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Influence of *t*-butylhydroquinone and β-naphthoflavone on formation and transport of 4-methylumbelliferone glucuronide in Caco-2/TC-7 cell monolayers

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Abstract

Human Caco-2 cells have been established as a model system for intestinal biotransformation and permeability. When grown on Transwell polycarbonate filters they develop morphologic and biochemical characteristics of enterocytes with well separated apical and basolateral surfaces. In addition, Caco-2/TC-7 cells have proven to be useful to study regulation of human UDP-glucuronosyltransferases (UGTs) by Ah receptor agonists and antioxidant-type inducers such as β-naphthoflavone (BNF) and *t*-butylhydroquinone (TBHQ). In the present investigation, formation and transport of 4-methylumbelliferone glucuronide was studied in intact Caco-2 cell monolayers. The following results were obtained: when loaded with 50–200 μM MUF either apically or basolaterally, MUF-GA was the major metabolite which was mostly released (80%) at the basolateral surface, probably via the multidrug resistance protein isoform MRP3; MUF sulfate formation was low (5 ± 2%). Pretreatment of cells with 80 μM TBHQ or 50 μM BNF for 72 hr before addition of 100 μM MUF enhanced basolateral secretion of MUF-GA 1.4- and 1.7-fold, respectively. However, at >200 μM MUF, MUF-GA secretion and induction was smaller, probably due to inhibition of intracellular UGT activity. MRP3 protein was localized to the basolateral surface of Caco-2 cells but was not induced by TBHQ or BNF. The results suggest that MUF-GA is mostly secreted basolaterally in Caco-2 cell monolayers. Treatment with TBHQ or BNF significantly enhanced MUF-GA formation in the intact cell. © 2002 Published by Elsevier Science Inc.

Keywords: Caco-2/TC-7 cells; Cell monolayers; 4-Methylumbelliferone glucuronide; Apical and basolateral transport; MRP3; *t*-Butylhydroquinone; β-Naphthoflavone

1. Introduction

Human Caco-2 cells have been established as a model system for intestinal biotransformation and permeability [1,2]. When grown on Transwell polycarbonate filters they develop morphologic and biochemical characteristics of enterocytes with well separated apical and basolateral surfaces. In addition, Caco-2/TC-7 cells have proven useful to study regulation of human UGTs by Ah receptor agonists such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or BNF and antioxidant-type inducers such as TBHQ [3–5]. Controversial findings have been published on the expression of UGT isoforms in Caco-2 cells, probably due to their heterogeneity: expression of UGT1A6 was found to be high in clone TC-7, low in clone PF-11 [3–5] and in clone P27.7 [6]. Expression of UGTs *in vivo* is regulated in a tissue-specific manner; some UGT isoforms are only expressed in the gastrointestinal tract and not in liver [7]. UGTs in intestinal epithelia may represent an important and inducible detoxification system for agents present in our plant diet.

Previously it has been shown that chrysin, a flavonoid present at high levels in honey and propolis, is extensively metabolized in Caco-2 cells, and chrysin glucuronides are preferentially secreted at the apical surface [8]. Pretreatment of these cells with chrysin resulted in a 4- and 14-fold induction of chrysin glucuronidation in intact cells and in cell homogenates, respectively [9]. 4-Methylumbelliferone (MUF, hymecromone) is a choleretic agent which is extensively metabolized *in vivo* by various UGTs and sulfotransferases. In rat liver MUF sulfate and in the intestine 4-methylumbelliferone glucuronide (MUF-GA) formation

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Abbreviations: MUF, 4-methylumbelliferone; MUF-GA, 4-methylumbelliferone glucuronide; UGT, UDP-glucuronosyltransferase; BNF, β-naphthoflavone; TBHQ, *t*-butylhydroquinone.

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predominated [10,11]. MUF is widely used as a substrate of UGTs. In polarized cells conjugates are secreted by ATP-binding cassette transporters of the multidrug resistance protein (MRP) family, for example, by the apical conjugate export pump MRP2 or basolaterally by MRP3 [12–14].

