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DIFFERENTIAL EFFECTS OF DISULFIRAM AND DIETHYLDITHIOCARBAMATE ON SMALL INTESTINAL AND LIVER MICROSOMAL BENZO[*a*]PYRENE METABOLISM

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Abstract—Microsomes isolated from rat small intestinal mucosa and liver were used to study the effects of disulfiram and diethyldithiocarbamate on benzo[*a*]pyrene monooxygenase activity. This activity was decreased in the intestinal microsomes to 25 per cent of control 24 hr after a single oral dose of disulfiram. In contrast, daily administration of disulfiram for 5 days produced a dose related increase of benzo[*a*]pyrene monooxygenase activity, above control level. The elevated activities were accompanied by a concomitant increase in the concentration of cytochrome P-450. This benzo[*a*]pyrene monooxygenase activity was further stimulated by addition of α-naphthoflavone to the incubation medium. Furthermore, the absorption maximum of this cytochrome was at 450 nm in the CO bound reduced difference spectrum. These observations indicate that the disulfiram induced cytochrome P-450 was of the control type. Daily pretreatment with diethyldithiocarbamate impaired both intestinal and liver microsomes at benzo[*a*]pyrene monooxygenase activities. Pretreatment with a single dose of 3-methylcholanthrene resulted in a more than 10-fold increase of intestinal benzo[*a*]pyrene monooxygenase activity after 24 hr. Administration of disulfiram 24 hr before treatment appeared to potentiate the 3-methylcholanthrene induced increase of intestinal benzo[*a*]pyrene monooxygenase activity. *In vitro* addition of disulfiram and diethyldithiocarbamate to incubates of intestinal or liver microsomes inhibited benzo[*a*]pyrene metabolism to various extents; the liver being more sensitive. Disulfiram was approximately 50-fold more potent as an inhibitor than diethyldithiocarbamate. The *in vitro* inhibition of intestinal benzo[*a*]pyrene monooxygenase activity obtained with disulfiram appeared to be caused both by direct interaction with the monooxygenase system and through NADPH dependent metabolic activation of disulfiram, while the inhibition of diethyldithiocarbamate may be a result of the latter process only.

The drug metabolizing enzymes of the gastrointestinal (GI) tract can be regarded as the body's first line of defence against xenobiotics taken orally. However, it has become apparent that this metabolism may lead not only to detoxification and decreased bioavailability, but also to activation of procarcinogens and other drugs [1–4]. The highest oxidative activity of the GI tract occurs in the small intestine [5, 6], where a variety of compounds are metabolized by the cytochrome P-450-dependent monooxygenase system [7, 8]. Although the small intestinal monooxygenase activity can easily be lost during subcellular fractionation procedures, methods are now available for the preparation of intestinal microsomes which minimize this loss and provide a stable preparation for the study of cytochrome P-450 and certain monooxygenase activities dependent in this hemoprotein [3]. A frequently used substrate is the chemical carcinogen benzo[*a*]pyrene (BP), which among several metabolites forms highly fluorescent phenols that can be accurately quantitated in low concentrations [9].

The small intestinal monooxygenase activity responds to drugs, environmental agents and nutritional factors [cf. 1, 2]. Pretreatment of rats with 3-methylcholanthrene (MC) rapidly increases the activity of intestinal BP monooxygenase to that comparable with normal liver [3]. Number of other xenobiotics and dietary constituents also are known to increase cytochrome P-450 dependent metabolism in the small intestine [10, 11]. Metabolism of polycyclic aromatic hydrocarbons to ultimate carcinogens in the GI tract may be highly significant since administration of these compounds results in tumor formation in various parts of the gut [12, 13].

Recently it has been reported that disulfiram (DS), a drug used in alcohol avoidance therapy, protects mice from BP-induced forestomach cancer [13] as well as dimethylhydrazin or azoxymethane-induced intestinal cancer [14, 15]. This protective effect could involve impaired metabolic activation of these compounds in the liver or other target organs since DS has previously been reported to impair certain oxidative as well as hydrolytic metabolic pathways in the liver [16–18].

