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Modifications By Lifestyle Factors of Metabolic Activation and Detoxification of Environmental Carcinogens in Chemically-Induced Carcinogenesis

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ABSTRACT

To elucidate the mechanism underlying modifications of experimental carcinogenesis by cigarette smoke (CS), ethanol, curcumin, α-naphthyl isothiocyanate (ANIT) and 6-methylsulfinyl isothiocyanate (6-MSITC), cytochrome P450 (CYP) levels, mutagenic activities of environmental carcinogens and UDP-glucuronyltransferase (UDPGT) activities were assayed in hamsters and rats. CS have a bifunctional action on CYP1A2 and UDPGT1A6 in both animal species; suppression of pancreatic carcinogenesis initiated with N-nitrosobis (2-oxopropyl) amine is attributed to an increased detoxification by UDPGT1A6 in hamster liver and enhancement of hepatocarcinogenesis initiated with 2-amino-3,8-dimethylimidazol[4,5-f] quinoxaline to an increase in metabolic activation by CYP1A2, without the contribution of increased detoxification by UDPGT1A6 in rat liver. The mutagenic activation of N-nitroso methylbenzylamine (NMBA) by CYP2A3 were selectively elevated by treatment with 10 or 50% ethanol. Ethanol did not affect three UDPGT activities; the enhancing effect on esophageal carcinogenesis induced by NMBA is explained by increase of the metabolic activation in esophagus. Intragastric treatment with 270mg/kg curcumin in rats decreased the mutagenic activations by esophagus CYP2B1 and CYP2A3; indicating that in suppression of the esophageal carcinogenesis, a decrease in metabolic activation of NMBA also in the target organ play an important role.

The mutagenic activations of five heterocyclic amines (HCAs), including 2-amino-1-methyl-6-phenylimidazol[4,5-b]pyridine (PhIP) by CYP1A2 were highly enhanced by intragastric treatment with 85mg/kg PhIP in female rats, and these enhanced activities of HCAs were significantly decreased in liver S9 from rats treated with PhIP plus feeding of 400 ppm ANIT, and UDPGT1A6 activity was markedly enhanced with the combination; chemoprevention by ANIT of PhIP-induced mammary carcinogenesis can be explained by a reduction in metabolic activation by hepatic CYP1A2 and an enhancement of detoxification by UDPGT1A6. The mutagenic activations of HCAs, aflatoxin B1 and N-nitroso compounds by constitutive CYPs were significantly decreased in the liver of male rats treated with 40 mg/kg 6-MSITC, and similar decreases were observed in the colon. No significant alterations were observed in UDPGT activities in the treated rats; suggesting that the protective effect on 1,2-dimethylhydrazine-induced colonic tumorigenesis may be attributed to a decrease in metabolic activation by colonic CYP2E1. Accordingly, all the lifestyle factors tested clearly modify the mutagenic activations by CYP isoform and/or detoxification by UDPGT in liver and/or target organ, which are closely related with modifications of chemically-induced carcinogenesis. The role of metabolic (in)activations by CYP and UDPGT in the prediction for chemical carcinogenicity is also discussed.

Abbreviations: CS: Cigarette Smoke; 6-MSITC: 6-Methylsulfinyl Isothiocyanate; CYP: Cytochrome P450; UDPGT: UDP-Glucuronyltransferase; NMBA: N-Nitroso Methylbenzylamine; NOCs: N-Nitroso Compounds; HCAs: Heterocyclic Amines; ITCs: Isothiocyanates; DMBA: Dimethylbenz[A]Anthracene; EROD: Ethoxyresorufin O-Deethylase; PROD: Pentoxyresorufin O-Dealkylase; AITC: Allyl ITC; BITC: Benzyl ITC; PEITC: Phenethyl ITC; ACF: Aberrant Crypt Foci; DMH: Dimethylhydratine; BCAC: β-Catenin-Accumulated Crypts; QR: Quinone Reductase; GST: Glutathione S-Transferase; MNNG: N-Methyl-N-Nitroso-Guanidine; BP: Benzo[A]Pyrene; 4-NQO: 4-Nitroquinoline N-Oxide; AOM: Azoxymethane; ENNG: N-Ethyl-N-Nitroso-Guanidine; DEN: N N-Dimethylnitrosamine; DMBA: 7,12-Dimethylbenz[A]Anthracene

Introduction

Lifestyle factors are considered major risk factors in human cancers, and cigarette smoking and consumptions of alcohol and diet contribute to about 30, 8 and 35% of the disease, respectively. Cigarette smoking is closely associated with an increased risk of cancers in many organs, including the lungs, liver, pancreas and esophagus, but attempts to induce lung or other cancers in experimental animals by inhalation of cigarette smoke (CS) have mostly been unsuccessful [1]. Alcohol consumption is mainly associated with cancers of the upper gastrointestinal tract, liver, large bowel, breast, lungs and pancreas, and excessive alcohol drinking is associated with an increased risk of esophageal cancer, a worldwide problem, and 75% of the esophageal cancers in US are attributable to the drinking [2]. Among many kinds of environmental factors, dietary habits have been regarded as the most important determinant for cancer development in humans. Individuals eating fried or broiled meat have a significantly elevated risk of intestinal cancer, and the ones in Japan have rising rates of colorectal, prostate and mammary cancers.

Human cancer has been closely associated with exposure to nitrate and nitrite, which are precursors of carcinogenic N-nitroso compounds (NOCs) [3,4], and exposure to N-nitroso methylbenzylamine (NMBA) is associated with an increased risk of human cancer in the esophagus in China. A number of carcinogenic heterocyclic amines (HCAs) occur in CS and cooked foods, and 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP) is the most abundant HCA at up to 480 ng/g cooked food, being detectable in 10 volunteers living in Tokyo (0.005-0.3 μg/person) and 3563 individuals in the US (0.72-1.11 μg/person). The chemical structures, together with the abbreviations routinely used, of HCAs are shown in Figure 1 and those of NOCs and other carcinogens are in Figure 2. All these carcinogens show mutagenicity in the Ames preincubation assay, and the carcinogenicity of HCAs has been demonstrated in many organs, including liver, colon and mammary of rodents. Environmental carcinogens are not genotoxic and carcinogenic per se, and almost all carcinogens require metabolic activation by cytochrome P450 (CYP) to elicit their carcinogenic effects. On the other hand, increased consumption of protective foods, such as vegetables, fruits and black or green tea, have been consistently associated with a low risk of colorectal cancer.

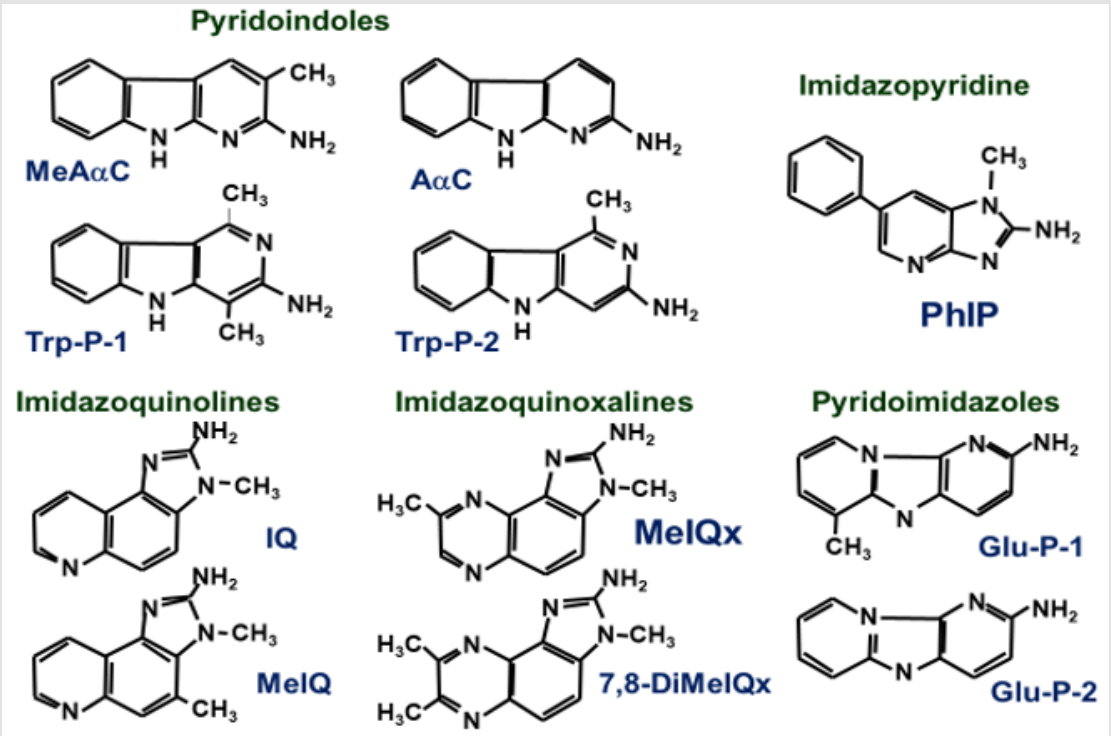

Figure 1: Chemical structures and their abbreviations of carcinogenic heterocyclic amines detected in cooked protein-rich food (food-born carcinogens) and in cigarette smoke.

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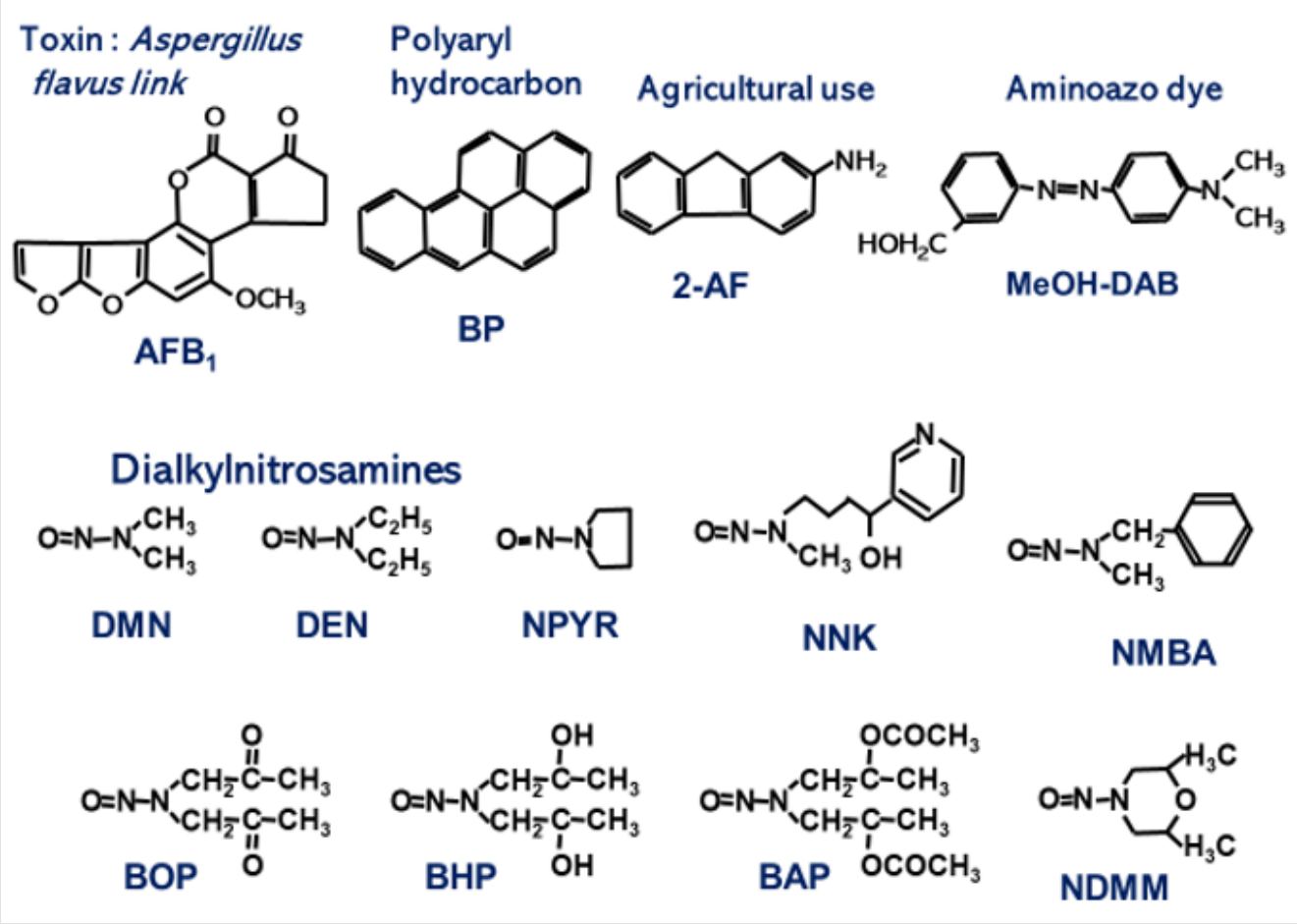
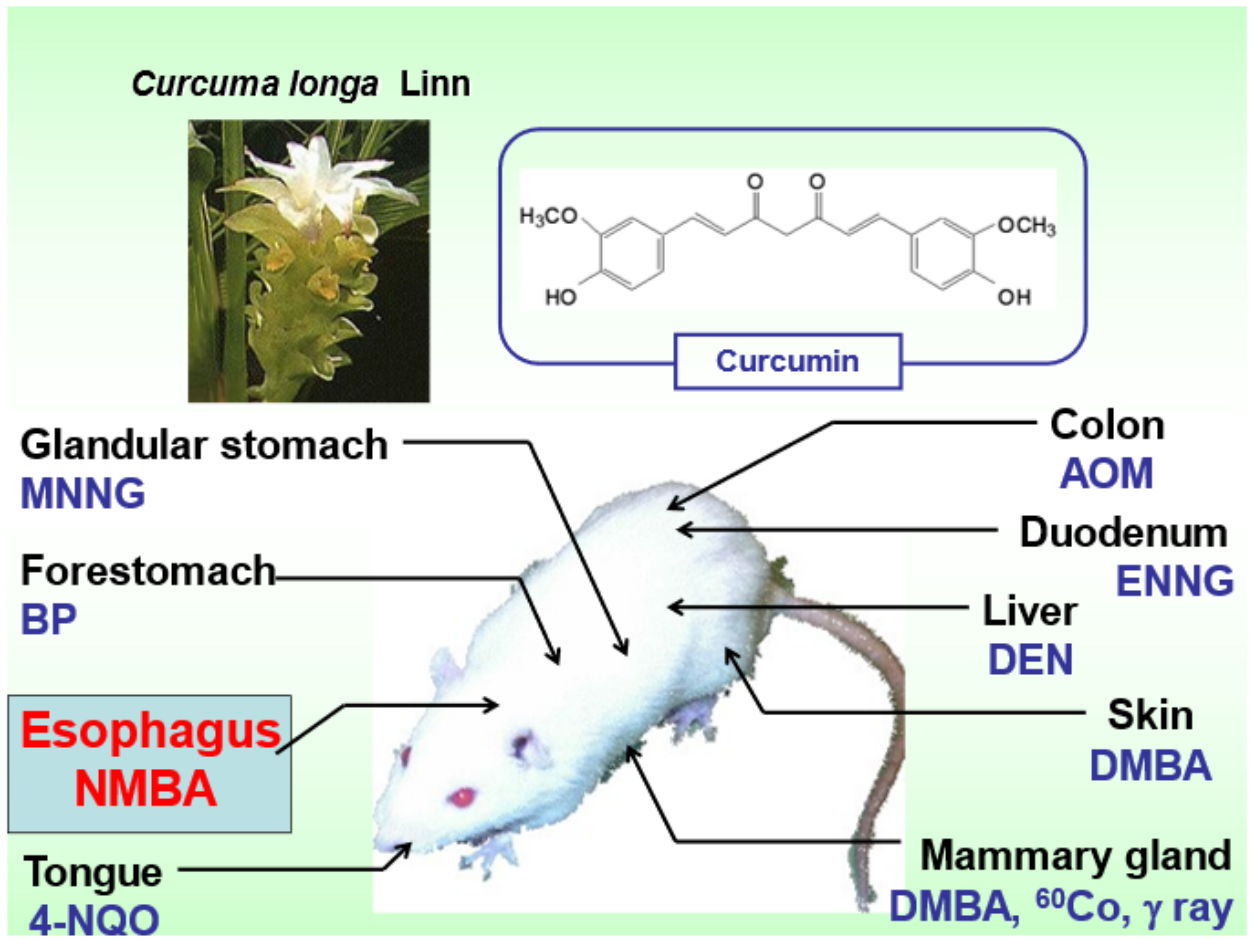


Figure 2: Chemical structures and their abbreviations of dialkylnitrosamines and other environmental carcinogens.

