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Differential Effects of Indole, Indole-3-carbinol and Benzofuran on Several Microsomal and Cytosolic Enzyme Activities in Mouse Liver[1]

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ABSTRACT

The effects of feeding indole, indole-3-carbinol and benzofuran (all at 5 mmole/kg body wt./day) on various hepatic microsomal and cytosolic enzyme activities involved in xenobiotic metabolism have been compared. Benzofuran was found to elevate the activities of many enzymes both in microsomes (e.g., aniline hydroxylase, 7-ethoxycoumarin O-deethylase, *p*-nitrophenol UDPGA-transferase and epoxide hydrolase) and in cytosol (e.g., glutathione reductase, glutathione S-transferase, NADH:quinone reductase and UDP-glucose dehydrogenase). The structures of indole and indole-3-carbinol are similar to benzofuran except for the substitution of nitrogen with oxygen atom within the furan ring. Results showed that the activities of UDPGA-transferase and NADH:quinone reductase were not elevated by these indole compounds. While the chemical structure of these two indole compounds are identical except for the presence of the carbinol (methanol) group in indole-3-carbinol, there were marked differences in the types and activities of microsomal enzymes that were enhanced. Among the microsomal enzyme activities determined, indole elevated only the NADPH:cytochrome c reductase, while indole-3-carbinol increased several mixed function oxidase and particularly the epoxide hydrolase activities. Based on the chemical structures of tested compounds and the observed results, possible explanations for the mechanisms involved in elevating epoxide hydrolase activity by benzofuran and indole-3-carbinol are discussed.

Key Words: indole, indole-3-carbinol, benzofuran, microsomal enzymes

INTRODUCTION

Phenolic antioxidant compounds which are commonly used as food preservatives (Branen, 1975; Chipault, 1966) are known to produce anticarcinogenic effects against tumor production inducible with variety of chemical carcinogens. Studies on the anticarcinogenic mechanisms of antioxidants have indicated that modulation of xenobiotic metabolizing enzyme activities are involved (Cha and Heine,

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1982: Grantham *et al.*, 1973: Miller, 1978; Talalay *et al.*, 1979; Ulland *et al.*, 1973; Wattenberg *et al.*, 1976; Wattenberg, 1980). Enzymatic changes brought about by antioxidants may then result in a reduction of the steady state levels of reactive carcinogenic metabolites either by efficient inactivation, or by decreasing its production from a suspected procarcinogen. For example, following a chronic dietary administration of the antioxidant 2(3)-*tert*-butyl-4-hydroxyanisole (BHA), elevations of glutathione-(Benson *et al.*, 1978; 1979; Cha and Heine, 1982; Cha *et al.*, 1982; 1983; Miranda *et at.*, 1981; Salocks *et al.*, 1981; Talalay *et al.*, 1979) and glucuronide conjugation enzyme systems (Cha and Bucding, 1979; Cha and Heine, 1982; Cha *et al.*, 1982; 1983; Grantham *et al.*, 1973; Moldeus *et al.*, 1982; Ulland *et al.*, 1973), increases of epoxide hydrolase activities (Benson *et al.*, 1979; Can and Heine, 1982; Cha *et al.*, 1979), as well as the alterations in the metabolic profiles of several carcinogens (Cha and Bueding, 1979; Cha and Heine, 1982; Cha *et al.*, 1982; 1983; Kahl and Netter, 1977; Speier and Wattenberg, 1975) were observed.

