UDP-glucuronosyltransferases mediate coffee-associated reduction of liver fibrosis in bile duct ligated humanized transgenic UGT1A mice

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Background: Coffee consumption has been shown to reduce the risk of liver fibrosis and is capable of inducing human UDP-glucuronosyltransferase (UGT) 1A genes. UGT1A enzymes act as indirect antioxidants catalyzing the elimination of reactive metabolites, which in turn are potent initiators of profibrotic mechanisms. The aim of this study was to analyze the role of UGT1A genes as effectors of the protective properties of coffee in bile duct ligation (BDL) induced liver fibrosis.

Methods: Fourteen days BDL with and without coffee pre- and co-treatment was performed in htgUGT1A-WT and htgUGT1A-SNP mice. Hepatic UGT1A mRNA expression levels, serum bilirubin and aminotransferase activities were determined. Liver fibrosis was assessed by collagen deposition, computational analysis of Sirius red tissue staining and expression of profibrotic marker genes. Oxidative stress was measured by hepatic peroxidase concentrations and immunofluorescence staining.

Results: UGT1A transcription was differentially activated in the livers of htgUGT1A-WT mice after BDL, in contrast to a reduced or absent induction in the presence of SNPs. Co-treated (coffee + BDL) htgUGT1A-WT-mice showed significantly increased UGT1A expression and protein levels and a considerably higher induction compared to water drinking WT mice (BDL), whereas in co-treated htgUGT1A-SNP mice absolute expression levels remained below those observed in htgUGT1A-WT mice. Collagen deposition, oxidative stress and the expression of profibrotic markers inversely correlated with UGT1A expression levels in htgUGT1A-WT and SNP mice after BDL and coffee + BDL co-treatment.

Conclusions: Coffee exerts hepatoprotective and antioxidative effects via activation of UGT1A enzymes. Attenuated hepatic fibrosis as a result of coffee-mediated UGT1A induction during cholestasis was detected, while the protective action of coffee was lower in a common low-function UGT1A SNP haplotype present in 10% of the Caucasian population. This study suggests that coffee consumption might constitute a potential strategy to support the conventional treatment of cholestasis-related liver diseases.

Keywords: Glucuronidation; cholestasis; liver fibrosis; coffee; oxidative stress

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Introduction

Coffee is believed to have been first discovered in a region of southwest Ethiopia called Kaffa and has seen as an unprecedented rise to become one of the most widely consumed beverages worldwide (1,2). With about 75% of the American population consuming coffee, the annual per-capita consumption in the United States 2015 exceeded 5 kg of green coffee (3). Besides its sought-after taste and stimulating effect, coffee has been associated with hepatoprotective properties. An increasing number of epidemiological studies have reported that coffee consumption is inversely associated with fibrosis progression, hepatic cirrhosis and hepatocellular carcinoma (HCC) (4-6).

Previous data from our own laboratory identified coffee as an efficient activator of UDP-glucuronosyltransferase (UGT) 1A expression (7). UGT1A enzymes eliminate a wide range of endo- and xenobiotic compounds, including many reactive metabolites, thereby acting as indirect antioxidants and contribute to cytoprotection (8). The UGT1A-mediated conjugation of substrates with glucuronic acid leads to the formation of water soluble, biologically inactive glucuronides and facilitates subsequent excretion via bile and urine (9-11). Transcription of UGT1A genes is known to be regulated by tissue-specific and ligand-activated transcription factors including bile acid activated farnesoid X-receptor (FXR), oxidative stress sensor Nrf2 (nuclear factor erythroid 2-related factor 2) and xenobiotic inducible aryl-hydrocarbon receptor (AhR) (12-14). UGT1A expression and activity is further influenced by the presence of single nucleotide polymorphisms (SNPs). With more than 100 identified UGT1A SNPs, which exhibit frequencies of up to 40% in the Caucasian population, a huge variety of genetic variants affecting protein function and transcriptional activation has been described so far (15,16). Polymorphisms leading to reduced UGT1A function have been identified as risk factors for cancer (17) and are associated with more severe fibrosis progression in patients with hepatitis C (18,19).

