Inhibition of UDP-Glucuronosyltransferase Enzymes by Major Cannabinoids and Their Metabolites

Shamema Nasrin, Christy J. W. Watson, Keti Bardhi, Gabriela Fort, Gang Chen, and Philip Lazarus

Department of Pharmaceutical Sciences, College of Pharmacy and Pharmaceutical Sciences, Washington State University, Spokane, Washington

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ABSTRACT

The UDP-glucuronosyltransferase (UGT) family of enzymes play a central role in the metabolism and detoxification of a wide range of endogenous and exogenous compounds. UGTs exhibit a high degree of structural similarity and display overlapping substrate specificity, often making estimations of potential drug-drug interactions difficult to fully elucidate. One such interaction yet to be examined may be occurring between UGTs and cannabinoids, as the legalization of recreational and medicinal cannabis and subsequent co-use of cannabis and therapeutic drugs increases in the United States and internationally. In the present study, the inhibition potential of the major cannabinoids Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN), as well as their major metabolites, was determined in microsomes isolated from HEK293 cells overexpressing individual recombinant UGTs and in microsomes from human liver and kidney specimens. The highest inhibition was seen by CBD against the glucuronidation activity of UGTs 1A9, 2B4, 1A6, and 2B7, with binding-corrected IC50 values of 0.12 ± 0.020 μM, 0.22 ± 0.045 μM, 0.40 ± 0.10 μM, and 0.82 ± 0.15 μM, respectively. Strong inhibition of UGT1A9 was also demonstrated by THC and CBN, with binding-corrected IC50 values of 0.45 ± 0.12 μM and 0.51 ± 0.063 μM, respectively. Strong inhibition of UGT2B7 was also observed for THC and CBN; no or weak inhibition was observed with cannabinoid metabolites. This inhibition of UGT activity suggests that in addition to playing an important role in drug-drug interactions, cannabinoid exposure may have important implications in patients with impaired hepatic or kidney function.

SIGNIFICANCE STATEMENT

Major cannabinoids found in the plasma of cannabis users inhibit several UDP-glucuronosyltransferase (UGT) enzymes, including UGT1A6, UGT1A9, UGT2B4, and UGT2B7. This study is the first to show the potential of cannabinoids and their metabolites to inhibit all the major kidney UGTs as well as the two most abundant UGTs present in liver. This study suggests that as all three major kidney UGTs are inhibited by cannabinoids, greater drug-drug interaction effects might be observed from co-use of cannabinoids and therapeutics that are cleared renally.

Introduction

UDP-glucuronosyltransferases (UGTs) are an important family of phase II metabolizing enzymes that facilitate the detoxification of a wide variety of endogenous and exogenous compounds, including steroid hormones, drugs, and environmental carcinogens (Meech et al., 2019). Mammalian UGTs are classified based on structural and amino acid sequence homology into two main families, the UGT1 and UGT2 families, which are further divided into three subfamilies UGT1A, UGT2A, and UGT2B, and catalyze the transfer of glucuronic acid from UDP glucuronic acid (UDPGA) to an electrophilic moiety of a given substrate, resulting in a more polar conjugate that is more easily excreted from the body in the urine or bile (Bushey and Lazarus, 2012). An additional subfamily, the UGT3A subfamily, contains two members, UGT3A1 and UGT3A2, which use the alternative sugar donors UDP-N-acetylgalactosamine, UDP-glucose, and UDP-xylene as co-substrates (MacKenzie et al., 2011). Mammalian UGTs are membrane-bound enzymes localized in the endoplasmic reticulum and expressed with a high degree of tissue specificity (Meech et al., 2019). Although many UGTs are highly expressed in the liver, some are also expressed in extrahepatic tissues, including kidney and tissues of the aerodigestive tract (Meech et al., 2019; Vergara et al., 2020). The UGTs that exhibit the highest level of hepatic expression are UGTs 2B7 (17% of total hepatic UGT expression), 2B4 (16.1%), 2B15 (11.2%), and 1A1 (11%) (Kasteel et al., 2020). A number of UGTs are also expressed in the