In an earlier study, we showed that dietary administration of dihydro-benzofuran (coumaran) prodeced marked increases of hepatic microsomal epoxide hydrolase activity, as with BHA (Heine *et al.*, 1984). It appears that the administered BHA could donate hydrogen and becomes a phenoxy radical (Sgaragli *et al.*, 1980). This BHA-radical may then undergo ring closure between the oxygen radical and the adjacent *tert*-butyl side chain, thus forming dihydro-benzofuran (coumaran) derivatives (Orlando *et al.*, 1966) which contain highly strained-bond-angle oxygen atom in its furan ring. Biological formation of such coumaran metabolites has been demonstrated in studies of metabolism with another phenolic antioxidant, 3,5-di-*tert*-butyl-4-hydroxyanisole (Topanol 354) (Daniel *et al*, 1973). Thus, we reasoned that compounds that are (i.e., coumaran) or could generate such heterocyclic structures (i.e., BHA) which contain highly strained-bond-angle oxygen atom may have been responsible for the elevation of epoxide hydrolase activity (Heine *et al*, 1984). Indole compounds, which contain nitrogen instead of oxygen atom, are structurally similar to the benzofuran compounds.

The basic structure of indole is an important chemical component of various compounds which are present in animal and plant tissues. Indoles are particularly abundant in cruciferous vegetables such as cabbage, brussels sprouts, cauliflower and broccoli, and thus often appear in significant amounts in the human diet (Wattenberg, 1975). In addition to being an important building block for the synthesis of tryptophan, certain indoles, especially the indole-3-carbinol, have been shown to be potent inducers of hepatic aryl hydrocarbon hydroxylase activity (Loub *et al*, 1975). In humans, increased ingestion of cabbage and brussels sprouts has been shown to alter the rate of metabolism of certain drugs (Pantuck *et al.*, 1979). In this connection, indole has been shown to protect rats and hamsters against the 2-acetylaminofluorene (AAF) and dibutylnitrosoamine (DBNA) induced hepatotoxicity and carcinoma of liver, esophagus and bladder (Hopp *et al.*, 1976; Kitajima *et al.*, 1975; Matsumoto *et al.*, 1977). Furthermore, dietary administration of indole-3-carbinol was observed to protect against the benzo(a)pyrene inducible neoplasia of the forestomach in female ICR/Ha mice and the 7,12-dimethylbenzanthracene inducible mammary tumor formation in female Sprague-Dawley rats (Wattenberg and Loub, 1978). Indole-3-carbinol has also been shown to reduce the covalent binding of reactive metabolites of benzo(a)pyrene and dimethylnitrosamine to hepatic macromolecules (Shertzer, 1982).

There are only few but limited studies involving the effects of indole compounds on the main xenobiotic metabolizing enzyme activities. Only in recent years, several investigators have studied, but in an isolated manner, the effects of indole on levels of cytochrome P-450, activities of few microsomal mixed function oxidases (Arcos *et al.*, 1980; Evarts and Mostafa, 1981; Shertzer, 1980; Watanuki *et al.*, 1982) and cytosolic glutathione S-transferases (Sparnins, 1980). In this paper, we have determined the effects of indole, indole-3-carbinol and benzofuran on several xenobiotic metabolizing enzymes of mouse liver in a comprehensive manner. Results obtained with these compounds were related to those previously obtained with BHA (Cha and Heine, 1982; Kahl and Wulff, 1979; Speier and Wattenberg, 1975; Talalay *et al.*, 1979; Wattenberg *et al.*, 1976) in an effort to understand the biochemical mechanisms for the observed anticarcinogenic actions of these compounds.

MATERIALS AND METHODS

Treatment of Animals

Female CD-1 mice (5 to 7 weeks old), purchased from Charles River Breeding Labs (Wilmington, MA), were housed in hanging stainless steel wire cages (5 mice per cage) with 12 hour light-dark cycle. During the 10 day acclimation period, mice had free access to tap water and Purina Laboratory Chow (Code 5001, Ralston Purina Co., St. Louis, MO). Subsequently, experimental mice were given diets conatining 0.33% indole or 0.41% indole-3-carbinol for 10 days. Benzofuran was dissolved in corn oil (Sigma) at 59.1 mg/ml and was injected orally at 0.1 mg/20 gm body weight twice a day for 10 days. These daily administered doses are 5 mmoles/kg body weight for each of these compounds which were all purchased from Aldrich Chemical Co. (Milwaukee, WI). The selection of this particular dose was based on the previously established average daily doses of BHA (for a 25 gm mouse on 0.75% BHA diet consuming 4.5 gm diet per day) (Cha and Bueding, 1979).