In the present investigation formation and transport of MUF-GA was studied in Caco-2/TC-7 cell monolayers. It was found that (i) MUF-GA was secreted at the basolateral surface most likely via MRP3; and (ii) treatment with TBHQ or BNF significantly enhanced MUF-GA secretion from intact cells.

2. Materials and methods

2.1. Chemicals

TBHQ, BNF and MUF, MUF-GA and MUF sulfate, saccharic acid–1,4-lactone and alamethicin were purchased from Sigma.

2.2. Cell culture

Caco-2 cells, clone TC-7 [1] were kindly provided by Alain Zweibaum (Institute National de la Santé et de la Recherche Médical, Villejuif, France) and grown on 100 mm × 20 mm Falcon tissue culture dishes (Becton Dickinson) in Dulbecco's modified Eagle's medium supplemented with 20% fetal bovine serum (heat-inactivated at 56° for 30 min), 25 mM glucose and 1% non-essential amino acids (Life Technologies). The medium was changed daily. Preconfluent cells (passages 35–39) were treated with 80 μM TBHQ and 50 μM BNF, dissolved in 0.1% DMSO. Solvent controls contained 0.1% DMSO. Exposures were continued for 72 hr after which cells were washed with 0.9% NaCl, harvested, and then stored at −80° prior to use. For studies with monolayers, cells were seeded in 12 mm i.d. Transwell polycarbonate inserts (Corning Costar Corp.) in 12-well plates at a density of 2×10^5 cells per insert. Cells were used at 20 days after seeding. MUF was added when the transepithelial electrical resistance exceeded 300 W cm^2.

2.3. Analysis of MUF, MUF-GA and MUF sulfate

MUF and its metabolites were measured in the medium by two methods:

(A) *HPLC separation*: It was carried out with modifications as described [11]. In brief, 50 μL samples were deproteinized by adding 200 μL methanol. The supernatant was injected into a system comprising of an analytical column (Prodigy 5 μm ODS/3, 100 Å, 250 mm × 4.6 mm; Phenomenex). Retention times for MUF-GA, MUF sulfate (MUF-S) and MUF were 5.5, 11.8 and 38 min, respectively. Fluorescence was recorded by fluorescence detector Jasco FP 920 and calibrated by external standards.

(B) *Simultaneous fluorimetric determination of MUF and MUF-GA*: MUF sulfate could be neglected since (i) its formation was low in Caco-2/TC-7 cells, as shown by HPLC separation (5 ± 2% of MUF-GA), and (ii) due to its quenched fluorescence. Samples were deproteinized by addition of 0.5 M $HClO_4$ (1:1). Supernatants were measured after addition of 1.6 M glycine–NaOH buffer, pH 10.3 (1:1). MUF-GA and MUF-S show the same blue-shift of MUF fluorescence but only MUF-S was markedly quenched. Fluorescence (FU) of MUF and its conjugates was determined at excitation and emission wavelengths of 315 and 445 nm for MUF, and of 315 and 365 nm for both MUF-GA and MUF-S in a Perkin-Elmer LS-5B fluorescence spectrophotometer [15]. By calibration with authentic standards relative fluorescence was found to be 1:0.36:0.024 for MUF, MUF-GA and MUF-S, respectively. Hence, when the low MUF-S concentration and its selectively quenched FU is taken into account, $FU_{MUF\text{-}S}$ was <0.5% of $FU_{MUF\text{-}GA}$ in medium samples and was therefore neglected. Methods A and B led to comparable results. Determinations obtained with method B are presented.

2.4. UDP-glucuronosyltransferase assay

For induction experiments cell monolayers were treated with 80 μM TBHQ or 50 μM BNF for 72 hr prior to MUF addition. Kinetic studies of alamethicin-activated UGT activity (MUF as substrate) were performed in cell homogenates of untreated, TBHQ- and BNF-treated cells [3,15]. Addition of the pore-forming peptide alamethicin (0.05 mg/mg protein) led to 1.6-fold activation of UGT activity. For statistical analysis Student's *t*-test was used.

For inhibition studies of UGT activity by MUF-GA, the UGT assay mixture [15] was used with the following modifications: the substrate concentration was reduced to 25 μM MUF, and saccharic acid–1,4-lactone (10 mM) and various concentrations of MUF-GA were included. The reaction was stopped by 0.2 M glycine–NaOH buffer, pH 10.3, and the time-dependent reduction of MUF fluorescence was measured.