In this study, we have evaluated the effects of the oral administration of DS and its reduced metabolite, diethyldithiocarbamate (DDTC), on BP metabolism by the small intestine and liver. In addition we also have studied the direct effects of these compounds on intestinal and hepatic microsomes *in vitro*. The results show that although DS and DDTC may inhibit BP metabolism in liver as well as intestine after oral

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administration or addition to microsomal incubations, important differences exist between these organs with regard to the actions of these compounds. The effects produced by repeated exposure to DS suggest that xenobiotic metabolism in small intestine may become relatively more important for the systemic bioavailability of orally administered compounds; increased oxidative enzyme activity in intestine while a substantially depressed activity in liver could occur.

MATERIALS AND METHODS

Chemicals. Benzo[*a*]pyrene (BP), disulfiram (DS, tetraethylthiuram disulfide) and 3-methylcholanthrene (MC) were obtained from Sigma Chemical Co. (St. Louis, MO), sodiumdiethyldithiocarbamate (DDTC) trihydrate from Fluka AG Buchs SG (Switzerland) and α-naphthoflavone (ANF) from Aldrich Chemical Co. Inc. (Milwaukee, WI). Other general chemicals were obtained from local commercial sources.

Animals and treatments. Male Sprague–Dawley rats, weighing 160–180 g, were kept in stainless steel cages and maintained on pelleted food (Anticimex Avelsfoder 213, Astra-Ewos AB, Södertälje, Sweden) and tap water *ad lib.* MC or DS were dissolved in corn oil and DDTC in water and administered by gavage with a stainless steel feeding tube. Rats received 0.5 ml solvent per 100 g body wt. MC was given at a dose of 20 mg/kg, DS at 100 mg or 200 mg/kg and DDTC at 500 mg/kg. Rats were treated with a single dose or once daily for 5 days except otherwise indicated, and killed 24 hr after the last dose. The rats showed no signs of toxicity with these drugs at the indicated doses and the weight gain was comparable to that of control groups.

Preparation of microsomes. All animals were decapitated between 8.00 and 9.00 a.m. to eliminate diurnal variations [19]. The procedure for isolation of intestinal microsomes was basically the same as previously reported [3] but with minor modifications. Microsomes were isolated from villous tip cells of mucosa from the upper part of small intestine. Beginning at pylorus, a consecutive 40 cm segment of small intestine was excised and rinsed with solution containing 0.15 M KCl and 0.05 M Tris–HCl buffer, pH 7.8. Any adherent mesenteric fat was carefully removed from the segment before it was slit open. Mucosal villous tip cells were removed from intestinal segments of two rats by light hand-pressured scraping with the edge of a glass slide and suspended in a total of 20 ml of the KCl–Tris buffer supplemented with glycerol (20 per cent v/v, final concentration), heparin (2 i.u./ml) and 25 mg trypsin inhibitor. The combination of glycerol and trypsin inhibitor has previously been shown to protect monooxygenase components from destruction and solubilization from endoplasmic reticulum while the addition of heparin resulted in an increased yield of microsomes [3]. The suspension was homogenized in a glass–teflon Potter–Elvehjem homogenizer at 500 r.p.m. and centrifuged at 600 *g* for 2 min and at 13,000 *g* for 10 additional min. Intestinal microsomes were isolated from supernatant by centrifugation at 105,000 *g* for 60 min. The microsomal pellet was washed and suspended in KCl–Tris buffer and recentrifuged at 105,000 *g* for 30 min. Intestinal microsomes contained cytochrome P-450 with negligible amounts of cytochrome P-420. Less than 20 per cent of the cytochrome P-450 content was lost during 8 hr storage on ice, whereas all experiments were performed within 2 hr after isolation of the microsomes. No spectrally detectable hemoglobin was present in the preparations which were also free from mitochondrial contaminations as established by the absence of succinate–cytochrome *c* reductase activity [20]. Livers were removed and chilled in ice cold KCl Tris–HCl buffer (pH 7.8). The hepatic microsomal fractions were prepared from 20 per cent homogenates in the same buffer with centrifugation and washing procedures as for intestinal microsomes.

