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Metabolomics evaluation of the effects of green tea extract on acetaminophen-induced hepatotoxicity in mice

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ABSTRACT

Green tea has been purported to have beneficial health effects including protective effects against oxidative stress. Acetaminophen (APAP) is a widely used analgesic drug that can cause acute liver injury in overdose situations. These studies explored the effects of green tea extract (GTE) on APAP-induced hepatotoxicity in liver tissue extracts using ultra performance liquid chromatography/quadrupole time-of-flight mass spectrometry and nuclear magnetic resonance spectroscopy. Mice were orally administered GTE, APAP or GTE and APAP under three scenarios. APAP alone caused a high degree of hepatocyte necrosis associated with increases in serum transaminases and alterations in multiple metabolic pathways. The time of GTE oral administration relative to APAP either protected against or potentiated the APAP-induced hepatotoxicity. Dose dependent decreases in histopathology scores and serum transaminases were noted when GTE was administered prior to APAP; whereas, the opposite occurred when GTE was administered after APAP. Similarly, metabolites altered by APAP alone were less changed when GTE was given prior to APAP. Significantly altered pathways included fatty acid metabolism, glycerophospholipid metabolism, glutathione metabolism, and energy pathways. These studies demonstrate the complex interaction between GTE and APAP and the need to employ novel analytical strategies to understand the effects of dietary supplements on pharmaceutical compounds.

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1. Introduction

The use of herbs and dietary supplements has increased greatly due to their purported beneficial health effects (Perlman et al., 2013). As a corollary, an increased interest in the use of such products means increased concurrent use with pharmaceuticals (Graham et al., 2008; Qato et al., 2008). It has been reported that nearly 20% of patients with chronic diseases or cancer use herbs or dietary supplements concurrently with prescription medications (Kaufman et al., 2002; Gardiner et al., 2006). Since these supplements do not go through a stringent approval process, information about adverse effects and drug interactions are limited (Ashar, 2010). The cytochrome P450 (CYP450) enzymes play a major role in the phase I metabolism of drugs and botanicals. It has been shown that the CYP450 enzymes can be induced or inhibited by herbal extracts altering the pharmacokinetic profile of a drug compound (Jang et al., 2004). The increasing trend of co-administering herbal supplements with over-the-counter or prescription drugs makes it critical to understand the effects of such supplements on drug metabolism.

Acetaminophen (APAP) is a widely used analgesic drug and a leading cause of drug-induced liver injury in the United States. An overdose of APAP can cause acute liver injury associated with hepatic centrilobular necrosis in animals and humans (Larson, 2007). APAP is predominantly metabolized by glucuronidation and sulfation with a small fraction of a therapeutic dose metabolized by CYP450 enzymes. In overdose situations, the predominant pathways are saturated and a larger fraction is metabolized by

Abbreviations: 3-HB, 3-Hydroxybutyrate; ALP, alkaline phosphatase; ALT, alanine aminotransferase; APAP, acetaminophen; AST, aspartate aminotransferase; ATP, adenosine-5′-triphosphate; CHOL, cholesterol; CTRL, control; CYP450, cytochrome P450; GGT, γ-glutamyl transferase; GLU, glucose; GSH, glutathione; GTE, green tea extract; LC/MS, liquid chromatography/mass spectrometry; LysoPC, lysophosphatidylcholine; NAD^+, nicotinamide adenine dinucleotide; NAPQI, *N*-acetyl-p-benzoquinone imine; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; PCA, principal component analysis; PLS-DA, partial least squares-discriminant analysis; QC, quality control; QTOF-POS, quadrupole time-of-flight positive mode; QTOF-NEG, quadrupole time-of-flight negative mode; ROS, reactive oxygen species; TBILI, total bilirubin; TRI, triglycerides; ULN, upper limit of normal; UPLC/QTof-MS, ultra performance liquid chromatography/quadrupole time-of-flight mass spectrometry; UPLC/MS, ultra performance liquid chromatography mass spectrometry.