High cruciferous vegetable consumption is inversely related to lung and bladder cancer risk, and the beneficial effects may be attributed to the high content of isothiocyanates (ITCs) which are known to occur in the broccoli, cabbage, califlowers, watercress etc. α-Naphthyl isothiocyanate (ANIT), one of the constituent present as glucosinolate precursor, is the first ITC the chemopreventive effect and is reported to inhibit liver and bladder tumors initiated by 3'-methyl-4-(N, N-dimethyl) aminoazobenzene and N-butyl-N-(4-hydroxybutyl) nitrosamine, respectively [5]. A typical Japanese condiment, wasabi (Wasabia japonica) is considered as a possible source of antimutagenic and anticancer agents, and one particular ingredient, 6-methylsulfinylhexyl isothiocyanate (6-MSITC), may be a candidate chemopreventive agent for human cancers [6]. Curcumin is the major yellow pigment in turmeric, widely used as a spice and coloring agents in several foods, such as curry, mustard and potato chips as well as cosmetics and drugs, and inhibit tumors in various organs of laboratory animals initiated with several carcinogens or radiation [7], as illustrated in Figure 3, together with the chemical structure of curcumin.



Note: MNNG; N-methyl-N-nitroso-guanidine, BP; benzo[a]pyrene, 4-NQO, 4-nitroquinoline N-oxide, AOM; azoxymethane, ENNG; N-ethyl-N-nitroso-guanidine, DEN; N,N-dimethylnitrosamine, DMBA; 7,12-dimethylbenz[a]anthracene.

Figure 3: Chemical structure of curcumin and its suppressive actions for various organs in rats or mice initiated with various carcinogens or radiation during initiation and promotion phases.

Mutagenic Activation of Environmental Carcinogens in Rodents and Humans

Most xenobiotics are known to be substrates for microsomal CYPs and UDP-glucuronyltransferases (UDPGTs). One possible mechanism underlying modification effects is the regulation of metabolic activation and detoxication by phase I and II enzymes, and it is necessary to clarify these reactions as basic information for understanding the modulation mechanism by lifestyle factors of chemically-induced carcinogenesis. For example, Figure 4 illustrates schematic relationship between effects on metabolic activation and inactivation by CYP, UDPGT, sulfotransferase (ST) or acetyltransferase and modifications of experimental carcinogenesis by CS, ethanol, curcumin or 6-MSITC. Metabolic activation of carcinogens is routinely estimated by determination of proximate and ultimate carcinogen or DNA alkylation levels, but it is relatively difficult to determine those levels which are chemically unstable compounds, especially when many carcinogens have to be treated.

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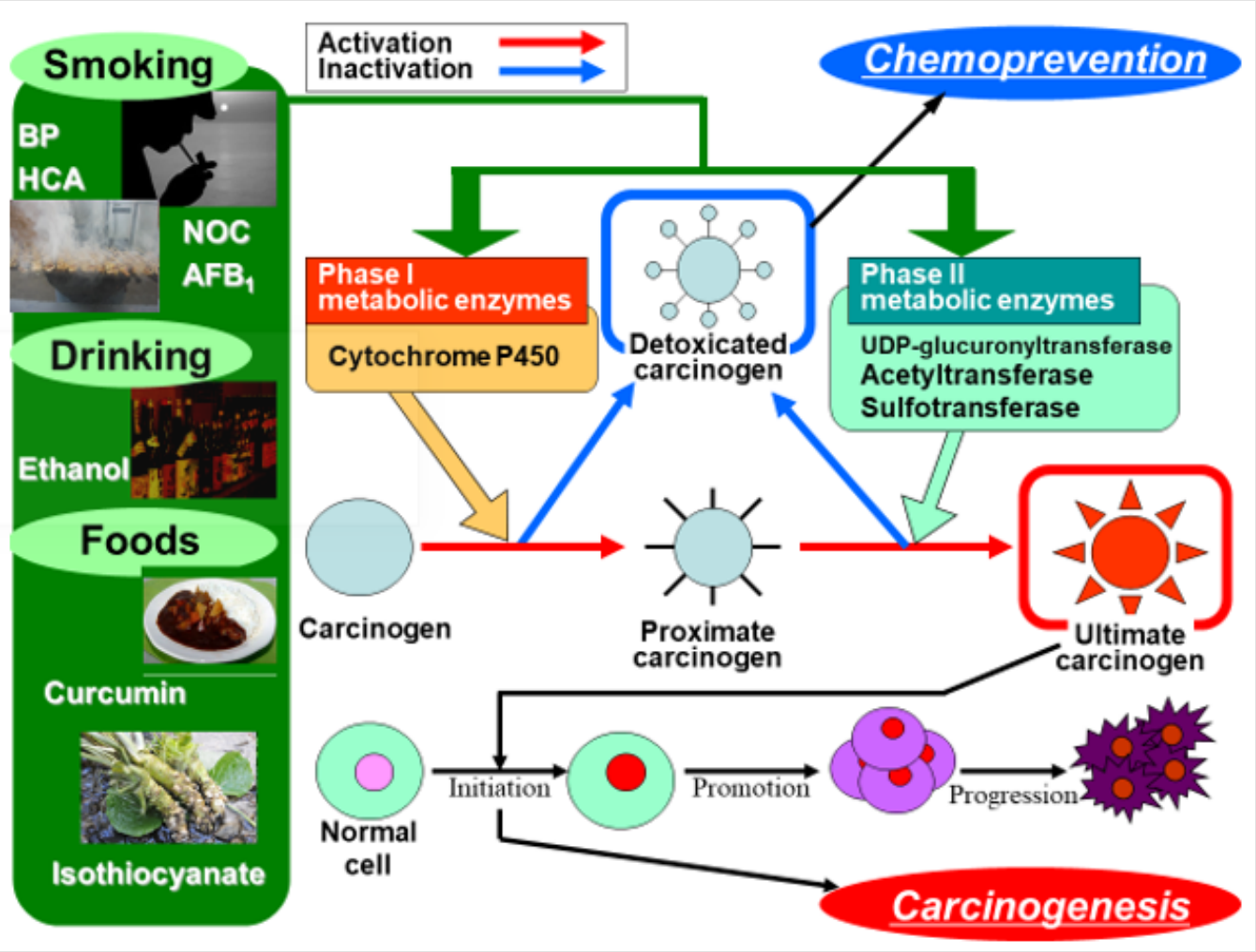

Figure 4: Schema for metabolic activation and/or inactivation of carcinogen by phase I and II enzymes and modifications of chemically-induced carcinogenesis by lifestyle factors used in this study.

In the place of the determinations by some chromatographic technique each, mutation assay with the Ames test in the presence of post mitochondrial (S9) fraction of cells can be used for estimation for the metabolic activation of various xenobiotics, together with assay of CYP isoform levels by Western blotting. Table 1 summarizes various carcinogens in the environment and hepatic CYP species involved in their mutagenic activations. NOCs show a positive mutagenicity in the presence of liver S9 from mice, rats, hamsters and man in S. typhimurium TA100 and HCAs and other carcinogens show in TA98 [8-10]. CYP2A, 2B and/or 2E species are specifically involved in the mutagenic activation of NOCs, and HCAs, except for MeA α C, are predominantly activated by CYP1A2 [11,12]; MeA α C, NNK and AFB1 predominantly by CYP2A subfamily. In the following studies, we assayed the hepatic and extrahepatic levels of microsomal CYP proteins, mutagenic activities in strain TA 98 or 100 of environmental carcinogens in the presence of tissue S9 and three types of hepatic UDPGT or ST activities in hamsters or rats.

Table 1: Environmental carcinogens and hepatic cytochrome P450 (CYP) isoform involved in expression of mutagenicity in Salmonella typhimurium tester strain, respective TA100 and TA98 for N-nitroso compounds (NOCs) and heterocyclic amines (HCAs) and other carcinogens, in the presence of liver S9 from rodents and humans.

Carcinogen	Abbreviation	CYP species
NOCs		
N-nitrosodimethylamine	DMN	2E1
N-nitrosodiethylamine	DEN	2E1
N-nitrosobis(2-acetoxypropyl) amine	BAP	2B1/2
N-nitrosobis(2-hydroxypropyl) amine	BHP	2B1/2
N-nitroso(2-hydroxypropyl) (2-oxopropyl) amine	HPOP	2B1/2
N-nitrosobis(2-oxopropyl) amine	BOP	2B1/2
N-nitroso-2,6-dimethylmorpholine	NDMM	2B1/2
N-nitrosomethylbenzylamine	NMBA	2A/2B
N-nitrosopyrrolidine	NPYR	2E1
4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone	NNK	2A1, 2A8
		1A2, 2B
HCAs		
2-amino-6-methyldipyridol[1,2-*a*:3′,2′-*d*]imidazole	Glu-P-1	1A2
2-aminopyridol[1,2-*a*:3′,2′-*d*]imidazole	Glu-P-2	1A2
2-amino-3-methylimidazol[4,5-*f*] quinoline	IQ	1A2
2-amino-3,4-dimethylimidazol[4,5-*f*]quinoline	MeIQ	1A2
2-amino-3,8-dimethylimidazol[4,5-*f*]quinoxaline	MeIQx	1A2
2-amino-1-methyl-6-phenylimidazol[4,5-*b*] pyridine	PhIP	1A2
3-amino-1,4-dimethyl-5*H*-pyrido [4,3-*b*]indole	Trp-P-1	1A2
3-amino-1-methyl-5*H* -pyrido [4,3-*b*]indole	Trp-P-2	1A2
2-amino-3-methyl-9*H*-pyrido [2,3-*b*]indole	MeA*a*C	1A1,2A8
Others		
aflatoxin B_1	AFB_1	2A8, 2B1,
		3A2
2-aminofluorene	2-AF	1A1/2
benzo[*a*]pyrene	BP	1A1
3′-hydroxymethyl-*N*, *N*-dimethyl-4-aminoazobenzene	MeOH-DAB	1A1/2

Modifications of Experimental Carcinogenesis and Metabolic Activation or Inactivation

Effect of CS on Mutagenic Activation, CYP Enzyme and UDPGT or ST Activities

Hepatic CYP1A2 content in smokers is shown to be 3.5-fold higher than that in non-smokers. We have shown that, in addition to CYP1A2 (3.0-fold above the control), CS exposure for 1 or 2 weeks induce CYP1A1 (to almost the same level of the constitutive CYP1A2) in the rat liver, but not in the hamster liver (CYP1A2; 3.9-fold above the control), in spite of 3 times higher exposure conditions than the case of rats, without significant change in the levels of CYP2E1, CYP2B and CYP3A in each species [8,9]. Hamburg type II smoking machine (Borgwardt, Hamburg, Germany) used in this study and the different conditions of CS exposure between rats and hamsters are illustrated in Figure 5. The enhancing effect of CS on CYP1A isoform in hamsters is the same as that in human; the hamster is a good model for studies on metabolic activation by CYP1A2 [13,14]. It has been shown that human liver has a potent activating capacity for PhIP, and caffeine shows to induce CYP1A2 and to enhance PhIP-induced carcinogenesis and mutagenic activation of various HCAs and BP.

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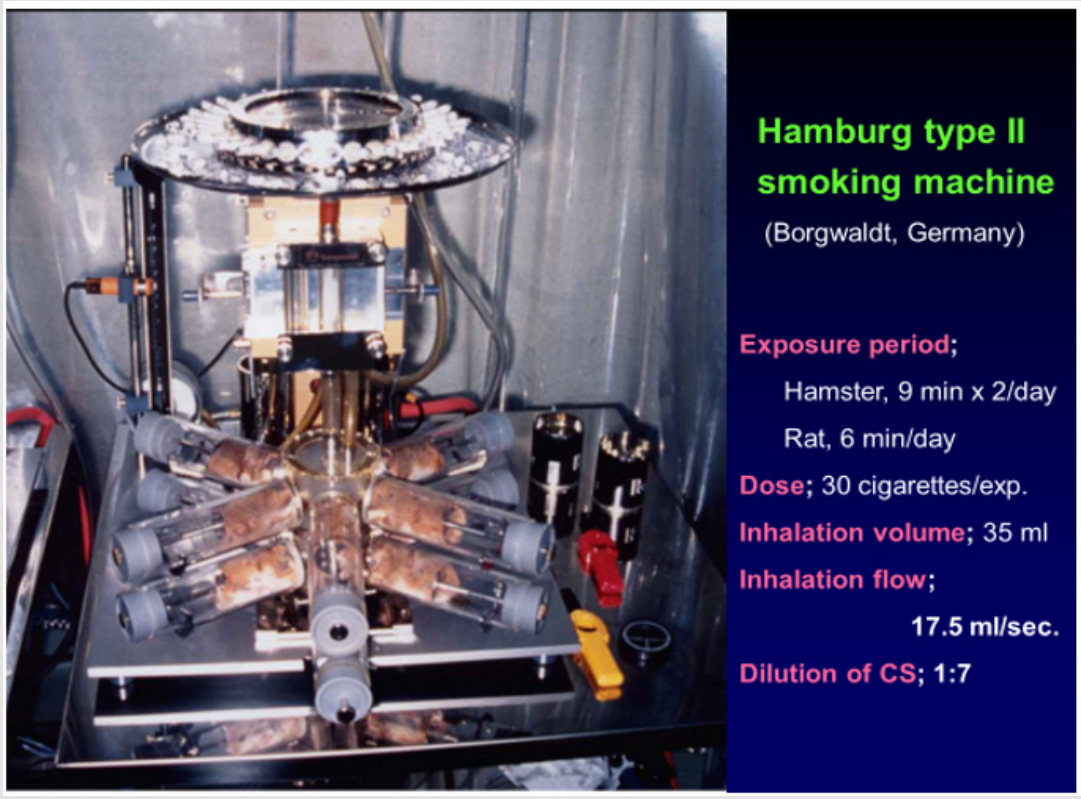

Figure 5: Ten Syrian golden hamsters exposed in the smoking machine and the different conditions of smoke generation between hamsters and rats.