Assays. Microsomal protein was measured according to Lowry *et al.* [21] with bovine serum albumin as standard. Reduced and CO-bound cytochrome

Table 1. Effects of disulfiram (DS) and diethyldithiocarbamate (DDTC) on benzo[*a*]pyrene (BP) monooxygenase activity and cytochrome P-450 content of rat intestinal and liver microsomes*

	Intestine		Liver	
Treatment	Cytochrome P-450 (pmoles/mg protein)	BP monooxygenase (pmoles product/mg protein per min)	Cytochrome P-450 (pmoles/mg protein)	BP monooxygenase (pmoles product/mg protein per min)
1 day				
Oil	24 ± 4 (100)	183 ± 13 (100)	495 ± 14 (100)	8505 ± 149 (100)
DS-100 mg/kg	25 ± 5 (104)	45 ± 10 (25)	460 ± 15 (93)	5788 ± 687 (68)
5 days				
Oil	24 ± 3 (100)	195 ± 21 (100)	464 ± 16 (100)	6887 ± 670 (100)
DS 100 mg/kg	41 ± 8 (171)	256 ± 28 (131)	380 ± 19 (82)	2905 ± 196 (42)
DS 200 mg/kg	44 ± 10 (183)	322 ± 28 (165)	276 ± 24 (60)	2004 ± 337 (29)
H_2O	23 ± 5 (100)	178 ± 15 (100)	420 ± 10 (100)	7622 ± 1143 (100)
DDTC 500 mg/kg	13 ± 3 (57)	90 ± 7 (51)	362 ± 14 (86)	3774 ± 450 (50)

* DS, DDTC or the appropriate vehicle was administered orally to adult male rats for 1 or 5 days. Intestinal and liver microsomal cytochrome P-450 concentrations and BP monooxygenase activities were determined as described in Materials and Methods. Values are the mean ± S.E. of at least four animals per group (livers) or six to ten per group (intestines). Numbers in parentheses are per cent of control.

Table 2. Effects of disulfiram (DS) and 3-methylcholanthrene (MC) pretreatment on benzo[a]pyrene (BP) monooxygenase activity of intestinal microsomes*

Treatment†	BP monooxygenase (pmoles product formed/mg protein per min)
Oil (24 hr)	180 ± 21
DS (24 hr)	30 ± 8
DS (48 hr)	49 ± 14
MC (24 hr)	2168 ± 225
DS (25 hr) + MC (24 hr)	2289 ± 157
DS (48 hr) + MC (24 hr)	2736 ± 116

* Results are expressed as mean ± S.E. of three different preparations.

† DS (100 mg/kg), MC (20 mg/kg) or the corn oil vehicle was administered orally. Numbers in parentheses indicate the time elapsed from the administration of the compound (DS or MC) until the animals were killed (i.e. DS 25 hr + MC 24 hr indicates that the rats were treated with DS 1 hr before MC administration and were killed 24 hr after MC administration).

P-450 difference spectra were measured and determined according to Omura and Sato [22], using an extinction coefficient of 91 $mM^{-1}cm^{-1}$. BP monooxygenase activity was assayed by the fluorometric method of Dehnen *et al.* [23], with 3-hydroxy-BP as reference standard (1 pmole product equals the fluorescence of 1 pmole of 3-hydroxy-BP). This method measures the formation of fluorescent BP-phenols, mainly 3-hydroxy- and 9-hydroxy-BP [24]. As was previously shown by separation of BP metabolites on high pressure liquid chromatography, these phenols represent 25–30 per cent of total BP metabolized by intestinal microsomes isolated from control or MC pretreated rats under the conditions used [3]. The incubation mixture, containing microsomes and a NADPH generating system in Tris–HCl buffer, pH 7.8, was preincubated for 2 min at 37° before BP (150 μM final concentration) was added. Incubations were carried out for 5 min [3] unless otherwise indicated. DDTC was added in aqueous solution and DS or α-naphthoflavone, in acetone, prior to the preincubation. Acetone slightly stimulated BP monooxygenase activity under these conditions. Results were corrected for this effect.