Apart from HCV infection, alcohol consumption and non-alcoholic steatohepatitis, cholestasis-related liver injury represents one of the major causes of hepatic fibrosis and cirrhosis in industrialized countries (20,21). Cholestasis induced fibrosis is characterized by a massive accumulation of extracellular matrix (ECM) proteins, an increase of inflammatory and other cytokines, as well as elevated oxidative stress levels (22-24). In a recent study, we were able to show that the coffee-mediated protective effects towards benzo(a)pyrene-induced oxidative stress are dependent on the presence of UGT1A enzymes (25). Aim of the study was therefore to show that the induction of UGT1As via coffee ameliorates the effects of chronic cholestatic liver injury. To this end an extreme model of advanced hepatic cholestasis was selected. Humanized transgenic (htg) UGT1A mice underwent surgical ligation of the common bile duct (bile duct ligation, BDL) leading to the subsequent retention of toxic bile acids and thus liver fibrosis within 14 days.

Methods

Surgery and treatment of humanized transgenic UGT1A mice

In this study two previously reported and characterized htg mouse lines were used. The htgUGT1A-WT line, containing the wild type (WT) UGT1A gene locus, and a line containing a haplotype of 10 common UGT1A SNPs (UGT1A1*28, UGT1A3 -66T>C, UGT1A3 W11R, UGT1A3 V47A, UGT1A6*2a (S7A/T181A/R184S), UGT1A7*3 (N129K/R131K/W208R/-57T>G)) (26). This SNP mouse model was created to simulate a frequent variant haplotype observed in 10% of the Caucasian population. Studies have shown that many of these SNPs are simultaneously present in Gilbert syndrome individuals and influence the glucuronidation by modifying the transcriptional activation (27) and/or by expressing proteins with altered enzymatic activity (28,29). A detailed overview of the SNPs included in the htgUGT1A-SNP mouse line and the respective minor allele frequency (MAF) is shown in Table 1. For animal experiments 8–12-week-old female htgUGT1A-WT and SNP mice were used. Mice of each genotype were divided into four groups of four to six animals and either underwent 14 days BDL or 14 days sham operation. In addition to BDL or sham operation, htgUGT1A-WT and SNP mice received pre- and co-treatment with coffee as their drinking water 14 days before and after surgical treatment.

BDL was performed using a standard technique as described elsewhere (35). Mice were anesthetized by inhalation of 4 vol% isoflurane in 100% oxygen at a flow rate of 4 L/min and 1.5–3 vol% isoflurane at a flow rate of 1 L/min was set to maintain anesthesia. After midline laparotomy (1.5 cm) the common bile duct was exposed and ligated twice with a surgical knot. In sham operated mice,
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the bile duct has not been ligated. 14 days after surgery, blood and organs were collected, shock-frozen in liquid nitrogen and stored at −80 °C until use.

Animals were housed in the Central Animal Facility of the University Hospital Bonn and were kept at 22 °C with a 12 h day/night cycle with ad libitum access to chow. All experiments were performed in accordance to the “German Animal-Protection Law” and the relevant guidelines of the Local Institutional Animal Care unit of our university (Haus für experimentelle Therapie, Bonn, Germany) and authorized by the relevant North Rhine-Westphalian state-agency for Nature, Environment and Consumer Protection (LANUV, Germany) under the file reference LANUV 84-02.04.2016.A483.

### Standardized preparation of coffee

A stock solution was prepared to represent the preparation mode of commonly used filter coffee. For this purpose 500 mL water (Aqua Irrigation Solution, DeltaSelect, Dreieich, Germany) were boiled in a beaker and cooled for 10 seconds. Ten grams of ground coffee powder (Jacobs Krönung, Kraft Foods, Bremen, Germany) were added and incubated for one min, followed by filtration through a paper coffee filter (Melitta, Minden, Germany).

### Measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin

Total serum bilirubin levels, ALT and AST were measured using a Fuji DRI-CHEM NX500i (Fujifilm Cooperation, Tokyo, Japan) serum analyser. After collecting blood, it was centrifuged at 13,000 g for 10 min and supernatant was subsequently stored at −20 °C until measurement.

