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An expressional system of human cytochrome P-450 CYP1A1 gene transcription

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KEY WORDS cytochrome P-450 CYP1A1; transfection; hepatoblastoma; *Brassica*; chloramphenicol acetyltransferase; glucosinolates; flavones; plasmids

AIM: To explore an expressional system of human cytochrome P-450 CYP1A1 (CYP1A1) gene transcription. **METHODS**: The plasmid pMC 6.3 K containing human CYP1A1 promoter was transiently transfected into Hep G2 cells. The expression of chloramphenical acetyltransferase (CAT) reporter gene was detected by ELISA. **RESULTS**: Both the CAT expression and CYP1A1 activity increased with the concentrations of β-naphthoflavone from 2.5 to 10 $\mu mol \cdot L^{-1}$. At 10 $\mu mol \cdot L^{-1}$ of β-naphthoflavone, the levels of CAT and CYP1A1 were 94-fold and 2.8-fold those of the corresponding control, respectively. Using this method, the study of 8 glucosinolates with various side chains on the induction of CYP1A1 gene transcription showed that none of the parent glucosinolates increased CAT expression, whereas the breakdown products of indol-3-yl-methyl glucosinolate (glucobrassicin), rather than indole-3-carbinol, increased the CAT expression. **CONCLUSION**: The CYP1A1 gene transcriptional system was more reliable and sensitive.

Cytochrome P-450 1A1 (CYP1A1) may serve as a potential indicator of carcinigenesis[1] and is a risk factor for lung cancer in smokers[2]. β-Naphthoflavon (β-NF) is an effective inducer of CYP1A1[3]. Human hepatoblastoma Hep G2 cells have been used in the study of drug-metabolism as model cells[4]. *Brassica* vegetables in the diet showed protective effects against carcinogenesis. This study was to establish an expressional system of human CYP1A1 gene transcription in Hep G2 cells to detect the effect of glucosinolates, the main bioactive components of *Brassica* vegetables on human CYP1A1.

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MATERIALS AND METHODS

Chemicals β-NF, indole-3-carbinol (I3C), NADPH, 7-ethoxyresorufin, resorufin, Me_2SO, and bovine serum albumin (BSA) were purchased from Sigma Co. β-NF, 7-ethoxyresorufin, and I3C were dissolved in Me_2SO just prior to use. Resorufin was dissolved in ethanol.

Plasmids CYP1A1 gene transcriptional expression was examined using a plasmid (pMC 6.3 K) containing a functional CYP1A1 promoter (the fragment spanning the BamHI site, 6.3 kb from the cap site to the Sau3A1 site (+2566), adjacent to the initiation codon) coupled to a reporter gene (chloramphenicol acetyltransferase, CAT) (Fig 1). This plasmid was kindly supplied by Dr Fujii-Kurigama Y of Tohoku University, Japan.

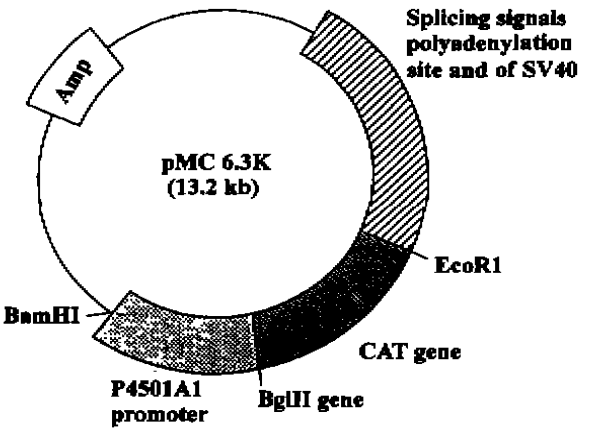

Fig 1. pMC 6.3 K

Transformation of pMC 6.3 K to the competent cells, HB 101, was performed using the routine method. The single HB 101 cell with the aim plasmid, pMC 6.3 K, was inoculated to LB medium with ampicillin and streptomycin for

overnight. The amplified plasmid DNA was isolated from HB 101 cells using Qiagen-tip 2500 kit supplied by Qiagen Co, UK.

Another plasmid, pRSV, containing the β-galactosidase gene was generously donated by Dr Siegmund WOLF, Institute of Food Research, UK. The pRSV as well as pMC 6.3 K was transfected to Hep G2 cells.

Cell culture and transfection Human Hep G2 cells supplied by The European Cell Culture Collection were grown in the Eagle's medium containing nonessential amino acid and 10 % fetal calf serum, and maintained in a humidified atmosphere of 5 % CO_2 at 37 ℃. Transfection was performed by the calcium phosphate method[5]. In brief, the cells were replanted at a density of $2 \times 10^5/cm^2$ in a 10-cm plate. After 24 h, the calcium phosphate-DNA suspension containing 15 μg of pMC 6.3 K and 15 μg of cotransfection pRSV was added to cells in 10 mL of culture medium. After 4.5 h, the cells were shocked with 15 % glycerol for 2.5 min and trypsinized. Transfection was normalized relative to a co-transfection of β-galactosidase. The transfected cells were distributed into 15 wells in a 24-well plate and treated with the tested compounds for 24 h. Four replicates were used. The cells were washed thrice with 1 mL of PBS and hydrolyzed with detergents and PBS buffer (Mg^{2+} and Ca^{2+} free) at 25 ℃ for 30 min. The cell extracts were centrifuged at 10 000 × *g* at 4 ℃ for 15 min. The supernatants were used in the assay of CAT and β-galactosidase.