RESULTS

Pretreatment of rats with disulfiram (DS) decreased benzo[a]pyrene (BP) monooxygenase activity and concentration of cytochrome P-450 in liver, but exerted a biphasic effect on these parameters in small intestine (Table 1). Administration of a single oral dose of DS produced an inhibition of intestinal BP monooxygenase activity to about 25 per cent of the control level after 24 hr. However, after daily administration for 5 days, activity had increased to about 130 per cent of control values. Administration of DS, 200 mg/kg, for 5 days produced an even larger increase in BP monooxygenase activity. Cytochrome P-450 concentrations were not diminished in intestinal microsomes during the inhibitory phase of DS actions. Furthermore, the levels were almost doubled after 5 days of DS treatment. In contrast to DS, DDTC administration for 5 days resulted in decreases both in cytochrome P-450 concentrations and BP monooxygenase activity. Only small and significant effects were observed on these parameters 24 hr after a single dose of DDTC (not shown).

In the liver, DS administered as a single oral dose decreased BP monooxygenase activity without altering P-450 concentrations, but after 5 days of treatment both cytochrome P-450 concentrations and BP metabolism were decreased. Administration of DDTC for 5 days also lowered BP metabolism and cytochrome P-450 concentrations. Although the effects of a single oral dose of DDTC were not determined in liver, it has recently been reported that DDTC significantly decreases P-450 concentrations already after 24 hr [25].

A single oral dose of DS administered either 1 or 24 hr before the rats were treated with 3-methylcholanthrene (MC) did not reduce the induction capacity of MC in intestine (Table 2). The combination treatment of DS 1 hr before MC treatment, timed so that DS would be present before and during early phases of MC induction, had no significant effect compared to when MC was administered alone. However, the treatment with DS 24 hr before MC was administered resulted in an increase that was even greater than when MC alone was given.

The addition of α-naphthoflavone (ANF)

Table 3. Influence of α-naphthoflavone on benzo[a]pyrene (BP) monooxygenase activity of intestinal microsomes isolated from control, disulfiram (DS) or 3-methylcholanthrene (MC) pretreated rats*

α-naphthoflavone	BP monooxygenase (pmoles product formed/mg per min) Control†	DS‡	MC§
	140 ± 17 (100)	234 ± 18 (100)	2236 ± 135 (100)
0.1 mM	274 ± 25 (196)	361 ± 29 (154)	514 ± 64 (23)

* Data are expressed as mean ± S.E. of experiments with three different preparations. Numbers in parentheses indicate per cent of the incubation without α-naphthoflavone.

† Control rats received corn oil orally once daily for 5 days and were killed 24 hr after the last dose.

‡ Rats were treated with DS (200 mg/kg) in corn oil once daily for 5 days and killed 24 hr after the last dose.

§ Rats were treated with a single dose of MC (20 mg/kg) in corn oil and killed 24 hr later.

Table 4. *In vitro* inhibition by disulfiram (DS) and diethyldithiocarbamate (DDTC) of benzo[*a*]pyrene monooxygenase activity in intestinal microsomes from control, DS, DDTC and 3-methylcholanthrene (MC) treated rats