These findings indicate that CS markedly stimulates metabolic activation of various food-derived carcinogens, which may contribute to the overall carcinogenic effects of cigarette smoking, and the smoking in combination with intakes of caffeine and heating protein-rich foods as a life style may contribute to human carcinogenesis by HCAs. The tobacco-specific NNK is mainly activated by CYP2A in rats, mice and humans, and CYP2A6 gene deletion due to genetic polymorphism was reported to reduce lung cancer risk among tobacco smokers in Japan and oral cancer risk in betel quid chewers in Sri Lanka. However, no data have been obtained on the influence of CS on CYP2A expression and its metabolic activity in any animal species. Meanwhile, CS is known to induce hepatic UDPGT1A6 activity in rats, mice and humans, suggesting that CS induces inactivation of carcinogens by glucuronidations. N-OH-HCAs and NOCs are known to be substrates for UDPGTs, and the human UDPGT1A and 2B subfamilies have been shown to be involved in glucuronidation of N-OH-PhIP. Three major ST isozymes catalyze sulphation of phenol (aryl STs) or alcohol (hydroxysteroid ST) in rats, and CS extract inhibits human ST activities in HepG2 cells and 3-methylchoranthrene(3-MC) markedly suppress ST2A2mRNA expression in the rat liver. We have shown that dehydroepiandrosterone (DHEA) sulphate, a hydroxysteroid ST (ST2A) inhibitor, significantly suppresses BOP-induced pancreatic carcinogenesis in hamsters, suggesting that sulphation of BOP metabolite is activation step in the initiation events [15].

Accordingly, it is suggested that CS might inhibit the carcinogenic action of BOP by both either enhancing glucuronidation or suppression sulfation. However, no data have been provided regarding the effect of CS on UDPGT1A1, 1A6 and 2B6 or on the three STs in either rats or hamsters. Nevertheless, the most definite differences between hamsters and rats were observed in conjugation with phase II enzymes; the glucuronidation of β -oxypropylnitrosamines is higher in rats than in hamsters and vice versa for sulfation. It has been shown that CS exposure increases the incidence of spontaneous lung tumors in A/J mice and promotes upper respiratory tract tumorigenesis in hamsters initiated with DEN; CS shows its modifying effects on endogenous and exogenous carcinogens. It is known that over 4000 xenobiotics are detected in CS and about 200 carcinogens and many promotors are present. Several constituents in CS are illustrated in Figure 6, together with major risk factors for human lung cancer. CS and its condensate contain various chemopreventive agents such as antioxidants, cembrane-type diterpenes, carotenoids and polyphe-

nol, in addition to many mutagenic, carcinogenic and cocarcinogenic substances such as polycyclic aromatic hydrocarbons (PAHs), HCAs, NOCs and norharman, and CS condensate and some constituents are reported to exhibit anti-mutagenicity. Nevertheless, influences of CS, but not the condensate or its constituents, on experimental tumorigenesis in other organs remained to be elucidated. We have shown that CS exposure inhibits pancreatic carcinogenesis induced by BOP when given in the initiation phase in Syrian golden hamsters [16], whereas CS enhances hepatocarcinogenesis when given in that in F344 rats induced with MeIQx [17].

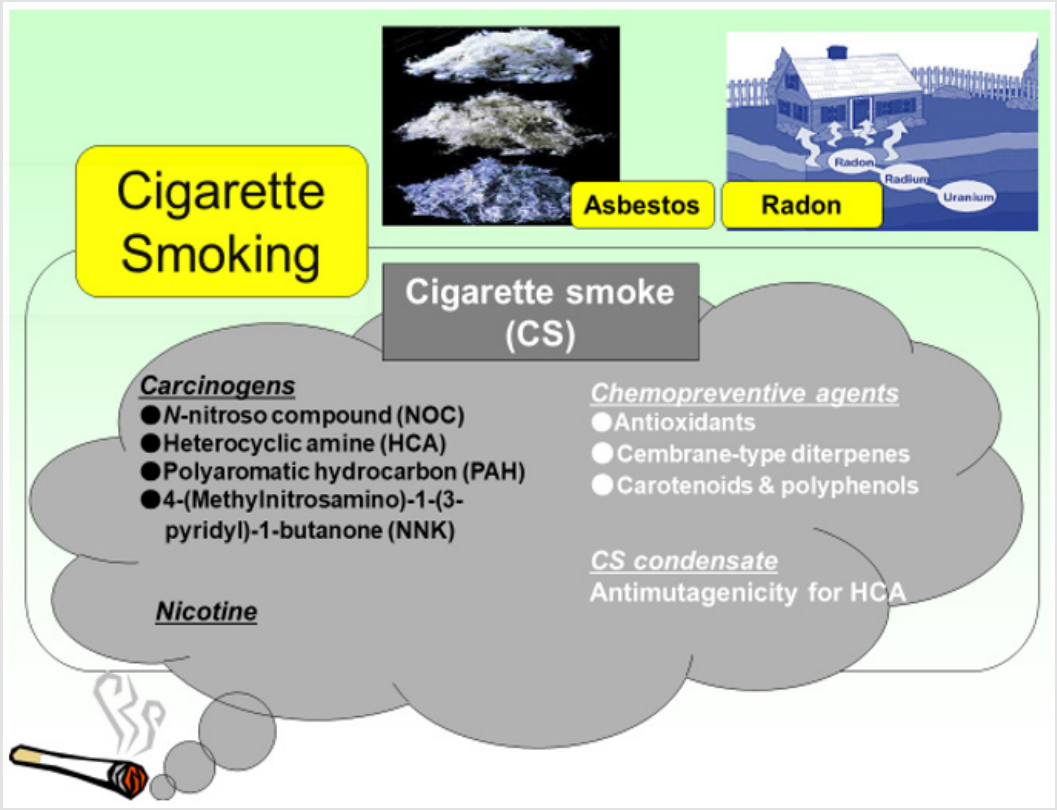

Figure 6: Several carcinogens and chemopreventive agents found in cigarette smoke, together with major risk factors for human lung cancer.

Inhibition of BOP- Induced Pancreatic Carcinogenesis in Syrian Golden Hamsters: CYP2A, 2B and 2E are known to play important roles in the activation of carcinogenic NOCs, including BOP. We have demonstrated that BOP-related NOCs are mutagenetically activated by phenobarbital (PB)-inducible CYP2B species [13,14]. In order to clarify modification of CYP2A, hepatic level of coumarin 7-hydroxylase (COH) activity was also assayed in male Syrian golden hamsters and F344 rats exposed to CS [1]. As summarized in Figure 7, constitutive CYP1A2 and CYP2A8 levels were increased to 2.6-and 4.0-fold increases, respectively, in hamsters and CYP1A1 and CYP1A2 (3.9-fold increase) were induced in rats following the CS exposure for 4 weeks; the enhancement of rat CYP1A2 was 4.9 times that of CYP1A1. The CS exposure enhanced the mutagenic activities of four HCAs in the presence of liver S9 in both species, and those of MeA α C, NNK and AFB1 with the rat liver S9 were not enhanced by CS, whereas those with the hamster liver S9 were markedly enhanced. However, the activities of other carcinogens, including BP, BOP, HPOP and BHP, were not induced by CS in either species. On the other hand, UDPGT activity towards 4-nitrophenol (4-NP) was enhanced by CS to 1.9- to 2.0-fold above the control, but not those towards bilirubin and testosterone, and three STs did not altered in both species. The degree of hamster CYP1A2 and rat CYP1A1/2 inductions are almost the same extent as that which occurs when both animals are exposed to CS for 2 weeks [13,14], and in rats exposed for 1 or 16 weeks [18]. Marked induction by CS of CYP2A8 but not CYP2A1/2, was consistent with the results on the induction of hepatic COH activity by CS.

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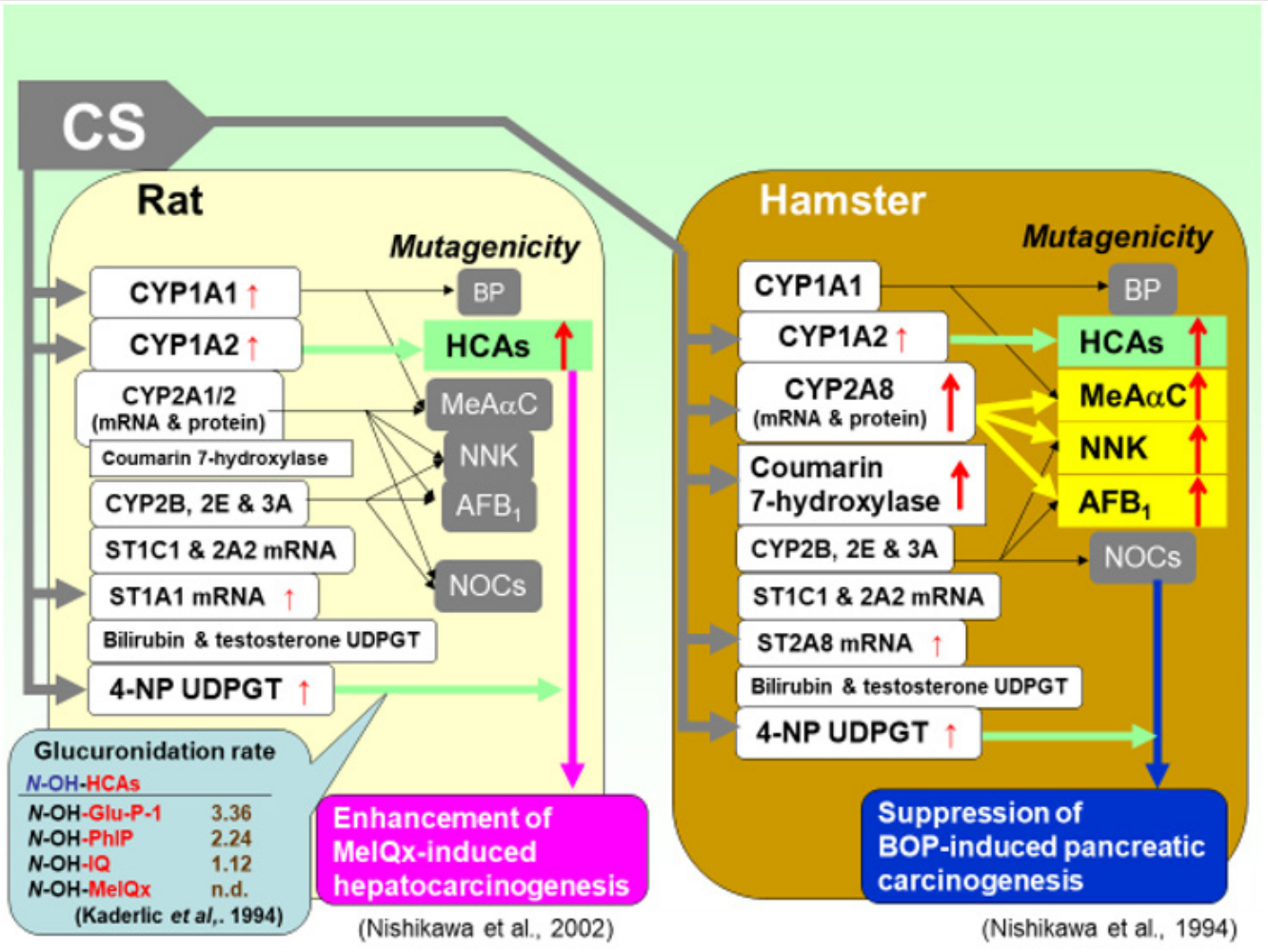


Figure 7: Enhancements by cigarette smoke (CS) of hepatic levels of CYP isoforms, coumarin 7-hydroxylase activity, mutagenic activities of various carcinogens, sulfotransferase (ST)mRNA or UDP-glucuronyltransferase (UDPGT) in rats and hamsters 4 weeks after CS exposure, together with glucuronidation rates with N-OH-heterocyclic amines (HCAs) in rats.

It is shown that CS inhalation triggers activation of the aryl hydrocarbon receptor (AhR)-xenobiotic response element (XRE) pathway; the region upstream of the CYP2A8 gene contains a positive regulatory element motif, which potentiates the activation of XRE. Together with the findings that it is not CYP1A1 but CYP2A8, selective CS induction of CYP1A2 and 2A8 in the hamster liver might be due to the AhR-XRE pathway activation. The NF-E2 p45-related factor (NRF2) regulates the transcription of several phase II enzymes, including UDPGT, and the NRF2 expression is also transcriptionally regulated by the AhR-XRE signaling pathway. In conjunction with the findings on induction of UDPGT1A6 activity in both species, CS might enhance both metabolic activation and inactivation via the AhR and NRF2 pathways. The mutagenic activity of MeA α C was markedly inhibited by methoxsalen and 7,8-benzoflavone (7,8-BF), but not by furafylline; indicating that the enhancing effect of CS on the activation of MeA α C is mainly performed by elevating CYP2A8 level [11,12]. It has been reported that 3-MC-inducible CYP2A8 has a potent capacity for activating AFB1, up to 170-fold higher than that of CYP1A2. Together with the potent inhibition by 7,8-BF rather than methoxsalen, mutagenic activation of AFB1 may mainly be attributed to hepatic CYP2A8, and the inhibition tests indicated the involvement of CYP1A2, 2A8 and 2B, but not CYP2E, in mutagenic activation of NNK with the hamster liver. The mutagenic activities of DEN and four pancreatic carcinogens were not induced by CS with liver S9 from hamsters and rats, reflecting no changes in levels of CYP2E1 and 2B proteins. Similarly, the activities of BHP, HPOP and NDMM with liver S9 from hamsters exposed to CS were markedly inhibited by metyrapone, but not methoxsalen and 4-methylpyrazole, indicating the contribution of CYP2B, but not CYP2A8 and 2E1, to mutagenic activities of BOP-related carcinogens. It is known that HPOP, N-OH-HCAs and a major metabolite of NNK are

substrates for UDPGTs. Since CS exposure clearly increased in hepatic 4-NP UDPGT activity with hamsters and rats, it is suggested that CS might detoxify many environmental carcinogens, through enhancement the excretion of carcinogens, resulting in decreased carcinogen DNA interaction and carcinogenesis. This induction is in line with previous findings in smokers, mice and rats, and glucuronidation of HPOP by the rat liver is reported to be clearly higher than that by the hamster liver both in vivo and in vitro, which is associated with the species difference in BOP-induced pancreatic carcinogenesis between hamsters and rats. Together with the contribution of UDPGT1A species to NOC, glucuronidation of NOCs is critical in detoxification and CS may suppress BOP-induced carcinogenesis partly through induction of hepatic UDPGT1A6 activity.

