Volume 6, Number 3 Summer 2008

Journal of the Society for Integrative Oncology

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ISSN 1715-894X

ARTICLE

Evaluation of Active Hexose Correlated Compound Hepatic Metabolism and Potential for Drug Interactions with Chemotherapy Agents

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Active hexose correlated compound (AHCC), a *Basidiomycotina* extract, is a well-tolerated nutritional supplement with no reported adverse effects. It has demonstrated potential antitumor activity and immune modulator activity. However, there is no current information regarding its metabolism and the potential for drug-drug interactions for AHCC in combination with chemotherapy. The objective of this study was to characterize AHCC hepatic metabolism, specifically involving the potential for drug interactions with selected chemotherapy agents. High-throughput cytochrome P-450 (CYP450) metabolism inhibition experiments were conducted in vitro evaluating CYP450 3A4, 2C8, 2C9, and 2D6 followed by an evaluation of AHCC as a substrate of these same isoenzymes. An ex vivo model of cryopreserved human hepatocytes was used to evaluate the CYP450 metabolism induction potential of AHCC for CYP450 3A4, 2C8/2C9, and 2D6. No inhibition of CYP450 activity was observed in presence of AHCC; however, AHCC was a substrate of CYP450 2D6. The CYP450 induction metabolism assays indicate that AHCC is an inducer of CYP450 2D6. AHCC does have the potential for drug-drug interactions involving CYP450 2D6, such as doxorubicin or ondansetron; however, the overall data suggest that AHCC would be safe to administer with most other chemotherapy agents that are not metabolized via the CYP450 2D6 pathway.

Key words: *active hexose correlated compound, cancer, inducer, metabolism, substrate*

Active hexose correlated compound (AHCC; Amino Up Chemical Co, Ltd., Sapporo, Japan) is an extract prepared from cultured mycelium of a Basidiomycetes rich in polysaccharides and fiber. The main component is acetylated α-glucan with a molecular weight of around 5,000 Da and contains < 0.2% β-glucans, which have a molecular weight of 10,000 to 500,000 Da, with the lower-molecular-weight α-glucans having much better absorption. Some uncharacterized proprietary elements result from the unique process by which AHCC is prepared that contribute to the product activity. AHCC has been used since 1989 in Japan, with no current reports of side effects or toxicities, and has been proposed to have many health benefits, including both immunomodulatory and antitumor effects. In clinical studies, AHCC has demonstrated the ability to decrease the side effects associated with anticancer chemotherapy.[1] In addition, previous studies have reported that the AHCC product has antidiabetic effects and antihyperlipidemia effects, as well as antihepatitis effects.[2–5] The functions of particular interest in the oncology arena are AHCC's immunomodulating and potential restorative effects on natural killer cells, macrophages, and cytokines after anticancer chemotherapy. There is also interest in AHCC's antitumor effects and the potential role for AHCC in the treatment of cancer.[6,7] However, the safety of combining this agent with anticancer agents has not been evaluated.

AHCC is proposed to have some of its antihepatitis activity by inducing hepatic enzymes to enhance the detoxification process and metabolism of toxic, offending agents.[3,5] This raises concerns for the possibility of drug interactions because of the likelihood of induction of

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Supported by a grant from Amino Up Chemical Co., Ltd., Sapporo, Japan.

Presented in part at the 15th International AHCC Symposium in Sapporo, Japan, July 22, 2007.

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DOI 10.2310/7200.2008.0022

hepatic cytochrome P-450 (CYP450) drug metabolism as well. Many commonly used anticancer agents undergo metabolism through the CYP450 pathway, such as doxorubicin, paclitaxel, cyclophosphamide, and irinotecan (Table 1). The objective of this study was to characterize AHCC hepatic metabolism, specifically involving the potential for drug interactions with selected chemotherapy agents.

Methods

Chemicals

The chemicals or reagents used were of the highest analytical grade available. AHCC was generously provided by Amino Up Chemical Co. All other chemicals and reagents were obtained from Sigma-Aldrich (St. Louis, MO).

Cytochrome P-450 Enzyme Microsomes

CYP450 3A4, 2C8, and 2C9 isoenzyme microsomes were obtained from BD Biosciences: Discovery Labware, BD Gentest (Woburn, MA). Each isoenzyme was packaged in 0.5 mL aliquots; the stated total protein content is 5.8 mg/mL in 100 mM potassium phosphate (pH 7.4), and the corresponding CYP450 content is 1,000 pmol/mL. These products are stable for two years; they were stored at −80°C and used in accordance with provided MSDS and technical bulletins.

