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Human pharmacokinetic study of tutin in honey; a plant-derived neurotoxin

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ABSTRACT

Over the last 150 years a number of people in New Zealand have been incapacitated, hospitalised, or died from eating honey contaminated with tutin, a plant-derived neurotoxin. A feature of the most recent poisoning incident in 2008 was the large variability in the onset time of clinical signs and symptoms of toxicity (0.5–17 h). To investigate the basis of this variability a pharmacokinetic study was undertaken in which 6 healthy males received a single oral dose of tutin-containing honey giving a tutin dose of 1.8 μg/kg body weight. The serum concentration–time curve for all volunteers exhibited two discrete peaks with the second and higher level occurring at approximately 15 h post-dose. Two subjects reported mild, transient headache at a time post-dose corresponding to maximum tutin concentrations. There were no other signs or symptoms typical of tutin intoxication such as nausea, vomiting, dizziness or seizures. Pharmacokinetic analysis using a two-site absorption model resulted in a good fit to the observed concentration data. A novel analytical method subsequently revealed the presence of glycoside conjugates of tutin in addition to unconjugated tutin in honey. These pharmacokinetic data will be important to better define a safe maximum tutin concentration in honey.

1. Introduction

Human toxicity and deaths from the consumption of 'wild honey' and some commercially produced honeys have occurred sporadically in New Zealand since the late 19th century. A series of papers published in the 1940s documented the research that was undertaken to identify the toxic agent(s) in such honey. Because of similarities with the clinical signs and symptoms resulting from the consumption of berries of the native New Zealand shrub tutu (*Coriaria arborea*) which is known to contain tutin, an acute neurotoxin, it was speculated that tutin may be the toxin responsible for honey poisoning (Sutherland and Palmer-Jones, 1947a). An initial investigation showed that various extracts of toxic honey samples were highly toxic to guinea pigs when administered orally, however no tutin was found in these extracts. Instead, "mellitoxin", was found at a concentration of approximately 150 mg per kg of honey, and isolated mellitoxin of unknown purity caused signs of toxicity identical to those observed when toxic honey was administered orally to guinea pigs. Mellitoxin, now known as hyenanchin, was subsequently shown to be a hydroxy derivative of tutin (Fig. 1) (Hodges et al., 1964).

An important observation reported in 1947 described how bees collected honeydew that had been excreted on the leaves of the tutu tree by a sap-sucking insect (passionvine hopper, *Scolypopa australis*) (Paterson, 1947). Hyenanchin could not be isolated from tutu but it was readily found in the honeydew excreted by *S. australis* feeding on tutu sap (Sutherland and Palmer-Jones, 1947b). Further research in the late 1940s identified the presence of tutin in honeydew, albeit at lower but unquantified levels relative to hyenanchin. Oral LD50 values in guinea pigs indicated that tutin was about 10-fold more toxic than hyenanchin, while in rats it was approximately 2–4-fold (Palmer-Jones, 1947). However, the purity of tutin and hyenanchin administered in these early studies is unknown. In recent mouse studies using purified substances, the difference in acute oral toxicity is more pronounced with no deaths or clinical signs being observed at hyenanchin doses 100 times the tutin LD50 (3.2 mg/kg bw) (Munday, 2008). Toxicity studies in guinea pigs dosed with various solvent extracts of honeydew suggested the presence of toxins other than tutin and hyenanchin. It was speculated that tutin is converted to hyenanchin and these other uncharacterized toxins on passage through the passionvine

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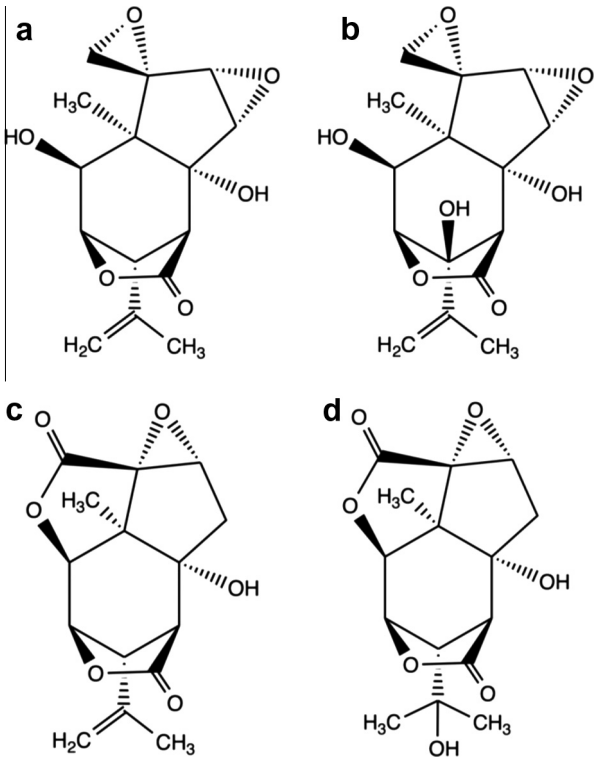

Fig. 1. Chemical structures of tutin (a), hyenanchin (b), picrotoxinin (c) and picrotin (d).