Detection of reporter gene expression The CAT expression was detected using an enzyme-linked immunosorbent assay (ELISA) method. The CAT ELISA supplied by Boehringer Mannheim Co was based on the sandwich-enzyme-immunoassay principle and antibodies to CAT (anti-CAT) were prebound to the surface of the microtiter plate modules. The peroxidase substrate 2,2′- amino-di-(3-ethyl-benzthiazoline sulforate) was finally added and absorbances were read at 410 nm against a standard curve (0 – 200 pg/well). Results were normalized to the expression of β-galactosidase. β-Galactosidase assay was performed[6] and the β-galactosidase enzyme assay system with Reporter Lysis Buffer kit was obtained from Promega Co. *O*-Nitrophenyl-β-*D*-galactopyranoside (ONPG) was used as the substrate of β-galactosidase. The extracted protein was determined by the Bradford method using reagents from Bio-Rad Laboratory and BSA as standard.

Assay of CYP1A1 activity CYP1A1 activity was detected using the modified fluorescence method[4] in the untransfected Hep G2 cells. The reaction mixture (0.5 mL final volume) included sodium phosphate buffer (pH 7.6) 0.1 mol·L^{-1}, NADPH 300 μmol·L^{-1}, 7-ethoxyresorufin 5 μmol·L^{-1} and cell extract (80 – 20 μg protein). Five replicates were used. The reaction was initiated by NADPH and stopped by the addition of ice-cold acetone 0.75 mL at 30 min. Fluorescence was read at λ_{em} 586 nm and λ_{ex} 576 nm against a standard of resorufin.

Glucosinolates The generic names, side chains, and final concentrations of 8 glucosinolates and their products used in this study were shown in Tab 1. Glucosinolates, with the exception of sinigrin, were purified from plant sources[7]. Concentration of the products was based on that of the parent compound before myrosinase (pH 6.6 for 1 h) treatment[7].

Tab 1. Glucosinolates and their breakdown products used for studying the induction of CYP1A1 gene transcription.

Generic name	Side chain	Final concentration/μmol·L^{-1}	
		No myrosinase	Myrosinase
Sinigrin	(1)	5, 10, 15	1, 5, 10
Gluconapin	(2)	5, 10, 15	1, 5, 10
Glucoiberin	(3)	5, 10, 15	1, 5, 10
Glucobrassicin	(4)	5, 10, 15	5, 7.5, 10
Progoitrin	(5)	5, 10, 15	1, 5, 10
Glucosinalbin	(6)	5, 10, 15	1, 5, 10
Glucotropaeolin	(7)	1.7, 3.4, 5	1, 5, 10
Gluconasturtiin	(8)	1.7, 3.4, 5	1, 5, 10

(1) allyl/prop-2-enyl; (2) but-3-enyl; (3) 3-methylsulphinylpropyl; (4) indol-3-yl-methyl; (5) 2-hydroxy-but-3-enyl; (6) *p*-hydroxybenzyl; (7) benzyl; (8) 2-phenylethyl.

RESULTS

Induction of human CYP1A1 gene transcription and enzyme activity by β-NF The CAT expression in the transfected cells and the CYP1A1 activity in the untransfected cells were markedly enhanced ($P < 0.01$) at 2.5 μmol·L^{-1} of β-NF. The CAT responses were

increased with β-NF concentration in the range of 2.5 – 10 μmol · L^{-1}. However, the CYP1A1 activity had the maximal levels in the range of 2.5 – 10 μmol · L^{-1} of β-NF. These results sufficiently demonstrated the concentration-dependent induction of β-NF on human CYP1A1 gene transcription and enzyme activity (Fig 2).

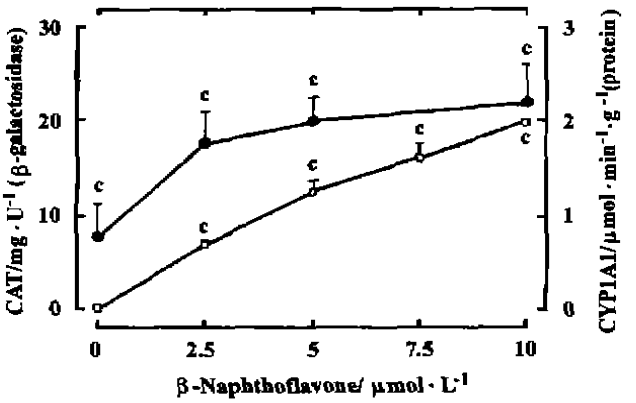

Fig 2. Effects of β-naphthoflavone on CYP1A1 gene transcription (○) and CYP1A1 activity (●) in Hep G2 cells. $n = 4$ for CAT or 5 for CYP1A1, $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