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CYP450 enzymes. It is well established that the metabolism of APAP by CYP450 enzymes results in the formation of the toxic metabolite, *N*-acetyl-p-benzoquinone imine (NAPQI), which is detoxified by conjugation with glutathione (GSH) at therapeutic doses (Wang et al., 1996). In overdose situations, GSH becomes depleted and excess NAPQI irreversibly binds to cellular proteins, especially mitochondrial proteins (Gibson et al., 1996; Jaeschke et al., 2012), which suppresses mitochondrial fatty acid β-oxidation (Bhattacharyya et al., 2013) and results in massive necrosis and apoptosis of hepatocytes (Chen et al., 2009a).

Green tea is used worldwide and is rich in polyphenolic catechins, such as epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate (Navarro, 2009). Of these, epigallocatechin gallate is the most abundant and the most pharmacologically active component with the highest antioxidant properties (Guo et al., 1996). Numerous studies have shown that green tea has potent free radical scavenging and iron chelating properties (Cabrera et al., 2006) and thus has many proposed health benefits, such as anti-cancer, anti-aging, anti-inflammatory activity, hepatoprotection and nephrotoxicity protective effects (Leung et al., 2001; Khan et al., 2009; Navarro, 2009; El-Mowafy et al., 2011; Fu et al., 2011). Adverse events possibly linked to green tea-related induction of oxidative stress that altered mitochondrial membrane potential have been reported (Navarro, 2009; Stickel et al., 2011). Stickel et al. (2011) noted that patients with reported adverse reactions to green tea also used other products.

Hepatoprotective effects of green tea or green tea extract (GTE) against liver injury induced by hepatotoxic compounds such as APAP (Oz and Chen, 2008; Chen et al., 2009b; Salminen et al., 2012), alcohol (Jin et al., 2008), or carbon tetrachloride (Chen et al., 2004) have been reported. Studies have indicated that GTE reduces the severity of liver injury by suppressing CYP450s expression at the protein and mRNA levels (Chen et al., 2009b), down-regulating cyclooxygenase and Bcl-2 activity (Oz and Chen, 2008), and down-regulating inducible nitric oxide-derived prooxidants (Chen et al., 2004). Sai et al. (1998) demonstrated that intake of green tea prevented hepatotoxicity, oxidative DNA damage and cell proliferation in the rat liver after repeated doses of *2*-nitropropane. Previous efforts to elucidate the pharmacological mechanism of GTE have focused primarily on gene expression, enzyme activity, and cell morphology. Published work from this laboratory has shown that GTE can potentiate the hepatotoxicity of APAP when given 6 h after a single dose of APAP (Salminen et al., 2012). The potentiation of the hepatotoxicity was proposed to be due to depletion of GSH by GTE.

Metabolomics approaches based on advanced analytical technologies and chemometric computation have been reported as important and effective tools to examine the changes in the endogenous metabolites of the whole system and potentially provide a better mechanistic understanding of the biochemical and cellular events (Coen et al., 2003, 2004; Nicholson and Wilson, 2003; Chen et al., 2007). Previous UPLC/MS and NMR-based metabolomics studies of APAP-induced toxicity in rats have been reported (Sun et al., 2008, 2009). Results showed a depletion of GSH and energy-related metabolites related to APAP overdose. Recently it was reported that metabolites involved in energy, urea and bile acid pathways in blood were found to have strong correlations to hepatic necrosis scores and elevated alanine aminotransferase (ALT) levels (Sun et al., 2013). The pathways associated with these metabolites were altered during the first 72 h but had generally recovered at 168 h after a single dose of APAP. In addition, a separate LC/MS-based metabolomics study of APAP metabolism in wild-type and *Cyp2e1*-null mice revealed that acylcarnitines, which are the intermediates in the mitochondrial β-oxidation of fatty acids, can function as complementary biomarkers for APAP-induced hepatotoxicity (Chen et al., 2009a).