hopper (Palmer-Jones and White, 1949). In the late 1970s, analysis of a sample of toxic honey showed the presence of dihydro-derivatives of tutin and hyenanchin, at levels 8-fold lower than those of tutin and hyenanchin (Blunt et al., 1979); however, no toxicity information is available on the dihydro-derivatives. Since that publication, no other compounds related to tutin and hyenanchin, or other chemically unrelated potential toxins in tutu honeydew honey have been reported. Tutin has also been found in Coriaria species that grow in other parts of the world, for example the Asian *Coriaria nepalensis* (Wei et al., 1998) and *Coriaria japonica* (Kinoshita et al., 2005), and the South American *Coriaria ruscifolia* (Fuentealba et al., 2007). Chemically tutin is a sesquiterpene lactone related to picrotoxin (a 1:1 mixture of picrotoxinin and picrotin), a central nervous system convulsant widely used in neuropharmacology studies (Olsen, 2006; Fig. 1). Tutin, like picrotoxin has been shown to possess inhibitory effects on receptors of the neurotransmitters gamma-amino butyric acid (GABA) and glycine (Curtis et al., 1973; Fuentealba et al., 2007 and Fuentealba et al., 2011).

In the most recent episode of poisoning in March 2008, there were 11 confirmed and 9 probable reported cases involving serious illness and hospitalisations following consumption of honeycomb honey containing tutin (Beasley, 2008; Goodwin, 2013). Confirmed cases were defined as those for which there was sufficient honey comb left over to confirm the presence of tutin. The probable cases are those which had clinical signs and symptoms consistent with the confirmed cases, but for which no honey remained for analysis. Tutin concentrations in honeycomb samples ranged from approximately 30–50 mg per kg of honey (NZFSA, 2008). Hyenanchin levels in these honey samples were consistently around 6-fold higher than the tutin levels. Reported amounts of honey consumed by confirmed cases ranged from one teaspoon to a quarter of a portion of comb, as packaged for retail sale. Clinical signs and symptoms of intoxication attributed to tutin include nausea, headache, vomiting and dizziness, and in severe cases seizures and coma. A common feature of poisoning from honey containing tutin is the large variability in the onset time of signs and symptoms of toxicity. In the cases reported in 2008 the onset of the first clinical sign or symptom ranged from 0.5 to 17 h with a median of 7.5 h (unpublished data). The primary objective of the study was to characterize the oral pharmacokinetics (PK) of tutin in honey to better understand the typically delayed onset of toxicity following acute exposure. This information would then aid the establishment of safe limits for tutin in honey. To simulate the exposure conditions of the incident, a small quantity of tutu honeydew honey containing a known concentration of tutin was ingested by healthy male subjects and their serum monitored for the presence of tutin.

2. Materials and methods

2.1. Study design

This was an open-label, single dose study in 6 healthy adult males. The study was conducted in accordance with Good Clinical Practice (GCP) and appropriate requirements with regards to Independent Ethics Committee review, informed consent, and regulations pertaining to the protection of subjects in research studies. The study protocol was reviewed and approved by the Lower South Regional Ethics Committee, Dunedin, New Zealand.

2.2. Test material

The test material was homogenized honey known to contain tutin and hyenanchin at concentrations of 5.1 and 23 mg per kg, respectively, as determined using a validated liquid chromatography-tandem mass spectrometry (LC–MS/MS) method by a laboratory accredited for the compliance testing of honey in New Zealand (AsureQuality, Wellington). Briefly, honey was dispersed in acetone:water, diluted with dichloromethane:hexane, centrifuged, and a portion of the upper phase evaporated, re-dissolved in acetone:hexane and subjected to solid-phase extraction (SPE). The resulting extract was analyzed by LC–MS/MS using an Agilent Model 1200 HPLC (column: Zorbax Eclipse XDB-C18, 100 mm × 4.6 mm × 1.8 μm) interfaced with an ABSciex API 4000QT mass spectrometer operated in atmospheric pressure chemical ionisation (APCI) mode. Specific identification of tutin was achieved using multiple reaction monitoring (MRM). Recovery data were collected from 21 replicates at each concentration of 1, 2 and 3 mg/kg. Estimates of LOD and LOQ were obtained from analyses of seven samples spiked at 0.02 mg/kg. Mean recovery for tutin was 80%, coefficient of variation (CV) was 8.6%, giving an expanded measurement uncertainty (95% CI) of 17%.

2.3. Dose selection

The selected tutin dose (1.8 μg/kg body weight) in this study was equal to that received by a high consumer of honey (97.5th percentile = 0.9 g honey/kg body weight) containing tutin at the current maximum level permitted under the Australia New Zealand Food Standards Code (2 mg/kg honey) (FSANZ, 2014). The mass of honey administered ranged from 25.0 to 32.0 g for subjects with the lowest and highest body weight, respectively. The tutin dose utilized in this study was slightly lower than the Acute Reference Dose (ARfD) of 2.5 μg/kg body weight, that had been established using purified tutin (purity = 95%) in an acute dosing study in mice (McNaughton and Goodwin, 2008). The only reported quantitative data relating to oral dosing of tutin in humans comes from a study into possible clinical applications in 1929. In that study the investigator self-administered single doses