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Detection of ECG effects of (2*R*,4*R*)-monatin, a sweet flavored isomer of a component first identified in the root bark of the *Sclerochitin ilicifolius* plant

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ABSTRACT

Enzymatically-synthesized (2*R*,4*R*)-monatin has, due to its pure sweet taste, been evaluated for potential use in foods. Non-clinical studies have shown that (2*R*,4*R*)-monatin is well tolerated at high dietary concentrations, is not genotoxic/mutagenic, carcinogenic, or overtly toxic. In a pharmacokinetic and metabolism study involving 12 healthy males, consumption of a single oral dose (2 mg/kg) of (2*R*,4*R*)-monatin resulted in a small reduction of heart rate and prolongation of the QTcF interval of 20–24 ms, corresponding to the time of peak plasma levels (t_{max}). These findings were evaluated in a cross-over thorough QT/QTc study with single doses of 150 mg (2*R*,4*R*)-monatin, placebo and positive control (moxifloxacin) in 56 healthy males. Peak (2*R*,4*R*)-monatin plasma concentration (1720 ± 538 ng/mL) was reached at 3.1 h (mean t_{max}). The placebo-corrected, change-from-baseline QTcF (ΔΔQTcF) reached 25 ms three hours after dosing, with ΔΔQTcF of 23 ms at two and four hours. Using exposure response (QTc) analysis, a significant slope of the relationship between (2*R*,4*R*)-monatin plasma levels and ΔΔQTcF was demonstrated with a predicted mean QT effect of 0.016 ms per ng/mL. While similarly high plasma levels are unlikely to be achieved by consumption of (2*R*,4*R*)-monatin in foods, QTc prolongation at this level is a significant finding.

Abbreviations: ADME, absorption, distribution, metabolism, and elimination; AE, adverse event; A_e, amounts excreted; $AUC_{0\text{-inf}}$, area under the plasma concentration–time curve to infinite time; $AUC_{0\text{-t}}$, area under the plasma concentration vs. time curve from time 0 to the last measurable concentration; BMI, body mass index; bpm, beats per minute; CFR, United States Code of Federal Regulations; CI, confidence internal; CL_R, renal clearance; C_{max}, maximum observed plasma concentration; CV, coefficient of variation; ΔHR, change-from-baseline heart rate; ΔQTcF, change-from-baseline QTcF; ΔΔQTcF, placebo-corrected ΔQTcF; ECG, electrocardiogram; EDI, estimated daily intake; EFSA, European Food safety Authority; FDA, US Food and Drug Administration; FSANZ, Food Standards Australia New Zealand; GCP, Good Clinical Practices; GLP, Good Laboratory Practice; HPLC, high pressure liquid chromatography; ICH, International Conference on Harmonisation; IRB, Institutional Review Board; IUT, Intersection Union Test; JECFA, Joint FAO/WHO Expert Committee on Food Additives; λ_z, terminal rate constant; MS, mass spectrometry; ms, millisecond; NOAEL, no-observed-adverse-effect level; OECD, Organisation for Economic Co-operation and Development; PK, pharmacokinetic; SAE, serious adverse event; SD, standard deviation; SE, standard error; SOP, standard operating procedure; $t_{1/2}$, terminal half-life; t_{max}, time to maximum observed plasma concentration; TQT, thorough QT.

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1. Introduction

The 2*R*,4*R*-isomer of 2-hydroxy-2-(indol-3-ylmethyl)-4-aminoglutaric acid (hereafter referred to as R,R-monatin) is a non-proteinogenic α-amino acid that exists naturally in small quantities in the root bark of the indigenous South African plant, *Sclerochitin ilicifolius* (Hlywka et al., 2011, 2013). *R,R*-Monatin has been reported to have a pure sweet taste, and sensory studies have revealed it to be approximately 3000 times sweeter than sucrose (Fry et al., 2012). As such, enzymatically-synthesized *R,R*-monatin was evaluated for its potential use as a food ingredient.

The safety testing program for *R,R*-monatin was developed based on the need to generate data to adequately support its safety for potential use in the diet. The program of studies to support the use of *R,R*-monatin as a food ingredient included a full battery of toxicology studies consistent with the guidelines of the U.S. Food and Drug Administration (FDA) (FDA Redbook 2000 (revised), 2007), the European Food Safety Authority (EFSA) (EFSA, 2012; SCF, 2001), Food Standards Australia New Zealand (FSANZ) (FSANZ, 2010), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (IPCS, 2009). To this extent, the safety of *R,R*-monatin has been evaluated in a comprehensive set of genetic, acute, short-term, subchronic, chronic, reproduction and embryo/fetal developmental toxicology, and carcinogenicity studies. These studies have indicated that R,R-monatin is not genotoxic or mutagenic, is not acutely or overtly toxic, is not a reproductive/developmental toxicant, is not carcinogenic, and is well tolerated when administered in the diet at high inclusion rates ((Brathwaite et al., 2013; Casterton et al., 2014a, 2014b; Hlywka et al., 2011, 2013) and *unpublished data on-file*). Regulatory guidance also suggests the conduct of pharmacokinetic and metabolism studies to better understand exposure and tissue distribution. Results of *in vivo* absorption, distribution, metabolism and elimination (ADME) studies in dogs and rats and *in vitro* experiments in rat liver microsomes have shown that *R,R*-monatin is readily absorbed, with the majority of the absorbed test article rapidly eliminated unchanged in the urine ((Casterton et al., 2014b) and *unpublished data on-file*).