### Gene expression analysis

Total RNA was isolated from shock-frozen liver tissue of sacrificed transgenic mice and homogenized in TRIzol (Invitrogen, Karlsruhe, Germany). For further analyses equal amounts from at least four animals per group were pooled. 5 μg of RNA was incubated with DNase I (Invitrogen) at room temperature for 15 min and subsequently inactivated at 65 °C for 10 min. DNase I treated RNA was then used for cDNA synthesis in an oligo(dT)-primed Superscript III reverse transcriptase reaction according to the manufacturer’s instructions (Fischer Scientific, Schwerte, Germany). For quantification
of gene expression, cDNA concentrations were determined by qPCR relative to mouse beta-actin. Using gene specific primers and probes qPCR reactions were performed in CFX96 real-time PCR detection system (Bio-Rad) with qPCR MasterMix (Eurogentec). All reactions were performed in triplicates and have been repeated three times. Bio-Rad CFX Manager 3.0 software was used to calculate the relative expression.

**Histological analysis**

Liver fibrosis was assessed by computational analysis of Sirius red stained areas. For the detection of collagen fibres, paraffin-embedded sections were trimmed to 2.0 µm slices and stained in Sirius red solution (saturated picric acid containing 0.1% DirectRed 80). The Sirius red positive area was quantified using ImageJ software (U.S. National Institutes of Health; http://rsb.info.nih.gov/ij/) and shown as percentage of the total section area. Images were analysed from four randomly picked pictures (magnification 100×) of each animal and averaged. The quantitative analysis of fluorescence intensity obtained from immunofluorescence images of UGT1A protein (magnification 200×) was also calculated with the ImageJ program and shown as relative fluorescence units (RFU).

For analyzation of UGT1A protein levels and for the determination of lipid peroxidation secondary immunofluorescence staining was performed. As described elsewhere (36) deparaffinization, rehydration and antigen retrieval of paraffin embedded tissue slides was accomplished by incubation of liver specimens in decreasing alcohol concentrations followed by 20 min heating in sodium citrate buffer pH 6.0 at 95–100 °C and then washed three times before being blocked with blocking buffer (1x PBS/5% goat serum) for 1 h. Overnight incubation with respective primary antibodies [anti 4 hydroxynonenal (4-HNE), Abcam ab46545, 1:50 and anti UGT1A, Santa Cruz sc-271268, 1:50] was carried out in TBS-T containing 5% goat serum. Appropriate secondary antibodies (Alexa Fluor® 488 Abcam ab150077 and ab150113, dilution 1:200 each) were added to tissue sample area for 1 h. A mounting medium with DAPI (Abcam) was applied according to manufacturer’s instructions. The specimens were visualized under a microscope (Axio Scope.A1, Zeiss) at the same day.

**Peroxidase assay**

For the colorimetric determination of total hepatic peroxidase concentrations, 100 mg liver tissue was homogenized and evaluated with the use of OxiSelect™ Hydrogen Peroxidase Assay Kit (Cell Biolabs, INC.) according to manufacturer’s protocol. Samples were analysed using Multiskan Go Reader (ThermoScientific).

**Statistical analysis**

Data are expressed as mean ± standard deviation (SD) determined by one-way analysis of variance followed by Students t-test to determine significance. A pool of four to six mice in each group was analysed, P values below 0.05 were considered as statistically significant.

**Results**

**Coffee reduces liver fibrosis in BDL-treated htgUGT1A-WT mice**

In order to examine coffee-mediated effects during obstructive cholestasis, BDL htgUGT1A-WT mice were analysed for hepatic collagen deposition, total serum bilirubin levels and aminotransferase activities. Sirius red staining in htgUGT1A-WT mice confirmed fibrosis development 14 days after BDL, as well as a clearly visible reduction of red stained collagen fibres in coffee co-treated BDL mice (Figure 1A). As expected, bile duct ligated htgUGT1A-WT mice showed significantly elevated total serum bilirubin levels (23.02 mg/dL) compared to sham operated mice (1.2 mg/dL). Interestingly, administration of coffee significantly reduced (31%) total serum bilirubin levels compared to water drinking BDL mice. Furthermore, measurement of serum aminotransferase levels revealed a significant elevation of AST (23.6-fold) and ALT (50.1-fold) levels after BDL, whereas coffee treatment led to a considerable reduction of AST (49%) and ALT (54%) levels in comparison to the water drinking BDL group (Figure 1B, C, D). Even though serum aminotransferase activities still were significantly elevated after coffee + BDL co-treatment (AST: 11.5-fold and ALT: 32.8-fold), these data demonstrate that coffee exerts a protective effect during obstructive cholestasis-induced liver damage.

