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Short Communication

GLUCURONIDATION IN THE CACO-2 HUMAN INTESTINAL CELL LINE: INDUCTION OF UDP-GLUCURONOSYLTRANSFERASE 1*6

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Abstract—The ability of the differentiated human intestinal cell line, Caco-2, to glucuronidate various endobiotic and xenobiotic molecules was investigated. Glucuronidation of hydroxylated or carboxylic acid compounds such as 1-naphthol, thymol, androsterone, estriol, hyodeoxycholic acid, lithocholic acid, chloramphenicol, paracetamol and morphine could be determined in microsomal fractions of Caco-2 cells. The activity toward 1-naphthol was the highest glucuronidation activity measured in Caco-2 cells. This activity was specifically increased four-fold upon addition of β-naphthoflavone into culture medium but not by rifampicine or clofibrate and was related to a biosynthesis of UDP-glucuronosyltransferase 1*6 (UGT1*6). α-Naphthoflavone did not affect the inducing property of β-naphthoflavone. 7-Ethoxyresorufin-*O*-dealkylation activity, supported by cytochrome P4501A1, was induced more than 1000-times in Caco-2 cells by β-naphthoflavone treatment, and this effect was partially abolished by α-naphthoflavone treatment. The results suggest that several isoforms, including UGT1*6, are expressed in Caco-2 cells.

Key words: Caco-2 cells, glucuronidation capacities; P4501A1 induction; UGT1*6 induction

Glucuronidation is one of the main routes for elimination of xenobiotic and endogenous compounds. The reaction is catalysed by UGTs, which are membrane-bound enzymes. UGTs are a multigenic family of enzymes and are divided into two distinct families, based on the cDNA sequence comparison [1]. The *UGT1* locus codes for four different UGT isoforms. Among them, UGT1*6 specifically catalyses the glucuronidation of planar phenols such as 1-naphthol and phenols of polycyclic aromatic hydrocarbons [2]. Glucuronidation is usually considered as a detoxifying pathway for xenobiotics and drugs. It takes place mainly in the liver, and to a lesser extent, in extra-hepatic tissues such as kidney, small intestine, skin and brain [3].

Intestinal glucuronidation represents the first metabolic line of defense against ingested toxic xenobiotics. It is known that rat intestinal glucuronidation can reduce the oral bioavailability of phenolic substances such as 1-naphthol [4]. Up to now, intestinal glucuronidation studies have mostly been carried out on rat intestinal segments or on isolated rat intestinal epithelial cells [4], but little is known about the glucuronidation capacities of human enterocytes, expression and regulation of the UGTs. The human intestinal cell line Caco-2 [5] is an enterocyte-like differentiated cell line. Caco-2 cells, at confluency, acquire the enterocyte phenotype of the small intestine with morphological (tight junctions and apical brush-border microvilli) and biochemical (hydrolase activities) characteristics [6]. However, until now, glucuronidation activities have not been reported in Caco-2 cells.

In this study, the glucuronidation of 17 different xenobiotic and endobiotic molecules, which is supported by several UGT isoforms, was assayed in the Caco-2 cell line. The specific activity towards these molecules was compared to that measured in human liver microsomes. The expression and regulation of UGT1*6 by inducers was investigated.

Materials and Methods

Chemicals. UDP-[U-^{14}C] glucuronic acid (285.2 mCi/mmol) was purchased from NEN (Dupont de Nemours, France) and UDP-glucuronic acid (disodium salt) from Boehringer Mannheim (France). Clofibrate, testosterone, 1-naphthol, dexamethazone, D-saccharic acid 1, 4-lactone and α-[1-^{14}C]naphthol (7.1 mCi/mmol) were obtained from Sigma (France). Chloramphenicol, thymol, stearic acid and myristic acid were from Fluka (Switzerland). 17β-Estradiol, androsterone, 6β-hydrocortisone, estriol, hyodeoxycholic acid and lithocholic acid were purchased from Aldrich (France). Paracetamol was obtained from Bottu (France), morphine hydrochloride from Cooperation Pharmaceutique Française (France) and lorazepam from Prophac (France). Lamotrigine was kindly supplied by Wellcome (France).

