Research Section

Induction of cytochromes P450 1A1 and 1B1 in human lung adenocarcinoma CL5 cells by frying-meat emission particulate

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Abstract

The effect of airborne frying-meat emission particulate (FMEP) on cytochrome P450 (P450)-dependent monooxygenase was determined using human lung adenocarcinoma cell line CL5 treated with organic extract of FMEP prepared from beef, fish or pork. Treatment with fish FMEP extract caused greater increases of intracellular peroxide production and glutathione content than did beef and pork FMEP extracts. Treatment with 200 µg/ml beef, fish or pork FMEP extract for 6 h increased benzo[*a*]pyrene hydroxylase, 7-ethoxyresorufin and methoxyresorufin *O*-dealkylases activities in S9. Immunoblot analysis of S9 proteins from control cells and cells treated with FMEP extracts revealed that the airborne particulates increased proteins immunorelated to CYP1A1 and CYP1B1. Northern blot analysis of total cellular RNA from controls and cells treated with FMEP extracts showed that the cooking by-products increased the levels of CYP1A1 and CYP1B1 mRNA. Treatment with 1 µM dibenzo[*a,h*]anthracene for 6 h increased monooxygenase activities, CYP1A1 and CYP1B1 protein and mRNA levels in CL5 cells. Beef FMEP extract and dibenzo[*a,h*]anthracene also induced CYP1A1 and CYP1B1 in human lung carcinoma NCI-H322 cells. The present finding demonstrates that airborne particulates generated during the frying of beef, fish and pork can induce carcinogen-metabolizing CYP1A1 and CYP1B1 in the human lung-derived cell line CL5. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Frying-meat emission particulate; CYP1A1; CYP1B1; Induction

1. Introduction

Cytochrome P450 (P450)-dependent monooxygenases are responsible for the oxidative and reductive metabolism of many drugs, carcinogens, steroid hormones and fatty acids. Among the P450 enzymes, CYP1A1 and CYP1B1 are involved in metabolic activation of poly-

cyclic aromatic hydrocarbons (PAHs) (Guengerich and Shimada, 1998). Frying of food can produce a variety of toxic chemicals in the airborne emission particulates (Lofroth, 1994). For example, frying-meat emission particulate (FMEP) from fish contained the PAH carcinogens benzo[*b*]fluoranthene, benzo[*a*]pyrene, benzo[*g,h,i*]perylene, benzo[*a*]anthracene and benzo[*k*]fluoranthene (Yang et al., 2000). Carcinogenic heterocyclic amines (HCAs) were present in FMEPs from frying beef patties, fish and bacon strips (Thiebaut et al., 1995; Yang et al., 1998).

The incidence of lung cancer in Chinese women is among the highest in the world. The cigarette smoking rate in Chinese women is low, compared to the rates in European and North American females, and may not be a major risk factor of lung cancer in these non-smoking Chinese women (Gao et al., 1987; Koo and Ho, 1990). Consequently, there is increasing interest in identifying other potential risk factors. Exposure to fumes emitted from heated cooking oils was associated with an

Abbreviations: AHR, aryl hydrocarbon receptor; DB[*a,h*]A, dibenzo[*a,h*]anthracene; DCFH-DA, dichlorofluorescein diacetate; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; DMSO, dimethyl sulfoxide; FMEP, frying-meat emission particulate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GC, gas chromatography; GC/MS, gas chromatography/mass spectrometry; HCAs, heterocyclic amines; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; P450, cytochrome P450; PAHs, polycyclic aromatic hydrocarbons; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.

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increased risk to lung cancer in women non-smokers in Taiwan, where lung cancer is the leading cause of cancer death (Ko et al., 2000). Treatment with fish FMEP extract resulted in the formation of benzo[*a*]pyrene 7,8-diol 9,10-epoxide *N*-deoxyguanosine adduct in human lung adenocarcinoma CL3 cells (Yang et al., 2000). These studies suggest a possible etiological role of exposure to cooking fumes in female lung cancer.

The ability of airborne cooking by-products to modulate carcinogen-metabolizing P450 enzymes has not been reported. Lung is a portal of entry and a possible target organ of airborne FMEPs. Modulation of pulmonary carcinogen-metabolizing enzymes by these airborne cooking by-products may be a major determinant of the toxicological fate of the carcinogenic PAHs and other chemicals produced during frying. The present study was carried out aiming to determine the inductive effects of FMEP on human pulmonary CYP1A1 and CYP1B1 using the cell line CL5 derived from a female human lung adenocarcinoma, which is the most common cell type of lung cancer in women and non-smokers (Valaitis et al., 1981; Gao et al., 1987). The induction study was extended to human lung carcinoma NCI-H322 cells.

2. Materials and methods

2.1. Cooking and FMEP collection

Commercial beef and pork slices measuring approximately 8×12×0.2 cm and pomfret 10×20×2 cm were used. 50–100 ml of soybean oil was preheated in a 30-cm diameter wok to 180 °C using a gas stove inside a chemical fumes hood. 150–200 g of beef or pork were placed in the preheated oil and stir-fried for 5–10 min. Fish weighing 100–250 g was fried for 5 min per side, twice each side. During the cooking of the food samples, the oil temperature in the wok varied from 130 °C to 220 °C. The fume produced by cooking was collected using a high volume sampler at a flow rate of 0.2 m³/min. An aluminium sampling module (Model GPS1–1, GRASEBY GMW, Village of Cleves, OH, USA), mounted on a kitchen ventilator hood, was placed 50 cm above the wok surface. The sampling module consisted of a chamber supporting a circular filter holder for high-temperature resistant 0.5 µm quartz filter and a chamber encapsulating a glass cartridge for polyurethane foam. FMEP was collected on the quartz filter. The filter was weighed before and after sampling. The following procedures were carried out in the dark. Five filters were cut into 1-cm squares, extracted three times using acetone in a sonicated water-bath. The organic extract of FMEP was filtered through Whatman No. 1 filter paper. The filtrate was concentrated and evaporated to dryness using a rotary evaporator. The FMEP extract was weighed and stored at –20 °C until analysis.

