Inhibition of Human Cytochrome P450-catalyzed Oxidations of Xenobiotics and Procarcinogens by Synthetic Organoselenium Compounds

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ABSTRACT

The effects of synthetic chemopreventive organoselenium compounds 1,2, 1,3, and 1,4-phenylenebis(methylene)selenocyanate (o-, m-, and p-XSC), benzyl selenocyanate (BSC), and dibenzyl diselenide (DDS) and inorganic sodium selenite on the oxidation of xenobiotics and procarcinogens by human cytochrome P450 (P450 or CYP) enzymes were determined in vitro. Spectral studies showed that BSC and three XSC compounds (but not sodium selenite or DDS) induced type II difference spectrum when added to the suspension of liver microsomes isolated from experimental animals and also possibly to humans, most studies have been done in experimental animals and also possibly to humans, most studies have been done using naturally occurring organoselenium compounds (e.g., selenomethionine and selenocysteine), examining them for abilities to prevent tumor formation caused by environmental carcinogens. The results collectively indicate that the naturally occurring organoselenium compounds are also active in inhibiting chemically induced tumor formation in laboratory animals, but these selenoamino acids had comparable chemopreventive activity to inorganic selenium compounds, and in some instances, they were more toxic than inorganic selenium compounds (5). Several synthetic organoselenium compounds have been developed recently, and El-Bayoumy et al. (9) have shown that the synthetic organoselenium compounds XSC inhibit 7,12-dimethylbenz[a]anthracene-induced tumors and DNA adduct formation in female Sprague Dawley rat mammary gland; further studies by Ip et al. (10) documented this observation. However, mechanisms underlying the effects of these selenium compounds on the inhibition of chemically induced tumor formation still remain unclear.

P450 comprises a superfamily of enzymes that catalyze oxidation of a great number of xenobiotic chemicals such as drugs, toxic chemicals, and carcinogens and endobiologic chemicals such as steroids, fatty acids, vitamins, and prostaglandins (11). A number of studies have suggested that most of the chemical carcinogens in the environment are not active themselves in interacting with intracellular DNA but induce tumors only after metabolic activation by a variety of drug-metabolizing enzymes including P450 (12, 13). During the past decade, significant roles of several human P450 enzymes in the activation of procarcinogens and promutagens have been determined in many laboratories; CYP1A1, 1A2, 1B1, 2A6, 2E1, and 3A4 have been reported to be the major enzymes involved in the activation of most of the procarcinogens and promutagens that are metabolized by P450 enzymes in humans (14, 15). Among these P450 enzymes determined, CYP1A1 and 1B1 have been shown to be expressed in extrhepatic tissues in experimental animals and humans (16, 17). Particular interests are the observation that both P450s have been shown to be expressed in mammary glands and to be able to activate mammary carcinogens such as 7,12-dimethylbenz[a]anthracene, fluoranthene-2,3-diol, benzo[c]phenanthrene-3,4-diol, dibenzo[a,l]pyrene-11,12-diol, and benzo[g]chrysene-11,12-diol. The resulting diol epoxides have been shown to be potent mammary carcinogens in the rat (18, 19).

In this study, we examined the effects of several organoselenium compounds as well as inorganic sodium selenite on the activities of xenobiotic oxidation and procarcinogen activation by human liver microsomes and by recombinant human CYP1A1, 1A2, and 1B1 enzymes. Selenium compounds used in this study include sodium selenite, BSC, DDS, and o-, m-, and p-XSC, and procarcinogenic substrates for the P450 enzymes used were 2-aminooanthracene, 2-aminofluorene, Trp-P-1, and MelQ.
MATERIALS AND METHODS

Chemicals. 7-Ethoxyresorufin, 7-ethoxycoumarin, coumarin, chloroxazone, and testosterone were purchased from Sigma Chemical Co. (St. Louis, MO), and S-warfarin and (±)-bufuralol were from UltraFine Chemicals Co. (Manchester, United Kingdom). Organoselenium compounds were prepared and purified as described previously (20). Other substrates, their oxidation products, and reagents used in this study were obtained from sources as described previously or of the highest qualities commercially available (21-23).