Cell culture and treatments. Caco-2 cells [5] were generously donated by Dr. Gueant (INSERM U-308, Nancy, France). The cells were grown in Dulbecco's modified Eagle's medium supplemented as previously described [7]. Cultures were maintained at 37° in a humidified atmosphere containing 5% CO_2. The cells were expanded in 75 cm^2 tissue culture flasks for 5 days, while the medium was renewed 2 days after seeding the cells and every day thereafter. After trypsinization, the cells were seeded at the density of 8000 cells/cm^2 in Petri dishes. They were allowed to grow for 10 days before the treatments were begun. β-NF, α-NF, rifampicin and clofibrate were

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† Abbreviations: UGT, UDP-glucuronosyltransferase (EC 2.4.1.17); UDP-GlcA, UDP-glucuronic acid; P450, cytochrome P450 (EC 1.14.14.1.); EROD, 7-ethoxyresorufin-*O*-dealkylase; β-NF, β-naphthoflavone; α-NF, α-naphthoflavone; RIF, rifampicin; TLC, thin-layer chromatography; CLO, clofibrate

Table 1. Pattern of glucuronidation in the human enterocyte cell line Caco-2 and in human liver microsomes.

	UGT activity (pmol/min/mg of protein)				
	Caco-2 microsomes				
	passage 78		passage 121		
Substrates	− SAC	+ SAC	− SAC	+ SAC	Human liver microsomes
1-Naphthol	2.5	7.1	97.6	195.1	891.3
Thymol	<1*	<1	<1	4.0	58.6
Testosterone	<1	<1	1.2	<1	40.6
17β-Estradiol	<1	<1	<1	<1	21.9
Androsterone	<1	<1	<1	1.4	34.3
Estriol	<1	4.8	6.2	6.6	285.2
6β-Hydroxycortisone	<1	<1	<1	<1	9.2
Hyodeoxycholic acid	1.3	5.5	3.1	2.7	222.4
Lithocholic acid	<1	<1	<1	1.4	9.1
Chloramphenicol	1.3	6.4	8.3	49.6	189.1
Paracetamol	<1	<1	<1	1.3	2.5
Morphine	<1	<1	<1	1.2	9.3
Lorazepam	ND†	<1	ND	<1	1.5
Lamotrigine	ND	ND	ND	<1	22.4
Clofibric acid	ND	1.5	ND	<1	9.7
Stearic acid	ND	<1	ND	<1	1.5
Myristic acid	ND	<1	ND	<1	22.6

Glucuronidation activities were determined in microsomes prepared from 11-day-old cultured cells with [U-^{14}C]UDP-GlcA, using 0.5 mM of aglycone and 0.125 of UDP-GlcA, by the thin-layer chromatographic method. − SAC, without D-saccharic acid 1,4-lactone; + SAC, with 5 mM D-saccharic acid 1,4-lactone as inhibitor of β-glucuronidase. The results expressed as means of duplicate measurements and the variations were less than 10%.

* Limit of detection was 0.5 pmol/min/mg of protein.

† ND, not detectable.

dissolved in DMSO and added to the culture medium to reach the final concentrations of 50 μM, 75 μM and 100 μM, whereas α-NF was used at the concentration of 25 μM. In control cultures, only 0.2% (v/v) DMSO was added. Cultures without inducers or solvent were also carried out simultaneously. The medium and inducers were renewed every 24 hr. After 72 hr of treatment, the cells were harvested by scraping in ice-cold PBS, centrifuged and stored at −80° until preparation of microsomes.

Enzymatic activity assays. Microsomes were prepared from cells at passages 78 and 121 by the method of Dragacci *et al.* [8]. Protein concentration was determined by the method of Bradford [9], using bovine serum albumin as standard. The glucuronidation pattern of Caco-2 cells was determined by using 17 aglycones at a final concentration of 0.5 mM, 0.125 mM UDP-GlcA and UDP-[U-^{14}C]GlcA (200,000 dpm/assay) as described by Pritchard *et al.* [10]. 1-Naphthol glucuronidation activity was measured by two methods: a TLC method and that described by Otani *et al.* [11] using 4 mM UDP-GlcA, 0.5 mM 1-naphthol and [1-^{14}C]naphthol (120,000 dpm/assay). EROD activity was determined as previously described [12].