2.2. Gas chromatography/mass spectrometry analysis of FMEP extract

Chemical analysis of FMEP extract was carried out using a Hewlett-Packard model 6890 gas chromatography (GC) and a 5973 mass spectrometer with an HP-5MS capillary column (30 m×0.25 mm). Helium was used as the GC carrier gas. Following sample injection, the GC column was held at 40 °C for 3 min, the temperature programmed to 295 °C at 10 °C/min, and held at 295 °C for 22 min. The total run time was 35 min with a flow rate of 1.4 ml/min. The mass spectrometer was operated under 280 °C detector temperature at 70 eV in the full scan and selected ion modes for qualitative and quantitative analyses.

2.3. Cells and treatments

The human lung cancer cell line CL5 was derived from a lung adenocarcinoma tumor specimen of a 40-year-old woman patient at the Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan. The cell line was single-cell cloned and maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO₂. The human lung carcinoma cell line NCI-H322 was purchased from American Type Culture Collection (Rockville, MD, USA) and maintained in the same medium. The cells were used when the monolayer had reached near confluence. FMEP extract was dissolved in dimethyl sulfoxide (DMSO) and added to the medium so that the DMSO concentration in the medium was less than 0.1%. Cell viability was determined using the colorimetric MTT method of Carmichael et al. (1987). Peroxide formation was determined using the oxidation-sensitive probe 2',7'-dichlorofluorescein diacetate (DCFH-DA) (Lebel et al., 1992) as described previously (Ueng et al., 2000). Total cellular glutathione content was determined with the enzymatic-recycling assay based on glutathione reductase following the method of Tietze (1969).

2.4. S9 fraction preparation and enzyme assays

Cells were harvested by scraping, and washed in a phosphate buffered saline solution. The following procedures were carried out at 4 °C. Cell suspension was centrifuged at 1500 rpm for 3 min, the cell pellet was washed and sonicated in 0.1 M potassium phosphate buffer, pH 7.4. Cell homogenate was centrifuged at 9000 g for 20 min and the resulting supernatant, S9 fraction, was stored at –70 °C prior to monooxygenase and immunoblot analyses. Benzo[*a*]pyrene hydroxylase (Nebert and Gelboin, 1968), 7-ethoxyresorufin *O*-deethylase (Pohl and Fouts, 1980), methoxyresorufin *O*-demethylase (Mayer et al., 1977), NADPH-cytochrome P450 reductase (Phillips and Langdon, 1962), glutathione *S*-transferase (Habig et al.,

1974) and *N*-acetyltransferase (Andres et al., 1985) activities were determined as described previously. All the enzyme assays were carried out under linear conditions with respect to time and protein concentration. Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

2.5. Gel electrophoresis and immunoblotting

S9 proteins were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting procedures as described elsewhere (Kang et al., 1997). Immunodetection of CYP1A1 was carried out using a mouse monoclonal antibody (MAB) 1–12–3 raised against rat CYP1A1 (Park et al., 1986), kindly provided by Dr Sang S. Park, Occupational Diseases Diagnosis and Research Center, Industrial Research Institute, KISCO, Inchon, Korea. Immunodetection of CYP1B1 was carried out using a rabbit polyclonal antibody prepared against a peptide corresponding to a putative surface loop region on the human CYP1B1 protein (Gentest Corporation, Woburn, MA, USA). The blot was washed and developed using an Amersham ECL detection system. β -Actin was used as an internal control for the amount of protein. Intensities of the immunoreactive bands were determined using a digital imaging system as described previously (Ueng et al., 2000).

2.6. Total RNA isolation and RNA blotting

Total RNA was isolated from CL5 and NCI-H322 cells following the method of Chomczynski and Sacchi (1987). Denatured RNA was subjected to RNA blot analysis using a CYP1A1 cDNA prepared from a human CYP1A1 3'-end cDNA clone (pHP1-450–3') (Jaiswal et al., 1985) as described previously (Ueng et al., 2000). A 360 bp RT-PCR product specific for CYP1B1 was prepared for the detection of human CYP1B1 mRNA, as described by Dohr et al. (1995). The CYP1A1 and CYP1B1 cDNA probes were 32 P-labeled using a commercial random primers DNA labeling system (Gibco/BRL Life Technologies, Inc., Gaithersburg, MD, USA). Following prehybridization, the membrane was reacted with the 32 P-labeled P450 probes, washed successively, and then subjected to autoradiography. The blot was deprobed and hybridized to a rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA probe, as an internal control for the amount of RNA. The intensities of RNA were quantified with the aid of a digital imaging analysis system.

2.7. Statistical analysis

The statistical significance of difference between control and treated cells was evaluated by the Student's *t*-test. A *P* value <0.05 was considered statistically significant.

