Induction of UDP-Glucuronosyltransferase UGT1A1 by the Flavonoid Chrysin in Caco-2 Cells—Potential Role in Carcinogen Bioinactivation

Alema Galijatovic,[1] Yoko Otake,[1] U. Kristina Walle,[1] and Thomas Walle[1,2]

Received October 13, 2000; accepted November 22, 2000

Purpose. Dietary flavonoids, present in fruits, vegetables and beverages have been demonstrated to be protective in cancer. Recently, we showed that the flavonoid chrysin induced UDP-glucuronosyltransferase (UGT) activity and expression in the human intestinal cell line Caco-2. In the present study, we determined the specific UGT isoform(s) induced and whether this induction facilitates glucuronidation and potential detoxification of the colon carcinogen 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (*N*-hydroxy-PhIP).

Methods. The induction was studied by immunoblot analysis with UGT isoform-specific antibodies, by Northern blot analysis and using quercetin as an isoform-specific catalytic probe. Glucuronidation of *N*-hydroxy-PhIP was characterized using both recombinant UGTs and control and chrysin-treated microsomes.

Results. Western blot analysis showed that pretreatment of Caco-2 cells with 25 μM chrysin induced UGT1A1 without affecting the expression of UGTs 1A6, 1A9 and 2B7. Northern blot analysis showed markedly increased expression of UGT1A1 mRNA after chrysin treatment. Similarly, glucuronidation of quercetin was greatly increased in a UGT1A1-specific way. The induction of UGT1A1 in the Caco-2 cells resulted in a 10-fold increase in the glucuronidation of *N*-hydroxy-PhIP.

Conclusion. Dietary flavonoid-mediated induction of intestinal UGT1A1 may be important for the glucuronidation and detoxification of colon and other carcinogens as well as for the presystemic metabolism of therapeutic drugs.

KEY WORDS: flavonoids; chrysin; enzyme induction; glucuronidation; UDP-glucuronosyltransferase; UGT1A1; intestinal cells; Caco-2 cells.

INTRODUCTION

UDP-glucuronosyltransferase (UGT) is an enzyme family that carries out major detoxification reactions in humans. Subdivided into two sub-families, UGT1A and UGT2, UGTs are involved in metabolism of bilirubin (1), steroids (2), bile acids, and drugs (3). In addition, UGTs metabolize carcinogens; reactions generally considered to be important in detoxification of carcinogens (4). Thus, induction of UGT isoforms that metabolize various carcinogens could facilitate bioinactivation and subsequent excretion of such toxic compounds.

While most inducers of UGTs are found to be rather toxic chemicals, such as TCDD (5) and β-naphtoflavone (6), there is evidence to suggest that dietary compounds such as bioflavonoids can induce UGTs (7,8). In a previous study we have shown that one such bioflavonoid, chrysin (5,7-dihydroxyflavone), induced its own glucuronidation (autoinduction) in the human colonic adenocarcinoma cell line Caco-2 (9). Pretreatment with 50 μM chrysin resulted in a 3.8-fold increase in the glucuronidation of chrysin and 14-fold in the cell homogenate. Western blot analysis using a non-specific antibody indicated that chrysin pretreatment resulted in substantial increase in the expression of UGT 1A isoform(s).

The focus of this study was to determine the specific isoform(s) that chrysin induces in Caco-2 cells. Isoform-specific antibodies revealed that UGT1A1 is one induced isoform. This was confirmed by Northern blot analysis using a UGT1A1-specific probe as well as by a catalytic assay. In addition, this study explored the role that the UGT1A1 isoform plays in glucuronidation of the colon carcinogen 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (*N*-hydroxy-PhIP) and whether increased expression of UGT1A1 in Caco-2 cells increased the glucuronidation of *N*-hydroxy-PhIP.

MATERIALS AND METHODS

Materials

Chrysin, uridine 5'-diphosphoglucuronic acid (UDPGA) and protease inhibitors were purchased from Sigma Chemical Co. (St. Louis, MO). Trifluoroacetic acid was of spectrophotometric grade from Aldrich Chemical Co. (Milwaukee, WI). Hanks' balanced salts solution (HBSS) was obtained from Cellgro, Mediatech, Fisher Scientific (Pittsburgh, PA). Recombinant human UGT1A1 and UGT1A9 were purchased from Gentest Corp. (Woburn, MA). Electrophoresis and blotting supplies and prestained molecular weight markers were purchased from Bio-Rad Laboratories (Hercules, CA). *N*-hydroxy-PhIP was obtained from NCI Chemical Carcinogen Reference Standard Repository at Midwest Research Institute (Kansas City, MO).

Caco-2 Cell Culture

The human colon adenocarcinoma cell line Caco-2 was obtained from American Type Culture Collection (Rockville, MD). The Caco-2 cells were cultured in Eagle's Minimum Essential Medium with Earle's salts and L-glutamine (Cellgro), supplemented with 1% non-essential amino acids (Cellgro), 10% fetal bovine serum (Summit Biotechnology, Ft. Collins, CO) and penicillin/streptomycin (Sigma Chemical Co.). Cells were grown in humidified air with 5% CO_2 in 100 mm petri dishes for preparation of microsomes and isolation of RNA. The cells were used at passage 35–75.