Animals were sacrificed on day 11 and livers were cleared of red blood cells and were individually homogenized in 35 ml of ice cold sucrose (0.25 M) using teflon-glass homogenizers (6 strokes). All subsequent steps for isolation of washed microsomes and cytosol were as previously described (Cha and Bueding, 1979). Protein concentrations were determined by the Lowry technique (Lowry *et al.*, 1951) using crystalline bovine serum albumin (Sigma) as the standard.

Enzyme Assays

In the isolated and washed microsomes, levels of cytochromes P-450 and b-5 were measured by the method of Omura and Sato (1964a; 1964b) utilizing DW-2a spectrophotometer (American Instrument Co., Silverspring, MD) operating in split-beam scanning mode. Assays of NADPH- and NADH-cytochrome c reductases were carried out according to Masters *et al.* (1965) using the spectrophotometer in double-beam time-base mode. Activities of microsomal mixed function oxidases were measured using either aminopyrine (8 mM), aniline (5 mM), and 7-ethoxycoumarin (147 uM) as substrates according to the methods described by Nash (1953), Schenkman *et al.* (1967) and Ullrich and Weber (1972), respectively. The epoxide hydrolase activity was measured radiometrically using the 14-C styrene oxide (New England Nuclear, Boston, MA) as the substrate (Cha *et al.*, 1978), and the activity of UDPGA-transferase was determined with *p*-nitrophenol as a substrate according to Mills and Smith (1978).

The activities of several cytosolic enzymes such as the glucose 6-phosphate dehydrogenase (Lohr and Waller, 1963), UDP-glucose dehydrogenase (Mills and Smith, 1963) and glutathione reductase (Racker, 1955) were also measured. Additionally, the activities of glutathione S-transferases (Habig *et al.*, 1974) and NADH:quinone reductase (Benson *et al.*, 1980) were determined using the spectrophotometer in double-beam time-base mode. The dicoumarol-sensitive NADH:quinone reductase activity was measured by determining the initial velocity of reduction of 2,6-dichloro-indophenol (Sigma) at 600-700 nm ($AM = 2.1 \times 10 \times 4\ M \times \text{-}1cm \times \text{-}1$). The glutathione S-transferase activities were measured using 1-chloro-2,4-dinitrobenzene (CDNB) or 1,2-dichloro-4-nitrobenzene (DCNB) (both from Eastman Organic Chemical, Rochester, NY) and reduced glutathione (Sigma) as the substrates.

The results were analyzed for significance using the Student's t-test with a P value of 0.01. Statistically significant differences are denoted in the tables by an astrisk (*)

RESULTS

The specific activities of enzymes investigated are listed in Tables 1 (microsomal) and 2 (cytosolic).

Table 1. Specific enzyme activities of microsomal fractions from mouse liversa

Enzymes	Control	Indole	Indole-3-Carbinol	Benzofuran
Cytochrome P-450	0.70 (±0.07)	0.65 (±0.09)	1.23[b] (±0.13)	0.49[b] (±0.08)
Cytochrome B_5	0.42 (±0.02)	0.50 (±0.06)	0.59[b] (±0.07)	0.39 (±0.06)
NADPH-cytochrome *c*-reductase	220.1 (±30.8)	357.3[b] (±42.0)	379.5[b] (±43.9)	270.7 (±19.6)
NADH-cytochrome *c*-reductase	2.07 (±0.13)	2.40 (±0.31)	1.45[b] (±0.30)	1.61 (±0.34)
Aminopyrine N-demethylase	34.5 (±6.6)	35.5 (±2.5)	102.9[b] (±21.0)	25.4 (±4.4)
Aniline Hydroxylase	56.4 (±13.6)	68.4 (±12.0)	161.0[b] (±47.7)	76.2 (±17.1)
7-Ethoxycoumarin O-deethylase	0.42 (±0.09)	0.58 (±0.08)	0.78[b] (±0.10)	1.05[b] (±0.21)
Epoxide Hydrolase	3.70 (±0.51)	4.53 (±0.42)	7.72[b] (±1.96)	8.08[b] (±1.64)
p-Nitrophenol UDPGA-transferase	16.3 (±3.0)	22.9 (±2.6)	24.0 (±7.8)	38.7[b] (±9.3)

a: Specific activities are in nmoles/min/mg except for cytochrome P-450 and cytochrome b_5 which are in nmoles/mg, and NADH-cytochrome *c*-reductase which is in μmoles/min/mg.