**Hepatic UGT1A expression during cholestatic liver fibrosis is differentially regulated in coffee exposed htgUGT1A-WT mice**

Coffee administration in sham operated htgUGT1A-
WT mice led to a significant hepatic mRNA induction of all investigated UGT1A isoforms (except for UGT1A7) compared to water drinking sham operated animals (Figure 2). After biliary obstruction, an upregulation of UGT1A1-, UGT1A6-, UGT1A7 and UGT1A9 mRNA expression compared to the sham operated group was observed. UGT1A gene expression in the livers of htgUGT1A-WT mice was significantly increased after coffee + BDL co-treatment and showed considerable higher induction compared to water drinking BDL mice for the isoforms UGT1A1 (7.1-fold), UGT1A3 (2.3-fold), UGT1A4 (1.8-fold), UGT1A6 (4.3-fold), UGT1A7 (5.9-fold) and UGT1A9 (3.2-fold). These data indicate that BDL-induced liver fibrosis differentially activates hepatic glucuronidation. Coffee co-treatment further points to a considerable responsiveness of transcriptional UGT1A activation, resulting in a synergistic inductive effect in htgUGT1A-WT mice. Immunofluorescent detection of UGT1A proteins confirmed the results measured at the mRNA level demonstrating the considerable induction of UGT1A protein after coffee administration and coffee + BDL co-treatment in comparison to equivalent treated
**Figure 2** Hepatic UGT1A mRNA expression in *htgUGT1A*-WT mice after sham operation (sham) or 14 days bile duct ligation (BDL) with and without coffee pre- and co-treatment. Graphs are expressed as means ± SD using 4 mice per sham group and 6 mice in each BDL group. Samples were analyzed with Students *t*-test. Means with different letters indicate significant differences at P<0.05, and columns sharing the same letter are not significantly different.

*htgUGT1A*-WT mice drinking water (Figure 3A). The quantitative analysis of fluorescence intensity obtained from the depicted UGT1A immunofluorescent images further substantiated these results. The calculated mean of intensity also basically corresponded to the determined UGT1A mRNA levels revealing the lowest relative fluorescence intensity after sham operation and the highest intensity in coffee + BDL co-treated *htgUGT1A*-WT mice (Figure 3B).

**Coffee reduces collagen deposition during cholestatic liver injury in *htgUGT1A*-WT and SNP mice**

To further elucidate the role of UGT1A expression in coffee-mediated fibrosis protection during BDL treatment, the *htgUGT1A*-SNP mouse line known for its reduced basal UGT1A expression and inducibility was used (26). Computational quantification of Sirius red stained tissues was performed and both *htgUGT1A* mouse lines were compared for their ECM content (Figure 4A). As expected, the direct comparison between BDL *htgUGT1A*-WT and SNP mice revealed significantly higher ECM deposition in mice carrying the low-activity SNP variant (WT 3.41% and SNP 4.94%). Furthermore, coffee-mediated reduction of fibrillar collagen was more pronounced in *htgUGT1A*-WT mice, whereas co-treated SNP mice also exhibited a reduced but still significantly higher content of positively stained areas and consequently showed a more severe fibrosis development evidenced by a higher total ECM deposition (WT 2.63% and SNP 4.08%). Expression levels of collagen type 1 alpha 1 (Col1a1) further supported the results obtained in computational Sirius red quantification. Although considerable upregulation of *Col1a1* expression was detected after BDL in both mouse lines (WT 12.4-fold and SNP 22.2-fold), *htgUGT1A*-SNP mice showed significantly higher mRNA expression levels (Figure 4B). After simultaneous treatment with coffee and BDL, a downregulation of *Col1a1* expression levels in both mouse lines was detected, even though mice containing multiple
genetic SNP variants still had higher expression levels (1.5-fold) compared to their equally treated WT counterparts. These findings were in line with the results observed in Sirius red staining (Figures 1A, 4C). In water and coffee drinking BDL htgUGT1A-SNP mice, Sirius red staining revealed advanced hepatic fibrosis, although a diminished proportion of red stained collagen fibres after coffee co-treatment could be observed.