To evaluate the effects of GTE on APAP-induced liver injury, UPLC/MS- and NMR-based metabolomic open profiling was utilized to examine the metabolic changes in the liver. UPLC/MS and NMR are complementary techniques that have some overlap in the metabolites detected but also detect unique metabolites. Therefore, use of both technologies provides a better coverage of the metabolome enhancing biomarker discovery, pathway analysis and elucidation of toxic mechanism(s). Previous altered metabolites identified in blood samples from rats treated with APAP alone (Sun et al., 2013) were evaluated to see how these metabolite levels changed when co-exposed to APAP and GTE. The metabolomics profiling data indicated that the timing of exposure to GTE in relation to APAP administration determines whether there will be protection against, or potentiation of, hepatotoxicity. GTE exposure prior to APAP appears to provide a protective effect by preventing APAP-induced inhibition of fatty acid β-oxidation. Previous results indicate that GTE may prevent the biotransformation of APAP to the toxic metabolite, NAPQI, by inhibiting CYP450 activity (Salminen et al., 2012). However, GTE exposure after APAP amplified hepatocellular damage, which may be due to further depletion of GSH by GTE (Salminen et al., 2012).

2. Material and methods

2.1. Chemicals and reagents

Optima LC/MS grade acetonitrile and water were purchased from Fisher (Pittsburgh, PA). APAP, methylcellulose, formic acid, leucine-enkephalin, imidazole, pentadecafluorooctanoic acid, *L*-tryptophan and all the MS standards were obtained from Sigma–Aldrich (St. Louis, MO). NMR solvents, d_6-2,2-dimethyl-2-silapentane-5-sulfonate (DSS-D_6) in 99.9% v/v D_2O was purchased from Chenomx (Edmonton, Alberta, Canada) and difluorotrimethylsilanylphosphonic acid (DFTMP) was obtained from Cambridge Isotope Laboratories (Andover, MA). GTE was provided by the US National Toxicology Program and the original manufacturer was Amax NutraSource Inc. (City of Industry, CA). The purity of green tea polyphenols was 99.25% (by UV–VIS) and the polyphenol contents in GTE were reported previously (Salminen et al., 2012).

2.2. Animal care and treatment

As described previously (Salminen et al., 2012), 8–9-week-old B6C3F1 mice were obtained from the FDA National Center for Toxicological Research (NCTR) breeding colony. The mouse was chosen for this study because it is a species that is commonly used for nonclinical toxicity evaluations. In addition, the B6C3F1 mouse is considered a good model of APAP-induced hepatotoxicity since the dose eliciting toxicity is similar to humans (Harrill et al., 2009; Liu et al., 2010; Martinez et al., 2010). The mouse has been used extensively for APAP-induced liver toxicity studies and a large database of classical toxicology measurements are available for this strain so that the results from this current study can be compared to past study data. Therefore, using the B6C3F1 mouse maximizes the likelihood of identifying responses that are quantitatively and qualitatively similar to those which may be expected in humans.

Animal rooms were maintained at 19–23 °C, 40–70% relative humidity, with a 12 h dark/12 h light cycle. Animals accessed feed and water *ad libitum*. Experiments were conducted in accordance to the National Institutes of Health (NIH) guidelines and reviewed and approved by NCTR's Institutional Animal Care and Use Committee (IACUC). Mice were fasted overnight for approximately 12 h before administration of APAP. In all studies, feed was given or returned to the animals 4 h after the administration of APAP. Three different *in vivo* studies were conducted to evaluate the effect of GTE on APAP-induced hepatotoxicity. The study designs and dose selection rationale were reported previously (Salminen et al., 2012). The dosing scenarios were selected to evaluate the different consumption patterns of GTE in humans as noted in Salminen et al. (2012). Doses of APAP and GTE were selected based on range finding studies (results not published); the GTE doses were chosen such that GTE alone would produce none to minimal effects. All doses were administered based on animal body weight via oral gavage. Fig. 1 provides a diagram of the three different dosing scenarios with the number of animals per dose group listed in parentheses. Briefly, the GTE pre-dose studies included 6 dose groups ($n = 8$ animals per group) which consisted of a control group (CTRL), APAP group (200 or 300 mg/kg), GTE group (500 mg/kg or 1000 mg/kg), GTE (500 mg/kg)/APAP (200 or 300 mg/kg) group and GTE (1000 mg/kg)/APAP (200 or 300 mg/kg) group. In the first GTE pre-dose study, the animals were fasted overnight and then received a single dose of GTE (500 or 1000) or its vehicle control (water). Three hours later the animals were administered a single dose of APAP (200) or its vehicle control (methylcellulose).