2.5. Immunoblot analysis of MRP3

Caco-2 cell samples were quickly thawed and homogenized in 10 mM Tris–sucrose (250 mM) buffer pH 7.4, containing 1 mM phenylmethanesulphonyl fluoride, aprotinin, pepstatin A and leupeptin (5 μg/mL each), and 0.5 mM benzamidine using a Dounce homogenizer. Homogenate proteins, determined according to Lowry *et al.* [16], were denatured with Laemmli buffer and heated at 95° for 10 min [17]. Proteins were separated by 7.5%

(w/v) SDS-PAGE. Thereafter, proteins were transferred electrophoretically to Immobilon™ polyvinylidene difluoride (PVDF) membranes (Millipore). The membranes were blocked with a solution of 0.66% (w/v) I-Block™ in PBS and reacted with selective antibodies: for quantitative immunoblot analysis of the isoform, the membranes were incubated overnight with two selective MRP3 antibodies, termed FDS and ALL [18]. The FDS antibody was raised in rabbits against 24 amino acids at the carboxyl terminus of MRP3, the ALL antibody was raised against 20 internal amino acids (876–895) of MRP3. The antibody was used at a dilution of 1:1000. After extensive washing with I-Block™ immunocomplexes were analyzed as previously described [4].

2.6. Immunofluorescence microscopy

Cryosections from Caco-2 cell layer rolls were fixed in 4% phosphate-buffered paraformaldehyde for 5 min at 4° [4,19]. Thereafter, the sections were washed in phosphate-buffered saline (PBS) for 5 min and reacted with primary antibodies against villin and MRP3. The two MRP3 antibodies FDS and ALL already described were used. The sections were first incubated with the mouse anti-villin antibody (1:200) overnight at 4° and then with the secondary antibody Cy3 (1:100) for 3 hr. After washing with PBS they were reacted with either anti-MRP3 FDS or ALL anti-serum (1:100) overnight and then for 3 hr with Cy2 (1:25). Nuclei were stained blue with TOPRO (Molecular Probes). Fluorescence microscopy was performed using a Leica TCS SP microscope. Mouse anti-villin antibodies were from Chemicon International Inc. Cy2-conjugated goat anti-rabbit antibody and Cy3-conjugated goat anti-mouse antibody were from Dianova (Hamburg, Germany).

3. Results

HPLC analysis of MUF metabolites in Caco-2 cell monolayers showed that MUF was mostly conjugated with glucuronic acid; MUF sulfate formation was low in the concentration range between 50 and 400 μM MUF ($5 \pm 2\%$; not shown). MUF (200 μM) rapidly disappeared with time from the apical and appeared in the basolateral chamber (Fig. 1). From the appearance of MUF in the receiving compartments during the first hour apical–basolateral and basolateral–apical fluxes (P_{app}) of 34.7×10^{-6} and 22.8×10^{-6} cm/s, respectively, were calculated using the equation given in a previous study [8]. MUF-GA was secreted into both chambers. It has to be noted that basolateral loading in the Transwell system represents addition of a 3-fold higher amount of MUF due to the 3-fold higher chamber volume. Preferential basolateral secretion of MUF-GA is obvious when the total amount of secreted MUF-GA is listed (Fig. 2). As can be seen in Figs. 1 and 2, apically-loaded 200 μM MUF (i.e.