Enzyme Preparation. The human CYP1B1 cDNA clone was introduced into Saccharomyces cerevisiae, and microsomes containing CYP1B1 protein were prepared as described (17, 24). Recombinant human CYP1A1 and 1A2 were purified to homogeneity from membranes of Escherichia coli in which modified P450 cDNAs had been introduced (25, 26). NADPH-P450 reductase and cytochrome b5 were purified from liver microsomes of phenobarbital-treated rabbits by the method of Yasukochi and Masters (27) as modified by Taniguchi et al. (28).

Human liver samples were obtained from organ donors or patients undergoing liver resection as described previously (22). Male Sprague Dawley rats (weighing about 200 g) obtained from Nihon Clea Co. (Osaka) were treated i.p. with β-naphthoflavone (50 mg/kg, daily for 3 days) or phenobarbital (80 mg/kg, daily for 3 days). Rats were starved overnight before killing on the fourth day. Liver microsomes were prepared as described and suspended in 10 mM Tris-Cl buffer (pH 7.4) containing 1.0 mM EDTA and 20% glycerol (v/v; Ref. 29).

Enzyme Assays. Standard incubation mixtures consisted of human P450 enzymes (2–10 pmol P450) with several substrates in a final volume of 0.25–1.0 ml of 100 mM potassium phosphate buffer (pH 7.4) containing an NADPH-generating system and 0.75 ml of bacterial suspension as described previously (16). Incubation conditions for yeast microsomal and reconstitution systems were described above. The induction of umu gene expression by activated carcinogens is presented as units of β-galactosidase activity/min/nmol P450 (21, 37).

RESULTS

Spectral Interaction of Selenium Compounds with Liver Microsomes of Rats Treated with βNF and Phenobarbital and of Humans. Spectral interaction of several organoselenium compounds with liver microsomes of βNF-treated rats showed that three XSC compounds and BSC induced type II spectral changes with P450 enzymes in liver microsomes (Fig. 1A). Inorganic sodium selenite, at 0.1 mM, did not cause spectral changes with rat liver microsomes. DDS was found to interact with liver microsomes causing unique, but uncharacterized, spectral changes, probably due to the turbidity of microsomes after interacting with DDS (Fig. 1B).

βNF treatment of rats was found to increase spectral changes with m-XSC, but phenobarbital did not (Fig. 2A). The same spectral change was seen with human liver microsomes (Fig. 2B).

Effects of Selenium Compounds on Xenobiotic Oxidations Catalyzed by Human Liver Microsomes. Effects of several selenium compounds on the xenobiotic-oxidizing activities were determined in human liver microsomes (Fig. 3). The substrates used included 7-ethoxyresorufin (marker substrate for CYP1A2), coumarin (CYP2A6), S-warfarin (CYP2C9), bufuralol (CYP2D6 for 1'-hydroxylation and CYP1A2 for 6-hydroxylation), 7-ethoxycoumarin (CYP2A6), and chlorzoxazone (CYP2C9). The oxidation of substrates by CYP1A1 and CYP1A2 was determined in reconstituted systems containing P450 (2–10 pmol) and NADPH-P450 reductase (2–10 pmol) and DLPC as described (16, 30). Liver microsomal incubations included microsomes (0.5 mg protein/ml) in 100 mM potassium phosphate buffer (pH 7.4) containing the NADPH-generating system and various concentrations of drug substrates (21, 22, 31).

The O-deethylation of ethoxyresorufin (10 μM), 7-hydroxylation of coumarin (50 μM), and O-deethylation of 7-ethoxycoumarin (50 μM) were assayed fluorometrically according to the methods as described (29, 32). The methods used for 1'-, 4-, and 6-hydroxylation of bufuralol (substrate concentration, 0.2 mM), 7-hydroxylation of S-warfarin (100 μM), 6-hydroxylation of chloroxazone (0.5 mM), and 6β-hydroxylation of testosterone (0.2 mM) were described previously (23, 33-36).
(CYP2E1 and CYP1A2), chlorzoxazone (CYP2E1), and testosterone (CYP3A4; Ref. 22). The effects of αNF on these monoxygenation reactions were also determined and compared with human liver microsomes.