ABBREVIATIONS: AZT, azithromycin; BSA, bovine serum albumin; CBD, cannabidiol; CBN, cannabinol; DDI, drug-drug interaction; HEK, human embryonic kidney; HKM, human kidney microsome; HLM, human liver microsome; IC50,u, binding-corrected IC50; 7-OH-CBD, 7-hydroxy-cannabidiol; 11-OH-CBD, 11-hydroxy-cannabidiol; 11-OH-THC, 11-hydroxy-Δ9-tetrahydrocannabinol; P450, cytochrome P450; rUGT, recombinant UGT; THC, (+)-trans-Δ9-tetrahydrocannabinol; THC-COOH, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol glucuronide; THC-COOH, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol glucuronide; UPLC, ultra-high-performance liquid chromatography; UPLC-MS/MS, UPLC–tandem mass spectrometry.
kidney, including UGT1A9 (45% of total renal UGT expression), UGT2B7 (41%), and UGT1A6 (7%) (Rowland et al., 2013). UGTs account for the metabolism of 15% of pharmaceuticals, and one-seventh of the drugs prescribed in the United States in 2002 are cleared by the UGTs (Williams et al., 2004). Although studies of drug-drug interactions (DDIs) are a major emphasis of research for phase I metabolizing enzymes including the cytochrome P450 enzyme family, UGT enzymes have historically received less scrutiny for their DDI potential, even though drug interactions via the inhibition of glucuronidation have been increasingly identified. Impaired glucuronidation activity can cause undesired effects resulting from the slow elimination of endogenous substances such as bilirubin (Sun et al., 2017) as well as the buildup of toxic drug metabolites, as has been documented in studies correlating individuals with UGT1A1-deficient phenotypes and irinotecan toxicity (Iyer et al., 1998; Tallman et al., 2007). DDIs between therapeutics and UGT inhibitors have also been observed in the case of UGT2B7 inhibition by both valproic acid and probenecid (Cimoch et al., 1998; Rowland et al., 2006).

The recent legalization of cannabis has caused a dramatic increase in the use of cannabis-derived products in both recreational and medicinal situations, where cannabis is frequently used or targeted for more chronic diseases like cancer, arthritis, and depression and often concurrently used with important groups of conventional medications including anticancer agents, antidepressants, and pain medications (Bridgeman and Abazia, 2017). Situations in which polypharmacy is occurring within a patient could result in deleterious DDIs between cannabinoids and any number of therapeutic agents. \(\Delta^8\)-tetrahydrocannabinol (THC) is the best described psychoactive constituent of cannabis, and plasma concentrations of THC and its active metabolite, 11-hydroxy \(\text{(OH)}\)-THC, quickly peak after use and decrease rapidly over a short duration, dependent on the specific mode of consumption (Fig. 1) (Sharma et al., 2012). In contrast, the inactive metabolites, 11-nor-9-carboxy-\(\Delta^8\)-tetrahydrocannabinol (THC-COOH) and 11-COO-\(\Delta^8\)-tetrahydrocannabinol-glucuronide (THC-COO-Gluc), peak much more slowly, to a lower level than the active cannabinoids, and remain present in plasma over a much longer duration of time (Huestis, 2007). Actual plasma levels of active and inactive cannabinoids are highly variable (in the micromolar to submicromolar range) and will vary widely depending on the user, dose, and method of ingestion. Cannabinol (CBN) appears to be a degradation product of THC within the \textit{Cannabis} plant (Russo and Marcu, 2017) and has been shown to be only weakly psychoactive. Cannabidiol (CBD) is often termed as medical marijuana and interacts with the CB1 and CB2 receptors in the brain with a much lower affinity as compared with THC and 11-OH-THC, resulting in extremely low psychoactive effects (Pertwee, 2008). However, CBD usage is rapidly expanding among many patient populations due in part to its good safety profile (Larsen and Shahinas, 2020). Recent clinical and preclinical trials have shown that CBD has a broad range of potential applications, displaying anti-inflammatory properties, antipsychotic, and antiepileptic effects, as well as modulation of the immune system and the central nervous system (Esposito et al., 2013; Boychuk et al., 2015; Campos et al., 2016; Devinsky et al., 2016). Similarly, CBD and its metabolites, 7-hydroxy-cannabinol (7-OH-CBD) and 7-carboxy-cannabinol (CBD-COOH), are present in the plasma after cannabis inhalation, with unchanged CBD and glucuronidated CBD (CBD-COO-Gluc), as the main excretion products in urine (Harvey and Mechoulam, 1990; Huestis, 2007). All cannabinoids are highly lipophilic and concentrate in tissues with slow release back into the bloodstream (Huestis, 2007). This leads to varying plasma concentrations of active and inactive cannabinoids that persist in the bloodstream, potentially incurring deleterious DDIs over a much wider time frame than that of the initial cannabis consumption.

Previous studies have shown that THC, CBD, and CBN can strongly inhibit several major hepatic cytochrome P450s (P450s) (Yamaori et al., 2010; Yamaori et al., 2011a; Yamaori et al., 2011b; Yamaori et al., 2011c; Jiang et al., 2013; Cox et al., 2019; Nasrin et al., 2021). In addition, the major active metabolite of THC, 11-OH-THC, and two major inactive metabolites, THC-COOH and THC-COO-Gluc, also exhibited strong inhibition of a number of hepatic P450 enzymes (Nasrin et al., 2021). In the present study, the inhibition potential of major cannabinoids and their metabolites against major hepatic and renal human UGT enzymes were evaluated.