Protein analysis. Electrophoresis of proteins was performed according to Laemmli [13]. Immunological detection was carried out using polyclonal anti-human *N*-terminal UGT1*6 antibody [14]. Microsomal fraction prepared from V79 cells transfected with human UGT1*6 cDNA (V79UGT1*6) [15] was used as reference. Analysis of P4501A1 expression in Caco-2 cells was performed with polyclonal anti-rabbit P4501A1 [16], a kind gift of Dr Maurel (INSERM U-128, Montpellier, France).

Results and discussion

The differentiation state of Caco-2 cells was evaluated by cellular growth and by the measurement of brush-border marker enzyme activities, alkaline phosphatase and gamma-glutamyltransferase (data not shown). Our results, which indicate that the differentiation process begins at confluency (day 5) and continues thereafter, are in agreement with previous reports [6, 17].

The ability of Caco-2 cells to glucuronidate various substrates carried out by different UGT isoforms was studied in microsomal fraction prepared from 11-day-old cells at passages 78 and 121. The obtained activities were compared to those measured in human hepatic microsomes. 1-Naphthol, thymol, chloramphenicol, paracetamol, morphine, lorazepam, lamotrigine, clofibric acid, testosterone, 17β-estradiol, androsterone, 6β-hydrocortisone, estriol, hyodeoxycholic acid, lithocholic acid, stearic acid and myristic acid were used as substrates. Glucuronidation activities are summarized in Table 1. The highest glucuronidation activity measured in these cells was 1-naphthol glucuronidation activity. To a lesser extent, Caco-2 cells could glucuronidate thymol, androsterone, estriol, hyodeoxycholic acid, lithocholic acid, chloramphenicol, paracetamol and morphine.

Glucuronidation activities were affected by cell passage number as shown in Table 1. 1-Naphthol glucuronidation was 28- and 39-fold higher in cells at passage 121 as compared to passage 78. However, all these glucuronidation activities remained lower than those measured in human liver microsomes. D-Saccharic acid 1,4-lactone was used as an inhibitor of β-glucuronidase, the enzyme able to hydrolyse the newly-formed glucuronide, resulting in an apparently low-level of UGT activity. Addition of this β-glucuronidase inhibitor to the incubation mixture increased glucuronidation activities markedly (Table 1). This result

