Journal of Ethnopharmacology 190 (2016) 313–318

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Effect of leaf digestion and artemisinin solubility for use in oral consumption of dried *Artemisia annua* leaves to treat malaria

Matthew R. Desrosiers [1], Pamela J. Weathers [*,1]

Department of Biology & Biotechnology, Worcester Polytechnic Institute, 100 Institute Rd., Worcester, MA 01609, USA

ARTICLE INFO

Article history:
Received 25 February 2016
Received in revised form
19 May 2016
Accepted 15 June 2016
Available online 20 June 2016

Classification:
Gastro-intestinal system

Keywords:
Malaria
Bioavailability
Flavonoids
Essential oils
Sesquiterpenoids
Artemisinin
Digestion
pACT

ABSTRACT

Ethnopharmacological relevance: Artemisia annua L. produces the antimalarial sesquiterpene lactone, artemisinin (AN), and was traditionally used by the Chinese to treat fever, which was often caused by malaria.

Aim of the study: To measure effects of plant-based and dietary components on release of artemisinin and flavonoids from *A. annua* dried leaves (DLA) after simulated digestion.

Materials and methods: Simulated digestion was performed on DLA in four types of capsules, or in conjunction with protein, and protein-based foods: dry milk, casein, bovine serum albumin, peanuts, peanut butter, Plumpy'nut®, and *A. annua* essential oils. Artemisinin and total flavonoids were measured in the liquid phase of the intestinal stage of digestion.

Results: After simulated digestion, peanuts and Plumpy'nut® lowered AN and flavonoids, respectively, recovered from the liquid digestate fraction. None of the compositions of the tested capsules altered AN or flavonoid release. Surprisingly, bovine serum albumin (BSA) increased both AN and flavonoids recovered from liquid simulated digestate fractions while casein had no effect. AN delivered as DLA was about 4 times more soluble in digestates than AN delivered as pure drug. Addition of a volume of essential oil equivalent to that found in a high essential oil producing *A. annua* cultivar also significantly increased AN solubility in simulated digestates.

Conclusion: These results indicate encapsulating DLA may provide a way to mask the taste of *A. annua* without altering bioavailability. Similarly, many peanut-based products can be used to mask the flavor with appropriate dosing. Finally, the essential oil fraction of *A. annua* contributes to the increased AN solubility in DLA after simulated digestion. Our results suggest that use of DLA in the treatment of malaria and other artemisinin-susceptible diseases should be further tested in animals and humans.

1. Introduction

In 2015, there were 214 million cases of malaria resulting in 438,000 deaths worldwide (WHO, 2016). Of these deaths, 91% occurred in sub-Saharan Africa and 70% of the victims were children under 5 (WHO, 2016). Since 2000, malaria incidence and death rates have decreased globally by 37% and 60%, respectively, however progress has been slower in sub-Saharan Africa (WHO, 2016). The foremost therapeutic used to quell malaria worldwide is artemisinin (AN, Fig. 1), but due to poor solubility, AN semi-synthetic derivatives, e.g. artesunate, dihydroartemisinin and artemether, are the preferred drugs. AN is a naturally occurring sesquiterpene lactone produced and stored in the glandular trichomes of the plant *Artemisia annua* L. (Ferreira and Janick, 1995). AN derivatives are combined with other antimalarials, e.g. artemether+lumefantrine (Coartem®) to slow the evolution of AN resistance, termed artemisinin combination therapies (ACTs), and are recommended by the WHO for treatment of malaria (WHO, 2015). Although ACTs are accepted as the frontline treatment for malaria, they are often too expensive or unavailable to those in need (Davis et al., 2013; Kyaw et al., 2014; Yeung et al., 2008). Indeed the highest malaria mortality rates occur in regions of the world with the highest proportions of people living on $<$ $1.25/day (WHO, 2012).

Recently, use of dried leaves of *A. annua* (DLA; aka pACT) to treat malaria has shown promise. This generally recognized as safe (GRAS) medicinal plant (Duke, 2001) has been used since 168 BCE in traditional Chinese medicine to treat a variety of conditions including "fever", which was likely caused by malaria (Cui and Su, 2009). Traditionally the plant was prepared as a tea infusion however, this mode of preparation is not recommended as it is difficult to control the many parameters, such as temperature and time, which dictate phytochemical extraction and stability (van

* Corresponding author.
E-mail address: weathers@wpi.edu (P.J. Weathers).
[1] Author contributions: MRD conducted all experiments and analyzed data; MRD and PJW designed experiments and wrote the manuscript.

http://dx.doi.org/10.1016/j.jep.2016.06.041
0378-8741/

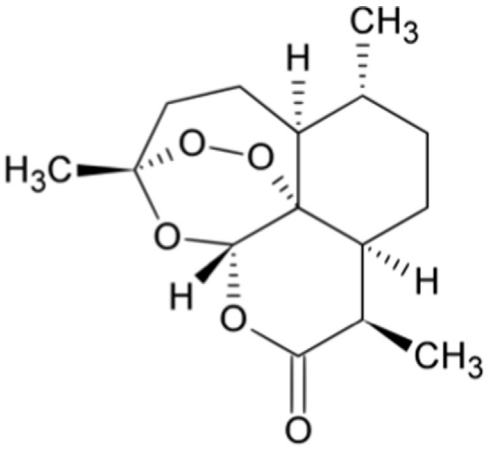

Fig. 1. Structure of artemisinin (AN).