![Fig. 1. Metabolic pathways and structures of major cannabinoids and their metabolites.](dmd.aspetjournals.org)
Material and Methods

Chemicals and Reagents. THC, 11-OH-THC, THC-COOH, THC-COO-Gluc, CBD, 7-OH-CBD, and CBN were purchased from Cayman Chemicals (Ann Arbor, MI) or Sigma-Aldrich (St. Louis, MO). Pooled human liver microsomes (HLMs) \( n = 50 \), mixed gender \( 21 \) female and \( 29 \) male, race \( 4 \) Caucasian, \( 4 \) Hispanic, \( 2 \) African American, and \( 2 \) Asian, and age \( 5-77 \) years and pooled human kidney microsomes (HKMs) \( n = 8 \), mixed gender \( 50\% \) each, race \( 3 \) African American, \( 3 \) Caucasian, and \( 2 \) Hispanic, and age \( 42-70 \) years) were obtained from Sekisui Xenotech, LLC (Lenexa, KS). 

MgCl\(_2\) (5 mM), 2% BSA, and 4 mM UDPGA in a column (BEH C\(_{18}\), 1.7 μm) were added to increase the solubility of cannabinoids as well as to sequester inhibitory fatty acids (Rowland et al., 2008; Patela-Vrana et al., 2019). 

Microsomal membranes fragmentations of UGTs 1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B10, 2B15, and 2B17 were determined and described previously (Dellinger et al., 2006). Microsomal membrane fractions of UGT-overexpressing cell lines were isolated using microsomes \( 50 \) μg/mL of cannabinoid or metabolite, probe substrate (Supplemental Table 1), 50 mM Tris-HCl buffer \( \mathrm{pH} 7.4 \), MgCl\(_2\) (5 mM), 2% BSA, and 4 mM UDPGA in a final reaction volume of 25 μL. All substrates were used at concentrations near their respective Michaelis–Menten constant (\( K_{\text{m}} \), Supplemental Table 1). As cannabinoids exhibit extensive nonspecific binding (70–90%) to protein and labware (Garrett and Hunt, 1974), microsomal incubation conditions were optimized to prevent underestimation of inhibitory potency \( (IC_{50}) \). To reduce nonspecific binding and adsorption to labware, low-binding microcentrifuge tubes were used for all reactions, with BSA added to increase the solubility of cannabinoids as well as to sequester inhibitor long-chain unsaturated fatty acids (Rowland et al., 2008; Patela-Vrana et al., 2019). 

Microsomes were preincubated with alamethicin (50 μg/mg of microsomal protein) on ice for 20 minutes prior to incubation. The reaction was initiated by the addition of UDPGA and incubated for 60–120 minutes (Supplemental Table 1) at 37°C. Reactions were terminated and proteins precipitated by the addition of an equal volume (25 μL) of ice-cold stop solution (acentonitrile-methanol; 1:1). Samples were mixed on a vortex mixer and centrifuged at 17,000 × g for 15 minutes. The supernatant \( (~50 \mu L) \) was transferred to an ultra-high-performance liquid chromatograph (UPLC) sample vial, and the probe metabolite was detected using a UPLC (Waters Acquity; Waters Corp, Milford, MA) coupled to a triple-quadrupole mass spectrometer (Waters Xevo TQD; Waters Corp) by multiple reaction monitoring analysis. As a positive control for every inhibition experiment, 10 μM or 100 μM probe inhibitors (ketonezolide-diclofenac) were added instead of the cannabinoid compounds. Reactions containing only vehicle (3% methanol) and without any inhibitor were used as an indicator of 100% activity for each substrate/enzyme combination. All IC\(_{50}\) analyses were performed in triplicate. Incubation conditions were optimized for HLMs, HKMs, and overexpressing cell lines for both microsomal protein and reaction time, with optimal conditions chosen based on the following criteria: 1) metabolite formation was linear with time and enzyme concentration, 2) substrate consumption was no more than 20% of the initial amount, and 3) metabolite formation was reliably and reproducibly detected by the UPLC-tandem mass spectrometry (UPLC-MS/MS) method used.