3. Results

The concentrations of FMEP in fumes generated from the stir-frying of beef and pork were about two- and three-fold higher than the concentration of FMEP from frying fish, respectively (Table 1). The yields of beef and pork FMEPs and their organic extracts were four- and three-fold higher than the respective yields of fish FMEP. These yields were similar to those of FMEPs from frying hamburger, herring and pork (Lofroth et al., 1991).

GC/MS analysis of FMEP extracts showed the presence of many organic compounds including cholesterol, squalene, phytosterols, unsaturated fatty acids and antioxidant γ -tocopherol (Table 2). In fish FMEP, the total content of these organic compounds was 25-fold higher than the total content of PAHs calculated from the report of Yang et al. (2000). In beef FMEP, the total content of organic compounds was about 60,000-fold higher than the total content of HCAs reported previously (Thiebaud et al., 1995).

Cytotoxic effects of FMEP extracts were determined by measuring cell viability, peroxide production, and glutathione content of CL5 cells following treatment with 200 μ g/ml FMEP extract for 2 and 24 h. Additional CL5 cells were treated with 1 μ M dibenzo[*a,h*]anthracene (DB[*a,h*]A) for comparison purposes. Beef and pork FMEP extracts caused 17 and 22% decreases at 2 h and 20 and 27% decreases at 24 h in cell viability, respectively (Table 3). The fish extract had no significant effect on cell viability at 2 h, but produced a 25% decrease at 24 h. Beef, fish and pork FMEP extracts caused 27, 59 and 18% increases of intracellular peroxide production at 2 h, respectively. The beef and pork extracts, but not the fish extract, resulted in 26% increases of peroxide production at 24 h. Fish FMEP extract produced a two-fold increase of glutathione content at 24 h, and in contrast the pork extract caused an 11% decrease at 2 h. Beef FMEP extract had no effect on glutathione content. DB[*a,h*]A did not alter cell viability, peroxide production or glutathione content, except that PAH caused an 11% decrease of cell viability at 24 h.

Treatment of CL5 cells with 100 μ g/ml fish FMEP extract for 6 h had no effect on 7-ethoxyresorufin *O*-deethylase activity in S9. Treatment with 200 μ g/ml fish FMEP extract for 1, 3, 6, 12 and 24 h caused no change, two-, five-, three- and two-fold increases of 7-ethoxyresorufin *O*-deethylase activity, respectively (data not shown). Based on these concentration- and time-dependent increases, CL5 cells were treated with 200 μ g/ml FMEP extract for 6 h in the following induction studies. Beef and fish FMEP extracts caused three-fold increases of benzo[*a*]pyrene hydroxylase activity in S9 (Table 4). Pork FMEP extract had no effect on benzo[*a*]pyrene hydroxylase activity. The beef, fish and pork extracts

Table 1
Preparation of FMEP extracts

	Beef	Fish	Pork
Concentration of FMEP in fume (mg/m ³)	27±3	12±1	32±5
Yield of FMEP (g/kg meat)	0.639±0.060	0.180±0.012	0.432±0.050
Yield of organic extract of FMEP (g/kg meat)	0.596±0.056	0.166±0.012	0.398±0.046

100–250 g beef, fish or pork sample was fried in preheated soybean oil in wok as described in Materials and methods. Frying-meat emission particulate (FMEP) was collected using 0.5-µm quartz filters in a high air volume sampler. Filters were pooled and extracted using acetone. The organic extract of FMEP was concentrated and evaporated to dryness. Each value represents mean±S.E.M. for at least five preparations.

produced four-, five- and four-fold increases of 7-ethoxyresorufin *O*-deethylase and 10-, 18- and five-fold increases of methoxyresorufin *O*-demethylase activities, respectively. Treatment of CL5 cells with 1 µM DB[*a,h*]A

Table 2
Chemical analysis of FMEP

Compound	Beef	Fish	Pork
	ng/g meat		
Organic			
Cholesterol	2736±74	2505±154	2565±166
Squalene	1091±76	645±245	413±65
β-Sitosterol	1019±292	822±371	532±9
Campesterol	607±231	516±214	292±33
Stigmasterol	396±240	348±191	256±21
Hexadecanoic acid	4009±659	1575±820	978±519
Oleic acid	2605±556	603±195	521±231
Octadecanoic acid	2591±130	1003±268	1272±161
9,12-Octadecadienoic acid	2144±352	638±316	451±131
γ-Tocopherol	481±47	230±129	272±174
Total	17679	8885	7552
PAH			
Benzo[<i>b</i>]fluoranthene	n.a. ^a	96.3±23.3	n.a.
Fluoranthene	n.a.	86.3±11.3	n.a.
Benzo[<i>a</i>]pyrene	n.a.	62.0±0.3	n.a.
Benzo[<i>g,h,i</i>]perylene	n.a.	70.7±14.3	n.a.
Benzo[<i>a</i>]anthracene	n.a.	24.3±2.7	n.a.
Benzo[<i>k</i>]fluoranthene	n.a.	19.7±0.3	n.a.
Total		359.3	
HCA			
PhIP	0.14±0.00	n.a.	1.00±0.20
MeIQx	0.14±0.01	2.5	n.d. ^b
DiMeIQx	0.01±0.00	n.a.	n.d.
Total	0.29		1.00

GC/MS analysis of organic compounds of beef, fish and pork FMEP extracts was carried out as described in Materials and methods. The contents of these compounds represent the average of three experiments. The data of HPLC analysis of PAHs of fish FMEP were taken from a previous study of Yang et al. (2000). Same fish pomfret was used and cooked under similar conditions in the present and previous studies. The contents of the HCAs 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx) in beef and pork FMEPs were the results of HPLC analysis of the particulates from frying beef patties and bacon strips, respectively (Thiebaut et al., 1995). The value of MeIQx in fish FMEP was estimated using the Ames mutagenicity assay (Yang et al., 1998).