der Kooy and Verpoorte, 2011; Weathers and Towler, 2012). Instead, *per os* (*p.o.*) consumption of DLA should be used to ensure administration of a consistent dose. In a rodent study, *p.o.* DLA delivery of AN was compared to *p.o.* delivery of pure AN (Weathers et al., 2011), and DLA provided ~45 times more AN in the serum than delivered from similar doses of pure AN. Furthermore, delivery as DLA was five times more effective at reducing parasitemia than pure AN in mice infected with *Plasmodium chabaudi* (Elfawal et al., 2012) and three times more resilient against emerging AN drug resistance (Elfawal et al., 2015).

A. annua is also rich in a variety of other compounds including flavonoids, phenolic acids, terpenes, coumarins, saponins, and essential oils (Elford et al., 1987; Ferreira et al., 2010; Lehane and Saliba, 2008; Suberu et al., 2013; Van Zyl et al., 2006). Many of these compounds have weak activity against malaria (Weathers and Towler, 2014) and some have been shown to synergize with AN (Liu et al., 1992; Suberu et al., 2013). For these reasons this plant-based artemisinin combination therapy (pACT) may provide an effective, inexpensive treatment option for those in extreme poverty.

One drawback to using DLA is the bitter taste associated with the dried leaves. Although preliminary data suggested about 61% of humans find the leaves distasteful, others actually like the taste (Supplemental Table 1). Nevertheless, masking the unpalatable taste with readily available food items or by encapsulation is desirable, especially for pediatric patients. Encapsulation or alternative taste masking is only feasible, however, if the capsules or foodstuffs do not significantly alter the bioavailability of the therapeutic compounds. In this study, we use a simulated human digestion system to investigate how various food items, pure proteins, and capsules affected AN and flavonoid content of intestinal stage digestates. We also used simulated digestion to investigate the solubility of AN in intestinal stage digestates of pure drug vs. DLA. Our results also suggest a possible partial mechanism for the increased bioavailabilty of AN when delivered as DLA vs. as pure drug.

2. Methods

2.1. Plant material

Two *Artemisia annua* L. clonal cultivars propagated by rooted cuttings (Towler and Weathers, 2015; Weathers and Towler, 2012) were used in this study: SAM (DLAS) (voucher MASS 317314), a high AN-producing cultivar (~1.4% w/w), and GLS (DLAG) (vouchers OR State Univ 171772 and 170353), a glandless AN-null mutant cultivar with no glandular trichomes that produces no AN (Duke et al., 1994) and 25% of the flavonoids found in SAM. SAM plant material used in protein and dietary constituent experiments was field-grown in Stow, MA, harvested at floral budding stage, dried and processed as previously described (Weathers et al., 2014b). SAM plant material used in solubility experiments was grown in the lab under glass-filtered sunlight, harvested at the vegetative stage, dried, and processed same as the field-grown SAM. GLS, a gift from Dr. Stephen Duke at University of Mississippi, was grown in the lab, under glass-filtered sunlight, harvested in the vegetative stage, dried, and processed same as SAM.

2.2. Chemicals and capsules

Chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified. Toluene was purchased from Thermo Fisher Scientific (Waltham, MA, USA) and *A. annua* essential oils from Bella Mira (Mannford, OK, USA). Vegetable capsules made from hydroxypropyl methylcellulose (HPMC) and water and gelatin capsules made from beef gelatin and water were purchased from Capsule Connection LLC (Prescott, AZ, USA). Vcaps®, Vcaps Plus®, and Plantcaps™ were a gift from Capsugel (Morristown, NJ, USA). Vcaps® are made of HPMC and a proprietary combination of gelling agents while Vcaps Plus® are made without the gelling agents. Plantcaps™ are made from pullulan, a polysaccharide polymer fermented from tapioca. All capsules were size "00." Evaporated milk, smooth peanut butter, and plain unsalted peanuts were store brand purchased from a local Shaw's Supermarket (Stow, MA USA). Plumpy'nut®, made from peanut based paste, is a Ready to Use Therapeutic Food (RUTF; USAID 2015, https://www.usaid.gov/what-we-do/agriculture-and-food-security/food-assistance/resources/ready-use-therapeutic-food Accessed 12-28-15) for treating malnutrition. Plumpy'nut® is produced locally by Edesia (Providence, RI, USA) and was a gift from Maternova Inc. (Providence, RI, USA).

2.3. Simulated digestion

Simulated digestion was performed according to Weathers et al. (2014a) (Fig. 2). All digestions were taken to the intestinal stage before being vortexed and filtered through Whatman #1 chromatography paper (0.16 mm thickness, porosity $< 10\,\mu m$) to separate solid and liquid digestate fractions. Liquid fractions were extracted in a sonicating water bath for 30 min with an equal volume of toluene to yield a clear two-phase separation to extract AN and flavonoids for analysis. Artemisinin but not all flavonoids are extracted by toluene. This solvent was required to obtain good

Fig. 2. Schematic for simulated digestion method.