For UPLC-MS/MS, samples \( 2-5 \) μL were injected onto an Acquity UPLC column (BEH C\(_{18}\), 1.7 μM, 2.1 × 100 mm; Waters Corp). A 9-minute gradient elution was used with mobile phases A (0.1% formic acid in water) and B (100% methanol) as follows: 1 minute at 95% A:5% B followed by a linear gradient for 7 minutes to 5% A:95% B, 1 minute at 5% A:95% B, and re-equilibration for 1 minute at 95% A:5% B. The flow rate was 0.4 ml/min, and the column temperature was 40°C. Analysts were detected using a Waters Xevo TQD tandem mass spectrometer equipped with a Zspray electrospray ionization interface operated in the positive ion mode for all the UGT metabolites tested in this study except fusidic acid glucuronide, which was analyzed in negative ion mode, with the capillary voltage at 0.6 kV. Nitrogen was used as both the cone and desolvation gas at 50 and 800 L/h, respectively. Ultrapure argon was used for collision-induced dissociation. The desolvation temperature was 500°C. For detection of the metabolite peaks, the mass spectrometer was operated in multiple reaction monitoring mode using the ion-related parameters for each transition. The following transitions were used for the detection of each probe metabolite: \( \beta \)-estradiol-3-glucuronide \( (m/z \) 447 > 271), acyl cheno-deoxycholic acid-24-glucuronide \( (m/z \) 567.5 > 391.5), trilufoherazine N-glucuronide \( (m/z \) 584 > 408.2), seratonin-glucuronide \( (m/z \) 352 > 160.02), propofol-O-glucuronide \( (m/z \) 354 > 177.02), codeine-6-glucuronide \( (m/z \) 476.2 > 300.2), AZT-5'-glucuronide \( (m/z \) 442 > 125.05), nicotine-N-glucuronide \( (m/z \) 339.15 > 163.124), S-oxazepam-glucuronide \( (m/z \) 463.3 > 269.1), and exemestane-17-O-glucuronide \( (m/z \) 475.23 > 281.19).

Determination of IC\(_{50}\) Values. For those cannabinoids or metabolites that inhibited UGT activity ≥50% at cannabinoid concentrations ≤100 μM, IC\(_{50}\) determinations were performed in HLMs, HKMs, and microsomes from HEK293 UGT-overexpressing cell lines, using multiple concentrations of cannabinoid inhibitor ranging between 0.5 and 120 μM. 

Experiments were performed to determine nonspecific binding constants \( (f_{\text{binding}}) \) for the individual cannabinoids in HEK293 microsomes, HLMs, and HKMs as previously described (NASIRI et al., 2021).

Statistical Analysis. Data were reported and analyzed using an Excel spreadsheet (Microsoft). The amount of metabolite formed at each concentration of inhibitor relative to the control (percent relative activity) was calculated as Peak area of metabolite with inhibitor/Peak area of metabolite without inhibitor × 100%.

IC\(_{50}\) values were calculated by plotting the percent relative activity of UGT enzymes versus the log concentration of the test inhibitors using GraphPad Prism 7.04 software (GraphPad Software Inc., San Diego, CA).

Results

Glucuronide metabolite peaks were detected by liquid chromatography–tandem mass spectrometry in incubations of each probe substrate analyzed in these studies (Fig. 2). Using recombinant UGT (rUGT)–overexpressing cell microsomes and probe UGT substrates, preliminary screening studies demonstrated that 100 μM THC decreased the relative activity of microsomes from rUGT 1A9, 2B4, and 2B7 overexpressing cells by 74%, 79%, and 69%, respectively, as compared with control reactions without added cannabinoid (Fig. 3). A similar pattern was observed for CBD, with 10 μM CBD exhibiting 25%, 91%, 66%, and 58% inhibition and 100 μM CBD exhibiting 54%, 98%, 94%, and 96% inhibition, against microsomes from rUGT1s 1A6, 1A9, 2B4, and 2B7 overexpressing cells, respectively, as compared with control reactions without added cannabinoid (Fig. 3). Similar to that observed for THC and CBD, CBN exhibited significant inhibition against rUGT1A9 and rUGT2B7 microsomes. Unlike that observed for THC and CBD, significant inhibition was not observed for rUGT2B4 microsomes with CBN. Although no significant inhibition was observed in rUGT1A1, rUGT1A3, rUGT1A4, and rUGT2B15 microsomes by THC, CBD, and CBN, marginal inhibition was observed for 100 μM CBD and CBN against rUGT2B17 microsomes (43% and 34%, respectively). Marginal inhibition (43% and 47%, respectively) was also observed for 100 μM CBN against rUGT1A6 and rUGT2B10 microsomes.

For THC and CBD metabolites, no significant inhibition was observed using up to 100 μM THC-COOH or THC-COO-Gluc against any of the UGT enzymes tested. However, 100 μM 11-OH-THC resulted in marginal inhibition of the activities of rUGT1A9 (41%),
rUGT2B4 (40%), and rUGT2B7 (53%) microsomes, whereas 100 μM 7-OH-CBD resulted in marginal decreases in the activities of rUGT1A9 (40%) and rUGT2B7 (45%) microsomes (Fig. 3).