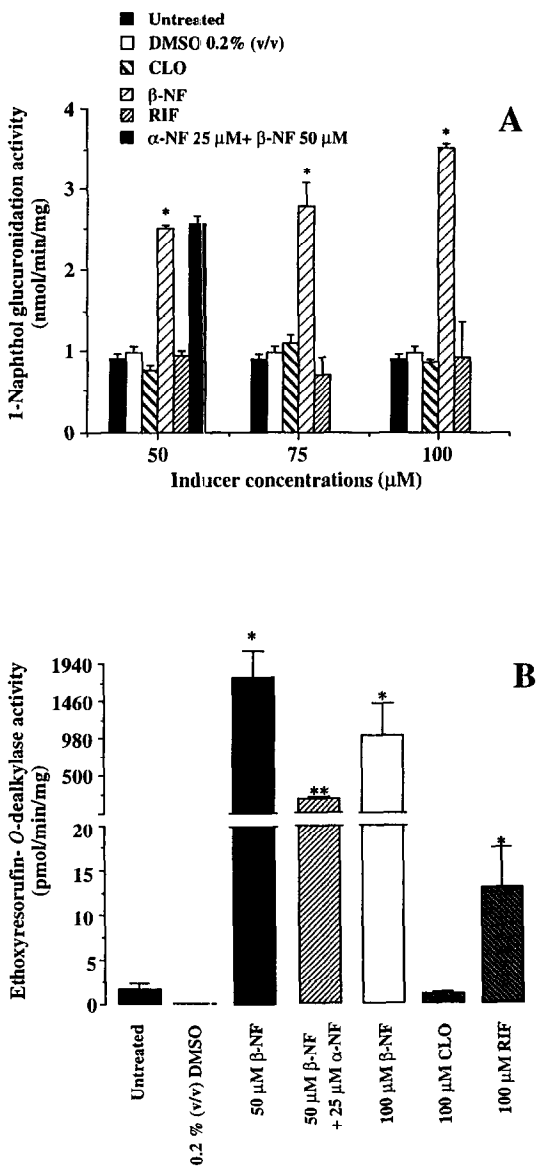

Fig. 1. Inducibility of 1-naphthol glucuronidation (A) and EROD (B) activities in Caco-2 cells. Caco-2 cells at passage 121 were treated at day 10 with 0.2% (v/v) DMSO, three concentrations of β-NF, CLO and RIF or with one concentration of α-NF. After 72 hr of treatment with renewal of the culture medium and inducers every 24 hr, the cells were harvested and microsomes prepared. 1-Naphthol glucuronidation activity was measured as described in the Materials and Methods section. Results were expressed as mean ± SD of triplicate measurements. * Significantly different from untreated cells ($P < 0.001$); ** significantly different from 50 μM β-NF treated cells ($P < 0.01$).

could indicate that β-glucuronidase is very active in Caco-2 cells.

In human liver, 1-naphthol is actively glucuronidated by UGT1*6, but this aglycone is a weak substrate of UGT1*7 [18]. On the other hand, thymol is mostly glucuronidated by UGT1*7 and not by UGT1*6 [18]. Thymol glucuronidation activity measured in Caco-2 cells was low, which could suggest that glucuronidation of 1-naphthol in Caco-2 cells is mainly related to UGT1*6. On the other hand, using anti-UGT1*6 antibodies [14] we found that UGT1*6 expression was higher in Caco-2 cells at passage 121 than at passage 78 (data not shown). These results corroborate the enzymatic activities measured and suggest that 1-naphthol glucuronidation in Caco-2 cells is carried out mainly by UGT1*6.

The inducibility of UGT1*6 by β-NF, RIF and clofibrate was also investigated. UGT1*6 dependent activity was monitored with 1-naphthol. The glucuronidation of 1-naphthol in these experiments was measured by a method different from that used for the determination of glucuronidation pattern (Table 1), where the UDP-GlcA concentration (0.125 mM) was not in saturation. This method uses radiolabelled UDP-GlcA, and the low concentration of UDP-GlcA makes it very sensitive. 1-Naphthol glucuronidation activity was not affected by 0.2% (v/v) DMSO treatment (Fig. 1A). β-NF significantly increased 1-naphthol glucuronidation activity in Caco-2 cells. This inducing effect was concentration-dependent and was maximal for 100 μM β-NF. 1-Naphthol glucuronidation activity was then increased 4-fold as compared to untreated or 0.2% DMSO-treated cells. The specificity of the induction was investigated using RIF and clofibrate. These inducers had no effect on 1-naphthol glucuronidation activity at any concentration used. The α-NF, an aryl hydrocarbon (*Ah*) receptor ligand known to antagonise the dioxin-induction of *CYP1A1* gene expression [19], was used in combination with β-NF. The inducing effect of β-NF on 1-naphthol glucuronidation activity in Caco-2 cells was not affected by the addition of 25 μM α-NF (Fig. 1A).

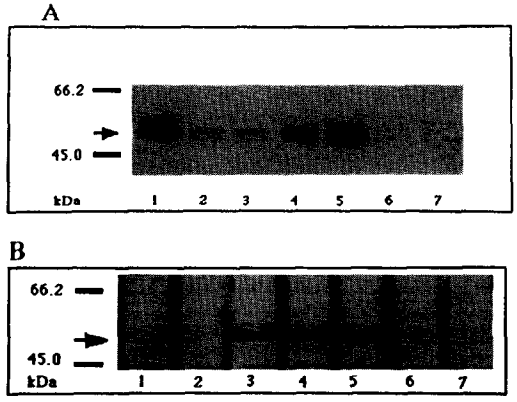

Fig. 2. Effects of inducers on the expression of UGT1*6 (A) and P4501A1 (B) in Caco-2 cells. Western blot analysis was performed on microsomal fraction prepared from either untreated cells, those treated with 100 μM of β-naphthoflavone (β-NF), CLO and RIF, or those treated with 50 μM β-NF in combination with 25 μM α-NF. Fifty micrograms of microsomal proteins was loaded in each line and electrophoresed on SDS-10% polyacrylamide gels. Immunoblot was developed with anti-human UGT1*6 and anti-rabbit P4501A1 antibodies. (A) 1, V79UGT1*6; 2, untreated; 3, 0.2% (v/v) DMSO-treated cells; 4, 100 μM β-NF-treated cells; 5, 100 μM β-NF + 25 μM α-NF-treated cells; 6, 100 μM CLO-treated cells; 7, 100 μM RIF-treated cells. (B) 1, untreated; 2, 0.2% (v/v) DMSO-treated cells; 3, 50 μM β-NF-treated cells; 4, 50 μM β-NF + 25 μM α-NF-treated cells; 5, 100 μM β-NF-treated cells; 6, 100 μM CLO-treated cells; 7, 100 μM RIF-treated cells.

