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Detection and Quantification of Glucuronidation of Ursolic Acid (UA) in Human Liver Microsomes (HLMs).

Kamola Tolliboeva
Virginia Commonwealth University

Philip M. Gerk
Virginia Commonwealth University

VIRGINIA COMMONWEALTH UNIVERSITY

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Kamola Tolliboeva, Phillip M. Gerk; Department of Pharmaceutics

Abstract

The purpose of this experiment was to establish and quantify the glucuronidation of ursolic acid (UA) in human liver microsomes (HLMs). We used three (100, 10, 1 uM) different UA concentrations incubated in 0.2 ug/uL HLMs for 1 hour. For each concentration of UA, we had an experimental (with UDPGA) and control group (without UDPGA). Aqueous samples were then treated with acetonitrile. Sample analysis was performed on a Cogent bidentate C18 column, with 90% methanol and 10% aqueous 10 mM ammonium acetate, and detection by electrospray mass spectrometry in negative ion mode ([M-H] = 455.3). The results confirmed the disappearance of UA in the presence but not absence of UDPGA.

Introduction

Ursolic acid (UA) is a lipophilic pentacyclic triterpenoid carboxylic acid that is widely found in food, herbs, fruits, and some medicines such as diabetic medications[1,2,3]. It is known to have range of biological activities such as serving as an antioxidant, anti-inflammatory, and an antibacterial[3]. Current research on the properties of UA has shown its potential for use in obesity, glucose intolerance, fatty liver disease[1], and tumor and cancer prevention[3,4]. Clinical trials administering UA on human subjects are currently underway, therefore, it's important to understand the metabolic process or glucuronidation of UA. Number of studies found the main UDP-glucuronosyltransferases (UGT) involved in the glucuronidation of UA were *UGT1A3 and UGT1A4*[3]. Based on the current research done on the metabolic mechanisms of UA, we hypothesize that UA is glucuronidated by HLMs as a source of UGT enzymes.

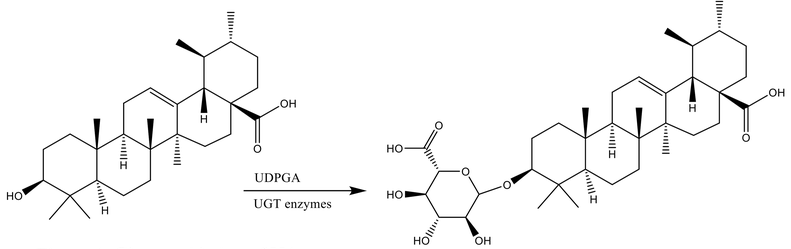
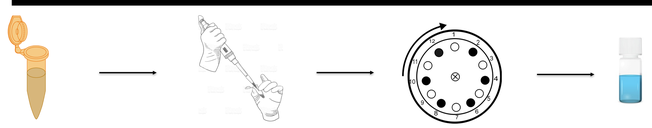


Figure 1: Glucuronidation of UA

Methods



Experimental and control groups were set up containing 3 different concentrations (100, 10, 1 uM) of UA in 50 mM Tris HCl (pH 7.4), 0.1mM saccharolactone, 10 mM $MgCl_2$, 0.2 ug/uL HLMs, 0.03125 mg/mL alamethicin, and distilled water.

The reactions were initiated with an addition of 2.5 mM UDPGA to samples in experimental group, and distilled water in the control group and incubated for 1 hour at 37°C. 50 uL samples from 3 concentrations of UA in both groups were collected at 1 and 60 minutes.

Each sample was treated with of 200 uL of aceto-nitrile, vortexed, and chilled on ice for 30 minutes, after which they were centrifuged at maximum speed (17000 RPM) and 4 °C for 10 minutes.

Supernatants were transferred to HPLC vials and analysis was performed on a Cogent bidentate C18 column, with 90% methanol and 10% aqueous 10 mM ammonium acetate, and detection by electrospray mass spectrometry in negative ion mode ([M-H] = 455.3).

Results

The results of our experiment showed disappearance of UA after 1 hour in the samples of the experimental group containing UDPGA compared to the control containing no UDPGA. This is consistent with glucuronidation of UA in the incubation mixture.

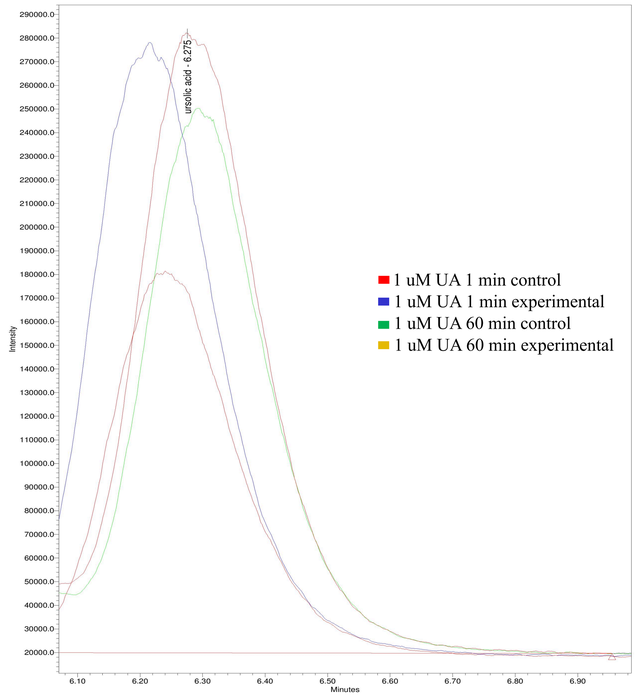


Figure 2: UA remaining after glucuronidation

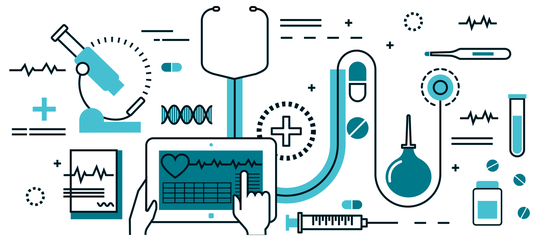
Additionally, we were able to quantify the glucuronidation of UA based on its peak height which showed gradual decrease with time indicating the loss of UA in the samples.

Table 1: Quantification of Glucuronidation of UA

Sample Name	Time (mins)	Height (uV)
1 uM experimental	1	262,035
1 uM control	1	262,214
1 uM experimental	60	165,437
1 uM control	60	230,565

Discussions

With numerous research studies pointing at the benefits of UA, many researchers are considering it as a potential therapeutic treatment for different diseases in humans. However, interaction of UA with metabolizing enzymes and other prescription drugs is largely unknown which creates several concerns regarding its administration to patients in clinical trials. In this experiment we were able to develop methods to establish the interaction of UA with UDPGA and UGT enzymes found in HLMs. We quantified the interaction by the peak height measured in uV indicating the disappearance of UA over time. Further refinement of these methods will help us understand how UA interacts with different UGT enzymes present in the human body (especially liver and intestine) allowing us to maximize its oral bioavailability and efficacy.



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