The inhibitory effects of THC, 11-OH-THC, CBD and CBN were extended to establish IC₅₀ values and binding-corrected IC₅₀ values (IC₅₀,u) for each cannabinoid against the UGT enzymes shown to be inhibited by ≥ 50% using 100 μM cannabinoid in the rUGT screening assays (described above). The unbound fraction in the incubation mixture were 0.042 ± 0.003, 0.038 ± 0.002, and 0.085 ± 0.005 in overexpressing HEK cell lines for THC, CBD and CBN respectively. For HKMs the unbound fraction were 0.042 ± 0.003, 0.038 ± 0.002, and 0.085 ± 0.005 in overexpressing HEK cell lines for THC, CBD and CBN respectively.

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For HKMs, the unbound fraction were 0.042 ± 0.003, 0.038 ± 0.002, and 0.085 ± 0.005 in overexpressing HEK cell lines for THC, CBD and CBN respectively. For HKMs, the unbound fraction were 0.042 ± 0.003, 0.038 ± 0.002, and 0.085 ± 0.005 in overexpressing HEK cell lines for THC, CBD and CBN respectively.

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reversed for THC inhibition of codeine glucuronidation in HKMs (IC50 = 55 ± 5.2 μM and IC50,u = 2.9 ± 0.27 μM) versus HLMs (IC50 = 13 ± 2.6 μM and IC50,u = 0.61 ± 0.13 μM). Again similar to that observed for CBD, more moderate inhibition was observed for THC inhibition of AZT glucuronidation in HLMs and HKMs, with IC50 values that were only slightly higher than that observed for rUGT2B7 microsomes (IC50 values = 59 ± 6.6 (IC50,u = 2.8 ± 0.32 μM) and 51 ± 12 μM (IC50,u = 2.6 ± 0.65 μM), respectively). The pattern of inhibition observed for CBN for propofol glucuronidation was virtually identical to that observed for both THC and CBD, with similar IC50 values observed for rUGT1A9 microsomes (IC50 = 6.0 ± 0.75 μM and IC50,u = 0.51 ± 0.063 μM) and HKMs (IC50 = 7.5 ± 1.7 μM and IC50,u = 0.90 ± 0.20 μM) and a higher IC50 value observed for HLMs (IC50 = 31 ± 4.1 μM and IC50,u = 2.9 ± 0.38 μM; Table 1). Similar to that observed for both THC and CBD, CBN exhibited more moderate inhibition of AZT glucuronidation, with similar IC50 values observed for rUGT2B7 microsomes (IC50 = 49 ± 12 μM and IC50,u = 4.2 ± 1.1 μM), HLMs (IC50 = 59 ± 8.6 μM and IC50,u = 5.5 ± 0.79 μM) and HKMs (IC50 = 57 ± 7.5 μM and IC50,u = 6.9 ± 0.090 μM). Since CBN did not exhibit inhibitory activity against codeine glucuronidation in the screening assays, IC50 values were not determined for CBN against codeine glucuronidation in rUGT2B4 microsomes, HLMs, or HKMs. The only THC metabolite that exhibited ≥ 50% inhibition for any UGT in the rUGT microsomal screening assays was 11-OH-THC for UGT2B7. This metabolite exhibited weak inhibition of rUGT2B7