**Differential oxidative stress levels after BDL treatment in htgUGT1A-WT and SNP mice**

Oxidative stress results from an imbalance in the production of reactive oxygen species (ROS) and the ability of the organism to scavenge them (37,38). UGT1As act as indirect antioxidants because of their ability to eliminate reactive metabolites capable of inflicting tissue injury (8). Since oxidative stress is a well-known initiator of fibrogenesis,
Figure 4 Deposition and expression of hepatic collagens in \textit{htgUGT1A-WT} and SNP mice. Quantification of the Sirius red positive areas of \textit{htgUGT1A-WT} and \textit{htgUGT1A-SNP} mice calculated with ImageJ (A). TaqMan PCR measured mRNA expression levels of \textit{Col1a1} after sham operation (sham) or 14 days bile duct ligation (BDL) with and without coffee pre- and co-treatment (B). Representative sections of Sirius red stained areas of \textit{htgUGT1A-SNP} mice (C, magnification 100×). Graphs are expressed as means ± SD using 4 mice per sham group and 6 mice in each BDL group. Samples were analyzed with Students\textit{t}-test. Means with different letters indicate significant differences at $P<0.05$, and columns sharing the same letter are not significantly different. \textit{Col1a1}, collagen type 1 alpha 1.

detection of 4-HNE was used as marker for lipid peroxidation and oxidative injury in liver tissue (39,40). As shown in Figure 5A, fluorescence intensity of 4-HNE was higher in BDL-treated \textit{htgUGT1A-SNP} mice compared to mice carrying the human wild type \textit{UGT1A} gene locus. Interestingly, coffee co-treatment nearly abolished the fluorescence signal of 4-HNE detection in \textit{htgUGT1A-WT} mice, whereas in the presence of the \textit{UGT1A} SNP variant merely a moderate reduction of lipid peroxidation compared to the water drinking BDL group was detected. These results indicate a coffee-mediated increase of the antioxidative capacity, which is more pronounced in mice carrying the \textit{UGT1A} wild type gene locus as indicated by lower lipid peroxidation-caused oxidative injury and confirm a role of UGT1A activity in cellular protection.

In addition, total hepatic peroxidase concentrations, which includes glutathione peroxidase as well-established indicator for oxidative stress (41) was investigated in \textit{htgUGT1A-WT} and SNP mice (Figure 5B). Following BDL, peroxidase concentrations significantly decreased in \textit{htgUGT1A-WT} mice (39.2%), whereas coffee pre- and co-treatment led to significantly higher hepatic peroxidase.
concentrations (1.47-fold) compared to water drinking BDL mice. However, peroxidase levels of BDL and coffee co-treated htgUGT1A-WT mice (65.5 and 96.6 mU/mL) were significantly higher as those observed in the presence of UGT1A SNPs (57.8 and 81.9 mU/mL). Although coffee co-treatment attenuated oxidative stress in both mouse lines, differences in 4-HNE immunofluorescence detection and total hepatic peroxidase concentrations indicate an essential role of UGT1A function for the coffee-mediated antioxidative effects. As a consequence, an altered modification of the metabolic antioxidative balance in htgUGT1A-SNP mice may result in enhanced fibrosis.

Figure 5 Oxidative liver injury and hepatic oxidative stress levels in htgUGT1A-WT and SNP mice. Representative pictures of lipid peroxidation detection by immunofluorescence staining with 4-HNE antibody (A, magnification 200x), and comparison of total hepatic peroxidase concentrations (B) in htgUGT1A-WT and SNP mice after sham operation (sham) or 14 days bile duct ligation (BDL) with and without coffee pre- and co-treatment. Graphs are expressed as means ± SD using 4 mice per sham group and 6 mice in each BDL group. Samples were analyzed with Students t-test. Means with different letters indicate significant differences at P<0.05, and columns sharing the same letter are not significantly different. 4-HNE, 4 hydroxynonenal.
The development of fibrosis is associated with an increased expression of various cytokines and chemokines, which serve as reliable biomarkers for the fibrogenic activity or hepatic inflammation. In comparison to sham operated mice, 14 days BDL led to a significant transcriptional activation of the profibrotic marker genes alpha smooth muscle actin (α-SMA, ACTA2), connective tissue growth factor (CTGF), beta-type platelet-derived growth factor receptor (PDGFRB) and platelet-derived growth factor subunit B (PDGFB) in the livers of htgUGT1A-WT and SNP mice (Figure 6). Furthermore, absolute expression levels of the proinflammatory markers tumour necrosis factor alpha (TNF-α) and C-C chemokine ligand 2 (CCL2) were also significantly upregulated after 14 days BDL. Comparing the transcriptional activation of profibrotic marker genes in water drinking BDL operated htgUGT1A-WT and SNP mice, significantly increased absolute expression levels for

