Effects of cigarette smoke and a heterocyclic amine, MeIQx on cytochrome P-450, mutagenic activation of various carcinogens and glucuronidation in rat liver

Yukio Mori2, Akihiro Koide, Yoshinori Kobayashi, Fumio Furukawa1, Masao Hirose1 and Akiyoshi Nishikawa3

Laboratory of Radiochemistry, Gifu Pharmaceutical University, 6-1 Mitahora-higashi 5-chome, Gifu 502-8585, Japan and 1Division of Pathology, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

In order to elucidate the mechanism underlying enhancement by cigarette smoke (CS) of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx)-induced rat hepatocarcinogenesis, hepatic levels of cytochrome P-450 (CYP) enzymes, mutagenic activation of various carcinogens and UDP-glucurononyltransferase (UDPGT) activities were assayed in male F344 rats. Immunoblot analyses for microsomal CYP proteins revealed induction of CYP1A1 and constitutive CYP1A2 (2.3- to 2.7-fold), but not CYP2B1/2, 2E1 or 3A2, by CS exposure for 1, 12 or 16 weeks using a Hamburg type II smoking machine; the enhancement of CYP1A2 was 4.7–5.7 times that of CYP1A1. CS exposure also elevated the mutagenic activities of MeIQx and five other heterocyclic amines (HCAs) 1.4- to 3.7-fold, but not those of benzo[a]pyrene (BP) and aflatoxin B1 in strain TA98 and N-nitrosodimethylamine, N-nitrosopyrrolidine and 4-(methyl)nitrosodimethylamine, 1-(3-pyridyl)-1-butanone in strain TA100. In contrast, feeding 300 p.p.m. MeIQx in the diet for 1 or 16 weeks produced no significant alterations in the levels of these CYP species and mutagenic activities. However, i.e., administration of 50 or 100 mg/kg MeIQx in a single dose selectively increased CYP1A1 and IA2 (2.6-fold) levels and mutagenic activities of five HCAs (1.7- to 3.3-fold), but not BP. On the other hand, feeding of MeIQx for 16 weeks enhanced UDPGT activities towards 4-nitrophenol and testosterone (2.9- and 1.5-fold, respectively), but not bilirubin, while CS exposure induced that towards 4-nitrophenol (1.6-fold); combined treatment with CS and MeIQx showed a summation effect on induction of UDPGT1A6 activity (3.5-fold). Consequently, these results demonstrate that CS and MeIQx have a bifunctional action, with similar induction patterns of specific CYP proteins, mutagenic activity and UDPGT activity. In conjunction with the finding of N-hydroxy-MeIQx being a poor substrate for rat liver UDPGT, our results clearly indicate that enhancement by CS of MeIQx-induced hepatocarcinogenesis in F344 rats can be attributed to an increase in metabolic activation of MeIQx by hepatic CYP1A2 during the initiation phase.

Introduction

Lifestyle factors such as diet, chronic cigarette smoking and alcohol consumption are considered major risk factors in human cancer (Doll and Peto, 1981). Epidemiological studies have suggested that cigarette smoking is closely associated with an increased risk of cancers in various organs, such as the lung, oropharynx, pancreas, stomach, liver and colon (Doll and Peto, 1976; Nagata et al., 1999). Hoffmann et al. (1979) and Homburger (1979) have reviewed evidence that cigarette smoke (CS) induces changes in the larynx and nasopharynx of hamsters, ranging from hyperplasia to papillomatous growths to occasional carcinomas, laryngeal carcinoma in susceptible inbred lines of hamsters and adenoma with occasional adenocarcinomas, but no squamous cell cancer, of the lungs in mice. However, attempts to produce lung cancer in experimental animals by inhalation of CS have mostly not been very successful (Henry and Kouri, 1986; IARC, 1986; Finch et al., 1996), indicating that CS is not a potent carcinogen per se. Therefore, the effects of CS may be explained in terms of its modifying the effects of endogenous and exogenous carcinogens. In fact, it has been shown that CS exposure increases the incidence of spontaneous lung tumors in A/J mice (Witschi et al., 1997) and promotes upper respiratory tract tumorigenesis in hamsters initiated with N-nitrosodimethylamine (DEN) (Takahashi et al., 1992). Nevertheless, influences of CS on experimental tumorigenesis in other organs remain to be elucidated. Recently, we found that CS exposure enhances hepatocarcinogenesis when given in the initiation phase and possibly colon carcinogenesis when given in the post-initiation phase in F344 rats induced with 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) (Nishikawa et al., 2002).