^a n.a.: not available.

^b n.d.: not detectable, i.e. <0.1 ng/g (Thiebaut et al., 1995).

for 6 h caused four-, 10- and 13-fold increases of benzo[*a*]pyrene hydroxylase, 7-ethoxyresorufin and methoxyresorufin *O*-dealkylases activities, respectively. FMEP extracts and DB[*a,h*]A had no effects on NADPH-cytochrome P450 reductase, *N*-acetyltransferase or glutathione *S*-transferase activities.

The catalytic activity data showed that treatment of CL5 cells with FMEP extract increased P450-dependent monooxygenase activities. To investigate this inductive effect further, S9 proteins from control CL5 cells and cells treated with FMEP extract or DB[*a,h*]A were subjected to gel electrophoresis and immunoblotting studies using antibodies to CYP1A1 and CYP1B1 (Plate 1), and the intensities of the protein bands were quantitated (Table 5). Immunoblot analysis using mouse MAb 1–12–3 to rat CYP1A1 (Park et al., 1986) revealed that

Table 3
Effects of FMEP extracts and dibenzo[*a,h*]anthracene on cell viability, peroxide production and glutathione content in human lung adenocarcinoma CL5 cells

	Control	FMEP extract			DB[<i>a,h</i>]A
		Beef	Fish	Pork	
	(% of control)				
Cell viability					
2 h	100±4	83±8 ^a	90±4	78±11 ^a	97±4
24 h	100±5	80±3 ^a	75±5 ^a	73±7 ^a	89±3 ^a
Peroxide production					
2 h	100±4	127±2 ^a	159±1 ^a	118±2 ^a	95±4
24 h	100±2	126±3 ^a	90±4	126±2 ^a	102±1
Glutathione content					
2 h	100±2	99±2	95±1	89±1 ^a	103±2
24 h	100±5	110±8	212±6 ^a	116±4	117±3

CL5 cells were treated with 200 µg/ml FMEP extract from beef, fish, or pork or 1 µM dibenzo[*a,h*]anthracene (DB[*a,h*]A) for 2 and 24 h. Control cells were treated with DMSO only. Cell viability was determined using the MTT method of Carmichael et al. (1987). Intracellular production of peroxide was analyzed using the oxidation-sensitive dye DCFH-DA (Lebel et al., 1992). Glutathione content of cell homogenate was determined following the enzyme recycling method of Tietze (1969). Glutathione contents of control cells were 34.9±0.5 and 41.3±2.1 nmol/mg protein at 2 and 24 h, respectively. Each value represents mean±S.E.M. for three experiments.

^a Value significantly different from the corresponding control value, *P*<0.05.

Table 4
Effects of FMEP extracts and dibenzo[*a,h*]anthracene on drug-metabolizing enzyme activities in human lung adenocarcinoma CL5 cells

Assay	Control	FMEP extract			DB[<i>a,h</i>]A
		Beef	Fish	Pork	
Benzo[<i>a</i>]pyrene hydroxylase (pmol 3-OHBP/min/mg protein)	1.1±0.2	2.9±0.6 ^a	3.3±0.5 ^a	1.8±0.9	4.6±0.9 ^a
7-Ethoxyresorufin <i>O</i> -deethylase (pmol RF/min/mg protein)	0.3±0.0	1.2±0.3 ^a	1.4±0.3 ^a	1.1±0.3 ^a	3.1±0.7 ^a
Methoxyresorufin <i>O</i> -demethylase (pmol RF/min/mg protein)	0.1±0.1	1.0±0.1 ^a	1.8±0.1 ^a	0.5±0.1 ^a	1.3±0.1 ^a
NADPH-cytochrome P450 reductase (nmol cyt.c/min/mg protein)	4.6±0.6	4.7±0.1	4.7±0.2	4.4±0.3	4.6±0.2
Glutathione <i>S</i> -transferase (nmol CDNB/min/mg protein)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
<i>N</i> -acetyltransferase (nmol PABA/min/mg protein)	19.4±0.5	18.2±0.8	18.9±0.5	19.1±1.2	21.4±1.8

CL5 cells were treated with 200 µg/ml FMEP extract from beef, fish or pork or 1 µM dibenzo[*a,h*]anthracene (DB[*a,h*]A) for 6 h. Control cells were treated with DMSO only. Cellular S9 fractions were prepared and drug-metabolizing enzyme activities were determined as described in Materials and methods. Each value represents mean±S.E.M. for three experiments.

^a Value significantly different from the corresponding control value, $P < 0.05$.

beef, fish and pork FMEP extracts increased the intensity of a CYP1A1-immunorelated protein band in CL5 cells and the FMEP-inducible CYP1A1 protein was not detectable in control cells (Plate 1, upper panel, lanes 1–4). DB[*a,h*]A also increased the intensity of a protein band cross-reactive with the antibody to CYP1A1 (lane 5). The CYP1A1 protein bands induced by FMEP extracts and DB[*a,h*]A showed similar electrophoretic mobilities (lanes 2–5). Protein blots probed using a rabbit

polyclonal antibody against human CYP1B1 showed an immunoreactive protein band in control cells (middle panel, lane 1). FMEP extracts caused three-fold increases in the intensity of the CYP1B1 protein band (lanes 2–4; Table 5). DB[*a,h*]A increased the intensity of the CYP1B1-immunorelated protein band, similar to the inductive effects of FMEP extracts (lane 5; Table 5).