**Figure 6** Hepatic expression of profibrotic marker genes in htgUGT1A-WT and SNP mice after sham operation (sham) or 14 days bile duct ligation (BDL) with and without coffee pre- and co-treatment. Graphs are expressed as means ± SD using 4 mice per sham group and 6 mice in each BDL group. Samples were analyzed with Students t-test. Means with different letters indicate significant differences at P<0.05, and columns sharing the same letter are not significantly different. ACTA2, alpha smooth muscle actin (α-SMA); CTGF, connective tissue growth factor; PDGFRB, beta-type platelet-derived growth factor receptor; PDGFB, platelet-derived growth factor subunit B; TNF-α, tumor necrosis factor alpha; CCL2, C-C chemokine ligand 2.
CTGF (1.2-fold), PDGFRB (2.3-fold), TNF-α (1.1-fold) and CCL2 (2.2-fold) in mice carrying the low-function UGT1A SNP haplotype were detected.

The antifibrotic potential of coffee in a wide-ranging spectrum of chronic liver diseases has been described in numerous studies (42,43). In this respect an inverse relationship between coffee consumption and fibrosis progression has also been shown in recently published data (44,45). In line with these data, coffee + BDL co-treatment reduced absolute expression levels of all depicted profibrotic marker genes (except for PDGFRB) in htgUGT1A-WT mice compared to the water drinking BDL group. A significant downregulation of mRNA expression has been detected for ACTA2 (0.43-fold), CTGF (0.36-fold), PDGFB (0.84-fold) and TNF-α (0.7-fold). Of note, in comparison to htgUGT1A-WT mice, coffee pre- and co-treatment showed less of a reduction of expression levels on fibrosis marker gene in the presence of UGT1A SNPs. Although coffee intake also resulted in a significant downregulation in htgUGT1A-SNP mice, higher mRNA expression levels for ACTA2 (2.2-fold), CTGF (2.3-fold), TNF-α (1.2-fold) and CCL2 (1.6-fold) compared to htgUGT1A-WT mice were measured. These data indicate a less pronounced protective effect of coffee in carriers of the UGT1A SNP haplotype. In combination, these data suggest that reduced UGT1A expression significantly attenuates the hepatoprotective effects of coffee on the expression of diverse biomarkers in the development of hepatic fibrosis.

Reduced hepatic UGT1A expression during cholestatic liver fibrosis in coffee drinking htgUGT1A-SNP mice

In order to investigate whether the observed hepatoprotective effect of coffee during biliary obstruction is based on differences in hepatic UGT1A expression, transcriptional UGT1A regulation in htgUGT1A-SNP mice was quantified (Figure 7). Except for the isoforms UGT1A7 and UGT1A9, coffee consumption resulted in a significant transcriptional activation of UGT1A genes in sham operated htgUGT1A-SNP mice, although the detected upregulation...
was less prominent as those obtained from equally treated htgUGT1A-WT mice. In contrast to the results observed in htgUGT1A-WT mice, UGT1A induction was reduced (UGT1A6 and UGT1A9) or absent in water drinking BDL mice carrying the UGT1A SNP haplotype. Even though a synergistic induction was detected after coffee + BDL co-treatment in htgUGT1A-SNP mice as well, absolute expression levels remained far below those observed in WT mice. In summary, htgUGT1A-SNP mice showed lower expression as well as a reduced responsiveness towards coffee during the development of cholestasis-induced liver fibrosis. As a consequence this may explain the reduced antioxidative and less protective effect of coffee during fibrogenesis observed in the presence of UGT1A SNPs.

**Discussion**

The beneficial effects of coffee in chronic liver diseases have been reported in numerous human and animal studies (46-49). Coffee is a potent inducer of the Nrf2 and AhR signalling pathways. Both are required for transcriptional UGT1A activation via xenobiotic response elements (XRE/AhR) and antioxidative response elements (ARE/Nrf2) binding motifs (50,51). The coordinated regulation between both transcription factors and UGT1A transcription links glucuronidation to xenobiotic-induced cellular protection and thus to the defence against oxidative stress (52). Oxidative stress represents a critical effector for the initiation of hepatocyte damage and hepatic fibrosis during cholestasis.