Since BOP-induced pancreatic carcinogenesis is suppressed by hydroxysteroid ST (ST2A) inhibition, it seems reasonable that ST2A2 candidate enzyme suppress BOP-induced carcinogenesis by CS. However, no significant differences were observed in the three ST activities including DHEA ST following CS exposure for 4 weeks in either species; further investigation is required to elucidate the effect of CS on ST expression. Consequently, it is concluded that suppression of BOP-induced pancreatic carcinogenesis in hamsters exposed to CS may be attributed to increased detoxification by UDPGT1A6 and not to decreased activation by CYP2B and ST2A2. Nevertheless, this is the first study to demonstrate that CS induces CYP2A species known to play an important role in lung and oral cancers among smokers; also suggesting that the hamster is a suitable animal species for studying CS-induced carcinogenesis. Furthermore, it seems reasonable to consider that CS might enhance carcinogenesis induced with MeAαC, NNK and AFB1 in hamsters through induction of CYP2A species. These observations show that CS is a bifunctional inducer for drug-metabolizing enzymes and a double-edged sword for modification of carcinogenesis. Thus, it is interestingly proposed that investigation into the modifying effect of CS on carcinogenesis induced by Glu-P-1 or PhIP, which are activated by CYP1A and inactivated by UDPGT, should be conducted.

Enhancement of MeIQx-Induced Hepatocarcinogenesis in F344 Rats: Hepatic CYP1A1/2 are known to be selectively involved in metabolic activation of various HCAs, including MeIQx in humans and rats [13,14]. Oral administration of MeIQx for 7 years causes no induction of neoplastic lesions in liver, colon, lung and gastrointestinal tract of cynomolgus monkey, lacking hepatic CYP1A2 expression. Further, it has been shown that modifying effects on IQ or PhIP-initiated carcinogenesis is closely associated with metabolic activation by the CYP1A subfamily. Immunoblotting analyses of liver microsomes revealed inductions of CYP1A1 and 1A2 (2.3- to 2.7-fold), but not CYP2B1/2, 2E1 or 3A2 in male F344 rats exposed to CS for 1, 12 or 16 weeks [18]. The mutagenic activities of MeIQx and five other HCAs were elevated 1.4-to 3.7-fold by this CS exposure, but not those of MeAαC, BP and AFB1 in strain TA98 and those of DMN, NPYP and NNK in strain TA100. Similarly, intragastric administration of 50 or 100 mg/kg MeIQx in a single dose selectively increased CYP1A1 and 1A2 (2.6-fold) levels and mutagenic activities of five HCAs (1.7- to 3.3 -fold), but not BP, although feeding 300 ppm MeIQx in the diet for 1 or 16 weeks produced no significant alterations in these CYP levels and mutagenic activities. In contrast, feeding of MeIQx for 16 weeks enhanced UDPGT activities towards 4-NP and testosterone to 2.9- and 1.5- fold, respectively, but not towards bilirubin, while CS exposure induced 4-NP UDPGT (1.6-fold); combined treatment with CS and MeIQx showed a summation effect on UDPGT1A6 activity (3.5-fold). The mutagenicities of MeAαC and BP were not enhanced by these treatments, indicating insufficient induction of the CYP species responsible.

DMN and NPYR are mutagenetically activated by rat CYP2E1, BHP and NDMM by 2B1/2, and AFB1 by CYP2B1 and 3A2 [8,9,13,14]. Therefore, it is reasonable that the mutagenicities of the seven carcinogens with liver S9 from CS- and/or MeIQx-treated rats were not enhanced, reflecting no induction of hepatic CYP2A, 2B1/2, 2E1 and 3A2. NNK is metabolically activated by hepatic CYP1A2, 2A1 and 3A2 in rats and a potent and wide CYP inducer, N-benzylimidazole considerably enhances the mutagenic activity of NNK in rat liver [11,12]. Therefore, 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; indicating that a higher exposure to CS predominantly induces CYP1A1 rather than CYP1A2. In addition to CYP1A, CYP2B1/2 and its metabolic activity are also induced in rats and CYP2E1 and 3A11/13 and the metabolic activities specific to these CYP isoforms are clearly induced in mice exposed to mainstream CS. In the present study, CYP1A2 was preferentially Induced by CS exposure for 1, 12 and 16 weeks to almost 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 [1,14]. 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 and side or main stream of CS, smoking apparatus, etc. However, our results are consistent with the finding in smokers, that CYP1A2 and its metabolic activity are highly induced, without induction of other CYP species.

A daily intake of 300 ppm MeIQx in the feed corresponds to 50 mg/kg MeIQx which is equivalent to 0.23 mmol/kg, and this intragastric treatment are in agreement with previous findings that intraperitoneal treatment with 0.22 mmol/kg of several HCAs, including MeIQx, induces the hepatic CYP1A subfamily, especially CYP1A2, in F344 rats. However, no induction of CYP1A proteins were observed in gavage of 300 ppm MeIQx for up to 16 weeks, suggesting the existent 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 up to 0.32 ng per filter-tipped cigarette. Based on this level, the daily intake of HCAs from CS in this study is

estimated to be under 1 ng/rat, being much less than the dose of 50 mg/kg MeIQx. Although the induction pattern of the CYP 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 HCAs, PAHs and nicotine present in CS are also known to induce hepatic CYP1A1/2, and hepatic CYP1A1 is 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. Further, hepatic CYP1A2 is highly induced in Sprague-Dawley rats fed 4.9 mg/kg nicotine for 30 days, while CYP1A1 induction is predominantly in the case of a high nicotine dose 15.4 mg/kg, in accordance with reported data for the induction pattern by CS. This is inconsistent with the present findings for CYP1A induction by CS, because the amount of nicotine per cigarette used in this study was 2.7 mg, giving an estimated daily dose of up to 40 mg/kg/day, based on all the CS produced being inhaled by the rat. Although the 1500- and 10000-fold excess of nicotine (0.8-3.0mg/cigarette) over PAHs and HCAs, respectively, in CS, a potential role of nicotine as a CS constituent in the induction of the CYP1A subfamily may be excluded.

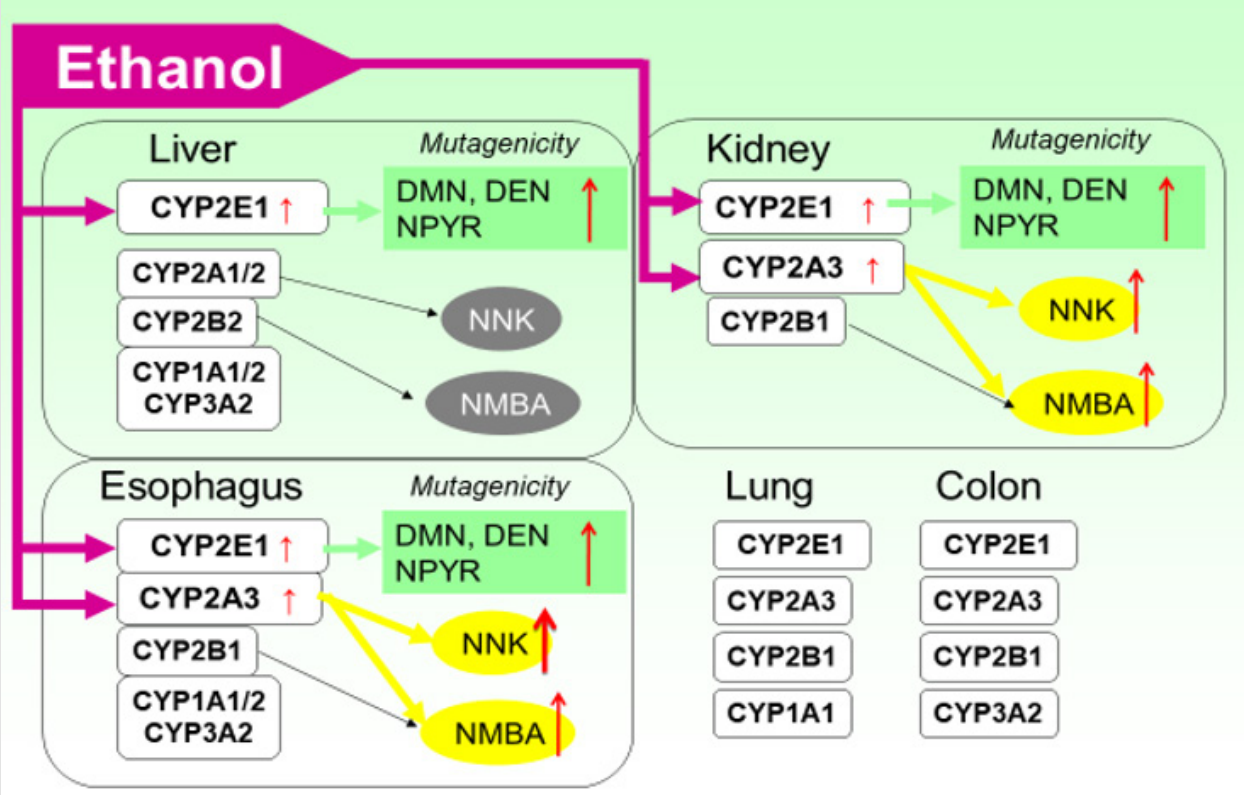
Formation of PhIP-DNA adduct and unscheduled DNA synthesis are increased in hepatocytes pretreated with the glucuronidation inhibitor D-galactosamine, indicating that N-glucuronidation of HCA is an important detoxification reaction. Rat UDPGT2B1 and rat, rabbit and human UDPGT1A6 are known to catalyze the glucuronidation of primary and/or secondary amines. Furthermore, it is suggested that human UDPGT2B isoforms are involved in glucuronidation of N-OH-PhIP, in addition to UDPGT1A species. Hepatic UDPGT2B1 and 1A6 activities were clearly increased in rats fed MeIQx for 16 weeks, without enhancement of UDPGT1A1 activity, suggesting the involvement in glucuronidation of N-OH-HCAs; anyway, this is the first study documenting induction of UDPGT activities by HCAs. On the other hand, induction by CS of UDPGT1A6 activity in rat liver microsomes is in line with previous findings in smokers and mice. As shown in Figure 7, the N-glucuronide of N-OH-MeIQx cannot be detected at all in rat liver, whereas much higher glucuronidation rates were observed in other HCAs. In fact, N-OH-PhIP-glucuronide is a major metabolite in bile (up to 45% of biliary metabolites) of F344 rats i.v. treated with PhIP, while N-OH-MeIQx-glucuronide is scarcely detected in bile of Sprague-Dawley rats orally treated with MeIQx. Consequently, 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, without the contribution of increased detoxification by UDPGT1A6 in rat liver. The present study has demonstrated that CS and MeIQx have a bifunctional action for phase I and II enzymes, with similar induction patterns of specific CYP proteins, mutagenic activities and UDPGT activity in rats. Therefore, the action of CS on metabolism apparently plays a dual role in the handling of and protection against environmental carcinogens. It has been reviewed evidence that regular intake of protective foods increases the detoxification of HCAs by UDPGT with induction of CYP1A2, and thus provides a lower risk situation in meat eaters. Again, 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.

Enhancement by Ethanol of NMBA-Induced Esophageal Carcinogenesis in F344 Rats

Administration of ethanol in the drinking water to F344 rats shows an enhancing effect on DEN-induced esophageal carcinogenesis during the initiation phase. We have shown that NMBA-induced esophageal tumorigenesis is weakly enhanced by treatment of ethanol in F344 rats [19]. Since ethanol per se is not genotoxic and carcinogenic, its effects may be seen also in terms of its modifying the effects of exogenous carcinogens. Various molecular mechanisms on the ethanol action are shown, including the genotoxic effects of acetaldehyde, the production of reactive oxygen species and the inhibition of S-adenosyl-L-methionine synthesis. Further, ethanol is well-known to be a CYP2E1 inducer in rodents, and the metabolic activation of environmental carcinogens by CYP2E1 is one of the routes by which ethanol exerts its carcinogenic action. Rat CYP2E1 predominantly activates DMN, DEN and NPYR, all of which occur in CS, whereas CYP2B and 2A activate NOCs possessing relatively long alkyl chains to produce proximate or ultimate carcinogens. Ethanol treatment increases the alkylation of esophageal DNA by DEN, but not that of liver and kidney DNA, in female Wistar-derived rats, indicating the importance of metabolic activation of DEN by esophagus CYP in esophageal tumorigenesis. UDPGT1A1, 1A6 and 2B1 are found as major enzymes which are inducible clofibrate, 3-MC and PB, respectively, in rat liver. It has been reported that chronic ethanol treatment reduces UDPGT2BmRNA, the protein levels and UDPGT activity in the rat liver, and UDPGT2B1 is likely to be the enzyme responsible for the glucuronidation of DEN and N-nitrosomethyl-n-pentylamine.

These results suggest that ethanol increases NOC-induced carcinogenesis by enhancing its metabolic activation or by suppressing its inactivation. NMBA is known to be the most potent carcinogens in the rat esophagus, and this is irrespective of its mode of administration. The metabolism of NMBA is inhibited up to 95% by CO and 70% by SKF-525A, indicating that there is a relationship between NMBA activation and CYP activity. We have shown that in a PB-induced rat liver, NMBA is mutagenetically activated by CYP2B1/2, but not by CYP2E1. In contrast, CYP2A3 or 2E1 is reported to contribute to the metabolic activation of NMBA, and the activation is observed not only in the liver but also in the esophagus, despite that the total CYP content in esophageal microsomes is only 7% of that in liver microsomes. This mucosal microsomes from male Sprague-Dawley rats can produce benzaldehyde and formaldehyde from NMBA at rates of 1/5 and 1/60 of their respective hepatic levels, but the O6-methylated guanine level in rats treated with NMBA is six times higher in esophageal DNA than in hepatic DNA. In addition, ethanol is known to increase the total CYP content and CYP2E1 in the rat esophagus, suggesting

that metabolic activation in the target organ may play an important role in ethanol-induced carcinogenesis. However, only limited data exist about the effect in extrahepatic tissues such as rat esophagus. As summarized in Figure 8, five weeks of treatment with 10% ethanol added to the drinking water or intragastric treatment with 50% ethanol twice, both resulted in elevated hepatic levels of CYP2E1 (up to 1.5- to 2.3-fold) and the mutagenic activities of DMN, DEN and NPY (1.5- to 2.4-fold) [2,20].



Note: Ethanol does not induce constitutive CYP proteins in the lung or colon, and UDPGT1A1, 1A6 and 2B1 activities in the liver.