To confirm the relevance of both animal toxicology and ADME study results to humans and assess its safety and tolerability, an ADME study was conducted wherein 12 healthy volunteers were administered a single oral dose of 2 mg/kg *R,R*-monatin. Analysis of 12-lead electrocardiograms (ECGs) recorded serially after dosing showed a modest reduction of the heart rate and prolongation of the QTcF interval (~20 ms), which coincided with peak plasma levels of *R,R*-monatin. This study did not include a placebo arm; hence, it was not possible to make a definitive conclusion based on the results in regard to the observed QT prolongation. It was therefore deemed appropriate to conduct a more thorough follow-up study. In this report, we describe the design and results of the single-dose ADME study, in which the QT prolongation was observed, and the subsequent thorough QT (TQT) study (ICH E14 Questions and Answers, 2014; ICH Harmonized Tripartite Guideline E14, 2005). The findings are discussed in relation to performed non-clinical assays/studies and potential implications of the findings.

2. Material and methods

2.1. ADME study

2.1.1. Study design

This study was conducted in 12 healthy male subjects with the primary objective to evaluate the pharmacokinetics and metabolism of *R,R*-monatin after a single oral dose; secondary objectives of safety and tolerability were also evaluated. Inclusion criteria for subjects were healthy males aged 18–45 years, with a body mass index (BMI) between 19.0 and 29.0 kg/m^2, and exclusion criteria were significant medical history and abnormal findings in ECG, physical examination, or laboratory tests. The study was conducted at the SNBL Clinical Pharmacology Center, Baltimore, MD, USA.

Blood, urine and fecal samples, for the assay of R,R-monatin and its lactone and lactam derivatives, were collected before and repeatedly after dosing, up to 120 h post-dose. Subjects remained confined to the unit from the morning of Day −1 (the day before dosing) until the morning of Day 6, approximately 120 h after dosing. Clinical laboratory tests, monitoring of vital signs, adverse events (AEs), physical examinations, and 12-lead ECG determinations were conducted to assess safety and tolerability of *R,R*-monatin in the subjects.

This study was conducted according to the United States (U.S.) Code of Federal Regulations (CFR), along with the applicable International Conference on Harmonisation (ICH) Guidelines, commonly known as Good Clinical Practices (GCPs), which are consistent with the Declaration of Helsinki (as modified by the 52nd World Medical Association General Assembly, October 2008). An Institutional Review Board (IRB) reviewed and approved the protocol and informed consent documentation prior to study initiation. Signed informed consent was obtained from each subject prior to any study procedures, and all subjects were informed of their right to withdraw from the study without prejudice at any time.

2.1.2. Test material and dosage

Enzymatically-synthesized, unlabeled *R,R*-monatin (sodium/potassium salt) (96.5% UV purity, 5.5% moisture) was supplied by Cargill, Incorporated. The test article was constituted, diluted, packaged, and labeled at the clinic.

All subjects consumed a single 2 mg/kg dose of *R,R*-monatin. Each dose was administered orally as a 120 ml aqueous solution, followed by a 60 mL rinse of the test article container to ensure ingestion of the total dose.

2.1.3. Pharmacokinetic sampling

Blood samples for determination of *R,R*-monatin plasma levels were collected pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 30, 36, 48, 72, 96 and 120 h after dosing. Urine samples for PK analyses were collected at −12 to 0 h before dosing and at 0 to 3, 3 to 6, 6 to 12, 12 to 24, 24 to 36, 36 to 48, 48 to 60, 60 to 72, 72 to 96, and 96 to 120 h post-dosing. Feces were collected for PK analyses as individual evacuations, from approximately 12 h prior to dosing until approximately 120 h post-dose.

2.1.4. Pharmacokinetic analysis

Non-compartmental methods were used to determine the following PK parameters: maximum observed plasma concentration (C_{max}), time to maximum observed plasma concentration (t_{max}), terminal half-life ($t_{½}$), terminal rate constant (λ_z), area under the plasma concentration *vs*. time curve from time 0 to the last measurable concentration (AUC_{0-t}), area under the plasma concentration–time curve to infinite time (AUC_{0-inf}), amounts excreted in urine and feces (A_e), and renal clearance (CL_R). PK parameters were determined using Win-Nonlin Version 5.2 validated using Validation Suite Version 1.2 (Pharsight Corporation). Analyses of *R,R*-monatin in the plasma, urine and feces were conducted by WIL Research Laboratories, LLC (Ashland, Ohio, USA) using validated high pressure liquid chromatography (HPLC), mass spectrometry (MS) assay procedure.

2.1.5. Safety and tolerability

The safety and tolerability of *R,R*-monatin were assessed on the