Abstract

Background: The evaluation of active hexose correlated compound (AHCC) on hepatic metabolism mediated-drug interaction is critical in current clinical setting as there is little published information on the potential effect on drug efficacy and safety. The primary objective of this study was to evaluate the potential phase II hepatic metabolism pathways associated with the metabolism of AHCC and to determine potential drug/AHCC interactions.

Methods: Four primary hepatic metabolism phase II pathways were evaluated: glutathione S-transferase (GST), quinone oxidoreductase (QOR), catechol-O-methyltransferases (COMT) and uridine diphosphate (UDP)-glucuronosyltransferase (UGT). Pooled human liver microsomes and human liver S9 fractions were utilized to evaluate QOR and UGT metabolism inhibition assays. The pool human liver S9 fractions were used to assess GST activity. Cryopreserved inducible human liver hepatocytes were used to evaluate potential induction of UGT and COMT metabolism. All experiments were carried out in triplicate.

Results: Data demonstrated that AHCC is not an inhibitor of GST or UGT pathways, but may be a potential inhibitor of QOR pathway. Evaluation of induction of the phase II pathways demonstrated that AHCC showed potential induction of the UGT 1A3 and 1A6 pathways. There was no induction of the COMT pathway.

Conclusion: Historically, drug interaction studies have only focused on Phase I metabolism pathways, so currently there is very limited information regarding the phase II metabolism of most commonly used medications. In conclusion, additional studies are warranted to determine potential of any phase II hepatic interactions with AHCC when administered with other medications or supplement that are substrates of these pathways.

Keywords: AHCC; Metabolism; Inhibition; Induction; GST; UGT; COMT; QOR; Drug interactions; Supplements

Background

Despite the known incidence of interactions of nutritional supplements with medications, the mechanism of most interactions remains unknown. Hepatic metabolic pathways play an essential role in the activation and elimination of a majority of medications and supplements. There are three primary pathways of hepatic metabolism described as phase I, phase II and phase III metabolism. The phase I and phase III are through cytochrome P450 (CYP450) enzymes function to mediate oxidation, reduction, or hydrolysis reactions, to activate or inactivate drugs. Typically phase II metabolism includes glucuronidation, acetylation, S-methylation, and glutathione- or sulfo-conjugation of drugs. Metabolism may involve one phase or combination of the three phases depending on the complexity of the compound. Multiple studies have also focused on the induction of phase I metabolism pathways to identify potential for supplements that induce the detoxification of drugs such as chemotherapy, which may lead to low drug efficacy. The likelihood of supplement-drug interaction can potentially be much higher than drug-drug interaction because most natural products usually contain more than one active chemical entity.

Active Hexose Correlated Compound (AHCC) (Amino Up Chemical Co, Ltd., Sapporo, Japan), is an fermented extract prepared from mycelia of a Basidiomycete mushroom (Lentinula edodes) that has been proposed to have many health benefits including both immunomodulatory and anti-tumor effects [1-5]. The primary active component is acetylated alpha-glucan (Figure 1) with an average molecular weight of around 5,000 Da and contains less than 0.2% β-glucans which have a molecular weight of 10,000 to 500,000, with the lower molecular weight α-glucans having much better absorption. There are some uncharacterized proprietary elements that result from the unique process involving cultivation, enzymatic decomposition, sterilization, concentration and freeze-drying by which AHCC is prepared that may also contribute to the product activity.

In clinical studies, AHCC has been shown to decrease the side effects associated with anticancer chemotherapy [6,7]. In addition, previous studies have reported that the AHCC product has anti-diabetic, anti-hyperlipidemia, as well as anti-hepatitis effects. The functions of particular interest in oncology are AHCC’s immune-modulating and
potential restorative effects on natural killer (NK) cells, macrophages and cytokines after anti-cancer chemotherapy. In a previous study evaluating the Phase I metabolism found that AHCC was a substrate as well as an inducer of the CYP450 2D6 pathway [8]. Otherwise, the overall data suggested that AHCC would not interact with the other CYP450 pathways and would be generally safe to administer with most other chemotherapy agents not metabolized via the CYP450 2D6 pathway [6].

The study on hepatic metabolism mediated drug-supplement interaction is very critical in current clinical setting as there are many patients using AHCC without knowing that there can be a potential effect on drug efficacy and safety. The primary objective of this study was to evaluate the potential phase II hepatic metabolism pathways associated with the metabolism of AHCC and to determine any potential for drug/AHCC interactions based on phase II metabolism pathways. Four primary hepatic metabolism phase II pathways were tested including: glutathione S-transferase (GST) that catalyzes conjugation of electrophilic substrates to glutathione, quinone oxidoreductase (QOR) reduces reactive quiones to hydroquinones, catechol-O-methyltransferases (COMT) catalyzes the methylation of catechol substrates for detoxification, and uridine diphosphos (UDP)-glucuronosyltransferase (UGT) catalyzes glucuronidation of substrates into more polar glucronides for detoxification.

Methods

Chemicals

The AHCC supplement was kindly provided by Amino Up Chemical Co., Ltd (Sapporo, Japan). All other chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were of the highest purity available.