High-Throughput CYP450 Inhibition Assays

The assay protocol was adapted from a validated protocol, High Throughput Method for Measuring CYP450 Inhibition (BD Gentest, version 4.2, 2000, BD).[8] Briefly, the positive controls (quercetin, sulfaphenazole, quinidine and ketoconazole), test compound (AHCC), and substrates (dibenzylfluorescein [DBF] and 3-[2-(*N*,*N*-diethyl-*N*-methylammonium)ethyl]-7-methoxy-4-methylcoumarin iodide (AMMC) were dissolved in acetonitrile, and working solutions were made by dilution in 0.5 M potassium phosphate buffer, pH 7.4. The common solutions, cofactor stocks, enzyme/substrate mixes, and positive control solutions were all prepared as recommended by the manufacturer. Each reaction well had final cofactor concentrations of 1.3 mM oxidized nicotinamide adenine dinucleotide phosphate, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, and 3.3 mM magnesium ion. The assay reactions were carried out at 200 µL total volume in 96-well titer plates. After the addition of the appropriate enzyme, substrate (DBF at 100 µM or AMMC at 500 µM) and inhibitor positive control (Table 2) or AHCC at a maximum concentration of 0.42 mg/mL was added to the reaction mixture and serially diluted 1:3 for eight wells (range 0.42 mL to 0.0002 mg/mL). The concentration of 0.42 mg/mL was chosen as an estimate of maximum achievable human serum concentration based on the current maximum recommended dosage of 3 g daily per the manufacturer's instructions, assuming 100% bioavailability, and then dividing by 7 L as the estimated total blood volume of the average adult. There are no current bioavailability data for AHCC; however, calculating the serum concentration in this manner would provide a ceiling, which would give the maximum concentration of AHCC expected in the serum. Higher AHCC serum concentrations may have more CYP450 inhibition or induction; however, if these concentrations are not achievable, the interaction becomes irrelevant. The reactions were incubated at 37°C for 30 to 60 minutes and were stopped with the addition of 75 µL of a 2 M NaOH solution or 80:20 acetonitrile:tris base solution (CYP450 2D6 only). In each reaction well, 4, 2, or 0.5 pmol was present of CYP450 2C8, 2C9, or 3A4, respectively. The extent of CYP450 inhibition was evaluated via calculation of the appropriate IC_{50} values. These data were produced by comparing the metabolism in assay reactions containing varied concentrations in the presence and absence of the known inhibitor. The amount of product metabolized for the control comparison reactions was determined via fluorescence emission detection at 528 nm (excitation 485 nm) of fluorescein (metabolite product of DBF metabolism by CYP450) or at 460 nm (excitation 360 nm) of 3-[2-(*N*,*N*-diethyl-*N*-methylammonium)ethyl]-7-hydroxy-4-methylcoumarin (AMHC) (metabolite product of AMMC). The relative amount of AHCC substrate metabolized was read by a FL600 Dual-Band plate reader from BioTek Instruments, Inc. (Winooski, VT).

Table 1. Common Anticancer Therapy and CYP450 Metabolism

Drug	*Substrate*	*Inhibitor*	*Inducer*
Cyclophosphamide	2B6, 2C19		
Ifosfamide	2B6		
Paclitaxel	2C8/2C9, 3A4		2C8/2C9
Tamoxifen	2C9, 2D6, 3A4		
Docetaxel	3A4		
Irinotecan	3A4		
Vincristine	3A4		
Interferon	1A2	1A2	
Imatinib	3A4	3A4	
Doxorubicin	3A4	2D6	

Table 2. Inhibitors Used in the In Vitro Inhibition Studies

CYP450 Inhibitor	*Fluorometric Substrate*	*Excitation/Emission Wavelength (nm)*	*CYP Inhibited*	*Inhibitor Concentration (μM)*	*Reaction Time (min)*
Ketoconazole	DBF	485/528	3A4	0–7.5	30
Quercetin	DBF	485/528	2C8	0–10	60
Quinidine	AMMC	360/460	2D6	0–0.75	45
Sulfaphenazole	DBF	485/528	2C9	0–15	60

AMMC = 3-[2-(*N*,*N*-diethyl-*N*-methylammonium)ethyl]-7-methoxy-4-methylcoumarin iodide; DBF = dibenzylfluorescein.

Human Hepatocytes

The cryopreserved human hepatocytes were obtained from BD Biosciences. Hepatocytes were replated using supplemented Hepatozyme SFM medium (Gibco, Invitrogen Corporation, Carlsbad, CA) containing 10% fetal bovine serum (Gemini Bio-Products, West Sacramento, CA) and 250 μM ascorbic acid (Sigma-Aldrich). Hepatocytes were then maintained in unsupplemented Hepatozyme SFM medium. Primary hepatocyte cells were plated into separate six-well collagen I–coated plates for the CYP450 induction and substrate experiments, respectively. The cultures were maintained in quadruplicate for each experimental time point. The cells were handled according to the supplier's instructions and were thawed, isolated, and plated according to the supplier's protocol. The cells were allowed 48 hours incubation at 37°C (5% CO_2) to allow recovery before use in any experimentation.