Figure 8: Selective induction by ethanol of the mutagenic activation of N-nitroso compounds in relationship with responsible CYP isoforms in five organs from F344 rats Ethanol does not induce constitutive CYP proteins in the lung or colon, and UDPGT1A1, 1A6 and 2B1 activities in the liver.

This was not the case with CYP2A1/2, 2B1/2, 1A1/2 or 3A2, nor with the activities of IQ, Trp-P-2, AFB1 or other NOCs, including NMBA and NNK. This elevations of CYP2E1 and CYP2A3 were observed in the esophagus (up to 1.7- and 2.3-fold) and kidney (up to 1.5- and 1.8-fold), but not in the lung or colon. The mutagenic activities of NMBA and four NOCs, including NNK, in the presence of esophagus or kidney S9 were significantly increased (1.3- to 2.4-fold) in treated rats. The application of CYP inhibitors revealed that CYP2A3 were likely to contribute to the enhancing effect of ethanol on NMBA activation in both organs, but that CYP2E1 failed to do so. In addition, ethanol did not affect three UDPGT activities, and no significant alterations were produced by subcutaneous treatment with 0.5 mg/kg NMBA three times per week for 5 weeks. This NMBA treatment also produced no obvious effects on any CYP levels and mutagenic activation by liver S9, confirming by our previous findings that no significant effects are observed in these expressions and activities by NMBA, BOP or N-butyl-N-(4-hydroxybutyl) nitrosamine [1,7,20,21]. Therefore, it is suggested that in the ethanol enhancing effect, activation of NMBA by esophageal CYP2A3 may play a key role, and this occurred independently of CYP2E1. The results on a 5-week treatment with 10% ethanol administered via drinking water are in consistent with our previous report [20], which demonstrated the mutagenic activation of DMN, DEN and NPYR by CYP2E1 after a 2-week treatment with ethanol. However, the levels of other hepatic CYPs and the activation of NMBA were not induced even 5-week treatment.

The mutagenic activity of NMBA with liver S9 from ethanol-treated rats was significantly inhibited by metyrapone, but not by 4-methylpyrazole; this supports our previous finding that NMBA was mutagenetically activated by hepatic CYP2B2, but not by CYP2E1 in PB-induced rats [19]. Together with the fact of the three forms of hepatic UDPGT, it is reasonable to suppose that ethanol does not affect

the activation or detoxification by these enzymes in rat liver. Conflicting results have been reported for the induction of UDPGT1A6 and 1A1 activities by ethanol;

1. Enhancing the MC-inducible activity in female Sprague-Dawley rats but not in male Wistar rats,
2. Enhancing the clofibrate-inducible activity in Wistar rats but not in Sprague-Dawley rats and humans.

Moreover, the enhancing of hepatic mRNA levels is reported for UDPGT1A1 and 2B1, but not UDPGT1A6, in male Sprague-Dawley rats. Since the 4-NP UDPGT activity in ethanol-treated male F344 rats is in agreement with previous findings on the same substrate, as is the mRNA level, it is concluded that ethanol has no effect on UDPGT1A6 activity in male rats. On the other hand, lack of UDPGT1A1 induction is consistent with findings in Sprague-Dawley rats, whereas lack of UDPGT2B1 induction is not in accord with previous findings for the mRNA in Sprague-Dawley rats. The reasons for these discrepancies are not clear and remain to be clarified. Nevertheless, our findings on hepatic UDPGT activities suggest that ethanol does not modify DEN- or NMBA-induced esophagus carcinogenesis via detoxification by these enzymes in rats. It is shown that ethanol enhances not only esophageal cancer but also pulmonary, renal and colorectal cancer in rodents. Ethanol treatments caused an increase in CYP2E1 level in esophageal and renal tissue, but not in the lung or colon, in consistent with other findings in which rats were fed with liquid diets containing ethanol. In contrast, it has been reported that pulmonary and colonic CYP2E1 is induced by ethanol treatment in rats.

The induction of CYP2E1 by ethanol can be attributed to protein stabilization by protection from cytosolic degradation, and furthermore, the ethanol induction of renal and hepatic CYP2E1 involves an additional transcriptional component when blood ethanol concentrations exceed a threshold value of 200-300 mg/dl. Therefore, this discrepancy may be due to lower ethanol induction of CYP2E1 in the lung (1.2- to 3.0-fold) compared with that in the kidney (5.0- to 9.0-fold). Together with reports that ethanol does not produce an increase in colonic CYP2E1, induction in the lung and colon may be undetectable under these experiments. CYP2A1/2 are predominantly expressed in the liver of rats, whereas CYP2A3 is in extrahepatic tissues, and esophageal and renal CYP2A3 were significantly increased by intragastric treatment with 50% ethanol, supporting previous findings that renal CYP2A3 was increased 1.9-fold by a combination of ethanol and a low-fat diet. However, clear enhancement of CYP2A3 was not observed in these tissues from rats treated with 10% ethanol in the drinking water; the reason for this discrepancy is entirely unknown, but ethanol induction of NMBA activation in rat esophagus performed on the initiation phase. Although other researchers have reported that CYP2A3mRNA was not detected in the kidney of untreated rats, we tested CYP2A activity in the rat kidney by using the inhibitory effect of coumarin and methoxsalen on the mutagenic activity of NMBA in the presence of kidney S9. Additionally, this is the first report in which ethanol has been shown to induce esophageal CYP2A protein in rodents. It is shown that esophageal CYP2A3 plays an important role in the metabolic activation of methyl-n-amylnitrosamine, DEN and NMBA, suggesting that ethanol may enhance the esophageal carcinogenesis that is initiated by these carcinogens.

Expression of the CYP2A3 gene is subject to a complex regulatory mechanism that varies from tissue to tissue. In conjunction with the finding that ethanol did not induce hepatic CYP2A1/2, further investigation of the mechanism of tissue-specific ethanol induction of CYP2A subfamily is required. Ethanol enhancement of the mutagenic activities of DEN, DMN and NPYR with tissue S9 was observed in the esophagus, kidney and liver, in addition, ethanol increased the mutagenicity of NMBA and NNK in the presence of esophageal or renal S9. These results are consistent with the finding that an addition of 5% ethanol to drinking water increases the mutagenicity of NMBA in the rat esophagus. Those are also in agreement with reports that NMBA-induced esophageal carcinogenesis is promoted by ethanol in drinking water or liquid diets containing ethanol. This enhancement would be associated with increases not in CYP2E1 but in CYP2A3 because this activation was suppressed by coumarin and methoxsalen but not 4-methylpyrazole. This is in accordance with findings that the CYP2A3 enzyme, expressed in baculovirus, catalyzes the conversion of NMBA to benzaldehyde and that the esophageal CYP2A3 level in the rat can be a determinant of its ability to metabolize NMBA in vivo. However, the inhibition by coumarin and methoxsalen was lower than that observed with SKF-525A in the esophagus or kidney, supporting the findings that a CYP subfamily other than CYP2A3 is responsible for catalyzing the methylene hydroxylation of NMBA. Metyrapone and orphenadrine produced a small but significant inhibition of the mutagenicities in these tissues.

The inhibition by SKF-525A was also observed in lung and colon, indicating that other unconfirmed CYP species were associated with activation in these tissues. In conjunction with these findings, multiple CYP species might be relevant to activation of NMBA and responsible enzymes depend on the class of the tissue. Anyway, this is the first demonstration that ethanol exerts an enhancing effect on the mutagenic activation of NMBA by an action on esophageal CYP2A in rats. In addition, activation of NMBA is predominantly associated with multiple CYP proteins including CYP2A and 2B, but not in the case for CYP2E1. CYP2A3 metabolizes NMBA predominantly by methylene hydroxylation and treatment with NMBA reduces the expression of CYP2A3mRNA in rat esophagus and lung. Deficient CYP2A6 activity due to genetic polymorphism reduces lung and oral cancer risk in male smokers. Further, methoxsalen is a strong chemopreventive agent against NNK-induced lung tumorigenesis. Therefore, CYP2A6 is one of the principal determinants affecting susceptibility to tobacco-related carcinogenesis. Meanwhile, ethanol and CS induce CYP2A protein in rodents and the ethanol induction are observed in esophagus and kidney, supporting that tobacco smoking and heavy alcohol consumption are associated with increased risk of human cancers, especially when these 2 exposures occur together.

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It has been demonstrated that ethanol administered either to drinking water or intragastrically results in an enhancing effect on levels of esophageal and renal CYP2A3 and 2E1 and hepatic CYP2E1, and on their mutagenic activation of five NOCs, including DEN and NMBA, in the esophagus and kidney. Consequently, it is indicated that this ethanol-mediated enhancement of NMBA-induced esophageal carcinogenesis in F344 rats can be attributed to an increase in the metabolic activation of NMBA by esophageal CYP2A3; this occurs during the initiation phase, without the contribution of metabolic activation or inactivation by glucuronidation in liver. The present data also suggest that treatment with ethanol may enhance the carcinogenesis initiated by DMN and NPYR or the esophageal and renal carcinogenesis initiated by other carcinogens activated by CYP2A3 or 2E1. Tissue-specific activation of N-nitrosopiperidine and NPYR contributes to organ-specific carcinogenicities, suggesting that CYP expression in the relevant organ may play an important role in tumorigenesis induced by NOCs during the initiation phase. Although ethanol induces the both levels of hepatic and extrahepatic CYP2E1, the metabolic activation of DEN by esophageal CYP2E1 may be predominantly involved in the ethanol-mediated enhancement of DEN-induced esophageal carcinogenesis. Alterations of xenobiotic-enzymes reflect the activation of environmental carcinogens and can be leading to predict their carcinogenic potential. Cancer predictions through evaluations with the enhancing and suppression effects on rodent CYP isoforms by CS, ethanol, curcumin or 6-MSITC are also described in the latter sections, including Figures 9 & 10.

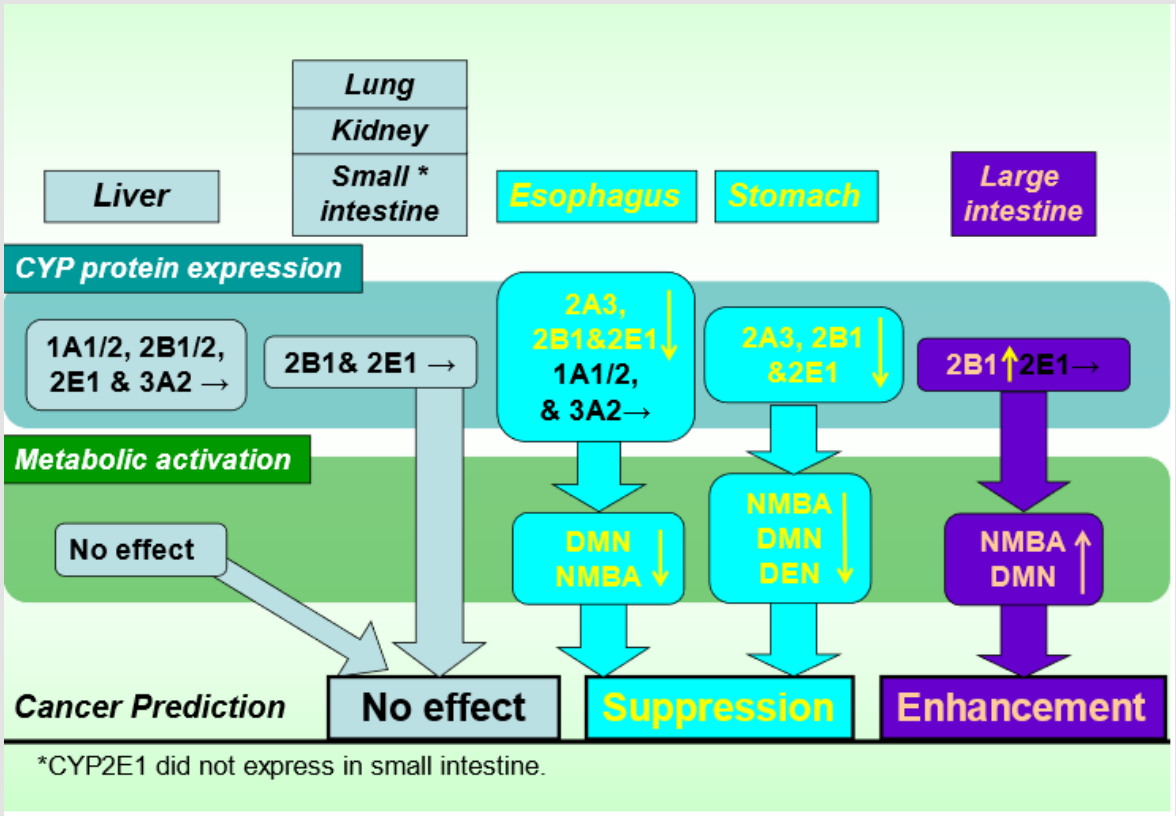

Figure 9: Suppression and enhancement by curcumin of CYP expression and mutagenic activities of N-nitroso compounds in the presence of hepatic and extrahepatic S9 in rats, and prediction for the modification of tissue-specific carcinogenesis initiated with N-nitroso compound.

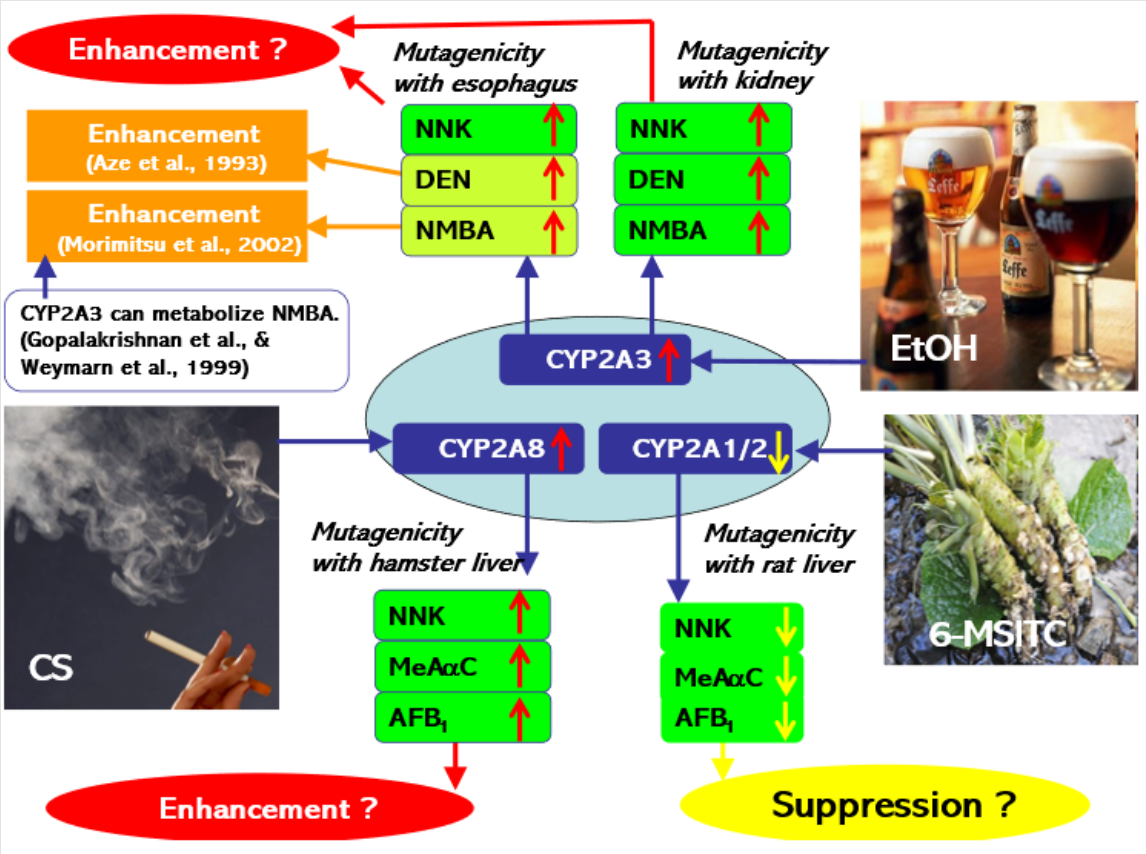

Figure 10: Enhancement and suppression by CS, ethanol and 6-MSITC of the mutagenic activation by CYP2A subfamily of NNK, DEN, NMBA, MeA α C and AFB1 in liver, esophagus and kidney, and prediction on modification of the chemically-induced carcinogenesis in laboratory animals [24].