Carcinogenic heterocyclic amines (HCAs) and N-nitrosamines occur in both CS (IARC, 1986; Manabe et al., 1991) and foods (Fine, 1982; Sugimura, 1985; Wakabayashi et al., 1992). N-nitrosodimethylamine (DMN) and N-nitrosopyrrolidine (NPR) are metabolically activated by cytochrome P-450 (CYP) 2E1 (Yang et al., 1987; Mori et al., 2002), while N-nitrosodialkylamines possessing relatively long alkyl chains are activated by CYP2B1 and 2B2 (Shu and Hollenberg, 1996). In addition, CYP1A2, CYP2A1 and CYP3A2 are reported to be responsible for metabolic activation of the tobacco-specific nitrosamine 4-(methyl)nitrosodimethylamine (NNK), 1-(3-pyridyl)-1-butanone (NNK) in rats (Guo et al., 1992; Mori et al., 2001). On the other hand, CYP1A1 and 1A2 are known to be selectively involved in metabolic activation of a number of HCAs, including MeIQx, in humans (Rich et al., 1992) and rats (Yamazoe et al., 1988). It has been reported that 7 year oral administration of MeIQx causes no induction of neoplastic lesions in liver, colon, lung and gastrointestinal tract of cynomolgus monkey, lacking hepatic CYP1A2 expression (Ogawa et al., 1999). Further, it has been shown that modifying effects on experimental carcinogenesis initiated by 2-amino-3-methylimidazo[4,5-f]quinolone (IQ) (Uehara et al., 1996) or PhIP (Tsuda et al., 1999) are closely associated with metabolic activation by the CYP1A subfamily. CS is known to be a hepatocarcinogenic inducer in smokers (Baker et al., 2001) and rodents (Kawamoto et al., 1993; Villard et al., 1994; Koide...
et al., 1999). Induction by CS of hepatic CYP2B, 2E1 or 3A proteins and metabolic activities specific to these CYP isoforms in rats (Wardlow et al., 1998) and mice (Seree et al., 1996) has also been reported.

In considering the generation of ultimate carcinogens, it is vitally important to determine both the bioactivation and detoxification processes that determine their concentrations, consequently leading to the expression of carcinogenicity. N-OH-HCAs (Kaderlik et al., 1994a) and N-nitrosoamines (Kokinakis et al., 1987; Wiessler and Rossnagel, 1987) are known to be substrates for UDP-glucuronyltransferases (UDPGTs). In rat liver, UDPGT1A1, A6 and 2B1 are found as major enzymes which are inducible by clofibrate, 3-methylcholanthrene (MC) and phenobarbital (PB), respectively (Narayanam et al., 2000). Rat UDPGT2B1 is suggested to be the probable enzyme responsible for glucuronidation of DEN and N-nitrosoethyl-N- pentyamine (Wiensch et al., 1992). The human UDPGT1A1 and 2B subfamilies have been shown to be involved in glucuronidation of N-OH-PhIP (Nowell et al., 1999), however, to date no studies have been reported on the UDPGT species responsible in rats and on the inducibility of UDPGT activity by HCA treatment in any animal species. Although CS exposure is known to induce hepatic UDPGT1A6 activity in mice (Villard et al., 1998) and humans (Fleischmann et al., 1986), no data have been provided about the effects of CS on UDPGT1A1, 1A6 and 2B1 activities in rats.

In order to elucidate the mechanism underlying enhancement by CS of MeIQx-induced rat hepatocarcinogenesis, hepatic levels of microsomal CYP enzymes known to activate typical environmental carcinogens, mutagenic activation of these carcinogens and three kinds of UDPGT activities were assayed in F344 rats treated with CS and/or MeIQx.