The effects of β-NF, RIF and clofibrate on the expression of UGT1*6 were investigated with antibodies directed against the human UGT1*6 [14]. This UGT isoform appeared to be constitutively expressed in Caco-2 cells and was inducible by β-NF as shown in Fig. 2A. However, RIF and clofibrate had no effect on the expression of UGT1*6. The inducibility of human UGT1*6 in Caco-2 cells has never been reported. The mechanism by which β-NF acts on the expression of UGT1*6 in human cells is not known. β-NF is an *Ah* receptor ligand inducing the gene expression of both *CYP1A1*, an effect antagonized by α-NF [19]. Santostefano *et al.* [19] have demonstrated that α-NF exhibits not only an antagonist activity via competition for receptor binding sites, but always expresses an agonistic property which results in the induction of CYP1A1 mRNA. In our study, β-NF induced UGT1*6 expression, whereas α-NF had no effect on β-NF induction. These data would suggest that the induction of UGT1*6 was mediated by the *Ah* receptor but the α-NF concentration (25 μM) used in this work was not efficacious enough to exert its antagonist property.

The effects of these inducers on EROD activity in Caco-2 cells were also investigated. As shown in figure 1B, this activity was tremendously increased (1113- and 796-fold) with 50 and 100 μM β-NF, respectively. The α-NF treatment partially abolished the inducing effect of β-NF by a factor of 9. This activity was also increased in Caco-2 cells by rifampicin by a factor of 5. Boulenc *et al.* [20] have demonstrated that EROD activity was supported only by P4501A1 in Caco-2 cells and this P450 isozyme was specifically induced by β-NF by a factor of 18. They also showed that the inducing effect of β-NF on EROD was completely abolished by α-NF. In our study, the induction factors were very high and may explain why α-NF could not completely antagonise the effect of β-NF. However, the reason for this discrepancy between the inducing factors is not clear, although it should be noted that our culture conditions differed from those previously reported [20]. Western blot analysis of P4501A1 expression in Caco-2 cells performed with polyclonal anti-rabbit P4501A1 is illustrated in Fig. 2B. P4501A1 was specifically induced by β-NF and not by clofibrate or RIF. These results are in agreement with those previously reported by Boulenc *et al.* [20]. Interestingly, EROD and 1-naphthol activities, as well as the expression of P4501A1 and UGT1*6, were co-induced by β-NF at the same concentrations. These results could suggest that UGT1*6 and P4501A1 are co-regulated by β-NF.

In summary, we have shown that Caco-2 cells are able to glucuronidate various aglycones, including xenobiotics, steroid hormones, bile acids and drugs, which are substrates of different UGT isoforms, and that the activity toward 1-naphthol was the highest. We also showed that UGT1*6, a 1-naphthol conjugating UGT isoform, was specifically induced by β-NF. Furthermore, this UGT isoform and P4501A1 are concomitantly increased by β-NF. The Caco-2 cell line can be a useful intestinal model for drug glucuronidation and UGT regulation studies.