The three XSCs were found to be the potent inhibitors of 7-ethoxyresorufin O-deethylation in liver microsomes of human sample HL-16; the potencies to inhibit the catalytic activities by these selenium compounds were almost the same as those by αNF (Fig. 3A). BSC and DDS also inhibited 7-ethoxyresorufin O-deethylation by human liver microsomes, whereas the inhibition effects were lesser than those by XSCs and αNF. Sodium selenite did not inhibit the 7-ethoxyresorufin O-deethylation at concentrations up to 1 μM.

Selenium compounds used in this study did not cause strong inhibition of coumarin 7-hydroxylation and bufuralol 1'-hydroxylation at concentrations up to 1 μM, although these compounds inhibited considerably S-warfarin 7-hydroxylation, 7-ethoxycoumarin O-deethylation, and chlorzoxazone 6-hydroxylation. Testosterone 6β-hydroxy-
Fig. 4. Effects of several organoselenium compounds on the kinetics of 7-ethoxycoumarin O-deethylation catalyzed by liver microsomes (10 pmol P450/ml of incubation mixture) of rats treated with βNF (A) and of a human sample HL-64 (B). The organoselenium compounds (final concentration, 0.5 μM) tested were BSC (○), DDS (•), o-XSC (△), m-XSC (△), p-XSC (□), and αNF (small ●). Control experiments (●) in the absence of these chemicals were also determined.

Fig. 5. Cytotoxic effects of several organoselenium compounds and αNF in S. typhimurium NM2009 tester strain. Different concentrations of these chemicals were added to the incubation mixture containing bacterial strain (absorbance at 600 nm of −0.3), and the bacterial suspension was incubated for 2 h at 37°C. The cytotoxicity was determined by measuring absorbance at 600 nm, using 100% as the reading in the absence of added chemicals. The organoselenium compounds tested were BSC (○), DDS (●), o-XSC (△), m-XSC (△), p-XSC (□), and αNF (small ●).
catalyzed by CYP1B1 were determined using selenium compounds and αNF and other typical P450 inhibitors (Fig. 6). The classical P450 inhibitors used were αNF (an inhibitor for CYP1 subfamily), metyrapone (CYP2B), piperonyl butoxide (CYP2B and others), sulfaphenazole (CYP2C9), quinidine (CYP2D), diethylidithiocarbamate and 4-methylpyrazole (CYP2E1 and CYP2A), and ketoconazole (CYP3A). These chemicals (at a final concentration of 50 μM), except for αNF and sulfaphenazole, did not cause severe inhibition of the activities of 2-aminoanthracene activation by CYP1B1 in S. typhi murium NM2009. In contrast, αNF and sulfaphenazole inhibited CYP1B1 activities more than 80% at a concentration of 50 μM. Because several selenium compounds were cytotoxic to the bacterial cells, we examined these compounds at 2 μM. All three XSCs were very effective in inhibiting activation of 2-aminoanthracene by CYP1B1, although sodium selenite and DDS did not cause inhibition of CYP1B1 activities at 2 μM. BSC inhibited ~50% of the CYP1B1 activities.

The abilities of the three XSCs to inhibit activation of 2-aminoanthracene by CYP1B1 were compared with those of Trp-P-1 by CYP1A1 and of MeIQ by CYP1A2 (Fig. 7). The three XSCs were all potent inhibitors for CYP1B1 and 1A1 in the activations of 2-aminoanthracene and Trp-P-1, respectively. However, these three XSCs, when compared with CYP1B1 and 1A1 activities, were less potent in inhibiting activation of MeIQ catalyzed by CYP1A2.

Comparison of the Inhibition of Effects of m-XSC and αNF on the 7-Ethoxycoumarin O-Deethylation catalyzed by CYP1B1, 1A1, and 1A2 and by Human Liver Microsomes. The effects of m-XSC and αNF on 7-ethoxycoumarin O-deethylation by CYP1B1, 1A1, and 1A2 and by human liver microsomes were compared (Fig. 8). αNF was found to be a potent inhibitor of 7-ethoxycoumarin O-deethylation catalyzed by CYP1B1, 1A1, and 1A2 and by human liver microsomes. m-XSC, on the other hand, was relatively weak in inhibition of activities by CYP1A2 and by human liver microsomes, although it significantly inhibited the activities catalyzed by CYP1B1 and 1A1.