To study the mechanism of P450 induction by these airborne cooking by-products, total RNA was prepared from control cells and cells treated with FMEP extract or DB[*a,h*]A and subjected to RNA blot analysis in which a human CYP1A1 3'-end cDNA (Jaiswal et al., 1985) and an RT-PCR product specific for human CYP1B1 (Dohr et al., 1995) were used to probe for hybridizable CYP1A1 and CYP1B1 mRNA species (Plate 2). The intensities of the mRNA bands in the RNA blots were quantitated (Table 5). RNA blots analysis using the human CYP1A1 cDNA probe showed a minimal intensity of a hybridizable CYP1A1 mRNA band in control cells; in contrast FMEP extracts caused 12- to 13-fold increases in the intensity of the CYP1A1 mRNA (Plate 2, upper panel, lanes 1–4; Table 5). DB[*a,h*]A produced a 37-fold increase of a CYP1A1 mRNA (lane 5, Table 5). The electrophoretic mobility of DB[*a,h*]A-inducible CYP1A1 mRNA band was similar to that of CYP1A1 mRNA band induced by FMEP extracts (lanes 2–5). Northern blot analysis using a human CYP1B1 cDNA probe revealed the presence of a CYP1B1 mRNA species in control cells (middle panel, lane 1). Beef, fish, and pork FMEP extracts caused four- to five-fold increases of the intensity of the CYP1B1 mRNA band (lanes 2–4, Table 5). DB[*a,h*]A caused a nine-fold increase of a CYP1B1 mRNA band with an electrophoretic mobility similar to the FMEP-inducible mRNA band (lane 5, Table 5).

In view of the fact that many P450 enzymes are polymorphic in nature, it was necessary to extend the induction study to another cell line. In this respect, human lung carcinoma cell line NCI-H322 was treated with 200 µg/ml beef FMEP extract or 1 µM DB[*a,h*]A

Plate 1. Immunoblots of CYP1A1 and CYP1B1 in human lung adenocarcinoma CL5 cells treated with organic extracts of frying-meat emission particulates and dibenzo[*a,h*]anthracene. CL5 cells were treated with 200 µg/ml organic extracts of frying-meat emission particulates (FMEPs) from beef, fish or pork and 1 µM dibenzo[*a,h*]anthracene (DB[*a,h*]A) for 6 h, respectively. Control (C) cells were treated with DMSO only. S9 proteins from the controls and treated cells were subjected to protein blot analysis in which a mouse Mab1–12–3 to rat human CYP1A1 (*top*) and a rabbit polyclonal antiserum to human CYP1B1 (*middle*) were used to probe for immunorelated proteins as described in Materials and methods. β-Actin was used as an internal standard (*bottom*). The S9 protein load in each lane was 200 µg.

Table 5

Effects of FMEP extracts and dibenzo[*a,h*]anthracene on CYP1A1 and CYP1B1 protein and mRNA levels in human lung adenocarcinoma CL5 cells

Assay	CYP	Control	FMEP extract			DB[<i>a,h</i>]A
			Beef	Fish	Pork	
(arbitrary unit)						
Protein	1A1	n.d. ^a	0.93±0.17 ^b	0.99±0.10 ^b	0.70±0.09 ^b	2.46±0.59 ^b
	1B1	0.39±0.10	1.26±0.22 ^b	1.31±0.23 ^b	1.17±0.23 ^b	1.03±0.03 ^b
mRNA	1A1	0.18±0.13	2.21±0.51 ^b	2.38±0.27 ^b	2.18±0.22 ^b	6.72±0.45 ^b
	1B1	0.80±0.14	2.79±0.37 ^b	3.88±0.56 ^b	3.51±0.58 ^b	6.99±0.73 ^b

CL5 cells were treated with 200 µg/ml FMEP extract from beef, fish or pork or 1 µM dibenzo[*a,h*]anthracene (DB[*a,h*]A) for 6 h. Control cells were treated with DMSO only. Protein and RNA blotting analyses of S9 and total RNA of CL5 cells were carried out as described in Materials and methods, respectively. Intensities of protein and mRNA bands were quantitated using a digital image analyzer. Intensity of mRNA band was normalized against an internal standard GAPDH. Each value represents mean±S.E.M. for three experiments.

^a n.d.: not detectable.

^b Value significantly different from the corresponding control value, *P* < 0.05.

for 6 h. The beef extract produced four-, five- and three-fold increases of benzo[*a*]pyrene hydroxylase, 7-ethoxyresorufin and methoxyresorufin *O*-dealkylases activities in S9, respectively (Table 6). The results of Northern analysis showed that the beef extract caused 12- and 3-fold induction of CYP1A1 and CYP1B1 mRNA levels, respectively (Plate 3). DB[*a,h*]A caused six-, three- and three-fold induction of monooxygenases activities towards benzo[*a*]pyrene, 7-ethoxyresorufin and methoxy-

resorufin, and 19- and five-fold induction of CYP 1A1 and CYP1B1 mRNA, respectively.