Human liver microsomes, human liver S9 fractions and human hepatocytes

Pooled human liver microsomes and human liver S9 fractions were purchased from Life Technologies (Grand Island, NY, USA). The pooled human liver microsomes (Catalog Number HMMC-PL) were packaged in 0.5 mL aliquots, with a stated protein concentration of 20 mg/mL were utilized to evaluate QOR and UGT inhibition. The pool human liver S9 fractions were used to evaluate GST activity. The S9 fractions were packaged in 1 mL aliquots, with a stated protein concentration of 20 mg/mL. Cryopreserved inducible human liver hepatocytes were purchased from BD Biosciences (San Jose, CA, USA). The hepatocytes were used to evaluate UGT and COMT induction. Hepatocytes were packaged in 1 mL aliquots, with a stated concentration of greater than five million cells per aliquot.
Phase II inhibition assay methods

GST activity was evaluated using a slightly modified method described by Mannervik and Guthenberg [9]. The assays were carried out in a 200 µL total volume with 96-well UV-Vis plates (Fisher Scientific, Waltham, MA, USA). Briefly, the reaction of 1 mM GSH with 1 mM CDNB in the presence of 2 µL human liver S9 fraction (20 mg/mL), diluted with 1 M potassium phosphate buffer (pH 6.5), was measured by UV absorbance on Synergy HT multi-detection microplate reader (Bio-Tek, Winooski, VT, USA) at 340 nm. An AHCC concentration of 0.42 mg/mL was added as a test agent and serially diluted 1:3 for eight wells. The 0.42 mg/mL concentration was selected as an estimate of the clinical relevant concentration based on the current maximum recommended dosage of 3 g daily as instructed by the manufacturer, assuming 100% bioavailability, and 7 L as the estimated total blood volume of an average adult. Ethacrynic acid (6 µM) is a broad inhibitor of GST activity thus was selected as a positive control inhibitor of GST activity [10] (Table 1).

UGT activity was evaluated following a method described by Liu and Franklin et al. [11]. Inhibition assays were conducted in a 200 µL total volume with 96-well UV-Vis plates. The three primary isoforms of UGT associated with drug metabolism were evaluated: UGT1A3, UGT1A6, and UGT2B17. Substrates for each isoform included estrone (UGT1A3), 1-naphthol (UGT1A6) and testosterone (UGT2B17) (Table 1). 2 mM of uridine 5’ dipospho-glucuronic acid (UDPGA) was reacted with each respective substrate in the presence of 2 µL human liver microsomes (20 mg/mL) and diluted with 50 mM potassium phosphate buffer (pH 8.0). The reaction was measured by UV absorbance on a FL600 Dual-Band plate reader (BioTek Instruments, Inc. Winooski, VT, USA) at either 220 nm (UGT1A6) or 230 nm (UGT1A3 and UGT2B17). AHCC was added as a test agent at 0.42 mg/mL and serially diluted 1:3 for eight wells. Removal of UDPGA was used as a negative control.

QOR activity was evaluated using the method described by Benson et al. [12]. Assays were carried out in a 200 µL total volume in 96-well UV-Vis plates. The reaction mixtures contained 0.7 bovine serum albumin, 125 mM Tris-HCl (pH 7.4), 0.2 mM NADPH, 40 µM 2,6-dichlorophenolindophenol (DCPIP) and control inhibitor discoumarol 10 mM (Table 1). AHCC was added as a test agent at 0.42 mg/mL and serially diluted 1:3 for eight wells. NADPH was measured by UV absorbance at 200 nm or DCPIP at 600 nm on a FL600 Dual-Band plate reader from BioTek Instruments, Inc. (Winooski, VT, USA).

Phase II enzyme induction assay

The cryopreserved human hepatocytes obtained from Corning (Gentest™ Discovery Labware Product, Union City, CA) were re-plated using supplemented Hepatocyte SFM media (Gibco™).
Invitrogen Corporation, Carlsbad, CA, USA) containing 10% fetal bovine serum (Gemini Bio-Products, West Sacramento, CA, USA) and 250 µM ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) in 6-well collagen-coated culture plates. Cells were allowed to adhere for 8 hours prior to removal of seeding media. Hepatocytes were maintained in un-supplemented Hepatocyte SFM media for at least 24 hours prior to the study being initiated. For the COMT method, primary hepatocyte cells were incubated with 5nM 17-β estradiol in the presence of 0.21 mg/mL and 0.42 mg/mL AHCC compared to control known inducer, folic acid 150 ng/mL [13] (Table 2). Estradiol concentrations remaining at each time point were evaluated by a validated high pressure liquid chromatography (HPLC) assay with ultraviolet (UV) absorbance detection according to parameters described in the CDER Guidance for Industry Bioanalytical Assay Method Validation detection [14].

In the phase II metabolism in vitro studies, four primary hepatic metabolism pathways commonly associated with drug metabolism were tested: glutathione S-transferase (GST), UDP-glucuronosyltransferase (UGT), quinone oxidoreductase (QOR) and catechol-O-methyl transferase (COMT). AHCC demonstrated no inhibitory effect on the GST or the UGT2B17, UGT1A3 or UGT1A6 pathways (p > 0.05) (Figures 2-5). AHCC did however inhibit in the QOR pathway that was statistically significant (p < 0.01) compared untreated enzyme and was comparable to the control inhibitor discoumarol (p > 0.05) (Figure 6).

