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Improved absorption and bioactivity of active compounds from an anti-diabetic extract of *Artemisia dracunculus* L☆

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ABSTRACT

An ethanolic extract of *Artemisia dracunculus* L. (PMI-5011) was shown to be hypoglycemic in animal models for Type 2 diabetes and contains at least 6 bioactive compounds responsible for its anti-diabetic properties. To evaluate the bioavailability of the active compounds, high fat dietary induced obese C57BL/6J male mice were gavaged with PMI-5011 at 500 mg/kg body weight, after 4 h of food restriction. Blood plasma samples (200 uL) were obtained after ingestion, and the concentrations of the active compound in the blood sera were measured by electrospray LC-MS and determined to be maximal 4–6 h after gavage. Formulations of the extract with bioenhancers/solubilizers were evaluated *in vivo* for hypoglycemic activity and their effect on the abundance of active compounds in blood sera. At doses of 50–500 mg/kg/day, the hypoglycemic activity of the extract was enhanced 3–5-fold with the bioenhancer Labrasol, making it comparable to the activity of the anti-diabetic drug metformin. When combined with Labrasol, one of the active compounds, 2′, 4′-dihydroxy-4-methoxydihydrochalcone, was at least as effective as metformin at doses of 200–300 mg/kg/day. Therefore, bioenhancing agents like Labrasol can be used with multicomponent botanical therapeutics such as PMI-5011 to increase their efficacy and/or to reduce the effective dose.

1. Introduction

PMI-5011 is a botanical extract prepared from *Artemisia dracunculus* L. (Russian tarragon), a culinary herb with anti-diabetic properties. PMI-5011 treatment decreases blood glucose concentrations in streptozotocin-induced diabetic rats and genetically diabetic KK.Cg-A^y/+ (KK-A^y) mice but does not effect blood glucose concentrations in non-diabetic mice or rats (Ribnicky et al., 2006). The historical use of the plant, together with recent chronic toxicology studies and AMES testing suggest that the extract is safe (Ribnicky et al., 2004). Biological activities associated with the anti-diabetic effects of PMI-5011 include the stimulation of insulin-mediated glucose uptake into cultured skeletal muscle cells (Cefalu et al., 2008), inhibition of PEPCK (regulator of hepatic glucose output) expression in cultured hepatocytes and in the liver tissue of diabetic animals (Cefalu et al., 2008; Ribnicky et al., 2006) and enhancement of insulin sensitivity via a reduction of phosphastase activities such as PTP1-B (Wang et al., 2006). Therefore, several modes of action may contribute to the anti-diabetic activity of PMI-5011 observed *in vivo*, suggesting that it contains multiple bioactive compounds.

Extensive bioactivity guided fractionation of PMI-5011 using *in vitro* assays, led to the isolation of 6 compounds which may contribute to the anti-hyperglycemic activity observed *in vivo* (Schmidt et al., 2008). The availability of the functional *in vitro* assays used for the characterization and standardization of the extract was also essential for the identification of the active components. Bioactivity observed *in vitro*, however, does not ensure a corresponding activity *in vivo*. Obtaining sufficient quantities of pure compounds from botanical mixtures for *in vivo* testing, however, can be difficult especially when the putative bioactives are present at low concentrations, thus complicating *in vivo* validation of bioactivity (Ribnicky et al., 2008). However, analysis of the active compounds in the blood plasma of treated animals helps to establish a correlation between the *in vitro* and *in vivo* activities. In addition, the relationship between plasma concentrations of active compounds and their associated bioactivity can be used to evaluate bioavailability.

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Many bioactive compounds from plants, including compounds contained in food, are poorly bioavailable because of weak absorption and/or rapid elimination (Scalbert and Williamson, 2000). For example, the anthocyanins from blackberry, one of the richest sources of anthocyanins that serve as natural antioxidants, are less than 1% bioavailable in rats (Felgines et al., 2002). In fact, the role of dietary antioxidants as defense against free radical damage is currently being questioned since they are found at only micro and nano molar concentrations *in vivo* (Holst and Williamson, 2008). Polyphenols, including anthocyanins, are a large class of phytochemicals that are the focus of research in many areas of disease prevention and treatment. A number of factors are noted to impact the bioavailability of polyphenols, such as growing conditions of the plants, cooking and processing conditions, the matrix in which they are presented and solubility (Manach et al., 2004). Botanical preparations containing active phytochemicals are similar to food in this respect. The administration of a pure bioactive compound, a compound contained within its original plant matrix, or a concentrated formulation of a compound in a modified matrix, may have distinct effects on the levels of active compounds in the blood.