b: notes values which are significantly different from control ($P < 0.01$).

These specific enzyme activities are expressed in moles of product formed per min per mg except where noted.

The dietary administration of large doses of indole (5 mmol/kg/day) resulted in the elevation (significant increase in the specific enzyme activity of treated over control) of only one microsmal enzyme activity. namely the microsomal NADPH-cytochrome c reductase. Based on the extensive metabolism of administered indole (King *et al.*, 1966), which will be discussed later, it was interesting to note that indole did not elevate any of the determined microsomal mixed-function oxidases (MFO) and epoxide hydrolase activities, whereas the indole-3-carbinol did (Table 1). The UDPGA-transferase activity was not significantly changed by either of these compounds. The feeding of indole-3-carbinol resulted in the elevations of cytochromes P-450 and b-5 contents as well as the NADPH-cytochrome c reductase (+72%), aminopyrine N-demethylase (+198%), aniline hydroxylase (+185%), 7-ethoxycoumarin O-deethylase (+88%),and epoxide hydrolase (+109%)activities. Conversely, the NADH-cytochrome c reductase activity was decreased by 30%. As for the cytosolic enzymes (Table 2), both the indole and indole-3-carbinol feedings produced similar increases of UDP-glucose dehydrogenase, glutathione reductase and glutathione S-transferase activities, but no significant changes in the glucose 6-phosphate dehyrogenase or the NADH-quinone reductase activities were observed.

The administration of benzofuran produced a decrease (−30%)of the cytochrome P-450 content without any changes of the cytochrome b-5 level or both the NADPH-and NADH-cytochrome c reduc-

Table 2. Specific enzyme activities of cytosolic fractions from mouse livers[a]

Enzymes	Control	Indole	Indole-3-Carbinol	Benzofuran
Glucose 6-phosphate Dehydrogenase	19.4 (±2.2)	21.2 (±4.9)	19.2 (±4.1)	22.2 (±8.5)
Glutathione (GSSG) Reductase	49.7 (±11.1)	89.9[b] (±16.7)	88.8[b] (±24.6)	97.9[b] (±14.9)
(CDNB)-Glutathione S-transferase	1.69 (±0.39)	2.68[b] (±0.65)	5.36[b] (±1.14)	11.93[b] (±1.79)
(DCNB)-Glutathione S-transferase	32.5 (±2.9)	64.6[b] (±8.8)	79.5[b] (±18.1)	119.6[b] (±18.6)
NADH: quinone Reductase	267.7 (±55.8)	365.3 (±81.2)	250.9 (±69.5)	615.2[b] (±109.1)
UDP-glucose Dehydrogenase	49.2 (±3.3)	82.7[b] (±8.8)	76.4[b] (±14.7)	69.7[b] (±5.3)

a: Specific enzyme activities are in nmoles/min/mg except for (CDNB)-glutathione S-transferase which is in μmoles/min/mg.

b: indicates values which are significantly different from control ($p < 0.01$).

tase activities (Table 1). Despite these results, the 7-ethoxycoumarin O-deethylase activity, among the three types of microsomal mixed-function oxidases investigated, was increased (+152%) Thus, benzofuran does not appear to have as great as an effect on the microsomal mixed function oxidase system as indole-3-carbinol did. However, the activities of microsomal epoxide hydrolase (+118%) and *p*-nitrophenol UDPGA-transferase (+137%) were increased. As with indole and indole-3-carbinol, the benzofuran treatment increased activities of cytosolic glutathione reductase, glutathione S-transferases and UDP-glucose dehydrogenase (Table 2). However, unlike the two former compounds, the NADH:quinone reductase activity was enhanced only by the latter.