Suppression by Curcumin of NMBA-Induced Esophageal Carcinogenesis in F344 Rats

Curcumin is not toxic to humans up to 8 g/day when taken by mouth for 3 months, being suggested as an effective chemopreventive agent. It is shown that turmeric and curcumin reduce BP- or 7,12-dimethylbenz[a]anthracene (DMBA)-derived DNA adducts in rat liver and hamster buccal pouch, and that NMBA-induced esophageal tumorigenesis is markedly suppressed by treatment with curcumin during the initiation and promotion phases in F344 rats. Further, turmeric and curcumin are a competitive inhibitor for ethoxyresorufin O-deethylase (EROD) and pentoxyresorufin O-dealkylase (PROD), and to decrease total CYP content and EROD, methoxyresorufin O-demethylase (MROD), and PROD activities in liver, lung and stomach microsomes from rats. Although curcumin is also reported to induce hepatic 4-NP UDPGT, there are no data with the effect of curcumin on hepatic levels of UDPGT activities towards bilirubin and testosterone, CYP enzymes and metabolic activation by CYP in rats, or on extrahepatic levels of CYP isoforms in any animal species. No significant alterations in the hepatic levels of constitutive CYP proteins, mutagenic activation by liver S9 of NOCs, HCAs, BP and AFB1 and three UDPGT activities were produced by subcutaneous treatment with 0.5 mg/kg NMBA three times per week for 5 weeks and/or feeding of 0.05 and 0.2% curcumin in the diet for 6 weeks [7].

In contrast, gavage of 0.05 and 0.2% curcumin decreased esophageal CYP2B1 by about 40 and 60%, respectively, compared with vehicle control, and that of 0.2% curcumin decreased esophageal CYP2E1 by 30%, as illustrate in Figure 9. Similarly, intragastric treatment with 270 mg/kg curcumin, corresponding to daily intake 0.2 % curcumin, decreased esophageal CYP2B1 and 2E1 by 60% and gastric CYP2B1 and 2E1 were to 70% of the control and to the undetectable level, respectively, but not in the cases of the lung, kidney and intestine. Conversely, large intestinal CYP2B1, but not CYP2E1, was increased to 2.8-fold higher in the treated rats than in control rats. Mutagenic activities of NMBA, DMN and/or DEN in the presence of esophagus and stomach S9 were markedly decreased in the treated rats, whereas those in the presence of large intestine S9 were increased to 2.2~3.0–fold above control. It has been reported that CYP1A1, but not CYP2B1, 2E1 and 3A1/2, is detected in esophageal microsomes from male

F344 and Wistar rats and CYP2E1 is in those from ethanol-treated rats. However, the present results indicate that CYP2B1, 2E1, 3A2 and 1A1/2 are constitutively detected in esophageal microsomes from male F344 rats.

The reasons for the discrepancies are currently unknown, but it is suggested that the differences might be due to experimental conditions, such as the transfer conditions, the antibodies used against rat or human CYP species or the detection method. In any case, this is the first demonstration of the presence of four CYP isoforms in the esophagus from uninduced rats; supporting the previous finding that CYP1A1, 2B6, 2E1 and 3A4 and CYP1A2mRNA are detected in esophageal mucosa from human and rats, respectively. In this study, it has been also demonstrated that CYP2B1 and 2E1 are constitutively detected in other four extrahepatic microsomes, except for CYP2E1 in the small intestine. Both CYP isoforms are known to be present in the lung, small intestine (except CYP2E1), kidney and large intestine (except CYP2B1) in rats, and CYP2E1 in the stomach from ethanol-induced rats. Accordingly, this is the first report on the presence of CYP2B1 protein in the stomach and large intestine and CYP2E1 protein in the stomach from uninduced rats, in agreement with the finding of CYP2B1mRNA expression in both tissues of rats.

CYP2A3 and its mRNA are known to be constitutively expressed in rat esophagus, and curcumin can decrease the NMBA activation in esophagus and stomach (Figure 9). Together with findings of the ethanol-induced NMBA activation by CYP2A3 in esophagus and kidney (Figure 8), it is reasonable to assume that curcumin could modify extrahepatic CYP2A3 levels, in addition to CYP2B1 and CYP2E1. We have shown that CYP2B1 and 2B2 are equally involved in mutagenic activation of NMBA by PB-induced liver, and CYP2B2 are in uninduced liver, in rats [20]. CYP2E1 activates NPYR and 1 mg dose of DMN and DEN to mutagens and CYP2B1/2 do BHP, NDMM and 10 mg dose of DMN and DEN. Thus, it seems reasonable that feeding for 6 weeks and intragastric treatment produce no effect on the mutagenic activation of these carcinogens by liver S9, reflecting no alteration of responsible CYP isoform each (Figure 9).

Differing results have been reported for the effect of curcumin or turmeric on metabolic activities specific to each CYP species in rodent liver, lung and stomach. Feeding of 2% curcumin for 2 weeks causes a modest reduction of hepatic EROD activity in female A/J mice, whereas 10% turmeric (equivalent to 0.5% curcumin) for 4 weeks shows no alteration of hepatic PAH hydroxylase activity in male Wistar rats. Feeding of 1% turmeric for 15 days produces no alteration of EROD and PROD activities, but exerts a suppressive effect on MROD activity, BP-induced EROD and MROD activities and PB-induced PROD activity in rat liver. In conjunction with present findings on the mutagenic activation of seven carcinogens by hepatic CYP in curcumin-treated rats, it is concluded that curcumin and turmeric exerts no suppression of metabolic activities specific to each CYP species in uninduced rodent liver, with the exception of MROD activity, but causes clear suppression in rats induced by PB and MC.

It is shown that feeding of 10% turmeric for 4 weeks clearly elevates 4-NP UDPGT activity but feeding of 5% turmeric (equivalent to 0.25% curcumin) does not. Therefore, it is reasonable that no alterations of three kinds of hepatic UDPGT activities were observed in rats fed 0.05 or 0.2% curcumin for 6 weeks. Together with the results of UDPGT activities in rats treated with 70 or 270 mg/kg curcumin as a single dose, treatment with a higher dose of curcumin might be needed for induction of UDPGT activities; suggesting that neither 0.05 nor 0.2% curcumin modify NMBA-induced esophageal carcinogenesis through detoxification by the enzymes under the experimental conditions used. However, curcumin caused a decrease in esophageal and gastric levels of CYP2B1 and 2E1 and the mutagenic activation of NMBA, DEN and DMN by the tissue S9, in agreement with the previous finding that curcumin suppresses the metabolic activity specific to CYP2B1/2 more strongly in stomach microsomes than in liver microsomes in rats. In contrast, curcumin increased in large intestinal CYP2B1, but not CYP2E1, and the mutagenic activations of NMBA and DMN. Together with the findings that high levels of esophageal DNA alkylation are induced by NOC, and DNA methylation by NMBA and N-nitrosomethyl-n-butylamine in rats occurs to a higher extent in esophagus than in liver, it suggests that modification of metabolic activation of NOC by the target organ plays a critical role in chemoprevention of NOC-induced in rats.

It has been reported that curcumin inhibits the expression of c-Jun and c-Fos/AP-1, a transcriptional factor that plays an important role in the expressions of CYP2B1/2 and 2E1, but not CYP1A and 3A, suggesting that the suppression of AP-1-induced transcription by curcumin might produce a decrease in CYP2B1 and 2E1 expressions in the esophagus and stomach. However, this is not consistent with the present findings of enhancement in mutagenic activation by large intestinal CYP and of no suppression in that by liver CYP. Since there are no reports on enhancement by curcumin of CYP2B1 or 2E1 expression in any tissue, the mechanism underlying modification of tissue-specific actions by curcumin remain to be elucidated, and further investigations are required. It has been shown that some ITCs markedly decrease the incidence and multiplicity of NMBA-induced esophageal tumors, with inhibition of esophageal DNA methylation in F344 rats, and some NOCs are shown to be metabolized in the esophagus at a relatively high rate, often leading to high levels of esophagus DNA alkylation, indicating the importance of metabolic activation of NMBA by the target organ during the initiation phase. In this study, it has been demonstrated that dietary feeding or intragastric treatment with curcumin shows a suppressive effect on the mutagenic activation of three NOCs, including NMBA, by esophageal or gastric CYP2B1, 2E1 and 2A3, but shows a promotive effect on the mutagenic activation of NMBA by large intestinal CYP2B1. Consequently, it suggests that suppression by curcumin of NMBA-induced esophagus carcinogenesis in F344 rats can be attributed to a decrease in the metabolic activation of NMBA by esophageal CYP2B1 and CYP2A3 during the initiation phase, without the contribution of metabolic activation or inactivation by glucuronidation in rat liver.

The present data also suggest that dietary exposure to curcumin might suppress esophageal carcinogenesis initiated with DEN, N-nitrosopiperidine and methyl-n-amylnitrosamine or gastric carcinogenesis initiated with other carcinogens activated by CYP2B1 and 2E1, but that curcumin might enhance large intestinal carcinogenesis induced by these carcinogens via CYP2B1 activation (Figure 9), although curcumin is known to suppress colon carcinogenesis initiated with azoxymethane, a direct carcinogen [7]. Together with findings that anti-inflammatory and antioxidant actions by curcumin are possible inhibitory mechanisms, it is reasonable to assume that curcumin could affect chemically-induced carcinogenesis through multiple mechanisms, including a bifunctional effect on metabolism, as illustrated in Figure 11.

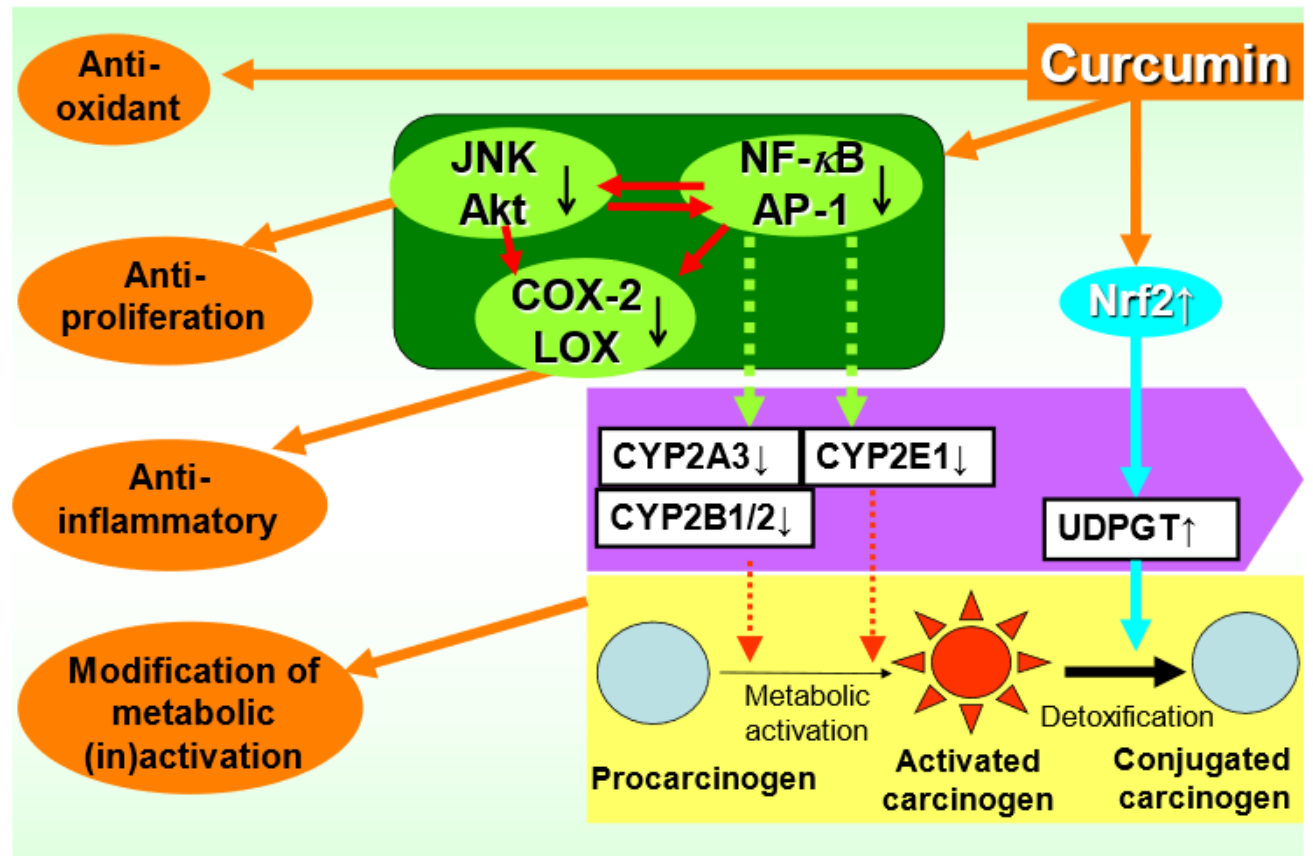

Figure 11: Proposed multiple mechanisms underlying suppression of experimental carcinogenesis by curcumin, including modification of metabolic (in)activation.