Preparation of Caco-2 Cell Microsomes

Caco-2 cells were plated in 100-mm petri dishes. Just prior to reaching confluency (6–8 days after cells seeding) the

[1] Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, 173 Ashley Avenue, P.O. Box 250505, Charleston, South Carolina 29425.

[2] To whom correspondence should be addressed. (e-mail: wallet@musc.edu)

ABBREVIATIONS: UGT, UDP-glucuronosyltransferase; HBSS, Hanks' balanced salt solution; UDPGA, uridine 5'-diphosphoglucuronic acid; *N*-hydroxy-PhIP, 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, PhIP; 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.

0724-8741/01/0300-0374$19.50/0

cells were incubated for 3 days with either 25 μM chrysin dissolved in ethanol:DMSO (80:20, v/v) or solvent. The medium was changed every 24 hr. After the final day of treatment, the cell monolayers (10 dishes/treatment) were washed twice with HBSS and scraped off the dishes in HBSS. After centrifugation the cell pellet was resuspended in 1 ml of 0.15 M KCl in 10 mM sodium phosphate buffer (pH 7.4) with protease inhibitors (2 mM PMSF, 50 μg/ml antipain, 2 μg/ml aprotinin, 0.2 mg/ml benzamidine, 0.5 μg/ml leupeptin and 1 μg/ml pepstatin). The cells were disrupted by sonication on ice (5 × 5 sec). The cell homogenate was centrifuged at 9000*g* for 20 min at 4°C to obtain the supernatant fraction (S9). The S9 fraction was further centrifuged at 100,000*g* for 60 min at 4°C. The microsomal pellets were resuspended in 400 μl of homogenization buffer. Microsomal suspensions, stored in aliquots at –80°C, were used for immunoblot analysis and catalytic activity assays.

Determination of UGT Protein Levels by Immunoblotting

Microsomal samples were heated at 90°C for 5 min with an equal volume of sample buffer and loaded on a 12% SDS-polyacrylamide minigel together with molecular weight markers and positive and negative controls. After electrophoresis (10), the proteins were transferred to a nitrocellulose membrane (11). The membrane was blocked and incubated with primary and secondary antibodies as described previously (9). Primary polyclonal antibodies raised against UGT1A6, UGT1A9 and UGT2B7 were all purchased from Gentest. Anti-UGT1A1, obtained from Dr. Ritter, was used as recently described (12), with the exception that incubations with primary and secondary antibodies were done in the presence of 5% nonfat milk.

Northern Blot Analysis of UGT mRNA

The mRNA from chrysin-treated and control Caco-2 cells was isolated using a FastTrack 2.0 kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Ten 100 mm Petri dishes were used for each isolation. Samples were combined with 3 volumes of RNA loading buffer, heated for 15 min at 65°C and 5 μg of mRNA was loaded on a 1% agarose gel with 3% formaldehyde. After electrophoresis, the RNA was transferred to a nylon filter (Hybond-N, Amersham Pharmacia Biotech, Piscataway, NJ). The filter was hybridized overnight at 65°C to a [^{32}P]-labeled probe prepared from a 0.7-kbp XhoI-EcoRI restriction fragment of pSK-UGT1A1 containing the 5' end of UGT1A1 (14). The probe was labeled by random primed synthesis using [α^{32}P]dCTP and a kit from Amersham Pharmacia Biotech. After hybridization, the filter was repeatedly washed with phosphate buffer containing SDS/EDTA and subjected to autoradiography using Hyperfilm MP (Amersham). The same membrane was stripped and reprobed with a 1 kbp glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (Ambion, Austin, TX) to confirm equal loading between control and chrysin-treated samples.

Quercetin Glucuronidation by Recombinant UGT1A9 and 1A1 and Caco-2 Microsomes

Quercetin (10 μM), dissolved in ethanol, was incubated with recombinant UGT1A9 or 1A1 (200 μg), control or chrysin-treated Caco-2 microsomes (125 μg, 213 μg respectively) in 50 mM Tris.HCl buffer (pH 7.4) containing 10 mM $MgCl_2$ and 1 mM UDPGA. The blank reactions were run without UDPGA. The reaction mixtures (500 μl) were incubated at 37°C for 60 min under argon gas. At the end of the reaction, 5 mg of ascorbic acid was added and the samples were extracted with 500 μl methanol by vortexing vigorously for 20 sec and centrifuging for 10 min at 14,000 g. The supernatant was subjected to HPLC analysis using a Symmetry C18 column (3.9 × 150 mm, Waters) with UV detection at 370 nm. The mobile phase was a linear gradient of 25–100% solvent B (5–40 min) at a flow rate of 0.9 ml/min. Solvent A was 0.1% trifluoroacetic acid in water and solvent B was methanol.