Materials and methods

Chemicals

Hydrochlorides of 2-amino-6-methylpyridinol-1,2,3′,4′-dimidazole (Glut-P) and PhIP, acetates of 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P) and 2-amino-3-methyl-5H-pyrido[2,3-b]indole (MeArdC) and IQ, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQx), benz[a]pyrene (BP), DMN, NPYR, 4-nitrophenol, testosterone, bilirubin and UDP-glucuronic acid were purchased from Wako Pure Chemicals (Osaka, Japan). Glucose 6-phosphate (G6P), G6P dehydrogenase (G6PDH), NADP⁺, NADPH, NADH and ATP were obtained from Oriental Yeast Co. (Tokyo, Japan). Aflatoxin B₁ (AFB₁) was purchased from Makor Chemicals (Jerusalem, Israel) and MeIQx was from Toronto Research Chemicals (Ontario, Canada). UDP-[14C(U)]glucuronic acid was purchased from American Radiolabeled Chemicals (St Louis, MO) and NNK was from Chemsyn Science Laboratories (Lenexa, KS). All other commercial products were of the purest grade available. N-nitrosobis(2-hydroxypropyl)amine (BHP) and N-nitroso-2,6-dimethylmorpholine (NDMM) were synthesized in our laboratory as described previously (Mori et al., 2001). Non-filter cigarettes were obtained from Japan Tobacco (Tokyo, Japan).

Animal treatment and tissue preparation

Male 3-week-old specific pathogen-free F344/DuCrj rats purchased from Charles River Japan (Kanagawa, Japan) were housed five animals per polycarbonate cage and maintained under standard laboratory conditions. After a 1 week acclimation period, the animals were divided into seven groups and were given CS and MeIQx in initiation and post-initiation phases using previously reported conditions (Nishikawa et al., 2002). Animals in Groups 1 and 3, each consisting of five rats, were fed the basal diet Oriental MF (Oriental Yeast Co., Tokyo, Japan) and underwent sham smoking (SS) or received CS, respectively, for 16 weeks. Groups 2 and 4 rats were fed basal diet supplemented with MeIQx at a concentration of 300 p.p.m. and simultaneously underwent SS or received CS exposure, respectively, for 16 weeks. Groups 5–7 rats were fed MeIQx at a dose of 500 p.p.m. for 4 weeks and exposed to CS for 12 weeks after the MeIQx treatment (group 5), were simultaneously exposed to MeIQx and underwent SS for 4 weeks and then underwent SS for 12 weeks (group 6) or were simultaneously exposed to MeIQx and CS for 4 weeks and then underwent SS for 12 weeks (group 7). CS was transnasally administered 7 days a week using a Hamburg type II smoking machine (Borgwaldt, Germany) under the following conditions: exposure period, 6 min/day; dose, 30 cigarettes/exposure; inhalation volume, 35 ml; inhalation flow, 17.5 ml/min; dilution of cigarette smoke, 1:7 (Koide et al., 1999). The SS treatment involved restricting animals to a chamber of the smoking machine under the same conditions as for CS treatment but without smoke. Alternatively, 20 male 6-week-old F344/DuCrj rats were divided into four groups and were given MeIQxs in the diet (300 p.p.m.) and simultaneously underwent SS or were exposed to CS for 5 days, while two other groups were fed basal diet and underwent SS or received CS for 5 days. Further, nine male 6-week-old F344/Nle rats were purchased from Japan SLC (Hamamatsu, Japan) and were divided into three groups. Two groups were given MeIQx dissolved in corn oil orally as a single dose of 50 or 100 mg/kg while the other rats were similarly treated with corn oil, serving as a control. All the animals were decapitated 24 h after treatment. Livers were perfused in situ with ice-cold sterile 1.15% KCl and 25% homogenates in 1.15% KCl were prepared. Liver S9 and microsomes were prepared using established procedures (Mori et al., 2002).