DISCUSSION

Present studies showed that three XSC compounds were very potent inhibitors of drug and xenobiotic oxidation activities catalyzed by various forms of human P450 enzymes. These chemicals were found to strongly inhibit 7-ethoxycoumarin O-deethylation and testosterone 6β-hydroxylation and moderately inhibit S-warfarin 7-hydroxylation, bufuralol 6-hydroxylation, 7-ethoxycoumarin O-deethylation, and chlorzoxazone 6-hydroxylation in human
liver microsomes. However, coumarin 7-hydroxylation and bufuralol 1′-hydroxylation (in human liver microsomes) were not severely affected by these three XSC compounds. One of the most important outcomes of this study was that these synthetic organoselenium compounds inhibited 7-ethoxyresorufin O-deethylation very markedly in human liver microsomes; the concentrations required to cause 50% inhibition of activities were below 0.25 μM. The inhibitory potencies of these compounds were found to be similar to αNF, which is well accepted to be a potent inhibitor of P450 proteins of CYP1A1 and 1B families (14, 21, 40).

With the use of recombinant human P450 enzymes, we found that the three XSCs used in this study had relatively higher abilities to inhibit xenobiotic and procarcinogen oxidations with CYP1A1 and 1B than CYP1A2 and other human P450 enzymes. Particularly, the XSCs very significantly inhibited the metabolic activation of Trp-P-1 by CYP1A1 and of 2-aminoanthracene by CYP1B1, at very low concentrations. CYP1A2 activities were also highly susceptible to these XSC compounds, e.g., 7-ethoxyresorufin O-deethylation and activation of MelQ by CYP1A2 and 7-ethoxyresorufin O-deethylation by human liver microsomes. The observation that the three XSC compounds and BSC caused greater spectral changes with liver microsomes of rats treated with βNF than those of untreated rats and phenobarbital-treated rats indicated that these selenium compounds may have higher affinities for βNF-inducible P450 enzymes in rat liver microsomes. m-XSC, which was most effective in inducing spectral changes with rat liver microsomes, also caused type II spectral change with liver microsomes of a human sample (HL-16), which had high levels of CYP1A2 in liver microsomes (Fig. 2B).

Recently, CYP1B1 cDNA was isolated and characterized in humans (17, 41), mice (42, 43), and rats (44, 45). CYP1B1 proteins have been isolated from a mouse embryo fibroblast cell line and from rat adrenal microsomes (46–48). Both the rat and mouse counterparts have been shown to catalyze the oxidation of carcinogenic polycyclic aromatic hydrocarbons such as 7,12-dimethylbenz[a]anthracene (46–48). The human CYP1B1 cDNA sequence has been reported to be about 80% similar to the mouse and rat orthologues, and the expression of the CYP1B1 mRNA has been observed in many organs including kidney, prostate, mammary gland, pituitary, thymus, spleen, adrenal, colon, ovary, uterus, brain, heart, lung, intestine, and testis (17, 45). CYP1B1 may be very important in understanding chemical carcinogenesis in humans, because this P450 enzyme can catalyze the activation of diverse procarcinogenic and promutagenic chemicals to genotoxic metabolites that induce the SOS response in Salmonella tester strains (16). Of particular interest is the observation that dibenzo[a]pyrene-11,12-diol, 5-methylchrysene-trans-1,2-diol and benz[a]pyrene-trans-7,8-diol, which are suggested to be groups of the most potent inducers of mammary tumors and lung cancers in experimental animals (18, 19, 49), have been determined to be activated extensively by human CYP1B1.