	Benzo[*a*]pyrene monooxygenase (pmoles of product formed/mg protein per min)							
	1 Day*			5 Days*				
Addition	Oil	DS 100 mg/kg	MC 20 mg/kg	Oil	DS 100 mg/kg	DS 200 mg/kg	H_2O	DDTC 500 mg/kg
None	183 ± 13 (100)	45 ± 10 (100)	2835 ± 61 (100)	195 ± 21 (100)	256 ± 28 (100)	322 ± 28 (100)	178 ± 15 (100)	90 ± 7 (100)
DS								
0.1 mM	104 ± 8 (57)	22 ± 3 (49)	1230 ± 65 (43)	74 ± 6 (38)	179 ± 20 (70)	210 ± 11 (65)	121 ± 9 (68)	69 ± 4 (77)
1.0 mM	54 ± 5 (29)	8 ± 1 (18)	1315 ± 71 (46)	37 ± 9 (19)	82 ± 5 (32)	104 ± 4 (32)	46 ± 7 (26)	31 ± 4 (34)
DDTC								
5 mM	105 ± 7 (57)	23 ± 5 (51)	2472 ± 81 (87)	78 ± 5 (40)	119 ± 19 (46)	157 ± 28 (49)	63 ± 11 (35)	43 ± 3 (48)

* Data are expressed as mean ± S.E. of at least three different preparations. Rats were treated with MC, DS, DDTC or the appropriate vehicle for the length of time indicated. Incubations were performed as described in Materials and Methods. Numbers in parentheses indicate the per cent of the corresponding control value.

increased BP monooxygenase activity of intestinal microsomes isolated from control or DS pretreated rats (Table 3). In contrast, ANF markedly inhibited the BP monooxygenase activity of MC microsomes as previously reported [3]. The CO-bound reduced difference spectrum of cytochrome P-450 in microsomes from DS treated rats showed an absorption maximum at 450 nm (not shown), indicating that the hemoprotein induced by repeated DS administration is similar to that of controls and not of the MC induced type, which exhibits an absorption maximum at 448 nm [3].

The inhibitory actions of DS and DDTC on BP monooxygenase activity *in vitro* were determined with microsomes isolated from intestine and liver after various treatments (Tables 4 and 5). DS exhibited a concentration dependent inhibition of intestinal BP monooxygenase activity in controls, DS and DDTC pretreated rats (Table 4). Similar inhibition was observed by DDTC. These indicated that the sensitivity of BP monooxygenase to the direct inhibition by DS or DDTC was not altered by prior exposure of rats to DS or DDTC. Disulfiram was 50-fold more potent than DDTC as an inhibitor of BP monooxygenase activity. However, after MC pretreatment, there appeared to be a fraction of BP monooxygenase resistant to the DS inhibition. Similarly, DDTC only slightly inhibited BP metabolism in intestinal microsomes isolated from MC treated rats. Both DS and DDTC inhibited BP metabolism in liver microsomes (Table 5) which appeared to be more sensitive than the intestinal microsomes. This was particularly evident with DDTC which consistently inhibited hepatic BP metabolism to about 15–20 per cent of control activity, whereas the intestinal metabolism was inhibited only to 40–50 per cent in

Table 5. *In vitro* inhibition by disulfiram (DS) and diethyldithiocarbamate (DDTC) of benzo[*a*]pyrene monooxygenase activity in liver microsomes isolated from control, DS or DDTC pretreated rats

	Benzo[*a*]pyrene monooxygenase (pmoles of product formed/mg protein per min)						
	1 Day*		5 Days*				
Addition	Oil	DS 100 mg/kg	Oil	DS 100 mg/kg	DS 200 mg/kg	H_2O	DDTC 500 mg/kg
None	8505 ± 149 (100)	5788 ± 687 (100)	6887 ± 670 (100)	2905 ± 196 (100)	2004 ± 337 (100)	7622 ± 1143 (100)	3774 ± 450 (100)
DS							
0.1 mM	2373 ± 77 (28)	1597 ± 93 (28)	1997 ± 83 (29)	944 ± 67 (33)	822 ± 12 (41)	2386 ± 91 (31)	1113 ± 79 (30)
1.0 mM	510 ± 178 (6)	301 ± 58 (5)	358 ± 28 (5)	218 ± 20 (8)	267 ± 56 (13)	313 ± 23 (4)	359 ± 60 (10)
DDTC							
5.0 mM	1344 ± 238 (16)	805 ± 75 (14)	930 ± 83 (14)	645 ± 46 (22)	471 ± 24 (24)	1120 ± 175 (15)	615 ± 34 (16)

* Data are expressed as mean ± S.E. of at least three different preparations. Rats were treated with DS, DDTC or the appropriate vehicle for the length of time indicated. Incubations were performed as described in Materials and Methods. Numbers in parentheses indicate the per cent of the corresponding control value.