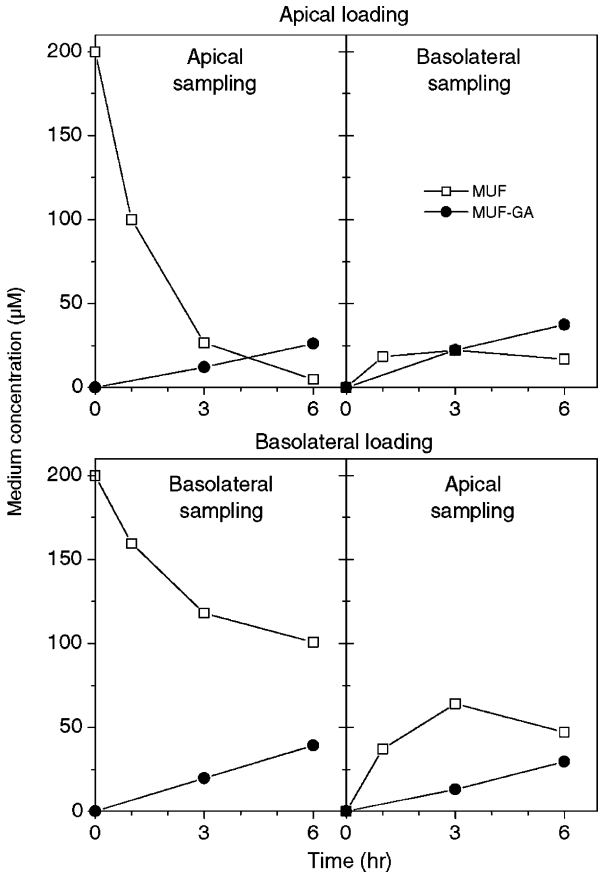

Fig. 1. Medium concentration of MUF (□) and MUF-GA (●) after apical and basolateral loading with 200 μM MUF. Means of three independent experiments are listed. Standard errors were <20%.

100 nmol) was mostly converted to MUF-GA within 6 hr; 56 nmol MUF-GA appeared in the basolateral and 13 nmol in the apical chamber. When various concentrations of MUF were loaded apically secretion of MUF-GA increased up to 400 μM. In contrast, basolateral loading of 400 μM MUF (corresponding to the 3-fold higher amount) led to lower secretion of MUF-GA, probably due to inhibition of UGTs by intracellular accumulation of MUF-GA.

Pretreatment of Caco-2 cell monolayers with TBHQ or BNF before addition of 100 μM MUF led to enhanced MUF-GA secretion (1.4- and 1.7-fold, respectively; Fig. 3). At 200 μM MUF, MUF-GA secretion and induction was smaller, again probably due to inhibition of intracellular UGTs. Kinetic studies of MUF-UGT activity in homogenates of TBHQ- and BNF-treated cells led to 1.8 and 2.2-fold increase of v_{max}, respectively (Fig. 4). Using hyperbolic regression analysis K_m values of 56, 60 and 72 μM were obtained with untreated, TBHQ- and BNF-treated cells, respectively. Moderate substrate inhibition was observed at 500 μM MUF. Therefore, it is assumed that inhibition of MUF-GA secretion at >200 μM MUF is mostly due to product inhibition by intracellularly accumulated MUF-GA. To substantiate this proposal we studied inhibition of MUF-UGT activity by MUF-GA as outlined in Section 2. It was found that indeed enzyme