CYP1A1 is reported to be expressed in extrahepatic organs including prostate, mammary gland, intestine, thymus, colon, adrenal, ovary, uterus, lung, and testis, whereas CYP1A2 is expressed primarily in microsomal fractions of the liver (22, 50, 51). Both enzymes play major roles in the metabolism of a variety of procarcinogens and promutagens, and recent studies have demonstrated that CYP1A1 and 1A2 are able to oxidize drugs such as theophylline, caffeine, bufuralol, chlorzoxazone, tamoxifen, zoxazolamine, acetaminophen, antipyrine, lidocaine, phenacetin, propranolol, and warfarin (22, 50, 52, 53). Very recently, we have shown that CYP1B1 catalyzes the oxidation of benz[a]pyrene, 7-ethoxyresorufin, theophylline, caffeine, bufuralol, and 7-ethoxycoumarin (30). These chemicals have been reported to be known substrates for human CYP1A1 and 1A2, except that bufuralol is catalyzed mainly by CYP2D6, whereas 4- and 6-hydroxylation of bufuralol are catalyzed by CYP1A1 and 1A2 enzymes (22, 23, 50–52, 54).

The three XSCs used in this study showed high toxicity in bacterial tester strain S. typhimurium NM2009. Because these compounds have been shown to be less toxic in experimental animals (9), the possibility exists that these selenium compounds may be metabolized by mammals to form less toxic metabolites. After absorption, sodium selenite is reduced by reduced glutathione through GSSeSG to the volatile and highly toxic H2Se, which can be incorporated into proteins and/or further methylated to dimethylselenide; the latter can be detected in the exhaled air (55). In contrast, however, in our preliminary investigation, we have demonstrated that less than 1% of selenium can be detected in the exhaled air, which may, in part, account for the lower toxicity of p-XSC as compared to sodium selenite (56).

In laboratory animals, we demonstrated that the organoselenium compounds can inhibit tumorigenesis induced by a variety of chemical carcinogens (9, 10, 20). In the rat mammary tumor model system using 7,12-dimethylbenz[a]anthracene, we found that p-XSC has a better chemopreventive index (more effective and less toxic) than those of BSC and sodium selenite (9, 10). Dibenzyl diselenide has never been tested in this animal system. Previous studies also indicate...
that \( \alpha \) - and \( m \)-XSC appear to be equally effective but both are better inhibitors of 7,12-dimethylbenz[\( \alpha \)]antracene-DNA addsuct formation in the rat mammary gland (9, 20, 56). Unfortunately, \( \alpha \)- and \( m \)-XSC have not yet been tested in efficacy studies. Collectively, these results suggest that organoselenium compounds inhibit 7,12-dimethylbenz[\( \alpha \)]antracene-induced mammary tumors in the rat either by inhibiting phase I activation or by inducing phase II detoxification enzymes that are involved in the metabolism of this mammary carcinogen. The present study clearly demonstrates that the efficacy of these organoselenium compounds is due, in part, to their inhibitory effects on P450s that are involved in the metabolic activation of 7,12-dimethylbenz[\( \alpha \)]antracene. However, on the basis of the \textit{in vitro} results obtained in this study, it would be premature to rank the \textit{in vivo} chemopreventive potency of the organoselenium compounds examined in this study. Moreover, we also demonstrated the efficacy of BSC and \( p \)-XSC against the development of colon cancer induced by azoxymethane and lung tumors induced by the tobacco-specific nitrosamine, 4-(\( \beta \)-nitrosoamino)-1-(\( \beta \)-3-pyridyl)-1-butanone (57).

In conclusion, the present results showed that several synthetic selenium compounds such as the three XSCs, BSC, and DDS inhibited activities of xenobiotic and procarcinogen oxidations catalyzed by human P450 enzymes \textit{in vitro}. Of these selenium compounds examined, the three XSCs were the most potent in inhibiting the xenobiotic and procarcinogen oxidation activities by P450 enzymes in human liver microsomes and by recombinant human P450 enzymes. Spectral studies showed that BSC and the three XSC compounds, but not sodium selenite and DDS, induced type II difference spectrum when added to liver microsomes isolated from \( \beta \)-naphthoflavone-treated rats, with \( m \)-XSC being most potent in inducing spectral interactions with P450 enzymes. \( m \)-XSC also caused type II spectral change with human liver microsomes. These results suggest that some of the synthetic organoselenium compounds inhibit the activation of environmental procarcinogens by P450 enzymes, particularly by CYP1 subfamily enzymes. These results suggest that some of the synthetic organoselenium compound, 1,4-phenylenediamine methylene selenocyanate. Cancer Res., 52: 2402–2407, 1992.

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