N-Hydroxy-PhIP Glucuronidation by Recombinant UGT1A1 and Caco-2 Microsomes

N-hydroxy-PhIP (0.1–50 μM), dissolved in ethanol:DMSO (80:20, v/v), was incubated with recombinant UGT1A1 (200 μg), control or chrysin-treated Caco-2 microsomes (100μg) in 100 mM Tris.HCl buffer (pH 7.4) containing 5 mM $MgCl_2$ and 3 mM UDPGA. The blank reactions were run without UDPGA or microsomes. The reaction mixtures (500 μl) were incubated at 37°C for 60 min. At the end of the reaction, the samples were cooled on ice and centrifuged for 2 min at 10,000*g*. The supernatant was solid-phase extracted as described previously (13), reconstituted in mobile phase and analyzed by HPLC using a Symmetry C18 3.9 × 150 mm column (Waters, Milford, MA). The mobile phase was 18% acetonitrile in 50 mM ammonium acetate buffer (pH 4.5). The samples were run at a flow rate of 0.9 ml/min with UV detection at 315 nm.

Identification of *N*-Hydroxy-PhIP Metabolites

Mass spectrometric identification of *N*-hydroxy-PhIP metabolites formed by UGT 1A1 used identical HPLC conditions as above. The effluent from the HPLC column was split and 10% of the flow was directed into the electrospray ionization (ESI) source of a Finnigan LCQ ion trap mass spectrometer operated in positive-ion mode. Instruments parameters were: ESI needle voltage, 4.2 kV; ESI capillary temperature, 220°C; isolation window, 2 amu; scan range 150–1000 amu. MS data were acquired with repetitive scanning with MS/MS data automatically acquired on the most intense precursor ion in each MS spectrum, using helium collision gas, with collision energy of 30 eV.

RESULTS

Our previous study on chrysin induction of glucuronidation in Caco-2 cells had shown maximum effect after 3–4 days of incubation with a concentration of 25–50 μM chrysin (9). Those were the experimental conditions used throughout the present study.

Chrysin-inducible UGT isoforms were in first hand identified by immunoblot analysis. Microsomes prepared from chrysin-treated and control Caco-2 cells were subjected to SDS-PAGE and probed with isoform specific antibodies, Fig. 1. As previously shown, an antibody selective for the whole subfamily of UGT1A isoforms showed a strong signal in the chrysin-treated vs. untreated cells (9). Thus, it was not unexpected to see that chrysin did not have any inducing effect on the UGT2B7 isoform. Surprisingly, there was no effect on the