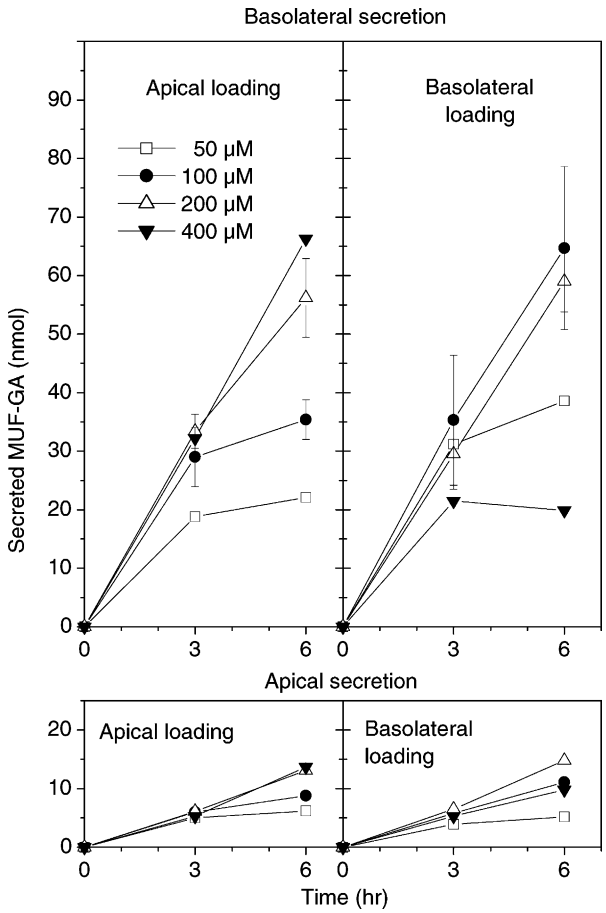

Fig. 2. Basolateral and apical secretion of MUF-GA after apical or basolateral loading with various MUF concentrations indicated. Means ± SD ($n = 3$) are listed.

activity was inhibited by MUF-GA with an apparent K_i of 135 µM (not shown).

Basolateral secretion of MUF-GA most likely occurs via MRP3. Therefore, immunoblot analysis was carried out using two selective MRP3 antibodies, directed against the

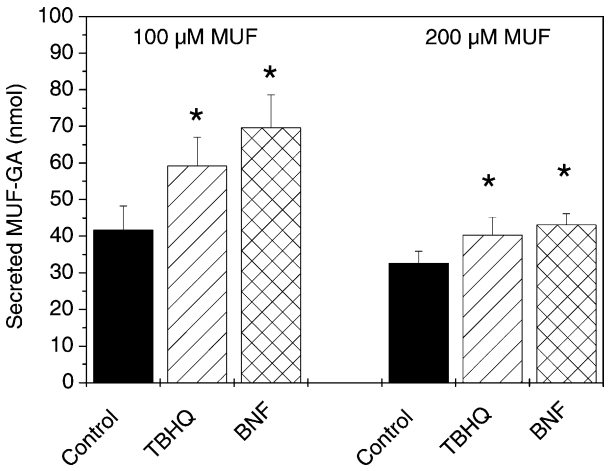

Fig. 3. Influence of TBHQ or BNF on formation and transport of MUF-GA in Caco-2 cell monolayers loaded basolaterally for 3 hr with 100 µM ($n = 3$) or 200 µM MUF ($n = 4$). Means ± SD are listed, * represents $P < 0.05$.

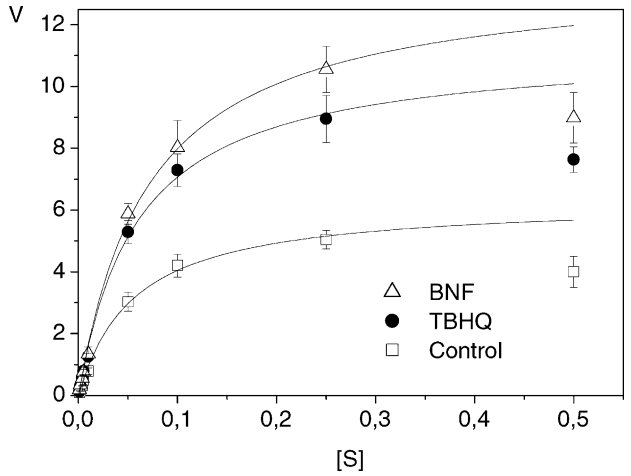

Fig. 4. Kinetic analysis of MUF-UGT activity in homogenates of untreated, TBHQ- and BNF-treated Caco-2 cells. Means ± SD ($n = 3$) are listed.

carboxyl terminus (FDS) and an internal peptide (ALL). With the FDS antibodies two characteristic bands were observed at 190 and 170 kDa (not shown) similar to findings with liver, MDCK and HepG2 cells [18,19]. With ALL antibodies, the 170 kDa band was predominant. Pretreatment of cells with TBHQ and BNF did not induce the MRP3 bands (not shown). In contrast to the apically localized villin (red fluorescence), basolaterally localized MRP3 was visualized with both FDS and ALL anti-MRP3 antibodies (Fig. 5A and B, respectively; green fluores-