### TABLE 1

<table>
<thead>
<tr>
<th>_probe substrate</th>
<th>Microsomes</th>
<th>THC</th>
<th></th>
<th>CBD</th>
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<td></td>
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<td>IC50</td>
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<td>Serotonin</td>
<td>rUGT1A6</td>
<td>10 ± 2.6</td>
<td>0.40 ± 0.10</td>
<td>17 ± 3.7</td>
<td>1.0 ± 0.23</td>
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<tr>
<td></td>
<td>HKM</td>
<td>28 ± 6.5</td>
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<td>1.4 ± 0.33</td>
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<td></td>
<td>HLM</td>
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<td>Codeine</td>
<td>rUGT2B4</td>
<td>58 ± 1.2</td>
<td>0.22 ± 0.045</td>
<td>39 ± 5.9</td>
<td>2.5 ± 0.37</td>
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<td>80 ± 1.1</td>
<td>0.40 ± 0.058</td>
<td>21 ± 3.9</td>
<td>0.82 ± 0.15</td>
<td>49 ± 12</td>
<td>4.2 ± 1.1</td>
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<td>90 ± 8.6</td>
<td>5.5 ± 0.79</td>
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<td>1.5 ± 0.21</td>
<td>57 ± 7.5</td>
<td>6.9 ± 0.90</td>
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<td>AZT</td>
<td>rUGT2B7</td>
<td>21 ± 3.9</td>
<td>0.82 ± 0.15</td>
<td>40 ± 10</td>
<td>2.2 ± 0.22</td>
<td>6.0 ± 0.75</td>
<td>0.51 ± 0.063</td>
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<td>35 ± 3.5</td>
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<td>50 ± 8.6</td>
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<td>Propofol</td>
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<td>0.12 ± 0.020</td>
<td>9.2 ± 4.8</td>
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<td>5.5 ± 0.56</td>
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<td>2.4 ± 0.38</td>
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<td>19 ± 4.6</td>
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<td>0.78 ± 0.18</td>
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<td>Furosemide</td>
<td>rUGT1A9</td>
<td>2.6 ± 0.66</td>
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<td>HLM</td>
<td>29 ± 4.0</td>
<td>1.5 ± 0.20</td>
<td>15 ± 0.77</td>
<td>1.9 ± 0.092</td>
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<td>Acetaminophen</td>
<td>rUGT1A9</td>
<td>1.9 ± 0.29</td>
<td>0.073 ± 0.11</td>
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<td>3.8 ± 0.82</td>
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<td>30 ± 4.5</td>
<td>2.7 ± 0.96</td>
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</table>

[IC50 values are presented as means ± S.D. of three independent experiments. IC50,u, binding-corrected IC50; NA, not analyzed.]
Inhibitory effects of CBD on the glucuronidation of UGT probe substrates in microsomes from UGT-overexpressing HEK293 cell lines (rUGT), HLMs, and HKMs. Shown are representative plots comparing CBD concentration with the percent glucuronidation activity against probe substrates in rUGT microsomes, HLMs, and HKMs. Incubations were performed for 60–120 minutes at 37°C using 80–90 μg of rUGT microsomes or 90–200 μg HLMs or HKMs with the following probe substrates: propofol, acetaminophen, and furosemide for UGT1A9; serotonin for UGT1A6; codeine for UGT2B4; and AZT for UGT2B7 (see Supplemental Table 1 for concentrations). Individual metabolites were analyzed by UPLC-MS/MS as described in the Materials and Methods.

To better validate the inhibitory effects of cannabinoids on UGT1A9-mediated glucuronidation, two additional UGT1A9 probe substrates, furosemide and acetaminophen, were examined. As shown in Table 1, the glucuronidation of both agents was strongly inhibited by THC, CBD, and CBN at levels similar to those observed for propofol glucuronidation in rUGT1A9 microsomes. The highest level of inhibition was again observed with CBD, with IC50 values in rUGT1A9 microsomes of 2.4 ± 0.66 μM (IC50_u = 0.090 ± 0.025 μM) and 1.9 ± 0.29 μM (IC50_u = 0.073 ± 0.011 μM) for furosemide and acetaminophen glucuronidation, respectively (Table 1). The IC50 values observed in HKMs were very similar to those determined in rUGT1A9 microsomes, with CBD exhibiting the highest level of inhibition at 3.6 ± 0.80 μM (IC50_u = 0.22 ± 0.049 μM) and 3.8 ± 0.82 μM (IC50_u = 0.24 ± 0.05 μM) for furosemide and acetaminophen glucuronidation, respectively, and less inhibition in HLMs, with IC50 values of 29 ± 4.0 μM (IC50_u = 1.5 ± 0.20 μM) and 12 ± 3.2 μM (IC50_u = 0.64 ± 0.16 μM), respectively. The decreased level of inhibition of furosemide and acetaminophen glucuronidation by CBD in HLMs versus HKMs and the similar inhibition pattern with both THC and CBN of HKMs and rUGT1A9 microsomes is apparent when examining plots of percent glucuronidation activity versus CBD concentrations (Supplemental Fig. 1).

**Discussion**

The present study is the first to conduct a comprehensive examination of the inhibitory effects of major cannabinoids (THC, CBD, and CBN) on the enzymatic activities of each of the primary hepatic UGT enzymes (UGTs 1A1, 1A3, 1A4, 1A9, 2B4, 2B7, 2B10, 2B15, and 2B17). In addition, the major metabolites of THC and CBD (11-OH-THC, THC-COOH, THC-COO-Gluc, and 7-OH-CBD) were also screened as potential inhibitors. The results from the present study indicate that the parent cannabinoids (THC, CBD, and CBN) exhibit strong inhibition of the glucuronidation activities of UGTs 1A6, 1A9, 2B4 and 2B7, and marginal inhibition of a number of additional UGTs including...
Manini et al., 2015). The IC50, u values observed in the present study was approximately 5 
UGT2B7, in vivo. Highly expressed UGT enzymes in human kidney, UGT1A9, and cannabinoids. These data support the possibility that the major cannabi-