Western blots

Goat anti-rat polyclonal antibodies for CYP1A1/2, 2B1/2, 1A6 and 3A2 (Daichi Pure Chemicals Co., Tokyo, Japan) were used as primary antibodies. Gel electrophoresis and blot analysis were performed as described in detail previously (Koide et al., 1999) according to the established methods of Laemmli (1970) and Towbin et al. (1979), respectively.

Mutation assay

All tests were carried out by the Ames preincubation assay (Yahagi et al., 1977). The N-nitrosoamines were dissolved in 100 µl of water and all the other carcinogens in 50 µl of dimethyl sulfoxide. The mutagenicities of MeIQ (dose 0.006 µg/plate), Trp-P, Glu-P and IQ (0.03 µg), MeIQx (0.3 µg), MeArdC (10 µg), PhIP and BP (5 µg), AFB₁ (1 µg), NPYR (0.25 mg), NNK (0.5 µg) and the other N-nitrosoamines (10 µg) were checked in the presence of liver S9, using established procedures (Mori et al., 2001, 2002). The amount of liver S9 fraction was 10 µl/plate for the HCA, 50 µl for BP and NNK and 150 µl for the other N-nitrosoamines and AFB₁. Salmonella typhimurium tester strains TA100 and TA98 were employed for all the N-nitrosoamines and the other carcinogens, respectively. The S9 mix contained 4 mM NADPH, 4 mM NAD, 0.5 µM G6PDH, 5 mM G6P and 5 mM ATP, except for the N-nitrosoamines, where 4 mM NAD³⁺ and 5 mM G6P were used.

Assay of UDPGT activity

UDPGT1A1 and 1A6 activities towards bilirubin and 4-nitrophenol in liver microsomes were assayed according to the methods described by Heiweg et al. (1972) and Isselbacher et al. (1962), respectively, and UDPGT2B1 activity towards testosterone was determined using UDP-[14C(U)]glucuronic acid as described by Matern et al. (1994).

Results

Figure 1 shows immunoblots and levels (pmol/mg protein) of microsomal CYP proteins in F344 rats treated with CS and MeIQx for up to 16 weeks. Hepatic CYP1A1 protein was induced in Groups 3–5 rats exposed to CS for 12 or 16 weeks and the constitutive CYP1A2 level was 2.3- to 2.7-fold higher (P < 0.01) in these groups than in Group 1 (SS treatment for 16 weeks). The level of CYP1A2 protein was 4.7–5.7 times that of CYP1A1, but there were no significant differences in either CYP1A1 or 1A2 level among the three groups. In contrast, feeding 300 p.p.m. MeIQx for 16 weeks in Group 2 and treatment for 4 weeks followed by SS for 12 weeks in Group 6 or simultaneous MeIQx and CS exposure for 4 weeks then SS for 12 weeks in Group 7 produced no significant effects on the expression of CYP1A1 and 1A2. CYP2B1 was not constitutively expressed and was not induced in any group of rats and there were no significant differences in hepatic levels of CYP2B2, 3A2 and 2E1 proteins among the seven groups.

Similar results were seen in rats exposed to CS for 5 days, as shown in Figure 2. CYP1A1 and 1A2 in rats exposed to CS with or without MeIQx were selectively induced, but at slightly higher levels than those observed in Groups 3–5 rats, lacking induction by MeIQx feeding. To clarify the induction potency of MeIQx for CYP expression, levels of CYP proteins

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Fig. 1. Immunoblots and densitometric determination of expression of CYP protein in liver microsomes from rats treated with CS, MeIQx or both for 4, 12 or 16 weeks. Liver microsomes were pooled from five rats each treated with SS 16w (lane 1), MeIQx 16w/H11001 SS 16w (lane 2), CS 16w (lane 3), MeIQx 16w + CS 16w (lane 4), MeIQx 4w + SS 4w→CS 12w (lane 5), MeIQx 4w + CS 4w→SS 12w (lane 7). Lane 8 contains CYP standards from Sprague–Dawley rats treated with MC (A), PB (B and C) or acetone (D). (A) and (B) contain 1.0 µg microsomal protein, (C) and (D) contain 0.4 µg microsomal protein. The values represent means of pmol/mg microsomal protein obtained from 4–7 experiments. *P < 0.01, compared with the SS group (lane 1) (Student’s t-test). n.d., not detected.