DISCUSSION

Dietary administrations of some synthetic food additive phenolic antioxidants such as butylated hydroxytoluene (BHT) and BHA, as well as some natural compounds like indole and indole-3-carbinol have all been demonstrated to protect against tumors inducible with diverse chemical carcinogens in a variety of animal tissues (Kitajima *et al.*, 1975; Matsumoto *et al.* 1977; Miller, 1978; Shertzer, 1982; Ulland *et al.*, 1973; Wattenberg, 1975; 1980; Wattenberg and Loub, 1978). While the chemical structures of these phenolic antioxidants and the indole compounds are vastly different, there may exist some common biochemical mechanisms for their demonstrated anticarcinogenic effects.

Associated with the uses of BHA and BHT as food additives by the food industry, there have been several studies dealing with their metabolism (Branen, 1975). These studies showed that BHA was metabolized rapidly to *tert*-butyl-hydroquinone (TBHQ) and excreted primarily as glucuronide conjugates of either BHA and TBHQ. In metabolic studies with another closely related phenolic antioxidant, 3,5-di-*tert*-butyl-4-hydroxyanisole (Topanol 354), the formation of dihydro-benzofuran (coumaran) metabolites have also been demonstrated (Daniel *et al.*, 1973). This suggested the possibility that coumaran metabolites could also be formed following the administeration of BHA which produces

the TBHQ. In support of this, it is well known that chemical synthesis of coumaran involves a simple pyrolysis of TBHO (Orlando *et al.*, 1966). This chemical synthesis utilizes the principle of ring closure between the phenoxy radical formed during pyrolysis with the adjacent t-butyl side chain. In connection with this, we have reasoned that some of the metabolites arising from BHA (inclusive of TBHQ) may have been responsible for some of the observed changes in several xenobiotic metabolizing enzymes thought to be involved in anticarcinogenic mechanisms of BHA. Thus, we have determined the effects of administering TBHQ, hydroxyanisole, coumaran, trimethylene oxide and trimethylene sulfide (Cha *et al.*, 1982; Heine *et al.*, 1984). These studies showed that the presence of *tert*-butyl side chain adjacent to hydroxyl group was required for the marked elavation of epoxide hydrolase activity seen with the BHA and BHT feeding. Results obtained with coumaran and related compounds further indicated that a preferential elevation of the epoxide hydrolase activity is produced by compounds which include the strained-bond-angle oxygen or sulfur atoms (Heine *et at.*, 1984).

Coumaran (dihydro-benzofuran) and benzofuran are structurally identical except that coumaran does not have double bond at the 2-3 position. The presence of 2-3 double bond in benzofuran could serve as an ideal substrate for the formation of 2,3-epoxide, which in turn, may serve as a potent inducer of the epoxide hydrolase activity. Comparing the results obtained upon administration of equimolar doses of these two compounds, the dihydro-benzofuran (without the double bond) (9.1 nmol/mg/min) (Heine *et al.*, 1984) was more effective in elevating the enzyme activity than the benzofuran (with the double bond) (8.1 nmol/mg/ml) (Table 1). Therefore, the presence of double bond does not appear to contribute toward any additional elevation of epoxide hydrolase activity. It was interesting to note that indole-3-carbinol increased the epoxide hydrolase activity, but indole did not (Table 1). Both of these compounds have the double bond at the 2-3 position. This result lends further support to the idea that the presence of double bond is not responsible for the observed increase of the enzyme activity.