Tissue and plasma concentrations of cannabinoids vary widely by user and are dependent upon a number of factors including dose, cannabis strain, mode of consumption and expertise of the user (Sharma et al., 2012). Average plasma concentrations of THC from a 10 mg dose by inhalation are 110 ng/mL (0.35 µM), which are about 3-fold higher than those observed for oral dosing (360 mg/mL; 1.1 µM). The average CBD plasma level after a 400 mg oral dose is 181 mg/mL (0.76 µM) (Manini et al., 2015). The IC50, u values observed in the present study for THC and CBD against several UGT enzymes are in the micromolar to submicromolar range, suggesting that unwanted DDIs with xenobiotics metabolized by the same UGT enzymes may occur in co-users of cannabis.

Of the cannabinoids tested, the strongest inhibition in rUGT micro-
somes was observed by CBD against the glucuronidation of propofol (UGT1A9), serotonin (UGT1A6), codeine (UGT2B4), and AZT (UGT2B7), followed by THC, which also exhibited strong inhibition against the same suite of enzymes. CBD was shown to be similarly effective at inhibiting the glucuronidation of propofol and AZT in rUGT1A9 and rUGT2B7 microsomes, respectively; however, unlike that observed for CBD and THC, no inhibition of UGT2B4 microsomal activity was observed by CBD using codeine as the probe substrate.

Although the liver is considered the most important organ for the metabolism of drugs and other xenobiotics, the kidney also plays an important role, especially when glucuronidation is a primary component of a drug’s metabolism and elimination (Margaillan et al., 2015). UGT protein expression in both the human liver and human kidneys has large interindividual variability; however, current literature estimates that 13 UGTs are expressed in significant amounts in liver, whereas only 3 UGTs are appreciably expressed in human kidney, including UGTs 1A9 and 2B7, which are expressed at similar levels, and UGT1A6, which is expressed at a much lower level; UGT2B4 shows negligible expression in human kidney (Margaillan et al., 2015; Basit et al., 2020). Consistent with the relatively high expression pattern of UGT1A9 in human kidney, the IC50, u values observed in HKMs for propofol, furose-
mide, and acetaminophen, all UGT1A9 substrates, were similar to that observed for each agent in rUGT1A9 microsomes for CBD, THC and CBN. This contrasts with HLMs, where the IC50, u values were higher (approximately 3-fold) than those observed in rUGT1A9 microsomes in all cases. In addition, the 6-fold lower IC50, u exhibited by UGT1A9 as compared with UGT2B7 in rUGT microsomes by THC, CBD, and CBN corresponds with the larger IC50, u values observed in HKMs using a UGT2B7 probe substrate (AZT) versus that observed for UGT1A9 probe substrates, reflecting the relative inhibition of the two enzymes by cannabinoids. These data support the possibility that the major cannabi-

Although the role of renal metabolism is still an underexplored area compared with hepatic metabolism, mounting evidence from recent publications indicates that the human kidney has significant metabolic capacity. Renal metabolism by UGT enzymes plays a major role in clearance of many drugs including acetaminophen and furosemide (assayed in this study) as well as carbamazepine, codeine, gemfibrozil, morphone, and the commonly used over the counter nonsteroidal anti-inflammatory drugs ibuprofen, ketoprofen, and S-naproxen (Knights et al., 2013). Preferential inhibition of the renal UGTs may have a larger effect on drugs that are mainly excreted by renal glucuronidation, and
interestingly, the two most highly expressed UGTs in human kidney (UGTs 1A9 and 2B7) were inhibited by CBD, THC, and CBN in the present study. Therefore, cannabinoids, and especially CBD, may significantly and disproportionately affect the 1.5 million people in the United States (Rein, 2020) who are diagnosed with chronic kidney disease and acute kidney injury. One-quarter to one-half of those patients also experience chronic symptoms such as pain, nausea, anorexia, sleep disturbance, anxiety, and depression (Rein, 2020), several of which are approved indications for medical cannabis (CBD). Additionally, chronic kidney disease is associated with decreased activity of drug metabolizing enzymes and transporters (Dreisbach and Lertora, 2008). Moreover, a recent study showed significant reduction in the glucuronidation capacity of drugs metabolized by UGT1A9 and UGT2B7 in patients with kidney tumors (Margaili et al., 2015). AZT and propofol metabolism were decreased 96- and 7.6-fold respectively, in a patient with neoplastic kidney when compared with normal kidney, suggesting that the use of CBD or cannabis in these patients may be deleterious.