Fig. 2. Immunoblots and densitometric determination of expression of CYP protein in liver microsomes from rats treated with CS, MeIQx or both for 5 days. Liver microsomes were pooled from five rats each treated with SS (lane 1), MeIQx + SS (lane 2), CS (lane 3) or MeIQx + CS (lane 4). Lane 5 contains CYP standards and the values represent the means as described in the legend to Figure 1. (A)–(D) contain 1.0 µg microsomal protein. *P < 0.01, compared with the SS group (lane 1) (Student’s t-test).

were checked in liver microsomes from rats orally treated with 50 or 100 mg/kg MeIQx as a single dose. As shown in Figure 3, there were no significant differences in hepatic levels of all the CYP proteins determined between treatment with doses of 50 and 100 mg/kg. CYP1A1 protein was clearly induced, but at less than one seventh of the constitutive CYP1A2 level, which was 2.6-fold higher (P < 0.01) in the two treated groups than in the vehicle group. CYP2B1 protein was not detected and three other CYP species were at the same levels in the three groups.

To confirm the mutagenic activation induction characteristics of CS and MeIQx, 14 carcinogens almost all of which are known to occur in CS and be metabolically activated by specific CYP enzymes were assayed in strains TA98 and TA100. Figure 4 shows the mutagenic activities of several HCAs, including MeIQx, BP, AFB1, and four N-nitrosamines, in the presence of liver S9 from rats treated with CS and MeIQx for up to 16 weeks in Groups 2–5. The numbers of revertant colonies/plate after subtraction of spontaneous rates (TA98, 19; TA100, 134) with liver S9 from the Group 1 rats were 30 ± 5 (mean ± SD) for Glu-P-1, 144 ± 13 for IQ, 148 ± 16 for PhIP, 471 ± 52 for MeIQ, 669 ± 60 for MeIQx, 56 ± 10 for Trp-P-2, 201 ± 21 for MeAαC, 310 ± 36 for
BP and 2180 ± 9 for AFB1 in strain TA98 and 100 ± 7 for NNK, 284 ± 28 for DMN, 604 ± 48 for NPYR and 89 ± 13 for BHP in strain TA100. Mutagenic activities of six HCAs, except for MeAαC, were significantly increased in rats exposed to CS for 12 or 16 weeks (Groups 3–5) to almost the same extents, 1.5- to 3.7-fold relative to those in Group 1 rats. On the other hand, no significant alterations in mutagenicity were observed with MeAαC, BP, AFB1, DMN, NPYR, NNNK and BHP in Groups 3–5 rats and also with all the carcinogens tested in Group 2 rats. Similarly, no enhancing effects on mutagenicity in strains TA98 and TA100 were observed with 13 carcinogens in Groups 6 and 7 rats (data not shown). The effects of CS exposure with or without MeIQx feeding for 5 days are shown in Figure 5. CS exposure increased the mutagenic activities of five HCAs 1.4- to 2.4-fold above the SS group, but not those of MeAαC, BP and five N-nitrosamines, including NDMM. On the other hand, feeding of 300 p.p.m. MeIQx exerted no enhancing effects on the mutagenic activities of 12 carcinogens. In contrast, mutagenic activities of the same five HCAs were increased in rats orally treated with 50 and 100 mg/kg MeIQx to almost the same extents (1.7- to 3.3-fold), but not those of MeAαC and BP, as shown in Figure 6.

Table I summarizes the effects of CS and MeIQx treatments for up to 16 weeks on three kinds of UDPGT activities in liver microsomes (Groups 1–7). There were no significant differences in UDPGT activity towards bilirubin among the seven groups. On the other hand, UDPGT activities towards 4-nitrophenol and testosterone in rats treated with MeIQx for 16 weeks (Group 2) were increased up to 2.9- and 1.5-fold above the SS control (Group 1), respectively. UDPGT activity towards 4-nitrophenol in Groups 3 and 5 rats (CS treatment for 12 or 16 weeks) was significantly increased up to 1.6-fold and the combined treatment with MeIQx (Group 4) showed a summation effect (3.5-fold). The activities towards 4-nitrophenol and testosterone in Groups 6 and 7 rats were at the same level as those in Group 1 rats.