4. Discussion

This report is the first to show induction of CYP1A1 and CYP1B1 by airborne particulates generated from frying meats. The induction of the P450 enzymes in CL5 cells is supported by several lines of evidence. First, treatment with FMEP extract increased monooxygenase activities towards benzo[*a*]pyrene and 7-ethoxyresorufin, which are the selective substrates of CYP1A1 and CYP1B1 (Ryan and Levin, 1990; Shimada et al., 1997). Secondly, the treatment induced P450 proteins cross-reactive with antibodies against rat CYP1A1 and human CYP1B1, respectively (Park et al., 1986). Thirdly, FMEP extracts increased mRNA species hybridizable to cDNA probes specific for human CYP1A1 and CYP1B1 (Jaiswal et al., 1985; Dohr et al., 1995). The NCI-H322 cell line is responsive to the inductive effects of FMEP and DB[*a,h*]A, similarly to CL5. To the best of our knowledge, this is also the first report of induction of CYP1B1 by DB[*a,h*]A. The major reasons for selecting DB[*a,h*]A over benzo[*a*]pyrene or other PAHs for the induction study were that direct evidence of induction of CYP1B1 by DB[*a,h*]A had remained not available and use of benzo[*a*]pyrene as an inducing agent would have interfered with the assay of benzo[*a*]pyrene hydroxylase activity in which the inducer was the enzyme substrate.

The PAHs of FMEP most likely are the inducing agents of CYP1A1 and CYP1B1. Frying fish generated benzo[*a*]pyrene and benzo[*a*]anthracene in FMEP at the concentrations of 62.0 and 24.3 ng/g meat (Table 2; Yang et al., 2000). These PAHs are potent aryl hydrocarbon receptor (AHR) agonists and CYP1A1 inducer in vivo and in vitro (Bigelow and Nebert, 1982; Nebert et al., 2000). Moreover, our monooxygenase activity,

Plate 2. RNA blots of CYP1A1 and CYP1B1 in human lung adenocarcinoma CL5 cells treated with organic extracts of frying-meat emission particulates and DB[*a,h*]A. CL5 cells were treated with 200 µg/ml organic extracts of frying meat emission particulates (FMEPs) from beef, fish or pork and 1 µM DB[*a,h*]A for 6 h, respectively. Control (C) cells were treated with DMSO only. Total RNA was isolated and subjected to RNA blot analysis in which a ³²P-labeled CYP1A1 or a CYP1B1 cDNA probe was used to probe for hybridizable mRNA species (*top* and *middle*) as described in Materials and methods. RNA blot was reprobed using a ³²P-labeled GAPDH cDNA probe as an internal control. GAPDH was used as an internal standard (*bottom*). The RNA load in each lane was 20 µg.

Table 6

Effects of FMEP extract and dibenzo[*a,h*]anthracene on drug-metabolizing enzyme activities and CYP1A1 and CYP1B1 mRNA in human lung carcinoma NCI-H322 cells

Assay	Control	Beef FMEP extract	DB[<i>a,h</i>]A
Benzo[<i>a</i>]pyrene hydroxylase (pmol 3-OHBP/min/mg protein)	0.6±0.1	2.2±0.2 ^a	3.4±0.4 ^a
7-Ethoxyresorufin <i>O</i> -deethylase (pmol RF/min/mg protein)	1.3±0.1	6.3±0.4 ^a	3.2±0.6 ^a
Methoxyresorufin <i>O</i> -demethylase (pmol RF/min/mg protein)	0.9±0.1	2.7±0.4 ^a	2.7±0.5 ^a
CYP1A1 mRNA (arbitrary unit)	0.2±0.1	2.5±0.1 ^a	3.8±0.4 ^a
CYP1B1 mRNA (arbitrary unit)	0.9±0.3	2.7±0.4 ^a	4.4±0.4 ^a

NCI-H322 cells were treated with 200 µg/ml beef FMEP extract or 1 µM dibenzo[*a,h*]anthracene (DB[*a,h*]A) for 6 h. Control cells were treated with DMSO only. Cellular S9 fractions and total RNA were prepared for drug-metabolizing enzyme activities and RNA blots analyses as described in Materials and methods, respectively. Intensity of mRNA band was normalized against an internal standard GAPDH. Each value represents mean±S.E.M. for three experiments.

^a Value significantly different from the corresponding control value, $P < 0.05$.

immunoblot, and RNA blot data have demonstrated that the characteristics of P450 induction by FMEP extracts were qualitatively indistinguishable from those of a prototypic PAH, DB[*a,h*]A. The mechanism of P450 induction by FMEP extracts and DB[*a,h*]A involves binding of the inducing agents to AHR to initiate a cascade of induction events. The present data

Plate 3. RNA blots of CYP1A1 and CYP1B1 in human lung carcinoma NCI-H322 cells treated with organic extract of frying-meat emission particulate from beef and DB[*a,h*]A. NCI-H322 cells were treated with 200 µg/ml organic extract of frying meat emission particulate (FMEP) from beef and 1 µM DB[*a,h*]A for 6 hr, respectively. Control (C) cells were treated with DMSO only. Total RNA was isolated and subjected to RNA blot analysis in which a ³²P-labeled CYP1A1 or a CYP1B1 cDNA probe was used to probe for hybridizable mRNA species (*top* and *middle*) as described in Materials and methods. RNA blot was reprobed using a ³²P-labeled GAPDH cDNA probe as an internal control. GAPDH was used as an internal standard (*bottom*). The RNA load in each lane was 20 µg.

do not exclude the possibility that AHR-independent induction mechanism(s) are involved.