Table 6. Effects of *in vitro* incubation conditions on the inhibition of intestinal benzo[a]pyrene (BP) monooxygenase by disulfiram (DS) and diethyldithiocarbamate (DDTC)*

Additions		BP monooxygenase (pmoles product formed/ mg protein per min)
Preincubation†	Incubation‡	
		134 ± 18 (100)
NADPH		115 ± 10 (86)
	0.1 mM DS	79 ± 11 (59)
0.1 mM DS		7 ± 3 (5)
NADPH + 0.1 mM DS		< 1 (0)
	1 mM DDTC	98 ± 13 (73)
1.0 mM DDTC		96 ± 8 (72)
NADPH + 1.0 mM DDTC		16 ± 4 (12)

* Results are expressed as mean ± S.E. of three different preparations. Numbers in parentheses indicate per cent of control rate.

† The 'preincubation' medium contained microsomes (0.7–1.0 mg/ml) in buffer plus the listed additions in the table. 'Preincubations' were performed for 10 min at 37°. NADPH refers to a NADPH generating system.

‡ The incubation medium contained the components in the preincubation medium, benzo[a]pyrene and the indicated additions plus the NADPH generating system if it had not been previously added. These mixtures were incubated for 5 min at 37°.

most cases. Pretreatment of rats with DS or DDTC had only small effects on the *in vitro* inhibition of hepatic BP monooxygenase by DS and DDTC.

Experiments designed to investigate modification of intestinal BP metabolism by DS and DDTC are presented in Table 6. Under similar experimental conditions as those inhibition studies shown in Table 4, 0.1 mM DS decreased the BP monooxygenase activity to 59 per cent of control. However, the addition of DS to the preincubation medium 10 min prior to additions of NADPH generating system and BP reduced BP metabolism to 5 per cent of control values. The preincubation with DS plus NADPH generating system completely abolished BP metabolism. In contrast, preincubation with DDTC did not affect BP metabolism unless the NADPH generating system was also included in the preincubation mixture. When microsomes were preincubated with only the NADPH generating system, a minor inhibitory action was observed.

DISCUSSION

Pretreatment with DS or DDTC has been shown to decrease the concentration of hepatic cytochrome P-450 and impair the metabolism of several xenobiotics dependent on this hemoprotein [16–17]. In this study, however, a 24 hr pretreatment with DS resulted in inhibition of hepatic BP monooxygenase activity but had no effect on cytochrome P-450 concentration (cf. Table 1). This supports a previous study with aniline hydroxylation [26]. However, continued treatments with DS for 5 days resulted in dose dependent decreases of both cytochrome P-450 and BP monooxygenase activity in the liver. In intestine, BP monooxygenase activity was markedly depressed by the same treatment without any effect on the concentration of cytochrome P-450 at 24 hr. The continued treatment for 5 days resulted in an increased, rather than decreased, concentration of cytochrome P-450 and BP monooxygenase activity (cf. Table 1). This is in contrast to liver. The increased activity of intestinal microsomes appears to be catalyzed by a cytochrome P-450 species of 'control' type; the DS-induced hemoprotein showed similar absorption characteristics and sensitivity to ANF as the cytochrome P-450 of control microsomes (cf. Table 3).

It has previously been proposed that the MC induced form of cytochrome P-450 might be the only inducible form present in the small intestinal mucosa [27]. However, spectral and metabolic studies have shown significant differences between control and MC induced forms of cytochrome P-450 [3, 8]. These findings are further supported by (1) the DS induced cytochrome P-450 is of the 'control' type and not sensitive to ANF (cf. Table 3) and (2) the BP monooxygenase activity of the intestinal microsomes from MC treated rats showed less sensitivity to inhibition by DS or DDTC in contrast to microsomes of control, DS or DDTC pretreated rats (cf. Table 4).