In the ex vivo cryopreserved human hepatocyte model, AHCC demonstrated no induction effects on the COMT metabolic pathway (p > 0.01). Both phase II metabolism in vitro studies evaluating UGT1A3 and UGT1A6 pathways and ex vivo cryopreserved human hepatocyte studies demonstrated the potential for induction of the UGT1A3 and UGT1A6 pathways (p > 0.05) (Table 3).

The integration of nutritional supplements and herbal products has become increasingly more frequent in Western oncology clinical practice. Progressively it is becoming more common for patients to integrate the use of nutritional supplements in the treatment of

### Table 2: Substrates used in the in vitro hepatocyte induction studies.

| Test Substrate | Pathway | Substrate Concentration | Wavelength |
|----------------|---------|-------------------------|------------|
| Estrone        | UGT1A3  | 150 µM                  | 230 nm     |
| 1-naphthol     | UGT1A6  | 600 µM                  | 220 nm     |
| 17-β-estradiol | COMT    | 500 ng/mL               | 200 nm     |

if the coefficient of variance was greater than 20%. Final results are described through appropriate summary statistics (e.g. means, standard deviations and correlation coefficients). ANOVA was employed to determine differences in metabolism activity for each respective metabolic pathway and Pearson’s correlation test was used to evaluate all correlations. A paired t-test was used to evaluate the viability in continuous data as appropriate. Results were considered to be significant when p < 0.05. The program GraphPad Prism 5.02 (GraphPad Software Inc., San Diego, CA, USA) was used to perform the analysis.

### Results and Discussion

The integration of nutritional supplements and herbal products has become increasingly more frequent in Western oncology clinical practice. Progressively it is becoming more common for patients to integrate the use of nutritional supplements in the treatment of

![Graph: GS-DNB concentration over time](image-url)
Curcumin
AHCC
1% DMSO
Reaction Buffer (RB)
Ethacrynic acid
No enzyme

Figure 3: UGT2B17 inhibition results.

Figure 4: UGT1A3 inhibition results.
various comorbidities including cancer. Spierings et al. previously had reported the safety and the acceptable tolerability of AHCC in healthy volunteers and multiple studies have been conducted evaluating the potential benefits of the integration of AHCC with conventional chemotherapy to improve treatment outcomes [15]. Hence while the use of AHCC is becoming more common in patients with cancer on multiple concomitant medications, the metabolic activity of AHCC was important to define to minimize potential for drug-drug interactions that could alter the effectiveness of the chemotherapy agents. The potential for drug interactions including either decreased activity or increased toxicity of chemotherapy agents used in the treatment of cancer is very concerning and needs to be minimized.

Figure 6: QOR inhibition results.

Figure 5: UGT1A6 inhibition results
In a study by Mach et al. that evaluated the phase I cytochrome P450 metabolism pathway interactions with AHCC was demonstrated only to have potential to interact with the CYP450 2D6 pathway [6]. The current study adds to safety information by demonstrating that AHCC is unlikely to interaction with the GST, UGT2B17 or COMT phase II pathways commonly associated with drug metabolism/detoxification. The observed potential for AHCC to interact with drugs/agents that are substrates of the UGT1A3, UGT1A6, or QOR pathways should be taken into consideration when co-administering AHCC with those drugs in Table 4 to avoid increased toxicity due to inhibition of the QOR pathway or potentially less efficacy due to induction of UGT1A6 or UGT1A3 pathway.

While the preclinical drug metabolism studies have a benefit to identifying the potential for drug interactions, confirmatory pharmacokinetic/pharmacodynamic studies are warranted to evaluate potential interactions. Many drug-drug interactions are concentration-dependent, so data always has to be assessed in the context of what is clinically achievable. In particular since there is no current data on plasma concentrations achieved with administration of AHCC thus this study had made the assumption of “100% bioavailability” to select the concentration 0.42 mg/mL of AHCC to use in each assay. Hence, the observed potential interactions in this study may not have clinical significance because it is unlikely AHCC has 100% bioavailability.
data the clinical judgment should be employed in considering the safety of use of AHCC of other substrates of the QOR and UGT1A6 or UGT1A3 pathways.

**Conclusion**

Based on the *in vitro* and *ex vivo* metabolism data from this study and our previous phase I metabolism studies, AHCC does not have potential for potential drug-supplement with drugs/agents that function as substrates of CYP450 3A4, C28, or C29, GST, UGT 2B17 or COMT pathways [6]. AHCC may have potential for drug-supplement interactions with drugs/agents that are substrates of the CYP450 2D6, UGT1A3, UGT1A6 or QOR pathways (Table 5). However, additional studies are needed to determine the clinical significance of potential interactions. Specifically, since there was a difference between the low and high concentration of AHCC, it will be important to determine the actual systemic concentration of AHCC that can be achieved with commonly used AHCC supplementation of three grams per day.

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