Effects of glucosinolates and their breakdown products on human CYP1A1 gene transcription Eight glucosinolates with various side chains on CAT expression were tested at the concentration of 1 – 15 μmol · L^{-1} in the system (Tab 1). None of the parent glucosinolate induced CAT expression. When the glucosinolates were hydrolyzed with myrosinase, only the breakdown products of indole-3-yl-methyl glucosinolate (glucobrasscin) increased CAT expression with the maximal induction (28-fold) at 7.5 μmol · L^{-1}. Meanwhile, a control incubation with myrosinase and without glucosinolate showed no induction. I3C, one of the breakdown products of indole-3-yl-methyl glucosinolate, did not induce CAT expression up to 10 μmol · L^{-1} (Fig 3).

These results showed that the breakdown products of indole-3-yl-methyl glucosinolate rather than I3C were responsible for the induction of CYP1A1 gene transcription.

DISCUSSION

Compared with CYP1A2, CYP1A1 is mainly distributed in extrahepatic tissues. But

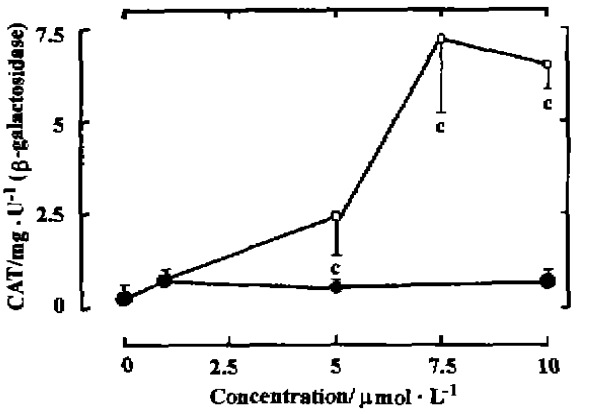

Fig 3. Effect of glucobrassicin breakdown products (○) and indole-3-carbinol (●) on CYP1A1 gene transcription in Hep G2 cells. $n = 4$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

CYP1A1 is highly inducible in liver (200-fold by dioxin in rats[8]). More and more evidences showed that CYP1A1 was implicated in carcinogen activation. The increases of CYP1A1 and P-450 reductase level accelerated the formation of DNA adducts with benzo(α)pyrene[9] and CYP1A1 was also a catalyst for the activation of aflatoxin B_1 to a DNA-binding species[10]. In this study, we established the expressional system of human CYP1A1 gene transcription using transient transfection and CAT ELISA technique. It offers a new and valuable means for studying drug-metabolism (including carcinogens and mutagens), evaluating the risk of carcinogens, and determining the active component of carcinogens.

The reliability of the system was studied by proving the induction of β-NF. The induction of β-NF had been demonstrated on the concentration- and time-effect relationship[11] study of CYP1A1 gene transcription, and supported further on the study of CYP1A1 enzyme activity. In our system, transfection of CYP1A1 promoter into Hep G2 cells got rid of the interference from endogenous CYP1A1 gene. Meanwhile, compared with the common method of detecting CAT activity, thin layer chromatography and radioactive CAT assay, the CAT ELISA technique have the advantages of being safer (no radioactivity), faster (approximately 4 h), more sensitive (0.1 pg CAT/well) and more accurate to quantify.

The induction of glucobrassicin breakdown

products on CYP1A1 gene transcription was also demonstrated in the system. The probable effective products are thiocyanate ions, indol-3-yl-acetonitrile and 3, 3′-diindolylmethane[12,13]. The latter 2 compounds proved to be potent inducers of CYP1A1 in rats[13].

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人细胞色素 P-450 CYP1A1 基因转录表达系统

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关键词 细胞色素 P-450 CYP1A1; 转染; 肝胚细胞瘤; 芥属; 氯霉素乙酰基转移酶类; 芥子油苷类; 黄酮类; 质粒

目的: 在人 Hep G2 细胞系上, 建立人细胞色素 P-450 CYP1A1 (CYP1A1) 基因转录的表达系统. **方法**: 瞬时转染含人 CYP1A1 启动子的质粒(pMC 6.3 K)、ELISA 法测定报道基因氯霉素乙酰转移酶(CAT)的含量和酶学测定 CYP1A1 活性. **结果**: β-萘黄酮 2.5 μmol·L^{-1}明显增强 CAT 表达和 CYP1A1 活性($P < 0.01$); 在 2.5 – 10 μmol·L^{-1}范围内, CAT 表达随浓度增高而不断增强, 而 CYP1A1 活性则接近最高水平; 10 μmol·L^{-1}时它们的作用强度分别为对照组的 94.3 和 2.8 倍. 用这种方法对八种含不同侧链的芥子油苷进行检测的结果表明, 芸苔苷的水解产物(而非吲哚-3-原醇)诱导 CYP1A1 基因表达. **结论**: CYP1A1 基因转录表达系统具有较高的可靠性和灵敏度.