Hepatic Metabolism Induction Assay

An ex vivo model of cryopreserved human hepatocytes was used to evaluate the ability of AHCC to induce CYP450 metabolism for CYP P450 3A4, 2C8/2C9, and 2D6. A known substrate for each isoenzyme was selected, including diclofenac (CYP450 2C8/2C9), dextromethorphan (CYP450 2D6), or docetaxel (CYP450 3A4). The experiment was set up in quadruplicate comparing the known substrate alone, to account for the intrinsic metabolism of the known substrate, using a control inducer, rifampicin 25 μM (Sigma-Aldrich), or AHCC 0.42 mg/mL as the test inducer and known substrate. Hepatocytes were induced for 72 hours with either the control inducer rifampicin or AHCC, with the substrate control plate receiving only medium changes every 24 hours; the medium containing the inducers was also changed every 24 hours. After 72 hours, the inducer was removed and the appropriate concentration substrate specific to the CYP450 isoenzyme of interest was added or test substrate AHCC was added (Table 3). Time points were taken at 0, 2.5, and 24 hours, and the absorbance was read at the appropriate wavelength for the test substrate (see Table 3) to determine if AHCC was an inducer and if its metabolism was induced by the control inducer, rifampicin.

Results

AHCC achieved no inhibition at any concentration in the CYP450 2C8, 2C9, or 2D6 in vitro studies. Only 7.9%, 2.1%, and 0.5% inhibition of CYP450 3A4 was achieved at AHCC 0.42, 0.14, and 0.046 mg/mL, respectively. The control inhibitor for CYP450 3A4, ketoconazole, achieved 90.2%, 87.6%, and 82.0% inhibition at a concentration of 7.5, 2.5, and 0.833 μM, respectively, continuing to decrease to a concentration of 0.005 μM, at which no inhibition was seen. Hence, AHCC is not an inhibitor of the selected CYP450 isoenzymes at what would be expected to be a physiologically achievable systemic concentration (0.42 mg/mL); therefore, IC_{50} values could not be determined.

Using the CYP450 in vitro isoenzyme metabolism assay, it was determined that AHCC is a substrate of the

Table 3. Substrates Used in the Ex Vivo Hepatocyte Induction Study

CYP450 Test Substrate	*CYP*	*Substrate Concentration*	*Wavelength (nm)*
Diclofenac	2C8/2C9	100 μM	280
Dextromethorphan	2D6	100 μM	280
Docetaxel	3A4	100 μM	230
AHCC	2D6	0.42 mg/mL	270

AHCC = active hexose correlated compound.

CYP450 2D6 pathway with 9% metabolism after 45 minutes of incubation. There was minimal metabolism of AHCC in the presence of the CYP450 2C8, 2C9, and 3A4 isoenzymes (Table 4).

In the ex vivo hepatocyte induction studies, no induction of CYP450 2C8/2C9 or CYP450 3A4 was observed. At 2.5 hours, there was a 55.5% induction in metabolism after AHCC treatment compared with a 62.9% induction by the control, rifampicin (Figure 1), with a p value of .48 indicating that there was no difference in the induction of CYP2D6 metabolism observed with AHCC compared with the induction seen with rifampicin.

A summary of the AHCC hepatic profile determined by these experiments is presented in Table 5.

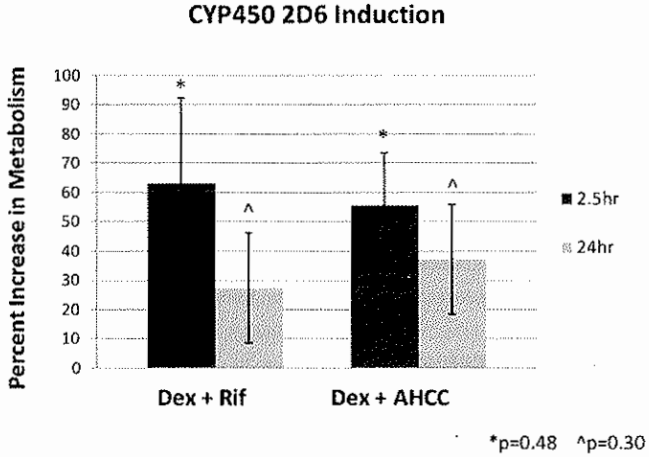

Figure 1. CYP450 2D6 induction. Percent increase in metabolism is compared with test substrate alone as the control. AHCC = active hexose correlated compound; Dex = dextromethorphan; Rif = rifampicin.