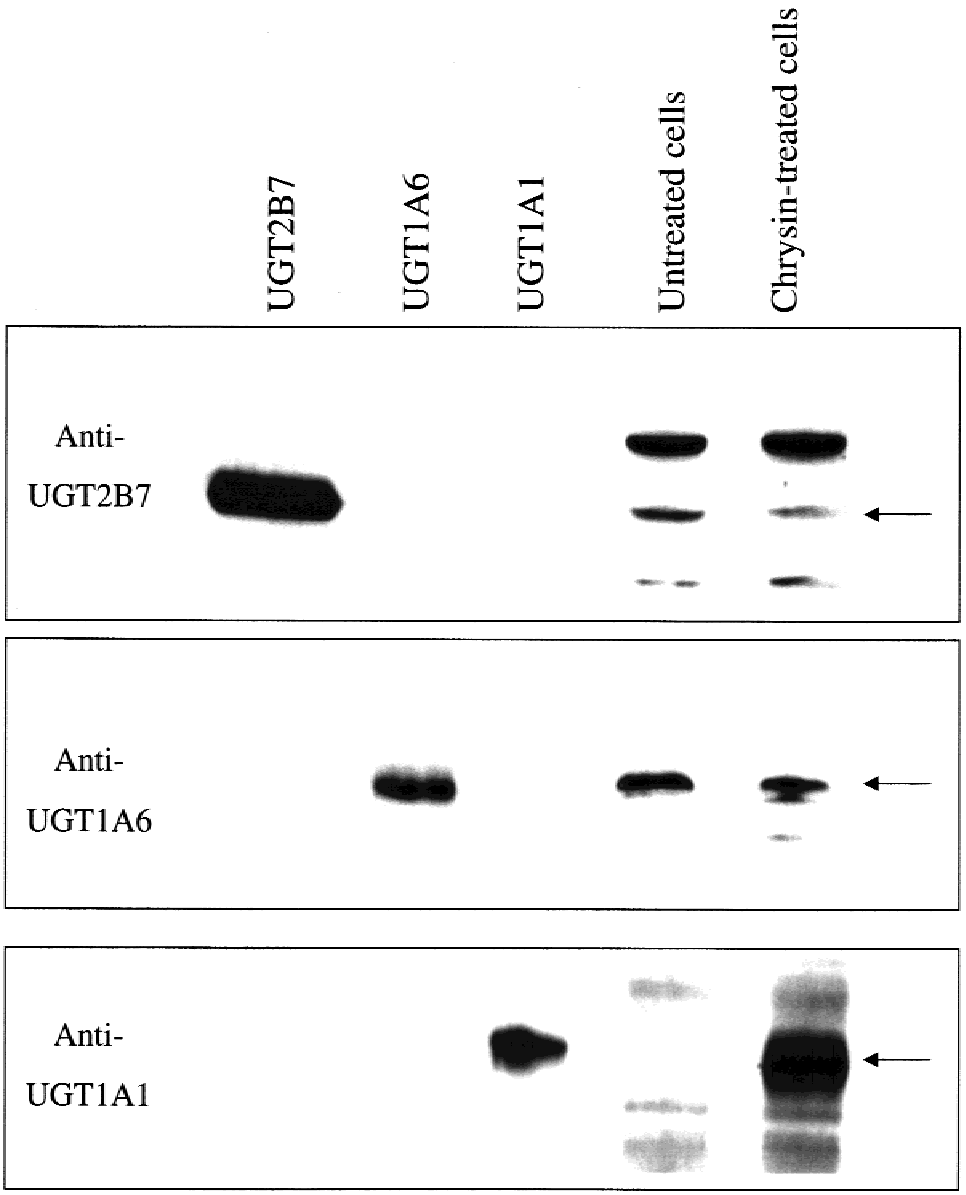

Fig. 1. Immunoblot analysis of Caco-2 cell microsomes from cells pretreated with 25 μM chrysin for 3 days as compared to untreated cells. Three different immunoblots were done with antibodies specific for UGT2B7, UGT1A6, and UGT1A1, respectively. Sample loading: lane 1, recombinant UGT2B7 protein; lane 2, recombinant UGT1A6 protein; lane 3, recombinant UGT1A1 protein; lane 4, microsomes from untreated Caco-2 cells; and lane 5, microsomes from chrysin-treated Caco-2 cells. The arrows designate UGT protein bands.

UGT1A6 protein, the isoform that has become known to use planar phenols, such as the flavones, as substrates. However, both of these isoforms were well expressed in the untreated Caco-2 cells. When a new and highly specific antibody towards UGT1A1 was used (12), a very strong induction was observed, Fig. 1, bottom. Only a very weak signal for this isoform could be seen in the untreated Caco-2 cells. The antibodies used displayed high specificity towards the various recombinant isoforms, i.e., UGT2B7, 1A6 and 1A1.

Poly(A)$^+$ RNA isolated from untreated and chrysin-treated Caco-2 cells subjected to agarose-formaldehyde electrophoresis and hybridized to a [^{32}P]-labeled cDNA fragment of pBluescript SK/UGT1A1 (12) showed a dramatic increase in UGT1A1 mRNA with the expected size (2.3 kb) in the chrysin-treated cells with no detectable amount in the untreated cells, Fig. 2, lane 2 compared to lane 1. A GAPDH cDNA probe was used to reprobe the stripped membrane and showed similar loading of chrysin-treated and untreated cell mRNA. These observations strongly confirmed the findings in Fig. 1 that the UGT1A1 isoform is induced by chrysin.

A previous study has shown that chrysin is a good substrate not only for UGT1A1 but also for UGT1A9 (14). Immunoblot analysis with several UGT1A9 antibodies was, however, equivocal. If induction of this isoform was occurring, it was weak. To learn more about the possible induction of this isoform, we used another, more complex, pentahydroxylated flavonoid, quercetin, as a molecular probe, Fig. 3. Recombinant UGT1A9 produced four distinct glucuronide

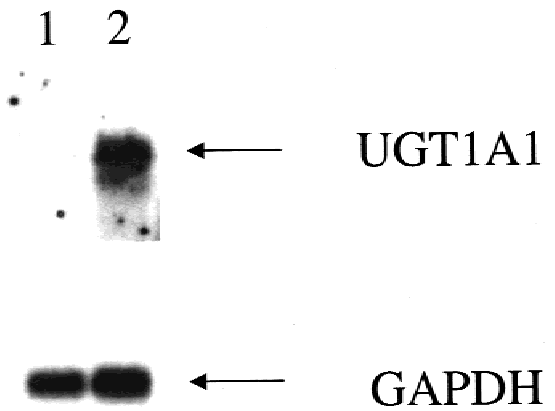

Fig. 2. Northern blot analysis of UGT1A1 mRNA expression in Caco-2 cells. Poly(A)$^+$ RNA (~5 μg) isolated from untreated and chrysin-treated cells (lanes 1 and 2, respectively) were subjected to agarose-formaldehyde electrophoresis, hybridization with a [^{32}P]-labeled UGT1A1 cDNA probe (14) and autoradiography. The blots were then stripped and hybridized to a GAPDH cDNA probe to confirm equal sample loading.

isomers (Fig. 3A), while UGT1A1 produced only one major and one minor isomer (Fig. 3B) and UGT1A4 and 1A6 did not use quercetin as a substrate. Interestingly, untreated Caco-2 cells only produced trace quantities of one isomer (Fig. 3C), whereas the chrysin-treated cells produced the same isomers and in the same ratios (Fig. 3D) as produced by recombinant UGT1A1. This again demonstrates induction of UGT1A1 by chrysin with no effect on UGT1A9 and the utility of quercetin as a probe for these two isoforms.

