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Nonlinear pharmacokinetics of visnagin in rats after intravenous bolus administration

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ABSTRACT

Ammi visnaga L. (syn. Khella, Apiaceae) preparations have traditionally been used in the Middle East for the treatment of kidney stone disease. Visnagin, a furanocoumarin derivative, is one of the main compounds of *Ammi visnaga* with potential effects on kidney stone prevention. To date, no information is available about the pharmacokinetic (PK) properties of visnagin. It was the aim of the study to characterize the PK properties of visnagin after intravenous (i.v.) bolus administration in rats and to develop an adequate model for the description of the observed data, including model parameter estimates.

Therefore, three doses of visnagin (1.25, 2.5, and 5 mg/kg) solubilized in 25% Captisol® were administered by i.v. bolus injection to male Sprague–Dawley rats. Plasma samples were extracted and subsequently analyzed using a validated LC–MS/MS method. Both non-compartmental and compartmental PK analyses were performed. A stepwise model building approach was applied including nonlinear mixed effect modeling for final model selection and to obtain final model estimates in NONMEM VI. The average areas under the curve (AUC_{0-last}) after doses of 1.25, 2.5, and 5 mg/kg were 1.03, 3.61, and 12.6 mg∗h/l, respectively. The shape of the plasma concentration-time profiles and the observed disproportionate increase in AUC_{0-last} with increasing dose suggested nonlinearity in the elimination of visnagin. A two-compartment Michaelis–Menten model provided the best fit with following typical values of the parameter estimates: 2.09 mg/(l∗h) (V_{max}), 0.08 mg/l (K_M), 0.175 l (V_C), 1.0 h^{-1} (k_{12}), and 1.22 h^{-1} (k_{21}). Associated inter-subject variability estimates (% CV) for V_{max}, K_M and V_C were 21.8, 70.9, and 9.2, respectively. Intra-subject variability (constant CV error model) was estimated to be 7.0%.

The results suggest the involvement of a saturable process in the elimination of visnagin, possibly an enzyme or transporter system.

1. Introduction

Ammi visnaga L. (syn. Khella, Apiaceae) fruit preparations, such as tea prepared from crushed or powdered seeds, have traditionally been used in the Middle East to ease urinary tract pain associated with kidney stones and to promote stone passage (Gunaydin and Beyazit, 2004). More than one million Americans develop new kidney stones each year and the risk for stone formation has been increasing over the past decades (Stamatelou et al., 2003). According to a recent survey almost 12% of white men and 6% of white women will have developed kidney stones by the age of 70 (Worcester and Coe, 2008). Once suffered from this condition the recurrence rate within the first 5–10 years is 50% and higher (Chandhoke, 2007). Despite great advancements in surgical removal of existing stones in the last three decades, a specific and satisfactory drug for prevention of recurrent stones in clinical therapy is still lacking.

Effective kidney stone prevention is dependent on the stone type and the identification of risk factors for stone formation, most notably supersaturation of the urine with crystal components. Patients are usually advised to follow dietary recommendations (i.e. increased fluid intake; sodium, protein and/or oxalate restriction) which should be preceded by a metabolic evaluation (Chandhoke, 2007; Worcester and Coe, 2010). Medical treatment is often required to accompany dietary changes. Depending on the chemical composition of the first stone, current common medical options for prevention include potassium citrate, off-label use of thiazide diuretics and the anti-gout drug allopurinol (Park and Pearle, 2007; Worcester and Coe, 2010). Calcium oxalate stones represent the majority of kidney stones. Allopurinol is not a typical option for calcium oxalate stones, leaving thiazides and potassium citrate for the prevention of this stone type. However, the benefit of these drugs is limited by side effects and tolerance in a great proportion of patients (Gonzalez et al., 1998; Park and Pearle, 2007; Preminger and Pak, 1987).

Herbal medicines could be useful as an alternative or complementary therapy for urolithiasis management as revealed by data

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from *in vitro* and *in vivo* experiments as well as clinical trials (Butterweck and Khan, 2009). The furanocoumarines visnagin and khellin are the main compounds of *Ammi visnaga* fruits with potential effects in kidney stone prevention. As previously demonstrated by our working group, the aqueous plant extract as well as the pure compounds visnagin and khellin decreased calcium oxalate deposition *in vivo* and reduced cell injury after calcium oxalate exposure *in vitro* (Vanachayangkul et al., 2010a,b). Based on these results visnagin seems to be slightly more promising in the prevention of kidney stones than khellin. Khan et al. reported a reduction of oxalate and calcium content in rat kidney after administration of khella tea preparation (Khan et al., 2001).

Pharmacokinetic (PK) characterization is a crucial step in drug development. Moreover, in order to gain wider credibility herbal medicines must undergo similar scrutiny to which synthetic drugs are subjected, including PK evaluation. Said (1982) investigated the PK properties of khellin in rats. Further studies about the PK characteristics of khellin were performed in rabbits (El-Yazigi, 1987). Even though the therapeutic use of visnagin has been studied for years (Duarte et al., 1995, 2000; Hudson et al., 1988; Schimmer, 1997) no information is available about its PK properties.