Discussion

The integration of nutritional supplements and herbal products has become increasingly more common in Western world oncology clinical practice. However, before we can define the role of each nutritional supplement and herbal product in the conventional treatment of cancer, more information is needed about the complex pharmacology of these agents in appropriate preclinical studies to confirm the safety and limit potential drug-drug interactions as we proceed to design and initiate prospective clinical studies to establish the role of herbal and nutritional therapies into current clinical practice.

Spierings and colleagues recently reported the safety and acceptable tolerability of AHCC in healthy volunteers.[9] However, prior to using AHCC in patients with cancer on multiple concomitant medications, the metabolic activity of AHCC was important to define to minimize the 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. Unfortunately, hepatic drug metabolism is unpredictable based on compound structure or drug class alone. Hence, the need to do metabolism studies on each compound is required to define its hepatic metabolism profile.

The results of this in vitro metabolism study indicated that AHCC is not an inhibitor of the major cytochrome P-450 isoenzyme pathways. AHCC is therefore unlikely to result in increased toxicity when used in combination with chemotherapy or supportive therapies such as antinausea medications or antidepressants. The results of the ex vivo hepatocyte induction studies indicated that AHCC may have the potential for interactions with drugs that are metabolized via the CYP450 2D6 pathway. CYP450 2D6 activity is generally regarded as noninducible; however, an increase in activity was seen with exposure to AHCC and has been seen with other herbal products.[10] Both rifampicin and AHCC had similar induction of the CYP450 2D6 pathway in this study. The drug interactions with rifampicin observed in clinical practice are significant, and close monitoring for drug interaction is generally recommended for patients on rifampicin. Hence, patients

Table 4. Active Hexose Correlated Compound CYP450 Substrate Determination

	Percent Metabolism			
Substrate Tested	*3A4*	*2C8*	*2C9*	*2D6*
Control substrate*	95	68	24	36
AHCC	−7	−0.5	−2	9

AHCC = active hexose correlated compound.
*See Table 2 for the appropriate fluorometric substrate.

Table 5. Summary of Active Hexose Correlated Compound CYP450 Metabolism Profile

CYP 450 Isoenzyme	*Substrate*	*Inhibitor*	*Inducer*
3A4	–	–	–
2C8	–	–	–
2C9	–	–	–
2D6	+	–	+

receiving AHCC should be monitored closely for drug interaction with substrates of the CYP 2D6 pathway.

Drugs that are substrates of the CYP450 2D6 pathway have the potential for increased metabolism that could be associated with decreased activity, especially if there is a narrow therapeutic window for plasma concentrations needed to achieve efficacy. Fortunately, the CYP450 2D6 pathway is not a predominant pathway for metabolism of the most commonly used chemotherapy agents except doxorubicin, which is used in both solid tumors and hematologic cancers and does inhibit the 2D6 pathway. The clinical significance of this potential interaction is unknown and warrants further in vivo evaluation. Other supportive agents used in the cancer setting, including antiemetics, antidepressants, and the steroid dexamethasone, also undergo some degree of metabolism through the 2D6 pathway; the clinical significance of this interaction with AHCC is unknown. Coadministration of these agents and AHCC could result in unexpected toxicities or decreased efficacy of the therapy.

There is growing evidence that herbal products, nutritional supplements, and functional foods are not completely benign when combining such agents with other medications. This emphasizes the importance for health care professionals to specifically discuss with patients what nutritional supplements and herbal products they might be consuming along with the prescription medications and chemotherapy agents. Furthermore, it is important that all nutritional supplements and herbal products undergo screening for drug interactions in a fashion similar to what is required for the approval of conventional prescription medications, to ensure that patients and providers have the most complete information possible to assist in the process of safe medication therapy. However, the feasibility of performing the necessary studies on the vast number of herbal products currently on the market is a daunting task. Often the drug interactions are discovered after patients have serious adverse events, and once these interactions are discovered, drug companies will add these known interacting herbal products to their package inserts. A lack of knowledge of possible drug interactions does not indicate safety; however, funding for such studies is lacking. The assumption that herbal products are safe and benign because they are natural is unfounded, and studies such as this study demonstrate that herbal or natural products may have potential interactions with common medications used in patients undergoing treatment for cancer. As this current study demonstrated, AHCC does have the potential for drug-drug interactions involving CYP450 2D6, such as doxorubicin or ondansetron; however, the overall data suggest that AHCC would be safe to administer with most other chemotherapy agents that are not metabolized via the CYP450 2D6 pathway.

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