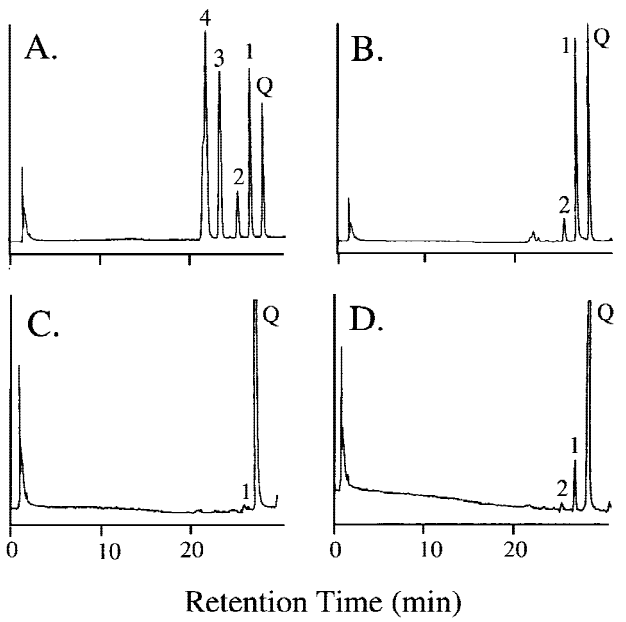

Fig. 3. Glucuronidation of quercetin as a marker of UGT1A9 and UGT1A1 activity. The enzyme sources were A. recombinant UGT1A9; B. recombinant UGT1A1; C. microsomes from untreated Caco-2 cells and D. microsomes from chrysin-treated Caco-2 cells. After incubations, samples were analyzed by isocratic reverse-phase HPLC. Q = quercetin. Arabic numerals 1–4 are isomeric quercetin monoglucuronides.

One of the potential benefits of flavonoid-induced glucuronidation in colon cells would be protection from carcinogens. One of the best known human colon carcinogens is the cooked-food mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and its more proximate carcinogen *N*-hydroxy-PhIP (15). To test whether the chrysin-inducible UGT1A1 can use *N*-hydroxy-PhIP as a substrate, we first employed recombinant UGT1A1 protein. A one-hr incubation with 25 μM *N*-hydroxy-PhIP produced a clear HPLC peak with a retention time of 7 min (Fig. 4A), a peak that was not observed in the absence of UGT1A1 enzyme or the cofactor UDPGA. HPLC/MS analysis of this peak demonstrated an $[M+H]^+$ ion of m/z 417 consistent with a glucuronic acid conjugate of *N*-hydroxy-PhIP. Upon collision-induced dissociation, product ions of m/z 241 and 225 were observed, indicative of *N*-hydroxy-PhIP (loss of 176 amu from the molecular ion) and PhIP, respectively. This is similar to a previous study (16). The UV absorption maximum for *N*-hydroxy-PhIP was shifted to 319 nm in the metabolite, characteristic of the *N*-hydroxy-PhIP-N^2-glucuronide as opposed to *N*-hydroxy-PhIP-N^3-glucuronide (16). When the kinetics of glucuronidation of *N*-hydroxy-PhIP (0.1–50 μM) by UGT1A1 was studied, we obtained an apparent K_m value of 13.8 μM and a V_{max} value of 63.4 pmol/min/mg protein.

When microsomes from untreated Caco-2 cells were incubated with *N*-hydroxy-PhIP, there was a minor HPLC peak at the expected retention time of 7 min for the *N*-hydroxy-PhIP-N^2-glucuronide (Fig. 4B). This peak was dramatically increased in the chrysin-treated cells (Fig. 4C). In repeated experiments (N = 5), there was an average 10-fold increase in the glucuronidation of *N*-hydroxy-PhIP in the chrysin-treated as compared to the untreated Caco-2 cells (Fig. 5).

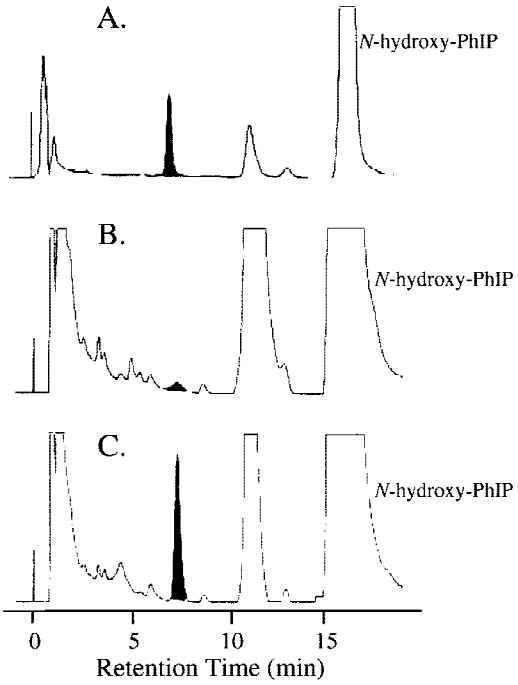

Fig. 4. Glucuronidation of the cooked-food mutagen/carcinogen *N*-hydroxy-PhIP by A. Recombinant UGT1A1, B. Microsomes from untreated cells and C. Microsomes from Caco-2 cells pretreated with 25 μM chrysin for 3 days. The peaks eluting at 10 to 12 min are impurities present in *N*-hydroxy-PhIP.

