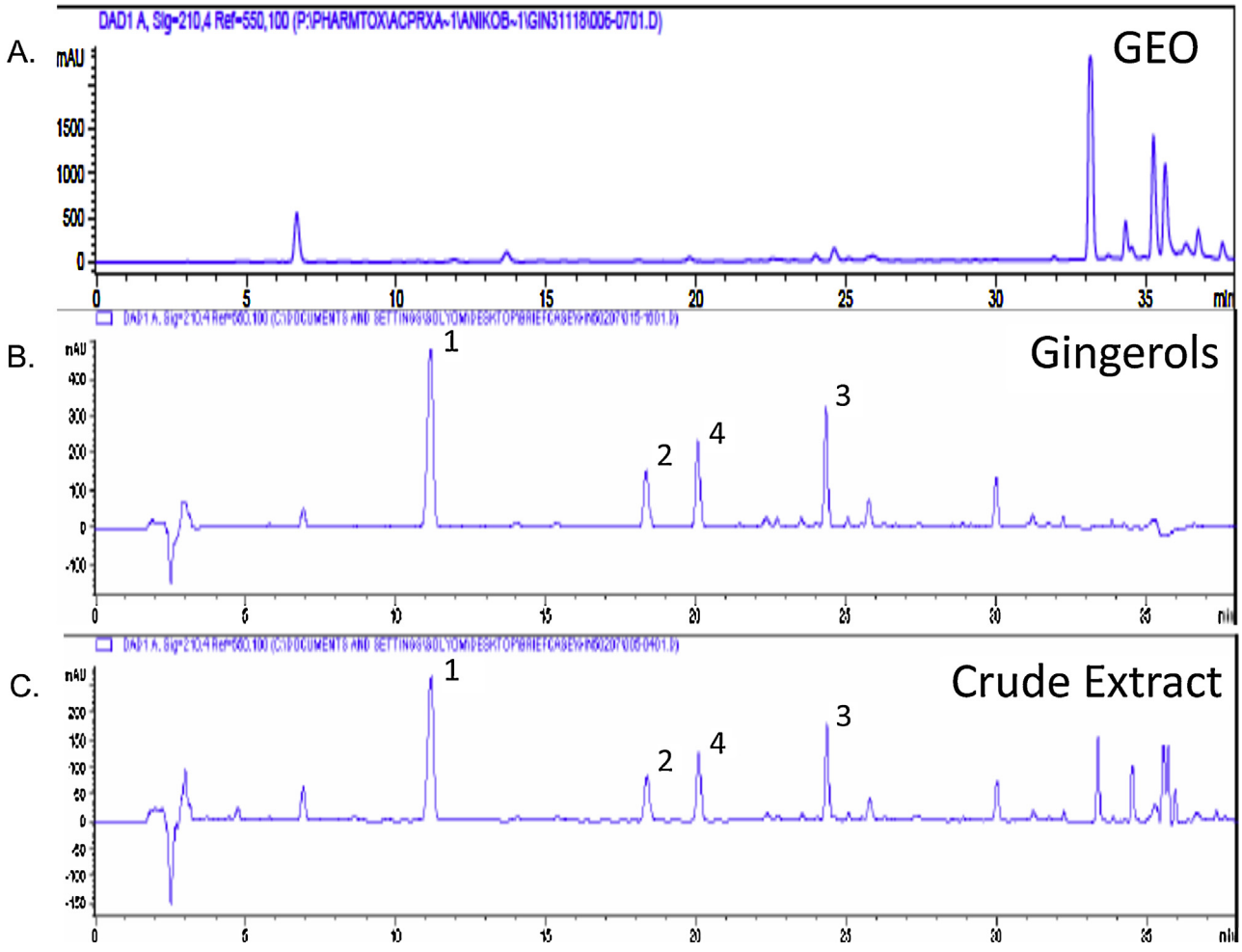

Fig. 1. HPLC-UV profiles (λ = 250 nm) of GEO (A), a gingerol-only fraction (B), and the crude DCM extract from which the GEO and gingerol fractions were derived (C). The 3 major gingerols (1, [6]-gingerol; 2, [8]-gingerol; 3, [10]-gingerol) and a primary gingerol degradation product (4, [6]-shogaol) are not present in the GEO fraction.