The present study investigated the influence of coffee on cytoprotective UGT1A regulation in the situation of severe cholestasis induced by BDL. We demonstrate the significant activation of UGT1A1, UGT1A6, UGT1A7 and UGT1A9 mRNA expression in htgUGT1A-WT mice as a result of BDL. These findings were expanded by studying the effects of coffee exposure. Coffee was found to further increase human UGT1A gene expression in BDL animals resulting in a significant decrease of total serum bilirubin levels and a considerable reduction of aminotransferase activities. These findings provide evidence for hepatic protection linked to the activation of the UGT1A genes by coffee. With the intention of examining the opposite effect and expanding this analysis a htgUGT1A-SNP mouse line with genetically reduced UGT1A expression because of the presence of 10 common UGT1A SNPs was used. In agreement with our hypothesis, expression levels were found to be lower and induction by coffee did not reach the levels observed with htgUGT1A-WT mice. Moreover, the direct comparison between htgUGT1A-WT and htgUGT1A-SNP BDL animals confirmed a lower rate of fibrosis in WT compared to SNP mice suggesting a protective role of UGT1A gene products for hepatic fibrogenesis in this situation. Because the retention of cytotoxic bile acids leads to severe cellular and tissue damage (53), the degree of activation of bile acid detoxifying mechanisms, including UGT1A-mediated conjugation with glucuronic acid, may be an important factor capable of influencing disease progression, and potential therapeutic interventions. Although the incidence of this variant SNP haplotype in other human populations than Caucasians is unknown, many of these polymorphisms were also found to exist at high frequencies in other ethnicities (Table 1). Therefore, an impaired UGT1A-mediated cytoprotection during severe cholestasis may also constitute a relevant risk factor for humans with another ethnic background.

In order to show the contribution of UGT1A enzymes in hepatoprotection by exposure to coffee, fibrosis development, collagen deposition and mRNA expression of profibrotic factors were studied in both htgUGT1A mouse lines. HtgUGT1A-SNP mice showed significantly more ECM deposition after BDL and coffee + BDL co-treatment compared to mice containing the human wild type UGT1A genotype. These results were accompanied by a comparable expression pattern of Col1a1 transcription, thereby confirming the results of the Sirius red staining fibrosis analysis. In line with these findings, the transcriptional induction of key genes related to fibrosis (CTGF, PDGFRB, CCL2 and TNF-α) was higher in the presence of UGT1A SNPs compared to htgUGT1A-WT mice, which showed a higher degree of protection. Since the only difference between both animal models is the expression level of the UGT1A genes, these data suggest that UGT1As and their transcriptional activation play a protective role for fibrogenesis in cholestasis. In addition, an attenuated protective effect of coffee in BDL mice carrying the UGT1A SNP haplotype further corroborates that the coffee-mediated protection against hepatic fibrosis is to a substantial extent attributable to the ability of inducing UGT1A gene products exerting antioxidative activity.

Recently published data also suggest that antioxidative properties of coffee leading to reduced oxidative stress in bile duct ligated Wistar rats can attenuate hepatic fibrosis (41). By sensing lipid peroxidation with 4-HNE antibody we demonstrated that UGT1A function and inducibility significantly affects the antioxidative protective effect of...
coffee during cholestasis resulting in a lower amount of ROS-caused alternations of macromolecules and oxidative injury. As a consequence, the mechanism of action behind the coffee-mediated hepatoprotective effects is closely related to the coffee-induced enhancement of the antioxidative defence system in which UGT1As constitute a major player. As a result, reduced cellular protection against oxidative stress in hgtUGT1A-SNP mice is likely to represent a crucial risk factor for enhanced fibrosis initiation and development.

In conclusion, we demonstrated that coffee exposure leads to protection against cholestasis-initiated liver fibrosis during BDL, which involves the regulation of UGT1A genes. The significant activation of human UGT1As is likely also associated with coffee exposure-mediated protective properties in other chronic liver diseases. Since coffee is a complex mixture containing a broad array of different chemical compounds (54), it would be of significant importance to identify the specific substances accountable for the detected UGT1A upregulation. A detailed examination of the UGT1A enzymes paired with the identification of the relevant key constituents in coffee responsible for UGT1A activation could provide substantial benefits for risk evaluation of low-activity variants and for the assessment of UGTs as potential new therapeutic target and additional option to support the treatment of patients with cholestasis-related liver diseases.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/hbsn-20-9). All authors have no conflicts of interest to declare.

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