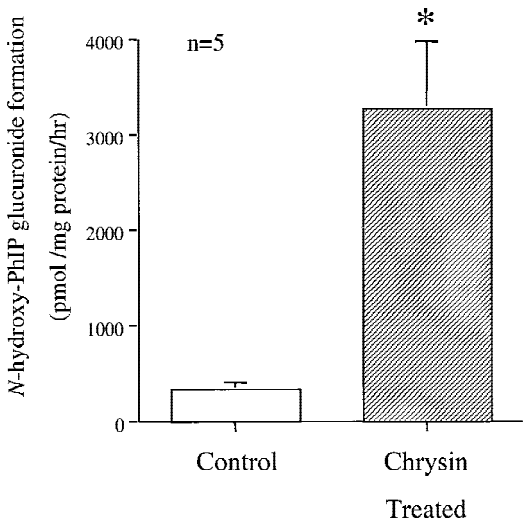

Fig. 5. Induction of *N*-hydroxy-PhIP glucuronidation by chrysin in Caco-2 cells. Cells were incubated with 25 μM chrysin for 3 days and microsomes were isolated as described. Microsomal incubates (50 μM *N*-hydroxy-PhIP for 60 min) were analyzed as in Fig. 5B and C. Mean values ± SEM from 5 experiments with chrysin-treated and untreated cells are shown. *Significantly higher than for untreated cells ($P < 0.01$).

DISCUSSION

In previous studies we have shown that the flavonoid chrysin is extensively metabolized by Caco-2 cells via glucuronidation and sulfation (13). Pretreatment of Caco-2 cells with chrysin induced its metabolism through glucuronidation, whereas sulfation was not affected (9). Immunoblot analysis with an antibody recognizing all UGT 1A isoforms demonstrated a dramatic increase in the expression of one or several of these proteins in treated cells.

In the present study, using UGT isoform-specific antibodies, we showed that chrysin treatment dramatically induced the expression of UGT1A1, while it had no effect on the expression of UGT2B7 and UGT1A6. The immunoblots also demonstrated clear expression of UGT2B7 and UGT1A6 in untreated Caco-2 cells with virtually no expression of UGT1A1. In addition, our data using quercetin as an isoform-specific probe of catalytic activity clearly demonstrated the absence of induction of UGT1A9 by chrysin but clear induction of UGT1A1. It should, however, be emphasized that additional UGT isoforms may be inducible. This has to await the development of additional antibodies, e.g., for UGT1A8 and UGT1A10, shown to be expressed in the intestine but not in the liver, or use of an approach such as DRT-PCR described by Strassburg et al. (17).

The induction of UGT1A1 protein in Caco-2 cells was further supported by examining the transcript levels using a UGT1A1-specific probe (12). Northern blot analysis indicated that chrysin treatment substantially induced the levels of UGT1A1 mRNA.

While flavonoids previously have been shown to induce hepatic glucuronidation *in vivo* in rodents (7,8), the only previous report of a flavonoid inducing UGTs in a cultured mammalian cell is that of biochanin A inducing the glucuronidation of UGT2B15 in a prostate cancer cell line (18). In a study parallel to the present, we recently showed that UGT1A1 is inducible by flavonoids in the human hepatoma cell line Hep G2 (14). However, as there are large qualitative and quantitative differences in the expression of the UGT isoforms in the normal human intestine vs. the liver, based on DRT-PCR (17), those findings could not necessarily be extrapolated to the intestinal Caco-2 cells. Whereas the uninduced Hep G2 cells had clear expression of the UGT1A1 mRNA (14), there was no such message in the uninduced Caco-2 cells. However, in a recent report Paine et al. showed by Western blotting clear but highly variable expression of UGT1A1 in fresh human intestinal tissue (19).

Some UGTs have previously been demonstrated to be inducible in the Caco-2 cells. Thus, UGT1A6 and UGT1A9 are inducible by TCDD, an aryl-hydrocarbon inducer (5). The antioxidant *t*-butylhydroquinone, which has a somewhat different spectrum of induction, induced UGT1A6, 1A9 as well as 2B7 (5). Interestingly, in a recent study it was demonstrated that the flavonoid quercetin at rather high concentrations was able to induce UGT1A6 (20). The magnitude of induction in these studies was relatively modest. Thus, there is no previous report on expression of UGT1A1 in Caco-2 cells.