As mentioned earlier, the results obtained with coumaran and related compounds suggested that the strained-bond-angle of the oxygen atom is responsible for the elevation of the enzyme activity (Heine *et al.*, 1984). Furthermore, the study also indicated that sulfur atom can substitute for the oxygen atom and cause the elevation of epoxide hydrolase activity. In this connection, we were interested to know if nitrogen atom could also subsitute for the oxygen atom. Sulfur and oxygen atoms are in the same group of the periodic chart and both atoms have six electrons in their outermost orbital. However, the nitrogen atom has one less electron, namely, five instead of six and requires an additional hydrogen atom. However, the presence of hydrogen atom on the nitrogen in indole may have prevented the enhancement of epoxide hydrolase activity (Table 1). This is not the case with indole-3-carbinol, however (Table 1). Carbinol (methanol) is an electron donating group and tends to donate an electron to contribute to the resonance structure of the indole ring. This may facilitate the dissociation of the N-hydrogen, through stabilization of the resulting anion. This nitrogen atom present in indole-3-carbinol would thus have an extra electron and would be very similar to the oxygen atom included in benzofuran. Comparison of the dissociation constants for the N-hydrogen of indole and indole-3-carbinol indicated that the N-hydrogen of the latter tends to dissociate more readily than the former (Yagil, 1967). In a recent study, Vaz *et al.* (1981) showed that if the nitrogen atom is removed further away spatially from the benzene moiety, similar results could be obtained. Thus, even with the hydrogen atom present, indole-3-carbinol was able to enhance the epoxide hydrolase activity (Table 1).

The metabolism of indole has been investigated by King *et al.* (1966). When a small dose of indole (^{14}C labled at the 2 position) was fed to rats, it was fairly rapidly metabolized and excreted. In 2 days, 81% of indole metabolites appeared in the urine, 11% in the feces, and 2.4% as carbon dioxide in the expired air. The major products excreted in the urine were indexyl sulfate(50% of ingested dose), indexyl glucuronide (11%), isatin (5.8%), exindole (1.4%), 5-hydroxyindole conjugates (3.1%) and others. Thus, the metabolism of indole involves mixed function oxidation enzymes, epoxide hydrolase, UDPGA-transferase and sulfotransferase and consequently, the activities of these enzymes were ex-

pected to be elevated in the present experiment. However, the administration of large doses of indole, in addition to what was normally present in the diet, did not elevate most of these microsomal enzyme activities (Table 1). This result may indicate that these hepatic microsomal enzyme activities were already in an elevated state by the constituent compounds present in normal diet (Wattenberg, 1975). While the metabolism of indole-3-carbinol has not yet been examined, administration of equimolar doses of this compound produced increases in several microsomal enzyme activities that were not elevated by indole (Table 1). It has also been reported that indole-3-carbinol elevates the aryl hydrocarbon hydroxylase activity (31). This leads to a speculation that the mechanism of anticarcinogenic actions between these two closely related compounds may be different.

Watanuki *et al* (1982) recently showed that indole may act as a competitive inhibitor of some mixed function oxidase enzymes which transform the inactive chemical carcinogens to reactive metabolites. In this connection, indole has also been shown to suppress the hepatotoxicity and carcinogenicity of acetylaminofluorene (AAF) in rats by decreasing the biliary secretion of the O-glucuronide of N-OH-AAF (Hopp *et al.*, 1976). This may possibly reflect the decreased formation of N-OH-AAF metabolite by competitive inhibition mentioned earlier (Watanuki *et al.*, 1982). Indole-3-carbinol, on the other hand, may act by elevating certain microsomal mixed function oxidase activities (Table 1) which could result in shifting the metabolic profile of carcinogens, so that less of the carcinogenic reactive metabolites are produced at the microsomal level.

Alternatively, as was observed with BHA, the elevation of glutathione S-transferases (GST) by both indole and indole-3-carbinol (Sparnins, 1980) may have been commonly responsible for the anticarciongenic mechanisms of these compounds (Table 2). The elevated GST may catalyze an increased formation of thioester conjugates of reactive electrophilic compounds in the presence of reduced glutathione (GSH). These conjugates are typically less toxic than the original reactive metabolites and are more readily eliminated from the body as mercapturic acids. In addition to this catalytic action, the binding actions of elevated GST may also represent an important function to serve as an expendable "buffer" protein for the inactivation of reactive carcinogenic metabolites (Jakoby, 1977). It was interesting to note that benzofuran increased the GST activities to an even higher level than those obtained with indole or indole-3-carbinol (Table 2).