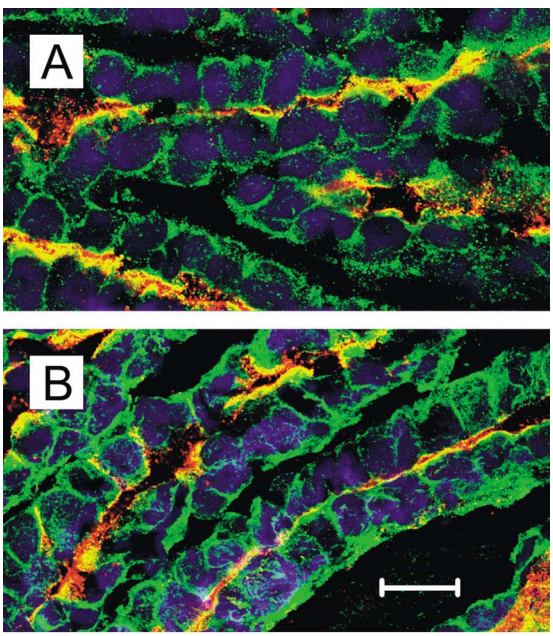

Fig. 5. Immunofluorescence localization of MRP3 in Caco-2/TC-7 cell monolayers. Cryosections from Caco-2 cell layer rolls were double-stained with MRP3 anti-serum (green fluorescence) and with red fluorescent anti-villin. Cell nuclei were stained blue. In contrast to anti-villin stained apical surfaces, the two anti-MRP3 antibodies FDS (A) and ALL (B) stained the rest of the cell surface. Bar, 20 µm.

cence). Note that two apical surfaces adhere in the analyzed cell monolayer rolls probably at their brush borders [4,20].

4. Discussion

Caco-2 cell monolayers represent a useful model since they retain many metabolic and transport characteristics of human intestinal epithelium. Glucuronidation was studied using the extensively conjugated MUF as UGT substrate. MUF sulfate formation was found to be low in Caco-2/TC-7 cells ($5 \pm 2\%$; not shown). Interestingly, MUF-GA was mostly released at the basolateral surface; apical secretion amounted to only 15–20%. This finding is in contrast to chrysin glucuronide secretion which is transported mostly at the apical surface [8]. Glucuronides are excreted from cells by a variety of multidrug resistance MRPs [12–14]. Caco-2 cells mostly express MRP2 and MRP3, whereas expression of MRP1 and MRP5 was found to be low [14]. MUF-GA is a substrate of both MRP3 and MRP2 [21]. Immunofluorescence analysis clearly showed basolateral localization of MRP3 in Caco-2/TC-7 cells (Fig. 5), whereas MRP2 was previously localized to the apical surface [4]. It is therefore conceivable that MUF-GA may be a preferred substrate of MRP3, explaining preferential release of MUF-GA at the basolateral surface, unless there is a marked difference in protein levels of MRP2 and MRP3. Chrysin glucuronide may be a preferred substrate of MRP2. However, more work is needed to characterize the substrate specificity of these transporters.

Caco-2/TC-7 cells express several UGTs which can be induced by Ah receptor agonists and antioxidant-type inducers [3–5]. These inducers received a lot of interest as dietary protectants against colon cancer [22–25]. Antioxidant-type inducers such as TBHQ and the flavonoid quercetin have been shown to induce UGT1A6, UGT1A9 and UGT2B7 [3] as well as MRP2 [4]. MUF is known to be an overlapping substrate of several UGT isoforms. However, UGT1A6 may play a major role in MUF glucuronidation since the apparent K_m of MUF-UGT activity in Caco-2 cell homogenates (56–72 μM) corresponds to that (58 μM) determined with heterologously-expressed human UGT1A6 (Harald Gschaidmeier; thesis, University of Tübingen, 1994). Pretreatment with TBHQ also induces UGT1A1 expression (Jutta Szillis[1]). This may explain induction of chrysin glucuronidation by chrysin which has been shown to be a substrate of UGT1A1 [26]. The present findings demonstrate that induction of MUF-UGT activity affects MUF-GA release from cells although to a lesser extent than the previously reported chrysin glucuronidation. MRP3 is not inducible by TBHQ or BNF in Caco-2 cells, in contrast to induction of MRP2 [4]. However, information on the regulation of MRPs is still scarce. MRP3 expression appears to be upregulated when MRP2 is genetically absent [12]. Moreover, the level of expression of transporters appears to differ in the small intestine and colon; for example, in inverted rat intestinal sacs 1-naphthol glucuronide (which may be transported similar to MUF-GA) was secreted in the small intestine mostly at the apical surface, whereas it was basolaterally secreted in the colon [27].