Suppression by Isothiocyanates of Mammary or Colon Tumorigenesis in Rats

Chemically-induced skin, mammary gland, lung, forestomach, esophagus, liver and bladder tumorigenesis have shown to be inhibited by naturally occurring ITCs, such as allyl ITC (AITC), benzyl ITC (BITC), phenethyl ITC (PEITC) and sulforaphane, and by synthetic ones. ANIT has been reported to induce cholestasis, bile duct proliferation and focal necrosis of hepatocytes, without the development of liver cancer, and to inhibit hepatocarcinogenesis in rats. ANIT was used in early studies of cancer chemoprevention, but it is toxic to the liver and is a cholestatic agent. Possibly as a result of this, it is now used rarely in chemoprevention studies and has not been scheduled for trial in humans. Although toxicity is a serious problem for trial of cancer chemopreventive agents, if lower doses of such compounds do not exert clear toxicity in any organs, these compounds should not be omitted from basic research on cancer chemoprevention, like developing medicinal drugs, in which many of them show some toxicity, depending on its dose. Therefore, we examined the effects of a low dose (400ppm in diet) of ANIT on PhIP-induced mammary carcinogenesis in female rats [5,22]; this dose of ANIT was too low to induce the side effects in rats, and histological examination revealed neither hepatotoxicity nor cholestasis.

An extract from wasabi was known to reduce the growth of the human monoblastic leukemia cells by inducing apoptosis, and its ingredient, 6-MSITC was found to inhibit cell proliferation in human breast cancer and melanoma cell lines. In addition, we have shown that the dietary administration of 400 ppm 6-MSITC during the initiation phase can significantly inhibit the induction of colonic aberrant crypt foci (ACF) and β-catenin-accumulated crypts (BCAC) by 1,2-dimethylhydratine (DMH) in F344 rats; immunohistochemically, 6-MSITC administration reduced the proliferating cell nuclear antigen labeling index in AFC and BCAC [6]. One of the mechanisms underlying the chemopreventive effects of these ITCs is related to the ability to attenuate DNA alkylation by NNK, NMBA or BOP; suggesting that it may be related to alteration of metabolic reactions. However, different results for the effects of ITCs on hepatic CYP have been reported; both increases and decreases in total CYP content, levels of CYP species and metabolic activities specific to each CYP species have been observed. In contrast, the activities of quinone reductase (QR) and glutathione S-transferase (GST) in the cytosol of cells of several organs and hepatocytes from rodents are reported to induce by five natural ITCs, and PEITC also enhances 4-NP UDPGT activity in rat liver microsomes, but BITC exhibits no significant effect on UDPGT activity towards 4-methylumbelliferone and chloramphenicol.

Effects of ANIT and PhIP on Mutagenic Activations by CYP in Female Sprague-Dawley Rats

PhIP has been demonstrated to produce colorectal, prostate and mammary cancers in rats. It is also shown that N-hydroxylation of PhIP by CYP1A2 followed by O-acetylation is the metabolic activation pathway, while ring hydroxylation by CYP1A1 and glucuronidation of N-OH-PhIP are detoxification pathways. Four natural ITCs, including BITC, and three synthetic norbornyl ITCs structurally related to sulforaphane have been reported to inhibit rat mammary tumorigenesis induced by DMBA, whereas that initiated by PhIP is not suppressed by BITC. We found that ANIT markedly suppresses mammary carcinogenesis induced by PhIP when given in the initiation phase in Sprague-Dawley rats [22]. It is shown that ANIT causes an increase in GST and QR activities and a decrease in total CYP content and the activities of ethoxycoumarin O-deethylase, benzphetamine N-demethylase, aminopyrine demethylase and aniline hydroxylase in rat liver [22]. However, no data have been provided on the effect of ANIT on hepatic levels of CYP species, mutagenic activities of environmental carcinogens and UDPGT activities in any animal species.

Inductions of CYP1A1, newly found 51 and 53 kDa proteins and constitutive CYP1A2 and 2B2 by intragastric treatment with PhIP eight times for 11 days were observed in female rats fed a high fat diet [5], as illustrated in Figure 12. Although the induction levels were not as high as in the case of PhIP, 3 weeks feeding of 400 ppm ANIT induced CYP1A1 and the 51 and 53 kDa proteins, but the constitutive CYP1A2 level was decreased by the feeding of ANIT. The mutagenicities in strain TA98 of PhIP, four other HCAs and BP were markedly elevated with the liver S9 fraction from treated with PhIP, but not with ANIT. Treatment with a combination of PhIP and ANIT significantly decreased in those of five HCAs, as compared with the case of PhIP alone, and the CYP1A2 level was consistently decreased in liver microsomes from rats administered a combination of PhIP plus ANIT. On the other hand, 4-NP UDPGT activity was markedly enhanced in liver microsomes from rats treated with the combined treatment as compared with the case of each alone. The mutagenic activity of PhIP has shown to be markedly induced by MC and to be dramatically inhibited by furafylline and 7,8-BF, indicating the involvement of CYP1A subfamily, predominantly CYP1A2 in the activation of PhIP by liver S9 from rats of both sexes [8]. Selective enhancement by PhIP of the mutagenicities for five HCAs reflects selective induction of hepatic CYP1A2, in agreement with previous findings that hepatic CYP1A1/2 are involved in metabolic activation of a number of HCAs and that several HCAs, including PhIP, induce hepatic CYP1A subfamily member, especially CYP1A2, in male F344 rats [8]. It is shown that 51 kDa protein in addition to CYP1A1/2, is induced by PhIP in liver microsomes from male F344 rats, but not by MeIQx, seven other HCAs and 4-aminoazobenzene, and that the 51 kDa protein induced by PhIP contribute to mutagenic activation of Glu-P-1 and Trp-P-2.

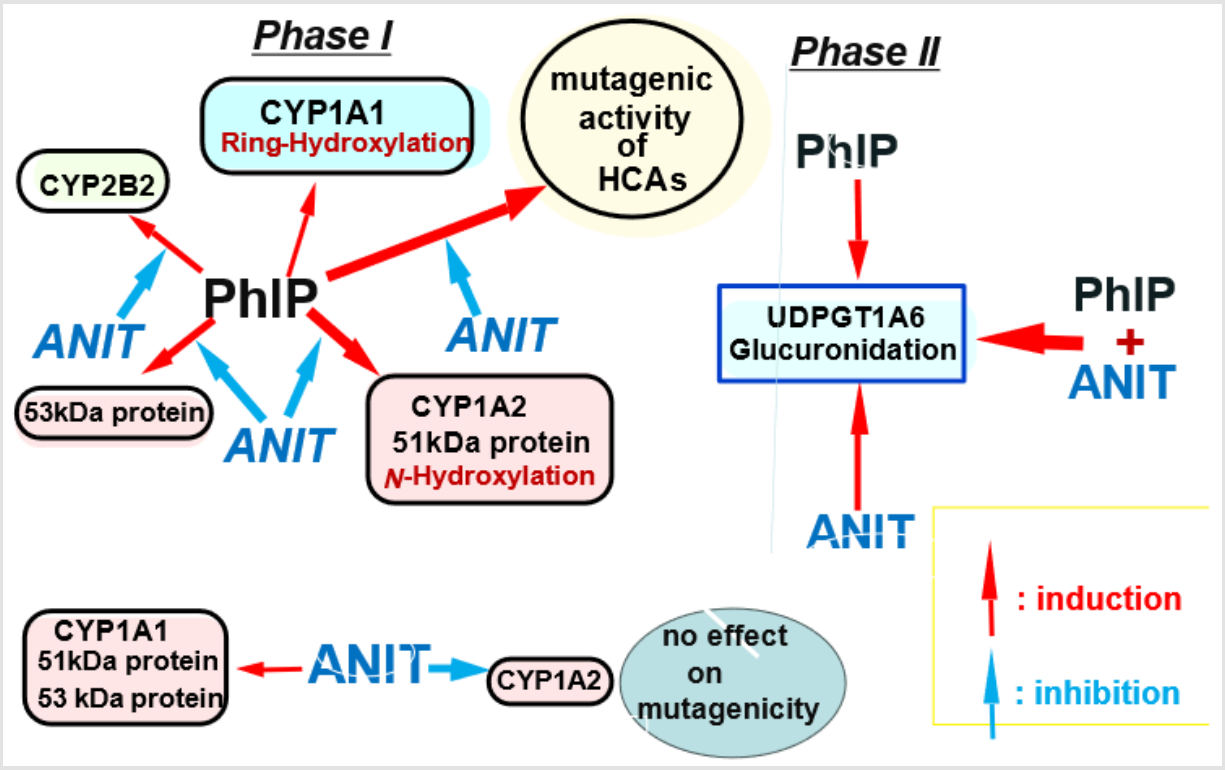

Figure 12: Induction and inhibition of hepatic level of CYP isoforms, mutagenic activities of HCAs and UDPGT1A6 activity by ANIT, PhIP and ANIT plus PhIP in female rats N-hydroxylation and ring hydroxylation of PhIP are catalyzed by CYP1A2 and CYP1A1, respectively. The UDPGT activity in rats treated with ANIT or PhIP was increased to 1.6- and 2.3-fold above the vehicle control, respectively, and the combined treatment showed much highly induction (4.7-fold).

In this study, it has been demonstrated that PhIP can induce the 53 kDa protein in addition to three CYP1A-related microsomal proteins in female Sprague-Dawley rats. As described in the above section (3.1.2), MeIQx enhances the mutagenic activities of several HCAs, but not MeAαC and BP, which are predominantly activated by rat CYP1A1, in spite that induction of hepatic CYP1A1 is one-fifth of the induced CYP1A2 level [18]. In contrast, PhIP caused a significant increase in the mutagenic activities of both carcinogens, although the hepatic CYP1A1 level in female rats treated with PhIP is almost equal to that in male rats treated with MeIQx. Accordingly, these findings suggest that the 53 and 51 kDa proteins may contribute to the activation of HCAs and BP. PB is shown to increase in in the mutagenic activity of BHP to 4.4-fold [23], hepatic CYP2B2 to 18.3-fold above control and CYP2B1 to 1.9-fold above the induced level of CYP2B2 in male rats [12]. PB increase also in PROD activity to 57.2-fold above the vehicle control in male rats, however, PhIP induced CYP2B2, but not CYP2B1, up to 4-fold and increased PROD activity up to 5-fold above the respective controls in female rats. Therefore, it is reasonable that liver S9 from female rats treated with PhIP repeatedly or as a single dose could not enhance the mutagenicity of BHP, indicating insufficient induction of CYP2B1/2 for activation. Nevertheless, this is the first demonstration that HCA can induce hepatic CYP2B2 and PROD activity in rats. DMN is known to be mutagenetically activated by rat CYP2E1 and 2B1/2 and AFB1 by CYP2B1 and 3A2.

Thus, it seems reasonable that PhIP has no effect on the mutagenic activation of two carcinogens, reflecting no induction of hepatic CYP2B1, 2E1 and 3A2 in female rats. Conflicting results have been reported for the induction of CYP species by PEITC; feeding treatment induces CYP1A1/2 and 2B1/2, but not CYP2E1 and 3A2, in male Fisher rats, and the intragastric treatment of male F344 rats also induces CYP2B1, but not CYP2B2 and 2E1, while intragastric treatment of female hamsters with PEITC had no effects on CYP1A2, 2B, 2E1 and 3A. However, there are no reports on either suppression or induction of CYP isoforms by other ITCs in any animal species. Feeding a 400 ppm ANIT-containing diet for 3 weeks and intragastric treatment as a single dose produced a decrease in hepatic CYP1A2 but a clear increase in three other CYP1A-related proteins; these did not affect the mutagenic activities of five HCAs and BP in ANIT-treated rats, indicating the insufficient induction for the activation.

On the other hand, no significant change was observed in hepatic levels of CYP2B1/2, 2E1 and 3A2, in accord with the observation of no induction of the mutagenic activities of BHP, DMN and AFB1. However, these results are not consistent with the previous finding that feeding of 220-1000 ppm ANIT for 2-6 weeks exerts a clear suppressive effect on metabolic activities specific to CYP1A1 and 2B1/2 in liver microsomes from male F344 rat. Further, PEITC causes a decrease in metabolic activities specific to CYP1A1/2, 2B1/2, 2E1 and 3A2 in male F344 rats 6 h after treatment, but an increase in those specific to CYP1A1/2 and CYP2B1/2 in the same animals 24 h after treatment. BITC, phenylbutyl ITC and phenylhexyl ITC show the similar effects on these metabolic activities, except for the CYP1A1/2 and 2B1 activities. The reasons for these discrepancies are currently unknown; it might be due to experimental conditions such as timing of death, treatment regimen (i.e. one or several applications, a conventional or high fat diet), age and/or sex of animals, metabolic substrates, etc. However, our results support the previous finding that feeding of 600 ppm ANIT for 24 weeks produces no significant effect on hepatocarcinogenesis in male rats initiated with DEN which is known to be activated predominantly by CYP2E1. The combination of ANIT and PhIP caused a marked decrease in the PhIP-induced level of hepatic CYP1A2, in accord with the observation of a marked decrease in those of mutagenic activities of Glu-P-1, IQ and PhIP. Although ANIT slightly induced CYP1A1 and the 51 and 53 kDa proteins, the combination with PhIP also caused a significant decrease in hepatic levels of these proteins and mutagenic activities of MeA α C and BP compared with those in PhIP-treated rats.

Thus, ANIT may suppress carcinogenesis by other HCAs or carcinogens which can induce CYP1A1/2 and are selectively activated by the CYP1A subfamily, including newly found proteins, through a dramatic inhibition of metabolic activation in the liver. ANIT also decreased PhIP-induced levels of CYP2B2 and PROD activity, suggesting the possibility of suppressing tumorigenesis initiated with carcinogens, including NOCs which are metabolically activated by CYP2B2. PhIP–glutathione conjugate is not found in bile and urine from rats, and N-OH-PhIP is not also conjugated by hepatic GST, whereas N-OH-PhIP N3-glucuronide and N-OH-PhIP N2-glucuronide are the major metabolites of PhIP in those of rats. D-Galactosamine markedly decreases the formation of the two N-glucuronides of N-OH-PhIP and increases the formation of DNA adducts and unscheduled DNA synthesis, indicating that N-glucuronidation of PhIP is an important detoxification reaction. The UDPGT1A subfamily is predominantly involved in the biotransformation of N-OH-PhIP, and PhIP clearly enhanced hepatic 4-NP UDPGT activity in female rats, but not bilirubin UDPGT activity, consistent with our finding in male rats treated with MeIQx [18]; suggesting that UDPGT1A6 is involved in glucuronidation of N-OH-HCAs. Induction of UDPGT1A6 activity by ANIT is in agreement with that by PEITC, however, the much higher induction of 4-NP UDPGT activity in combination with PhIP implies promotion of detoxification of N-OH-PhIP. BITC is reported to suppress rat mammary tumors initiated with DMBA, but not those initiated by PhIP. DMBA is detoxified by QR, GST and UDPGT, and BITC clearly induces hepatic GST and QR activities, but not UDPGT1A6. Therefore, BITC is unable to inhibit PhIP-induced rat mammary tumorigenesis due to a lack of ability to induce UDPGT1A6.