**Discussion**

Selective enhancing effects of CS exposure and i.g. administration of MeIQx on the mutagenic activation of five or six HCAs, including MeIQx, by liver S9 are in accord with observations for selective induction of hepatic CYP1A1 and 1A2 species. However, the mutagenicities of MeAαC and BP, which are predominantly activated by rat CYP1A1 (Degawa et al., 1988), were not enhanced by these treatments, indicating insufficient induction of the CYP species responsible (Koide et al., 1999; Mori et al., 2001). DMN and NPYR are mutagenetically activated by rat CYP2E1, BHP and NDMM by CYP2B1/2 and AFB1 by CYP2B1 and CYP3A2 (Mori et al., 2001, 2002). Therefore, it is reasonable that the mutagenicities of the five carcinogens with liver S9 from CS- and/or MeIQx-treated rats were not enhanced, reflecting no induction of hepatic CYP2B1, 2B2, 2E1 and 3A2. NNK is metabolically activated by hepatic CYP1A2, 2A1 and 3A2 in rats (Guo et al., 1992) and a CYP inducer, N-benzylimidazole, considerably enhances the mutagenicity of NNK, with marked induction of CYP1A2 and 3A2 and probably CYP2A1 in rat liver (Mori et al., 2001). Together with the fact that the induced levels of CYP1A2 protein observed in this study are much lower when compared with the case of N-benzylimidazole-treated rats, it also seems reasonable that CS and/or MeIQx had no effect on the mutagenic activation of NNK, suggesting no induction of hepatic CYP2A1 under the conditions used.

Differing results have been reported for CS-inducible CYP species in rodent liver. Either CYP1A1 or CYP1A2 protein is preferentially induced in liver microsomes from Wistar rats, depending on different conditions of inhalation of sidestream CS for 5 days using a stainless steel chamber (0.1 m³ volume) (Kawamoto et al., 1993). CYP1A1 protein, but not CYP1A2, is markedly induced in F344 rats exposed to mainstream CS for up to 8 weeks using an AMESA type 1300 automated smoking machine (Geneva, Switzerland) (Wardlaw et al., 1998). These results indicate that a higher exposure to CS predominantly induces CYP1A1 rather than CYP1A2, and these reports also describe induction of hepatic CYP2B1/2 and its metabolic activity. In addition to CYP1A1, hepatic CYP2E1 and 3A11/13 and the metabolic activities specific to these CYP isoforms are clearly induced in mice exposed to mainstream CS using a Hamburg type II smoking machine (Villard et al., 1994; Seree et al., 1996). In the present study CYP1A2 was preferentially induced by CS exposure for 1, 12 and 16 weeks to almost the same extent, but CYP2B1/2, 2E1 and 3A2 were not inducible, consistent with our previous findings in hamsters.
and Wistar rats exposed for 2 weeks (Koide et al., 1999). The reasons for these discrepancies with CS-inducible CYP species are not clear, but it is suggested that the differences might be in part due to experimental conditions, such as the brand of cigarettes, inhalation levels of CS, smoking apparatus, etc. However, our results are consistent with the findings in smokers that CYP1A2 protein and its metabolic activity are highly induced, without induction of other CYP species (Baker et al., 2001).

MeIQx at 50 mg/kg corresponds to a daily intake of 300 p.p.m. MeIQx in the feed and is equivalent to 0.23 mmol/kg. The present observations with this i.g. treatment are in agreement with previous findings that i.p. treatment with 0.22 mmol/kg of several HCAs, including MeIQx, induces the hepatic CYP1A subfamily, especially CYP1A2, in F344 rats (Degawa et al., 1989). However, neither CYP1A1 nor 1A2 proteins were induced by gavage of 300 p.p.m. MeIQx for up to 16 weeks, suggesting the existence of a threshold in the daily intake for induction of the CYP1A subfamily by HCA. It has been reported that eight HCAs are detected in CS and the total level amounted to ~0.32 ng per filter-tipped cigarette (Manabe et al., 1991). Based on this level, the daily intake of HCAs from CS in this study is estimated to be <1 ng/rat, being much less than the dose of 50 mg/kg MeIQx. Although the induction pattern of the CYP1A subfamily by CS is the same as that by HCAs, it is reasonable to assume that HCAs in CS may not contribute to induction.