Transporters such as the apical export pump MRP2 and the basolateral export pump MRP3 clearly determine the site of secretion of glucuronides, for example into the lumen of the intestine or into the portal blood [12–14]. However, the relative roles of UGTs and of transporters on glucuronide secretion from cells are still unclear. The present findings suggest that at low MUF concentration metabolism may be a major factor determining glucuronide secretion while at high substrate concentration transporters may become rate limiting. UGT activity was clearly inhibited by MUF-GA. It is therefore conceivable that intracellularly accumulating MUF-GA may inhibit UGT activity. Inhibition of MUF-GA secretion at high MUF concentrations was observed both in untreated and in TBHQ- or BNF-treated cells. It remains to be resolved why basolateral loading with MUF leads to stronger inhibition. It is conceivable that higher intracellular MUF concentrations may be reached by passive diffusion when MUF is added to the larger basolateral chamber. However, MUF may also be taken up by selective basolateral uptake transporters, as in the case of bilirubin uptake into hepatocytes [28].

In conclusion, the present study suggests: (i) that MUF-GA—in contrast to other glucuronides—is mostly excreted at the basolateral surface most likely via MRP3; (ii) pretreatment with TBHQ and BNF significantly enhances MUF-GA formation in Caco-2 cell monolayers.

[1] Unpublished results.

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References

[1] Caro I, Boulenc X, Rousset M, Meunier V, Bourrie M, Julian B, Joyeux H, Roques C, Berger Y, Zweibaum A, Fabre G. Characterisation of a newly isolated Caco-2 clone (TC-7), as a model of transport process and biotransformation of drugs. Int J Pharmacol 1995;116: 147–8.