The present study has demonstrated that PhIP and ANIT have a bifunctional action, with induction of CYP1A proteins and UDPGT activity, and suppression by ANIT of PhIP-induced mammary carcinogenesis in rats can be attributed to a dual action mechanism: a decrease of metabolic activation of PhIP predominantly by hepatic CYP1A2 to its N-hydroxylation and an increase in detoxification by UDPGT1A6, without contribution of the inactivation by CYP1A1 to the ring hydroxylation. Together with the findings that PEITC and sulforaphane block the increase in cell proliferation induced by BOP in its target organs in hamsters and induce apoptosis, it is suggested that ITCs are expected to affect chemically-induced carcinogenesis through multiple mechanisms.

Effect of 6-MSITC on Mutagenic Activations of Carcinogens by CYP in F344 Rats

To induce the colonic preneoplastic lesions, rats were given four weekly subcutaneous injections of 40 mg/kg DMH. ACF have been widely used as intermediate biomarkers of colon carcinogenesis in experimental animal models, and a positive correlation has been described between the effects of chemopreventive agents on ACF and tumor development. BCAC, another mucosal lesion, was evaluated as a surrogate biomarker of colon carcinogenesis. Significantly, AFC and BCAC are considered to be independent and distinct, as they differ in biology, genetics and morphology. In this context, the finding that 6-MSITC inhibited the formation of two different lesions can be regarded as convincing [6]. Furthermore, rats fed the diets containing 6-MSITC showed no adverse effects and no clinical signs of toxicity. Collectively, these findings suggest that 6-MSITC is a new chemopreventive agent against colon cancer development. 6-MSITC is known to activate the ARN/Nrf2-dependent detoxification pathway, and oral administration resulted in the induction of hepatic GST and QR in mouse liver. Nrf-2 also regulated UDPGT in the mouse, however, no data have been provided regarding the effects of 6-MSITC on the levels of CYP enzymes, mutagenic activation of environmental carcinogens and UDPGT activities in the livers and other tissues of any animal species.

All the constitutive CYP proteins were decreased by 10-40 %, compared with the vehicle control, in the liver of male F344 rats subcutaneously injected with 40 mg/kg DMH and fed with 200 or 400 ppm 6-MSITC for 5 weeks. as summarized in Figure 13. Similar decreases in these CYP isoforms were also observed in rats 24 hours after single intragastric treatment with 20 or 40 mg/kg 6-MSITC; the treatment with 40mg/kg significantly decreased CYP1A2, 2A1/2, 2B2, 2E1 and 3A2 proteins by 20, 35, 44, 41 and 20%, respectively, relative to the vehicle group, whereas that with 20 mg/kg 6-MSITC decreased hepatic CYP1A2, 2B2 and 2E1 protein levels. The intragastric treat-

ment decreased the mutagenic activities of HCAs (Glu-P-1, Trp-P-2 and PhIP) and AFB1 in strain TA98 and NOCs (BHP, DMN and NNK) in TA100 by 30-50% and 20-40% of the control, respectively. In the rat colon, 6-MSITC decreased in constitutive CYP1A1 (60% decrease), CYP2A3 (20-30%), 2B1 (40-80%), 2E1 (40-50%) and 3A2 (30-40%), together with a decrease in the mutagenic activities of DMN (55-65% decrease) and BHP (35-65%) in the presence of colon S9. On the other hand, there were no significant alterations on the three UDPGT activities in liver microsomes from rats fed with 400 ppm 6-MSITC for up to 5 weeks. The ST activities towards 4-NP at pH 5.5 (ST1C1) and pH 6.5 (ST1A) were slightly (1.3-fold above the controls) but significantly increased in the liver cytosols from rats 24 hours after the intragastric treatment with 20 or 40 mg/kg 6-MSITC, whereas the ST activity towards dehydroepiandrosterone (ST2A) and three UDPGT activities were not altered in the same rats.

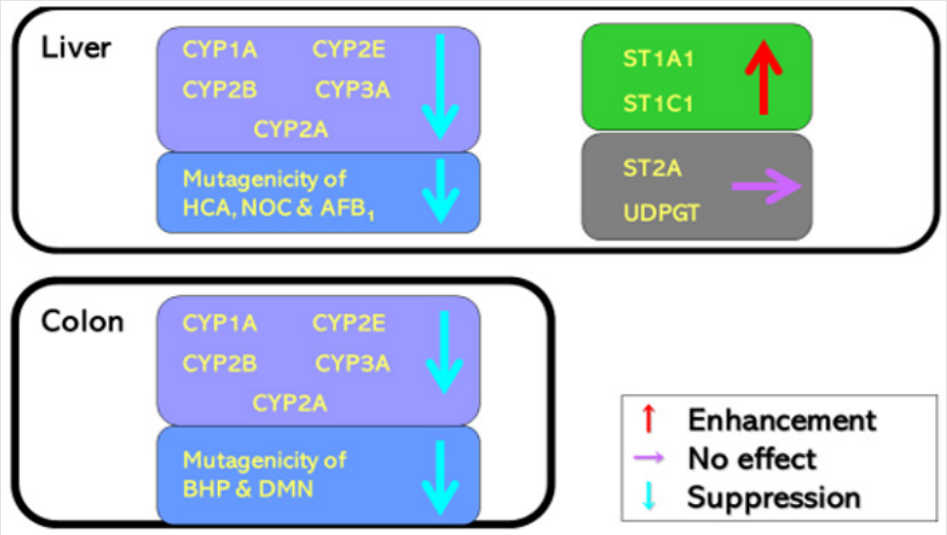

Figure 13: Suppression and enhancement by 6-MSITC of CYP expression, mutagenic activities of various carcinogens and UDPGT and ST activities in rat liver and colon.

CYP2E1 has been reported to be the enzymes catalyzing the conversion of azoxymethane and methylazoxymethanol, which are metabolites of DMH, to alkylating species capable of causing initiation events. In this context, clearly decreased levels of hepatic and colonic CYP2E1 by 6-MSITC and the mutagenic activities of DMN with both tissue S9 may be expected to result in the reduction and/or slowing of the transformation of DMH metabolites to proximate or ultimate carcinogens. We tried to assay the effect of 6-MSIC on mutagenic activation of DMH by CYP enzymes, but DMH could not be commercially available and the try to synthesize this compound was failed, because of its chemical stability, and the effect on metabolic activation of DMH remains to be clarified. The present results demonstrate that the dietary administration of 6-MSITC can significantly inhibit the induction of colonic ACF and BCAC induced with DMH by reducing cell proliferative activity, and 6-MSITC decrease in the mutagenic activations of HCAs, NOCs and AFB1 by constitutive CYP each, whereas 6-MSITC increase in the ST1A1 and 1C1, but not ST2A and any of UDPGTs in the rat liver. Since the reduction by 6-MSITC of mutagenic activation by the constitutive CYPs are also observed in the target tissue, suppression by 6-MSITC of DMH-induced colonic carcinogenesis may be attributed to a decrease in metabolic activation of DMH presumably by colonic CYP2E1.

Sulfation of BOP metabolites is shown to be an activation pathway [15], as described in the Section 3.1; suggesting that 6-MSITC may enhance BOP-induced pancreatic carcinogenesis. Although conjugation by STs is known to be activation pathway also in other carcinogens, there are no data on a relationship between modification of the tumorigenesis and alteration of hepatic STs; the role of slight induction of STs by 6-MSITC in chemically-induced carcinogenesis is not clearly known, and remains to be clarified. Interestingly, the reduction by 6-MSITC of mutagenic activations were observed in all the environmental carcinogens tested; strongly suggesting the widely chemopreventive action by 6-MSITC on chemically-induced carcinogenesis in laboratory animals. Nevertheless, these findings indicate that 6-MSITC is an important candidate as a new chemopreventive in human cancers for not only the colon cancer but various organ cancers. Together with the findings that actions of antioxidation, apoptosis, COX-2 expression and tumor metastasis by 6-MSITC are possible inhibitory mechanisms, it is reasonable to assume that 6-MSITC could affect chemically-induced carcinogenesis through multiple mechanisms, including modifications of metabolic (in)activation, as illustrated in Figure 14.

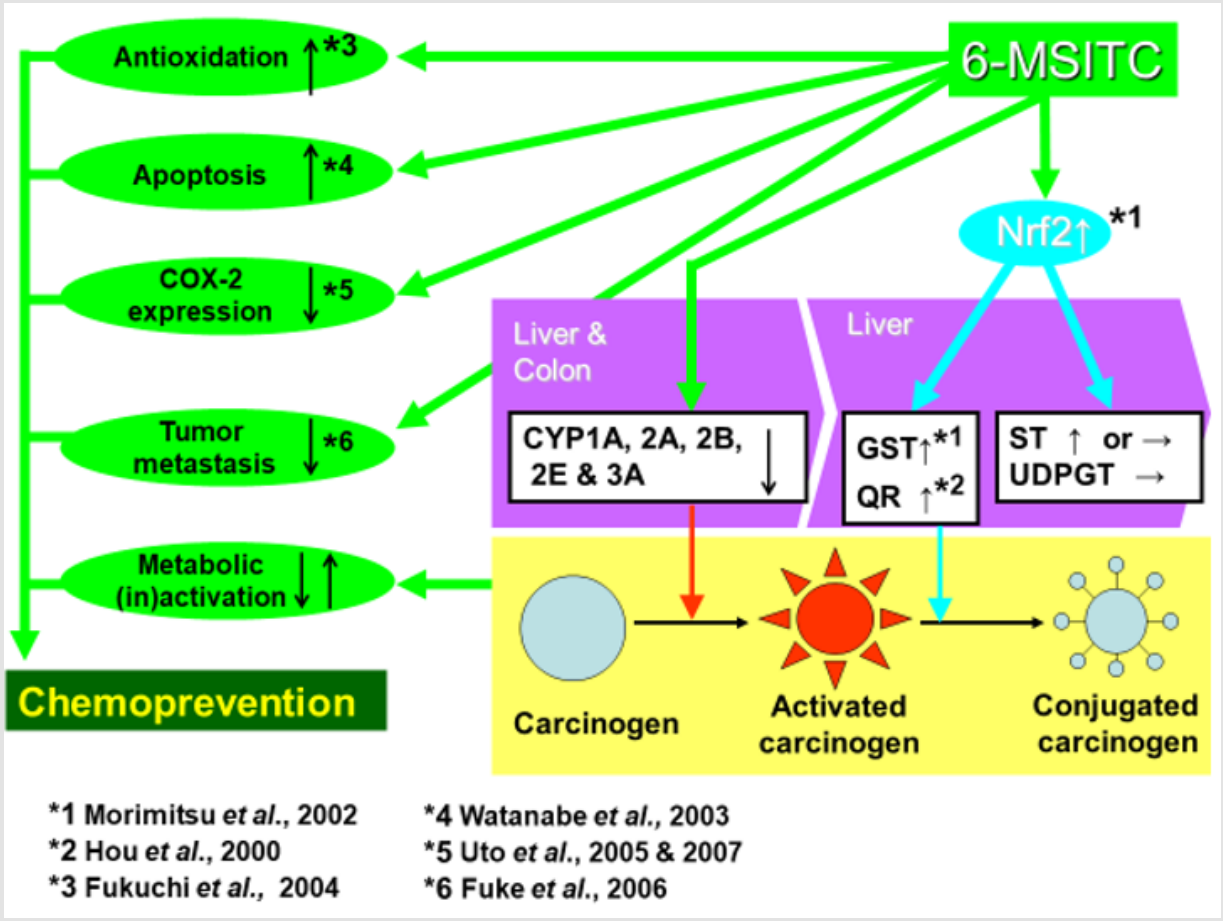

Figure 14: Proposed multiple mechanisms underlying suppression by 6-MSITC of chemically-induced carcinogenesis, including modification of metabolic (in)activations by phase I and II enzymes.

Conclusion

Lifestyle factors such as cigarette smoking, alcohol consumption and dietary habits are closely associated with an increased or decreased risk of human cancers in various organs. The modifications by CS, ethanol, curcumin, ANIT and 6-MSITC on mutagenic activation and inactivation of environmental carcinogens by phase I and/or II enzymes are also associated with enhancement or suppression of experimental carcinogenesis in rodents. In modification by CS of pancreatic or hepatic carcinogenesis, CS is a double-edge sword; CS suppresses BOP-induced pancreatic carcinogenesis in hamsters while CS enhances MeIQx-induced hepatocarcinogenesis in rats. CS have a bifunctional action on CYP1A1/2 and UDPGT1A6 enzymes in both animal species; the suppression of BOP-induced pancreatic carcinogenesis can be attributed to increased detoxification by UDPGT1A6 without CYP2B activation in hamster liver, and the enhancement of MeIQx-induced hepatocarcinogenesis to an increase in metabolic activation of MeIQx by CYP1A2, without the contribution of increased detoxification by UDPGT1A6 in rat liver. In enhancement by ethanol of DEN- or NMBA-induced esophageal carcinogenesis in rats, the enhancing effects can be attributed to an increase in the metabolic activation of DEN by hepatic CYP2E1 and that of NMBA by esophagus CYP2A3, and in the latter activation, this occurred independently of hepatic and esophageal CYP2E1, without a contribution of detoxification by hepatic UDPGT enzymes. In suppression by curcumin of NMBA-induced esophageal carcinogenesis in rats, the modifying effect can be attributed to a decrease in metabolic activation of NMBA by CYP2B1 and 2A3 in the target organ, without the contribution of metabolic activation and inactivation by liver.

In suppression by ANIT of PhIP-induced mammary carcinogenesis in female rats, the chemoprevention by ANIT is able to explain by a dual action mechanism, i.e. a decrease in the metabolic activation of PhIP by hepatic CYP1A2 and an increase in detoxification by UDPGT1A6. 6-MSITC can decrease in all the mutagenic activations of HCAs, NOCs and AFB1 by constitutive CYP each, without enhancement of UDPGTs in rat liver; suppression by 6-MSITC of DMH-induced colonic carcinogenesis might be attributed to a decrease in metabolic acti-

vation of DMH presumably by colonic CYP2E1. Since the reduction by 6-MSITC does not specifically occurs and are also observed in the target tissue, widely chemopreventive action of 6-MSITC on chemically-induced carcinogenesis could be produced in various organs of animal models. Consequently, these lifestyle factors clearly modify the metabolic activation of NOCs, HCAs, PAH or AFB1 by specific CYP isoform and/or detoxification by UDPGT, which are closely related with carcinogenesis initiated with these environmental carcinogens in liver and other organs, including target organs. Finally, it suggests that modification by lifestyle factors of chemically-induced carcinogenesis could be predicted by evaluation with metabolic (in)activation of environmental carcinogens. For example, findings on modification by CS, ethanol and 6-MSITC of mutagenic activations of NOCs, MeA α C and AFB1 by CYP2A subfamily are leading to predict for unknown modifications of the chemical carcinogenesis, as illustrated in Figure 10 [24], in addition to the case of NOC activation by extrahepatic CYP2A, 2B and 2E, as shown in Figure 9.

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