Glucuronidation, catalyzed by the microsomal UDP-glucuronyl transferase, in the presence of UDP-glucuronic acid (UDPGA) of cytosol, represents one of the major conjugation reactions for many of the phenolic intermediary metabolites produced by the microsomal mixed function oxidases. The water-soluble glucuronide metabolites, which are biologically inactive, are excretable in urine or bile. The concentration of UDPGA, a cofactor required for such glucuronidation, is normally maintained at low level (Moldeus, 1978). The biosynthesis of this cofactor involves an oxidation of UDP-glucose which is catalyzed by the UDP-glucose dehydrogenase (UDPGH) upon reduction of NAD to NADH within cytoplasm. This enzyme reaction is, however, inhibited by the high ratio of NADH:NAD (i.e., during ethanol oxidation) (Moldeus, 1978). A cytosolic enzyme that could lower this ratio, thus promoting the UDPGA synthesis especially when the quinone substrates are provided, is the NADH dependent quinone reductase. It is interesting to note that only the benzofuran treatment produced elevations for all three enzyme activities involved in the glucuronide conjugation system (Tables 1 and 2). These results may indicate that the administered benzofuran could dissociate to form quinone compounds, which in turn, could serve as potent inducers of not only the UDPGA-transferase but also the NADH: quinone reductase activites (Cha *et al.*, 1982). Therefore, the replacement of oxygen atom in benzofuran with nitrogen atom (i.e., indole compounds) may reduce the potential to serve as inducers of glucuronidation enzyme system.

In summary, the results indicate that benzofuran may also serve as an anticarcinogenic agent as with BHA, indole, and indole-3-carbinol. While there may exist several different mechanisms for their observed anticarcinogenic actions, the enhancement of detoxification enzyme pathway involving GST appear to be common among many tested compounds. For the elevation of glucuronidation system en-

zyme activities (i.e., NADH:quinone reductase), the presence of oxygen atom appears to be required. However, for the increase of microsomal epoxide hydrolase activity, the oxygen atom of benzofuran could be replaced with the nitrogen atom provided that the electronic requirements are met, as is the case with the indole-3-carbinol.

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=국문초록=

Indole, Indole-3-calbinol 및 Benzofuran이 간장 microsome과 cytosol의 약물대사 효소 활성도에 미치는 영향

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이물질 **(xenobiotics)** 대사에 관여하는 간장 microsome과 cytosol 효소 활성에 indole, indole-3-carbinol 및 benzofuran이 미치는 영향을 검색하기위하여 마우스에 이들 약물을 각각 5 mmole/kg씩 10 일간 투여하여 다음 몇가지의 성적을 얻었다.

Benzofuran은 microsome 효소인 aniline hydroxylase, 7-ethoxycoumarin O-deethylase, *p*-nitrophenol UDPGA-transferase, epoxide hydrolase와 cytosol 효소인 glutathione S-tranferase, NADH : quinone reductase, UDP-glucose dehydrogenase의 활성도를 증가시켰다. 그러나 benzofuran과는 구조적으로 furan ring내의 N원소가 O원소로 치환되었을 뿐 주된 구조가 유사한 indole과 indole-3-carbinol 투여로는 UDPGA-transferase와 NADH : quinone reductase의 활성도 증가를 볼 수 없었으며, 특히 indole은 NADPH : cytochrome C reductase만을 증가시킨데 비하여 구조상 indole에 carbinol (methanol)기가 붙은 indole-3-carbinol은 수종의 mixed function oxidase와 아울러 특히 **epoxide** hydrolase의 활성도 역시 증가시켰다.

이러한 결과는 benzofuran과 indole-3-carbinol에 의한 **epoxide** hydrolase 활성도 증가의 기전의 일부를 설명할 수 있을 것으로 생각된다.