[2] Hidalgo IJ, Raub TJ, Borchardt RT. Characterization of human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology 1989;96:736–49.
[3] Münzel PA, Schmohl S, Heel H, Kälberer K, Bock-Hennig BS, Bock KW. 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 Disp 1999;27: 569–73.
[4] Bock KW, Eckle T, Ouzzine M, Fournel-Gigleux S. 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 2000;59:467–70.
[5] Sabolovic N, Magdalou J, Netter P, Abid A. Nonsteroidal anti-inflammatory drugs and phenols glucuronidation in Caco-2 cells: identification of the UDP-glucuronosyltransferases UGT1A6, 1A3 and 2B7. Life Sci 2000;67:185–96.
[6] Paine MF, Fisher MB. Immunochemical identification of UGT isoforms in human small bowel and in Caco-2 cell monolayers. Biochem Biophys Res Commun 2000;273:1053–7.
[7] Tukey RH, Strassburg CP. Human UDP-glucuronosyltransferases: metabolism, expression and disease. Annu Rev Toxicol 2000;40:581–616.
[8] Walle UK, Galijatovic A, Walle T. Transport of the flavonoid chrysin and its conjugated metabolites by the human intestinal cell line Caco-2. Biochem Pharmacol 1999;58:431–8.
[9] Galijatovic A, Walle UK, Walle T. Induction of UDP-glucuronosyl transferase by the flavonoids chrysin and quercetin in Caco-2 cells. Pharmacol Res 2000;17:21–6.
[10] Mulder GJ, Brouwer S, Scholtens E. High-rate intestinal conjugation of 4-methylumbelliferone during intravenous infusion in the rat *in vivo*. Biochem Pharmacol 1984;33:2341–4.
[11] Zimmermann CL, Ratna S, Leboeuf E, Pang KS. High-performance liquid chromatographic method for the direct determination of 4-methylumbelliferone and its glucuronide and sulfate conjugates: application to studies in the single-pass *in situ* perfused rat intestine–liver preparation. J Chromatogr 1991;563:83–94.
[12] König J, Nies AT, Cui Y, Leier I, Keppler D. Conjugate export pumps of the multidrug resistance protein (MRP) family: localization, substrate specificity, and MRP2-mediated drug resistance. Biochim Biophys Acta 1999;1461:377–94.
[13] Kool M, van der Linden M, de Haas M, Scheffer GL, de Vree ML, Smith AJ, Jansen G, Peters GJ, Ponne N, Scheper RJ, Oude Elferink RPJ, Baas F, Borst P. MRP3, an organic anion transporter able to transport anti-cancer drugs. Proc Natl Acad Sci USA 1999;96: 6914–9.
[14] Hirohashi T, Suzuki H, Chu X-Y, Tamai I, Tsuji A, Sugiyama Y. Function and expression of multidrug resistance-associated protein family in human colon adenocarcinoma cells (Caco-2). J Pharmacol Exp Ther 2000;292:265–70.
[15] Lilienblum W, Walli AK, Bock KW. Differential induction of rat liver microsomal UDP-glucuronosyltransferase activities by various inducing agents. Biochem Pharmacol 1982;31:907–13.
[16] Lowry OH, Rosebrough HJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
[17] Lämmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1990;227:680–5.
[18] König J, Rost D, Cui Y, Keppler D. Characterization of the human multidrug resistance protein isoform MRP3 localized to the basolateral hepatocyte membrane. Hepatology 1999;29:1156–63.
[19] Cantz T, Nies AT, Brom M, Hofmann AF, Keppler D. MRP2, a human conjugate export pump, is present and transports fluo 3 into apical vacuoles of Hep G2 cells. Am J Physiol Gastrointest Liver Physiol 2000;278:G522–31.
[20] Carrière V, Lesuffleur T, Barbat A, Rousset M, Dussaulx E, Costet P, de Waziers I, Beaune P, Zweibaum A. Expression of cytochrome P-450 3A in HT29-MTX cells and Caco-2 clone TC7. FEBS Lett 1994;355:247–50.
[21] Hirohasi T, Suzuki H, Sugiyama Y. Characterization of the transport properties of cloned rat multidrug resistance-associated protein 3 (MRP3). J Biol Chem 1999;274:1581–5.
[22] Talalay P, Fahey JW, Holtzclaw WD, Prestera T, Zhang Y. Chemoprotection against cancer by phase 2 enzyme induction. Toxicol Lett 1995;82/83:173–9.
[23] Buetler TM, Gallagher EP, Wang C, Stahl DL, Hayes JD, Eaton DL. Induction of phase I and phase II drug-metabolizing enzyme mRNA, protein and activity by BHA, ethoxyquin, and Oltipraz. Toxicol Appl Pharmacol 1995;135:45–57.
[24] O'Dwyer PJ, Szarka E, Yao KS, Halbherr C, Pfeiffer GR, Green F, Gallo JM, Brennan J, Frucht H, Goosenberg EB, Hamilton TC, Litwin S, Balshem M, Engstrom PF, Clapper ML. Modulation of gene expression in subjects at risk for colorectal cancer by chemopreventive dithiolethione Oltipraz. J Clin Invest 1996;98:1210–7.
[25] Hayes JD, Chanas SA, Henderson CJ, McMahon M, Sun C, Moffat GJ, Wolf CR, Yamamoto M. The Nrf2 transcription factor contributes both to the basal expression of glutathion-*S*-transferases in mouse liver and to their induction by the chemopreventive synthetic antioxidants, butylated hydroxyanisole and ethoxyquin. Biochem Soc Trans 2000;28:33–42.
[26] Walle T, Okate Y, Galijatovic A, Ritter JK, Walle UK. Induction of UDP-glucuronosyltransferase UGT1A1 by the flavonoid chrysin in the human hepatoma cell line HEP G2. Drug Metab Disp 2000;28:1077–82.
[27] Inoue H, Yokata H, Taniyama H, Kuwahara A, Ogawa H, Kato S, Yuasa A. 1-Napthol ß-D-glucuronide formed intraluminally in rat small intestine mucosa and absorbed into the colon. Life Sci 1999;65:1579–88.
[28] Cui Y, König J, Leier I, Buchholz U, Keppler D. Hepatic uptake of bilirubin and its conjugates by the human organic anion transporter SLC21A6. J Biol Chem 2001;276:9626–30.