In addition to HCA, polyaromatic hydrocarbons (PAHs) (Brooks et al., 1999) and nicotine (Iba et al., 1999) present in CS (IARC, 1986) are also known to induce hepatic CYP1A1 and 1A2. Hepatic CYP1A1 was predominantly expressed in rats treated with PAH, but the total PAH concentration in CS is considered to be insufficient to account for the CYP1A1 induction by CS (Koide et al., 1999). In addition, the half-life of pulmonary CYP1A1 induced by CS is similar to that of pulmonary CYP1A1 induced by nicotine, but not by PAHs (Iba et al., 1998). Further, hepatic CYP1A2 is highly induced in Sprague–Dawley rats fed 4.9 mg/kg nicotine for 30 days, while CYP1A1 induction is predominant in the case of a high nicotine dose (15.4 mg/kg) (Iba et al., 1999), in accordance with reported data for the induction pattern by CS (Kawamoto et al., 1993). This is inconsistent with the present findings for CYP1A1 induction by CS, because the amount of nicotine per cigarette used in this study was 2.7 mg, giving an estimated daily dose of ~40 mg/kg/day, based on all of the CS produced being inhaled by the rat. However, in view of the 1500- and 10 000-fold excess of nicotine (0.8–3.0 mg/cigarette) over PAHs and HCAs, respectively, in CS, a potential role of nicotine as a CS constituent in the induction of the CYP1A subfamily cannot be excluded.

Further, CYP1A2 protein and its metabolic activity are highly induced, without induction of other CYP species (Baker et al., 2001). MeIQx at 50 mg/kg corresponds to a daily intake of 300 p.p.m. MeIQx in the feed and is equivalent to 0.23 mmol/kg. The present observations with this i.g. treatment are in agreement with previous findings that i.p. treatment with 0.22 mmol/kg of several HCAs, including MeIQx, induces the hepatic CYP1A subfamily, especially CYP1A2, in F344 rats (Degawa et al., 1989). However, neither CYP1A1 nor 1A2 proteins were induced by gavage of 300 p.p.m. MeIQx for up to 16 weeks, suggesting the existence of a threshold in the daily intake for induction of the CYP1A subfamily by HCA. It has been reported that eight HCAs are detected in CS and the total level amounted to ~0.32 ng per filter-tipped cigarette (Manabe et al., 1991). Based on this level, the daily intake of HCAs from CS in this study is estimated to be <1 ng/rat, being much less than the dose of 50 mg/kg MeIQx. Although the induction pattern of the CYP1A subfamily by CS is the same as that by HCAs, it is reasonable to assume that HCAs in CS may not contribute to induction.

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<th>Group</th>
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<th>UDPGT activity (nmol/min/mg protein)</th>
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<td>1</td>
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<td>3</td>
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<td>7</td>
<td>MeIQx 4w + CS 4w + SS 12w</td>
<td>1.05 ± 0.07 (1.2)</td>
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Each test was carried out with liver microsomes pooled from five rats. The results are expressed as means ± SD of 3–6 experiments. Values in parentheses show the ratio to the activity obtained with Group 1.

*p < 0.01, compared with Group 1 (Student’s t-test).

**p < 0.05, compared with Group 2 (Student’s t-test).
et al., 1987). Weisburger (1999) has reviewed evidence that regular intake of protective foods, such as vegetables, tomatoes and black or green tea, increases the detoxification of HCAs by UDPGT with induction of CYP1A2, and thus provides a lower risk situation in meat eaters. Accordingly, it suggests that CS might have a chemopreventive action against tumorigenesis initiated with HCAs, such as Glu-P-1 and PhIP, which are highly susceptible to glucuronidation in the rat liver.

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