UGT1A1 is the isoform that has been shown to be primarily involved in metabolism of bilirubin (1), an endogenous toxic waste product. Thus, chrysin and potentially other flavonoids may increase glucuronidation of bilirubin in conditions of hyperbilirubinemia, such as neonatal jaundice. This is likely to be more important in the liver (14). In the present study we demonstrate for the first time that not only is this isoform induced in the colonic Caco-2 cells but it is also involved in the metabolism of the potent colon carcinogen *N*-hydroxy-PhIP. We have identified that the glucuronide conjugate formed by UGT1A1 is the *N*-hydroxy-PhIP-N^2-glucuronide, using HPLC/MS as well as characteristic UV absorption. The enzyme kinetics for its formation demonstrated a favorably low K_m value of 13.8 μM. These findings are in sharp contrast to those previously made by Nowell et al. (21) but are supported by observations made by Malfatti and Felton in a preliminary communication (22).

Our finding that *N*-hydroxy-PhIP glucuronidation is mediated by recombinant UGT1A1 is further strongly supported by our observations in the Caco-2 cells. Thus, there was as much as a 10-fold increase in the glucuronidation of *N*-hydroxy-PhIP by the chrysin-treated Caco-2 microsomes. *N*-hydroxy-PhIP-N^2-glucuronide, a clear detoxification product that is not susceptible to hydrolysis by β-glucuronidase, is the major metabolite excreted in human urine upon PhIP ingestion (23). Thus, induction of UGT1A1 in the colon by flavonoids such as chrysin could increase the conversion to *N*-hydroxy-PhIP-N^2-glucuronide followed by efflux by the MRP2 transporter, as previously described in Caco-2 cells (24). This may decrease the mutagenic/carcinogenic potential of *N*-hydroxy-PhIP.

UGT1A1 has also been shown to conjugate the contraceptive drug ethinylestradiol (25) as well as the antitumor drug irinotecan (26). It may be possible that intestinal metabolism by this isoform could be important for other drugs as well. Overexpression of UGT1A1, such as after flavonoid consumption, may lead to diminished therapeutic response to such drugs. Thus, UGT1A1 should perhaps be added to CYP3A4 and P-glycoprotein (27) as an important intestinal barrier to drug bioavailability and a source of interindividual variability in drug response.

In conclusion, considering the extraordinary utility of Caco-2 cells as a model of human intestinal absorption, the establishment of the flavonoid induction response in these cells is essential. The enterocyte is indeed the cell that will see the highest concentrations of dietary chemicals like chrysin.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health grant GM55561. We gratefully acknowledge Dr. Joseph Ritter for providing us with UGT 1A1 antibody and UGT 1A1 cDNA and the USDA grant CSREES 0035200-9071. The use of the Medical University of South Carolina Core Mass Spectrometry Facility in our structure identification work is greatly acknowledged.