Frying beef patties produced 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) in FMEP at 0.14 ng/g meat (Table 2; Thiebaud et al., 1995). Induction of CYP1A1 and CYP1B1 by these HCAs in CL5 cells was less likely, relative to PAHs, because HCAs were far lower than PAHs in terms of concentration (Table 2) and ability to activate DNA-binding activity of AHR (Kleman et al., 1992). In a different experiment, treatment of CL5 cells with 100 µM MeIQx for 6 h caused a minimal induction of CYP1A1 and CYP1B1 protein and monooxygenases activities. Treatment with lower MeIQx concentrations had no effect on P450 (data not shown). These data indicated that CL5 cell line was not responsive to the HCA treatment, in comparison to the marked inductive effect of DB[*a,h*]A. The ability of fatty acid or steroid to induce CYP1A1 or CYP1B1 has not been reported. Chemical-chemical interaction in the complex mixture may modulate the toxicological effect of FMEP. It is not inconceivable that relatively high concentrations of steroids, fatty acids, or their oxidative products in FMEP might modulate CYP1A1 and CYP1B1 induction by PAHs.

The key metabolizing system for HCAs is CYP1A2, which appears to be expressed only in liver (Guengerich and Shimada, 1998). The present study did not attempt to investigate the ability of FMEP to induce the HCA-metabolizing enzyme in human lung adenocarcinoma CL5 cells mainly because expression of CYP1A2 in lung has not been clearly defined at this time. For instance, CYP1A2 was not detectable in the human lung adenocarcinoma cell line A549 and lung samples from smokers (Antilla et al., 1991; Hukkanen et al., 2000). However, catalytically functional CYP1A2 was detected along with CYP1A1 in histologically normal human lung specimens in other studies (Wei et al., 2001). It will be of interest to determine and compare the effects of FMEP on CYP1A2 and other important HCA-metabolizing

enzymes such as *N*-acetyltransferase in liver and lung in future investigations.

In Taiwanese women non-smokers, those who cooked when they were 20–40 years of age without using a fume ventilator had a higher risk of lung cancer at the age of 40 years and over than the hospital controls (Ko et al., 2000). It seems reasonable to speculate that at the younger ages high FMEP exposure induced pulmonary CYP1A1 and CYP1B1 and increased metabolic activation of PAHs, which played a role as an important metabolic determinant of the initiation stage of carcinogenesis. To test this possibility, additional studies are required to determine the association between CYP1A1 and CYP1B1 induction and lung cancer incidence in high FMEP exposure groups such as cooks or individuals who cook more meals than usual, and particularly those with genotypes showing high inducibility of CYP1A1 and CYP1B1.

In conclusion, the present report demonstrates that emission particulates from frying beef, fish and pork have the ability to induce CYP1A1 and CYP1B1 protein and mRNA in the human lung tumor-derived cell line CL5 and the induction mechanism involves a pretranslational event. The ability of the emission particulates from frying meats to induce pulmonary carcinogen-activation enzymes may be an important factor to consider in assessing the role of exposure to airborne cooking by-products in lung cancer of non-smokers.