The metabolism of BP by liver microsomes was much more sensitive to inhibition with DS or DDTC than intestinal microsomes (Tables 4 and 5). Thus, DS or DDTC may be used as tools to differentiate between monooxygenase activities of small intestinal and hepatic microsomes, as well as of the activities dependent on different species of cytochrome P-450 within the small intestine. These differences may be of use for similar studies with other (extra)-hepatic monooxygenase activities.

The results suggest that differences exist between the liver and intestine with regard to the regulation of the monooxygenase enzymes. Administration of various enzyme inducers have previously revealed differences between liver and intestinal monooxygenase activities [2, 8], which are further illustrated by the different responses to DS treatment in these tissues. A simultaneous increase of intestinal monooxygenase activity during a decrease of activity in liver has also been observed after treatment with $CoCl_2$, which was explained on the basis of differential effects on heme oxygenase in these tissues

[28]. Administration of DDTC has recently been shown to stimulate heme oxygenase and δ-levulinic acid synthetase in liver [29]. Thus, changes of heme regulating enzyme activities could account for the observed effects after DS and DDTC treatments.

Pretreatment of rats with MC results in a large and rapid increase of small intestinal BP monooxygenase activity which is accompanied by an elevated concentration of microsomal cytochrome P-450 (P-448) [3, 20]. The administration of DS (1 or 24 hr) before the MC treatment did not interfere with these MC dependent increases (cf. Table 2). This indicates that the MC mediated induction of intestinal monooxygenase activities is not affected. Therefore, it seems likely that the normal influence of dietary factors on intestinal monooxygenase [11] would not be impaired during disulfiram therapy.

Several mechanisms have been suggested to explain the impairment of hepatic drug metabolism by DS and DDTC. In this regard, DS was approximately 50-fold more potent than DDTC as *in vitro* inhibitor of BP monooxygenase activity both with intestinal and liver microsomes (cf. Tables 4 and 5). DS binds to hepatic cytochrome P-450 producing a type 1 spectral change [26] and may cause competitive inhibition of drug metabolism [17]. The primary metabolite of DS, DDTC, is itself cleaved to diethylamine and CS_2 [30]. The latter may then undergo cytochrome P-450 dependent oxidation to carbonylsulfide [31]. This leads to a release of atomic sulphur which can covalently bind to microsomal proteins and destroy cytochrome P-450 [32]. Thus, DS can be reduced, metabolically activated and bound causing irreversible inactivation of cytochrome P-450. Both the intestine [33] and liver [34] have been shown to convert parathion to paraoxon in a similar manner, which in the liver has been shown to decrease the level of cytochrome P-450 [35]. The importance of NADPH dependent activation of DDTC for the *in vitro* inhibition of intestinal BP monooxygenase activity (DDTC does not seem to bind to cytochrome P-450 [26]), and to a lesser extent by DS, is apparent (cf. Table 6). Differences between intestine and liver may depend on the differential ability to activate these compounds to metabolites destroying cytochrome P-450 in respective organs.

The protective effect of DS and DDTC on GI tract carcinogenesis has been attributed in part to its antioxidant effect [13]. Treatment with DS or DDTC prevented the carcinogenic action of 1,2-dimethylhydrazine in the intestine [14], presumably by inhibiting the hepatic *N*-oxidation of the intermediary metabolite, azomethane [36, 37]. Independent of these mechanisms, the effects of DS and DDTC on BP metabolism may also be of importance. Inhibition of such procarcinogen metabolism may occur in several target tissues; DS also inhibits BP monooxygenase activity in forestomach and colon (unpublished observations). Also, the effects on the levels of cytochrome P-450 both in liver and in other target tissues (i.e. intestine) should not be excluded. A changed balance of oxidative capacity dependent on a variability in concentrations and in forms of cytochrome P-450, could lead to either a decreased formation of reactive metabolites and/or increased formation of inactive metabolites from procarcinogens, which in turn could explain the observed inhibition of gastrointestinal tract carcinogenesis.

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