Therefore, the aim of this study was to evaluate the PK characteristics of visnagin in rats after intravenous (i.v.) bolus administration. First, focus was directed to the question if visnagin possesses linear or nonlinear PK in the tested dosing range. Thereafter, an adequate PK model was developed for the description of the observed data, including model parameter estimates.

2. Material and methods

2.1. Chemicals and reagents

Visnagin (>97%), warfarin (98%), heparin sodium salt (202 U/mg), D-(+)-Glucose (>99.5%) and ammonium acetate (>99%) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Captisol® (β-cyclodextrin sulfobutyl ethers, sodium salts) was purchased from Cydex Pharmaceutlicals (Lenexa, KS, USA). Methanol, ethyl acetate and formic acid (>88%) were obtained from Fisher Scientific (Pittsburgh, PA, USA). All chemicals used were analytical grade. HPLC grade deionized water was prepared using a Barnstead Nanopure Diamond UV ultra-pure water system (Dubuque, IA, USA).

2.2. Instrumentation and LC–MS/MS conditions

2.2.1. Chromatographic conditions

Chromatographic separation was performed on a Symmetry® C_{18}, 4.6 mm × 50 mm, 3.5 μm analytical column (Waters, Milford, MA, USA) at ambient temperature. The mobile phase was comprised of 0.1% formic acid, 5 mM ammonium acetate in deionized water and methanol (15:85, v/v). Prior to use, mobile phase was filtered through an Express Plus® Membrane filter (GPWP, 0.22 μm; Millipore, Cork, Ireland) and degassed utilizing helium gas. Mobile phase was delivered at a flow rate of 0.6 ml/min and the injection volume of samples was 10 μl. Auto-sampler rinse solution consisted of 50% methanol.

2.2.2. Mass spectrometry conditions

The LC–MS/MS system was composed of a PerkinElmer Series 200 LC pump and auto-sampler, coupled with a Micromass® Quattro LC triple quadrupole mass spectrometer. The Micromass® Quattro LC was equipped with an electrospray ionization source and operated in the positive ion mode. Data was acquired and processed using MassLynx® V3.5 software (Micromass Limited, Manchester, UK). Following instrument parameters were applied: capillary voltage of 3.7 kV, source block temperature of 120 °C, desolvation temperature of 400 °C, desolvation gas flow of 706 l/h, cone gas flow of 65 l/h and collision energy of 22 eV. Quantification was carried out using multi reaction mode of the transitions of m/z 230.8 → 216.1 for visnagin and 309.2 → 162.9 for warfarin, with a scan time of 0.4 s per transition for visnagin and 0.1 s per transition for warfarin.

2.3. Sample preparation

2.3.1. Preparation of visnagin calibration standards and quality control samples

Visnagin stock solution (1 mg/ml) was prepared in methanol. Visnagin stock solution was demonstrated to be stable for at least 4 weeks at 4 °C. Working solutions for a seven point calibration curve ranging from 1 to 100 ng/ml were prepared by adequately diluting the stock solution in 50% methanol. Calibration standards were prepared by spiking nine parts of blank rat plasma with one part of respective working solutions. Independent quality control (QC) samples were prepared at concentrations of 1, 5, 50, and 100 ng/ml in the same manner.

2.3.2. Plasma extraction

Calibration standards and QC samples were extracted prior to LC–MS/MS analysis. Warfarin solution (100 μl, 200 ng/ml prepared in 50% methanol) was added as internal standard (IS) to each sample (100 μl). Subsequently, 1 ml ethyl acetate was added to extract visnagin and warfarin from the plasma to the organic solvent. Therefore, the samples were mixed for 5 min on a vortex shaker and centrifuged at 9300*g* for 15 min at room temperature. Clear supernatant (950 μl) was transferred to borosilicate glass vials and ethyl acetate was completely evaporated at room temperature utilizing a vacuum centrifuge. Residues were stored at 4 °C overnight until reconstitution in 60 μl of mobile phase (Section 2.2.1). Reconstituted samples (50 μl) were transferred to auto-sampler vials and analyzed within 4 h after reconstitution.

2.4. Method validation

Method validation included determination of calibration curve, recovery, accuracy and precision according to the criteria of the United States Food and Drug Administration (FDA) Bioanalytical Method Validation Guidance (FDA, 2001). For evaluation of the relationship between instrument response and nominal visnagin concentrations, two sets of calibration standards were prepared per run. The calibration curve was fitted by simple linear regression of the average peak area ratio of visnagin to warfarin on the nominal standard visnagin concentration in ng/ml. A weighting factor of 1/y was used.

Accuracy and precision were determined on three independent days utilizing four concentrations of independent QC samples (n = 5): two low QCs (1 and 5 ng/ml), one middle QC (50 ng/ml), and one high QC (100 ng/ml). Calculation of QC sample concentrations was based on the fitted regression equation.

Recovery and matrix effects were determined in triplicate for three different concentrations. For evaluation of extraction recovery, peak area ratios of visnagin to warfarin of spiked plasma samples were compared to those of blank plasma samples spiked with visnagin after extraction. Matrix effects were determined by comparison of peak area ratios of visnagin to warfarin of blank plasma samples spiked with visnagin after extraction with those of compound spiked injection solutions.

Blank samples (blank plasma processed without IS) and zero samples (blank plasma processed with IS) were positioned in-between the calibration samples and analyzed to assess possible carry-over.