In the present study, the first is to demonstrate that the major cannabinoids present in Cannabis are able to inhibit several of the primary UGT enzymes involved in phase II metabolism. CBD was shown to be the most potent cannabinoid inhibitor, exhibiting IC50 values 2–3-fold lower than that observed for THC. Although this is the first study to specifically address the inhibition of UGTs by CBD and other cannabinoids, previous reports indicate that CBD, THC, and several other cannabinoids are potent inhibitors of several major P450 enzymes (Yamaori et al., 2011a; Yamaori et al., 2011b; Yamaori et al., 2011c; Bansal et al., 2020; Nasrin et al., 2021). Results from this study now show that two major hepatic UGTs and three of the most highly expressed UGTs present in kidney are strongly inhibited by these cannabinoids, suggesting that deleterious drug-drug interactions may be more likely to occur in patients in whom reduced hepatic or kidney function and cannabis use are occurring simultaneously. In light of the rising acceptance of cannabis use in the United States and internationally, further in vivo studies examining cannabinoid-drug interactions of both phase I and phase II are warranted.

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Authorship Contributions

Participated in research design: Nasrin, Lazarus. Conducted experiments: Nasrin, Watson, Bardhi, Fort. Performed data analysis: Nasrin, Watson, Chen, Lazarus. Wrote or contributed to the writing of the manuscript: Nasrin, Watson, Lazarus.

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**Address correspondence to:** Dr. Philip Lazarus, Department of Pharmaceutical Sciences, College of Pharmacy and Pharmaceutical Sciences, Washington State University, 412 E. Spokane Falls Blvd., Spokane, Washington 99202-2131. E-mail: phil.lazarus@wsu.edu
Inhibition of UDP-glucuronosyltransferase enzymes by major cannabinoids and their metabolites

Shamema Nasrin*, Christy J.W. Watson*, Keti Bardhi, Gabriela Fort#, Gang Chen, Philip Lazarus

Department of Pharmaceutical Sciences, College of Pharmacy and Pharmaceutical Sciences, Washington State University, Spokane, Washington, USA.

#Present Address: Department of Oncological Science, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA
Supplemental Table 1. Assay conditions used for the experiments.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Metabolite</th>
<th>Total protein (µg)</th>
<th>Incubation time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A1</td>
<td>β-estradiol-3-glucuronide</td>
<td>11 µM</td>
<td>80</td>
</tr>
<tr>
<td>UGT1A3</td>
<td>acyl CDCA-24-glucuronide</td>
<td>15 µM</td>
<td>80</td>
</tr>
<tr>
<td>UGT1A4</td>
<td>trifluoperazine-N-glucuronide</td>
<td>40 µM</td>
<td>80</td>
</tr>
<tr>
<td>UGT1A6</td>
<td>serotonin-glucuronide</td>
<td>6 mM</td>
<td>80</td>
</tr>
<tr>
<td>UGT1A9</td>
<td>propofol-O-glucuronide</td>
<td>98 µM</td>
<td>100</td>
</tr>
<tr>
<td>UGT2B4</td>
<td>codeine-6-glucuronide</td>
<td>1 mM</td>
<td>90</td>
</tr>
<tr>
<td>UGT2B7</td>
<td>AZT-5'-glucuronide</td>
<td>500 µM</td>
<td>90</td>
</tr>
<tr>
<td>UGT2B10</td>
<td>nicotine-N-glucuronide</td>
<td>900 µM</td>
<td>100</td>
</tr>
<tr>
<td>UGT2B15</td>
<td>S-oxazepam-glucuronide</td>
<td>900 µM</td>
<td>200</td>
</tr>
<tr>
<td>UGT2B17</td>
<td>exemestane-17-O-glucuronide</td>
<td>15 µM</td>
<td>100</td>
</tr>
</tbody>
</table>
Supplemental Figure 1. Inhibitory effects of THC and CBN on the glucuronidation of UGT probe substrates in microsomes from UGT-overexpressing HEK293 cell lines (rUGT), HLM and HKM. Shown are representative plots comparing THC (panel A) and CBN (panel B) concentrations with the percent glucuronidation activity against probe substrates in rUGT microsomes, HLM and HKM. Incubations were performed for 60-120 min at 37°C using 80-90 μg of rUGT microsomes or 90-200 μg HLM or HKM with the following probe substrates: propofol, acetaminophen, and furosemide for UGT1A9; serotonin for UGT1A6; codeine for UGT2B4; and zidovudine (AZT) for UGT2B7 (see Supplemental Table 1 for concentrations). Individual metabolites were analyzed by UPLC-MS/MS as described in the Materials and Methods.