Poorly water soluble bioactive compounds are often noted for low bioavailability because of inefficient dissolution, dispersion, absorption and circulatory retention and are often formulated with lipid-based vehicles. The beneficial effects of dietary fats on the bioavailability of hydrophobic drugs has guided the development of lipid-based formulation vehicles (Humberstone and Charman, 1997). A wide range of commercial products that improve solubility and bioavailability of pharmaceuticals and related products are currently available and designed for a variety of chemical classes with a wide range of dissolution characteristics. Many bioenhancing agents consist of an oil and a surfactant (or some combinations of each) that, when formulated with a bioactive agent, form an emulsion on contact with water. These combination products are often called self emulsifying drug delivery systems (SEDDS) and yield emulsion droplets that range in size, membrane permeability and thermostability. Labrasol is a commercial bioenhancer consisting of a mixture of glyceride esters of polyethylene glycol and fatty acids. Labrasol enhances both membrane permeability and intestinal absorption of cephalexin, a widely used β lactam antibiotic (Koga et al., 2002) and significantly improves the efficacy of Vancomycin, a water soluble glycopeptide antibiotic with poor absorption characteristics (Prasad et al., 2003). In addition to enhancing solubility, permeability and absorption, bioenhancers, such as Labrasol that contains certain types of surfactants, are also know to further improve the bioavailability of absorbed compounds by acting as p-glycoprotein inhibitors and thereby decreasing intestinal efflux (Yu et al., 1999). In this study we develop methods to determine the bioavailability of the active compounds from a complex botanical therapeutic used for diabetes and identify a formulation with improved solubility, bioavailability and bioactivity in a murine model.

2. Materials and methods

2.1. Materials

The seeds of *Artemisia dracunculus* L. were purchased from Sheffield's Seed Co., Inc. (Locke, New York). The plants were grown in hydroponics and their shoots harvested when plants were beginning to flower after about 4 months. The harvested plants were frozen and stored at −20 °C prior to extraction. The chalcone, 2′, 4′-dihydroxy-4-methoxydihydrochalcone (DMC-2), was produced by custom synthesis by Gateway Biochemical Technology, Inc. to 99% purity. Labrasol, Labrafil M 1944 CS and Capryol 90 were obtained from Gattefosse Corp., Westwood, NJ. Capmul MCM was obtained from ABITEC Corp., Paris Il and cyclodextrin from Sigma, St Louis MO.

2.2. Preparation of extract

To produce PMI-5011, 4 kg of the harvested shoots were heated to 80 °C, with 12 L of 80% ethanol (v/v) for 2 h. The extraction continued for an additional 10 h at 20 °C. The extract was then filtered through cheesecloth and evaporated with a rotary evaporator to 1 L. The aqueous extract was freeze-dried for 48 h and the dried extract was homogenized with a motor and pestle. PMI-5011C was prepared in a similar fashion using 95% ethanol as the initial extraction solvent.

2.3. Liquid chromatography-mass spectrometry analysis

Extracts were fractionated and analyzed with the Waters (Milford, MA) LC-MS Integrity™ system consisting of a W616 pump and W600S controller, W717plus auto-sampler, W996 PDA detector and a Varian 1200L (Varian Inc., Palo Alto, CA) triple quadrupole mass detector with electrospray ionization interface (ESI), operated in either positive, or negative ionization mode. The electrospray voltage was −4.5 kV, heated capillary temperature was 240 °C, sheath gas air for the negative mode, and electrospray voltage 5 kV and sheath gas nitrogen for the positive ionization mode; mass detector scanning from 110 to 1400 atomic mass units. Data from the Varian 1200L mass detector was collected and compiled using Varian's MS Workstation, v. 6.41, SP2. Substances were separated on a Phenomenex® Luna C-8 reverse phase column, size 150 × 2 mm, particle size 3 μm, pore size 100 Å, equipped with a Phenomenex® SecurityGuard™ pre-column. The mobile phase consisted of 2 components: Solvent A (0.5% ACS grade acetic acid in double distilled de-ionized water, pH 3–3.5), and Solvent B (100% Acetonitrile). The mobile phase flow was adjusted at 0.25 ml/min with a gradient from 5% B to 95% B over 35 min.

2.4. Formulation of the extract

PMI-5011, PMI-5011C, and metformin were dissolved in 10% DMSO for the initial evaluation of bioactivity. For the study comparing the effect of excipients on bioactivity, PMI-5011 was formulated with 100% DMSO, Labrasol, Labrafil M 1944 CS, Capryol 90, Capmul MCM or 10% cyclodextrin. PMI-5011, PMI-5011C and DMC-2 were formulated with 66% Labrasol for the remaining studies. Compounds or extracts were dissolved into the delivery vehicle at 10–100 mg/ml in order to provide a dose to the animals of 50 mg/kg B.W. to 500 mg/kg B.W. in a gavage volume of 200–250 uL.

2.5. Plasma analysis of the blood from the treated mice

Trunk plasma was prepared from blood that was collected after carbon dioxide inhalation and decapitation of treated animals. The plasma from treated mice (200 uL, stored at −20 °C prior to analysis) was mixed with 200 uL phosphate buffer, Ph 5.5, and 500 uL water and 20 uL enzyme solution (glucuronidase/sulfatase β glucuronidase, TypeHP-2, from Helix pomatis 101400 units/mL) and incubated at 37 °C for 15 h for hydrolysis of bound forms (Richelle et al., 2002). The samples were then cooled and diluted with 1 mL water and defatted with 2 mL hexane in a glass screw top test tube and partitioned with 3 × 2 mL ethyl acetate. The pooled ethyl acetate partitions were dried by speed vac and resuspended in 125 uLof 80% ethanol, transferred to an insert lined HPLC sample vial and analyzed as described above.