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REFERENCES

1. Burchell B, Nebert DW, Nelson DR, Bock KW, Iyannagi T, Jansen PLM, Lancet D, Mulder GJ, Chowdhury JR, Siest G, Tephly TR and Mackenzie PI, The UDP glucuronosyltransferase gene superfamily: suggested nomenclature based on evolutionary divergence. *DNA Cell Biol* **10**: 487–494, 1991.
2. Bock KW, Forster A, Gschaidmeier H, Brück M, Münzel P, Schareck W, Fournel-Gigleux S and Burchell B, Paracetamol glucuronidation by recombinant rat and human phenol UDP-glucuronosyltransferases. *Biochem Pharmacol* **45**: 1809–1814, 1993.
3. Mulder GJ, Glucuronidation and its role in regulation of biological activity of drugs. *Ann Rev Pharmacol Toxicol* **32**: 25–49, 1992.
4. Koster ASj, Meewisse CPJ and Noordhoek J, Glucuronidation in rat intestinal cells. Dependence of glucose supply and resistance to inhibition by ethanol and fasting. *Arch Toxicol* **55**: 123–126, 1984.
5. Fogh J, Fogh JM and Orfeo T, 127 Cultured human colon cell lines producing tumors in nude mice. *J Natl Acad Sci USA* **59**: 221–226, 1977.
6. Pinto M, Robine-Leon S, Appay MD, Kedinger M, Triadou N, Dussaulx E, Lacroix B, Simon-Assmann P, Haffen K, Fogh J and Zweibaum A, Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. *Biol Cell* **47**: 323–330, 1983.
7. Abid A, Sabolovic N, Batt AM, Siest G, Maurel P, Guillemin F and Magdalou J, Induction of UDP-glucuronosyltransferases and cytochrome P450 in human hepatocytes. *Cell Pharmacol* **1**: 257–262, 1994.
8. Dragacci S, Thomassin J, Magdalou J, Souhaili-El-Amri H, Boissel P and Siest G, Properties of human hepatic UDP-glucuronosyltransferases: relationship to the other inducible enzymes in patients with cholestasis. *Eur J Clin Pharmacol* **32**: 485–491, 1987.
9. Bradford M, A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
10. Pritchard M, Fournel-Gigleux S, Siest G, Makenzie P and Magdalou J, A recombinant phenobarbital-inducible rat liver UDP-glucuronosyltransferase (UGT2B1) stably expressed in V79 cell catalyzes the glucuronidation of morphine, phenols and carboxylic acids. *Molec Pharmacol* **45**: 42–52, 1994.
11. Otani G, Abou-El-Makarem MM and Bock KW, UDP-glucuronosyltransferase in perfused rat and in microsomes. III. Effects of galactosamine and carbon tetrachloride on the glucuronidation of 1-naphthol and bilirubin. *Biochem Pharmacol* **25**: 1293–1297, 1976.
12. Burke MD, Thompson S, Elcombe CR, Halpert J, Haaparanta T and Mayer RT, Ethoxy-, pentoxy- and benzoxyphenoxazone and homologues: a series of substrates to distinguish between different induced cytochrome P-450. *Biochem Pharmacol* **34**: 3337–3345, 1985.
13. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**: 680–685, 1970.
14. Ouzzine M, Pillot T, Fournel-Gigleux S, Magdalou J, Burchell B and Siest G, Expression and role of the human liver UDP-glucuronosyltransferase UGT1*6 analysed by specific antibodies raised against a hybrid protein produced in *Escherichia coli*. *Arch Biochem Biophys* **310**: 196–204, 1994.
15. Fournel-Gigleux S, Sutherland L, Sabolovic N, Burchell B and Siest G, Stable expression of two human UDP-glucuronosyltransferases cDNAs in V79 cell cultures. *Molec Pharmacol* **39**: 177–183, 1991.
16. Daujat M, Pichard L, Dalet C, Larroque C, Bonfils C, Pompon D, Li D, Guzelian PS and Maurel P, Expression of five forms of microsomal cytochrome P-450 in primary culture of rabbit hepatocytes treated with various classes of inducers. *Biochem Pharmacol* **36**: 3597–3606, 1987.
17. Jumarie C and Malo C, Caco-2 cells cultured in serum-

free medium as a model for study of enterocytic differentiation *in vitro*. *J Cell Physiol* **149**: 24–33, 1991.
18. Ebner T and Burchell B, Substrate specificities of two stably expressed human liver UDP-glucuronosyltransferases of the UGT1 gene family. *Drug Metab Dispos* **21**: 50–55, 1993.
19. Santostefano M, Merchant M, Arellano L, Morrison V, Denison MS and Safe S, α-naphthoflavone-induced CYP1A1 gene expression and cytosolic aryl hydrocarbon receptor transformation. *Molec Pharmacol* **43**: 200–206, 1993.
20. Boulenc X, Bourrie M, Fabre I, Roque C, Joyeux H, Berger Y and Fabre G, Regulation of cytochrome P4501A1 gene expression in a human intestinal cell line, Caco-2. *J Pharmacol Exper Ther* **263**: 1471–1478, 1992.