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References

- Andres, H.H., Klem, A.J., Szabo, S.M., Weber, W.W., 1985. New spectrophotometric and radiochemical assays for acetyl-CoA: arylamine *N*-acetyltransferase applicable to a variety of arylamines. *Analytical Biochemistry* 145, 367–375.
- Anttila, S., Hietanen, E., Vainio, H., Camus, A.M., Gelboin, H.V., Park, S.S., Heikella, L., Karjalainen, A., Bartsch, H., 1991. Smoking and peripheral type of cancer are related to high levels of pulmonary cytochrome P4501A in lung cancer patients. *International Journal of Cancer* 47, 681–685.
- Bigelow, S.W., Nebert, D.W., 1982. The Ah regulatory gene product. Survey of nineteen polycyclic aromatic compounds' and fifteen benzo[*a*]pyrene metabolites' capacity to bind to the cytosolic receptor. *Toxicology Letters* 10, 109–118.
- Carmichael, J., DeGraff, W.G., Gazdar, A.F., Minna, J.D., Mitchell, J.B., 1987. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Research* 47, 936–942.
- Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Analytical Biochemistry* 162, 156–159.
- Dohr, O., Vogel, C., Abel, J., 1995. Different response of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-sensitive genes in human breast cancer MCF-7 and MDA-MB 231 cells. *Archives of Biochemistry and Biophysics* 321, 405–412.
- Gao, Y.-T., Blot, W.J., Ershaw, A.G., Hsu, C.-W., Levin, L.I., Fraumeni, J., Zheng, W., 1987. Lung cancer among Chinese women. *International Journal of Cancer* 40, 604–609.
- Guengerich, F.P., Shimada, T., 1998. Activation of procarcinogens by human cytochrome P450 enzymes. *Mutation Research* 400, 201–213.
- Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione *S*-transferases. The enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* 249, 7130–7139.
- Hukkanen, J., Lassila, A., Paivarinta, K., Valanne, S., Sampo, S., Hakkola, J., Pelkonen, O., Raunio, H., 2000. Induction and regulation of xenobiotic-metabolizing cytochrome P450s in the human A549 lung adenocarcinoma cell line. *American Journal of Respiratory Cell and Molecular Biology* 22, 360–366.
- Jaiswal, A.K., Gonzalez, F.J., Nebert, D.W., 1985. Human P₁-450 gene sequence and correlation of mRNA with genetic differences in benzo[*a*]pyrene metabolism. *Nucleic Acids Research* 13, 4503–4520.
- Kang, J.-J., Wang, H.-W., Liu, T.-Y., Chen, Y.-C., Ueng, T.-H., 1997. Modulation of cytochrome P-450-dependent monooxygenases, glutathione and glutathione *S*-transferase in rat liver by geniposide from *Gardenia jasminoides*. *Food and Chemical Toxicology* 35, 957–965.
- Kleman, M.I., Overvik, E., Mason, G.G.F., Gustafsson, J.A., 1992. *In vitro* activation of the dioxin receptor to a DNA-binding form by food-borne heterocyclic amines. *Carcinogenesis* 13, 1619–1624.
- Ko, Y.-C., Chen, L.-S., Lee, C.-H., Huang, J.-J., Huang, M.-S., Kao, E.L., Wang, H.-Z., Lin, H.-J., 2000. Chinese food cooking and lung cancer in women nonsmokers. *American Journal of Epidemiology* 151, 140–147.
- Koo, L.-C., Ho, J.-H., 1990. Worldwide epidemiological patterns of lung cancer in nonsmokers. *International Journal of Epidemiology*, 19, S14–S23.
- Lebel, C.P., Ischiropoulos, H., Bondy, S.C., 1992. Evaluation of the probe 2',7'-dichlorofluorescein as an indicator of reactive oxygen species formation and oxidative stress. *Chemical Research in Toxicology* 5, 227–231.
- Lofroth, G., Stensman, C., Brandhorst-Satzkorn, M., 1991. Indoor sources of mutagenic aerosol particulate matter: smoking, cooking and incense burning. *Mutation Research* 261, 21–28.
- Lofroth, G., 1994. Airborne mutagens and carcinogens from cooking and other food preparation processes. *Toxicology Letters* 72, 83–86.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193, 265–275.
- Mayer, R.T., Jermyn, J.W., Burke, M.D., Prough, R.A., 1977. Methoxyresorufin as a substrate for the fluorometric assay of insect microsomal *O*-dealkylase. *Pesticide Biochemistry and Physiology* 7, 349–354.
- Nebert, D.W., Gelboin, H.V., 1968. Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. I. Assay and properties of induced enzyme. *Journal of Biological Chemistry* 243, 6242–6249.
- Nebert, D.W., Roe, A.L., Dieter, M.Z., Solis, W.A., Yang, Y., Dalton, T.P., 2000. Role of the aromatic hydrocarbon receptor and [Ah] gene battery in the oxidative stress response, cell cycle control, and apoptosis. *Biochemical Pharmacology* 59, 65–85.
- Park, S.S., Miller, H., Klotz, A.V., Kloepper-Sams, P.J., Stegeman, J.J., Gelboin, H.V., 1986. Monoclonal antibodies to liver microsomal cytochrome P-450E of the marine fish *Stenotomus chrysops* (*scup*): cross reactivity with 3-methylcholanthrene induced rat cytochrome P-450. *Archives of Biochemistry and Biophysics* 249, 339–350.

- Phillips, A.H., Langdon, R.G., 1962. Hepatic triphosphopyridine nucleotide-cytochrome c reductase: isolation, characterization, and kinetic studies. *Journal of Biological Chemistry* 237, 2652–2660.
- Pohl, R.J., Fouts, J.R., 1980. A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. *Analytical Biochemistry* 107, 150–155.
- Ryan, D.E., Levin, W., 1990. Purification and characterization of hepatic microsomal cytochrome P-450. *Pharmacology and Therapeutics* 45, 153–239.
- Shimada, T., Gillam, E.M., Sutter, T.R., Strickland, P.T., Guengerich, F.P., Yamazaki, H., 1997. Oxidation of xenobiotics by recombinant human cytochrome P450 1B1. *Drug Metabolism and Disposition* 25, 617–622.
- Thiebaud, H.P., Knize, M.G., Kuzmicky, P.A., Hsieh, D.P., Felton, J.S., 1995. Airborne mutagens produced by frying beef, pork and a soy-based food. *Food and Chemical Toxicology* 33, 821–828.
- Tietze, F., 1969. Enzymatic method for qualitative determination of nanogram amounts of total and oxidized glutathione. *Analytical Biochemistry* 27, 502–522.
- Ueng, T.-H., Hu, S.-H., Chen, R.-M., Wang, H.-W., Kuo, M.-L., 2000. Induction of cytochrome P-450 1A1 in human hepatoma HepG2 and lung adenocarcinoma NCI-H322 cells by motorcycle exhaust particulate. *Journal of Toxicology and Environmental Health, Part A* 60, 101–119.
- Valaitis, J., Warren, S., Gamble, D., 1981. Increasing incidence of adenocarcinoma of the lung. *Cancer* 47, 1042–1046.
- Wei, C., Cacavale, R.J., Kehoe, J.J., Thomas, P.E., Iba, M.M., 2001. CYP1A2 is expressed along with CYP1A1 in the human lung. *Cancer Letters* 164, 25–32.
- Yang, C.-C., Jenq, S.-N., Lee, H., 1998. Characterization of the carcinogen 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in cooking aerosols under domestic conditions. *Carcinogenesis* 19, 359–363.
- Yang, S.-C., Jenq, S.-N., Kang, Z.-C., Lee, H., 2000. Identification of benzo[a]pyrene 7,8-diol 9,10-epoxide N2-deoxyguanosine in human lung adenocarcinoma cells exposed to cooking oil fumes from frying fish under domestic conditions. *Chemical Research in Toxicology* 13, 1046–1050.