REFERENCES

1. P. J. Bosma, J. Seppen, B. Goldhoorn, C. Bakker, R. P. J. Oude Elferink, J. R. Chowdhury, N. R. Chowdhury, and P. L. Jansen. Bilirubin UDP-glucuronosyltransferase 1 is the only relevant bilirubin glucuronidating isoform in man. *J. Biol. Chem*. **269**:17960–17964 (1994).
2. M. D. Green, E. M. Oturu, and T. R. Tephly. Stable expression of a human liver UDP-glucuronosyltransferase (UGT2B15) with activity toward steroid and xenobiotic substrates. *Drug Metab. Dispos*. **22**:799–805 (1994).
3. B. Burchell, C. H. Brierley, and D. Rance. Specificity of human UDP-glucuronosyltransferases and xenobiotic glucuronidation. *Life Sci*. **57**:1819–1831 (1995).
4. R. H. Tukey, and C. P. Strassburg. Human UDP-glucuronosyltransferases: Metabolism, expression, and disease. *Ann. Rev. Pharmacol. Toxicol*. **40**:581–616 (2000).
5. P. A. Münzel, S. Schmohl, H. Heel, K. Kälberer, B. S. Bock-Hennig, and K. W. Bock. Induction of human UDP glucuronosyltransferases (UGT1A6, UGT1A9, and UGT2B7) by *t*-butylhydroquinone and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Caco-2 cells. *Drug Metab. Dispos.* **27**:569–573 (1999).
6. F. K. Kessler and J. K. Ritter. Induction of a rat liver benzo[*a*-]pyrene-*trans*-7,8-dyhydrodiol glucuronidating activity by oltipraz and β-naphthoflavone. *Carcinogenesis* **18**:107–114 (1997).
7. M. C. Canivenc-Lavier, M. F. Vernevaut, M. Totis, M. H. Siess, J. Magdalou, and M. Suschetet. Comparative effects of flavonoids and model inducers on drug-metabolizing enzymes in rat liver. *Toxicology* **114**:19–27 (1996).
8. M. H. Siess, J. P. Mas, M. C. Canivenc-Lavier, and M. Suschetet. Time course of induction of rat hepatic drug-metabolizing enzyme activities following dietary administration of flavonoids. *J. Toxicol. Envir. Health* **49**:481–496 (1996).
9. A. Galijatovic, U. K. Walle, and T. Walle. Induction of UDP-glucuronosyltransferase by the flavonoids chrysin and quercetin in Caco-2 cells. *Pharm. Res.* **17**:21–26 (2000).
10. U. K. Laemmli. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**:680–685 (1970).
11. H. Towbin, T. Staehelin and J. Gordon. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**: 4350–4354 (1979).
12. J. K. Ritter, F. K. Kessler, E. T. Thompson, A. D. Grove, D. J. Auyeung, and R. A. Fisher. Expression and inducibility of the human bilirubin UDP-glucuronosyltransferase UGT1A1 in liver and cultured primary hepatocytes: Evidence for both genetic and environmental influences. *Hepatology* **30**:476–484 (1999).
13. A. Galijatovic, Y. Otake, U. K. Walle, and T. Walle. Extensive metabolism of the flavonoid chrysin by human Caco-2 and Hep G2 cells. *Xenobiotica* **29**:1241–1256 (1999).
14. T. Walle, Y. Otake, A. Galijatovic, J. K. Ritter, and U. K Walle. Induction of UDP-glucuronosyltransferase (UGT) 1A1 by the flavonoid chrysin in the human hepatoma cell line Hep G2. *Drug Metab. Dispos.* **28**:1077–1082 (2000).
15. K. H. Dingley, K. D. Curtis, S. Nowell, J. S. Felton, N. P. Lang, and K. W. Turteltaub. DNA and protein adduct formation in the colon and blood of humans after exposure to a dietary-relevant dose of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine. *Cancer Epidemiol. Biomark. Prev.* **8**:507–512 (1999).
16. K. R. Kaderlik, G. J. Mulder, R. J. Turesky, N. P. Lang, C. H. Teitel, M. P. Chiarelli, and F. F. Kadlubar. Glucuronidation of *N*-hydroxy heterocyclic amines by human and rat liver microsomes. *Carcinogenesis* **15**:1695–1701 (1994).
17. C. P. Strassburg, N. Nguyen, M. P. Manns, and R. H. Tukey. UDP-glucuronosyltransferase activity in human liver and colon. *Gastroenterology* **116**:149–160 (1999).
18. X. Y. Sun, C. A. Plouzek, J. P. Henry, T. T. Y. Wang, and J. M. Phang. Increased UDP-glucuronosyltransferase activity and decreased prostate specific antigen production by biochanin A in prostate cancer cells. *Cancer Res.* **58**:2379–2384 (1998).
19. M. F. Paine, and M. B. Fisher. Immunochemical identification of UGT isoforms in human small bowel and in Caco-2 cell monolayers. *Biochem. Biophys. Res. Comm.* **273**:1053–1057 (2000).
20. K. W. Bock, T. Eckle, M. Ouzzine, and S. Fournel-Gigleux. Coordinate induction by antioxidants of UDP-glucuronosyltransferase UGT1A6 and the apical conjugate export pump MRP2 (multidrug resistance protein 2) in Caco-2 cells. *Biochem. Pharmacol*. **59**:467–470 (2000).
21. S. A. Nowell, J. S. Massengill, S. Williams, A. Radominska-Pandya, T. R. Tephly, Z. Q. Cheng, C. P. Strassburg, R. H. Tukey, S. L. MacLeod, N. P. Lang, and F. F. Kadlubar. Glucuronidation of 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine by human microsomal UDP-glucuronosyltransferases: Identification of specific UGT1A family isoforms involved. *Carcinogenesis* **20**:1107–1114 (1999).
22. M. A. Malfatti and J. S. Felton. The glucuronidation of the cooked-food carcinogen intermediate *N*-OH-PhIP by human UDP-glucuronosyltransferases. *Proceed. Am. Assoc. Cancer Res*. **41**:835–836 (2000).
23. M. A. Malfatti, K. S. Kulp, M. G. Knize, C. Davis, J. P. Massengill, S. Williams, S. Nowell, S. MacLeod, K. H. Dingley, K. W. Turteltaub, N. P. Lang and J. S. Felton. The identification of [2-^{14}C]2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine metabolites in humans. *Carcinogenesis* **20**:705–713 (1999).
24. U. K. Walle, A. Galijatovic, and T. Walle. Transport of the flavonoid chrysin and its conjugated metabolites by the human intestinal cell line Caco-2. *Biochem. Pharmacol*. **58**:431–438 (1999).
25. T. Ebner, R. P. Remmel, and B. Burchell. Human bilirubin UDP-glucuronosyltransferase catalyzes the glucuronidation of ethinylestradiol. *Mol. Pharmacol.* **43**:649–654 (1993).
26. L. Iyer, C. D. King, P. F. Whitington, M. D. Green, S. K. Roy, T. R. Tephly, B. L. Coffman, and M. J. Ratain. Genetic predisposition to the metabolism of irinotecan (CPT-11): Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. *J. Clin. Invest.* **101**:847–854 (1998).
27. P. B. Watkins. The barrier function of CYP3A4 and P-glycoprotein in the small bowel. *Advan. Drug Deliv. Rev*. **27**:161–170 (1997).